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FORMULATION AND EVALUATION OF CO-CRYSTALS OF POORLY WATER SOLUBLE DRUG

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Keywords:

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ABSTRACT: The present study is an attempt to formulate and evaluate cocrystals of poorly water soluble drug Darunavir by cooling crystallization method using succinic acid as coformer. Cooling crystallization method used to prepare the cocrystals of darunavir with succinic acid. The prepared cocrystals were characterized by Microscopic characters, Product yield, Surface morphology. (SEM), Differential scanning calorimetry, X-ray diffraction, Fourier Transform Infrared Spectroscopy, Micromeretic properties, Dissolution study of cocrystals, stability studies, Thus, cocrystals using water soluble polymer like Succinic acid can be prepared using cooling crystallization method for a poorly water soluble drug such as Darunavir.

INTRODUCTION: Crystalline forms of active pharmaceutical ingredients, API's, have traditionally been limited to salts, polymorphs, and solvates (including hydrates). Given the high intrinsic value of API's and the importance of structure and composition in the context of both intellectual property and bioavailability, it is perhaps surprising that systematic approaches to the development of a new broad class of API, pharmaceutical cocrystals, have only been attempted in recent years.

Co-crystals represent a long known class of compounds, a prototypal example of which is quinhydrone, which was reported at least as early as 1844 and 1893 [1].

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Co-crystals are solids that are crystalline materials composed of two or more molecules in the same crystal lattice (or) Pharmaceutical co-crystals have, however, been defined as 'co-crystals that are formed between a molecular or ionic active pharmaceutical ingredient (API) and a benign co-crystal former that is a solid under ambient conditions [2]. Solubility is an important parameter for evaluating the properties of a pharmaceutical cocrystal. Traditional methods for improving solubility of poorly water soluble drugs include salt formation, solid dispersion (emulsification), and particle size reduction (micronisation).

However there are practical limitations with these techniques [4]. A pharmaceutical cocrystal is a novel approach to improve the physicochemical properties such as solubility of compounds [1, 3, 4, 5, 6]. Darunavir, BCS Class II drug having low solubility and high permeability was selected as the drug taking into consideration the above given parameters.

The drug with a molecular weight of 547.66 g/ml has a bitter taste with a maximum upto 400-600 mg/day in case of HIV infection [7].

MATERIALS AND METHODS:

Materials: Darunavir was received as gift samples from Mylan laboratories Ltd., Hyderabad, India. Succinic 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)

2. Formulation of cocrystals: [8] 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.

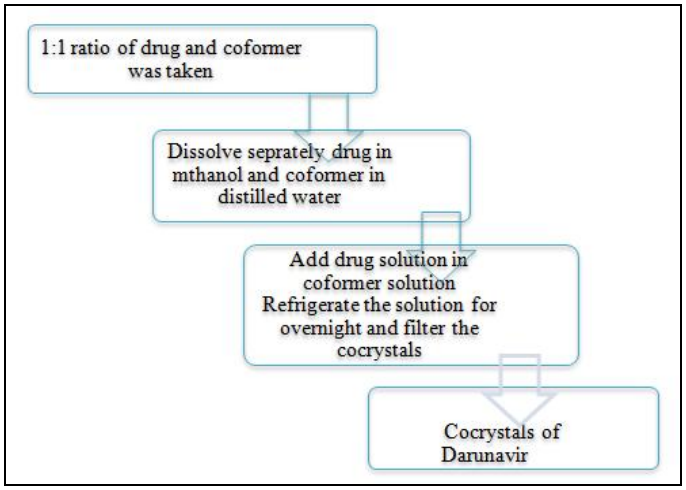


FIG. 1: METHOD OF PREPARATION OF COCRYSTAL

TABLE 1: FORMULATION OF COCRYSTALS

Ingredients	Formulation batches of cocrystals (Drug: Coformer)			
	C1 (1:1)	C2 (1:2)	C3 (1:3)	C4 (5:1)
Drug(DRV)	100 mg	100 mg	100 mg	500 mg
Succinic acid	100 mg	200 mg	300 mg	100 mg
Methanol	10 ml	10 ml	10 ml	20 ml
Water	10 ml	10 ml	10 ml	20 ml

*DRV- Darunavir; C1, C2, C3, C4- Formulation batches which are prepared by (drug: conformer) ratio

3. Evaluation /Characterization of Co-Crystals:

- **Physical appearance:** Cocrystals were characterize 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 (EI product- 181).

- **Solubility study:** [9] To determine the aqueous solubility of darunavir, 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 at 264 nm. The results obtained from saturation solubility studies were statistically validated.

- **Differential scanning calorimetry (DSC):** [10] Thermal analysis was done to know the interaction of coformer with the darunavir which is evident from the changes in endothermic peaks of the drug and cocrystals.

- **X-ray diffraction (XRD):** [10] XRD studies of cocrystals were performed using Philips Analytic X-Ray— PW 3710 (Philips, Almelo, The Netherlands) diffractometer.

- **Scanning electron microscopy (SEM):** [10] The surface morphological properties of cocrystals of succinic acid were investigated by scanning electron microscopy (SEM-Jeol Instruments, JSM-6510, Japan).

- **Infrared spectroscopy:** [10] IR spectroscopy was carried out to check compatibility. This was done with 03A26, Shimatzu, Japan.

