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Research Paper

Dense Gas Processing of Micron-Sized Drug Formulations Incorporating Hydroxypropylated and Methylated Beta-Cyclodextrin

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Purpose. Because of their importance in pharmaceutical applications, hydroxypropyl-β-cyclodextrin and methyl-β-cyclodextrin have been selected to study the formation of micronized complexes incorporating active pharmaceutical ingredients (APIs) and cyclodextrins (CDs) by dense gas (DG) processing.
Methods. A single-step DG technique was used as an alternative to conventional methods for the manufacturing of API/CD complexes. The DG technology is highly attractive in the pharmaceutical industry because of its potential to generate micronized particles with controlled particle size distributions at moderate operating conditions. The effect of the aerosol solvent extraction system (ASES) processing on the dissolution performance of naproxen (NPX) was examined.
Results. The CDs were produced as microspheres smaller than 3 μm. The coprecipitation of each CD with NPX resulted in the production of microparticles with enhanced dissolution rates.
Conclusions. The ASES was operated under mild conditions and generated micron-sized spherical particles that could be of particular interest in formulations for pulmonary delivery.

Particular advantages of the technique are that (1) nontoxic solvents are used, and (2) it is suitable for the processing of thermally labile compounds. The proposed process can create opportunities to improve current administration routes and exploit novel delivery systems for drug formulations incorporating CDs.

KEY WORDS: carbon dioxide; cyclodextrin; dense gases; pulmonary drug delivery; supercritical fluids.

INTRODUCTION

The potential advantages of introducing cyclodextrins (CDs) into therapeutic formulations cover a variety of aspects in relation to the properties of the active pharmaceutical ingredient (API) and the administration route. Enhancement of API solubility and dissolution rate in aqueous-based solutions, stability against hydrolysis, oxidation, photolysis, and dehydration are examples of CD complexation features. Improving pharmaceutical formulations through the formation of complexes with CDs can overcome the need for structural changes to the APIs, which has various disadvantages. The approval of an improved formulation of a currently marketed API by the relevant authorities requires less time and financial effort compared with a newly developed API, thus facilitating marketability and patent life extension (1). A further advantage of utilizing accepted carriers relates to the phenomenon of drug absorption. The hydrophobic nature of many APIs is a disadvantage in approaching sufficient solubility in water-based biological fluids, but is favorable to their permeation across biological membranes. Cyclodextrins enhance the availability of hydrophobic APIs in biological fluids without affecting their molecular properties and hindering the intrinsic ability of the molecules to permeate biological membranes (2). The interaction between CDs and APIs is a function of the guest-molecule hydrophobicity and steric compatibility and is not dependent on the therapeutic class of the active. Reported API/CD inclusion complexes released in the market include steroids, cardiac glycosides, nonsteroidal anti-inflammatories, antidiabetics, antibiotics, peristaltic stimulants, and antifungal agents (3).

The primary application of CDs is as carriers for hydrophobic molecules in polar media. The efficacy of CDs as solubilizers for hydrophobic molecules is dependent on their solubility in the solvent with a limited solubility of the CD being a hindrance to a significant solubilizing effect. Of the natural CDs, β-CD exhibits an aqueous solubility that is significantly lower than the others. The solubility of β-CD in water decreases with increasing temperature, resulting in a further limitation of this carrier for the enhancement of API solubility. Despite the low aqueous solubility of β-CD, considerable interest is still focused on its applications. Beta-cyclodextrin has long been the most available of the CDs, and it demonstrates superior complexing ability for a number of compounds. The chemical modification of β-CD

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ABBREVIATIONS: API, active pharmaceutical ingredient; ASES, aerosol solvent extraction system; CD, cyclodextrin; DELOS, depressurization of an expanded liquid organic solution; HP-β-CD, hydroxypropyl-β-cyclodextrin; M-β-CD, methyl-β-cyclodextrin; NPX, naproxen; PGSS, particles from gas-saturated solutions; SAA, supercritical assisted atomization.

may reduce the parenteral toxicity of the molecule. The toxicity of β-CD has been attributed to the crystallization of insoluble complexes with cholesterol in the kidneys (4) and to the damaging of intercellular functions or extraction of membrane components (5). Because pulmonary or nasally administered β-CD enters systemic circulation, its use in lung and nasal formulations is not advisable (6).

The insertion of groups in the CD is also favorable to the formation of inclusion complexes because of the extension of the hydrophobic cavity. Of the many CD derivatives developed, some are available on the market in sufficient quantity and quality for pharmaceutical applications on the commercial scale. Such modified CDs include hydroxypropylated and methylated CDs (6).

Hydroxypropyl-cyclodextrins (HP-CDs) and methyl-CDs (M-CDs) belong to the group of alkyl ether derivatives of CDs with HP-β-CD being one of the more commonly used. The structure of hydroxypropyl and methyl CDs is shown schematically in Fig. 1. Both derivatives of β-CD are very effective as solubility and dissolution enhancers and as stabilizers (7).

