

PREPARATION AND CHARACTERIZATION OF CO-CRYSTALS OF DIACEREIN

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ABSTRACT

Diacerein, an anti-inflammatory drug used in the treatment of osteoarthritis. Being a BCS class II drug, it has poor solubility, dissolution rate, and other physicochemical properties. Thus the aim of present study was to prepare co-crystals of diacerein to improve solubility and dissolution rate. The diacerein co-crystals were prepared using urea and tartaric acid as conformer by Solvent drop grinding method. The diacerein co-crystals were characterized by scanning electron microscopy (SEM), Fourier Transform Infrared Spectroscopy (FT-IR), Differential scanning calorimetry (DSC) and X-ray diffractometry (XRD). The co-crystals were evaluated for solubility, dissolution rate, and other physicochemical properties and compared with commercial diacerein. The co-crystals exhibit the difference in the size and shape of crystals. The FT-IR spectra of diacerein co-crystals showed slightly different in the characteristic peaks compared to commercial diacerein sample. DSC data indicate the decrease in the melting endotherm of co-crystals compared to diacerein. The co-crystals with urea showed an increase and intense peak and co-crystals with tartaric acid showed decreased number of peaks compared to commercial diacerein. The co-crystals of diacerein formulated into the Tablet and evaluated for tablet properties. The tablet formulation showed improved tablet characteristics as well as dissolution rate compared to commercial diacerein.

Keywords: Co-crystals, Diacerein, physicochemical properties, FT-IR

INTRODUCTION

Crystal habit and internal structure of a drug can affect bulk and physicochemical properties, which range from the flowability to chemical stability. Crystals are characterized by the repetitious spacing of constituent atoms or molecules in a three-dimensional array, whereas amorphous forms have atoms or molecules randomly placed as in a liquid. Crystallization is a spontaneous arrangement of particles to a repetitive ordered array i.e. regular geometrical pattern (Subrahmanyam *et al.*, 2000). When applied solid the adjective crystalline, implies an ideal crystal in which structural units termed unit cells are repeated regularly and indefinitely in three dimensions in a space (Vippagunta *et al.*, 2001). An ideal crystal is constructed by the regular spatial repetition of identical structural units (Saifee *et al.*, 2009) Crystal form can be

crucial to the performance of dosage form (Hickey *et al.*, 2007)

Over the last decade, there has been growing interests in the design of pharmaceutical co-crystals, which has emerged as a potential method for enhancing the bioavailability of drug with low solubility (Bittain *et al.*, 2006). To start with, it is necessary to important definitions: co-crystal and pharmaceutical co-crystals. Co-crystal can be defined in a number of ways. A restrictive definition utilized by that co-crystals are structurally homogenous crystalline materials containing two or more components present in definite stoichiometric amounts. The co-crystals components are discrete neutral molecular reactants which are solid at ambient temperature (Gagniere *et al.*, 2009). Based on this definition of co-crystal, a pharmaceutical

co-crystal means co-crystals with one of the co-crystal components as an Active Pharmaceutical Ingredient (API) and other components are called conformers. From these definitions, it is clearly shown that an API hydrate is not co-crystal; however solid-state API hydrate can cocrystallized with a solid conformer to form co-crystal (Lachman *et al.*, 1991). Co-crystal represents a class of compound which offers huge opportunities for the pharmaceutical industry (Yadav *et al.*, 2012). Diacerein, an anti-inflammatory drug used in the treatment of osteoarthritis. Being a BCS class II drug, it has poor solubility, dissolution rate, and other physicochemical properties. Thus project had focused on the improvement of physicochemical properties of diacerein by co-crystal techniques.

MATERIALS AND METHODS

Diacerein obtained as a gift sample from Lupin Pharmaceutical Ltd, Mumbai, India. Urea and tartaric acid were purchased from SD Fine Chemical Mumbai. All the solvents used are of analytical grade and purchased from Qualigens, Mumbai.

Preparation of co-crystal

Co-crystal prepared with urea as coformer

Pharmaceutical co-crystal of diacerein was prepared with different co-formers using solvent drop grinding methods. Diacerein-Urea co-crystal was prepared by grinding diacerein and urea in 1:1 molar ratio in a pestle and mortar for 90min with the addition of few drops of ethanol (approximately 10% of weight). The solid powder was then scratched from walls of mortar and stored in a container (Maski *et al.*, 2009).

