International Journal of Pharmaceutical Development & Technology

www.ijpdt.com

e ISSN - 2248 - 910X

Print ISSN - 2248 - 9096

NOVEL APPROACH OF PHARMACEUTICAL CO-CRYSTALS FOR POORLY SOLUBLE DRUGS

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ABSTRACT

Curcumin a polyphenolic active pharmaceutical ingredient, extracted from curcuma longa possess a wide variety of pharmacological activities. The utility of curcumin is limited by its, lack of water solubility, and relatively low invivo bioavailability. In this context, Co-crystal approach are non-ionic supramolecular complexes and can be used to address physical property issues such as solubility, stability and bioavailability in pharmaceutical development without changing the chemical composition of the API. Novel co-crystal of curcumin with methyl paraben was obtained by liquid assisted grinding method by (1:1) molar ratio. This formulation was evaluated for Anti-inflammatory activity. FT-IR, DSC, PXRD results delineation of the hierarchy of hydrogen bonding between complementary functional groups or supramolecular heterosynthons can be accomplished. The percentage of inhibition in paw edema after 3 h were recorded 67.71 % in case of Indomethacin, 55.73% in case of 100 mg/kg of Curcumin, 66.67 % in case of 100 mg/kg of Curcumin co-crystals respectively.

Keywords: Curcumin Co-crystal, Anti-inflammatory activity, Curcuma longa.

INTRODUCTION

Curcumin has been speculated to have promising chemotherapeutic and preventive activities, which could approve avenues for alternative treatment of many diseases. Currently, studies attributed its low availability into the blood and target sites to its poor solubility, very low GIT dissolution rate, low absorption, and its extensive systemic metabolism. It is one of the investigational new drug substances that have great clinical potential. Several of these approaches seem to be either successful or partly successful to improve the solubility, stability and bioavailability of curcumin. There is still a lacuna at the end and thus there is still a need for further elaborate studies. This studies is the challenging aspects in the development such drug molecules are associated with their slow dissolution in biological fluids; thus, such APIs receive insufficient and inconsistent systemic exposure and consequently have sub-optimal clinical efficacy.

Co-crystal systems have emerged as an interesting material to exploit in order to design drugs with enhanced functionality. The ability to alter molecular interactions, composition, and structure in materials of pharmaceutical relevance using crystal engineering principles and strategies has led to the discovery of a large number of pharmaceutical cocrystals [1–6]. Formation of pharmaceutical cocrystals has gained attention offering another option that has the potential to provide new, stable solid structures which may improve the properties of the API and which is also applicable to non-ionizable drugs [7-9].

A number of co-crystals of APIs with different co-formers formed by different methods have been reported and it was shown that the solid-state interactions between the two compounds are mainly based on hydrogen bonds [10-16]. In this conte Curcumin xt the presence of the phenolic group of methylparaben is, able to compete with carbonyl group of curcumin keto and enol group trimer.

MATERIALS AND METHODS

Curcumin was procured from ITC Guntur, Methylparaben was purchased from Himedia Laboratories pvt ltd, Mumbai.All the other chemicals and excipients used were of analytical grade.

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Preparation of co-crystals

Liquid-assisted grinding method

Addition of a few drops of a solvent as lubricant (here EtOH) to accelerate molecular mobility during grinding or kneading, referred to as liquid-assisted grinding,30 facilitated quantitative formation of the product cocrystals. Curcumin methylparaben (1:1) .cocrystals were obtained by liquid-assisted manual grinding of the individual solid components for 30 min in a mortar pestle after adding 5-6 drops of EtOH.

Characterisation of crystals

FT-IR (Fourier Transform Infra-red Spectroscopy) Studies, Differential Scanning Calorimetry (DSC) studies, X-Ray Powder Diffraction (XRPD), Scanning Electron Microscopy (SEM) studies,and the solubility studies of pure drug, co-former and crystals were subjected to the above methods.

Fourier Transform Infrared (FT-IR) Studies

For the pure drug, co-former, co-crystals Fourier Transform Infrared (FT-IR) spectra were obtained. The spectra were recorded in a Thermo-IR 200 FT-IR spectrophotometer. Potassium bromide pellet method was employed and background spectrum was collected under identical conditions. Each spectrum was derived from 16 single average scans collected in the range of 400-4000 cm^{-1} at the spectral resolution of 20 cm^{-1}.

Differential Scanning Calorimetry (DSC) studies

Thermal analysis of pure curcumin, co-former, crystals were recorded on a DSC (NETZSCH DSC 204). The temperature axis and cell constant of DSC were previously calibrated with indium. A heating rate of 10 C/min was employed with nitrogen pursing. Powder samples (15- 30 mg) was weighed into an aluminum pan and analyzed as sealed with pin holes and an empty aluminum pan was used as reference.

X-Ray powder Diffraction (XRPD) studies

X-ray powder diffractometry (XRPD) is a powerful technique for the identification of the crystalline solid phases. Every crystalline solid phase has a unique XRPD pattern, which can form the basis for its identification. The study was carried out using X-Ray Diffractometer using Cu kα radiation. The tube operated at 45 kV, 9mA and data was collected over an angular range from 0 to 500 2θ of the diffraction angle in continuous scan mode using a step size of 0.050 2θ and a time of 0.1 s. The X-ray powder diffraction (XRD) spectra of curcumin ,methylparaben, and crystals (figure) shows characteristic peaks .