- **Drug content analysis:** The percent drug content of Darunavir 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:** [11] 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 20 mg 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 at 298nm. The data obtained from dissolution studies were statistically validated.

RESULTS AND DISCUSSION:

1. Preformulation study:

TABLE 2: PHYSICOCHEMICAL PROPERTIES OF DRUG

Drug name	Organoleptic properties	pH measurement	Melting point	Solubility
Darunavir	White to light brown colour powder.	3	74 - 76 ºC	Methanol

2. Microscopic characterization of cocrystals: Microscopic characteristics of prepared cocrystals were observed cocrystals by light microscope. Microscopic images are shown in following **Fig. 2**.

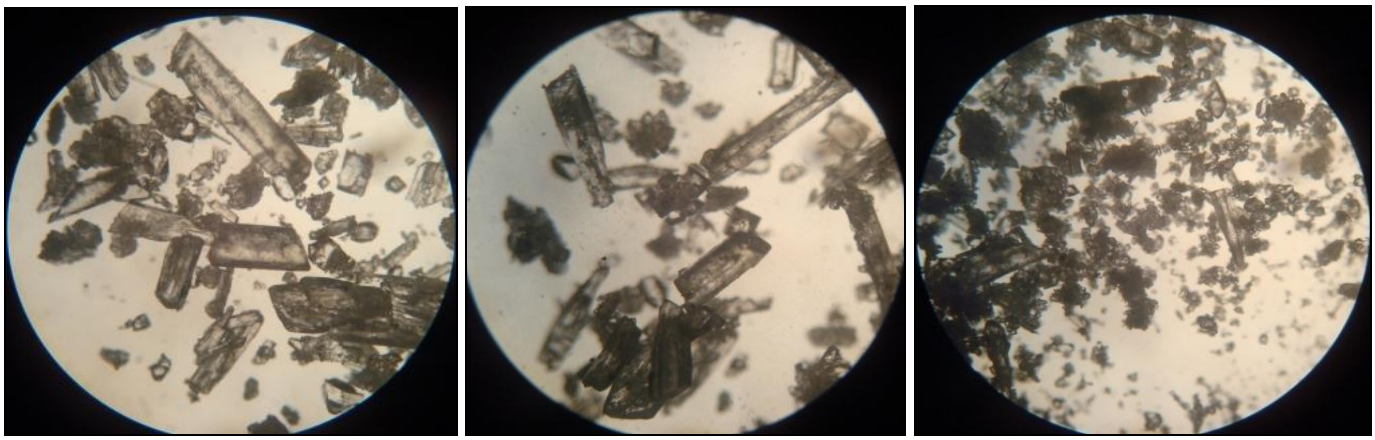


FIG. 2: MICROSCOPIC IMAGES OF COCRYSTALS.

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

TABLE 3: RESULT OF DRUG CONTENT

System	% Drug content (w/w)
Cocrystals	83.91%

The results of drug contents were found satisfactory with 83.91%. This is quite important with respect to formulation development of cocrystals in the suitable form.

4. Flowability studies: The micromeritic properties such as flowability of cocrystals are shown in **Table 4**.

TABLE 4: RESULTS OF FLOWABILITY STUDIES

System/parameters	Hausnar's ratio	Carr's index(%)	Angle of repose(θ°)
Darunavir(Drug)	1.30	21-25	38.49
Cocrystals	1.42	27-32	40

5. 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, physical mixtures and cocrystals were shown in **Fig. 3-6.**

