RESEARCH ARTICLE

Amorphous Mixtures of Albendazole with Carboxylic Acids By Cogrinding Technique: Solid State Characterizations and *In Vitro* Efficacy Study

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ABSTRACT

Purpose: To improve the physicochemical properties of poorly aqueous soluble albendazole by formulating its amorphous mixtures by different techniques. **Methods:** cogrind amorphous mixtures were successfully formulated with different carboxylic acids like citric acid, benzoic acid, salicylic acid and succinic acid in equimolar ratios by grinding and solvent drop grinding technique without formation of cocrystals. The physicochemical properties of pure albendazole and corresponding cogrind mixtures were assessed in terms of melting point, drug content, saturation solubility and dissolution studies, Infrared spectroscopy (IR), X-ray powder diffractometry (XRPD) and Differential scanning colorimetry (DSC). **Results:** The results indicated a marked improvement in flow properties and saturation solubility of amorphous mixtures. Further, in case of dissolution experiments, the amorphous mixtures showed a significant enhancement in the dissolution profiles as compared to pure albendazole. In-vitro anthelmintic as well as in In vitro antifungal activity was also improved. It could be concluded that better improvement of physicochemical properties of albendazole could be possible with salicylic acid and citric acid using cogrinding technique. **Conclusion:** It is possible to formulate amorphous mixtures of albendazole with different carboxylic acids without forming cocrystals still strong agreement to form cocrystals by solubility parameters, structural features.

Keywords: Albendazole, Amorphous, Dissolution rate, Mxtures, ΔpKa.

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INTRODUCTION

In drug discovery, solubility information is widely used to enhance the quality of drug candidates to diagnose early solubility issues of a new chemical entity and further modifications. In drug development, the solubility of the active pharmaceutical ingredient (API) is a crucial attribute with respect to selecting the dosage forms for clinical trials, designing experiments to identify potential salt forms, cocrystal forms, polymorphic forms, solvates, and hydrates, developing analytical procedures and aiding drug manufacturing. The solubility of new chemical entities (NCE) is emerging as a significant issue in drug discovery and development as it affects many aspects of drug discovery and development, including in vitro/in vivo assay quality, formulation, human PK, clinical trial design and biowaivers. Various strategies have been developed to overcome solubility issues using medicinal chemistry approaches and formulation techniques throughout the pharmaceutical industry to de-risk the solubility impact on the drug candidate.[1]

Molecular complexation is of two type's inclusion complexes, and coordination assisted lattice-type complexes former depends on stereochemical host, and guest interaction and latter depends on noncovalent interaction between drug and coformer and their complementary functional groups. Coordination type of molecular complexes is also known as molecular crystal, and it comes under the multicomponent system, includes the formation of hydrates, solvates, salts,

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and cocrystal through the intermolecular interactions. Cocrystal formation involves two solid-state structures drug and coformer (selected on complementary structural features), which self assembles by noncovalent interaction such as electrostatic interactions (ion-ion, ion-dipole, and dipole-dipole interactions), hydrogen bonding, halogen bond in a well-defined stoichiometry. The basic difference in traditional & an alternative and potentially fruitful approach crystal engineering is the formation of a cocrystal of an API with more stable (maintain the chemical integrity of API), improved physiochemical properties such as stability, dissolution rate, solubility, hygroscopicity, and compaction behavior. [2-4]

If we look at the methods of preparation of amorphous mixtures mechanochemistry based approach is also preferred. Cogrind amorphous mixtures without forming cocrystals are one of the interests of the pharmaceutical industry by considering this, we have screened albendazole with dicarboxylic acids.

Albendazole is **[**Methyl l [(5-propylthio)-1H-benzoimidazoly2yl] Carbamate is a widely used antihelminthic agent against human and animal parasite.[5] Albendazole is a Benzimidazole derivative that belongs to BCS class-II having low aqueous solubility (0.01 mg/mL in the water at 25°C).[5] Due to this amount of drug reaching to systemic circulation is very low to indicate effective pharmacological activity which directly results in administration of high dose (400mg) to achieve desired therapeutic action and may leads to adverse effect like fever, skin rash or itching, sore throat, thinning or loss of hair, unusual tiredness and granulocytopenia, pancytopenia, agranulocytosis, thrombocytopenia, hepatotoxicity, etc.

Different strategies have been reported, such as vehiculization of ABZ in, cyclodextrin Complexation, cogrinding, solid dispersions, and the synthesis of new analogs with higher solubility.[6-9] The purpose of the present investigation was to evaluate whether the formation of an amorphous mixture of abendazole with carboxylic acid is possible or not and to study the effect of formed material on solubility, dissolution rate, and pharmacodynamic activity.

MATERIALS AND METHODS

Materials

Albendazole was a gift sample from Cipla Pvt.Ltd.Mumbai. All the other chemicals and solvents were of analytical grade procured from Merck (India) and Loba Chemie Mumbai (India).

Theoretical screening of Albendazole for solid-solid miscibility

Theoretical prediction of miscibility between solute –solute (drug and coformer) was estimated by three methods namely Fedors group contribution, Vankrevalen, and Hoy's molar attraction method. Calculation of difference between ∂ values ($\Delta\partial$) of drug & carboxylic acids.[10]

Preparation of cogrind mixtures

Cogrinding and solvent drop cogrinding technique in short, Albendazole (265mg) and selected conformers like Citric acid (192.1mg), Salicylic acid (138mg) Benzoic acid (122mg), Succinic acid (118mg) were mixed together in 1:1 molar ratio without and with and addition of a few drops of volatile solvent acetone to promote the formation of mixture or to increase the kinetics of interaction by using a clean mortar and pestle.

