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ENHANCEMENT OF ACECLOFENAC'S PHYSICOCHEMICAL PROPERTIES BY DEVELOPING ITS COCRYSTALS

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Abstract
The design, development and characterisation of aceclofenac cocrystals is the focus of this study. Co-crystallisation known as key method for improving drug's solubility in aqueous medium as it modifies crystal structure by co-former incorporation in formulation. In the present work, for co-former and drug selection ΔpKa method as well as CSD software were used. Solvent evaporation method for cocrystal formation was used. Cocrystals were analysed by using XRD, FTIR and DSC methods. Anti-inflammatory activity (in vivo) was performed on Albino Wistar rats. In present study, after comparison of cocrystals with marketed formulations and pure drug dissolution studies (in vitro) demonstrated enhanced solubility profile of cocrystals. In vivo study showed increased anti-inflammatory effects in comparison to standard drug. Based on results of present study we found that co-crystallization improves physicochemical properties of APIs of BCS class II without altering their pharmacological effects.

KEYWORDS: Cocrystals, Aceclofenac, Chitosan, DSC, XRD, Dissolution

1. INTRODUCTION

To develop an appropriate formulation with better physicochemical qualities is a challenging task for researchers. The co-crystallisation process is commonly used on a variety of APIs that have problems with their medicinal and physicochemical features, including poor flow qualities, reduced solubility profiles, and heat instability. Although co-crystallisation is a well-known process, it has recently become very significant in the pharmaceutical industry as a new technique for improving physicochemical properties such as bioavailability, stability, solubility, tabletability and permeability.[1] A conformer and an active ingredient are present in a stoichiometric ratio in cocrystals, which are multicomponent systems linked by non-covalent interactions inside the crystal lattice.[2,3] Cocrystal formation occurs when API engage in a fixed stochiometric ratio with any pharmaceutically acceptable conformer through hydrogen bonding or other intermolecular interactions.[4] Technique cocrystallization works with all kinds of APIs including nonionizable, basic and acidic. Two factors are responsible for novel cocrystals designing, first one is hydrogen bonding. A newer synthon system is generated when two components engage through hydrogen bonding.[5] One of the most widely used drug worldwide is Aceclofenac utilised as anti-inflammatory agent. It acts as selective inhibitor of COX-2 (cyclooxygenase-2) and has higher GIT tolerance than other NSAIDS like naproxen.[6] As per BCS (Biopharmaceutical classification system) aceclofenac comes under class II, due to its poor aqueous solubility and high permeability. The presence of ether and carbonyl oxygens enables hydrogen bonding with conformer as well as carboxylic acid group provides proton donor site.[7] Lattice formation is promoted by presence of aromatic rings which supported by π-π stacking and van der waals interactions.[8] The melting point range of aceclofenac from 150-155^{0}C making it suitable for crystallisation with thermal stability and logP value 2.9-3.2 enables the selection of hydrophobic and hydrophilic co-formers.[6] It is evident from the recent published research co-crystallization has become a cutting-edge method for modifying various physicochemical properties of formulations. Co-crystals can have different physicochemical characteristics upon comparison with their constituents such as hygroscopicity, flow property, solubility, compressibility and dissolution rate etc.[9] There have been found recent reports of pharmaceutical cocrystals of aceclofenac with various co-formers including ibuprofen, lysine, l-cystine etc.[10,11] The selection of an appropriate conformer is crucial in cocrystal synthesis. Because it may affect the final properties of synthetic cocrystals. Additionally, selecting a conformer from GRAS list makes sure that it is safe for human use.[12,13] Different techniques can be applied for suitable conformer selection. In this study, co-former selected by calculating ΔpKa value.[11] However, there are various methods to prepare co-crystals but in this research solvent evaporation method is used as it is readily scalable, inexpensive, and easy to operate.[13,14] For characterization of co-crystals different analytical methods like FTIR, DSC, XRD, HSM were used by thorough evaluation of literature. Thus, the purpose of this work was to enhance rate of dissolution and solubility of aceclofenac by co-crystallization. Chitosan, a recognised biocompatible co-former utilised in cocrystal formation which enhances the rate of dissolution of less soluble drugs by improving surface morphology, wettability reducing particle size. Chitosan polymer deposition over the drug particles enhanced when it forms bridges between the polymeric chains of drug. Therefore, cocrystals with chitosan can increase the drug's solubility in gastrointestinal fluid, but bioavailability enhancement is still difficult with less variability.[15] Here, stoichiometric ratio (1:1) of both drug and conformer was selected for co-crystallization. DSC, FTIR

and XRD methods were used to analyse and characterise pure drug, conformer, physical mixture and cocrystals. Drug release profile of cocrystals, pure drug, marketed formulations was analysed by anti-inflammatory (in vivo) activity as well as in vitro dissolution study. Results of this research found that co-crystals showed enhanced bioavailability as compared to pure drug.

2. MATERIAL AND METHODS

Aceclofenac and chitosan were procured from Sigma-Aldrich chemical limited. Analytical grade ethanol was obtained from Rankem chemicals. Carrageenan was got from CDH Ltd, New Delhi. Cocrystals were prepared by using solvent evaporation method. Animal study performed as per IAEC guidelines and approved by Institutional Animal Ethics Committee. Twenty-eight Albino Wistar rats weighing about 190 g body weight were selected for study from animal house of Subharti medical college. Study was authorised through (AEC) animal ethics committee of institute under proposal/reference No. 1204/PO/ReBiBt/S/2008/CCSEA/25-16 dated 05-08/2024. Twenty-eight Albino Wistar rats were divided in seven groups having 04 animals in each group.

2.1 Selection and screening of co-former

Selection of a suitable conformer is critical for cocrystal formation. Synthon formation between two molecules finally leads to co-crystallisation. Due to limited bioavailability and low water solubility aceclofenac is selected for co-crystallisation to improve its physicochemical properties. Various approaches can be employed for conformer selection such as intramolecular hydrogen bonding evaluation, supramolecular synthon analysis with CSD (Cambridge Structural Database), lattice energy calculations, pKa-based models etc. Among these strategies, ΔpKa value technique was used to predict cocrystal formation.[16] Here, the ΔpKa value 0.9 was obtained i.e. between 0 to 1, which is the most suitable range of cocrystal formation. This ΔpKa value was obtained by subtracting pKa value of less acidic component (chitosan pKa 5.6) from pKa value of more acidic component (aceclofenac pKa 4.7).[17] CSD software was utilised to determine suitable functional group for synthon formation. CSD study revealed that -COOH group of aceclofenac was the most favourable functional group and chitosan having amide functional group. (fig 1) So, heterosynthon formation took place between two components through hydrogen bonding.

