

Introduction

Crystal engineering is the design and synthesis of molecular solid-state structures with desired properties, based on an understanding and exploitation of intermolecular interactions.¹ The two main strategies currently in use for crystal engineering are based on hydrogen bonding and coordination complexation.² With increase advancement in power technology, different attempts are taken to design primary and secondary particles of substances for several application. Enlargement of particle size is an important process in manufacturing of tablets and is to impart some degree of functionality to particle such as improvement of in flowability, solubility, dissolution, micromeritic, compression and compressibility properties. Different technique for enlargement of particle size are an important tool are modifying primary and secondary properties of pharmaceutical substances³. Nowadays several new techniques combining granulation and crystallization are being developed to improve particle properties. There are various conventional process which are used to enlarge the particle size and involve the wider acceptability but recently non conventional technique of particle size enlargement are being developed which include extrusion-spheronization, melt solidification, melt granulation, melt extrusion and spherical crystallization. These techniques are advantageous due to less number of unit operations and economic in term of processing cost and depend on the desired properties of enlarged particle and physico-chemical properties of drug and excipients.⁴ Crystal engineering design techniques are widely used in pharmaceutical industries to modify primary (Particle size, shape, crystal habit, crystal form, density, porosity, dust generation etc) as well as secondary (flowability, compressibility, compactibility, consolidation, reduced adhesion of formulation to the processing equipment, reduction in air entrapment during processing, etc) properties of pharmaceuticals. Especially, improvement in the efficiency of the manufacturing process and high degree of partial functionality can be achieved by these techniques⁵. CCA is a novel particle engineering technique, which aggregates crystals of drugs in the form of small spherical particles using excipients and solvents to develop an intermediate material with improved micromeritic and mechanical properties, solubility, and dissolution. The rate of dissolution of the drug from the agglomerates or compacts thereof can be improved and

modified by using suitable excipients during the process of preparation of agglomeration⁶. The present work reports a CCA technique used to prepare agglomerates of ritonavir, an antiviral drug, the crystalline form consisting of long needles, which otherwise has low bulk density, very poor flow property as well as compressibility, and very low solubility in water which makes direct compression difficult. Excipients to be incorporated in the formation of agglomerates should have an affinity toward the bridging liquid. Talc, due to its hydrophobicity, undergoes preferential wetting with bridging liquids and is a suitable excipients for incorporation in agglomerates. Apart from talc, various hydrophilic and hydrophobic polymers have been used to study their effect on physicochemical and physicomechanical properties.

Materials and methods

Ritonavir was obtained as a gift sample from Emcure Pvt. Ltd, Pune, HPMC K-15, PEG-6000 PVP K-30, talc, acetone and dichloromethane were purchased from Lobachem, Mumbai, India. All the solvents used are of analytical grade.

Preparation of Crystallo-co-agglomerates

Different agglomerates were prepared of the compositions shown in Table-1. Ritonavir agglomerates were prepared using a three solvent system comprising acetone: dichloromethane: water (acetone as good solvent, dichloromethane as bridging liquid and water as bad solvent, respectively). In a vessel, polymers were dissolved in sufficient amount of distilled water. Then, talc and tween 80 were added under stirring condition at 600 rpm maintained at 10°C. Ritonavir was dissolved in acetone. The latter solution was added immediately to the dispersion containing dissolved polymer under constant stirring conditions (600rpm) kept at room temperature. The stirring was continued for 20 min and bridging liquid dichloromethane was added drop wise to obtain agglomerates, which were then set aside overnight. Then obtained agglomerates were filtered and dried. Three batches(A-1, A-2, A-3, B-1, B-2, B-3, C-1, C-2, C-3) were prepared by changing the concentration of excipients (0.5, 0.75 and 1 % w/v).

Characterization of Agglomerates

Saturation solubility studies

To evaluate the increase in the solubility of agglomerates, saturation solubility measurements were conducted. Excess amount drug or agglomerates was added to 50 ml conical flask containing distilled water. The system was agitated on a rotary shaker for 48 h at 100 rpm maintained at room temperature and filtered. The filtrate was suitably diluted and analyzed at 201 nm by using UV visible spectrophotometer (UV-1800, Shimadzu, Japan)⁷.

Micromeretic study

The flow properties of agglomerates were determined in terms of angle of repose, bulk density, tapped density, Carr's Index and Hausnar's ratio. Angle of repose was determined by fixed funnel method whereas Carr's Index and Hausner ratio were calculated from bulk and tapped densities. Hausner ratio was taken as a ratio of tapped density to bulk density. Carr's Index was calculated according to the following equation⁷.