Determination of Drug Content

Prepared crystals (10 mg) were triturated with 0.1 N HCl. Finally, the volume was made up to 100 ml with the same, the solution was filtered through a membrane filter (0.45µm) and analyzed spectrophotometrically (UV-1601, Shimadzu, Japan) at 291nm and drug content was calculated.

Saturation Solubility analysis

Saturation solubility studies were performed in triplicate according to a method reported by Higuchi and Connors.[11] For saturation solubility, an excess quantity of albendazole prepared co-crystals was placed in the vials containing 10 ml of 0.1N HCl. The vials were agitated in incubator shaker (100 agitations/min) for 24 hours. The solution was then filtered through a membrane (0.45µm) and drug solubilized was analyzed spectrophotometrically (UV-1601, Shimadzu) at 291nm.

Infra-red Spectroscopy

IR spectroscopy was conducted using a Shimadzu FTIR 8300S Spectrophotometer (Shimadzu, Tokyo, Japan), and the spectrum was recorded in the wavelength region of 4000–400 cm^{-1}. The procedure consisted of dispersing a sample (drug, physical mixtures, and prepared cogrind mixtures) in KBr and compressing into discs by applying a pressure of 5t for 5 minutes in a hydraulic press. The pellet was placed in the light path, and the spectrum was recorded. All spectra were collected as an average of three scans at a resolution of 2 cm^{-1}.

Differential Scanning Calorimetry (DSC)

The DSC was performed using DSC-60A (Shimadzu, Tokyo, Japan) calorimeter to study the thermal behavior of drug, physical mixtures, and prepared cogrind mixtures. The samples were heated in hermetically sealed aluminium pans under nitrogen flow (30 mL/min) at a scanning rate of 3°C/min from 30 to 250°C. Empty aluminium pan was used as a reference.

Powder X-Ray Diffraction (P-XRD)

The X-ray diffraction patterns of pure drug and the optimized formulations were recorded using a Philips Analytical XRD PW 3710 diffractometer. The radiation was generated by a Cu Kα lamp. The instruments was operated in the continuous scan mode with the scanning speed at 2°/min. Scan range was 3–70° 2 θ/θ with a scan speed 0.066° 2 θ.

In-Vitro Dissolution Study

Dissolution tests of all formulations were performed using an USP Type-II dissolution apparatus. The rotational paddle speed was set at 50 rpm and the temperature was kept constant at 37 ± 0.5°C and dissolution medium 900 mL 0.1 N HCl and Five-milliliter aliquots were withdrawn at pre-determined time intervals of 5, 10, 15, 30, 45, 60, 75……..120 minutes

during 2hours and the same amount of fresh medium was added in order to keep the volume constant throughout the test. The samples were filtered, and the concentration of dissolved drug was measured at 291nm using the UV–vis spectrophotometer (shimatzu1601). The measurements were performed in triplicate.

In-vitro Anthelmintic Activity[12-13]

Indian earthworms (*Phrentima posthuma*) were used to study the anthelmintic activity of cocrystals. Earthworms were collected from humid soil from the farms of Ghogaon and washed with normal saline to remove waste and fecal matter adhered to their body. Earthworms of size 4-5 cm in length and 0.1–0.2cm in width were used for the in-vitro anthelmintic activity of novel formulation. The earthworms were divided into the respective groups containing six-earth worms of similar size in each group as follows; Control group; Earthworms in normal saline and 2% tween 80, Standared group: Earthworms in drug solution, Treatment Group: Earthworms in formulation solutions and coformers solutions. All the prototypes and standard drug solutions were prepared before the commencement of experiments. Hence prototypes and standard drug solutions were dissolved in a vehicle 2% v/v Tween 80 in normal saline and volume was adjusted up to 10 ml normal saline for making a concentration of 5, 10, and 20 mg/mL. Then all the earthworms were released in respective formulation as normal saline and 2% tween 80, Albendazole 20mg/mL, cogrind formulations (5mg/ml, 10mg/ml, 20mg/ml) Anthelmintic activity was determined by analyzing spontaneous motility or time taken to paralysis as well as death evoked response which was done by observing the paralyzing time (indicated by fading of color or color change) and death time was analyzed by reduction in motility or immobilization.

In-vitro Antifungal Activity

Albendazole belongs to benzimidazole derivative and has some antifungal activity due to its chemical nature, which has been reported in literature[6]. In-vitro antifungal activity was carried out on *Aspergillus niger,* procured from Krishna Medical Sciences, Karad to compare the in-vitro antifungal activity of drug with Formulation by well plate method. The antifungal ability of the Albendazole mixtures was tested on fungal strain growth inhibition on a lawn of *Aspergillus niger* in a saobaroud dextrose agar plate.[14] Three different treatments (10 µL) were pippetted onto the separate plates: Albendazole and cogrind formulations at 1,3,5 µg/mL, prepared in DMSO, suspension without drug or DMSO as control was used. The plates were incubated at 37ºC for 48 to 72 hours. Circled areas on culture plates indicate treatment placement.[14] The zone of inhibition was measured by using an antibiotic zone reader. Digital photos of the cultures were taken after treatments to assess the antifungal activity.

Stability Studies of Cogrind Mixtures

After the determination of drug content, the optimized formulations were charged for stability studies according to ICH guidelines (30 ± 2 ◦C and 65 ± 5% RH) for 6 months in the stability chamber (Thermo Lab, Mumbai, India). The samples were placed in USP type-1 flint vials and hermetically sealed with bromobutyl rubber plugs and aluminum caps. Samples (n = 3) were taken out at 0, 30, 60, 90, and 180 days, and evaluated for the drug content and physical changes.