Scanning electron microscopy (SEM):

The surface characteristic of prepared crystal was studied by SEM (ZEISS Electron Microscope, EVO MA 15). Powder samples was mounted onto aluminum stub using double sided adhesive tape and sputter coated with a thin layer of gold at 10 Torr vacuum before examination. The specimens were scanned with an electron beam of acceleration potential of 20 kV and the images were collected as secondary electron mode.

Preparation of capsules:

Single-unit capsules were formulated with the help of different Excipients, which upon administration would attain a density of less than that of the gastric fluids and therefore would float . Exactly **60 mg** of curcumin methylparaben crystals was weighed and physically blended with excipients in a glass mortar and pestle and filled in a **hard gelatin capsule # 00.** The drug and excipients blend was transferred into the empty capsule shells manually. The composition of the capsules is given in Table1

IN-VIVO STUDIES

Prevention of carrageenan-induced paw edema in rats and mice is frequently used for evaluation of anti-inflammatory activity. In this study, the anti-inflammatory activity of curcumin and co-crystals (100 mg/kg) in comparison with that of indomethacin was investigated by testing the inhibitory effects of these compounds on paw edema in rats to provide evidence for a potential role of curcumin in the prevention and treatment of various proinflammatory chronic diseases.

Materials

Curcumin, Co-crystals, Carrageenan, Indomethacin, 1% v/v Tween 80.

Animals

Adult Albino Wistar rats weighing about 150-180 g of either sex were procured from the animal house. The animals were maintained in a well-ventilated animal house approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), with 12:12 hour light/dark cycle in polypropylene cages with 27±2°C temperature. The animals were kept under laboratory conditions for one week before start of the experiments and allowed food and water *ad libitum*. Six animals were used in each treatment group.

Experimental Design

Group I received 1% w/v CMC (10 ml/kg, p.o.) considered as negative control group, Group II received Indomethacin (10 mg/kg, p.o) considered as Standard group, Group III received 100 mg/kg of Curcumin suspended in 1% w/v CMC, p.o. and Group IV received 100 mg/kg of Curcumin Co-Crystals suspended in 1% w/v CMC,p.o.

Methodology

The anti-inflammatory activity of the extracts was determined using carrageenan induced rat paw edema assay Winter CA, Risley EA, [17] .After 30 mins of the treatment, 0.1ml of 1% carrageenan in saline was injected

into the sub plantar region of the left hind paw of each rat to induce edema. The paw volume was measured initially and at intervals of 30, 60, 120, 180 mins. after carrageenan injection by volume displacement method using Plethysmometer by immersing the paw in mercury cell. The percentage inhibition of paw volume in drug treated group was compared with control group. Indomethacin (10 mg/kg) was used as standard drug. The percentage inhibition of paw edema was calculated by using the following formula;

$$\text{Percentage inhibition} = \left[1 - \frac{V_t}{V_c}\right] \times 100$$

Vc- Volume of paw edema in control group
Vt- volume of paw edema in treated group

Statistical analysis

The statistical analysis was carried out using Graph pad prism 4.0 software. All values were expressed as Mean ± S.E.M. Data analysis was done by one-way ANOVA followed by Dunnett's multiple comparison tests. Difference level at $P<0.05$ was considered as statistically significant condition.

RESULTS AND DISCUSSION

FT-IR Studies

FT-IR studies have been performed for the pure drug, co-formers and the prepared co-crystals. From the FT-IR results [Figure 1] it can be confirmed that there is interaction between the pure drug and excipient. The peak of C=O stretching is observed at 1614.47 of pure curcumin and in methylparaben at 1681.98, whereas in curcumin-methylparaben crystals this peak has been shifted to 1589.40.

In curcumin – methylparaben crystals the decrease in frequency implies that the functional groups participate in strong hydrogen bond. As, there is significant shift of wavelength only to lower numbers in both curcumin and methylparaben and in crystals, it can be confirmed that a proton transfer did not occur. So, the formation of the co-crystal can be confirmed.

Differential Scanning Calorimetry (DSC) studie

DSC experiments were carried out to study the thermal behavior of the crystal form in relation to the individual components. DSC thermal data are shown in figure. DSC study of curcumin and methyl paraben shows endothermic peak at 175.67 C and 112.02 C while DSC study of prepared co-crystal shows sharp endothermic value at 117.86 C, the sharp endothermic values of crystal form and the individual components agreed with the measured melting range in the melting point determination. The thermal profile of crystal form was distinct, with a different melting transition from that seen with either of the individual components. This indicates the formation of novel crystal phase: crystal form of Curcumin with Methyl paraben (1:1 molar ratio). This single endothermic transition indicates the absence of any unbound or absorbed solvent or water and also demonstrates the stability of the phase until the melting point.

X-Ray powder Diffraction (XRPD) studies

Powder diffraction is a scientific technique using Xray, neutron, or electron diffraction on powder or microcrystalline samples for structural characterization of materials Structure determination of organic solids from powder The powder x-ray diffraction (XRD) was performed by X'pert Prowith Spinner PW3064 using Nifiltered, CuKα radiation, a voltage of 45 kV, and a current of 40 mA with a scintillation counter. The diffraction spectra of Curcumin and Methylparaben show numerous distinct peaks indicating that both are present in a highly crystalline state. The XRD pattern of co crystal of curcumin and methylparaben exhibits all the characteristic diffraction peaks of curcumin and methylparaben

Scanning Electron Microscopy

SEM analysis has been performed for the pure drug, excipients and co-crystals. The pure drug exhibited irregular shape with smooth surface. The co-crystals also exhibited irregular shape but the shape was different with those of the pure drug and excipient. From the SEM analysis, the crystals showed reduced particle size.

