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Preparation of olanzapine and methyl-β-cyclodextrin complexes using a single-step, organic solvent-free supercritical fluid process: An approach to enhance the solubility and dissolution properties.

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Graphical abstract

ABSTRACT

The purpose of this study was to evaluate a single-step, organic solvent-free supercritical fluid process for the preparation of olanzapine-methyl-β-cyclodextrin complexes with an express goal to enhance the dissolution properties of olanzapine. The complexes were prepared by supercritical carbon dioxide processing, co-evaporation, freeze drying and physical mixing. The prepared complexes were then analysed by differential scanning calorimetry, X-ray powder diffraction, scanning electron microscopy, solubility and dissolution studies. Computational molecular docking studies were performed to study the

formation of molecular inclusion complexation of olanzapine with methyl-β-cyclodextrin. All the binary mixtures of olanzapine with methyl-β-cyclodextrin, except physical mixture, exhibited a faster and greater extent of drug dissolution than the drug alone. Products obtained by the supercritical carbon dioxide processing method exhibited the highest apparent drug dissolution. The characterisation by different analytical techniques suggests complete complexation or amorphisation of olanzapine and methyl-β-cyclodextrin complexes prepared by supercritical carbon dioxide processing method. Therefore, organic solvent-free supercritical carbon dioxide processing method proved to be novel and efficient for the preparation of solid inclusion complexes of olanzapine with methyl-β-cyclodextrin. The preliminary data also suggests that the complexes of olanzapine with methyl-β-cyclodextrin will lead to better therapeutic efficacy due to better solubility and dissolution properties.

ABBREVIATIONS

Me-β-CD: Methyl-β-cyclodextrin

SC-CO_2: Supercritical carbon dioxide

SEM: Scanning electron microscopy

DSC: Differential scanning calorimetry

XRPD: X-ray powder diffraction

DP: Percent drug dissolved

DE: Dissolution efficiency

Keywords: Olanzapine, methyl-β-cyclodextrin, inclusion complexes, freeze drying, supercritical carbon dioxide

1. Introduction

Olanzapine is a second-generation atypical neuroleptic drug approved by the Food and Drug Administration as a first-line therapy for the treatment of schizophrenia and mania associated with bipolar disorder (Abdelbary and Tadros, 2013). It suffers from poor aqueous solubility (12-44 μg mL^{-1}) and a low dissolution rate leading to an erratic bioavailability (Kulkarni *et al*., 2010; Dixit *et al*., 2011; Raman *et al*., 2013). Moreover, the drug undergoes extensive hepatic first-pass metabolism and is required in high doses (Sood *et al*., 2013).

Several approaches have been reported to enhance the solubility and dissolution rate of olanzapine, *e.g.* solid-dispersions (Krishnamoorthy *et al*., 2011), nano-emulsions (Raman *et al*., 2013), solid-lipid nanoparticles (Sood *et al*., 2013), freeze dried tablets (Dixit *et al*., 2011) and inclusion complexation with cyclodextrins (Kulkarni *et al*., 2010; de Freitas *et al*., 2012).

Cyclodextrins, also known as cyclomaltoses, cycloamyloses and Schardinger dextrins, are macrocyclic oligomers of α-D-glucose with a hydrophilic exterior and a relatively non polar central cavity (Appel *et al*., 2012; Kfoury *et al*., 2014; Kfoury *et al*., 2015; Rudrangi *et al*., 2015). Cyclodextrins can form inclusion complexes by taking up the entire or a part of lipophilic drug molecule in its hydrophobic interior cavity (Loftsson and Duchêne, 2007; Salústio *et al*., 2009). Through formation of inclusion complexes, cyclodextrins are known to enhance the aqueous solubility and dissolution rate of poorly soluble drugs (Trapani *et al*., 2000; Pose-Vilarnovo *et al*., 2001; Latrofa *et al*., 2001; Jain and Adeyeye, 2001; Riekes *et al*., 2010).

The study published by de Freitas *et al*. (2012) reported that olanzapine and methyl-β-cyclodextrin (Me-β-CD) complexes prepared in the 1:1 molar ratio using rotary evaporation

method exhibit a higher dissolution profile than the active alone or in a state of physical mixture. Despite their success, the complex preparation by the stated method required an organic solvent which is not desirable. Removal of environmentally harmful organic solvents from the drug product to the levels approved by the Food and Drug Administration is very challenging and therefore conventional techniques used for the preparation of inclusion complexes (co-evaporation, spray drying and kneading) involve several drying steps for considerable time, which may also affect the drug stability (Al-Marzouqi *et al.*, 2006). Hence, it is highly recommended to eliminate the use of organic solvents in the preparation of drugs or drug-cyclodextrin complexes.

The aim of the present study was to produce olanzapine-Me-β-CD complexes in the same stoichiometric ratios (1:1 molar) without using organic solvents or auxiliary agents. Therefore, application of supercritical fluid processing was studied as an alternative to conventional methods in the current work.

A supercritical fluid is defined as a substance that exists above its critical pressure and temperature. Supercritical fluids feature densities like liquids and viscosities and diffusivities like gases and hence offer excellent mass transfer and solubilising properties (York, 1999; Kompella and Koushik, 2001; Sunkara and Kompella, 2002; Bandi *et al.*, 2004). Carbon dioxide becomes supercritical above 31.25 °C and 73.8 bar. Supercritical carbon dioxide (SC-CO_2) is environmentally benign and is considered to be green. SC-CO_2 is a non-combustible, non-toxic, recyclable and environment-friendly solvent (Palakodaty and York, 1999; Lang and Wai, 2001; Lee *et al.*, 2008; Deshpande *et al.*, 2011; Girotra *et al.*, 2013; Rudrangi *et al.*, 2015) and has provided an appealing alternative to toxic organic solvents or conventional complexation media. SC-CO_2 has been successfully employed in the preparation of inclusion complexes between various drugs and cyclodextrins in dynamic or static modes (Table 1).