RESULTS AND DISCUSSION

Theoretical Screening of Albendazole for Cocrystallization

Calculation of ∂ (cohesive energy density) value for Albendazole and coformers was done by Fedors group contribution, Hoys molar attraction and vankrevalen method as shown in Table 1. In first method cohesive energy density "energy needed to break all these interactions, allowing atoms or molecules to detach and resulting in solid to liquid/gas or liquid to gas transformations.[15-19] The ratio of latent heat of vaporization to molar volume was taken to determine miscibility. Fragmentation of drug molecule was done, and then the value of latent heat of vaporization and molar volume for each fragmented volume was calculated experimentally. Constituent were taken from literature to calculate ∂ value. In Hoys molar attraction method, molecular attraction between two components was taken for calculating solubility parameters. In the Vankrevalen method contribution of three forces, namely polar force, dispersion force and hydrogen bonding forces between molecules, contributes to the solubility parameter. Subtraction of ∂ values of two gives idea regarding their miscibility.

This theoretical prediction of miscibility between drug and coformer helps in the selection of coformer, which reduces the use of a large number of coformers in trial batches. The results of theoretical prediction reveal that the difference between ∂ values of drug and coformer was less than 7 for Benzoic acid by three methods; hence, it is miscible with Albendazole. Also the results of miscibility with other coformers indicate miscibility by only two methods. A theoretical prediction confirms the formation of cocrystals.

Table 1: Theoretical predictions of miscibility

Formulation code	*Method*	*∂ value*	*Miscibility*
ABZ	Fedor's	11.89	-
	Hoys M.A.	12.39	-
	Vankrevalen	15.72	-
ABZ-CA	Fedor's	10.71	Imiscible
	Hoys M.A.	3.58	Miscible
	Vankrevalen	6.6	Miscible
ABZ-SA	Fedor's	4.38	Miscible
	Hoys M.A.	2.8	Miscible
	Vankrevalen	8.28	Imiscible
ABZ-BA	Fedor's	1.93	Miscible
	Hoys M.A.	1.21	Miscible
	Vankrevalen	1	Miscible
ABZ-SU	Fedor's	8.88	Imiscible
	Hoys M.A.	2.07	Miscible
	Vankrevalen	4.55	Miscible

ABZ: Albendazole CA: Citric acid SA: Salicylic acid BA: Benzoic acid SU: Succinic acid

Drug Content Analysis

Table 2 reveals that practical yield was ranged from 86.8 % to 94.6%. The drug content was found to be good and uniform among the different batches of crystals prepared and ranged from 97.82 to 99.18%. In a cogrind formualtions, the solubility of albendazole was enhanced from 0.502mg/mL to 1.81mg/mL. Saturation solution in a qualitative way defined as, spontaneous interaction of two substances to form homogeneous molecular dispersion. An increase in interaction between two components in saturated solution was due to more amount of solute is in equilibrium with its solid phase.[11] Hence the enhancement in solubility was mainly due to greater solute-solvent interaction.

In-vitro Dissolution Study

In vitro dissolution study of physical mixtures showed that the interaction of Albendazole is better with Salicylic acid, Citric acid than Benzoic, and Succinic acid. Because percentage drug released was found to be 84.57%, 52.60%, 53.15%, 31.05% respectively (Figure 1 and 2).

It happens because of the opening of interaction sites is not prominent in physical mixing. Results of dissolution study by cogrinding method indicate 96.49, 74.69, 50.85 and 35.46% drug released within 2hrs for salicylic acid, Citric acid, Benzoic acid, Succinic acid, respectively (Figure 3). In case of solvent drop cogrinding percentage drug released obtained within 2hrs for salicylic acid, Citric acid, Benzoic acid, Succinic acid were 97.57%, 62.50%, 60.80%, and 17.56% (Figure 4) respectively. The dissolution rate mainly depends on strength, crystal lattice, and solvation component. The enhanced dissolution rate was observed and it may be due to the lowering of lattice energy and increased solvent affinity, which is maintained by formulation.

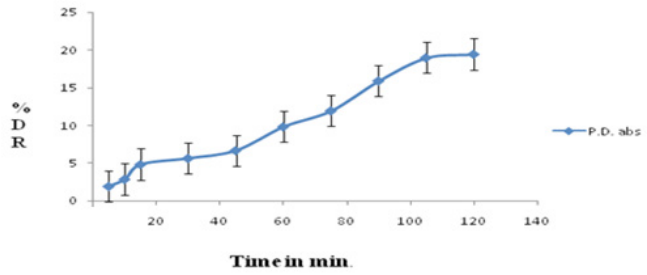

Figure 1: Drug release profile of pure albendazole in 0.1 N HCl.

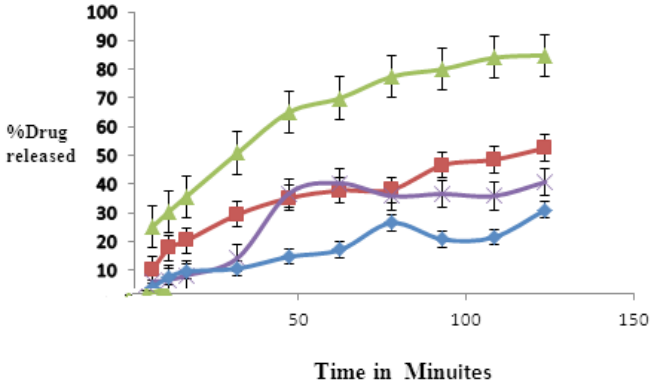

Figure 2: Drug release profile of physical mixtures in 0.1 N HCl. Citric (—■—), Salicylic (—▲—), Benzoic (—×—) and Succinic acids (—♦—)

