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RESEARCH ARTICLE

Novel Approaches in Co-Crystal Formation of Asunaprevir for Improved Physicochemical Properties

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ABSTRACT

In the current study, we attempted to improve the physicochemical properties of antiviral drug asunaprevir through the cocrystal synthesis. The neat cooling crystallization method to study the effect of coformer-hydroxybenzoic acid (FA) on solubility and dissolution of asunaprevir, which can serve as the green cocrystal synthesis approach. The prepared cocrystals were characterized for characteristics like powder flow properties, aqueous saturation solubility, *in vitro* powder dissolution study. The synthesized cocrystals were characterized by Fourier transform infrared spectroscopy, differential scanning calorimetry, powder X-ray diffraction. The formation of a cocrystal of asunaprevir and 2,5-dihydroxybenzoic acid was confirmed by the characterization techniques which suggests the interactions between drug and coformer acid leads to cocrystal formation. The powder flow properties, solubility, and dissolution profile of drug are significantly improved by its cocrystallization.

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CONTENTS

1. Introduction

Co-crystals are crystals containing two or more components, present at stoichiometric amounts, and where the components are solid at ambient conditions. Co-crystals are employed in a diverse number of applications, including non-linear optics, organic conductors, dyes and particularly pharmaceuticals. In the pharmaceutical industry, it is the poor biopharmaceutical properties rather than toxicity or lack of efficacy that are the main reasons why less than 1%

of active pharmaceutical compounds eventually appear into the marketplace. Among these biopharmaceutical properties, solubility remains a key issue, with drugs often discarded during commercial production due to their low solubility. Improving the solubility of drugs is currently one of the main challenges for the pharmaceutical industry. Many approaches have been adopted for improving the aqueous solubility of drugs including micronisation, salt formation, emulsification, solubilisations using co-solvents, and the use of polymer drug vehicles for delivery of poorly soluble drugs. Although these techniques have been shown to be effective at enhancing oral bioavailability, success of these approaches is dependent on the specific physicochemical nature of the molecules being studied. Over the last decade, there has been growing interests in the design of pharmaceutical cocrystals, which emerges as a potential method for enhancing the bioavailability of drugs with low aqueous solubility.

Pharmaceutical cocrystal design strategies:

Pharmaceutical cocrystals have rapidly emerged as a new class of API solids demonstrating great promise and numerous advantages. Much work has focused on exploring the crystal engineering and design strategies that facilitate formation of cocrystals of APIs and cocrystal formers. In order to get a desirable cocrystal product of an API with limited aqueous solubility, the first step is to study the structure of the target API molecule and find out the functional groups which can form intermolecular interaction with suitable coformers. As explained before, these intermolecular interactions include van der Waals contacts, stacking interactions, and the most common interaction in cocrystal structure of the hydrogen bonding. The next step is to choose a cocrystal former. The primary request for a coformer is to be pharmaceutically acceptable, for example, pharmaceutical excipients and compounds classified as generally as safe (GRAS) for use as food additives. Coformer selection is the crucial step for designing a cocrystal. For most pharmaceutical cocrystal structures, hydrogen bonds take an important role in directing intermolecular recognition between an API and a coformer molecule.

Steps for Co Crystal Design and Preparation

1. Selection and research of APIs
2. Selection co-formers
3. Characterization of Cocrystals
4. Screening of Cocrystals
5. Development of pharmaceutical formulation

Cocrystal formation methods

To date, many ways of producing cocrystals have been reported. The most common formation methods given below. are based on solution and grinding (He et al., 2008). The solution method is of great importance due to most of the cocrystals which qualify for single X-ray diffraction (SXRD) testing can only be prepared through this method. Solution methods include evaporation of a heterometric solution method, reaction crystallisation method, and cooling crystallisation. Grinding methods include neat grinding and solvent drop grinding. Apart from solution and grinding methods, there are also many newly emerging methods, such as cocrystallization using supercritical fluid, hot-stage microscopy, and ultrasound assisted cocrystallization.

1. Solution methods
    a. Reaction crystallization
    b. Cooling crystallization
2. Grinding method

Cocrystal characterization techniques

Cocrystal characterization is an important constituent part within cocrystal research. The basic physicochemical properties of cocrystal can usually be characterized by powder X-ray diffraction (PXRD), single crystal X-ray diffraction (SXRD), infrared spectroscopy (IR), Raman spectroscopy, differential scanning calorimetry (DSC), solid state nuclear magnetic resonance spectroscopy (SSNMR), scanning electron microscopy (SEM), and terahertz spectroscopy.