Whereas HP-β-CD presents good safety properties, the systemic administration of M-β-CD causes higher toxicity than the native CD, probably because of the more pronounced affinity for cellular lipids (5). Hydroxypropyl-β-CD and M-β-CD are significantly absorbed through the lungs. Once the CDs are in the systemic system, they have similar

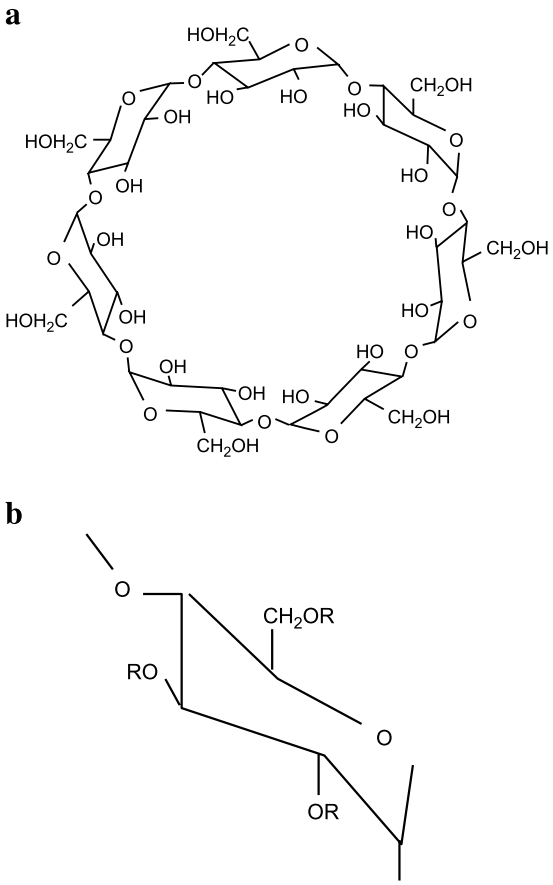

Fig. 1. (a) Chemical structure of β-CD; (b) chemical structure of alkylated cyclodextrins: methyl-CD, R = H or CH_3; hydroxypropyl-CD, R = H or –[CH_2–$CH(CH_3)$–O]H.

toxicological issues to parenterally administered dosages (4,8). Only HP-β-CD was found to be safe for parenteral administration and thus can be a candidate for pulmonary formulations. Whereas M-β-CD is not safe for parenteral administration, it is safe and has peculiar abilities as a permeation enhancer in nasal and transdermal delivery (3,9).

Micronized CDs can be effective in improving the aerodynamic performance of respiratory API particles (10). The use of CDs as hygroscopic growth inhibitors in drug formulations for lung delivery has been proposed (11). The reduction of moisture sorption may improve the stability of inhalable formulations toward hygroscopic growth during storage and in the lung region. The reduction of the particle growth favors the deposition of the formulation in the deep region of the lungs and drug bioavailability (11).

The commercial viability of HP-β-CD and M-β-CD formulations has been established, and different products have reached the market or are undergoing advanced clinical trials (3). Hydroxypropyl-β-CD and M-β-CD are easily accessible in the market and can bring substantial advantages to drug formulations. Because they have been accepted by regulatory authorities worldwide, newly processed pharmaceutical formulations including such CDs would be likely to be accepted for commercialization. Any novel technique that would add enhanced features to the processing of CD coformulations with APIs has the potential to impact on a significant section of the pharmaceutical market.

Dense gas (DG) processing can be a single-step alternative to conventional methods for processing drug/CD complexes and removing organic solvent residues. In particular, the DG technology could be a feasible alternative to liquid–liquid antisolvent crystallization, freeze-drying, and coevaporation for the production of drug/CD systems. Additionally, DG technology presents the potential to generate micronized particles at moderate operating conditions with a controlled particle size distribution, which is highly attractive for the micronization of pharmaceuticals. The formation of drug/CD micronized complexes can create opportunities to improve current administration routes and exploit novel delivery systems.

Dense gases are fluids that are near or above their critical points, generally with reduced temperature and pressure between 0.9 and 1.2. Near-critical fluids exhibit distinctive properties such as low viscosity, high diffusivity, low surface tension, and solvation power. Because the properties of DGs are dependent on pressure and temperature, they offer a unique environment with tunable properties that is ideal for the formulation of pharmaceuticals. Cyclodextrins have low solubility in DGs and can be processed by DG antisolvent techniques.

Dense gases can expand solutions, thereby lowering the solvent power of the solvent and triggering the precipitation of the solute. Dense gas antisolvent processes have been widely used for pharmaceutical applications because of the possibility of operating under mild conditions, thus preserving the properties of labile compounds and because of their ability to generate microsized particles with optimum morphology for various drug applications. A major distinction is made between batch and semicontinuous processes. In a batch process, a liquid solution is introduced in a high-pressure vessel. Subsequently, the system is pressurized with

a DG that expands the solution to an extent that is dependent on the operating conditions. Stirring devices can be used to improve mass transfer in the system. Once the final pressure is reached and the precipitate is formed, the expanded solvent is purged from the system, and the residual solvent is removed from the product by a flux of DG antisolvent. The system is then depressurized and the product collected.

Gas antisolvent processes can also be performed in a semicontinuous mode by introducing the solution and the antisolvent in a high-pressure chamber continuously, until the desired amount of product is formed. The solution flow is then stopped, and the residual solvent is extracted from the system by a flow of DG. The system is finally depressurized to enable the collection of the product. To promote the prompt expansion of the solution and the formation of small particles, the solution is generally introduced through an atomization nozzle.

In this study, the feasibility of utilizing a semicontinuous DG antisolvent technique to process HP-β-CD and M-β-CD systems, including the hydrophobic model compound naproxen, was examined. Naproxen belongs to a class of active pharmaceutical ingredients (nonsteroidal anti-inflammatory drugs) for which dissolution and solubility properties can be enhanced by combination with CDs. Therefore, both the drug absorption via administration sites where a limited amount of biological fluid is present, and a more rapid onset of action, are promoted (12).

In previous studies (unpublished data), the same process was applied to the model drug generating crystalline products with lamellar or needle-like morphology. The solvents selected were ethanol, dimethyl sulfoxide (DMSO), DMSO/ethanol (unpublished data), and acetone (13). In all cases, the process produced negligible yields because of the solubility of NPX in the CO_2–solvent systems.

In this work, the effects of the concentration, pressure, and CD derivatives on the characteristics of the precipitate were studied. The dissolution performances of API/CD coprecipitated systems by the aerosol solvent extraction system (ASES) method were compared to samples of similar composition produced from coevaporation and physical mixing.