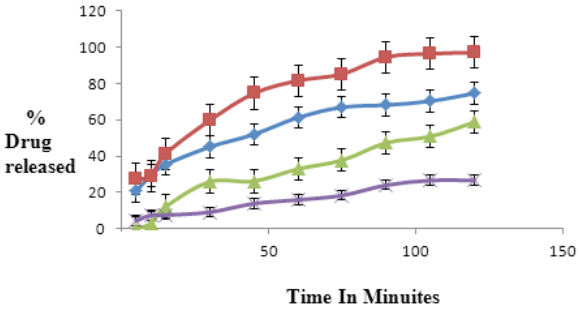

Figure 3: Drug release profile of amorphous mixtures prepared by cogrinding method in 0.1 N HCl.
Citric (—♦—), Salicylic (—■—), Benzoic (—▲—) and Succinic acids (—×—)

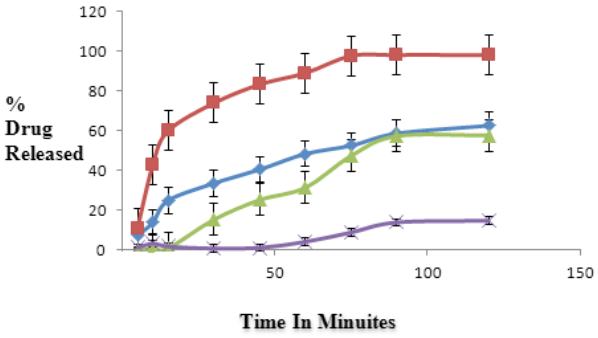

Figure 4: Drug release profile of amorphous mixtures prepared by solvent drop cogrinding method in 0.1 N HCl. Citric (—♦—), Salicylic (—■—), Benzoic (—▲—) and Succinic acids (—×—)

Table 2: % yield, saturation solubility & % drug content of the formulations

Sr. No.	*Formulation Code*	*% Yield*	*% Drug Content*	*Solubility in mg/ml*
1	Albendazole (ABZ)	-	-	0.502 ± 0.0351
2	Cogrinding citric acid (GCA)	92.35 ± 1.3	98.12 ± 0.12	0.99 ± 0.015
3	Cogrinding salicylic acid (GSA)	94.6 ± 0.8	99.18 ± 0.3	1.813 ± 0.015
4	Cogrinding benzoic acid (GBA)	97.1 ± 0.54	98.08 ± 0.5	1.227 ± 0.037
5	Cogrinding succinic acid (GSU)	90.65 ± 0.26	97.12 ± 0.5	0.661 ± 0.018
6	Solvent drop cogrinding citric acid (SDC)	90.8 ± 0.043	98.02 ± 0.7	1.291 ± 0.025
7	Solvent drop cogrinding salicylic acid (SDSA)	92.05 ± 0.87	97.92 ± 0.6	1.672 ± 0.056
8	Solvent drop cogrinding benzoic acid (SDBA)	93.5 ± 0.88	96.82 ± 0.4	0.830 ± 0.575
9	Solvent drop cogrinding succinic acid (SDSU)	86.8 ± 0.024	96.05 ± 0.12	0.663 ± 0.055

Cogrinding method improves the dissolution rate than solvent drop cogrinding and physical mixture. Cogrinding induced changes such as molecular collision, molecular defects and molecular diffusion between to molecules that will enhance interaction and indirectly dissolution rate. Also a possibility of amorphization due to the energy input method. In the case of solvent drop cogrinding, acetone act as catalyst for cocrystal formation and enhance rate of crystallization. Also, the addition of solvent leads to opening of conformational and orientational freedom to molecule at various interfaces for intermolecular interaction also might be possibility of partial crystallization of albendazole.[20]

Infra-red Spectroscopy

The results revealed considerable changes in the IR peaks of Albendazole in the prepared cogrind formulations when compared to pure drug, thereby indicating the presence of interaction. Effect of cogrinding time on ineraction between drug and coformer was elucidated by FTIR.

In the case of citric acid cogrinding for 15min induces the interaction,[21] which is indicated by the disappearance of the characteristic peak of O-H stretching of Citric acid at 3228-3495cm-1 that might be involved in interaction. Another effective interaction was observed after 45 min, due to dimerisation ability of citric acid, it forms a dimer with albendazole Fand is indicated by shifting of characteristic dimerization peak of citric acid at 1655–1738 cm^{-1} from 1711 cm^{-1}. There might be chances of merging of the carbonyl group of citric acid molecule in N-H group of albendazole (Figure 5).[22] In FTIR spectrum of salicylic acid cogrind mixture as shown in Figure 6 pronounced changes were seen in stretching of Ar-O-H group at 1633cm^{-1} and carbonyl functionality at 1711–1725$cm^{-1}$of salicylic acid. The most prominent changes were seen after 45 minutes cogrinding reason for this is cogrinding induces interaction between albendazole and salicylic acid, which is indicated by shifting of peaks for hydrogen bonding more towards lower side wave numbers. This indicates presence of intermolecular hydrogen bonding as intramolecular hydrogen bonding shifts the peaks to higher wave numbers.[22-23] This confirms presence of weak hydrogen bonding with salicylic acid. FTIR spectrum of Benzoic acid and Succinic acid cocrystal shown in Figure 7 and 8 indicates pronounced changes after 30min of cogrinding this may be due to the availability of few chemical interaction sites in initial