Co-crystal prepared with tartaric acid as coformer

Pharmaceutical co-crystal of diacerein was prepared with different co-formers using solvent drop grinding methods. Diacerein-Tartaric acid co-crystal was prepared by grinding diacerein and urea in 1:1 molar ratio in a pestle and mortar for 90min with the addition of few drops of ethanol (approximately 10% of weight). The solid powder was then scratched from walls of mortar and stored in container (Maski *et al.*, 2009).

Micromeritic properties of co-crystal

Melting point

To determine the melting point of a co-crystal, a small amount of the sample is placed into a thin-walled capillary tube that is sealed at one end. The capillary tube is inserted into a melting point apparatus in which the temperature can be measured when heated (Deshmukh *et al.*, 2010).

Angle of repose

It was determined by fixed funnel method. Accurately weighed quantity (5gm) of the sample was taken in a funnel; the height of the funnel is adjusted such that the tip of the funnel just touches the apex of the heap of the blend. Then the drug is allowed to flow through the funnel freely onto the surface. The diameter is then measured and angle of repose was calculated by following equation (Deshmukh *et al.*, 2010).

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

Where θ is the angle of repose, h is the height of the cone and r is the radius of the cone base.

Bulk density

It was determined by pouring a weighed quantity (5gm) of the sample in to a graduated cylinder. The cylinder was dropped at 2s interval on hard wood surface three times from the height of one inch. It was then calculated by the equation given below (Deshmukh *et al.*, 2010).

$$\text{Bulk density} = \frac{\text{the weight of the powder}}{\text{bulk volume}}$$

Tapped density

It was determined by pouring a sample (5gm) in a measuring cylinder. The cylinder was dropped at 2s interval on hard surface 100 times from the height of 1 inch. Then the final volume occupied by the drug was measured (Deshmukh *et al.*, 2010).

$$\text{Tapped density} = \frac{\text{weight of the blend}}{\text{final volume}}$$

Compressibility index

The compressibility index (Carr's index) is a measure of a powder to be compressed.

$$\text{Carr's compressibility index} = \frac{\text{tapped density} - \text{bulk density}}{\text{tapped density}} \times 100$$

Hausner ratio

The Hausner ratio of powder was calculated according to the equation given below.

$$\text{Hausner ratio} = \frac{D_t}{D_f}$$

Where, D_t = tapped density, D_f = bulk density

**Characterization of co-crystal
Infrared spectroscopy (FTIR)**

The co-crystals of Diacerein and commercial sample were scanned and recorded in the range of $4000\text{--}400\text{cm}^{-1}$ by using an Infrared spectrophotometer, (Bruker, Alfa-T, Germany). The co-crystal samples were triturated with dried potassium bromide using mortar and pestle. The mixture after grinding into fine powder was kept uniformly in a suitable die and compressed into a pellet form by using a hydraulic press. The resultant pellet was mounted in a suitable holder in the IR spectrophotometer.

Differential scanning calorimetry (DSC)

After calibration, thermograms were obtained by heating (5mg) of commercial sample and its co-crystals prepared with coformer at a constant heating rate of $10^\circ\text{C}/\text{min}$ with chart speed of $20\text{mL}/\text{min}$ under an atmosphere of nitrogen. The exact peak temperatures, melting point and heat of fusion were automatically calculated. The temperature range for the scan was 20°C to 400°C for all the samples.

Powder X-ray diffraction spectroscopy (PXRD)

X-ray diffraction pattern of Diacerein and its various co-crystals prepared with coformer were obtained using the X-ray diffractometer (BRUKER D8 ADVANCE, Germany) at 40kV, 30mA and a scanning rate of $1^\circ/\text{min}$ at the diffraction angle 2θ over the range of $10\text{--}60^\circ$ using Cu (as anode) radiation of wavelength 1.5406 \AA .

**Solubility and dissolution study
Solubility studies**

Solubility of diacerein as well as co-crystal was studied in medium such as (distilled water). The excess quantity (52.63 mg) of co-crystal was added in to glass stopper containing 50 mL of

the medium at $37\pm 0.5^\circ\text{C}$ for 24h on a magnetic stirrer at speed 150rpm. Then the solution was filtered using Whatman filter paper no 1. Appropriate dilution was made and concentration was determined spectrophotometrically (Shimadzu, Japan) at 340nm.