MATERIALS AND METHODS

Materials

Naproxen [(*S*)-6-methoxy-α-methyl-2-naphthaleneacetic acid, purity 99+%] was purchased from Sigma (St. Louis, MO, USA). Hydroxypropyl-β-cyclodextrin (2-hydroxypropyl-β-cyclodextrin, degree of substitution 0.58–0.73) and M-β-CD (cyclomaltoheptaose, methyl ether, degree of substitution 1.7–1.9) were kindly donated by Wacker-Chemie (Munich, Germany). The water content of the untreated CDs was measured by thermal gravimetric analysis and was found to be 9 and 4% for HP-β-CD and M-β-CD, respectively. Carbon dioxide (BOC Gases Food Grade, 99.95% purity) was used as antisolvent. Acetone (Unichrom, Sydney, Australia), ethanol (Merck Chemicals, Darmstadt, Germany), and DMSO from Univar (Rotterdam, The Netherlands) were of analytical grade and were used as received. Distilled water was used throughout this work.

Analytical Methods

Scanning Electron Microscopy

Scanning electron microscopy (SEM) was used to observe the product morphology and particle size. Specimens were prepared by placing the samples on metal stubs covered with double-sided carbon tape (ProSciTech, Queensland, Australia). Specimens were gold-coated using a Polaron Sputter coater. The voltage and the exposure time were set at 50 mV and 4 min, respectively. Samples were analyzed with a Hitachi S4500 Field Emission SEM high-resolution (1.5 nm), tilting stage, Robinson Back-Scatter Detector, Oxford Cathodoluminescence Detector (MonoCL2/ISIS), Link ISIS 200 Microanalysis System.

Dissolution Studies

Dissolution tests were conducted utilizing a VK 7000 dissolution apparatus (Varian Inc., Palo Alto, USA). The shaft rotation was 100 rpm, and the bottom of the shaft was 2.5 cm above the bottom of the vessel. The solute was dissolved in 900 ml distilled water at 37°C. At suitable time intervals, 5-ml aliquots were withdrawn through a 0.7-μm filter (Varian). Samples were withdrawn at the midway point between the bottom of the vessel and the surface of the dissolution medium. The amount of drug in the filtered samples was assessed by high-performance liquid chromatography (HPLC) analysis. The dissolution test was run under sink conditions.

Thermal Analysis

Differential scanning calorimetry (DSC) was performed using a 2010 Differential Scanning Calorimeter (TA Instruments, Newcastle, DE, USA) to investigate the interaction between the drug and the CD in the samples. The analysis was run on 5- to 7-mg samples in uncovered aluminum pans under a 12-ml/min nitrogen flow. The heating rate was set at 10°C/min.

Thermogravimetrical analysis was performed using a 2050 Thermogravimetric Analyzer (TA Instruments). The weight loss of 4- to 8-mg samples was measured under a heating program of 10°C/min.

Refractive Index

The concentration of M-β-CD in ethanol solutions was determined at 17°C by refractive index measurements. An ATAGO 1T Abbe refractometer coupled to an ATAGO digital thermometer was used.

High-Performance Liquid Chromatography

High-performance liquid chromatography was used to quantify the NPX content in NPX/CD mixtures using UV detection. The HPLC system used consisted of a Waters 600 HPLC pump, a Waters 717 plus autosampler, and a Waters 996 photodiode array detector. The mobile phase (50:49:1 acetonitrile/water/glacial acetic acid) was passed through a C_{18} LiChrosorb (Phase Separation) column (4.6 × 250 mm,

5 μm) at 1 ml/min at room temperature. In each analysis, 10 μl of a NPX/CD solution was injected, and NPX concentration was detected at a wavelength of 260 nm. The API loading in NPX/CD samples was determined by dissolving a known amount of the sample in distilled water and measuring the NPX concentration by HPLC analysis. The amount of CD was calculated as the difference between the sample weight and the NPX content.

Sample Preparation

Coevaporation

Coevaporated samples were prepared by dissolving NPX/CD mixtures of the appropriate composition in the minimum amount of 0.22 g/l aqueous ammonia solution. The solvent was evaporated in a rotary evaporator (RV 05S5 from Janke & Kunkel GmbHu.CoKG, Staufen, Germany) at 50°C under vacuum. The presence of residual ammonia was tested using the Nessler test (14,15).

Dense Gas Technique

Melting-Point Depression Study

A study of the melting-point depression of M-β-CD was carried out using a static technique (16). In summary, a glass tube (5.8-mm i.d.) loaded with the solute was placed inside a high-pressure view cell (Jerguson sight gauge series no. 32). The vessel was placed in a constant temperature water bath. After air was purged from the system, the pressure was increased incrementally by introducing CO_2. The system was isolated at each interval to approach equilibrium, and changes in the physical status of the CD were observed visually.

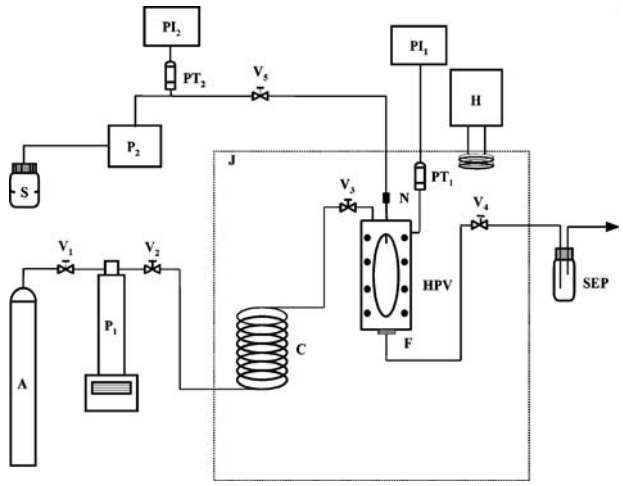

Fig. 2. Schematic diagram of the experimental apparatus for the ASES technique. **A** Gas cylinder, **C** Heating coil, **F** Filter, **H** Heater, **HPV** High pressure vessel, **J** Water bath, **N** nozzle, $\mathbf{P_n}$ Pump, $\mathbf{PI_n}$ Pressure indicator, $\mathbf{PT_n}$ Pressure transducer, **S** Solution, **SEP** Separator, $\mathbf{V_n}$ Valve.