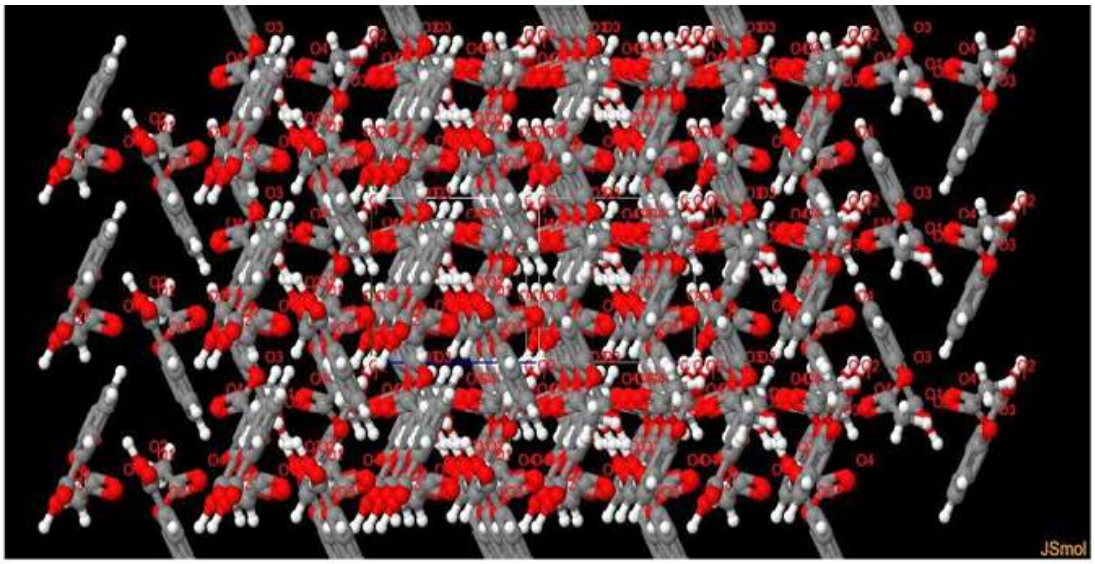

Fig. 1: Packing of ACF molecules

2.2 Analytical method development

2.2.1 Absorption maxima determination (λ_{max})

UV spectroscopy was used for the determination of absorption maxima in this study. To prepare stock solution, first dissolve 200 mg pure drug in 1000 ml of 0.05M buffer solution (sodium acetate) pH 4.5. 6µg/ml amount used for scanning in the range of 200nm to 400nm using UV spectrophotometer.[18]

2.2.2 Constructing calibration curve

Stock solution was used to create various concentrations. Methanol used as solvent. Further following concentrations were obtained by diluting stock solution i.e. (2, 4, 6, 8, 10µg/ml). Samples were examined using Ultra Violet spectroscopy. (UV 1800, Shimadzu corporation Japan)[18]

2.3 Development of Aceclofenac: Chitosan Cocrystals

The technique solvent evaporation was finalised to prepare CCs based on thorough review of literature.[19] In fixed stoichiometric ratio, aceclofenac and chitosan were utilised as the drug and conformer in equimolar amounts, respectively. Aceclofenac was accurately weighed and added to the mortar then carefully added chitosan to same mortar. After stirring these powders together with spatula, a pestle was used to properly mix them. Ethanol was used to dissolve aceclofenac (354mg) and chitosan (150mg) in the predetermined stoichiometric ratio 1:1. Both components were dissolved in adequate

amount of ethanol. Solution was kept in desiccator at room temperature to allow to evaporate. After drying, the binary combinations were obtained for further study shown in figure 2.[20]

Figure 2: Cocrystals of aceclofenac and chitosan

2.4 Characterization: Characterization of ACF, chitosan, their PM and CCs

Aceclofenac, chitosan, their physical mixture and cocrystals were utilised for analytical purpose. In order to prepare physical mixture, equal amounts of each aceclofenac (50mg) and chitosan (50mg) were mixed without use of any solvent.

2.4.1 Differential Scanning Colorimetry (DSC)

A US instrument DSC Q10 V9.9 Build 303 was used for DSC analysis of aceclofenac, chitosan, physical mixture and cocrystals. Samples were placed in metal pans (aluminium) that were crimped and vented. Sample size for analysis was 2 mg. Samples were heated between a range of temperature 20 to 60^0C in presence of dry nitrogen with 60ml/min flow rate. Empty aluminium pan was referred as reference.[21]

2.4.2 Fourier-Transform Infrared Spectroscopy (FTIR)

KBr disc technique was used to study FTIR of aceclofenac, chitosan, their physical mixture and cocrystals using instrument FT-IR Alpha Bruker 1206 0280, Germany. The spectrum was observed between a scanning range of 4000-400cm^{-1}. The obtained peaks in the graph were interpreted.

2.4.3 XRD Analysis

The instrument model 'XPERT PRO' with continuous scanning type at 2θ angle position was used to perform XRD analysis of aceclofenac, chitosan, physical mixture and co-crystals. To confirm the formation of new solid phase the diffraction profiles were compared with standard pattern of pure drug and physical mixture.[22]

2.4.4 Dissolution Studies

The paddle method described in USP was followed for dissolution study of aceclofenac and chitosan cocrytals, pure aceclofenac as well as marketed formulation (Aceclo SR 200mg tablets; Aristo Pharmaceuticals Pvt Ltd).[23] USP type II apparatus (ELECTROLAB) was used. 100mg amount of drug used for study.[24] 0.05M sodium acetate buffer solution of 4.5pH (500ml) was employed as dissolution medium for each test. Cocrystals were filled in empty capsule shells before dissolutio test. Paddle speed and temperature of apparatus were set at 150 rotation per minute and 37±0.5^0C respectively. Volumes of 5ml were taken out after every 20 minutes and replaced the same with fresh dissolution medium for consistent level. After diluted ten times, samples were passed through membrane filters (0.45μm) and forward for spectrophotometric analysis. To determine amount of drug release, samples were examined at 275nm using UV spectrophotometer (UV-1800, Shimadzu Corp., Japan).[25]

2.4.5 Anti-inflammatory activity (in vivo)

In vivo animal activity were conducted at institutional animal house as per IAEC guidelines after approval of committee. As previously reported, the carrageenan-induced rat paw edema model was used to analyse and evaluate anti-inflammatory effect of pure aceclofenac and cocrystals. Wistar rats were used for experiment after divided into four groups, each group with five animals (Table 1). Before start of experiment all animals were kept on fasting for 24 hours (overnight) and kept under airconditioned housing facilities. Pure aceclofenac was used as standard at dose 50mg/Kg. Group I was injected with carageenan (0.05ml of 1% solution), group II injected with polymer chitosan at 50mg/kg, group III received 50mg/kg of pure drug (aceclofenac) and group IV injected 50mg/kg dose of cocrystals to evaluate antiinflammatory activity.[26] Because of enhanced dissolution profile of drug after cocrystallization, half dose (50mg/kg) of cocrystals was used. The rats were given a subcutaneous injection of 0.05 ml of 1% carageenan solution in planer side of left hind paw after 30 minutes of treatment. Paw was submerged in mercury. Paw volume was measured by using Plethysmograph just after carageenan administration and upto five hours at every one hour interval for inflammation progression. % inhibition was calculated by using formula given,

% Inhibition= paw volume of control (mean)- paw volume of treated (mean)/paw volume of control (mean) × 100

Table 1: Anti-inflammatory study (in vivo)

Sr. no.	Groups	Treatment	Dose	Route of administration
1	I (control)	Carageenan	0.05 ml of 1% solution	Injected in planar side of left hind paw
2	II	Chitosan	50mg/kg	Oral route
3	III	Aceclofenac Pure	50mg/kg	Oral route
4	IV	Aceclofenac: Chitosan cocrystals	50mg/kg	Oral route

*no. of rats= 24 (six in each group)

3. RESULTS

3.1 Absorption maxima determination

A UV spectrophotometer was used to scan a solution of concentration 6µg/ml between 400 and 200nm. At 275nm, absorption maxima (λmax) was found. Absorption maxima for aceclofenac was showed in figure 3.