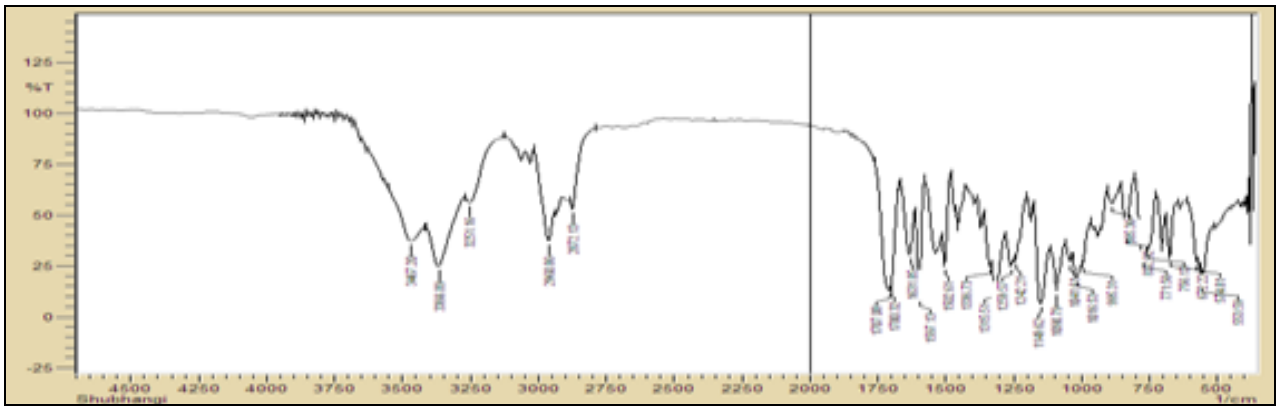


FIG. 3: FT-IR SPECTRUM OF DRV

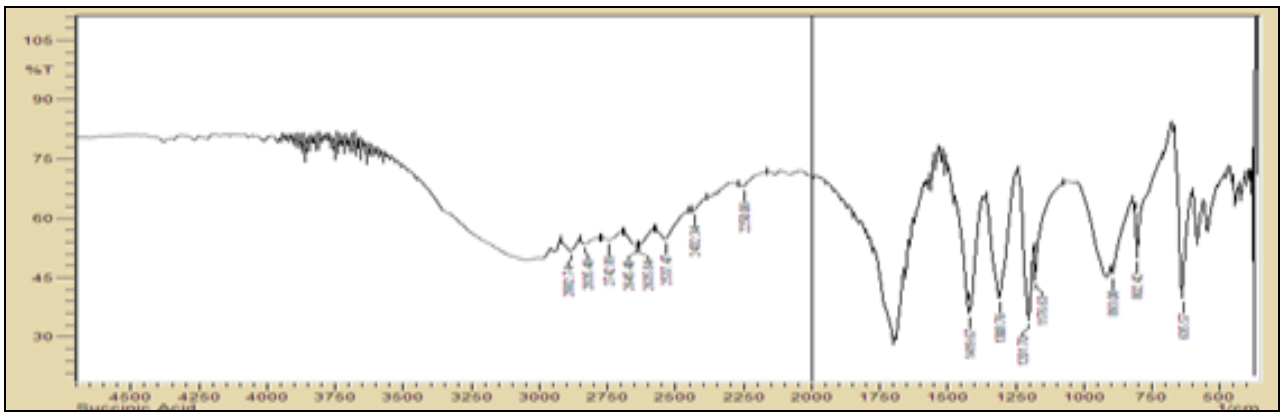


FIG. 4: FT-IR SPECTRUM OF SUCCINIC ACID (COFORMER).

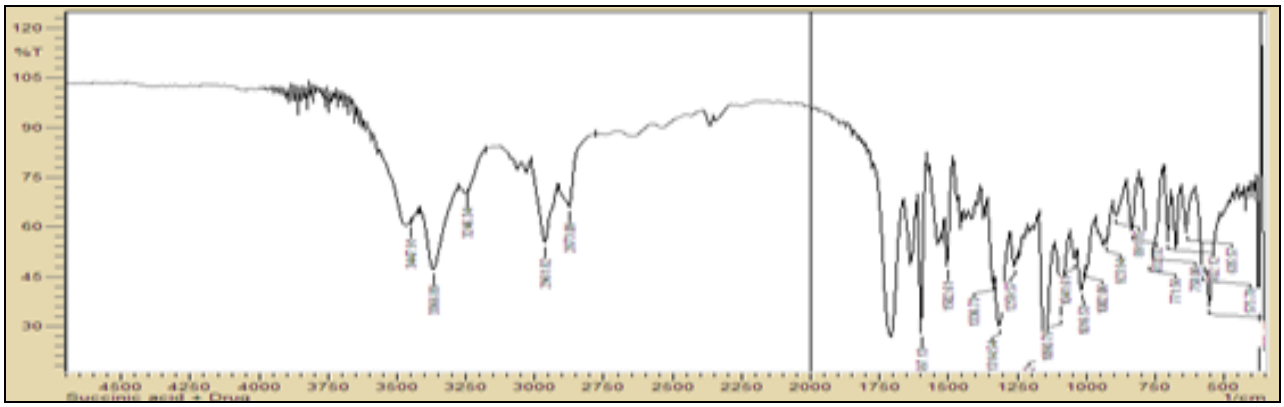


FIG. 5: FT-IR SPECTRUM OF PHYSICAL MIXTURE OF DRUG AND COFORMER

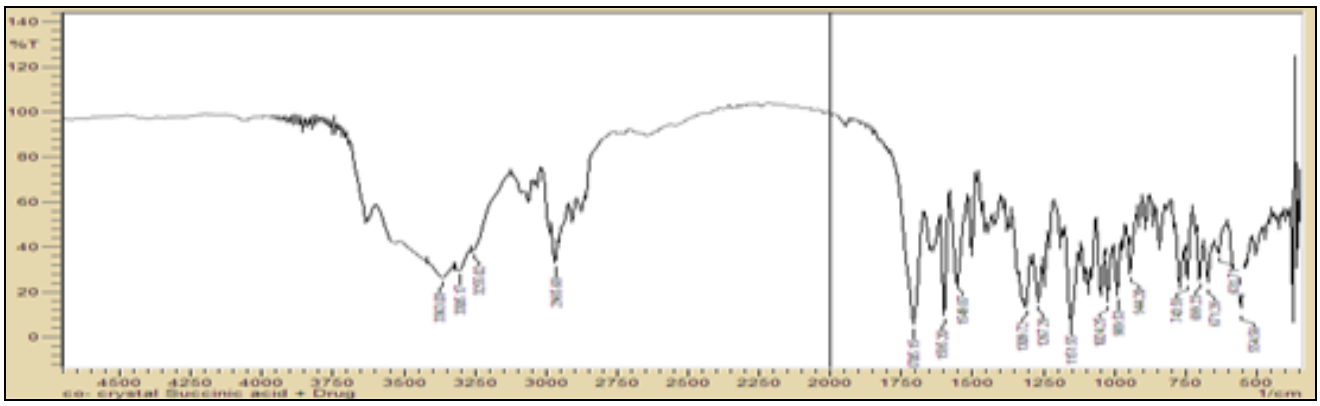


FIG. 6: FT-IR SPECTRUM OF COCRYSTALS

b) Scanning electron microscopy (SEM): SEM of drug, coformer, physical mixture and cocrystals were shown in **Fig. 7-9**.