Applications of Co-Crystals

Compared to other solid-state modification techniques employed by pharmaceutical industry, co-crystal formation appears to be an advantageous alternative for drug discovery (e.g. new molecule synthesis, nutraceutical co-crystals), drug delivery (solubility, bioavailability) and chiral resolution. Experts are of the opinion that pharmaceutical intellectual property landscape may benefit through co-crystallization

Asunaprevir, The drug with a molecular weight of 748.3 g/ml, is indicated in combination with other agents for the treatment of chronic hepatitis C in adult patients with hepatitis C virus genotypes 1 or 4 and compensated liver cirrhosis. Hepatitis C is a liver disease caused by the hepatitis C virus. The chronic state of this condition accounts for 60-80% of the cases from which the risk of cirrhosis of the liver within 20 years is of around 15-30%. The genotype 1 is the most common type of hepatitis C in the United States and the most difficult to treat. In preclinical studies, asunaprevir showed a high liver-to-plasma AUC ratio. It is rapidly absorbed within 30 minutes of administration. Clinical pharmacokinetic studies showed a tmax of 2-4 hours. The pharmacokinetic profile act in a dose-proportional manner and in a dose of 100 mg the steady-state Cmax and AUC was 572 ng/ml and 1887 ng.h/ml. The absolute bioavailability is reported to be 9.3%. The absorption of asunaprevir is increased if it is accompanied by a high-fat diet.

Aim & Objective:

The main objective of current research work is to design and evaluate the pharmaceutical co crystals of Asunaprevir, BCS Class II drug having low solubility and high permeability.

2. Materials and Methods

Materials: Asunaprevir was received as gift samples from Mylan laboratories Ltd., Hyderabad, India. 3-methoxy-4-hydroxybenzoic acid, 2,4-dihydroxybenzoic acid, and 2,5-dihydroxybenzoic acid and Methanol was obtained from Loba Chemicals, Mumbai.

Methods: 1. Drug and Coformer Compatibility

Studies: Drug coformer compatibility studies were carried out using Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared Spectroscopy (FTIR)

Formulation of cocrystals:

Till date many methods are adopted for the formulation of cocrystals. The most common method are based on solution method and grinding method. In this work the co-crystals were prepared by cooling crystallization method.

1:1 ratio of drug and co former was taken

Dissolve separately drug in methanol and conformer in water

Add the drug solution to co former solution

Refrigerate the solution for overnight and then filter the obtained co crystals by vacuum filtration

Dry the cocrystals by vacuum drying

Table 1: Formulation of Cocrystals

Formulation Code	Drug	Co former	Ratio
F1	Asunaprevir	3-methoxy-4-hydroxybenzoic acid	1:1
F2	Asunaprevir	2,4-dihydroxybenzoic acid	1:1
F3	Asunaprevir	2,5-dihydroxybenzoic acid	1:1

Evaluation /Characterization of Co-Crystals:

Physical appearance:

Cocrystals were characterized visually to study its texture, color, odor, taste etc. Microscopic characterization also done to see the shape of crystals. These are the identification tests for cocrystals.

Determination of pH & melting point:

For melting point determination drug was filled into capillary tube and tied in such a way it remains dipped in liquid paraffin bath in Thiel's tube and temperature was noted. The pH determination was done by the pH meter.

Solubility study:

To determine the aqueous solubility of drug, saturation solubility study has been carried out. The excess amount of cocrystals were dissolved in a water for 24 hrs on the rotary shaker. Appropriate aliquots were then withdrawn and filtered through Whatman filter paper no. 41 and analyzed spectrophotometrically. The results obtained from saturation solubility studies were statistically validated.

Differential scanning calorimetry (DSC): Thermal analysis was done to know the interaction of coformer with the drug which is evident from the changes in endothermic peaks of the drug and cocrystals.

X-ray diffraction (XRD): XRD studies of cocrystals were performed using Philips Analytic X-Ray—PW 3710 (Philips, Almelo, The Netherlands) diffractometer.

Scanning electron microscopy (SEM):

The surface morphological properties of cocrystals of succinic acid were investigated by scanning electron microscopy (SEM-Jeol Instruments, JSM-6510, Japan).

Infrared spectroscopy:

IR spectroscopy was carried out to check compatibility. This was done with 03A26, Shimatzu, Japan.

