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Development and Comparative Assessment of Crystal Engineered Pantoprazole with Improved Gastric Acid Stability

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Abstract: ***The majority of proton pump inhibitor (PPI) drugs are susceptible to gastric acid degradation and are therefore available as delayed-release enteric products. In this study, Co-crystals (CCRs) and Crystallo-co-agglomerates (CCAs) of Pantoprazole (PNZ) were prepared and compared for the improvement of micromeritic and physicochemical properties with special emphasis on gastric acid stability. A gastric acid stability study was carried out using HPLC method by using 0.1N HCl. New absorption bands were observed in the CCRs batches in FT-IR spectrum which supports the possibility of the formation of the hydrogen bond between PNZ and co-formers. SEM and XRD confirmed the transformation of PNZ to the crystalline form. DSC data showed two endothermic peaks corresponding to the polymorphic forms I and II of PBC and NCT, respectively. The CCAs batch B-1(PNZ-PBC) and B-2 (PNZ-DCP) showed the acid degradation of PNZ up to 95% in simulated gastric fluid. Whereas, CCRs batch A-1 shows the highest acid stability of up to 81%. Thus, it was concluded that the mere development of agglomerates with alkaline material will not able to avoid the gastric degradation of the drug. However, being new solid-state entity, CCRs of PNZ can be an effective strategy for improving the acid stability of the gastric acid-labile drugs.***

Keywords: Pantoprazole, Gastric acid stability, Crystal engineering, Co-crystals

1. Introduction

Crystal engineering is a technique used for creating new structures in drug molecules through intermolecular interactions. Intermolecular interactions of either neutral or ionic building blocks have been used as a design strategy in crystal engineering techniques. It originated from the field of organic chemistry and physical chemistry [1], this method has been explored in the pharmaceutical crystal engineering to exploit the non-covalent intermolecular interactions, to generate new solid forms with tailored crystal packing to improve functional characteristics. In the pharmaceutical field, several crystal engineering techniques have been reported, which aim to modulate the primary properties like particle shape, size, crystal form, density, porosity, etc. It can potentially be applied to improve the mechanical properties of powder mass, like flowability, compressibility, consolidation, etc [2]. Besides, it was reported for the improvement of functional properties like solubility, dissolution, and bioavailability of modified drugs [3]. There are various methods involved in crystal engineering, of which co-crystallisation and crystal-co-agglomeration are well-known techniques for changing the physical state of APIs for the improvement of various micromeritic, solubility, dissolution properties, photostability, thermal stability and also the chemical stability [4,5]. The majority of PPIs are weakly basic and reported to be acid-labile. They tend to be rapidly degraded in the stomach, usually within a few minutes, because of the acidic environment of the stomach. Delayed-release enteric-coated products are available in the market for all PPI category drugs. The drug can be protected from gastric acid degradation by enteric coating. However, delays in the absorption and hence peak plasma concentration (Cmax) have been reported in the literature. Following the oral administration of these products, Cmax was not found to be attained for a considerable period [6]. Also, patients taking enteric-coated, delayed-release PPIs may experience nocturnal gastric acidity or nocturnal acid breakthroughs along with nighttime symptoms of heartburn in some cases [7].

Buffer tablets or some PPI granules with sodium bicarbonate coating are reported to have some drawbacks such as limited shelf life, systemic alkalosis and contraindicated for the patient on a restricted sodium diet [8]. Hence, it was a long-awaited need to prepare solid PPI formulations with comparable stability in a gastric environment. Co-crystallisation by using suitable co-formers has been reported for the improvement of chemical stability [9]. To check the possibility and suitability of improving gastric acid stability of the PPI drug, PNZ, the crystal engineering technique was used in this study. CCRs and CCAs of PNZ were prepared by using suitable co-formers and compared for the physicochemical and mechanical properties with special emphasis on gastric acid stability.

2. Materials and Methods

Pantoprazole was obtained as a gift sample from Enaltech Lab, Mumbai. Nicotinamide, Potassium Bicarbonate, Dibasic Calcium Phosphate, Ethanol and Dichloromethane (DCM) were purchased from S. D. Fine Chem, Mumbai. All the solvents used were of analytical grade.