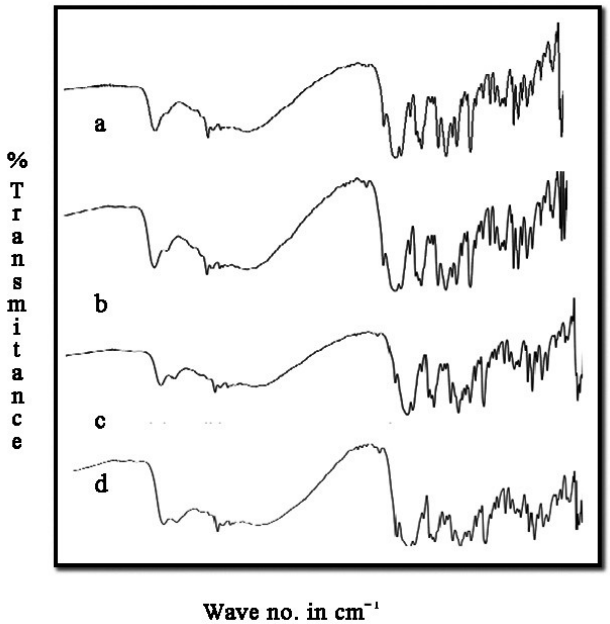

Figure 6: FTIR IR Spectrum of salicylic acid Formulations: a) PS b) PS-15 c) PS-30 d) GS-45

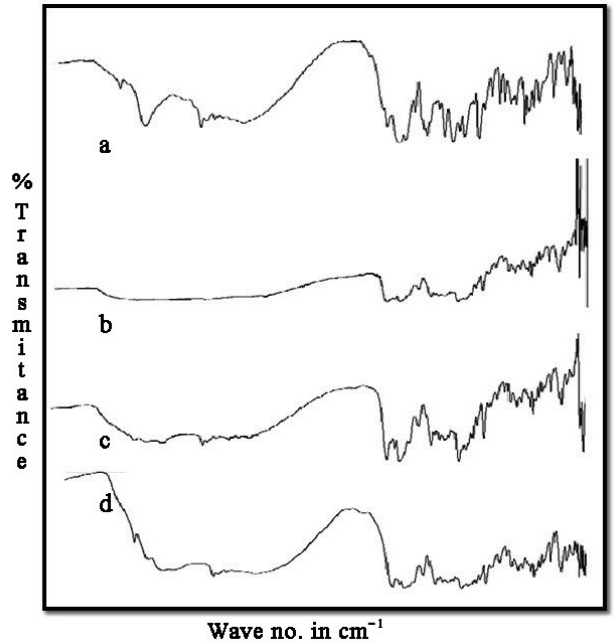

Figure 5: FTIR Spectrum of Citric acid formulations: a) PC b) PC-15 c) PC-30 d) GC45

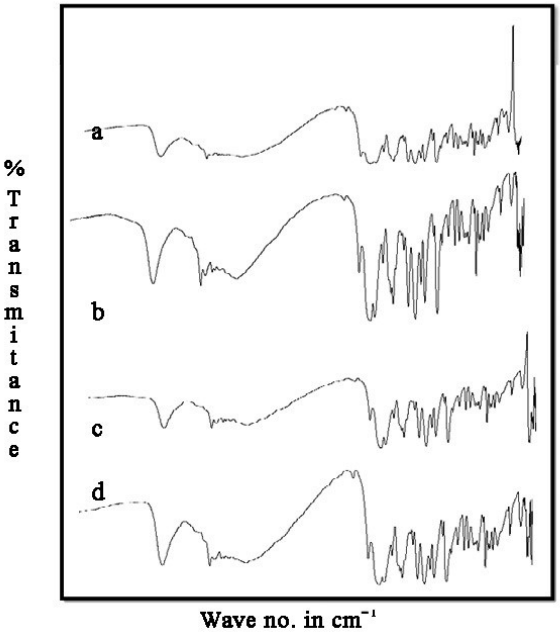

Figure 7: FTIR IR Spectrum Benzoic acid formulations: a) PB b) PB-15 c) PB-30 d) GB-45

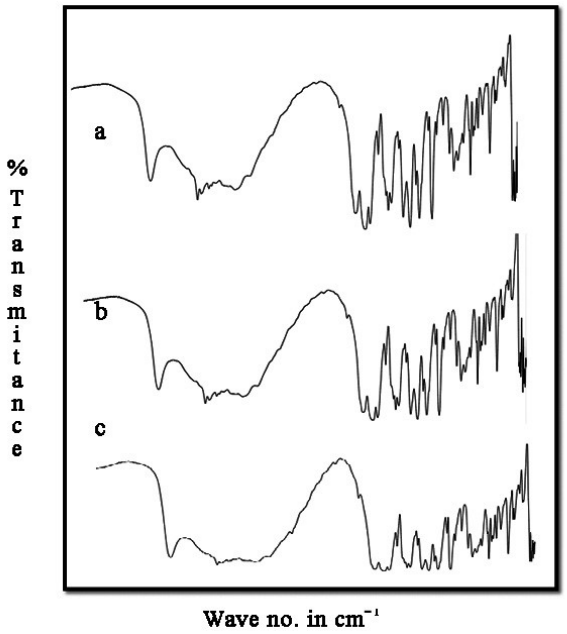

Figure 8: IR Spectrum of Succinic acid formulations: a) PU-15 c) PU-30 d) GU-45

steps. Cogrinding after 45 minutes showed sharp and intense peak in between 2500-3000 cm^{-1} due to possible hydrogen bonding. In Solvent drop cogrinding method there might be a possibility of solvate formation. Still, results of IR spectroscopy shown in Figure 8 suggest that after 45 min cogrinding intermolecular hydrogen bonding was increased as peaks of 2500-3000cm-1 shifted more to lower side due to strong hydrogen bond formation. These results of IR were supported by dissolution enhancement in salicylic acid cocrystal by the solvent drop cogrinding method. FTIR strongly reveals the possibility of cocrystal formation.