The use of the SC-CO_2 processing has already been investigated in the preparation of drug-Me-β-CD inclusion complexes (Charoenchaitrakool *et al.*, 2002; Banchero *et al.*, 2013; Rudrangi *et al.*, 2015). A significant improvement in the dissolution rate of drug was observed in all cases. It was suggested by Banchero and co-workers (Banchero *et al.*, 2013) that the liquefaction of Me-β-CD in SC-CO_2 favours the complexation of drug and cyclodextrin without any addition of water or auxiliary agents as the drug molecules would better reach the cavity of the cyclodextrin in the molten or liquid state.

The effect of supercritical carbon dioxide processing on the preparation of olanzapine-Me-β-CD complexes has not yet been reported. Inclusion complexes were prepared by physical mixing, freeze drying, co-evaporation and SC-CO_2 processing at various working (temperature and pressure) conditions. The prepared complexes were then characterized by solubility studies, differential scanning calorimetry, X-ray powder diffraction, scanning electron microscopy and dissolution studies.

2. Materials and methods

2.1. Materials

Olanzapine (≥99%, molecular weight: 312.44, CAS number: 132539-06-1) was obtained from Dr. Reddy's Laboratories Ltd. (Hyderabad, Telangana, India). Me-β-CD (average molecular weight: 1310, CAS number: 128446-36-6, extent of labeling: 1.6–2.0 mol CH_3 per unit anhydroglucose) was purchased from Sigma–Aldrich (Gillingham, Dorset, UK). Carbon dioxide (99.9%) was obtained from BOC Ltd. (Guildford, Surrey, UK). All chemicals were used as received without further purification.

2.2. Preparation of binary mixtures of olanzapine with Me-β-CD

All binary mixtures of olanzapine with Me-β-CD were prepared in a 1:1 molar ratio. The processed samples were stored in a desiccator over solid calcium chloride until submitted for analysis.

2.2.1. Physical mixing

Physical mixture was obtained by tumble-mixing an accurately weighed equimolar mixture of olanzapine and Me-β-CD at 100 rpm for 15 minutes using a TURBULA® T2F mixer (Willy A. Bachofen AG – Maschinenfabrik, Muttenz, Switzerland).

2.2.2. Freeze drying

Olanzapine was added to the aqueous solution of Me-β-CD under constant stirring. The mixture was agitated in an orbital shaker at room temperature until equilibrium was attained (48 h). The resultant suspension was frozen at −60 °C and then lyophilized in a freeze-dryer (ScanVac CoolSafe, UK) for 48 h. The obtained product was sieved through 0.150 mm sieve.

2.2.3. Co-evaporation

An ethanolic solution of olanzapine was added to an aqueous solution of Me-β-CD and the mixture was agitated in an orbital shaker for 24 h. The solvents were then evaporated under reduced pressure to yield a pale yellowish, dry powder.

2.2.4. Supercritical carbon dioxide process

The complexes were prepared using an extraction apparatus supplied by Thar Process Inc., USA in the static mode. The schematics of SC-CO_2 processing have been previously described in detail (Rudrangi *et al.*, 2015).

The physical mixtures of olanzapine and Me-β-CD (100.01 mg and 412.28 mg; 100.03 mg and 412.27 mg; 100.02 mg and 412.28 mg; 100.01 mg and 412.30 mg, respectively) were placed in a sample cell. Carbon dioxide was pumped from a cylinder via a cooling unit into

the sample cell. The physical mixtures were processed at four different working conditions [45 °C-100 bar, 45 °C-200 bar, 55 °C-100 bar and 55 °C-200 bar] in order to study the influence of pressure and temperature on the formation of inclusion complexes.

The desired pressure was achieved by pumping carbon dioxide against an automated back-pressure regulator. The sample cell in the reaction vessel was heated to the desired temperature and held for 1 h before recovering the solid complex by depressurisation at a rate of 7–8 bar min^{-1}. The product was then homogenised in a mortar prior to further analysis.

2.3. Analysis of the prepared binary mixtures

2.3.1. Differential scanning calorimetry analysis (DSC)

Thermal analysis of pure materials and the binary mixtures was carried out using a differential scanning calorimeter (Mettler-Toledo, LLC, UK). The equipment was periodically calibrated with indium. Accurately weighed samples (5 mg) were hermetically sealed in aluminium pans and heated at a rate of 10 °C min^{-1} from 50°C to 200 °C.

2.3.2. X-ray powder diffraction analysis (XRPD)

X-ray powder diffraction analysis of pure materials and the binary mixtures was carried out at room temperature using a D8 Advance X-ray Diffractometer (Bruker, Germany) in theta–theta Bragg–Brentano geometry using reflection mode. The diffractograms were collected between 2–40° 2θ, with a step size of 0.006° and a counting time of 0.5 s per step using Cu Kα radiation. The degree of crystallinity (% Crystallinity) was determined using the amorphous subtraction method (Suryanarayanan, R. and Mitchell, A.G., 1985).

2.3.3. Scanning electron microscopy analysis (SEM)

Micrographs of pure materials and the binary systems were collected using a Hitachi SU-8030 scanning electron microscope. The samples were securely mounted on aluminium stubs using double-sided adhesive tape and made electrically conductive by coating in vacuum

with a thin layer of chromium (~300Å) at 30 W for 30 s. The photomicrographs were obtained at an excitation voltage of 2.0 kV and a magnification of ×350.

2.3.4. Solubility studies

Saturation solubility of olanzapine was measured in triplicate by adding excess amounts of the drug to 10 mL of deionised water in sealed glass containers. The solutions were agitated for 72 hours at 37 ± 0.5 °C. The solutions were then filtered (0.45 µm filter pore size) and assayed for drug concentration by ultra-violet spectroscopy (Cary 100 UV-vis, Agilent Technologies, USA) after dilution in 10 mm quartz cuvettes.

Phase-solubility studies were carried out in triplicate as described by Higuchi and Connors (1965). Excess amounts of olanzapine (*i.e.* in amounts above its solubility limit) were added to 10 mL of de-ionised water (pH 7.1) containing successively increasing concentrations (0, 5, 10, 15, 20, 25, 37.5 and 50 × 10^{-3} M) of Me-β-CD in sealed glass containers. The resultant solutions were agitated (100 rpm) at 37 ± 0.5 °C until equilibrium was attained (72 h). The suspensions were then filtered and assayed for drug concentration as described above. The apparent stability constant ($K_{1:1}$) for olanzapine-Me-β-CD complexes was calculated from the slope of the linear portion of the phase solubility diagram.