Evaluation of capsules

The weight variation for F1 to F6 formulations were found to be 197.4 ± 0.04 to 200.6 ± 0.05. The percentage deviation is coming within 3% to 5% range for this test accepted % deviation should be 5 % for 200 mg capsules..F1 to F6 batches come within limit and passed the test.

Disintegration was determined. The results were illustrated in table. The disintegration time for F1 – F6 were ranging 3.20 ± 1.23 to 3.98 ± 1.32 seconds. Out of all formulations F1 given quick disintegration (i.e with 3 minutes 20 seconds). Uniformity of dispersion was carried .The results were illustrated in tab. The residue remaining on the screen was found to be nil hence all the formulations from F1 to F6 passed the test.

In vitro Dissolution studies

Dissolution studies were carried out by USP type II (Basket apparatus) at 50 rpm in P^H-1.2 buffer. Temperature was maintained at 37 ±0.5 ℃. Aliquots of dissolution media was withdrawn at 5,10,15,30,45,60,90 minutes of time intervals and the sample was filtered. Same quantity of fresh media was replaced. The filtered solution was used to determine the drug content. Absorbance was we as used at 431 nm by UV/Visible spectrophotometer.

In vivo studies

In this study, curcumin was demonstrated to inhibit inflammation in the carrageenan-induced rat paw

edema model. The onset and duration of action suggest that the anti-inflammatory mechanism of curcumin occurs through inhibition of prostaglandin synthesis via cyclooxygenase pathway. The pharmacological activity combined with the lack of toxicity render curcumin a valuable candidate for further investigation as an agent for treatment of various disorders associated with inflammation. Anti-inflammatory effect of Curcumin and Co-crystals were observed and found to be significant at the level of $p<0.001$ when compared with the vehicle 1% CMC (control group) and indomethacin (Standard). The percent inhibition in paw edema after 3 h were recorded 67.71 % in case of Indomethacin, 55.73% in case of 100 mg/kg of Curcumin, 66.67 % in case of 100 mg/kg of Curcumin Co-crystals respectively.

Table 1. Formulae of curcumin methylparaben capsules

S.NO.	INGRDIENTS	F1	F2	F3	F4	F5	F6
1	CO-CRYSTALS	200	60	60	60	60	60
2	LACTOSE	0	35	45	34	40	30
3	SODIUM LAURYL SULPHATE	0	35	30	35	35	40
4	PVP30	0	32	24	35	25	34
5	CARBOXY METHYL CELLULOSE	0	32	35	30	34	30
6	MAGNESIUMSTEREATE	0	4	4	4	4	4
7	TALC	0	2	2	2	2	2
TOTAL		200	200	200	200	200	200

Table 2. Comparison of interpretation of IR spectrum of Curcumin, methylparaben and co-crystals

PEAKS	PURE DRUG	METHYL PARABEN	CRYSTAS BY L.A.G. METHOD
O-H stretch	3504.77	3306.10	3508.63
C=O stretch	1614.47	1681.98	1589.40
Aromatic C=C stretch	1585.54	1593.25	1504.53
Phenol C-O stretch	1440.87	1510.31	1274.99
Enol C-O stretch	1197.83	1438.94	1026.16

Table 3. Evaluation of capsules

Formulation	Uniformity content		Weight variation	Disintegration time
	Residue remaining on screen	Result		
F1	Nil	pass	200.6± 0.05	3.20 ± 1.23
F2	Nil	pass	199.2 ± 0.01	3.45 ± 1.13
F3	Nil	pass	198.5± 0.02	3.78 ± 0.92
F4	Nil	pass	199.3±0.04	3.83 ± 0.95
F5	Nil	pass	198.7±0.01	3.90 ± 1.19
F6	Nil	pass	197.4.±0.04	3.98 ± 1.32

Table 4. Comparative drug release profile of pure drug and capsules

Time interval (mts)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
5	30.914	29.254	31.121	30.256	31.451	7.523
15	45.536	46.231	45.263	44.874	45.556	12.565
30	57.141	55.254	55.213	54.698	55.231	21.869
45	69.259	68.245	66.212	65.356	64.521	30.97
60	76.855	74.65	73.235	74.584	73.258	38.748
75	85.45	84.991	83.236	82.256	83.145	42.45
90	**95.35**	94.131	94.256	94.002	93.339	**47.8**