Dissolution studies

Dissolution studies of diacerein as well as co-crystal were performed using the USP dissolution test apparatus with basket rotating at 50rpm using 900mL medium at $37\pm 0.5^\circ\text{C}$. Co-crystal equivalent to 50 mg of diacerein were taken and filled in to capsule. The capsule was kept in basket & dissolution was carried out for 60min and the sample (5mL) was withdrawn at 10, 20, 30, 40, 50, 60min interval and the same volume was replaced to maintain sink condition. The absorbance of solution (after filtering through Whatman filter) was observed by using UV Spectrophotometer (Shimadzu, Japan) at 340nm (Nanajawade *et al.*, 2012).

Formulation of Co-crystals into Tablet

Accurately weight quantity of co-crystal and various excipients such as lactose monohydrate, starch, and talc are properly mixed, following addition of magnesium stearate as lubricant. The powder mixture was subjected to compression in to tablet.

**Evaluation of tablet
Hardness**

The resistance of tablet during shipping to breakage, under condition of storage, transportation and handling before use depends on its hardness. For each formulation, the hardness of 3 tablets was determined using the Pfizer hardness tester. The tablet was held along its oblong axis in between two jaws of taster. At this point, reading should be zero kg/cm^2 . Then constant force was applied until the tablet fractured. The value at this point was noted in kg/cm^2 .

Friability

Friability is the measure of tablet strength. Electrolab friabilator was used for testing the friability. For each formulation, the friability of 20 tablets was determined. This test subjects a number of tablets to the combined

effect of shock abrasion by utilizing the plastic chamber which revolves at the speed of 25rpm, dropping the tablet to a distance of 6 inches in each revolution. A sample of pre-weight 20 tablets was placed in friabilator which was then operated for 100 revolutions i.e. 4min. tablets were then dedusted and reweight. A loss of less than 1% in weight is generally considered acceptable. Percent friability (%F) was calculated as follows.

$$\% F = \frac{(\text{Initial weight} - \text{Final weight})}{\text{Initial weight}} \times 100$$

Weight variation test

The weight variation test was done by taking 20 tablets and weight accurately. The average weight of tablet was calculated.

Drug Content

The drug content was carried out by taking 20 tablets and triturate. Take equivalent weight of powder (5mg) were dissolved in 5mL dimethyl sulfoxide (DMSO) and volume was made up to 50mL with water by sonicating for 15min. The solution was filtered through Whatman filter paper no.41. After appropriate dilutions with water it was analyzed spectrophotometrically at 340nm (Shimadzu 1800, Japan).

Disintegration time

The process of breakdown of a tablet into smaller particles is called as disintegration. The in-vitro disintegration time of a tablet was determined using disintegration test apparatus as per I.P. specifications. Place one tablet in each of the 6 tubes of the basket. Add a disc to each tube and run the apparatus using distilled water maintained at $37 \pm 2^\circ\text{C}$. The assembly should be raised and lowered between 28 to 32 cycles per minute in the distilled water maintained at $37 \pm 2^\circ\text{C}$. The time taken for complete disintegration of the tablet with no palpable mass remaining in the apparatus was measured and recorded.

RESULT AND DISCUSSION

Preparation of Co-crystal

Diacerein showed good solubility in Dimethyl-sulfoxide. It was practically insoluble in distilled water, ethanol, methanol,

chloroform, ethyl acetate, and acetone. Thus co-crystals were prepared by solvent drop grinding method using ethanol as a solvent of choice because of its easy availability, cheaper in cost as well as coformer is soluble. Diacerein co-crystal was prepared by simple solvent drop grinding method because amongst its other methods like Solution co-crystallization, slurry conversation, anti solvent addition fails formation of co-crystal due to its solubility problem hence this method has use for formation of co-crystal in presence of co-former such as Urea, Tartaric acid with 1:1 molar ratio. The coformer has selected by its selection criteria high water solubility, hydrogen bond donor & acceptor group. These newly formed Co-crystals along with the pure drug were subjected to further characterization and various evaluation tests such as solubility, dissolution, etc.