2.3.5. Dissolution studies

Dissolution studies of olanzapine from all binary systems and olanzapine alone were performed in triplicate using USP Type II paddle method (Hanson G2 Vision® Classic 6, Chatsworth, CA). Accurately weighed samples of drug or binary mixtures, equivalent to 10 mg of olanzapine were dispersed into 900 mL of deionised water (pH 7.1) at 37 ± 0.5 °C and stirred at 50 rpm. At predetermined time points (2, 5, 10, 20, 45, and 60 min); 5 ml aliquots of the samples were drawn, filtered (0.45 µm filter pore size) and assayed for drug concentration by UV spectroscopy. The dissolution curves were characterized by the

percentage of drug dissolved and the dissolution efficiency at 30 minutes. Dissolution efficiency was evaluated according to the method reported by Khan (1975).

2.3.6. Computational details

Molecular docking calculations were conducted using Glide (grid-based ligand docking) application implemented in the Maestro 9.3 software package (Schrodinger, LLC, New York, 2012). The Me-β-CD structure was prepared by adding hydrogens, followed by an energy minimisation to a convergence of RMSD 0.30Å using OPLS_2005 as force field. Olanzapine was energetically minimised and ionization considered at pH 7 using ionizer subprogram of LigPrep 2.6. The 'Generate grid' sub application of the Glide tool was utilised for the generation of grid by selecting the whole Me-β-CD structure as a receptor site to locate coordinates of the receptor centre. The generated grid was then utilised as a receptor for docking of olanzapine using the 'standard precision' (SP) flexible docking method, located in the Glide tool. Docked Me-β-CD and olanzapine complexes were visualised and molecular surface complex pictures were generated using Maestro.

3. Results and discussions

3.1. Differential scanning calorimetry analysis

Fig. 1 presents the DSC thermograms of olanzapine, Me-β-CD and olanzapine-Me-β-CD binary systems prepared by various processing methods.

The thermogram of olanzapine exhibited a sharp melting endotherm at ~198 °C, indicating the crystalline nature of the drug. DSC studies also indicated that processing the drug with SC-CO_2 has not altered the crystallinity of the drug. Similar results were reported by Rudrangi *et al.* (2015) for indomethacin when processed with SC-CO_2. Thermogram of Me-β-CD revealed a broad endothermic peak between 60 and 120°C attributed to the release of water molecules as explained by Banchero *et al.* (2013).

Thermograms of the binary mixture prepared by physical mixing showed a similar diffraction pattern to that of the respective drug and cyclodextrin. It displayed the melting endotherm of olanzapine, indicating the retention of the crystalline structure of the drug and suggests the absence of interaction between the drug and the cyclodextrin.

Thermograms of the binary mixtures prepared by freeze drying, co-evaporation and SC-CO_2 processing at 45 °C-100 bar, 45 °C-200 bar and 55 °C-100 bar displayed broad endothermic peaks of reduced intensity (as represented by arrows) compared with the melting endotherm of olanzapine and shifted to lower temperature. This may be ascribed to the partial inclusion of the drug into cyclodextrin as explained by Marques *et al.* (1990). On the other hand, thermogram of the complexes prepared by SC-CO_2 processing at 55 °C-200 bar revealed a complete disappearance of the drug endotherm which may be ascribed to the transformation of drug from crystalline to an amorphous state, or the formation of inclusion complexes (Charoenchaitrakool *et al.*, 2002).

3.2. X-ray powder diffraction analysis

Fig. 2 presents the X-ray powder diffraction patterns of pure olanzapine, Me-β-CD and olanzapine-Me-β-CD binary systems prepared by various processing methods.

The diffraction pattern of olanzapine displayed sharp and intense characteristic peaks at a diffraction angle of 2θ equal to 8.63°, 10.36°, 12.44°, 14.64°, 17.03°, 17.81°, 18.84°, 19.85°, 21.02°, 21.50°, 22.32°, 23.94°, 25.24°, 26.39° and 29.71° confirming the crystalline nature of drug. In agreement with DSC analysis, XRPD analysis also indicated that SC-CO_2 process had not altered the crystallinity of the drug.

The diffraction pattern of Me-β-CD showed two broad halos at 2θ equal to 11° and 18° confirming its amorphous nature. Diffraction pattern of the binary mixture prepared by physical mixing showed a similar diffraction pattern to that of the respective drug and

cyclodextrin. It displayed all the principal peaks of olanzapine, indicating the retention of the crystalline structure of drug and suggests the absence of interaction between the drug and the cyclodextrin.

In agreement with the DSC results, the diffraction patterns of the complexes prepared by SC-CO_2 processing at 45 °C-200 bar and 55 °C-100 bar revealed significant diminution of the diffraction peaks suggesting the interactions between drug and Me-β-CD. The diffraction pattern of the complexes prepared by SC-CO_2 processing at 55 °C-200 bar was characterised by the complete disappearance of the drug peaks. However, it displayed two broad features similar to that of the pure Me-β-CD suggesting the formation of inclusion complexes in which the drug was entrapped in the cavity of cyclodextrin (Charoenchaitrakool *et al*., 2002; Banchero *et al,*. 2013; Rudrangi *et al.*, 2015).

The increase in the amorphous content of the drug was observed in the following order: SC-CO_2 processing at 55 °C-200 bar (0% crystalline) > 55 °C-100 bar (4.34% crystalline) ≈ 45 °C-200 bar (6.49% crystalline) > 45 °C-100 bar (6.49% crystalline) > Co-evaporation (78.79% crystalline) > Freeze drying (80.17% crystalline) > Physical mixing (89.41% crystalline).

3.3. Scanning electron microscopy analysis

Morphology of olanzapine, Me-β-CD and olanzapine-Me-β-CD binary systems prepared by physical mixing, freeze drying, co-evaporation and SC-CO_2 processing was analyzed by SEM and is presented in Fig. 3.

From SEM analysis, pure olanzapine (Fig. 3a) and olanzapine processed with SC-CO_2 at 55 °C-200 bar (Fig. 3b) appeared as small to large irregularly sized and shaped crystals with a tendency to self-agglomerate while pure Me-β-CD (Fig. 3c) appeared as perforated hollow

spheres. In agreement with DSC and XRPD analyses, SEM analysis also indicated that SC-CO_2 process had not altered the crystallinity of the drug.