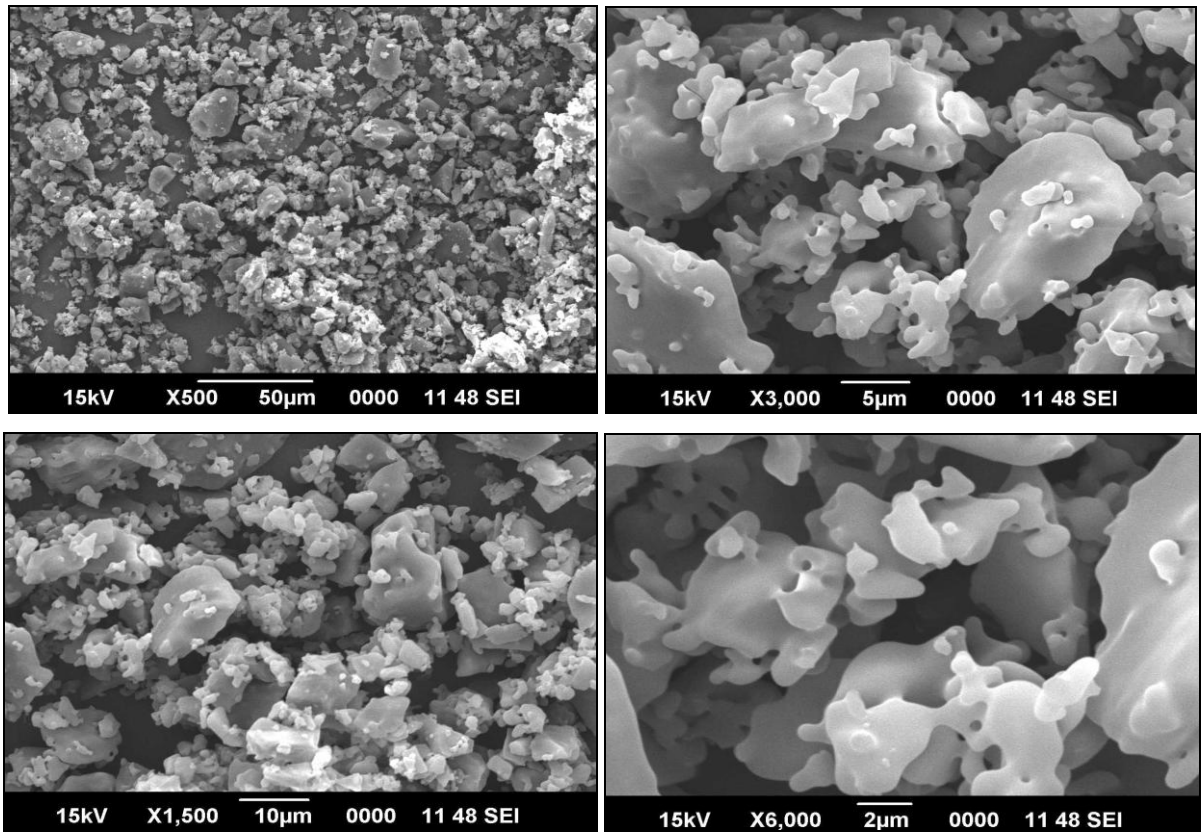
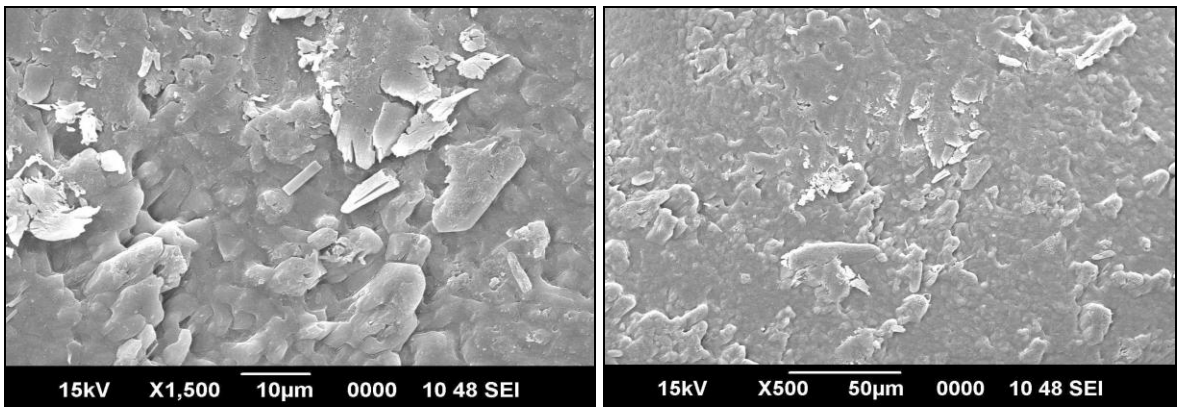