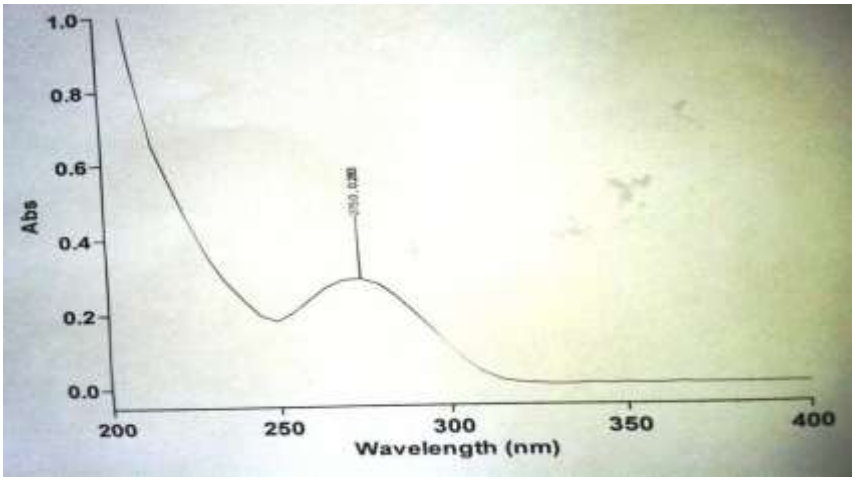

Figure. 3: λmax of aceclofenac

3.2 Constructing calibration curve

Different concentrations of aceclofenac in 0.05M sodium acetate buffer were taken. At 267nm absorbance of each concentration was observed by using UV spectrophotometer. Graph plotted between concentration of drug and absorbance (Figure 4). R^2 value was found to be 0.995. Data for absorbance versus concentration displyed in table 2.

Table 2: sample concentrations vs absorbance data

Sr. No.	Drug amount in 100ml of stock solution	Concentration (µg/ml)	Absorbance
1	2ml	2	0.129± 0.14
2	4ml	4	0.282±0.22
3	6ml	6	0.398±0.17
4	8ml	8	0.589±0.38
5	10ml	10	0.719±0.21

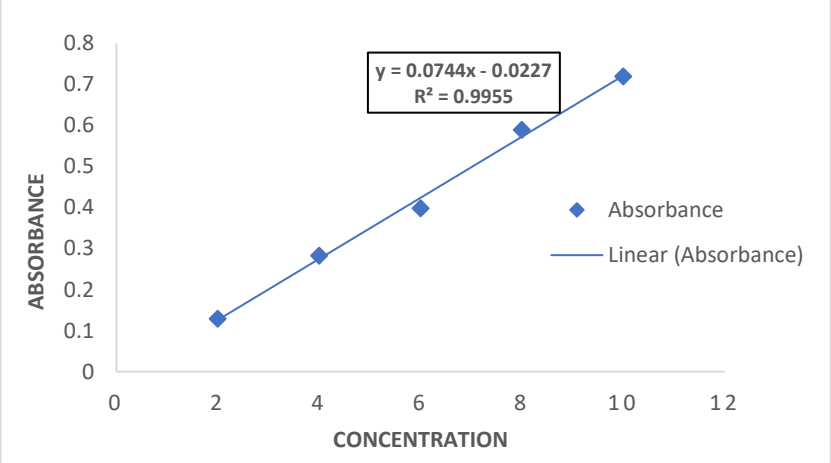

Figure 4: Calibration curve of Aceclofenac

3.3 Characterization of aceclofenac, chitosan, physical mixture, cocrystals

ACF, Chitosan, ACF: Chitosan and their cocrystals were utilised for characterization. Both aceclofenac and chitosan were used in equal amount i.e. 100mg for physical mixture.[14]

3.3.1 Differential Scanning Colorimetry Analysis

DSC is the only important method that provide information on the co-crystallization phase. DSC Q10 V9.9 Build 303, a US instrument was used for DSC analysis. A distinct endothermic peak in DSC thermogram of aceclofenac (ACF) (Figure 5) is shown at melting point 157.01^{0}c. A sharp endothermic peak observed at 65.23^{0}c in thermogram of chitosan (Figure 6) indicates its melting point. Both thermograms showed no additional peaks, indicating that obtained samples were pure. The thermogram of physical mixture (Figure 7) shown a peak at 153.69^{0}c that represents physicochemical interaction of aceclofenac and chitosan instead corresponding peaks of both chitosan and aceclofenac. A distinct endothermic peak is visible at 148.32^{0}c in DSC thermogram of cocrystals (Figure 8). This thermogram showed no further peaks, indicating that drug was fully co-crystallized.