The physical mixture (Fig. 3d) showed the presence of olanzapine crystals, mixed with, or adhered to the surface of broken hollow spheres of Me-β-CD. In agreement with the XRPD analysis, the complexes prepared by freeze drying (Fig. 3e) and co-evaporation (Fig. 3f) showed the presence of crystalline olanzapine. Co-evaporated product appeared as aggregates of drug particles and broken spheres. The binary mixtures prepared by SC-CO_2 processing at 55 °C-200 bar (Fig. 3g) revealed the disappearance of the original morphology of the drug and Me-β-CD. The product appeared as heterogeneous aggregates and it was not possible to differentiate between the raw materials.

3.4. Solubility studies

Aqueous solubility of olanzapine at 37 ± 0.5 °C was found to be 1.96 ± 0.24 µg mL^{-1} and 23.49 ± 1.53 µg mL^{-1} at the end of 1 h and 72 h, respectively. No further improvement was observed in the drug solubility after 72 h. Kulkarni *et al*. (2010), Dixit *et al*. (2011) and Raman *et al*. (2013) reported a drug solubility of 13.13 ± 1.6 µg mL^{-1}, 34.3 ± 11.1 µg mL^{-1} and 43.4 ± 1.74 µg mL^{-1} in distilled water at 37 °C, respectively.

Fig. 4 presents the phase solubility diagram for the complex formation between olanzapine and Me-β-CD in deionized water at 37 ± 0.5 °C. The studies revealed that the apparent solubility of olanzapine increased linearly as a function of Me-β-CD concentration over the entire concentration range studied. Linearity is a characteristic of A_L-subtype system, suggesting the formation of water soluble complexes in solution as explained by Higuchi and Connors (1965). Moreover, the linear (olanzapine and Me-β-CD) correlation with slope of less than 1 (0.0223) suggested the formation of 1:1 complexes over the concentration range (0–50 mM) investigated. The apparent stability constant ($K_{1:1}$), obtained from the slope of the

linear phase solubility diagram was 304 M^{-1}.Similar phase solubility profile was reported for olanzapine-Me-β-CD complexes in distilled water by de Freitas *et al*. (2012).

Phase solubility studies of olanzapine-hydroxypropyl-β-cyclodextrin complexes were carried out by Kulkarni *et al*. (2010) in distilled water and the authors reported a stability constant of $K_{1:1}$ = 242 M^{-1}. Phase solubility studies of indomethacin-Me-β-CD complexes were carried out by Rudrangi *et al.* (2015) in phosphate buffer medium (pH 7.4) and the authors reported an A_N-subtype phase solubility diagram with a stability constant of $K_{1:1}$ = 167 M^{-1}.

3.5. Dissolution studies

Fig. 5 presents the dissolution profiles of olanzapine from drug alone and from drug-Me-β-CD binary systems in deionised water at 37 ± 0.5 °C. All binary systems other than the physical mixture exhibited better dissolution profiles than the drug alone. The results in terms of percent of olanzapine dissolved at 30 min and the dissolution efficiency at 30 min are presented in Table 2.

The increase in the dissolution properties of olanzapine was observed in the following order: SC-CO_2 processing at 55 °C-200 bar > 55 °C-100 bar ≈ 45 °C-200 bar > 45 °C-100 bar > co-evaporation > freeze drying > physical mixing.

The amount of olanzapine dissolved from unprocessed drug alone and drug processed with SC-CO_2 at 55 °C-200 bar was very low with 17.62 ± 1.59% and 18.84 ± 1.44% dissolving at the end of 60 min, respectively. The dissolution studies confirmed that SC-CO_2 processing alone had not affected the dissolution of the drug.

The binary mixture obtained by physical mixing exhibited no increase in the drug dissolution with 16.75 ± 1.72% of olanzapine dissolving at the end of 60 min. Similar dissolution profile was reported for olanzapine-Me-β-CD physical mixture in distilled water by de Freitas *et al*. (2012).

Improvement in the drug dissolution was found to be dependent both on the processing method and the processing conditions used for the preparation of complexes. The binary mixtures prepared by freeze drying and co-evaporation exhibited an increased drug release with 34.44 ± 2.61% and 68.67 ± 3.11% of olanzapine dissolved after 60 min, respectively. On the other hand, binary mixtures prepared by SC-CO_2 processing resulted in more than 80% of drug dissolution within the first 30 min irrespective of the temperature and pressure employed. However, the binary mixtures prepared by SC-CO_2 processing at 55 °C and 200 bar showed highest drug dissolution with more than 90% of the drug dissolved within the first 10 min. These results suggested that both the temperature and pressure have influence on the formation of the inclusion complexes in SC-CO_2 at studied parameters.

The improved dissolution characteristics of the binary mixtures can be attributed to the improved drug wettability, high aqueous solubility (greater than 2000 mg mL^{-1}) and surfactant-like properties of Me-β-CD as suggested by Banchero *et al.* (2013), Cirri *et al.* (2005) and Guyot *et al.* (1995). Moreover, the greater improvement obtained with all the SC-CO_2 processed systems could also be a result of reduced crystallinity of the binary mixtures (El-Badry, M. *et al.*, 2009) and the formation of inclusion complexes between olanzapine and Me-β-CD in the solid state (Rudrangi *et al.*, 2015).

3.6. Docking studies

Computational molecular docking studies were conducted to study the possibility of molecular arrangement of inclusion complexes between olanzapine and Me-β-CD. Fig. 6 presents the best pose of olanzapine docked in the cavity of Me-β-CD.

The three-dimensional structure of olanzapine is depicted in stick form with a mesh representing the molecular surface (grey with reddish tint in the cavity); the hydrophilic area (cyan mesh) and the hydrophobic area (orange mesh at the mid cavity depth of Me-β-CD). The figure shows the binding of olanzapine in the cavity of Me-β-CD through anchorage by

the methyl group of the piperazine due to its hydrophobicity. The interactions responsible for the inclusion seem to be purely hydrophobic in nature, as no indications of hydrogen bonding can be found in the optimal complex geometries. The binding affinity (GLIDE energy), van der Waals energy and docking score for inclusion of olanzapine in Me-β-CD are -24.13 kcal mol^{-1}, -21.57 kcal mol^{-1} and -3.09 kcal mol^{-1}, respectively.