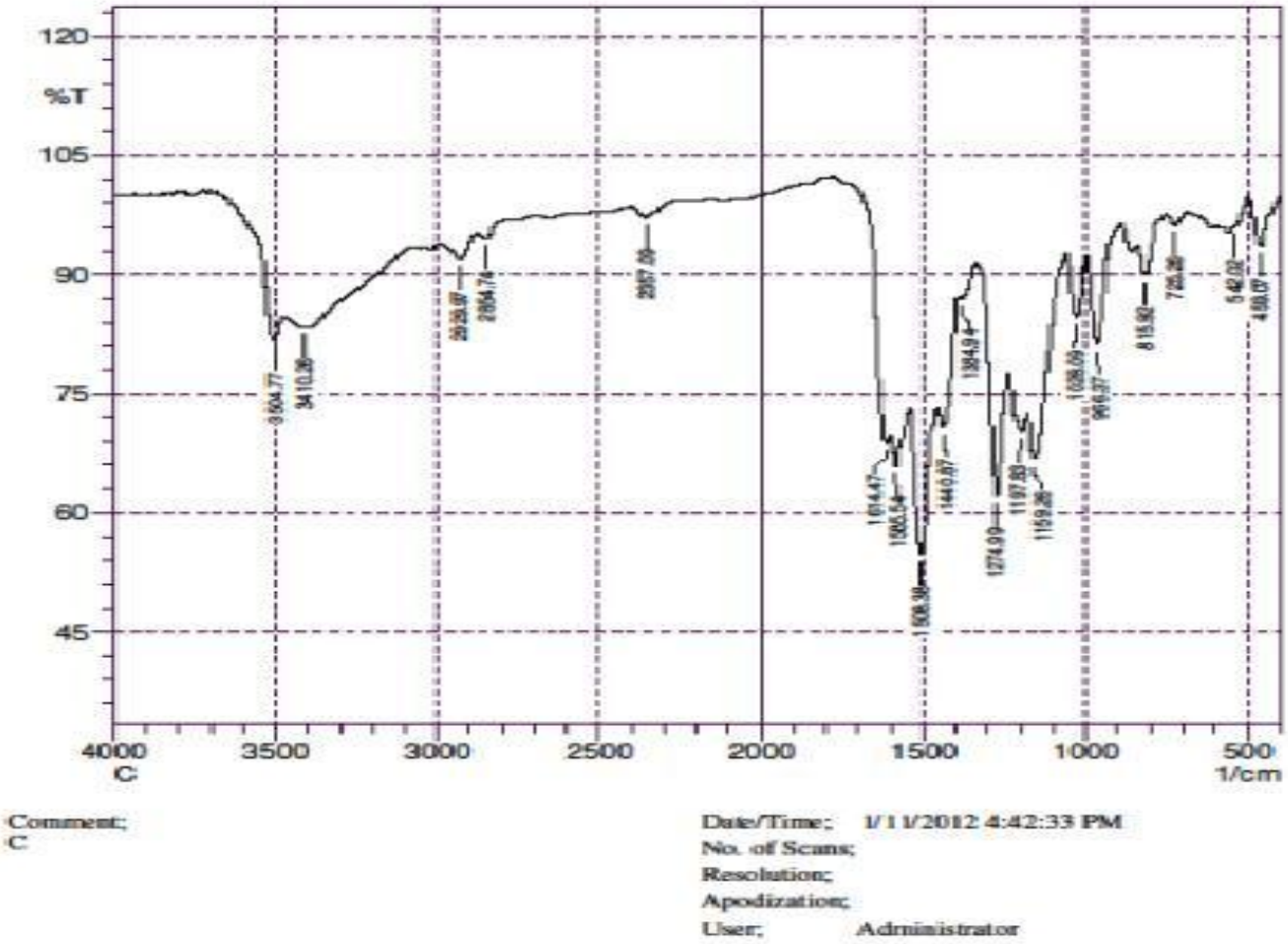
Table 5. Effect of Curcumin and Curcumin Co-crystals on carrageenan induced paw Edema method

Groups	Paw edema volume (ml)					Percentage of inhibition
	0min	30min	60min	120min	180min	
Group I (Negative Control)	0.81±0.01	1.27±0.01	1.37±0.02	1.79±0.02	1.92 ±0.04	_
Group II (standard)	0.82±0.02	1.12±0.02*	1.23±0.02*	1.13±0.01**	0.62±0.02**	67.71
Group III (Curcumin-100)	0.80±0.01	1.21±0.02*	1.25±0.02	1.06±0.02*	1.83±0.02**	4.65
Group IV (Curcumin co-crystals-100)	0.83 ±0.01	1.03±0.02*	1.15±0.02*	0.95±0.02**	0.64 ±0.03**	66.67

*Values are expressed as mean ± SD (n = 6); *p < 0.01, **p < 0.001 When compared with control (One way ANOVA test).*

Figure 1. FT-IR studies of (A) curcumin, (B) methyl paraben, (C) crystals

(A)



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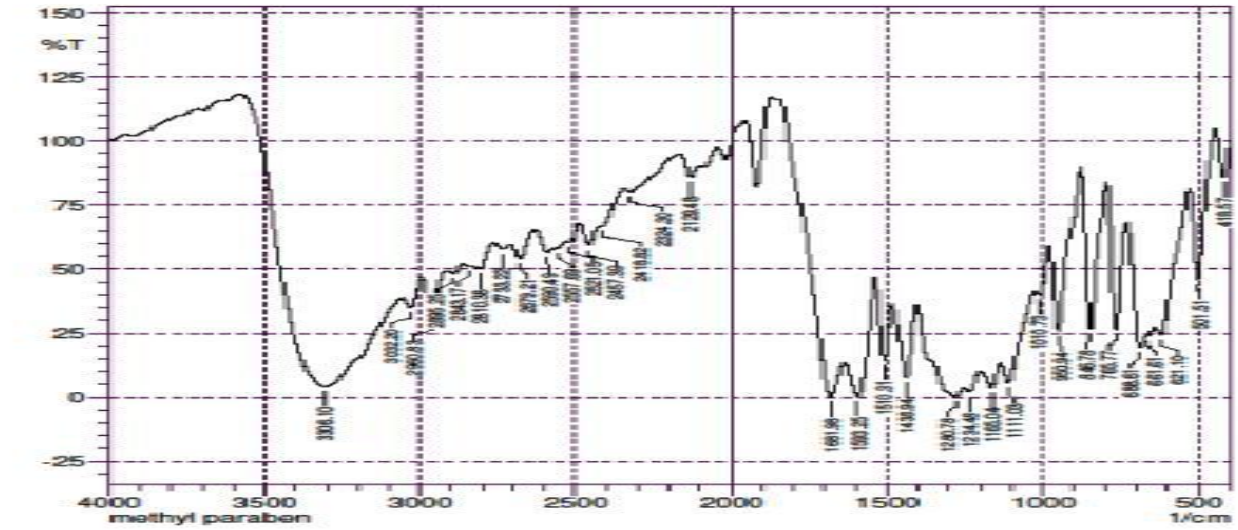