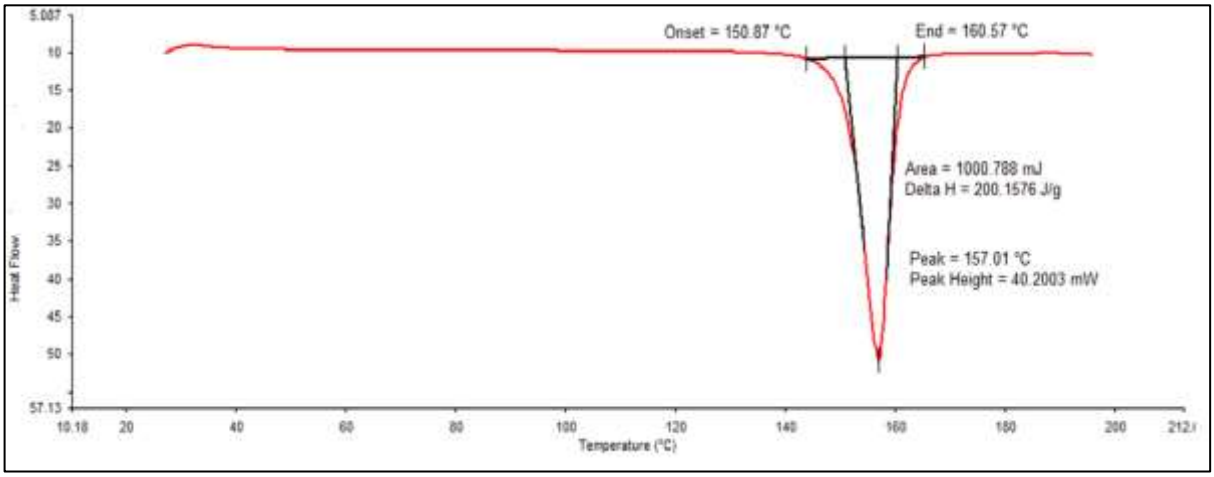

Figure 5: DSC thermogram of ACF

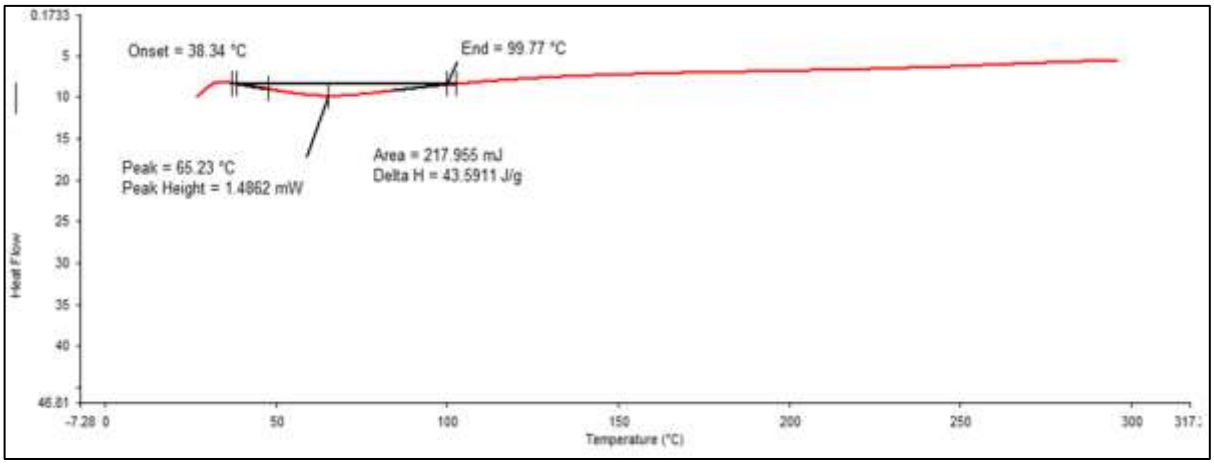

Figure 6: DSC thermogram of chitosan

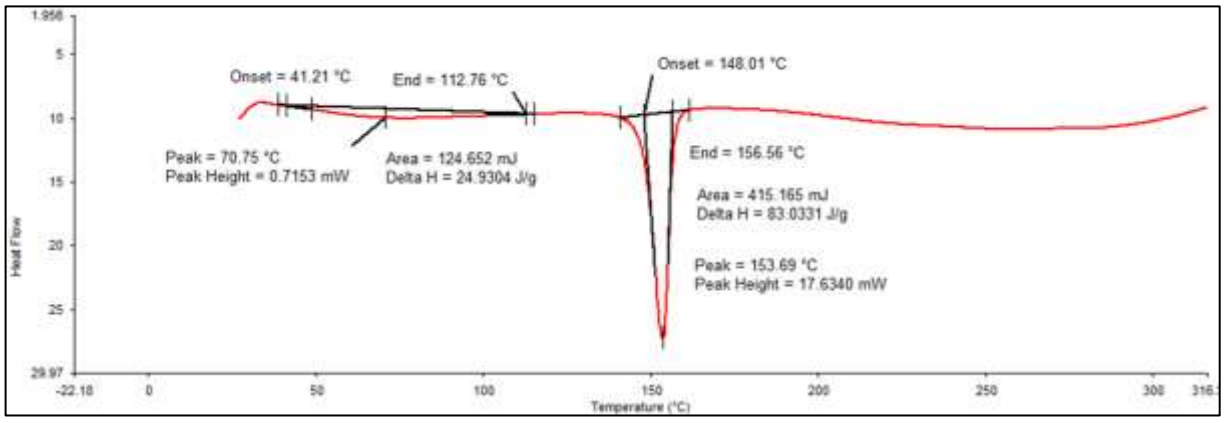

Figure 7: DSC thermogram of Physical Mixture

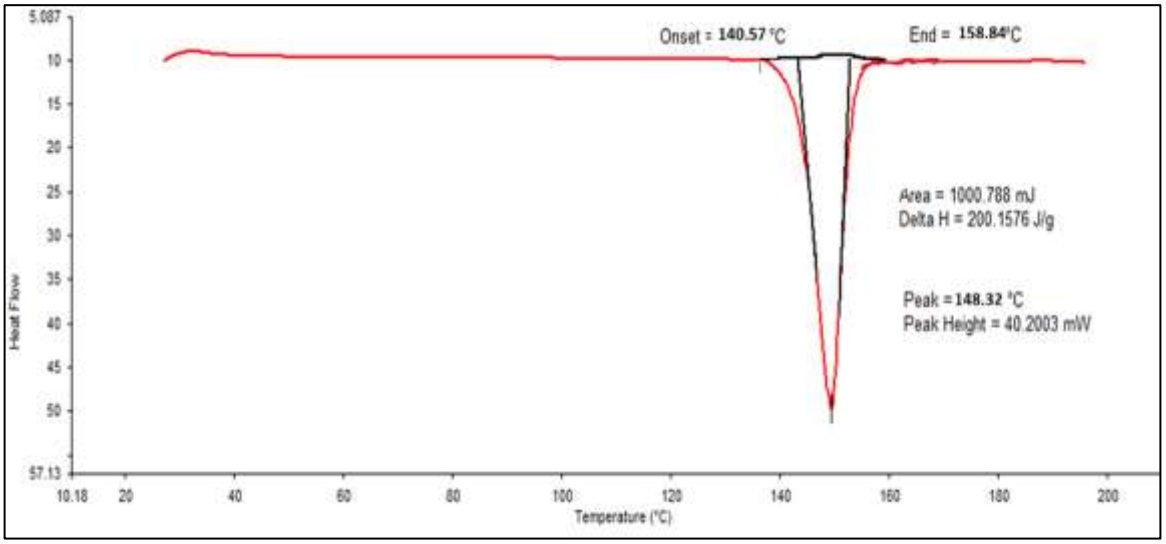

Figure 8: DSC thermogram of Cocrystals

3.3.2 Fourier Transform Infrared Spectroscopy

The characteristic peaks of aceclofenac, chitosan, their physical mixture and co-crystals are showed in figure 9, figure 10, figure 11, figure 12 respectively. characteristic peak in the FTIR spectra of aceclofenac obtained at 1770.73cm^{-1}, 3317.71cm^{-1}, 3475.88cm^{-1}, 1588.55cm^{-1}, 717cm^{-1} can be attributed to C=O and O-H stretching of carboxylic group and N-H stretching and C=C stretching as well as C-Cl stretching. These spectrum characteristics confirmed the presence of carboxylic acid group in aceclofenac structure.[27] In case of chitosan prominent peaks obtained at 1658.85cm^{-1}, 3481.66 cm^{-1}, 3267.55 cm^{-1} for C=O stretching, O-H stretching, O=C-NH stretching respectively and 1552.76 cm^{-1} for N-H bending also. Most of the abovesaid peaks observed in physical mixture of aceclofenac and chitosan with minor shifting because of van der waals forces i.e. 1694.64cm-1 for C=O stretching, 3418.01 cm-1 for O-H stretching and 1583.63 cm-1 for N-H stretching.[27] This confirmed that there was no chemical interaction between drug and conformer physical mixture without solvent addition. After solvent addition in aceclofenac and chitosan physical mixture in ratio1:2, the FTIR spectrum of co-crystals showed the formation of homosynthon by hydrogen bonding between COOH group and NH_2 group present in drug and co-former. Characteristic peak for O-H and -NH stretching in cocrystal's FTIR spectrum observed at 1296cm^{-1} and 3279 cm^{-1}.