Computational molecular docking studies of indomethacin with Me-β-CD were carried out by Rudrangi *et al.* (2015) using the Glide application and the authors reported the binding affinity, van der Waals energy and docking score of 27.880 kcal mol^{-1}, -28.941 kcal mol^{-1} and -4.882 kcal mol^{-1} respectively.

4. Conclusions

Complexation of olanzapine with Me-β-CD was accomplished successfully using a single-step, organic solvent-free supercritical fluid process. The phase solubility diagram with Me-β-CD in de-ionised water was classified as A_L–subtype, indicating the formation of 1:1 stoichiometric inclusion complexes. All the binary mixtures with Me-β-CD, except physical mixture, exhibited a faster and greater extent of drug dissolution than the drug alone.

Information obtained from the differential scanning calorimetry, X-ray powder diffraction, scanning electron microscopy and dissolution studies suggest complete complexation or amorphisation of olanzapine and Me-β-CD prepared by SC-CO_2 processing method.

Different degrees of crystallinity and dissolution rate were observed in the products processed by SC-CO_2 at various processing conditions, suggesting the possibility of olanzapine-Me-β-CD interactions of different efficiencies in the solid state. Products obtained by the SC-CO_2 processing method exhibited the highest apparent drug dissolution followed by co-evaporation, freeze drying and physical mixing. Therefore, a solid inclusion method using SC-CO_2 proved to be a novel and efficient complexation method for olanzapine into

Me-β-CD. Furthermore, since this method has no toxic solvent residue, products obtained by this method should provide minimal side effects in humans, compared to those obtained by techniques, which require the use of organic solvents.

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Figure captions

Fig. 1. DSC thermograms of olanzapine, Me-β-CD and olanzapine-Me-β-CD binary systems prepared by various processing methods

Fig. 2. X-ray powder diffractograms of olanzapine, Me-β-CD and olanzapine-Me-β-CD binary systems prepared by various processing methods

Fig. 3. SEM photomicrographs of olanzapine-unprocessed (a), olanzapine processed with SC-CO_2 at 55 °C-200 bar (b), Me-β-CD (c) and olanzapine-Me-β-CD binary systems prepared by physical mixing (d), freeze drying (e), co-evaporation (f) and SC-CO_2 processing at 55 °C-200 bar (g).

Fig. 4. Phase solubility studies of olanzapine with increasing concentrations of Me-β-CD at 37 ± 0.5 °C and in deionized water-pH 7.1.

Fig. 5. Dissolution profiles of olanzapine and olanzapine-Me-β-CD binary systems prepared by various processing methods at 37 ± 0.5 °C and in deionised water (*n*=3).

Fig. 6. Representation of the complex between Me-β-CD (orange) and olanzapine (stick representation with surface meshes) obtained by computational molecular docking (1:1 stoichiometry).

Table 1. Drug-cyclodextrin complexes prepared by SC-CO_2 processing in static or dynamic modes.

Drug	**Cyclodextrin**	**Mode**	**Reference**
Acetaminophen	β-CD	Not reported	Giordano *et al.*, 1996.
Benzocaine	β-CD	Static	Al-Marzouqi *et al.*, 2007a
		Static	Al-Marzouqi *et al.*, 2007b
Budesonide	HP-β-CD[a]	Static	Bandi *et al.*, 2004
	γ-CD	Static	Toropainen *et al.*, 2006
Bupivacaine	β-CD	Static	Al-Marzouqi *et al.*, 2007b
Econazole	β-CD	Static	Al-Marzouqi *et al.*, 2007c

		Static	Al-Marzouqi *et al*., 2009
Eflucimibe	γ-CD	Not reported	Papet *et al*., 2003
	γ-CD	Static	Rodier *et al*., 2005
Fluconazole	β-CD	Static	Al-Marzouqi *et al*., 2009
Flurbiprofen	TMe-β-CD[b]	Not reported	Moribe *et al*., 2007
Ibuprofen	Me-β-CD	Static	Charoenchaitrakool *et al*., 2002
	β-CD	Static	Türk *et al*., 2007
		Static	Hussein *et al*., 2007
Imazalil	β-CD	Static	Lai *et al*., 2003
Indomethacin	HP-β-CD	Static	Bandi *et al*., 2004
	Me-β-CD	Static	Rudrangi *et al*., 2015
Itraconazole	β-CD	Static	Al-Marzouqi *et al*., 2006
		Static	Al-Marzouqi *et al*., 2009
		Static	Hassan *et al*., 2007
Ketoprofen	Me-β-CD	Static	Banchero *et al*., 2013
Mepivacaine	β-CD	Static	Al-Marzouqi *et al*., 2007b
Miconazole	β-CD	Static	Van Hees *et al*., 2002
	HP-γ-CD[c]	Static	Barillaro and Bertholet, 2004.
Naproxen	TMe-β-CD	Not reported	Moribe *et al*., 2007
	β-CD	Dynamic	Junco *et al*., 2002
Piroxicam	β-CD	Static	Van Hees *et al*., 1999
		Static	Van Hees *et al*., 2002
		Static	Grandelli *et al*., 2012
Simvastatin	HP-β-CD	Dynamic	Jun *et al*., 2007

*In static mode, the contents of the cell are exposed to carbon dioxide, pressurized and allowed to equilibrate; while carbon dioxide is circulated continuously through the cell in the dynamic mode.

[a]HP-β-CD: Hydroxypropyl-β-cyclodextrin; [b]TMe-β-CD: Trimethyl-β-cyclodextrin; [c]HP-γ-CD: Hydroxypropyl-γ-cyclodextrin.

Table 2: Percent olanzapine dissolved (DP) at 30 min and dissolution efficiency (DE)* at 30 min from olanzapine and olanzapine-Me-β-CD binary systems prepared by various processing methods (USP Apparatus II; deionised water-pH 7.1; 37 ± 0.5 °C; 100 rpm; n=3).