FIG. 7: SEM IMAGES OF DRUG



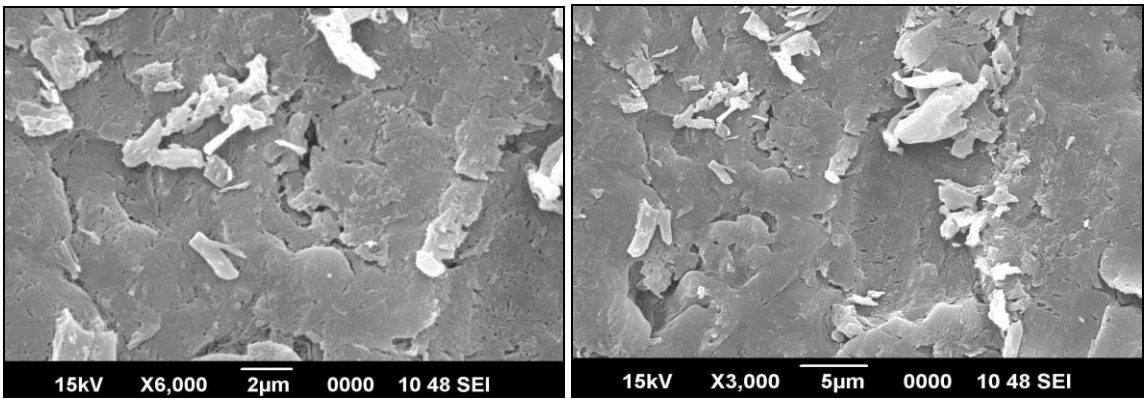


FIG. 8: SEM IMAGES OF CONFORMER

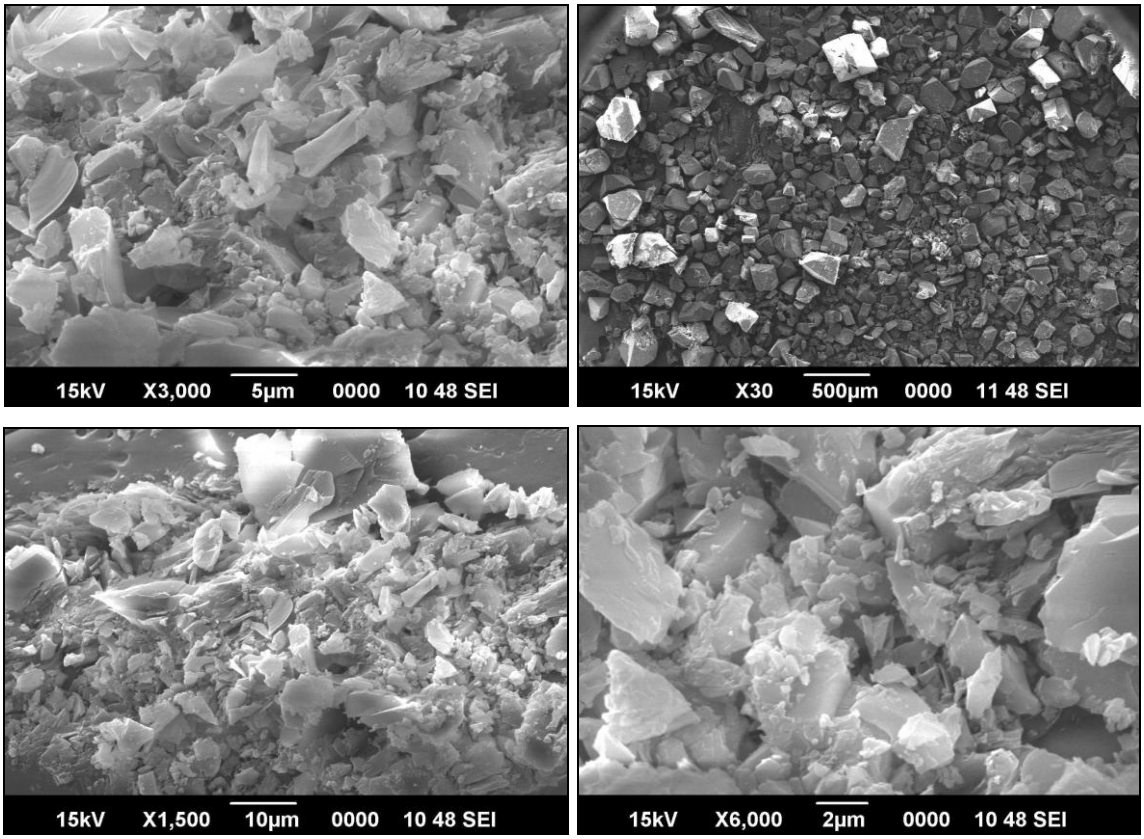


FIG. 9: SEM IMAGES OF COCRYSTALS

c) X-ray powder diffraction: X-RD patterns of pure drug, coformer and cocrystals were shown in **Fig. 10-12.**