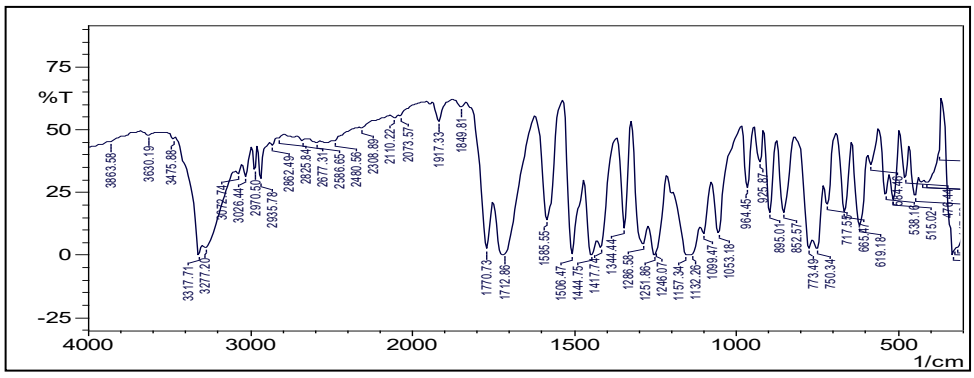

Figure 9: FTIR spectrum of aceclofenac

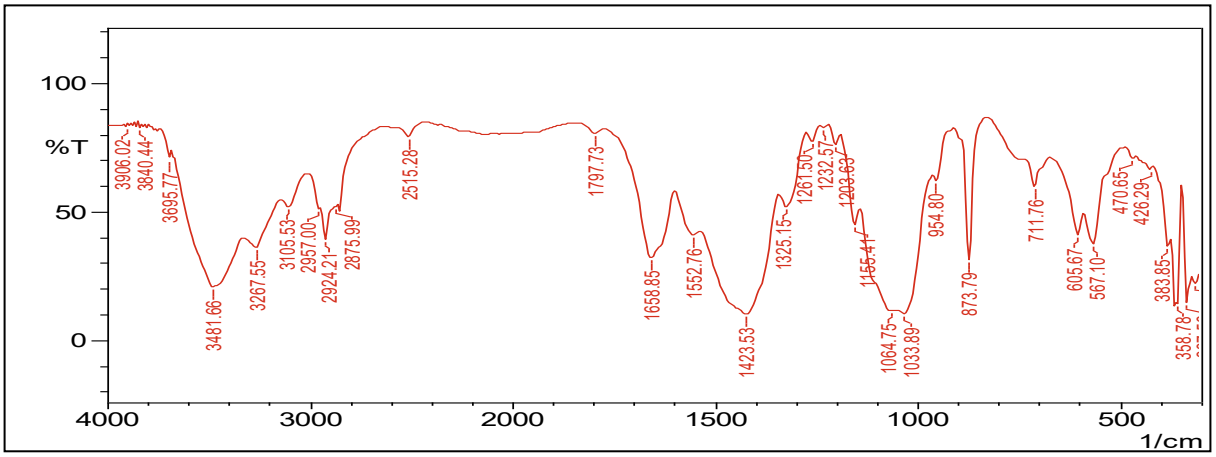

Figure 10: FTIR spectrum of chitosan

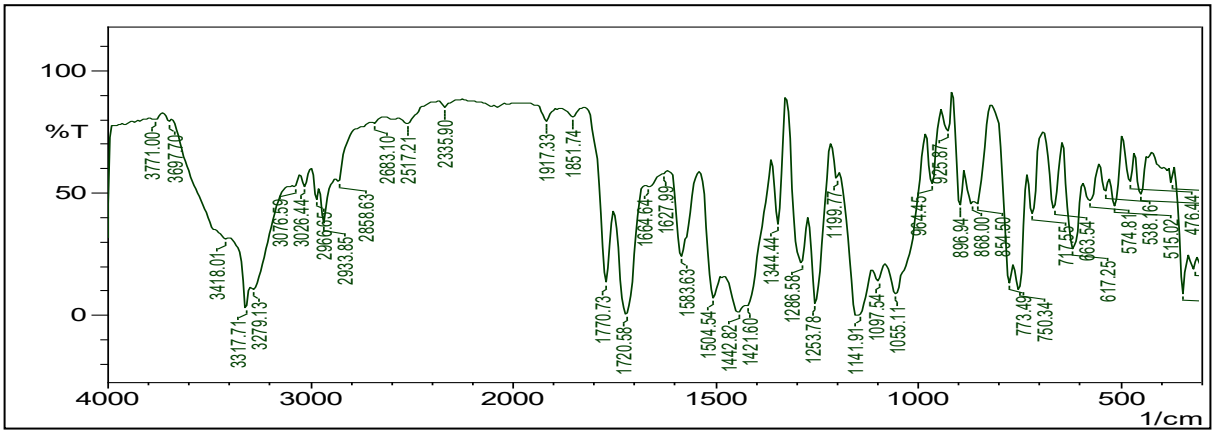

Figure 11: FTIR spectrum of physical mixture

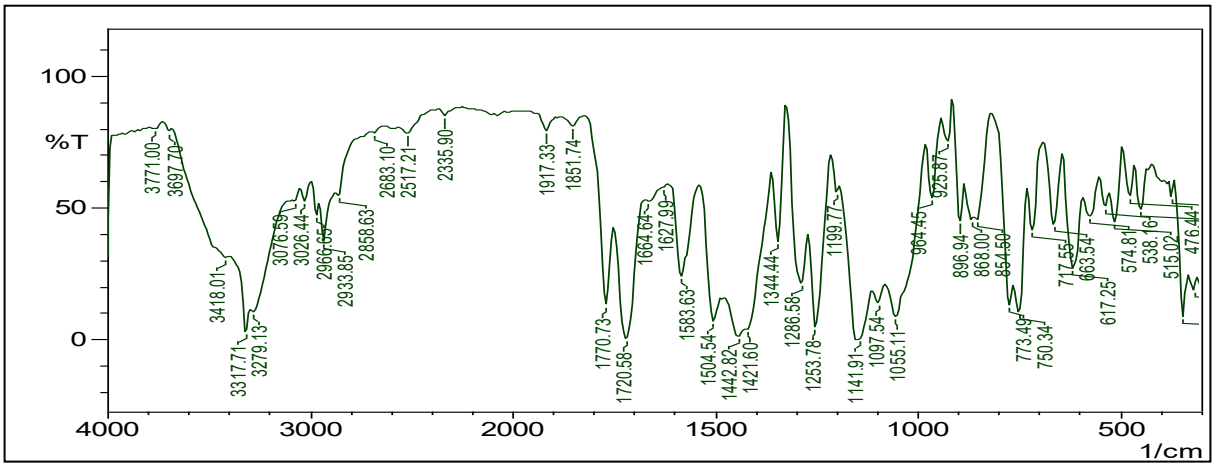

Figure 12: FTIR spectrum of co-crystals

3.3.3 X-ray Diffraction Study

The XRD spectrum of aceclofenac displayed the distinctive peaks on position of 2θ angle at 7.9101, 15.8692, 21.5188, and 26.1809 having relative intensity (in percentage) of 77.88, 100.00, 29.63, and 23.53 respectively (Table 2). XRD spectrum of chitosan showed distinctive peaks on 2θ angle position on 7.6624, 8.0021, 17.1132, and 18.0394 with relative intensity (in percentage) 71.60, 84.22, 63.74, and 100.00 respectively (Table 2). The XRD spectrum of physical mixture of aceclofenac and chitosan showed main distinctive peaks on 2θ angle position at 9.1112, 17.6892, and 18.3005 with relative intensity (in percentage) 100.00, 28.46, and 41.06 respectively (Table 2). The purity of drug and co-former was demonstrated by relative intensities of the peaks. Co-crystals XRD spectrum showed distinctive peaks on 2θ angle position at 6.2106, 7.9123, 13.1426, 14.3963, and 20.4027 with relative intensity (in percentage) of 68.15, 35.91, 36.91, 96.01, and 100.00 respectively (Table 3).[29]

Table 3: XRD data of aceclofenac, chitosan, Physical mixture and co-crystals

Position (^{0}2θ)	FWHM total (^{0}2θ)	d-spacing (^{0}A)	Relative intensity (%)	Area [cts*2 θ]
Aceclofenac				
7.9101	**0.0987**	**11.86892**	**77.88**	**2282.47**
15.8692	**0.1389**	**5.62691**	**100.00**	**4016.83**
16.7008	0.1598	5.32889	3.98	171.38
20.0556	0.2001	4.01109	8.01	461.84
21.5188	**0.1698**	**3.90616**	**29.63**	**15.1868**
23.0045	0.1192	3.38595	18.26	808.41
23.8902	0.0897	3.69014	5.98	98.12
26.1809	**0.2641**	**3.13287**	**23.83**	**2079.12**
Chitosan				
7.6624	**0.1489**	**10.08670**	**71.60**	**712.12**
8.0021	**0.1401**	**10.90312**	**84.22**	**921.48**
17.1132	**0.2923**	**5.16936**	**63.14**	**1027.17**
17.0021	**0.1389**	**5.49282**	**62.14**	**687.64**
18.0394	**0.0801**	**5.19203**	**100.00**	**685.06**