2.1 Selection of co-formers

Co-former selection is the most crucial step for CCR design. Physicochemical properties and in-vivo performance of

obtained CCRs are dependent on the co-formers used [10-12]. A variety of co-formers like pharmaceutical excipients, food additives, and generally regarded as safe GRAS-listed APIs can be utilised for the preparation of CCRs [13,14]. Intermolecular bonding and molecular arrangement in the crystal lattice are dependent on the drug-co-formers interaction. NCT is a well-known co-former as it has an amide group capable of forming hydrogen bonds, and the carbonyl group can establish ion-induced dipole interactions with S=O and C=O of PNZ, respectively. Alkaline material like PBC may increase the micro-environment pH, thereby saving the drug from acidic degradation, thus, it was used as a co-former.

2.2 Phase solubility study

Phase solubility analysis has been reported to be used for quantitatively determining the molecular interactions of system components by the application of solubility measurements. To depict the stoichiometric modalities of co-former and drug in CCR formation, a phase solubility study was conducted by the Higuchi and Connors method. A solvent mixture of ethanol and distilled water in the ratio of 3:7 was used for the determination of the solubility of the drug with increasing molar concentration of co-formers. PNZ was added in excess quantity to a 20 ml solvent mixture of ethanol: distilled water (3:7) that contains an incremental molar concentration of co-former (0–0.01 M). This dispersion was then allowed to equilibrate by shaking for 48 hrs using a rotary orbital shaker at 37±2 °C. Samples were then subjected to centrifugation at 3000 rpm for 10 min and filtered through a 0.45µm cellulose acetate membrane filter. The concentration of PNZ in each sample was determined by using UV spectrophotometry at 300 nm. Phase solubility diagrams were constructed to assess the stoichiometry of CCR formation [15].

2.3 Preparation of PNZ – PBC CCRs:

PNZ CCRs were prepared by taking equimolar (1:1 molar ratio) quantities of the drug and PBC as a co-former, using two solvent systems comprising ethanol and water. 3.83 gm (1M) of PNZ was dissolved in a sufficient amount of ethanol. To get a clear saturated solution, the drug dispersion was gently warmed in a water bath at about 40°C. A saturated solution of PBC was separately prepared by dissolving 1 gm in a sufficient amount of water to receive a 1M concentration. PNZ solution was added to the PBC solution in an ice bath, and the solvent was allowed to evaporate at room temperature. Obtained CCRs were filtered, dried and stored at room temperature under ambient conditions [16].

2.4 Preparation of PNZ – NCT CCRs:

PNZ CCRs were prepared by taking equimolar (1:1 molar ratio) quantities of the drug and NCT as a co-former, using two solvent systems comprising ethanol and water. 3.83 gm (1M) of PNZ was dissolved in a sufficient amount of ethanol. To get a clear saturated solution, the drug dispersion was gently warmed on a water bath maintained at about 40°C. 1.22 gm (1M) of NCT was dissolved separately in a sufficient amount of water to get the saturated solution. The drug solution was then added to the co-former solution in an ice bath, and the solvent was allowed to evaporate at room temperature. CCRs thus obtained were filtered, dried and stored at room temperature under ambient conditions [16].

2.5 Preparation of PNZ- PBC CCAs:

Three solvents system was followed for the preparation of PNZ agglomerates. It comprises ethanol as a solvent, DCM as a bridging liquid, and distilled water as a non-solvent. After dissolving PBC in a sufficient amount of distilled water, 1/3 amount of the total talc was uniformly dispersed in it. Talc, being physiologically inert and having the least adsorption capacity, was an obvious choice for the preparation of agglomerates [17,18]. Besides, it was used as a substrate for size enlargement based on its hydrophobicity and ability to easily undergo wetting with bridging liquid. It helps the uniform distribution of HPMC and is reported to decrease crystallinity, and assists particle deformation under pressure. A clear solution of PNZ was obtained by dissolving it in a sufficient amount of ethanol at 40°C. The remaining 2/3 amount of talc was dispersed with continuous stirring for about 20 min. PNZ – Talc dispersion was then added to the PBC – Talc dispersion with constant stirring at 600 rpm. Finally, DCM was added drop wise while stirring was continued for 20 min to obtain agglomerates. Then obtained agglomerates were filtered, dried and stored under ambient conditions [19].