Drug content analysis:

The percent drug content of in cocrystals was estimated by dissolving cocrystals and put in a volumetric flask containing 100 ml of simulated stomach fluid at pH 1.2. The samples were sonicated using ultrasonicator (Remi Equipments, Mumbai) for 15 min and the sample were filtered through Whatman filter paper (No. 41) analyzed using UV spectrophotometer (1601, Shimadzu Corporation, Japan) and absorbance were taken.

Dissolution study of co-crystals:

The dissolution rate studies were conducted in 900 mL of phosphate buffer (pH 1.2) at 50 rpm maintained at 37±0.5°C in a dissolution apparatus Electrolab, Navi Mumbai using the basket method. Quantity equivalent to unit dose of cocrystals was added to dissolution medium and the samples were withdrawn at appropriate time intervals. The samples were immediately filtered through Whatman filter paper no. 41, suitably diluted and analyzed spectrophotometrically. The data obtained from dissolution studies were statistically validated.

3. Results & Discussion

Microscopic characterization of cocrystals: Microscopic characteristics of prepared cocrystals were observed cocrystals by light microscope. Microscopic images are shown in following **Fig.**

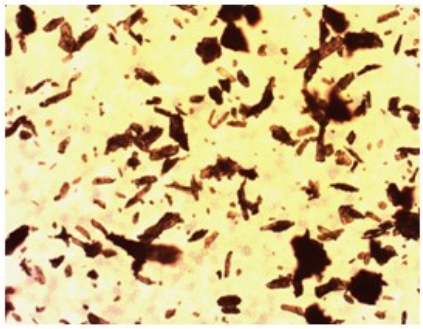


Figure: Cocrystals of F1

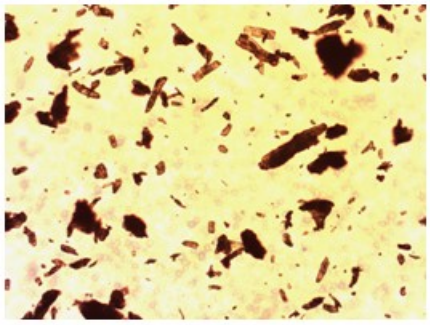


Figure: Cocrystals of F2

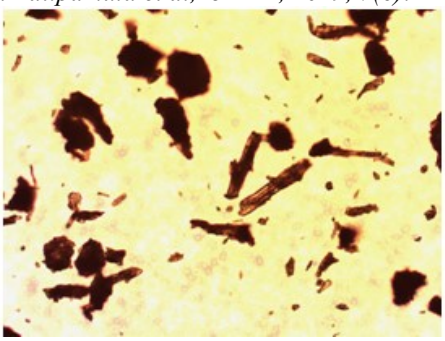
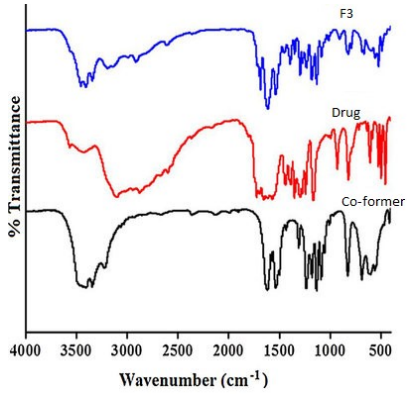


Figure: Cocrystals of F3

Solid state characterization

a) Fourier transformation infrared spectroscopy (FTIR): The FT-IR spectrum was measured in the solid state as potassium bromide mixture. FTIR spectrum of pure drug, coformer, and optimized cocrystals were shown in **Fig.**



b) Scanning electron microscopy (SEM): SEM of drug, coformer, and optimized cocrystals were shown in figures below.