18.9904	0.3129	4.70296	9.02	71.29
20.0225	0.0792	4.22247	8.87	145.97

Physical Mixture (1:1)				
7.8561	0.0715	11.24545	19.71	2005.41
9.1112	**0.1139**	**10.78591**	**100.00**	**10448.21**
15.3594	0.1308	5.68445	25.73	3498.12
17.6892	**0.1214**	**5.42355**	**28.46**	**2889.21**
18.3005	**0.1816**	**4.87101**	**41.06**	**4666.20**
16.8054	0.1201	5.2681	8.02	791.01
17.2044	0.0828	5.11201	5.67	28.84
17.7101	0.1719	4.98401	0.18	56.51
Cocrystals				
6.2106	**0.1391**	**14.14181**	**68.15**	**701.92**
6.4105	1.3161	9.70326	5.47	248.18
7.9123	**0.1666**	**12.30547**	**35.91**	**1174.85**
13.1426	**0.2146**	**10.90924**	**36.91**	**127.16**
14.3963	**0.5234**	**6.34346**	**96.01**	**3809.12**
15.2950	0.1802	5.68662	5.67	1421.88
17.1745	0.1287	5.11203	0.75	2856.51
20.4027	**0.2234**	**4.29101**	**100.00**	**7819.02**
21.0047	0.1904	4.21580	0.87	64.36
22.1273	0.2451	4.19021	1.18	72.14

3.3.4 Dissolution Study

Drug release (in vitro) data for aceclofenac, their cocrystals and marketed formulations (Aceclo SR 200mg tablets; Aristo Pharmaceutical Pvt Ltd) in sodium acetate buffer (500 ml, pH 4.5) was reported. Over various intervals of time 5, 10, 15, 30, 45, 60, and 90 minutes, pure aceclofenac showed 6.69%, 11.56%, 16.60%, 21.81%, 24.21%, 27.89%, and 34.41% and physical mixture showed 10.32%, 14.16%, 19.29%, 23.17%, 29.19%, 33.92%, and 41.23% release. Cocrystals exhibited release about 21.02%, 42.22%, 65.10%, 83.40%, 90.92%, 91.49% and 91.84%, while marketed formulation showed release 23.66%, 45.59%, 65.65%, 77.58%, 84.88%, 86.69%, and 87.58% respectively in figure 13.[30] The readings were recorded three times and mean of standard deviation (n=3) of data reported. Percentage release of drug aceclofenac (pure), physical mixture, cocrystals and marketed formulation represented in Table 4.

Table 4: % drug release data for aceclofenac, physical mixture, cocrystals and marketed formulation

Time (minute)	Pure drug (%)	Physical mixture (%)	Cocrystals (%)	Marketed formulation (%)
0	0	0	0	0
5	6.59	10.32	21.02	23.66
10	11.56	14.16	42.22	45.59
15	16.60	19.29	65.10	65.65
30	21.81	23.17	83.40	77.58
45	24.21	29.19	90.92	84.88
60	27.89	33.92	91.49	86.59
90	34.41	41.23	91.84	87.58