Sample (Method)	**DP_{30}**	**DE_{30}**
Olanzapine	11.31 ± 1.52	5.7
Physical mixing	11.51 ± 1.57	7.6
Freeze drying	21.75 ± 2.42	17.3
Co-evaporation	65.42 ± 3.04	62.1
Supercritical carbon dioxide processing		
45 °C-100 bar	80.15 ± 3.06	71.1
45 °C-200 bar	86.23 ± 3.42	80.8
55 °C-100 bar	85.47 ± 3.18	79.6

55 °C-200 bar	93.28 ± 2.79	88.8

*DE_{30} was calculated from the area under the dissolution curve at 30 min and expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time of the total amount added.

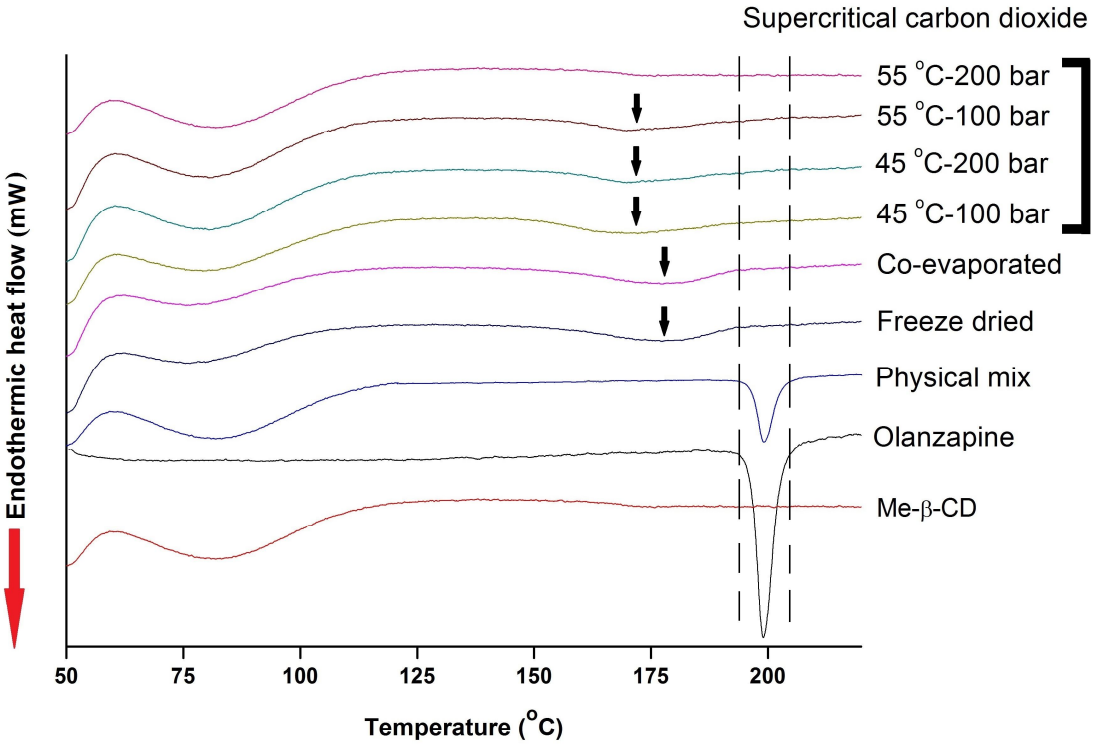


Fig. 1

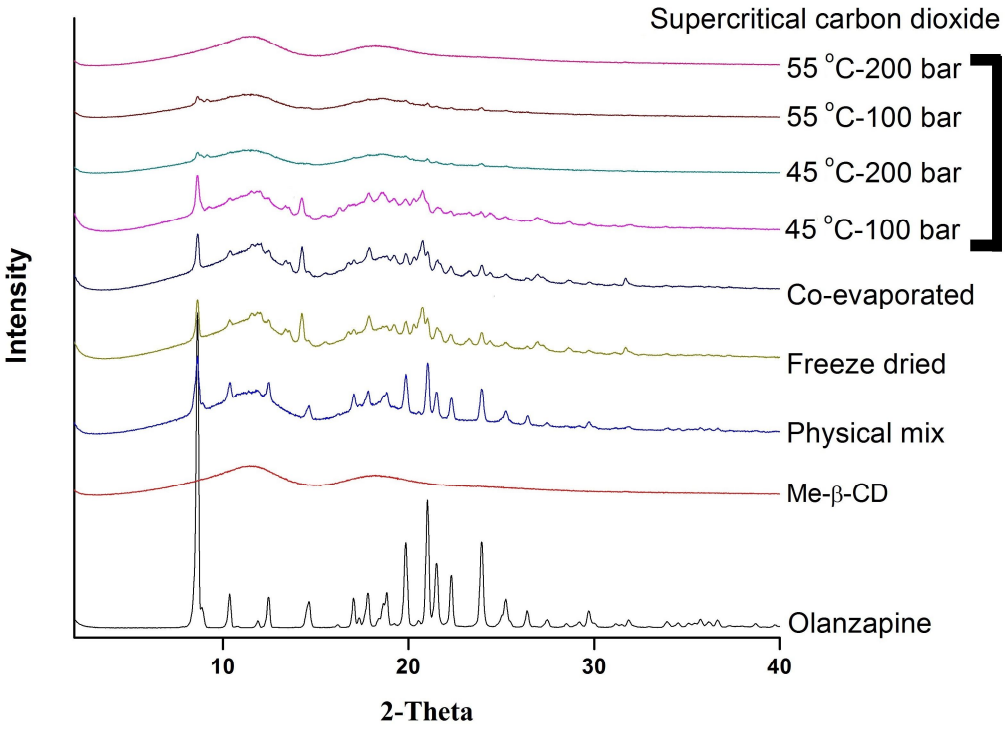


Fig. 2

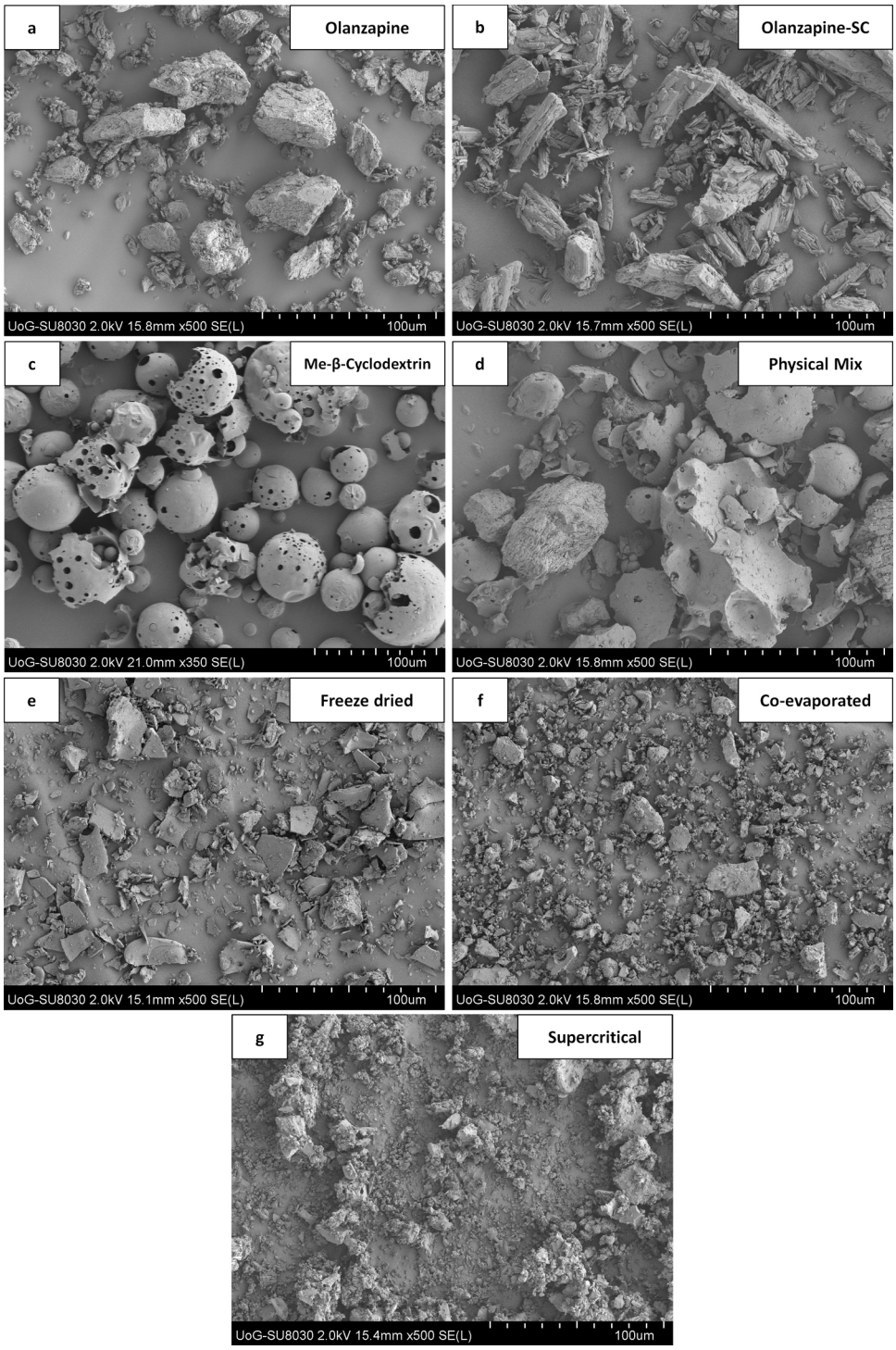
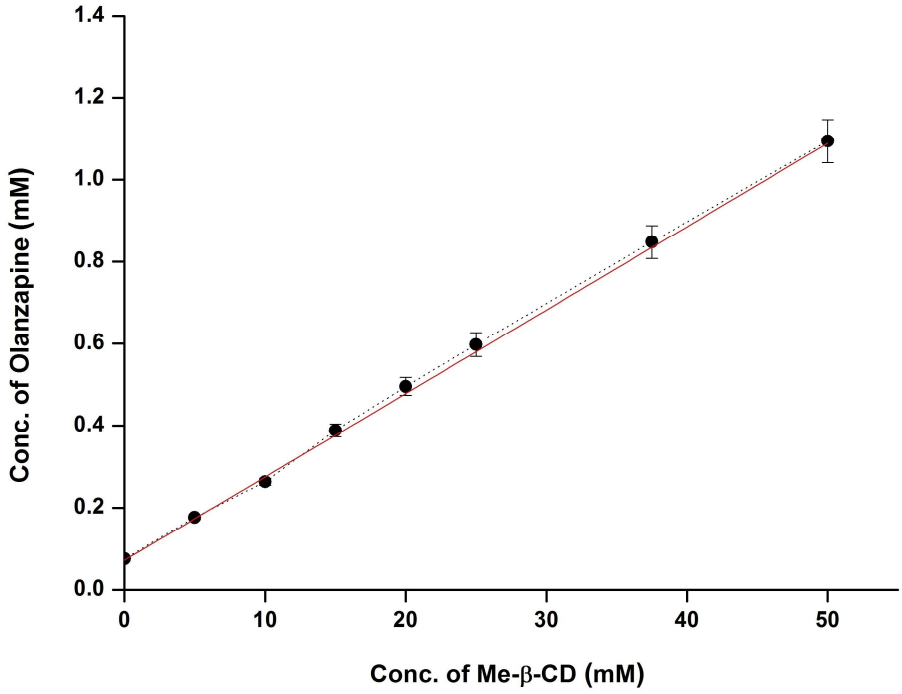