The values of micromeritic properties were mentioned in the table I. The values indicates, that prepared co-crystals showed good flowability as well as compressibility. The angle of repose of co-crystals prepared with urea and tartaric acid showed 27.08 ± 0.85 and 28.77 ± 0.586 respectively compared to commercial diacerein 36.75 ± 0.303 , indicates improvement in the flowability.

The bulk and tapped density value of co-crystals prepared with urea and tartaric acid showed 0.363 ± 0.016 , 0.346 ± 0.024 and 0.436 ± 0.02 , 0.423 ± 0.01 respectively considered to commercial diacerein 0.331 ± 0.012 and 0.391 ± 0.02 showed good compactibility. The Carr's index and Hausners ratio values also supports the flowability and compressibility of diacerein.

Characterization of co-crystals

Infrared spectroscopy

Characteristic peaks of Diacerein appeared at 1417.68cm^{-1} (C=C stretching), 1668.43cm^{-1} (C=O stretching), 1026.1cm^{-1} (C-O-C stretching) and 1369.46cm^{-1} (Acidic OH) were observed. IR spectra of commercial drug when compared with the various Co-crystals of Diacerein obtained in the presence of coformer shows slight change in their characteristic peaks because due to the by donating and accepting hydrogen to form the hydrogen bonding in

Table I. Micromeritic properties of co-crystal

Formulation	Angle of Repose	Bulk density	Tapped density	Carr's index	Hausner's ratio	Melting Point
Pure Drug	36.75 ±0.303	0.331 ±0.012	0.391 ±0.02	14.45	1.37	217°C
Drug-urea co-crystal	27.08 ±0.805	0.363 ±0.016	0.436 ±0.02	11.96	1.25	150°C
Drug-TA co-crystal	28.77 ±0.586	0.346 ±0.024	0.423 ±0.01	11.20	1.27	177°C

Table II. FTIR data of pure drug & co-crystal

Sr. no	Crystal codes	C=C stretching	C=O stretching	C-O-C stretching	O-H Stretching
1	Pure drug	1417.68	1668.43	1026.13	1369.46
2	Drug-urea co-crystal	1435.04	1678.07	1157.29	1255.56
3	Drug-TA co-crystal	1450.47	1693.5	1193.94	1290.38

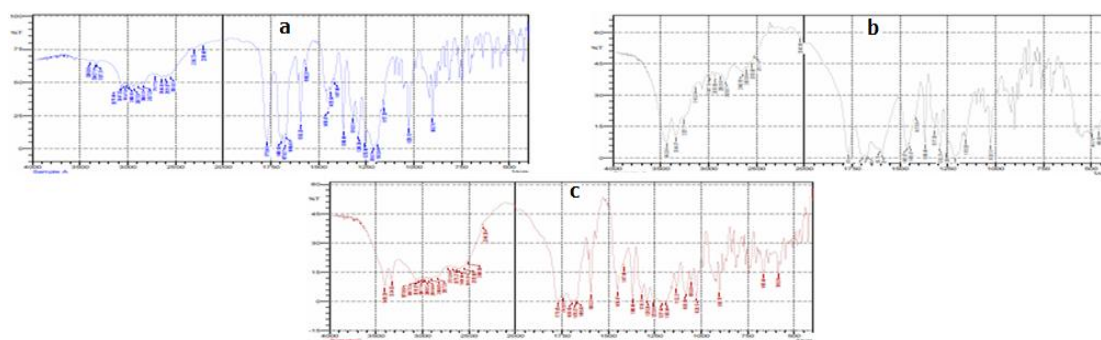


Figure 1. (a) FTIR spectra of pure drug (Diacerein), (b) FTIR spectra of Drug-urea co-crystal, (c) FTIR spectra of Drug-TA co-crystal

drug and coformer. These results indicated that there is slight interaction between drug and coformer when formed as co-crystals,

Differential scanning calorimetry

All the co-crystals prepared by solvent drop grinding method showed characteristic endothermic peak of diacerein. DSC is showed usually co-crystal composition of pharmaceutical powders, when the co-crystal presents different melting points. The thermal behaviour of commercial diacerein and co-crystals are shown below. The DSC curve showed that Diacerein appeared on sharp endothermic peak at about 236.0°C corresponding to its melting point. However the co-crystals obtained with urea and tartaric acid as coformer showed the

shift of endothermic peak towards lower temperature at 133.6°C and 170.2°C respectively. Shift of endothermic peak towards lower temperature indicates the decrease in the melting point of drug in co-crystals. This decreased melting point accounts for increased solubility of drug.