Differential Scanning Calorimetry (DSC)

Albendazole showed a melting endotherm at 209.5ºC. As shown in Figure 9, after a physical mixture of Citric acid and albendazole sharp endothermic peak was observed at 153ºC which may be due to degradation of citric acid molecule (involved in dimer formation) surrounding the albendazole molecule[21], second broad peak was observed it was of melting of albendazole in presence of citric acid. DSC of cogrinding showed endothermic peak at 167.40ºC (melting of formed new phases) and second peak at 209.20 this indicates the melting endotherms of albendazole. In case of cogrinding with salicylic acid similar phenomenon but shifting of melting endotherm to 187°C indicates the reduction in crystallinity of albendazole as shown in Fgure 10. Cogrinding with benzoic acid and succinic acid complex peaks were observed above 200°C, which may lead to the formation of a structure having high lattice energy that results in high energy to removal of molecule from its crystal lattice which ultimately affects dissolution rate.[23] Thus these results of DSC were supported by results of the dissolution study. The entire DSC spectrum's showed more than one endotherms so we can conclude that it's very difficult to say formed material is a cocrystal it may be reduction in crystallanity of albendazole

Powder X-Ray Diffraction (P-XRD)

The XRD pattern of prepared formulations exhibited a reduction in both number and intensity of peaks compared to albendazole at the specific angles indicating the decrease in

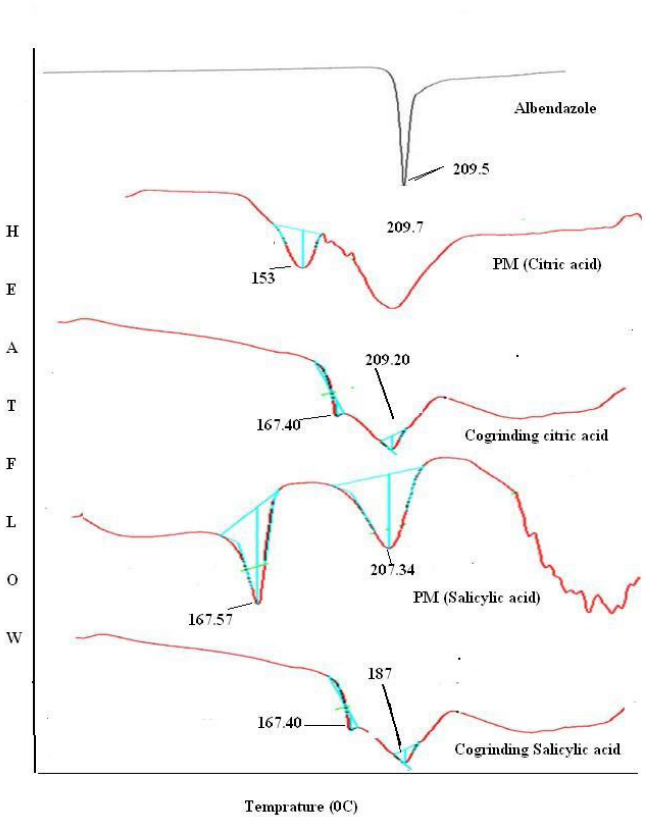

Figure 9: Differential Scanning Colorimetry (DSC) of formulations with citric acid and salicylic acid

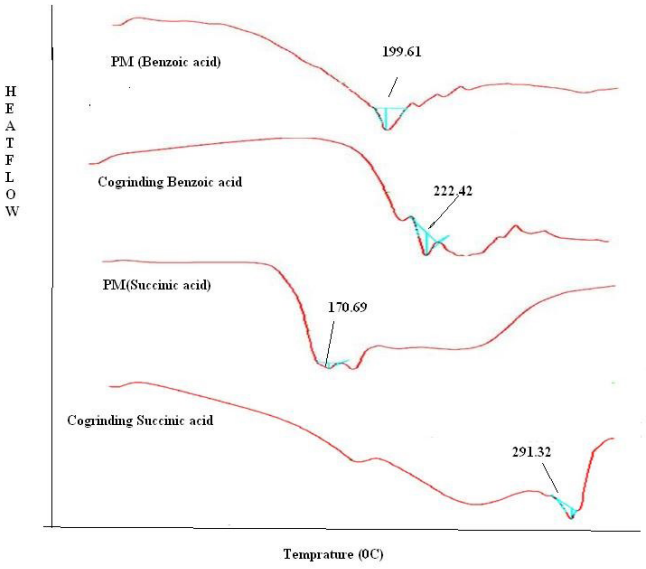

Figure 10: Differential Scanning Colorimetry (DSC) of formulations with benzoic acid and succinic acid

crystallinity or partial amorphization of the drug. The relative degree of crystallinity (RDC) of Albendazole in prepared formulations was calculated according to the equation RDC= Isam/ Iref, whereas Isam is the peak height of the sample under investigation and Iref is the peak height at the same angle for the reference with the highest intensity.RDC was calculated for each formulation. In case of Albendazole possibility of amorphization is less in 1:1 stoichiometric ratios theoretically. Specific electrostatic interactions with accurate positioning of the acidic or basic functional groups of the Albendazole (H-bond acceptor host) with guests (dicarboxylic acids) near regions of negative or positive charge density in the host will shift the pKa on account of electrostatic repulsions or attractions.[24-25] PXRPD data shows that the physical mixing of the API and carboxylic acid has resulted in the formation of physical mixtures with varying amounts of amorphous and crystalline character. The albendazole appears to be primarily amorphous, while some amount of the carboxylic acids is retained in a crystalline state, as shown in Figure 11. Hence practical results confirm that the possibility of formation cocrystals is very less and amorphous albendazole greater also it doesn't fulfil the rule for Pka difference to form cocrystals.[26]