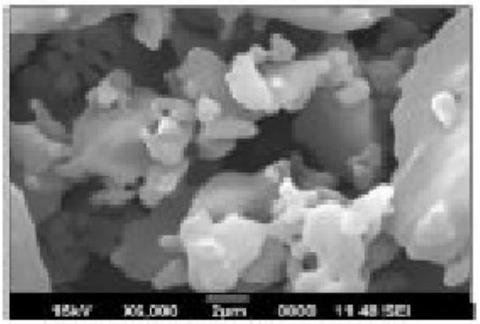


FIG. SEM IMAGES OF DRUG

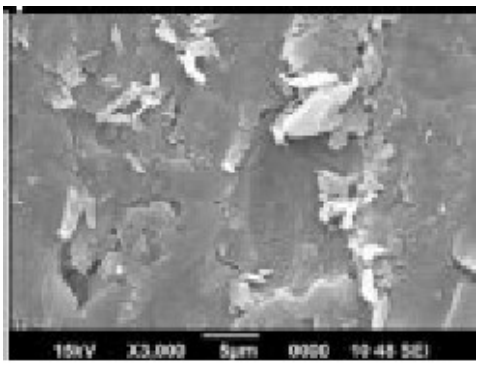


FIG. : SEM IMAGES OF CONFORMER

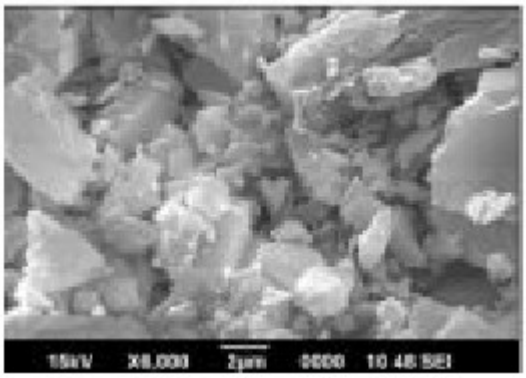


FIG. : SEM IMAGES OF COCRYSTALS

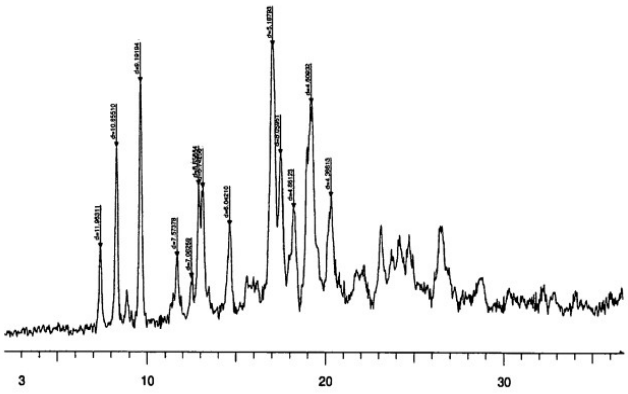


Figure: XRD of Pure Drug

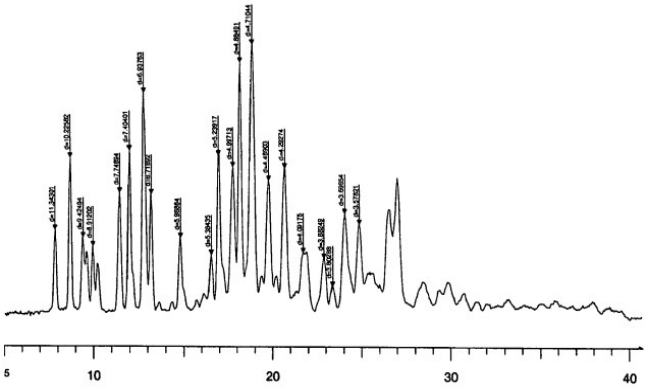


Figure: XRD of Co-Former

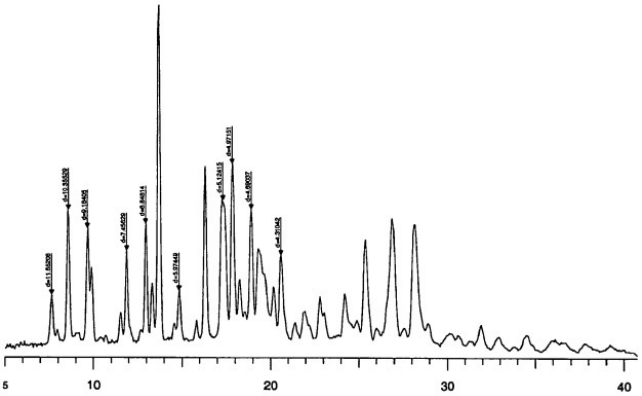


Figure: XRD of Optimized Co-Crystal

D) Differential Scanning Calorimetry (DSC): The DSC thermographs of pure drug, co former and cocrystals were shown in figure**.**