Antisolvent Precipitation of Cyclodextrin Systems

The DG antisolvent technique utilized in this work was the ASES. A schematic diagram of the apparatus is shown in Fig. 2. The temperature was controlled by immersing the apparatus in a water bath incorporating a Thermoline Unistat heater circulator. The pressure was monitored with a Druck pressure transducer (model PDCR 911) coupled to a Druck pressure indicator. Once thermal equilibrium was attained, the system was pressurized with CO_2 using a Series 500D ISCO syringe pump. The CO_2 flow rate was then adjusted between 10 and 15 ml/min via a metering valve located prior to the exit. Solutions of the compounds to be processed were sprayed into the precipitation chamber cocurrently with the CO_2 flow rate through a 20-cm-long, 0.16-cm o.d. tube (180-μm i.d.) by an HPLC pump (Waters M6000A) at a flow rate of 0.3 ml/min.

Hydroxypropyl-β-cyclodextrin and NPX/HP-β-CD (1:1 mol ratio) were precipitated from DMSO/acetone (1:1 vol ratio) or ethanol. Methyl-β-cyclodextrin and NPX/M-β-CD equimolecular mixtures were precipitated from DMSO/acetone (1:1 vol ratio), ethanol, and acetone. The 1:1 DMSO/acetone mixture was used because it can be used for the DG antisolvent processing of β-CD, thus enabling further investigations about the dependence of product morphology on the chemical structure of the CD. The solubility of β-CD in some CO_2-expandable solvents, such as ethanol, methanol, and acetone, is negligible. Beta-cyclodextrin is, however, freely soluble in DMSO, which can be expanded by CO_2. Formation of fine droplets and atomization may be hindered when a viscous solvent such as DMSO is used (13). Consequently, a mixture of DMSO with a volatile solvent such as acetone was utilized to improve the atomization and generate fine particles.

In systems containing DMSO, alternate spray/wash stages were used, which involved spraying a predetermined volume of the solution and purging the total volume of the system with neat CO_2 at the experimental conditions. The alternate spray/wash mode was implemented to limit the concentration of DMSO in the precipitation vessel because, as evidenced in other studies (17,18), the extraction of DMSO during DG antisolvent processing can be otherwise unsatisfactory. The spray/wash stages were repeated until the desired volume of solution was delivered to the system. Acetone-based solutions were sprayed continuously, whereas ethanol solutions were processed either continuously or according to a spray–wash sequence. Subsequently, the solution flow was stopped, and the product was rinsed with CO_2 to remove residual solvent. Finally, the system was depressurized, and the precipitate was collected.

Samples were characterized in terms of particle size, morphology, and drug loading. The dissolution rate of DG-processed samples was compared to coevaporated and physically combined mixtures of the same composition.

Solubility of Methyl-β-Cyclodextrin in Modified Carbon Dioxide

The solubility of CD in CO_2 was measured by a dynamic technique (19). The apparatus for solubility measurements is depicted in Fig. 3.

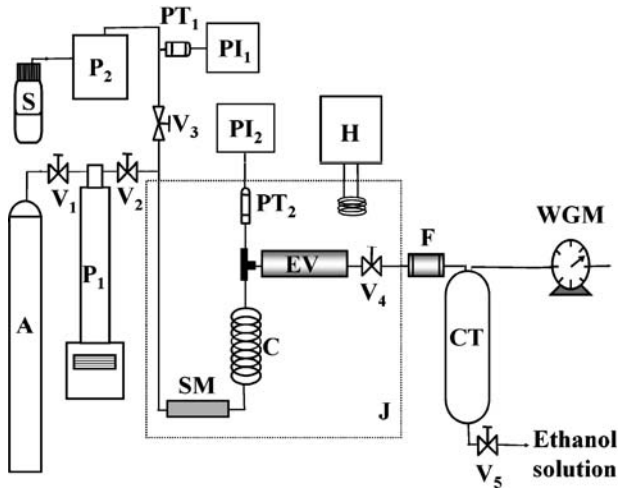

Fig. 3. Schematic diagram of the apparatus used to measure M-β-CD solubility in ethanol-modified CO_2. **A** Gas cylinder, **C** Heating coil, **CT** Cold trap, **E** Ethanol, **EV** Extraction vessel, **F** Filter, **H** Heater, **J** Water bath, $\mathbf{P_n}$ Pump, $\mathbf{PI_n}$ Pressure indicator, $\mathbf{PT_n}$ Pressure transducer, **SEP** Separator, **SM** Static mixer, $\mathbf{V_n}$ Valve, **WGM** Wet gas meter.

The experiments were performed by passing an ethanol/CO_2 mixture through an extraction vessel loaded with M-β-CD. Carbon dioxide was delivered to the system with a 500D ISCO pump. The extraction vessel was a 20-cm-long stainless-steel tube (6.35-mm i.d.) loaded with alternated layers of M-β-CD and glass wool. A 0.45-μm sintered filter (inline Swagelok filter F series) was placed in line to avoid entrainment of undissolved solid during the solubility measurement. During each experiment, the ISCO pump was operated in the constant pressure mode, and the flow rate was adjusted by a metering valve (31 series union bonnet metering valve from Whitey) placed before the cold trap. Ethanol was pumped into the system using a HPLC pump (Waters M6000A) and was mixed on-line with the CO_2 using a static mixer (Koflo part no. 1/2-.095-3-21H-1TIG). The system was placed in a constant temperature water bath maintained at 25 ± 0.1°C with a Thermoline Unistat heater circulator.