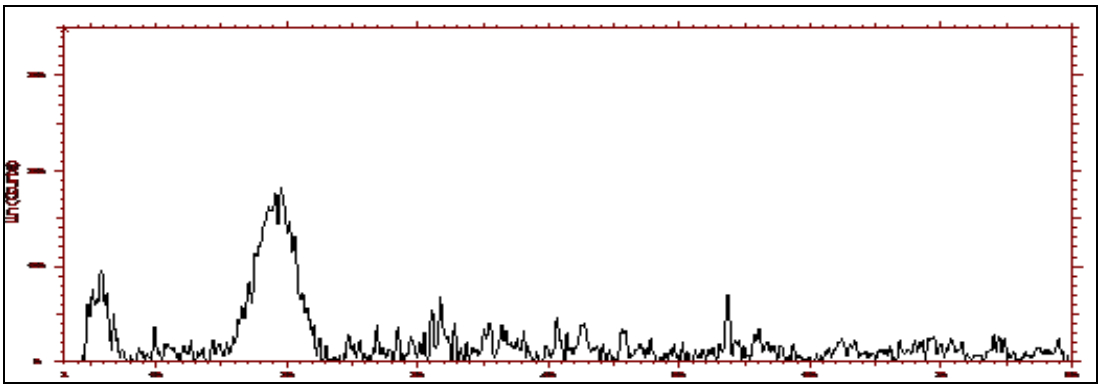


FIG. 10: XRD PATTERN OF DRUG DARUNAVIR

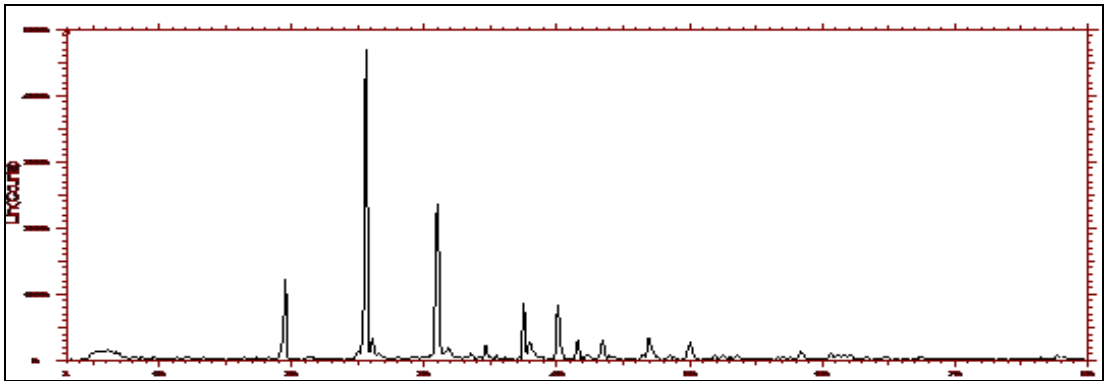


FIG. 11: XRD PATTERN OF CONFORMER (SUCCINIC ACID)

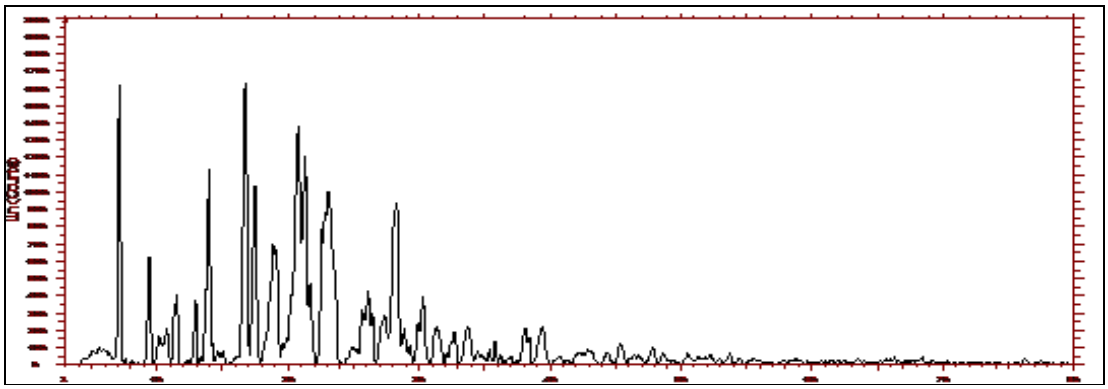


FIG. 12: XRD PATTERN OF COCRYSTALS

D) Differential Scanning Calorimetry (DSC): The DSC thermographs of pure drug, physical mixtures and cocrystals were shown in **Fig. 13-16.**