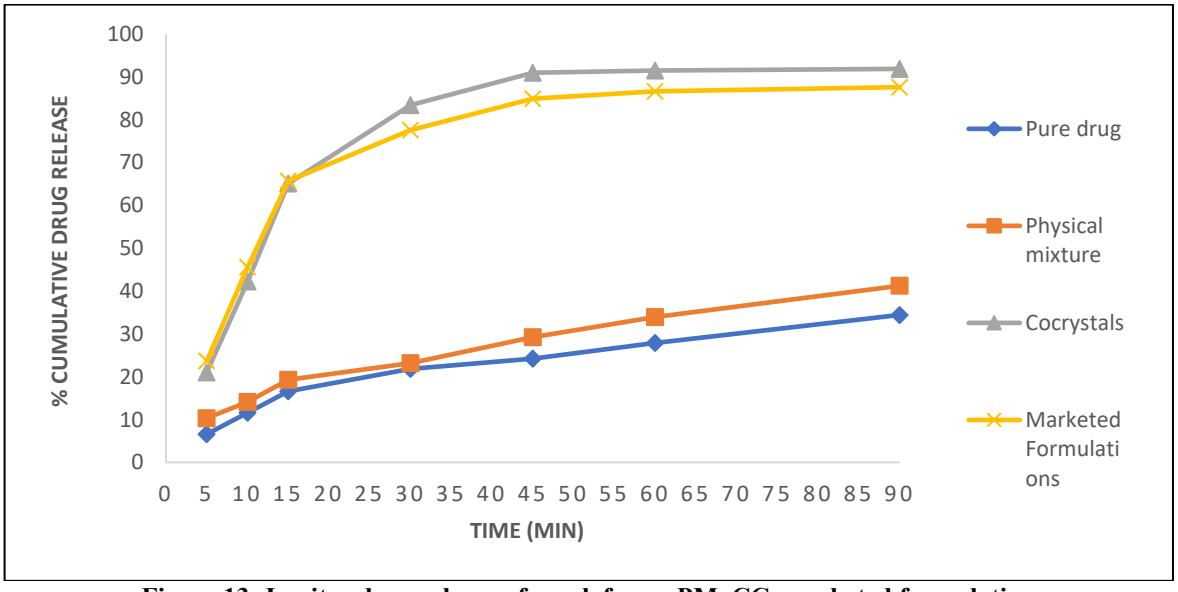

Figure 13: In vitro drug release of aceclofenac, PM, CC, marketed formulation

3.3.5 Anti-inflammatory Study (in vivo)

Anti-inflammatory potential of cocrystals on carrageenan induced paw oedema in rats' hind paws is represented in Table 5 and figure 14, figure 15. Group 2 received a 50mg/Kg dose of polymer chitosan compared to the standard drug whereas group 3 and group 4 received equivalent dose of pure drug and cocrystals. In group 1 (control group) no reduction in inflammation was found. However, all dose treated groups exhibit inflammation reduction but cocrystals showed maximum reduction in inflammation. After five hours of carrageenan administration, drop in volume of each paw exhibited. The values of paw volumes are 0.998 ±0.03, 0.728 ±0.04, 0.811 ±0.01, and 0.608 ±0.01 respectively. The carrageenan group showed a significant value of **p<0.01 when data of in vivo anti-inflammatory study was applied for one way ANOVA test.

Table 5: Anti-inflammatory activity

Time	Group 1*	Group 2*	Group 3*	Group 4*
1	0.587 ±0.04**	0.516 ±0.02**	0.518 ±0.01**	0.496 ±0.02**
2	0.701 ±0.04**	0.576 ±0.01**	0.579 ±0.01**	0.526 ±0.06**
3	0.815 ±0.03**	0.750 ±0.02**	0.741 ±0.02**	0.656 ±0.03**
4	0.941 ±0.03**	0.801 ±0.03**	0.799 ±0.02**	0.611 ±0.03**
5	0.958 ±0.02**	0.788 ±0.03**	0.804 ±0.01**	0.676 ±0.02**
6	0.998 ±0.003**	0.728 ±0.04**	0.811 ±0.01**	0.608 ±0.01**

Group 1: Control; Group 2: Chitosan(50mg/Kg); Group 3: Pure drug (Aceclofenac); Group 4: Cocrystal (50mg/Kg); *Mean ±S.D. (n=6). **p<0.01compared to carrageenan control group.

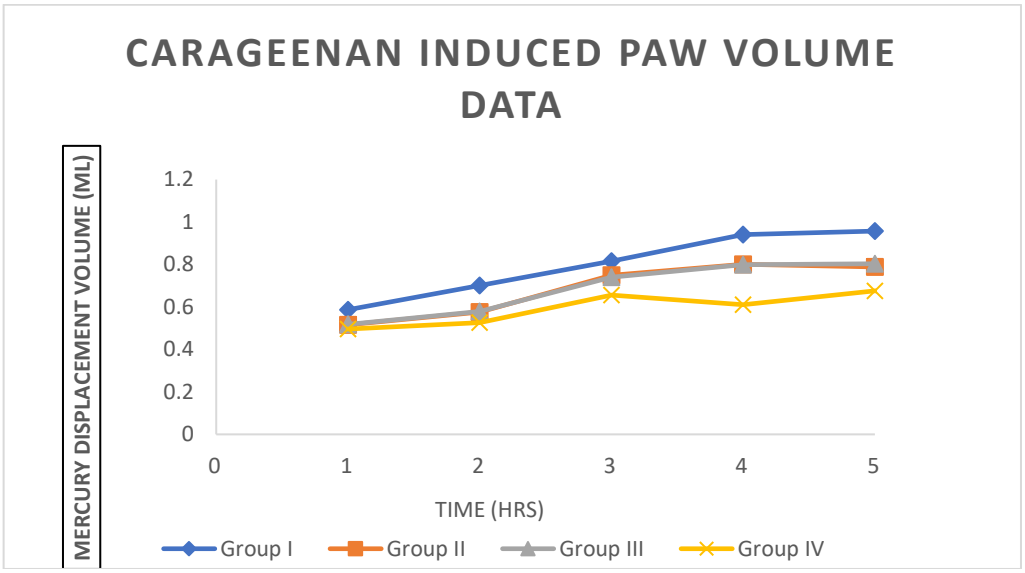

Figure 14: Paw volume with time

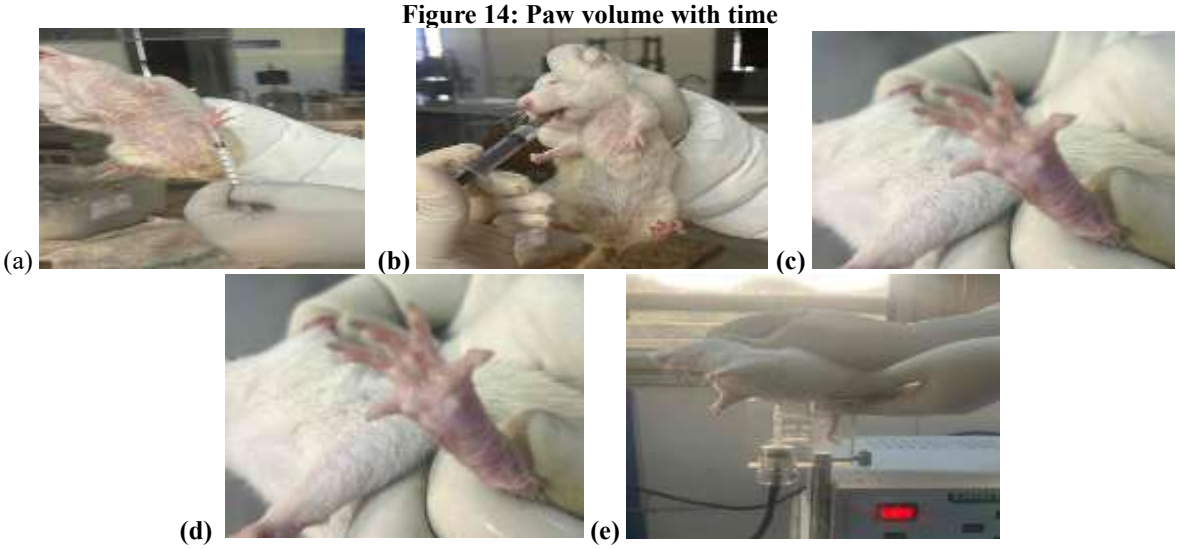

Figure 15: (a, b) injection of 0.05ml of 1% solution of carrageenan in hind paw (c and d) Inflammation after carrageenan (e) measurement of inflammation by plethysmometer