The system was first pressurized with CO_2 to the operating pressure, and the DG flow was adjusted. Subsequently, ethanol was pumped into the system, mixed on line with the CO_2, and passed through the system to purge neat CO_2. After reaching equilibrium, the ethanol/CO_2 mixture was passed through the extraction vessel. The ethanol/CO_2-laden solution separated upon depressurization through a metering valve. The mass of CO_2 utilized was measured at ambient conditions with a wet gas meter (Alexander Wright DM3A). The organic solution was collected in a 100-ml stainless-steel vessel that was refrigerated by immersion in acetone and dry ice and was assessed for M-β-CD content by refractive index measurement.

RESULTS AND DISCUSSION

Melting-Point Depression Study

The phase behavior of the M-β-CD/CO_2 system was observed at 45°C and pressures up to 110 bar. The CD maintained the solid state at pressures below 60 bar. Partial liquefaction of the M-β-CD was observed between 60 and 100 bar, and the CD changed to a clear viscous gel at 110 bar.

Table I. List of the Experimental Conditions for the ASES Precipitation of HP-β-CD and NPX/HP-β-CD systems

[NPX]/[HP-β-CD] (M)	Pressure (bar)
Solvent: DMSO/acetone 1:1 (v/v)	
0/0.05	80
Solvent: ethanol	
0/0.02	65
0/0.02	70
0.02/0.02	80(a)
0.02/0.02	80(b)
0.02/0.02	80(c)

The solution flow rate was 0.3 ml/min, the temperature was 25°C, and the CO_2 flow rate was 10 ml/min measured at the operating conditions. The nozzle size was 180 μm. Spray time: (a) 3 min, (b) 6 min, and (c) 9 min.

Antisolvent Precipitation of Naproxen/Cyclodextrin Systems

Hydroxypropyl-β-Cyclodextrin

The experimental conditions adopted for the processing of HP-β-CD systems are presented in Table I. Hydroxypropyl-β-cyclodextrin was precipitated from 0.05 M DMSO/acetone at 80 bar producing spherical particles with a broad size distribution. The precipitation of HP-β-CD from ethanol, alone or in an equimolecular mixture with NPX, generated discrete spherical particles with a diameter of about 200 nm and very narrow particle size distribution as shown in Fig. 4.

The product recovery and drug loading, obtained from 0.02 M ethanol solutions of NPX/HP-β-CD equimolecular mixtures, were evaluated as a function of the spray time. Three spray times were tested: 3, 6, and 9 min, corresponding to the processing of 1, 2, and 3 ml, respectively. The spray–washing stages were repeated four times in each experiment. The results are reported in Fig. 5.

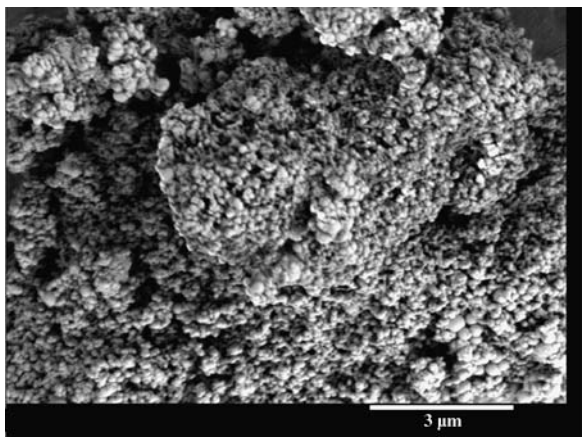

Fig. 4. Particles produced by ASES processing NPX/HP-β-CD equimolecular solutions in ethanol at 80 bar. Solution concentration: 0.02 M; solution flow rate: 0.3 ml/min; nozzle size: 180 μm; temperature: 25°C.

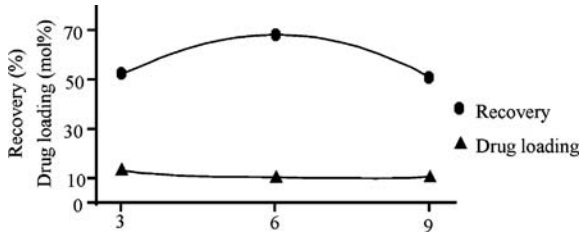

Fig. 5. Product recovery and drug loading *vs.* spray time in ASES-processed NPX/HP-β-CD samples produced at 80 bar and 25°C from 0.02 M equimolecular solutions in ethanol.

Product recovery varied between 51 and 68% with the higher product recovery being obtained for a spray time of 6 min. The trend of product recovery with the spray time can be explained on the basis of the filter efficiency in retaining the precipitate and of the solvent/antisolvent ratio in the precipitation chamber. Issues related to the efficiency of filtering devices to retain nanoparticles generated by the ASES process have been observed in different systems. The primary particle size of the ASES-manufactured CD is below the nominal porosity of the filtering element used to retain the precipitate in the system. Consequently, single particles and small particle aggregates could not be recovered. A longer spray time caused a higher particle density in the precipitation chamber that, in turn, favored particle aggregation and retention in the system. Longer spray times also resulted in higher solvent/antisolvent ratios in the precipitation chamber because, initially, the solution flow entering the precipitation chamber came across neat antisolvent, and with the continuation of the spraying, the solvent accumulated in the systems until a steady ratio was attained. Increments of the solvent/antisolvent ratio are unfavorable to the attainment of high recoveries because a lower level of supersaturation can be achieved.