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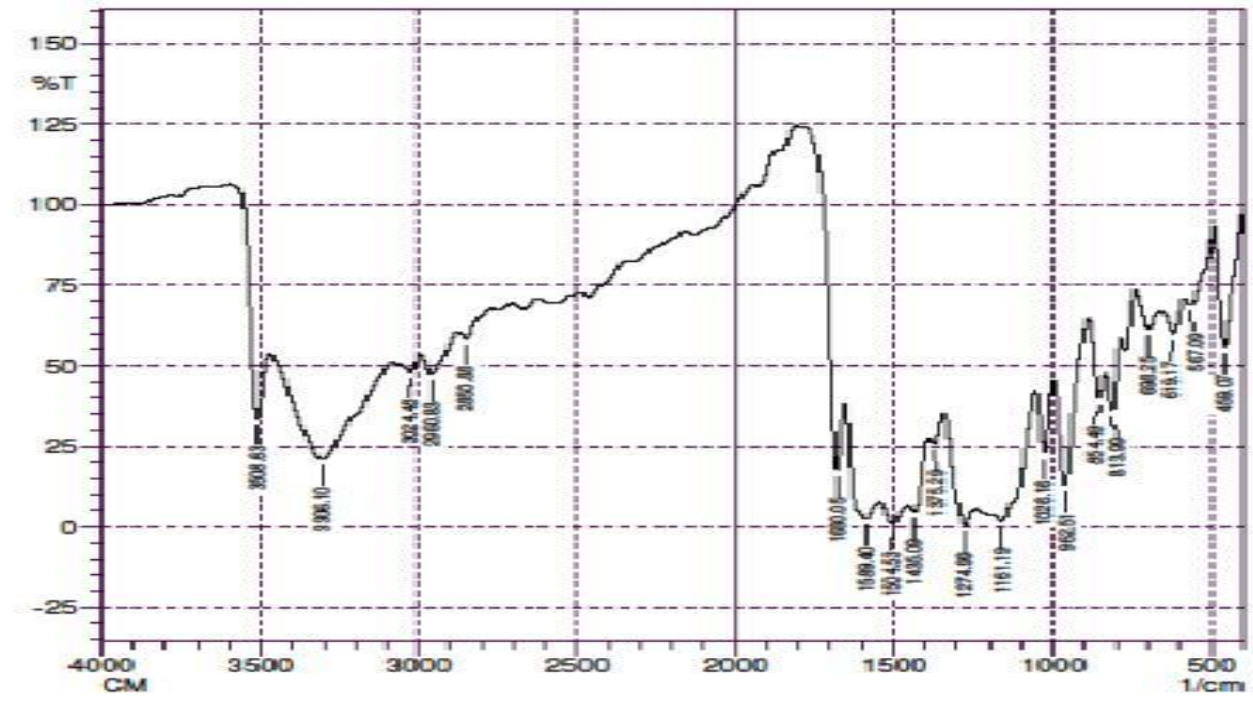

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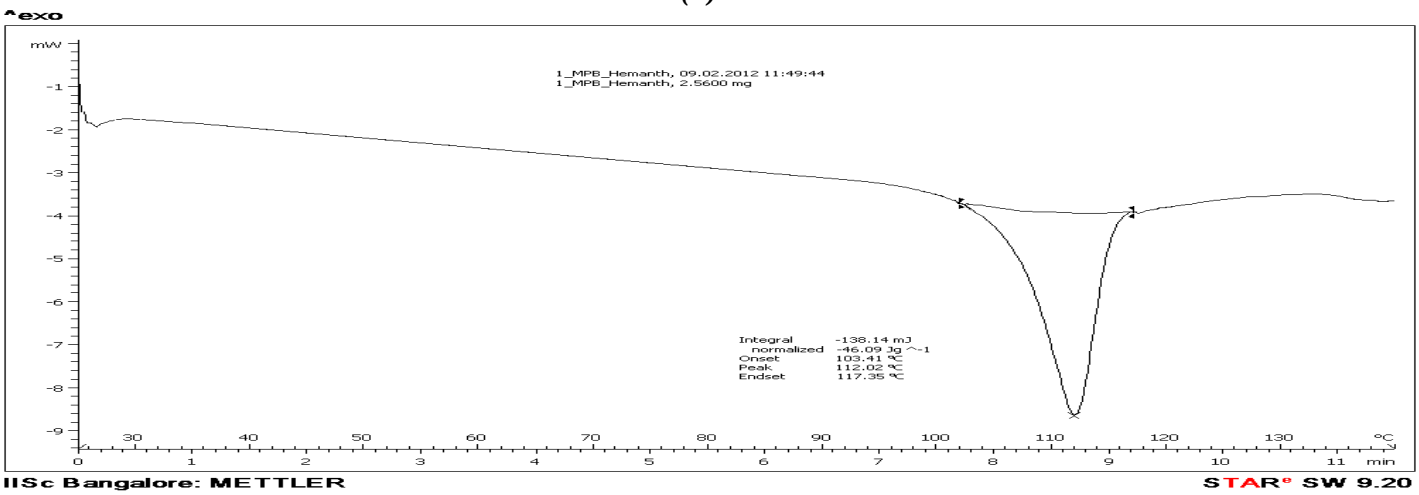
Figure 2. Comparative DSC studies