Fig. 3



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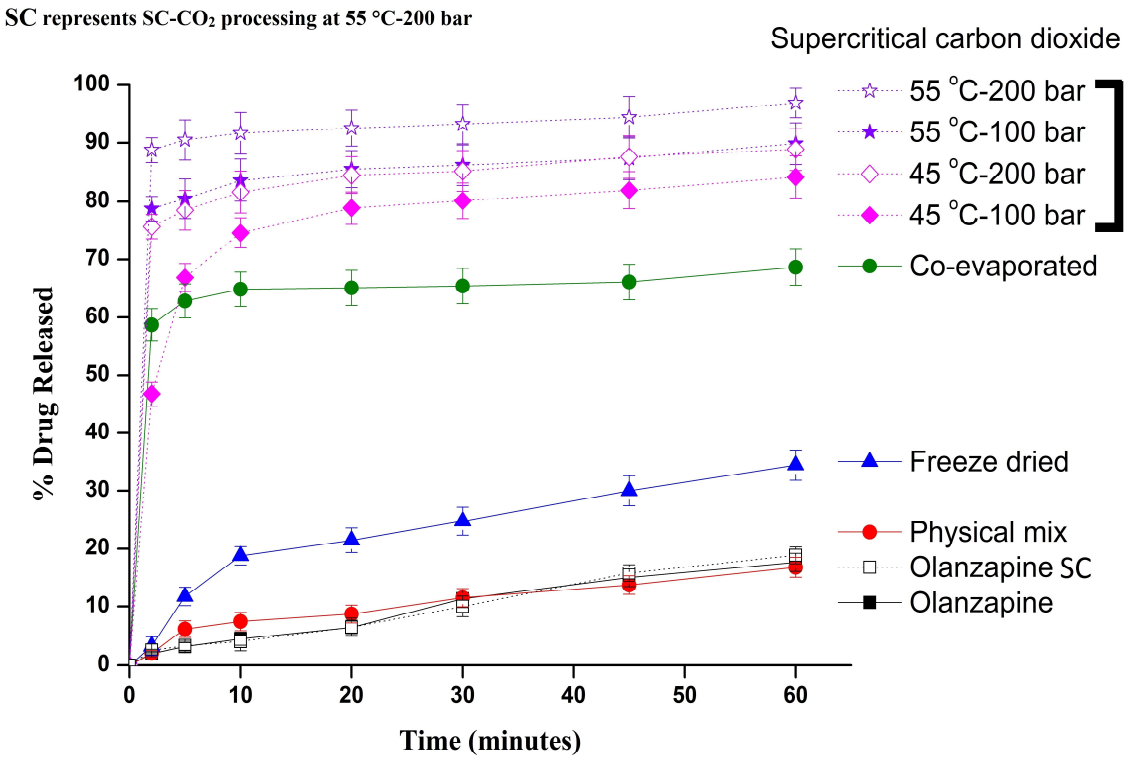

Fig. 5

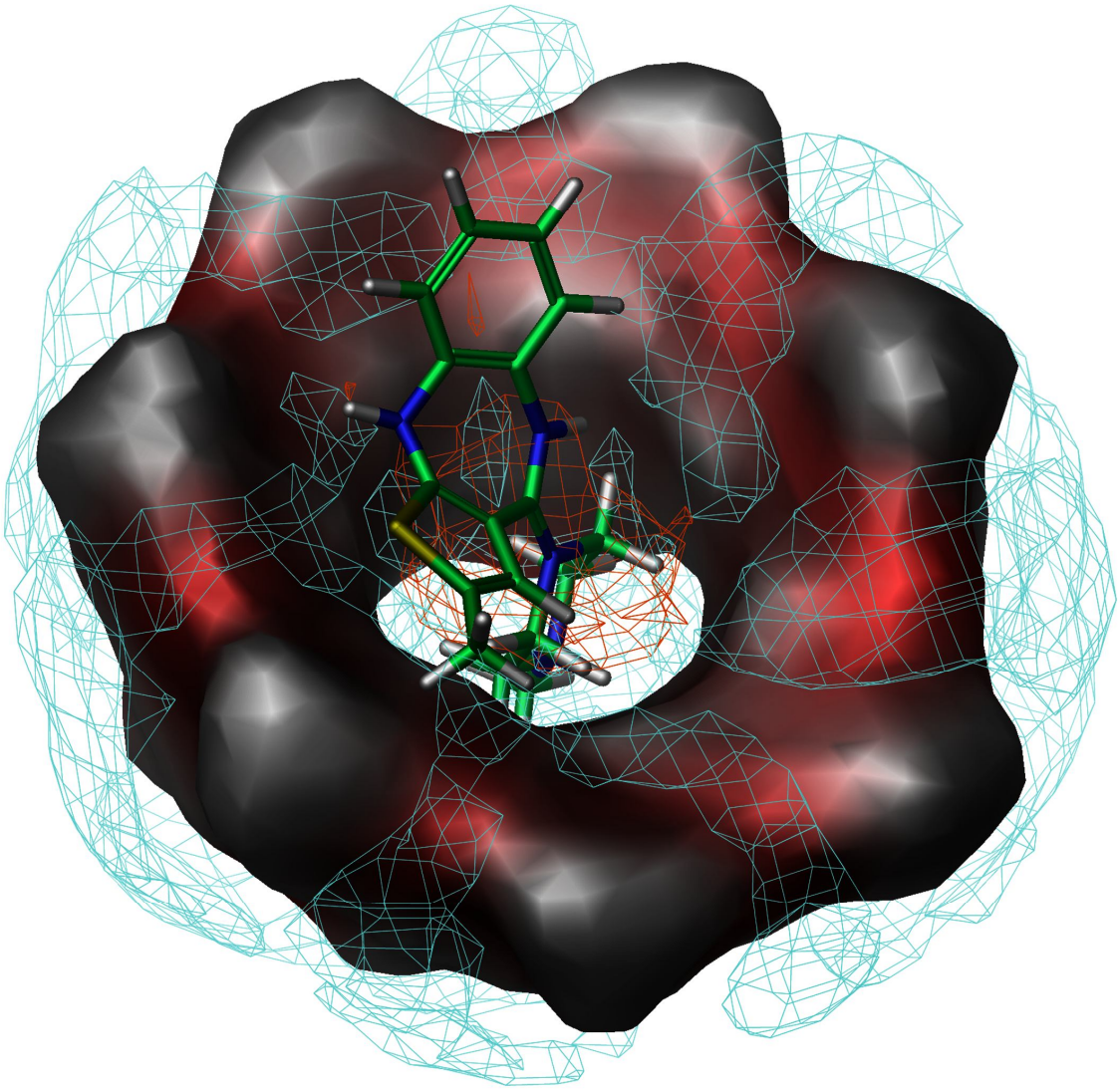

Fig. 6