The drug loading was generally unaffected by the spray time and was about 11 mol%. As mentioned in the introduction, when NPX was processed under similar experimental conditions, negligible yields were attained, whereas about 10% of the drug was recovered in the ASES-processed NPX/CD systems. The scenario suggests that only NPX that had interacted with HP-β-CD in the solution, i.e., which was included in the CD cavity, would be recovered in the treated material. The NPX/CD ratio in the processed sample did not change with the efficiency of HP-β-CD precipitation, thus corroborating this hypothesis.

The drug loading in the ASES-coprecipitated sample was lower than in the original solutions. The result may be caused by the extraction of NPX from the precipitate by the DG phase present in the precipitation chamber during processing or to the precipitation of only a small fraction of the drug in the solution. Samples obtained at higher spray times were exposed longer to an ethanol-rich phase. If the precipitated drug was effectively solubilized into the ethanol-rich phase, the drug loading obtained at 9-min spray time would have been lower than at 3- and 6-min spray time. The consistency of the API loading suggests that the solubility of NPX in the ethanol/CO_2 environment was not a major factor in determining the NPX content in the processed material. The NPX content in the ASES-processed samples may be related to the drug/CD interaction in the solution. The stability constant of the NPX/HP-β-CD in aqueous solutions at 25°C is quite high (2083 M^{-1}) (15). Nevertheless, the API/CD interaction in ethanol solution may be much less favorable. Factors affecting the NPX/HP-β-CD complex formation may be a better drug/solvent interaction, which is unfavorable to the complex formation, and the replacement of the crystallization water in the CD cavity with the less polar ethanol (1). A driving force in the formation of CD complexes is the energy gained when less polar compounds replace existing guest molecules in the CD cavity (1); the replacement of ethanol molecules with NPX may give a less significant energy gain than the replacement of water molecules.

Methyl-β-Cyclodextrin

To observe the effect of the CD type on particle morphology and size, M-β-CD was precipitated from a 0.05 M DMSO/acetone solution at 80 bar. The experiment produced submicron spheres, which were highly agglomerated as can be observed in Fig. 6.

The effect of solvent, pressure, and concentration on the precipitation of M-β-CD and NPX/M-β-CD was studied on samples produced with a 180-μm nozzle and 0.3-ml/min solution flow rate. A list of the operating conditions adopted is presented in Table II.

The effect of solvent has been observed on 0.05 M M-β-CD ethanol or acetone solutions, processed at 65 bar. The particles obtained from the ethanol solution were quite discrete microspheres between 0.3 and 3 μm, whereas the acetone solution produced more aggregated microspheres sized about 0.2 μm.

Particle aggregation in samples produced from ethanol solutions was dependent on the operating pressure. The effect of pressure was studied between 70 and 80 bar on 0.02 M NPX/M-β-CD equimolecular solutions and between 70 and 160 bar on 0.05 M M-β-CD solutions. The pressure did not affect the particle size, between 1 and 2 μm in all the systems, but both the M-β-CD and the NPX/M-β-CD systems exhibited a less dramatic particle aggregation at higher pressures.

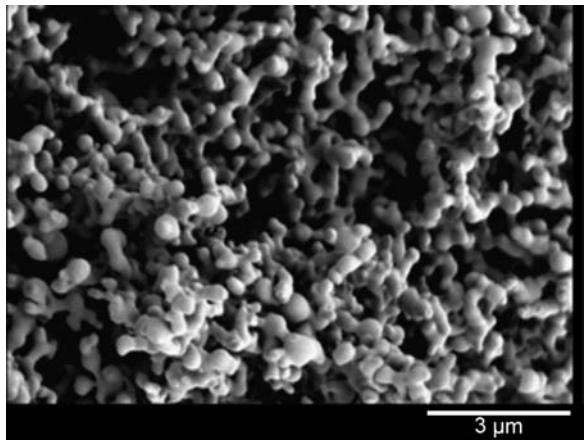

Fig. 6. Particles produced by ASES processing NPX/M-β-CD equimolecular solutions in ethanol at 80 bar. Solution concentration: 0.05 M; solution flow rate: 0.3 ml/min; nozzle size: 180 μm; temperature: 25°C.

Table II. List of the experimental conditions for the ASES precipitation of M-β-CD and NPX/M-β-CD systems

[NPX]/[M-β-CD] (M)	Pressure (bar)
Solvent: DMSO/acetone 1:1 (v/v)	
0/0.05	80
Solvent: acetone	
0/0.1	65
0.05/0.05	66
0.02/0.02	70
0.05/0.05	74
Solvent: ethanol	
0/0.05	65
0/0.05	70
0/0.05	160
0.02/0.02	80
0.05/0.05	70
0.05/0.05	80

The solution flow rate was 0.3 ml/min, the temperature was 25°C, and the CO_2 flow rate was 10 ml/min measured at the operating conditions. The nozzle size was 180 μm.

The effect of solution concentration was observed on NPX/M-β-CD systems precipitated from 0.02 and 0.05 M ethanol solutions at 80 bar. Similar to the operating pressure, solution concentration affected the particle agglomeration more than the particle size. Particles produced from 0.05 M solutions were agglomerated to a significant extent, whereas individual particles formed from the 0.02 M solutions.