(A)



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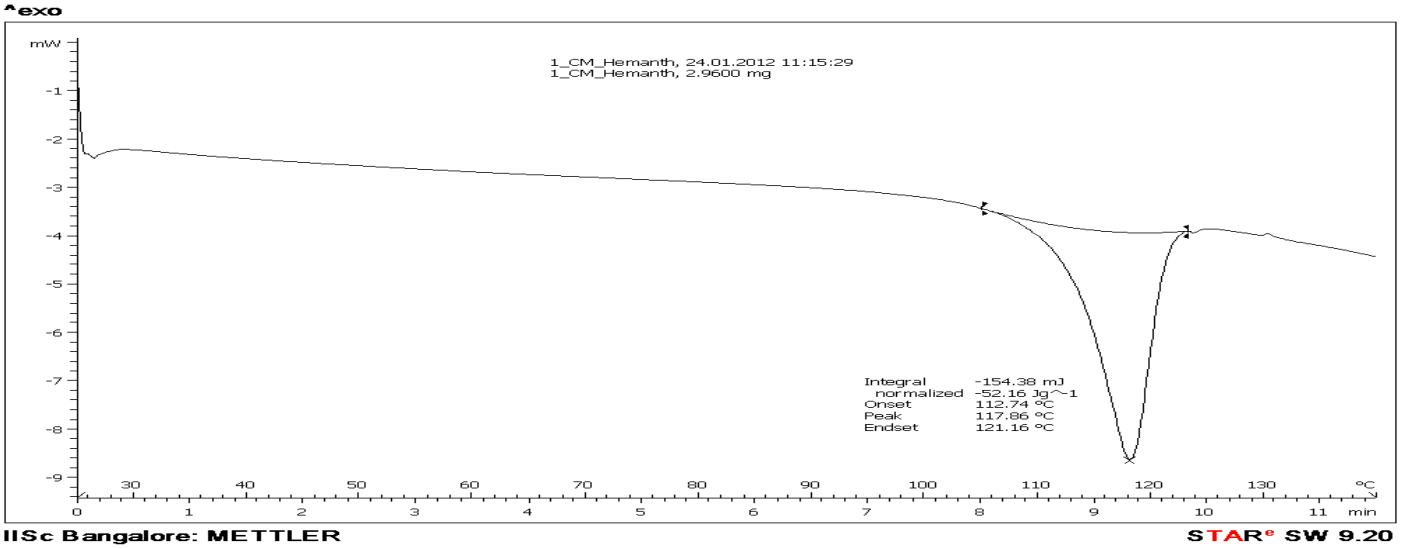


(C)

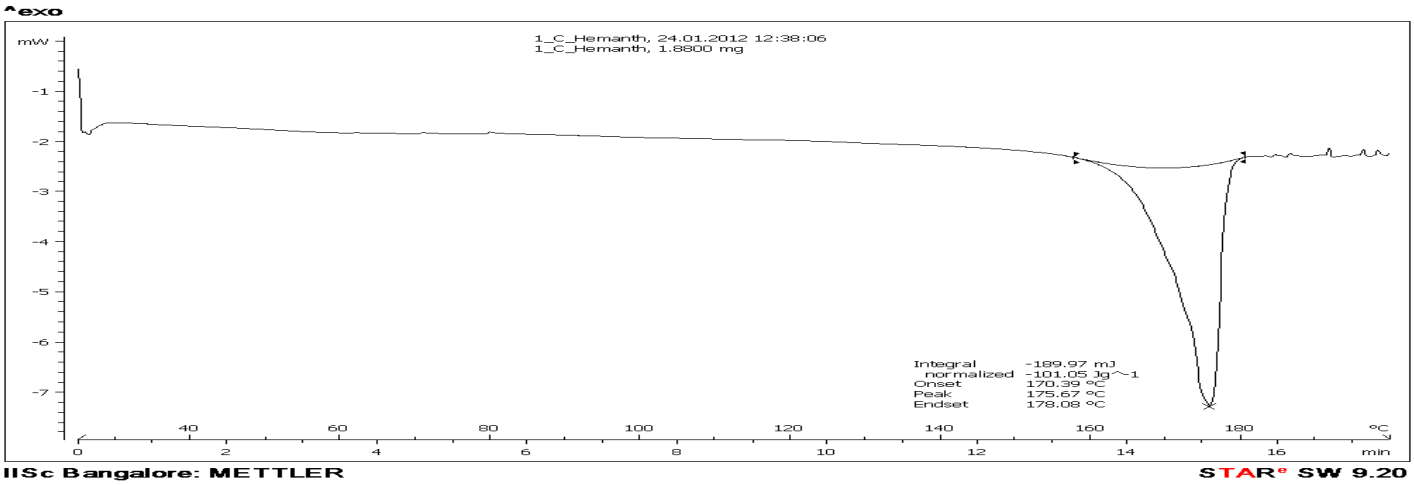
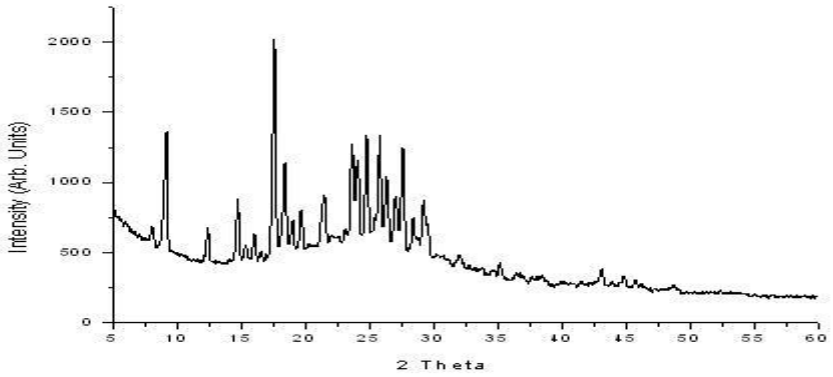
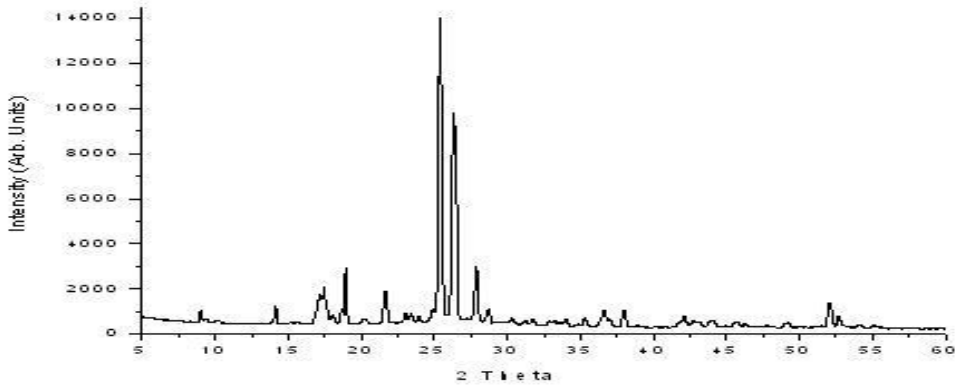