2.6 Preparation of PNZ – DCP CCAs:

A similar set of solvents was used for the preparation of PNZ agglomerates with DCP which were used for the preparation of PNZ- PBC CCAs. DCP and 1/3 amount of total talc were uniformly dispersed in distilled water with constant stirring. A clear solution of PNZ was obtained by dissolving it in a sufficient amount of ethanol at 40°C. Remaining 2/3 amount of talc was dispersed in this PNZ solution with constant stirring at 20 min. PNZ – Talc dispersion was then added to the DCP – Talc dispersion with constant stirring at 600 rpm. Finally, DCM was added dropwise while stirring was continued for 20 min to obtain agglomerates. Then obtained agglomerates were filtered, dried and stored under ambient conditions [19]. All the formulations of CCRs and CCAs are tabulated in Table 1.

Table 1: Formulation of different batches of CCRs and CCAs of PNZ

Sr. No.	The material used/ Batches	A-1 CCRs	A-2 CCRs	B-1 CCAs	B-2 CCAs
1	PNZ	3.83 gm	3.83 gm	1.00 gm	1.00 gm
2	PBC	1.00 gm	--	1.00 gm	--
3	NCT	--	1.22 gm	--	--
4	DCP	--	--	--	1.00 gm
5	Talc	--	--	0.15 gm	0.15 gm
6	DCM	--	--	2 ml	2 ml
7	Ethanol	q.s	q.s	q.s	q.s
8	Water	q.s	q.s	q.s	q.s

3. Evaluation of CRRs and CCAs

3.1 Saturation Solubility Study:

A saturation solubility study was done to determine the aqueous solubility of different PNZ crystals. It was performed

in distilled water as per Higuchi and Connors's method. Both CCRs and CCAs of PNZ were added to 20 ml of distilled water in excess quantity. Allowed to equilibrate in vials for 24 h by rotating on an orbital shaker at room temperature. Aliquots were filtered and analyzed spectrometrically at 300 nm. The concentration of the drug in aliquots was determined in triplicate to get saturation solubility values [20,21]. Physical mixture in 1:1 proportion of PNZ and PBC and NCT in case of CCR and physical mixture of PNZ with PBC and DCP in case of CCA were also subjected to saturation solubility. This study was conducted to check whether a change in the solubility is contributed by a new crystal entity or simply because of a physical mixture. pH was checked during each sample withdrawal to ensure whether the improvement in solubility was due to co-crystal formation or a change in pH.

3.2 Micromeritic Properties

Micromeritic properties of CCRs and CCAs were evaluated to know the effect of crystallization on the flowability and compressibility of drug, PNZ. Flow property was assessed by determining the angle of repose. Compressibility of obtained crystals was evaluated in terms of Carr's Index and Hausner's ratio, calculated based on practically determined values of bulk and tap densities [22,23].

3.3 Compressibility of Pure PNZ and Crystal Forms

To develop a solid dosage form good compactibility and compressibility are very important perquisites. These attributes of CCRs and CCAs were evaluated by powder compaction analysis and compared with pure drugs. All samples of crystals and drugs were passed through a 60-mesh sieve to minimize the particle size difference. Approximately, 40 mg quantity of crystals and drugs were compressed at a variable pressure range of 4.9-15.4 kN in a tablet compression machine (Karnavati Mini Press model). The diameter, thickness and hardness of the prepared tablets were measured in triplicate and compared to know the effect of crystal formation on the compressibility of the drug [24-26].

3.4 Production yield (%):

Production yield was calculated as weight per cent of practical mass i.e. dried crystals and the theoretical mass in terms of the amount of drug and co-former used for the preparation of crystals.

$$\text{Production yield \%} = \frac{\text{Practical mass (Crystals)}}{\text{Theoretical mass (Co} - \text{former} + \text{Drug)}} \times 100$$

3.5 Drug content determination of crystal forms

10 mg of PNZ crystals were accurately weighed, crushed and transferred to a 10 ml volumetric flask. It was dissolved in 10 ml of ethanol and further diluted to get 100 µg/ml solutions of crystals, serving as a test solution. A similar procedure was followed for the preparation of the PNZ standard solution. Both test and standard solutions were analyzed spectrophotometrically by reading the absorbance at 300 nm using a UV-VIS spectrophotometer [27].