The results suggest that the solvent retained part of its ability to interact with the CD in the presence of CO_2 (20). At the higher operating pressure, the interaction between the CD and the ethanol-rich DG phase would be less strong because of the reduction in the solvent power of ethanol (20). As a possible consequence, particle coalescence could be less dramatic. At the lower solution concentration, the coalescence phenomenon would be reduced because of the improved dispersion of the precipitate particles in the precipitation chamber. Experiments performed on M-β-CD ethanol solutions were characterized by wide fluctuations of the antisolvent flow. A mechanism related to such phenomenon could be the passage of small CD particles through the filter and their accumulation in the small clearance of the needle valve. The phenomenon could be common to the other CD systems, but in the processing of M-β-CD, the blockage of the needle valve would have been more considerable because of the adhesive properties of this CD. Alternatively, the CD could have been solubilized in the ethanol-rich phase and, subsequently, precipitated in the valve as a result of the sharp cooling effect related to the pressure reduction. In this case, a reasonable amount of the CD should have been soluble in expanded ethanol at the experimental conditions. The issue of fluctuations in the CO_2 flow was addressed by investigating the solubility of the CD in ethanol/CO_2 mixtures.

The solubility of M-β-CD in ethanol/CO_2 mixture was measured at 80 bar and 25°C. The mol fraction of ethanol in the DG mixture was 0.06. The composition of the ethanol/CO_2 mixture mirrored the solvent/antisolvent ratio existing in the precipitation chamber during the ASES process. It was found that 17 mg of M-β-CD was extracted for 1 ml of the organic solvent. Such a noticeable solubility in the ethanol/CO_2 mixture supports the hypothesis that the valve blockage was determined by the depressurization and cooling of the expanded ethanol solution of the CD.

The results demonstrate that the expanded solvent retains its solvation power toward M-β-CD, which is a hindrance to the application of the ASES as a micronization process. The solvation power of expanded ethanol may be an advantage in other DG processes. Dense gas techniques that are potentially suitable for the manufacturing of API/M-β-CD systems, and thus we indicate for further studies, are the depressurization of an expanded liquid organic solution (DELOS) (21) and the supercritical assisted atomization (SAA) (22,23) processes. In the DELOS process, solute precipitation occurs upon rapid depressurization of expanded solutions. Processing M-β-CD solutions in expanded ethanol could bring satisfactory yields. The SAA process is operated spraying a DG-expanded solution through a nozzle into a heated chamber. The presence of the DG enhances the jet atomization through a nozzle. Other DG processes of interest may exploit the phase transition that M-β-CD undergoes at mild temperatures when exposed to CO_2 (i.e., 45°C and 110 bar). The DG processes include the particles from gas-saturated solutions (PGSS) (24), hot melt extrusion (25), and blending (26). The PGSS process consists of the rapid depressurization of compounds melted by exposure to DGs and would be limited to those API/M-β-CD systems in which the active also liquefies at the experimental conditions. Hot melt extrusion can take advantage of the low-temperature phase transition induced by the CO_2 and of the foaming properties of the DG. The process may enable the production of API/M-β-CD blends with high-specific surface. For

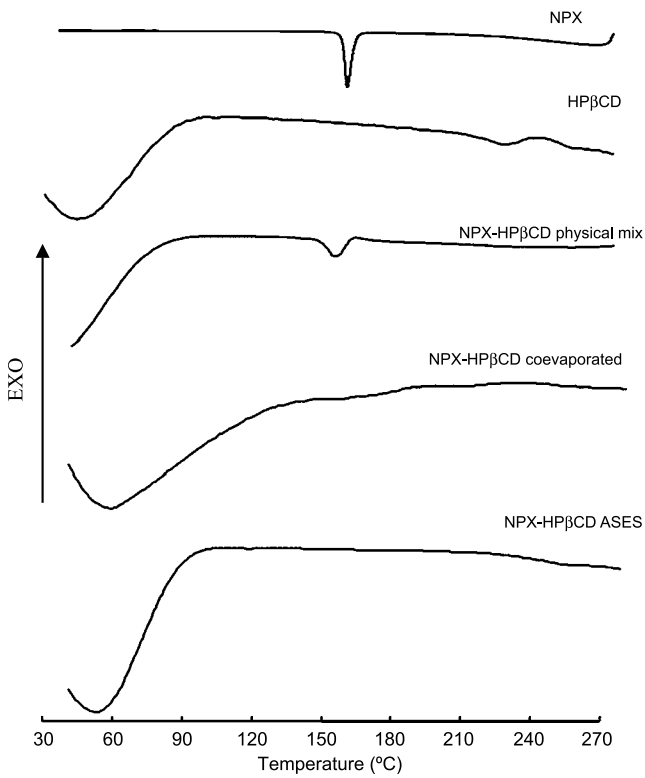

Fig. 7. Differential scanning calorimetry (DSC) diagrams of NPX and HP-β-CD systems.

applications in which the particle size is not a crucial point, API/M-β-CD blends could be produced by melting the CD by exposure to CO_2 in a stirred vessel in the presence of the active compound. A similar process has been used in the formation of blends of bioactive ingredients with polymers (26).

Naproxen–Cyclodextrin Interaction

Upon heating, HP-β-CD and M-β-CD lose the adsorbed water and water of crystallization. Evidence for the phenomenon is a broad endothermic peak below 100°C in their DSC diagrams (27). Thermal degradation takes place at temperatures above 280°C (27).

Crystalline NPX melts at 160°C as indicated by a single sharp peak in the DSC diagram. The signal was detected in the DSC diagram of NPX/HP-β-CD physical mixtures, indicating the presence of crystalline drug. The exothermic phenomenon that takes place subsequently to the NPX melting is the formation of an inclusion complex between the liquefied drug and the CD (28).

Crystalline NPX was not detected by the DSC analysis of NPX/M-β-CD physical mixtures and NPX/CD samples produced by ASES and coevaporation. The DSC diagrams are presented in Figs. 7 and 8. Derived CDs are amorphous and can reduce the crystallinity of blended compounds. Crystallinity reduction in CD blends can be induced during DSC analysis because of the administration of thermal energy as can be deduced from the DSC results of the NPX/M-β-CD physical mixtures. While reduced in intensity, the NPX melting peak is still evident in the DSC diagram of NPX/HP-β-CD physical mixtures, whereas it is absent in coevaporated and ASES-processed samples, indicating that both processes either reduce the crystallinity of the drug or induce the formation of inclusion complexes.