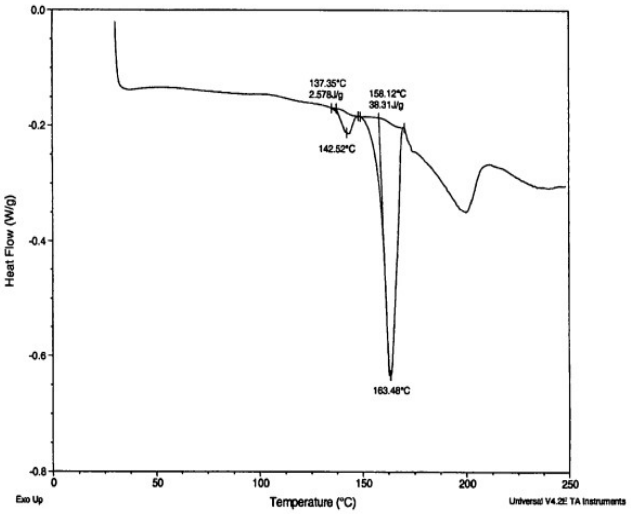


Figure: DSC of Pure Drug

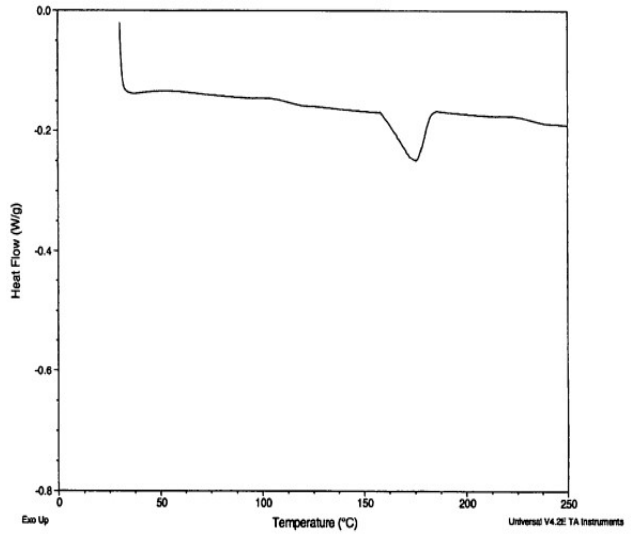


Figure: DSC of Co-former

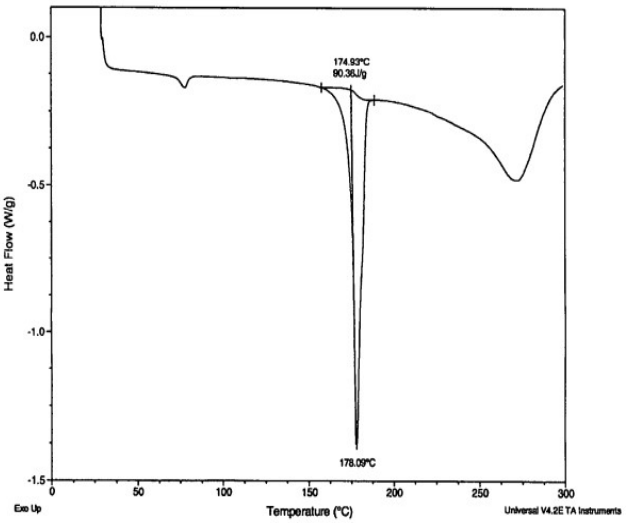
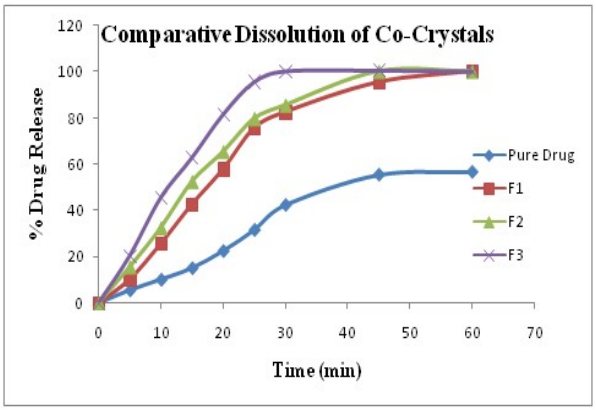


Figure: DSC of Optimized Cocrystal

Dissolution studies: *In vitro* dissolution study of prepared cocrystals were done by using U.S.P. Paddle type apparatus at 37 ºC.