In-Vitro Antifungal Activity

From the results of In-vitro anthelmintic activity, it was found that time required to paralyze and death of the earthworm by pure albendazole was more (45 min) as compared to formulations 20-30 minutes concentration-dependent. Thus Complexation increases solubility as well as the permeability of ABZ. These results suggest that pharmacodynamics of drug was improved due to the formation of amorphous albendazole as inhibition of roundworm ß-tubulin, inhibits polymerization,

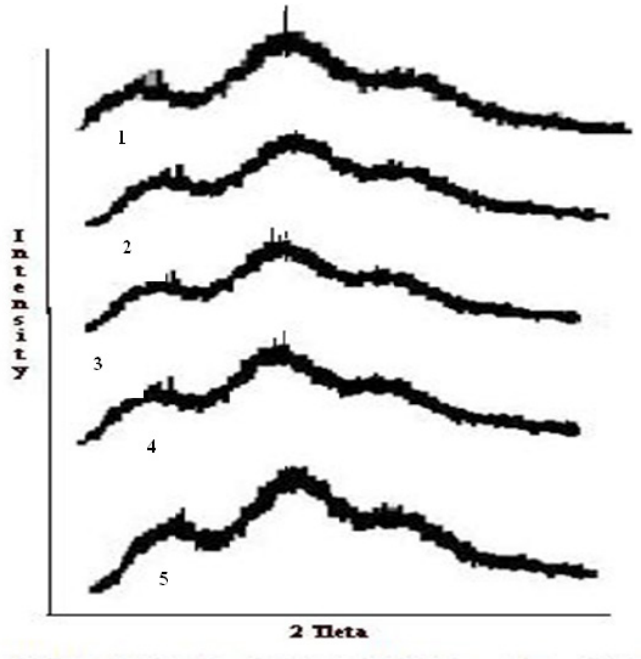

Figure 11: PXRD Analysis PXRD of 1) Albendazole 2) Cogrinding with citric acid 3) Solvent assisted grinding with citric acid 4) Cogrinding with salicylic acid 5) solvent drop grinding with salicylic acid

thus preventing the formation of microtubules and so stops cell division. The loss of the cytoplasmic microtubule leads to impaired uptake of glucose by the roundworm and depletes their glycogen leads to paralysis of worm Degenerative changes in the endoplasmic reticulum, the mitochondria of the germinal layer, and the subsequent release of lysosomes result in decreased production of adenosine triphosphate (ATP), which is the energy required for the survival of the helminthic.[27-29] Due to diminished energy production, the parasite is immobilized and eventually dies.

In the case of *in-vitro* antifungal activity it was observed that the diameter of zone of inhibition was more for cogrind mixture as compared to pure albendazole and at lower concentrations (This happens because coformers used for molecular complexation have antifungal activity as well as they enhance diffusion of formulation on the surface of media, which indirectly inhibits the growth of fungal species. Another interesting reason for lower MIC of amorphous mixtures was the structural features of Albendazole assist in reduction of growth of fungal species. Presence of imidazole nitrogen at third position in Albendazole structure binds to heme protein (Fe) of Cyt-P-450. Azole derivatives act by inhihibits 14-α lanosterol demethylase encoded by the ERGp-11 gene. They inhibit the cytochrome P450 14- α lanosterol demethylase, encoded by the ERG11 gene (also known as *CYP51*), which is the rate-limiting step of the ergosterol biosynthetic pathway. Inhibition of Erg11p depletes the membranes of ergosterol and results in the increase of toxic sterol pathway intermediates, which inhibit growth.[30]

Stability Studies of cogrind Mixtures

A result of stability studies indicates that amorphous mixtures were stable at room temperature. Drug content was uniform among all the formulations after preparation and after storage for 45 days (table 3). Cogrind mixtures were visually observed for color, odo,r and appearance also. Noney considerable change was observed in previous results.

CONCLUSION

In present investigation difference in theoretical predictions and practical results revealed by PXRD. Theoretical screening is needed to be supported by practical results. The present investigation makes stable amorphous solids of poorly soluble compounds by physically mixing API with a suitable coformer

Table 3: Stability studies of amorphous mixtures

Sr.No.	*Formulation code*	*% Drug Content*
1	GCA	97.12±0.6
2	GSA	97.98±0.5
3	GBA	97.82±0.8
4	GSU	97.96±0.45
5	SDC	97.96±0.16
6	SDSA	97.12±0.25
7	SDBA	94.18±0.98
8	SDSU	92.08±0.2

G: Cogrinding S: Solvent assisted cogrinding, CA: Citric acid, SA: Salicylic acid, BA: Benzoic acid, SU: Succinic acid

that does not form a cocrystal. Saturation solubility, dissolution rate was improved by cogrinding method than solvent drop cogrinding. The results of in-vitro anthelmintic activity and in-vitro antifungal activity revealed that cogrinding improves pharmacodynamic performance of albendazole.