Angle of Repose

Angle of repose has been used to characterize the flow properties of solids. It is a characteristic related to inter particulate friction or resistance to movement between particles. This is the maximum angle possible between surface of pile of powder or granules and the horizontal plane.

$$\tan\theta = h / r$$

$$\theta = \tan^{-1} h / r$$

Where, θ = angle of repose, h = height of heap, r = radius of base of heap circle.

Method: A funnel was fixed at a height approximately 2-4 cm over the platform. The drug powder was slowly passed along the wall of funnel, till the tip of powder cone so formed just touched the tip of funnel stem. Angle of repose was then determined by measuring the height of the cone of powder and radius of the circular base of powder heap⁸.

Compressibility Index and Hausner's ratio

In recent years, compressibility index and the closely related Hausner ratio have become the simple, fast and popular methods of predicting powder flow characteristics.

Compressibility index has been proposed as an indirect measure of bulk density, size and shape, surface area, moisture content, and cohesiveness of materials because all of these can influence the observed compressibility index. The compressibility index and Hausner's ratio are determined by measuring both bulk density and the tapped density of agglomerates⁹.

$$\text{Compressibility Index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Production yield (%)

The production yields were calculated as the weight percentage of the final product after drying, with respect to the initial total amount of ritonavir and polymer used for the preparations⁸.

$$\text{Production yield \%} = \frac{\text{Practical mass (CCA)}}{\text{Theoretical mass (polymer + drug)}} \times 100$$

Drug Content

10 mg of agglomerates were accurately weighed in a 100 ml volumetric flask & adjust the volume to 100 ml with methanol (100µg/ml), serving as a test solution. Standard solution was sonicated for 5 min & analyzed at 238 nm using a UV spectrophotometrically¹⁰.

Fourier Transformation Infrared Spectroscopy (FTIR)

The study was conducted with an intention to check the compatibility of polymers like HPMC K15M, PEG-6000 and PVP K-30 with ritonavir. Also, it helps to check the suitability of polymer for the preparation of agglomerates. FTIR spectra were obtained using a Shimadzu FTIR spectrometer (Thermo Fisher, Japan). The samples of pure drug and physical mixture such as Ritonavir and HPMC K-15, PEG-6000, PVP K-30 were prepared. The scanning range was kept from 4000 to 500 cm⁻¹⁸.

Scanning electron microscopy (SEM)

The surface morphology of the optimized formulations was studied using a scanning electron microscope (JSM 6390, JEOL) operated at an accelerating voltage of 10 kV and obtained micrographs were examined at different magnifications¹¹.

X-Ray Diffraction of Powder (XRD)

The X-ray powder diffraction patterns were recorded on the X-ray diffractometer (PW 1729, Philips, Netherland). The samples were irradiated with monochromatised Cu K- α radiation (1.542\AA) and analyzed between $10\text{--}50^\circ 2\theta$. The voltage and current used were 30kV and 30mA, respectively. The range and chart of speed were 1×10^4 CPS and 5mm/2 respectively¹¹.

Differential scanning calorimetry (DSC)

The thermal behavior of drug loaded agglomerates was studied using a differential scanning calorimeter (Mettler Toledo) at a heating rate of $10^\circ\text{C}/\text{min}$. The measurements were performed at a heating range of $20\text{--}250^\circ\text{C}$ under nitrogen atmospheres¹¹.

Dissolution Studies

The dissolution rate studies were conducted in 900 mL of pH 6.8 phosphate buffer (Simulated Intestinal fluid) at 50 rpm maintained at $37 \pm 0.5^\circ\text{C}$ in a dissolution apparatus (Model Electrolab Dissolution tester USP TDT-08L) using the paddle method. 100mg equivalent quantity agglomerates was added to dissolution medium and the samples were withdrawn at appropriate time intervals up to 90 minutes. The samples were immediately filtered through $0.45\ \mu\text{m}$ membrane filter, suitably diluted and analyzed spectrophotometrically at 201 nm. The data obtained from dissolution studies were statistically validated¹².

Result and discussion

Ritonavir was crystallized from acetone–water and agglomerated with diluents. In this process, the crystallization of the drug was performed by the addition of a solution to the anti-solvent phase (water). Acetone served as good solvent and the bridging liquid and aqueous phase as the non-solvent. The saturation solubility of prepared agglomerate with HPMC K-15 showed highest solubility compared to pure ritonavir as shown in Table-2.