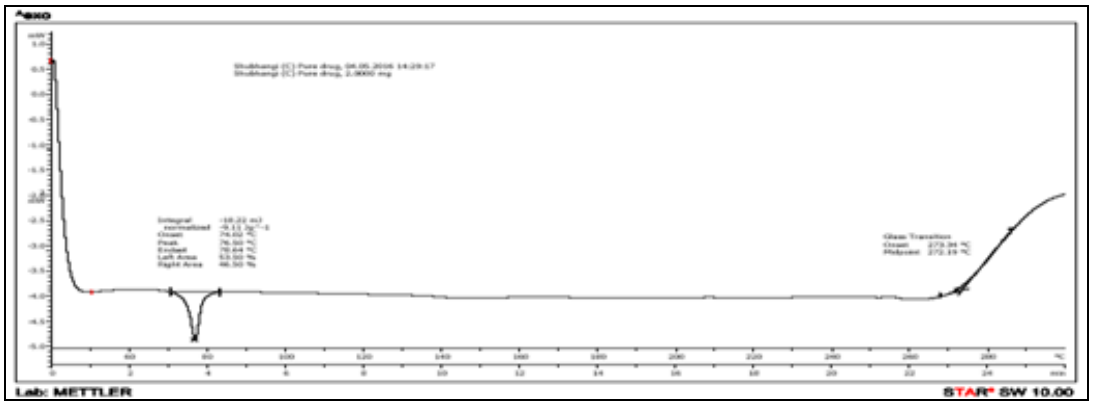


FIG. 13: DSC THERMOGRAM OF DARUNAVIR

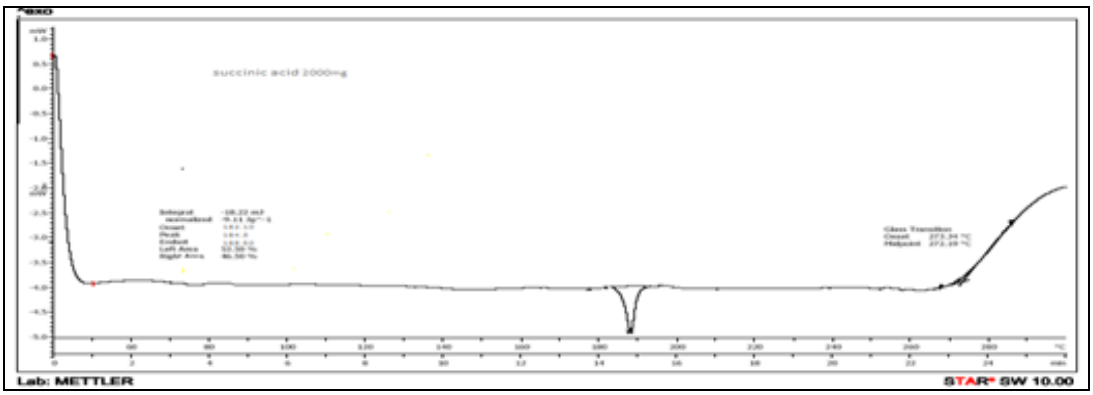


FIG. 14: DSC THERMOGRAM OF SUCCINIC ACID

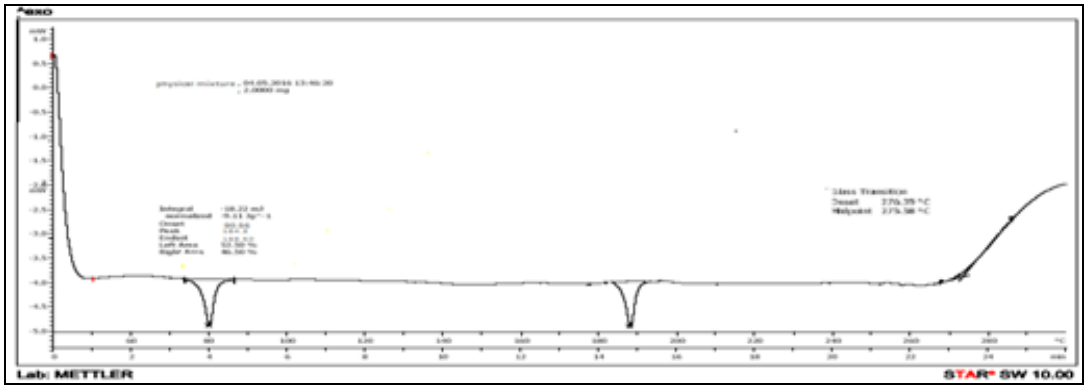


FIG. 15: DSC THERMOGRAM OF PHYSICAL MIXTURE

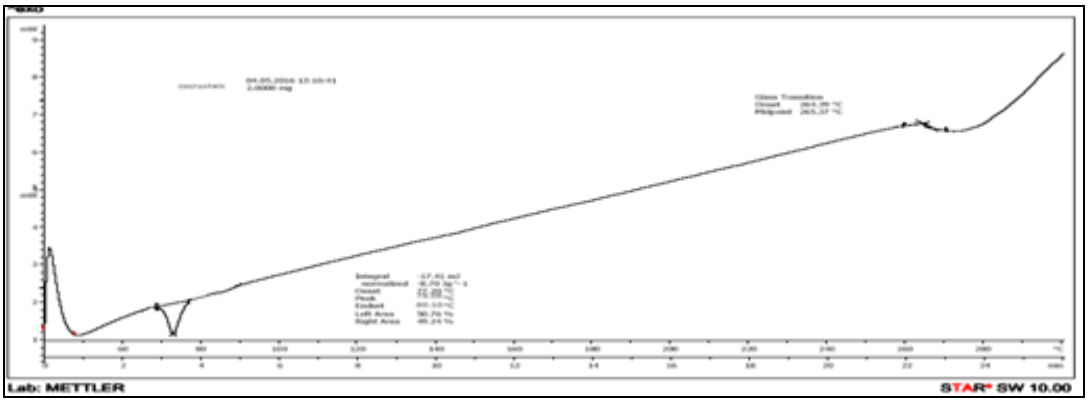


FIG. 16: DSC THERMOGRAM OF COCRYSTALS

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

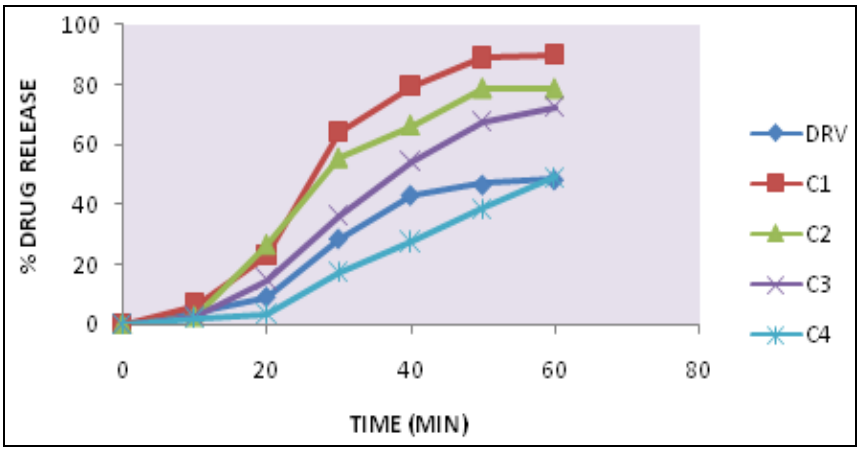


FIG. 17: DRUG RELEASE PATTERN OF COCRYSTALS

7. Stability studies: Optimized formulation (C1) was filled into vials as the final optimized batch which was subjected to stability studies to determine its physical stability.

TABLE 5: STABILITY DATA OF OPTIMIZED BATCH C1

Period Parameters	Initially (0 Day)	1 Month	2 Months	3 Months
Bulk density(g/cc)	0.504	0.501	0.50	0.49
Tapped density(g/cc)	0.462	0.46	0.45	0.45
Angle of repose	40°	40°	40°	40°
Drug content (%)	83.91%	83.89%	83.88%	83.87%
Drug release (%)	89.65%	89.64%	89.60%	89.59%

From the preformulation study the melting point of drug Darunavir was found to be 74-76 ºC. Darunavir shows poor aqueous solubility (299.53µg/ml). Cocrystallization technique shows significant improvement in the aqueous solubility of darunavir. Incorporation of coformer like succinic acid enhances solubility of darunavir by the process of cocrystallization increased the aqueous solubility of darunavir to (575.503 µg/ml) i.e. 1.92 fold increase in saturation solubility. The flowability represented in terms of the angle of repose, Carr's index and Hausnar's ratio of cocrystals was not much more improved compared to those of the original drug. These results however were contrary to those reported by earlier workers. So the prepared cocrystals does not have the good flow properties.

The results of drug contents of optimized batch were found satisfactory with 83.91%.

Pure DRV spectrum showed sharp characteristic peaks at 3467, 3366, 1708, 2872, 1242 cm^{-1}. All the above characteristic peaks of drug appeared in the spectra of the physical mixture at the same wave number indicating no modification or interaction between the drug and the coformer. It showed that darunavir was compatible with coformer succinic acid. There is a shift in the C-H (alkyl) functional groups at peak 2965. FT-IR frequency indicate the formation of Hydrogen bond synthons in the Drug and Coformer. Prepared Cocrystal of Darunavir and Succinic acid as coformer were Characterized by FTIR.