4. STABILITY STUDY

Samples were taken out at intervals of months (0, 3, and 6) and content of drug was assessed using the HPLC technique. Different concentrations of drug and their AUC were used to create calibration curve (Table 6). The AUC for Aceclofenac

was 51448, 35957, and 24117 as well as for cocrystals 51241, 47871, and 38451 at an interval of 0, 3, and 6 months respectively. To obtain AUC, all samples were analysed three time. After six months, stability profile of pure drug was 47% and cocrystals stability profile was 74%. The drug content was analysed by AUC (Table 7, figure 16). According to stability studies, co-crystallization technique improved the stability profile of aceclofenac. Pure drugs and cocrystals both followed first order kinetics. Drug degradation rate is contingent on the remaining drug concentration in sample.[30]

Table 6: Concentration and AUC of samples

Concentration (µg/ml)	AUC
2	8753
4	17562
6	25816
8	34072
10	41924
12	52467

Table 7: Stability study data at 0, 3 and 6 months interval for aceclofenac and cocrystals

Time (Month)	% Drug Remaining (Cocrystals)*	% Drug Remaining (Aceclofenac)*
0	97.86±0.397	98.66±0.025
3	93.81±0.786	70.67±0.86
6	74.28±1.613	47.49±0.86
Mean± S.D (n=3)		

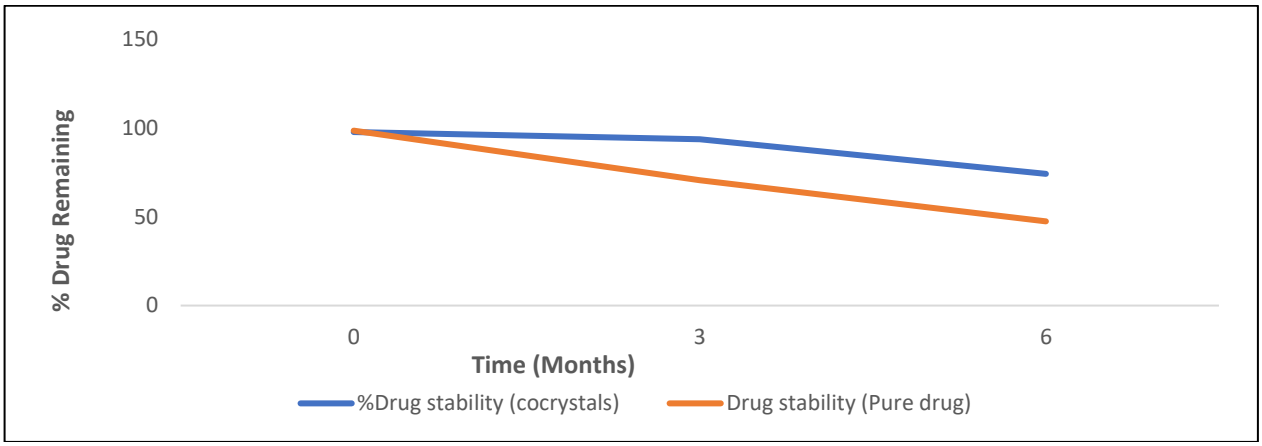

Figure 16: Stability study of aceclofenac and cocrystals

5. DISCUSSION

The process of co-crystallization relies on the ΔpKa value method. In this study, for the selection of API and co-former we used ΔpKa and CSD value methods. In DSC thermogram of aceclofenac, chitosan, and their physical mixture different distinct peaks observed at 157.01^{0}C, 65.23^{0}C, and 153.69^{0}C respectively. Instead of corresponding peaks of aceclofenac and chitosan in thermogram of their cocrystals and physical mixture there is distinct peak at 148.32^{0}C and 153.59^{0}C which conformed complete co-crystallization of drug and conformer due to elevated temperature in DSC pan. When samples are heated moisture content in conformer and drug functions as solvent resulted in automatic co-crystallization, no trace remained non crystallized. Consequently, only single peak obtained. In FTIR analysis, corresponding distinct peaks of aceclofenac and chitosan were observed with minor shifting in physical mixture. These shifting of peaks in 1:1 physical mixture was due to van der Waals interactions between aceclofenac and chitosan. Hydrogen bonding was confirmed by characteristic peak shifting of aceclofenac unbounded hydrogen from 3317.71cm^{-1} to 3279.13 cm^{-1}. The shift of carbonyl stretch of aceclofenac and chitosan from 1770.73 to 1627.98 due to homosynthonic interaction of conformer and drug.[26] Cocrystals do not have distinctive peaks found in XRD spectrum of aceclofenac and chitosan. There are various novel peaks present in cocrystals that are differ than physical mixture spectrum. New crystal structure with distinct characteristics has been generated because of H-bonding between carboxylic acid group and amine group of drug and conformer.[32] The results of drug release study (in vivo) showed the enhanced dissolution rate of cocrystals in comparison to marketed formulation and aceclofenac (figure 13). As aceclofenac is categorised under BCS class II, therefore we conclude, based on dissolution study that co-crystallisation process could enhance dissolution profile of BCS class II drugs. Cocrystals showed dose dependent anti-inflammatory activities (in vivo) (Table 5). In control group, a progressive increase observed in oedema paw volume. Whereas, in group aceclofenac (100mg/kg), half dose (50mg/kg) cocrystals group, and 100mg/kg cocrystals group significantly reduced paw oedema volume (figure 14).

6. CONCLUSION

In this research, aceclofenac and chitosan have been co-crystallised by using solvent evaporation technique in fixed stoichiometric ratio (1:1). The drug and co-former interaction were confirmed through H-bonding between -COOH and

NH2 group of both. Cocrystals showed enhanced dissolution profile and effective potential as anti-inflammatory in carrageenan induced paw oedema model as compared to standard pure drug. Therefore, co-crystallization of aceclofenac allows to develop formulation with improved pharmaceutical and physicochemical properties. This technique is less costly and less laborious also. Further, bioavailability, toxicological and stability studies are required to highlight the benefits of co-crystallization technique. Animals used for research was approved by IAEC of Subharti Medical College under vide project proposal no. 1204/PO/ReBiBt/S/2008/CCSEA/25-16 dated 31/05/2025.

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8. AUTHORS CONTRIBUTION
SA: Contribution to conceptualization, initial drafting. GV: Assisted in data validation and supervised the study design and finalized the manuscript. RS: Reviewed, edited and refined the work. All authors approved the final version and agreed to accountability for the work.

9. CONFLICT OF INTEREST
The authors declare no conflict of interest.

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