The spherically agglomerated crystals, produced in yields generally within the range 55–80% (Table-2), were produced simultaneously as crystallization was completed. As both phases (acetone and aqueous) contain the diluents, then it is likely that it is distributed both inside the agglomerates (intragranularly) and outside the agglomerate (extragranularly), attached to the surface.

Micromeritic of agglomerates

The micromeritic properties such as flowability of agglomerates are shown in Table-2. It shows that the flowability represented in terms of the angle of repose, Carr's index and Hausnar's ratio of agglomerates was much improved compared to those of the original drug. Statistical analysis showed that the angle of repose and Carr's index for both the agglomerates reduced significantly in comparison with the original drug. The Hausnar's ratio for both the agglomerates was found to be less than 1.52 indicating improvement in their flow properties. The poor flow properties of pure ritonavir might be attributed to amorphous in nature. These findings proved that the flowability of agglomerates was preferably improved as compared to pure drug.

Morphology of agglomerates

An examination of the SEM, confirmed that the starting material was markedly smaller in particle size than any of the treated crystals. Similar results were obtained in other studies using crystallo-co-agglomeration procedures for other drugs. Ritonavir exhibit platy crystal habit which was distributed at crystallo-co-agglomeration (CCA) formation. Prominent changes were observed with formulation C-1 as compared to A-1 and B-1. Although all formulations show formation of agglomerates in the SEM images in fig. A.1 - A.4. as evident from the adherence of the polymer and talc on to the crystal surface of drug.

Fourier Transformation Infrared Spectroscopy (FTIR):

There was no considerable change in the positions of characteristic absorption bands and bonds of various functional groups present in the drug. This observation clearly suggests that the drug remains in its normal form with no prominent change in its characteristics even in its physical mixture and formulation. The results of FTIR spectra

indicated the absence of any well defined interaction between drug, diluents and polymer.

X-ray diffractometry (XRD):

XRD is a powerful technique for determining the presence of polymorphs, crystal habit modification in drug crystals and generation of new crystals form during crystallo-co-agglomeration technique. Every crystalline solid phase has a unique (XPRD) pattern, which can form the basis for its identification. The X-ray powder diffraction pattern (XRPD) in the 10-80°, 2 θ range showed that the diffraction peaks, characteristics of ritonavir were detectable in crystalline sample i.e. crystallo-co-agglomeration (CCA) sample, fig. C-1 to C-4 suggesting that the particles crystallized in the presence of polymers and talc did not undergo structural changes or modification. However, the difference in the relative intensities of their peak and some new identification peak over extended range may be attributed to the difference in the crystallinity or particle size of sample.

Differential Scanning Calorimetry (DSC):

DSC can be combined with XRPD to determine the polymorphic composition of pharmaceutical powders, if two or more polymorphs are present. The uniformity of crystalline structure in all the batches was confirmed by the DSC. All the formulation irrespective of polymer used and concentration, showed a sharp melting endotherm started at 120-121°C with flat baseline, which indicated that material was not degraded by hydration, salvation or any crystalline changes. Also, it shows no interaction of drug with polymers during crystalline changes take place. There was no appreciable change in the melting endotherm of Crystallo-co-agglomerates compared to that of pure drug. The DSC results (Fig. D-1 to D-7) also revealed little amorphization of ritonavir when prepared in the form of agglomerates with HPMC K-15, PEG-6000 and PVP K-30. This is evident by a decrease in the enthalpy changes of agglomerates when compared with that of pure drug (ritonavir) -408.25 mJ/mg and that for Crystallo-co-agglomerates of formulation of A-1, B-1 and C-1 were -27.75 mJ/mg , -32.1818 mJ/mg and -49.77 mJ/mg respectively.

Conclusion

Crystallo-co-agglomerates of ritonavir with different hydrophilic polymers such as PEG 6000, PVP K-30 and HPMC K-15 showed an improvement in the solubility, dissolution rate and flowability as compared with pure drug. Solid state characterization of drug and CCA showed satisfactory results such as; FTIR proves compatibility, SEM showed enlarged size with signs of porosity, XRD proves crystallinity while DSC showed thermal evaluation. The altered size and shape of Crystallo-co-agglomerates indicated modified crystal habit which could be responsible for dramatic improvement in flowability, solubility and dissolution properties of ritonavir. Crystallo-co-agglomeration of ritonavir is an alternative and effective approach for improvement in physicochemical and micromeritic properties of ritonavir.

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No Conflict of Interest