Figure 3. Comparative XRPD graphs.

Curcumin



Methyl Paraben



Curcumin Methyl Paraben Crystals

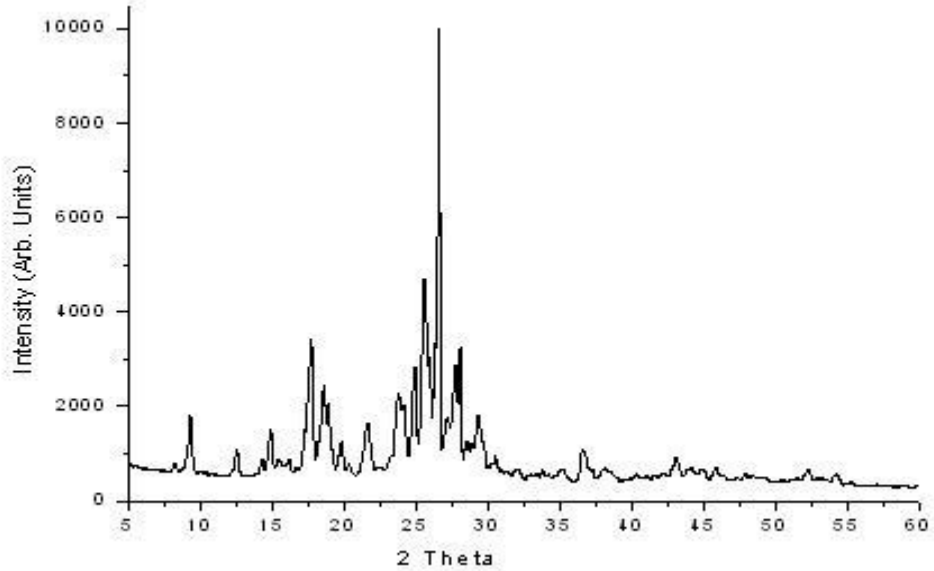
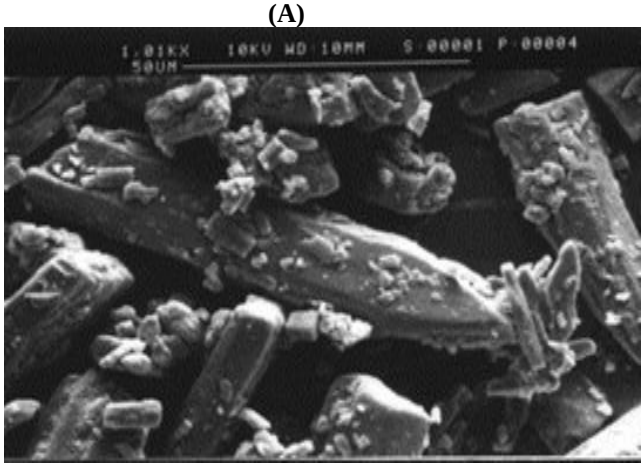
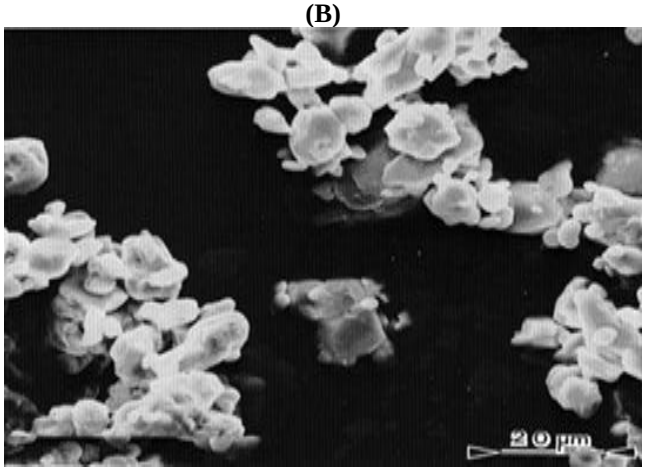