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REFERENCES

1. Dil, Li. and Paul, V.F, Takashi M. (2012). Bridging solubility between drug discovery and development. *Drug Discovery Today,* 7(9), 86-495.
2. Yadav, A.V, Shete, A. S, Dabake, A.P, Kulkarni, P.V and Sakhare, S.S. (2009). Co-Crystals: A Novel Approach to Modify Physicochemical Properties of Active Pharmaceutical Ingredients. *Indian J Pharm Sci* , 71 (4), 359-370.
3. Yadav, A.V, Shete, A.S and Dabake, A.P. (2010). Crystal engineering an approach to modify physicochemical characteristics of Mefloquine Hydrochloride. *Drug Dev Ind Pharm,* 36 (09), 1036- 1045.
4. Sekhon, B.S. (2009). Pharmaceutical co-crystals- a review. *ARS Pharmaceutica*, 50(3), 99-117.
5. Silvina, G.C, Sergio, S.B, Carlos, E.L. and Daniel, A.A. (2010). Improved Albendazole Dissolution Rate in Pluronic-188 Solid dispersions. *AAPS pharmascitech,* 11(4), 1518-25.
6. Evrard, B, Chiap, P and Detullio, P. (2002). Oral bioavailability in sheep of albendazole From a solution containing hydroxypropyl β-cyclodextrin. *J Control Release*, 85(1–3), 45-50.
7. Vogt, M, Kunath, K and Deessman, J.B. (2008). Dissolution improvement of four poorly water soluble drugs by cogrinding with commonly used excipients. *Eur J Pharm Biopharm*, 68(2), 330–337.
8. Torrado, S, Torrado, J.J and Cardoniga, R, (1996), Preparation, dissolution and characterization of albendazole solid dispersions. *Int J Pharm,* 140,247–250.
9. Palomares, AF., Jung, CH., Pérez, VJ., Piliado, JC., Rodríguez, MS. and Palencia, HG. (2009). Synthesis and in vitro cysticidal activity of new benzimidazole derivatives. *Eur J Med Chem ,* 44(4),1794–1800.
10. Mohammada, A.A and Velagaa, SP. (2011) Hansen solubility parameter as a tool to predict cocrystal formation. *Int J Pharm*, 407, 63–71.
11. Sinko, PJ. (2010). Editor. Martin's Physical Pharmacy and Pharmaceutical Sciences, Baltimore USA: Lippincott Williams & Wilkins;
12. Baskar, L and Papiya, MM. (2011). In -Vitro Anthelmintic Activity of Some 1-Substituted Imidazole Derivatives**.** *Acta Parasitological Globalis*, 2 (1),01-05
13. Aswar, M, Aswar, U, Watkar, B and Vyas, M. (2008). Anthelmintic activity of *Ficus benghalensis*. *International Journal of Green Pharmacy,* 170-172.
14. Setia, A, Goyal, S and Shrivastava, B. (2011). In Vitro Antifungal Evaluation of Albendazole Solid Dispersion. *Asian Journal of Biochemical and Pharmaceutical Research*, 4(1), 164-68.
15. Sathesh Babu, P.R. and Subrahmanyam, CVS. (2008). Solubility Behaviour of Rofecoxib in Individual Solvents using the Concept of Partial Solubility Parameters. *Ind J pharm Edu Res* 42(2), 161-69.
16. Sathesh Babu, P.R, Subrahmanyam, C.V.S, Thimmasetty, J and Manavalan, R. (2007). Extended Hansen's Solubility Approach: Meloxicam in Individual Solvents. J Pharm Sci 20 (4), 311-16.
17. Zdemir, C.O. and Guner, A. (2007). Solubility profiles of poly(ethylene glycol)/solvent systems I: Qualitative comparison of solubility parameter approaches .*Eur Polym J* , 43, 3068–3093.
18. Segarceanua, O. and Lecab, M. (1997). Improved method to calculate Hansen solubility parameters of a polymer. *Progress in Organic Coatings*, 31, 307–310.
19. Ning, Q, Mingzhong, L, Walkiria, S and Nazneen, M.D. (2011). Pharmaceutical cocrystals: An overview. *Int J Pharm*, 419 (1-2), 1– 11.
20. Po-Chun, H, Hong-Liang, L, Shun-Li, W. and Shan-Yang, L. (2012). Solid-state thermal behaviour and stability studies of theophylline–citric acid cocrystals prepared by neat cogrinding or thermal treatment. *J Solid State Chem* , 192, 238:245.
21. Takkaki, M, Yasuo Yshi, *et al.* (2012). Cocrystallization and amorphnisazation induces by drug – excipient interaction improves the physical properties of acyclovir. *Int J Pharm* (422)160-69.
22. Chatwal, G.R. and Sham, K. A. Instrumental methods of chemical analysis. Nagpur: Himalaya publishing house; 2008.
23. Aulton, ME. (2007). The Science of Dosage Form design. China: Churchill Livingstone Elsevier.
24. Ghosh, I and Werner, M.N. (2012). The strategic use of supramolecular pKa shifts to enhance the bioavailability of drugs. *Adv Drug Deli Rev*, 64,764–783.
25. Kalaiselvan, R, Mohanta, G.P. and Manna, P.K. (2006). Studies on mechanism of enhanced dissolution of Albendazole solid dispersion with crystalline carrier. *Ind J Pharm Sci,*68, 599-07
26. David, P. and Eldera, R.H. (2012). Use of pharmaceutical salts and co crystals to address the issue of poor solubility. *Int J Pharm*, 453 (1), 88-100.
27. Lekme, T.L. and Williams, D.A, (2013). editors, Foye's Principles of Medicinal Chemistry. New Delhi: Wolters Kluwer.
28. Richard, D.C., Erwin, L. and Ann, R.H, (2009). Efflux-Mediated Antifungal Drug Resistance *Clinical microbiology reviews*, 22(2), 291–211
29. Andrew, V, Trask, W.D, Samuel, M. and William, J. (2004). Solvent-drop grinding: green polymorph control of crystallisation. *Chem Commun (Camb)*, 7(7), 890– 891.
30. Santiago, T. (1996). Formulation parameters of Albendazole solution. Int J Phar, 140, 45-50.