Discussion of Results:

From the preformulation study the melting point of drug was found to be 155-165ºC. Asunaprevir shows poor aqueous solubility (50mg/L). Cocrystallization technique shows significant improvement in the aqueous solubility of Asunaprevir. Incorporation of coformer like 3-methoxy-4-hydroxybenzoic acid, 2,4-dihydroxybenzoic acid and 2,5-dihydroxybenzoic acid enhances solubility of asunaprevir by the process of cocrystallization increased the aqueous solubility of API to 125mg/L, 153mg/L & 225.5mg/L i.e. 2-4 fold increase in saturation solubility. The flow ability represented in terms of the angle of repose, Carr's index and Hausnar's ratio of cocrystals was improved compared to those of the original drug. So the prepared cocrystals have the good flow properties.

The results of drug contents of optimized batch were found satisfactory with 97.36-99.35%. All the characteristic peaks of purer drug appeared in the IR spectra of the co crystal at the same wave number indicating no modification or interaction between the drug and the coformer. It showed that drug was compatible with coformer. There is a shift in the C-H (alkyl) functional groups in FT-IR frequency indicate the formation of Hydrogen bond synthons in the Drug and Coformer. Prepared Cocrystal of drug and 2,5-dihydroxybenzoic acid as coformer were Characterized by FTIR. Absence of crystalline structure in SEM images of drug indicates its amorphous nature. Whereas, SEM image of coformer shows big crystals and cocrystals showing aggregates of crystals. An examination of the SEMs shows surface morphological properties of drug and crystals confirm that drug particles crystallized from methanol-water system containing coformer. The generation of cocrystalline material was confirmed by recording X-Ray powder diffraction of pure drug, coformer and optimized cocrystals. XRPD is a powerful technique for determining the presence of polymorphs, crystal habbit modification in drug crystals and generation of new crystals form during cocrystallization technique fig., unique XRPD pattern distinguishable from the host (drug) and the guest (coformer). The thermal analysis of drug and cocrystals were studied using differential scanning calorimetry (DSC)

shown in Figure and Sharp melting endotherm of drug. Coformer showing no mmelting endotherm. Thermogram of cocrystals shows melting endotherm of drug at similar melting point which may be because of adsorbed drug and coformer on cocrystals showing melting endotherm. The cocrystal described here show's melting temperature reduced from that of Drug, suggesting that the cohesive energy of cocrystal is decreased from that of the pure Drug.

The release data obtained for formulations are shown in Figure which shows the plot of cumulative percent drug released as a function of time for different formulations. The *in-vitro* release of all the three of cocrystals showed an immediate release with an initial burst effect. Among all the formulation F3 has shown faster release than all other formulations and hence has been selected as best formulation.

TABLE 2: PHYSICOCHEMICAL PROPERTIES OF DRUG

Drug name	Organoleptic properties	pH measurement	Melting point	Solubility
Asunaprevir	White to light brown colour powder.	3.2	155 - 165 ºC	Methanol

Determination of drug content: Drug contents in cocrystals were shown in **Table 3.**

TABLE 3: Result of Drug Content

Formulation	% Drug content (w/w)
F1	97.36
F2	98.26
F3	99.35

The results of drug contents were found satisfactory with 97.36-99.35%. This is quite important with respect to formulation development of cocrystals in the suitable form.

Flow ability studies: The micromeritic properties such as flow ability of cocrystals are shown in **Table 4**.

Table 4: Results of Flow ability Studies

System/parameters	Hausnar's ratio	Carr's index (%)	Angle of repose(θ°)
Asunaprevir (Drug)	1.32	27-32	38.49
F1	1.22	27-32	27.32
F2	1.15	25-27	25.36
F3	1.11	23-25	22.32

4. Conclusion

In the present work prepared drug cocrystals exhibited excellent physicochemical properties (solubility and dissolution) and micrometric properties when compared with pure drug. From the conducted study, we can conclude that cocrystals with 2,5-dihydroxybenzoic acid prepared by the use of cocrystallization technique showed an improvement in the solubility, dissolution rate and flow ability as compared with pure drug. Solid state characterization of drug and cocrystals showed satisfactory results such as FTIR proves compatibility; SEM showed enlarged size with signs of porosity, while DSC showed thermal evaluation. The altered size and shape of cocrystals indicated modified crystal habit which could be responsible for dramatic improvement in flow ability, solubility and dissolution properties of asunaprevir from the cocrystals. On the basis of these results, it could be concluded that, cocrystals of asunaprevir with 2,5-dihydroxybenzoic acid could be possible and served as an alternative and effective approach for improvement in physicochemical and micromeretic properties of Asunaprevir. By virtue of improved stability, immediate release or conventional dosage form can be prepared by using such cocrystals which could be the best alternative for the prompt delivery as compared to orally administered products available in market.

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