Powder X-ray diffraction

The X-ray diffraction pattern of various co-crystals were studied by X-ray diffractometer. The X-ray diffraction pattern of pure drug has more number of peaks when compared to various co-crystals. XRD spectra of drug-tartaric acid co-crystal have decreased number of peaks but increase in intensity was observed. The XRD spectra of drug-urea co-crystal have increase in the intensity of peaks. Characteristic

Table III. DSC data of pure drug and modified co-crystals

Sr. no.	Crystal form	DSC data		
		Melting point (°C)	Peak fusion point (°C)	Heat of fusion (J/g)
1	Pure drug	238.5	236.0	36.73
2	Drug-urea co-crystal	137.5	133.6	129.8
3	Drug-Tartaric acid co-crystal	174.4	170.2	138.4

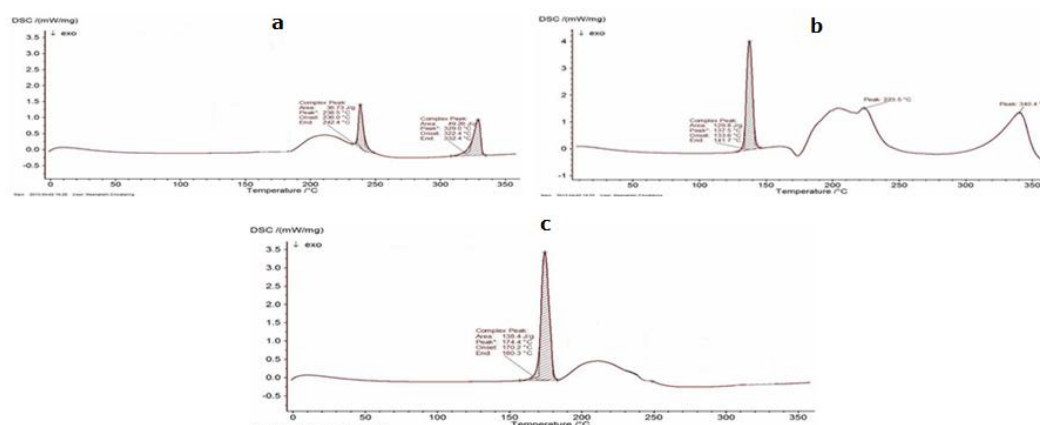


Figure 2. (a) DSC Thermogram of pure drug (Diacerein), (b) DSC Thermogram of Drug-Urea co-crystal, (c) DSC Thermogram of Drug-tartaric acid co-crystal

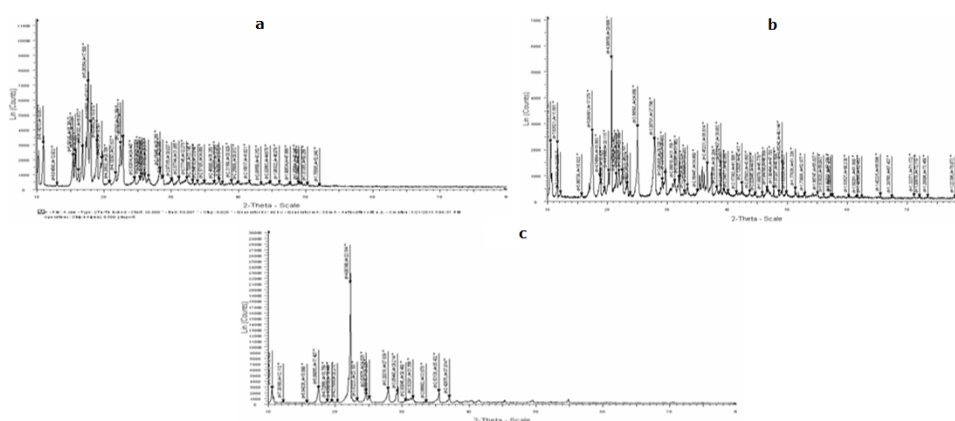


Figure 3. (a) XRD Spectra of pure drug (Diacerein), (b) XRD Spectra of Drug-Urea co-crystal, (c) XRD Spectra of Drug-Tartaric acid co-crystal

diffraction peaks were observed at $2\theta = 15.843$ and 16.673 . The XRD spectra of Pure drug and co-crystals obtained in presence of co-crystal coformer exhibited essentially similar diffraction patterns (2θ values), suggesting that particles crystallized in the presence of co-coformer by solvent drop co-

grinding method did not undergo structural modification.