Absence of crystalline structure in SEM images of Darunavir 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 Darunavir particles crystallized from methanol-water system containing succinic acid as coformer. The generation of cocrystalline material was confirmed by recording X-Ray powder diffraction of pure Darunavir, coformer and 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 darunavir at 76 ºC. Coformer showing melting endotherm at 184 ºC. Physical mixture of 1:1 Darunavir and succinic acid showing intact endothermic peak at 80 ºC and 184 ºC. Thermogram of cocrystals shows melting endotherm of darunavir at 76 ºC and succinic acid at 184 ºC which may be because of adsorbed drug and coformer on cocrystals showing melting endotherm peak at 74 ºC. 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 C1, C2, C3 and C4 are tabulated in table no. and 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 four batches of cocrystals showed an immediate release with an initial burst effect. In the first 10 mins, drug release was 6.43%, 2.54%, 2.65 % and 1.78 % for batch C1, C2, C3 and C4 respectively. The *in vitro* dissolution performance of Cocrystals, which was significantly higher in C1 batch than the plain drug i.e 89.65%. Optimized formulation (C1) was filled into vials as the final optimized batch which was subjected to stability studies to determine its physical stability. It was found that there is no significant changes in bulk density, tapped density, angle of repose, drug content and drug release in the duration of four months.

CONCLUSION: In the present work prepared darunavir 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 succinic acid prepared by the use of cocrystallization technique showed an improvement in the solubility, dissolution rate and flowability and stability 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 flowability, solubility and dissolution properties of darunavir from the cocrystals. On the basis of these results, itcould be concluded that, cocrystals of darunavir with succinic acid could be possible and served as an alternative and effective approach for improvement in physicochemical and micromeretic properties of darunavir. 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 of darunavir as compared to orally administered products available in market.

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