Figure 4: SEM Pictures of pure (A) Curcumin, (B) Methyl paraben, (C) Crystals

(A)



(B)



(C)

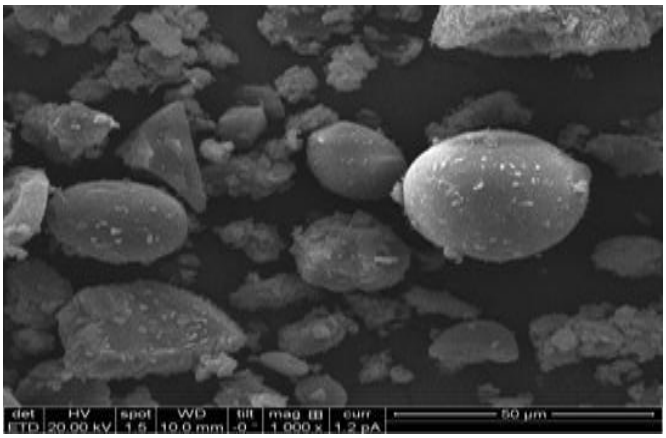
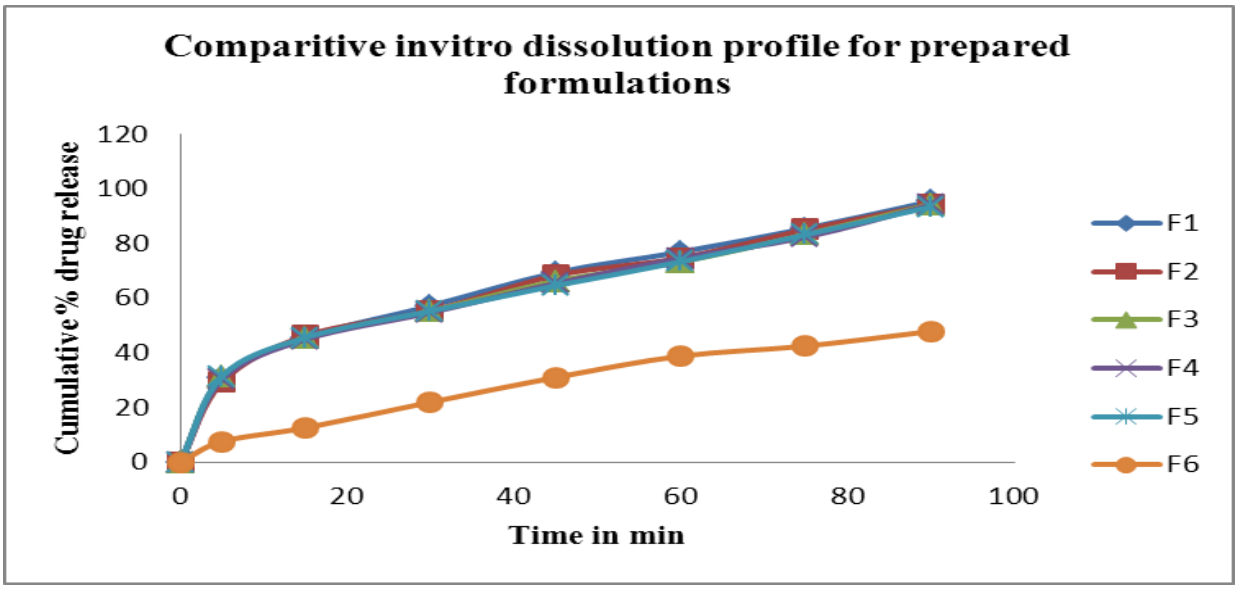


Figure 5: Drug release profile of pure drug and capsules



CONCLUSION

The relevance of crystal engineering in API formulation includes the ability to fine-tune physical properties without changing the molecular structure of the API, identification of novel forms of polymorphic API's, and the opportunity to generate a broader range of intellectual property than with present methods. In this context, co-crystallization of an API with a co-former can be thought as a more-radical strategy to address the poor solubility and physical stability of moisture-sensitive pharmaceutical materials. The aim of the current study was to develop a formulation which can overcome the limitation of curcumin being so poorly soluble in aqueous medium. In this research work co-crystals of curcumin with methylparaben was prepared by liquid –Assisted grinding method. Techniques used for their characterisation are FT-IR, DSC, XRPD, SEM. Low doses of pure curcumin (100 mg/kg) gave less inhibitory effect of 4.65%, and prepared co-crystals shows significant inhibition effect of 66.67%. The reduction of edema by Indomethacin, Curcumin and Co-crystals at 3 h or more after Carrageenan injection suggests that both compounds produce anti-inflammatory effects in the second phase of edema, indicating inhibition of prostaglandin synthesis. In effect, this ability to understand supramolecular heterosynthons, along with knowledge of optimal crystallization and characterization techniques, reasonably design co-crystals with a high rate of success. However, the downstream implications for later stage development must be carefully weighted in light of the total picture of corporate needs.

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