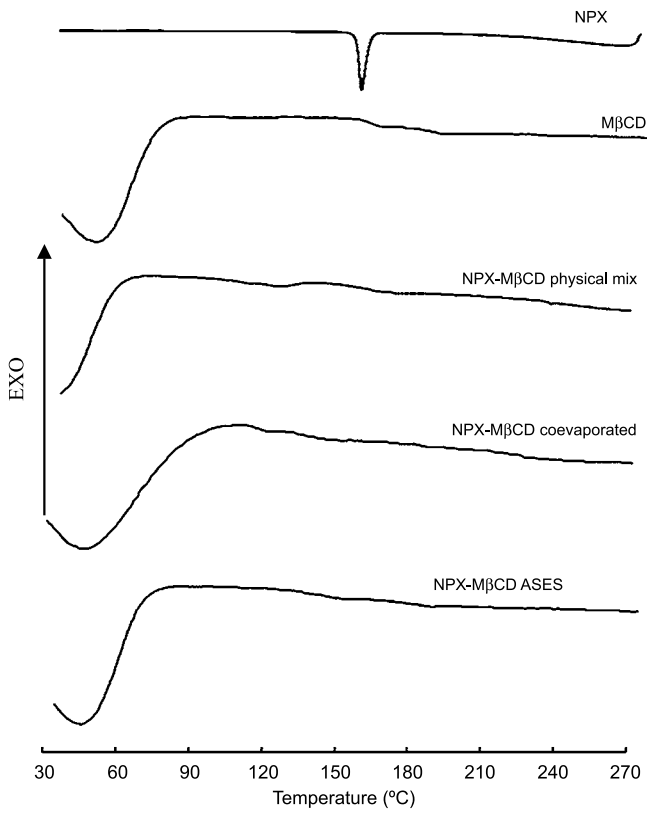

Fig. 8. DSC diagrams of NPX M-β-CD systems.

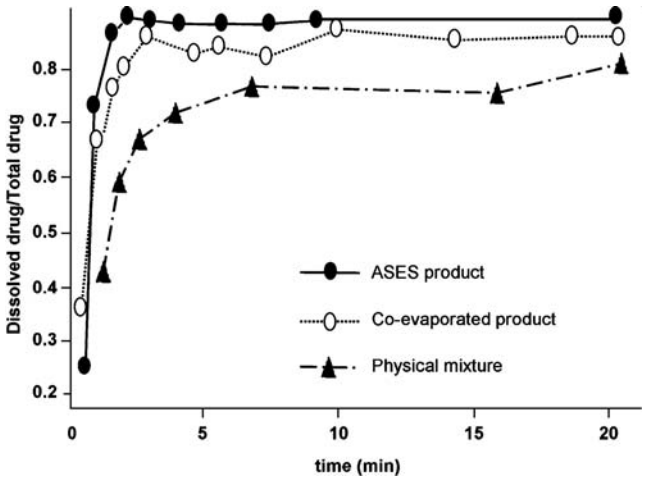

Fig. 9. Dissolution profile of NPX from NPX/HP-β-CD mixtures prepared by physical mixing, coevaporation, and ASES precipitation.

Dissolution Studies

Dissolution studies were conducted on ASES-manufactured samples, on physical mixtures, and on coevaporated samples of similar composition. The drug loading in the selected systems was 14 and 15 mol% for NPX/HP-β-CD and NPX/M-β-CD, respectively. The deviation between the results of the three tests was below 3%.

The dissolution profiles of NPX from all systems showed a clear dependence on the process. In particular, the dissolution rate of NPX from the ASES-treated samples is similar to coevaporated samples and faster than from physical mixtures, as can be observed by the dissolution profiles of the NPX/HP-β-CD systems presented in Fig. 9. The results show that particle size reduction did not have a significant impact on the drug dissolution because coevaporation did not produce micronized particles.

CONCLUSIONS

The results demonstrate that ASES precipitation is a viable technique for the micronization of CDs. Hydroxypropyl-β-cyclodextrin and M-β-CD were precipitated as microspheres smaller than 3 μm. The coprecipitation of each CD with NPX resulted in the production of microparticles with enhanced dissolution rates. The technique can be particularly convenient for processing the derivatized CDs because a nontoxic solvent such as ethanol can be used, whereas the use of DMSO would be required in the ASES processing of the native CD. The application of the ASES technique can be especially advantageous compared to the coevaporation method for those systems containing CD in which one or more components are heat-sensitive. Moreover, the ASES method can be utilized to manufacture therapeutic formulations in which the particle size is an important aspect. Hydroxypropyl-β-cyclodextrin systems were robust toward changes in the experimental conditions, which would be a

positive aspect in scale-up of the process. Further emphasis should be focused on systems containing HP-β-CD that can improve the hydrodynamic properties of micronized APIs and that are considered safe for lung administration.

The ASES processing of M-β-CD was limited by its solubility in expanded polar solvents such as ethanol. Because of the importance of API/M-β-CD formulations in topical and nasal drug delivery, there is justification to undertake further studies involving DG processing of systems containing M-β-CD. Dense gas processes that may be appropriate for the production of M-β-CD formulations include DELOS, PGSS, and blending.

The coprecipitation of API/CD systems from organic solutions is limited to those actives that are soluble and stable in organic solvents. The precipitation of API/CD systems from water solutions could extend the applicability of ASES to the preparation of CD formulations including water-soluble APIs such as some therapeutic proteins.

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