However, the differences in the relative intensities of their peaks may be attributed to differences in the crystal size and habits of the sample, which may be attributed to the different solubility of the drug in the media.

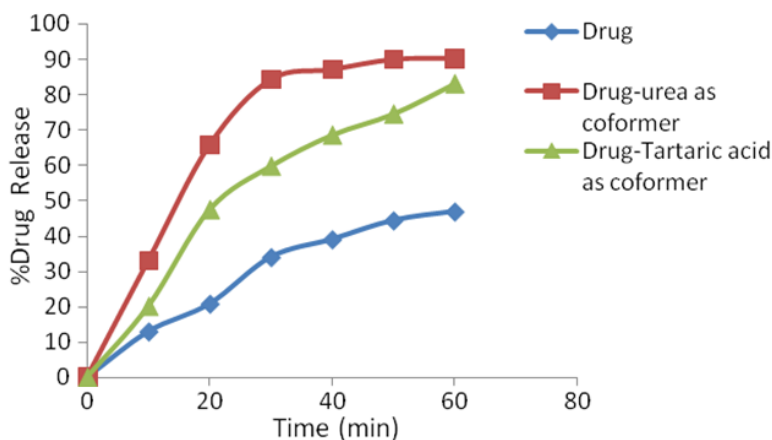


Figure 4. Dissolution study of Diacerein co-crystal

Table IV. Post compression parameter

Formulation	Hardness (Kg/cm ²)	Friability (%)	Weight Variation	Drug Content (W/W)	Disintegration Time (Sec)
Drug	4.7±0.4	0.453±0.15	Passed	98.23 ± 0.02	190 ± 3
Drug-urea co-crystal	4.5±0.35	0.410±0.10	Passed	98.05 ± 0.95	182 ± 2
Drug-T.A co-crystal	5.1±0.55	0.443±0.08	Passed	99.09 ±1.01	153 ± 1

Solubility studies

The solubility study showed that the pure drug Diacerein was the least soluble (2.625µg/mL) and the co-crystals grown in presence of urea as coformer was soluble (21.0µg/mL) with 7.84-fold increase in solubility and the co-crystal in presence of tartaric acid was soluble (13.125µg/mL) with 4.80-fold increase solubility. The increase in the solubility of co-crystals due to melting of coformer which are associated with the co-crystals when compared to pure drug (Diacerein).

Dissolution studies

The dissolution curves of the different co-crystals showed differences in the rate of dissolution. The result of dissolution studies are presented as percent release vs. time. The highest dissolution rate was found for the drug-urea co-crystal. The dissolution rate co-crystal in presence of Urea, Tartaric Acid as coformer showed 91.31% and 83.13% compared to commercial diacerein 46.88% in 60min.

The general appearance of tablets, its visual identity and overall elegance is essential for acceptability, the shape of all the formulation remained off white, smooth, flat faced circular and no visible cracks. The friability was measured by Roche friabilator and was found to be drug-0.453%, Drug-urea co-crystal 0.410%, Drug-Tartaric acid co-crystal 0.443% friability and this parameter given the satisfactory mechanical resistance of the tablet.

The entire tablet passed the weight variation test as the weight variation was within the pharmacopoeias limits of 7.5% of the weight.

Percentage drug contents of prepared co-crystals were found to be in the range of 98.05±0.95w/w to 99.09±1.01 w/w. Disintegration time of tablet formulation was found to be in the range 153±1 to 190±3s.

CONCLUSION

Diacerein co-crystal was successfully prepared by solvent drop method. Solubility and dissolution rate of co-crystals were

markedly increased compared to untreated Diacerein. Thus the co-crystals techniques were helpful to improve the physicochemical properties of Diacerein.

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