3.6 Fourier Transformation Infrared Spectroscopy (FT-IR)

To check the compatibility of co-formers like PBC, NCT, and DCP with PNZ, FT-IR was conducted. It also helps to check the possibilities of co-formers forming bonds with the drug during crystallization, particularly in the case of CCRs. FT-IR spectrums were taken on the Shimadzu IR Affinity 1S model spectrometer. Samples were prepared by mixing and dispersing them with KBr in a 1:9 ratio. The powder dispersion was placed in the DRS assembly and the spectrum was recorded in the scanning range 4000 to 500 cm^{-1} [28].

3.7 Scanning Electron Micrographs (SEM)

Surface morphology of CCRs and CCAs of PNZ was performed by using a Hitachi Tabletop microscope TM-1000 with an electron beam voltage of 15 kV. The dried sample was fixed on aluminum stubs using double-sided copper tape and coated with gold palladium. Obtained images of PNZ pure drug and crystals were examined at x100, x500, x1000, and x2000 magnifications

3.8 Differential Scanning Calorimetry (DSC)

A differential scanning calorimeter was used for the thermal analysis of PNZ and its crystals. Samples of PNZ CCRs and CCA (1-3 mg) were crimped in an aluminum pan and analyzed from 20 to 250°C with a heating rate of 10 °C/min. Nitrogen was purged at a flow rate of 50 ml/min to maintain an inert atmosphere during thermal scanning.

3.9 X-ray Diffraction of Powder (XRPD)

X-ray powder diffraction (XRPD) measurements of PNZ and its crystal forms were analyzed on a powder diffractometer. 0.7 mm glass capillaries were filled with samples and mounted on a goniometer head. These data were collected using CuK-α radiation (=1.5418A˚) and tube voltage and current of 30kV and 30mA, respectively. The scanning parameter range was 2-40° angle at a continuous scanning rate of 3 deg/min. The monochromator slit was set to 2 mm.

3.10 Dissolution study of crystal forms

In-vitro drug dissolution studies of crystal forms of PNZ were conducted by using USP Type I, eight-stage dissolution test apparatus. It was operated at 50 rpm, in 900 ml of phosphate buffer (PBS) pH 6.8 maintained at 37° ± 0.5°C. The amount of crystals equivalent to 40 mg of PNZ was placed and tied in a muslin cloth and kept in a dissolution basket. Samples were withdrawn at regular time intervals and replaced with fresh dissolution medium. The samples were filtered through a 0.45 µm membrane filter, suitably diluted and immediately analyzed spectrometrically at 300nm.

3.11 Acid stability study of pure PNZ and PNZ crystals by HPLC method:

3.11.1. Preparation of standard stock solution

A stock solution of 1mg/ml was prepared by dissolving an accurately weighed 25 mg of PNZ in 25 ml of ethanol and filtered through 0.45 µm Teflon membrane [29].

3.11.2. Chromatographic conditions

C_{18} (Grace) 5µm, 4.6×250 mm HPLC cartridge column was used as a stationary phase. A mixture of 0.05 M potassium dihydrogen phosphate, ethanol and acetonitrile in the ratio of 5:3:2 (v/v/v) was used as a mobile phase. The pH was adjusted to 7.0±0.2 with 1.0 M potassium hydroxide and filtered through a 0.45mm Teflon membrane filter. The flow rate was adjusted to 1.5 ml/min with an injection volume of 20µl. The separation was monitored at a wavelength of 280 nm [29,30].

3.11.3 Preparation of the acid degradation product of drug and crystal forms

For the intentional degradation of PNZ and its crystals, 20 mg of PNZ and weight equivalent to 20 mg of crystals were weighed accurately and transferred into a 50 ml conical flask. 25 ml of 0.1 M hydrochloric acid was added to it and this solution was set aside at room temperature (25 ± 2^0C) for 90 min. It was then neutralized with 0.1 M sodium hydroxide solution, then quantitatively transferred into a 100 ml volumetric flask and completed to 100 ml with methanol. The drug degradation was calculated by using the formula;

1) $$\% \text{ Area retained(AR)} = \frac{\text{Area of test}}{\text{Area of standard}} \times 100$$

2) % Drug degradation = 100 - % AR

4. Results

4.1 Phase Solubility Study

The phase solubility curves of PNZ-PBC and PNZ-NCT are presented in Fig. 1 (a) and Fig. 1 (b). The curves show that the aqueous solubility of PNZ was increased linearly as a function of concentration PBC and NCT respectively. So, these phase solubility curves can be classified as A_L type according to Higuchi and Connors. This indicates an increase in the solubility of PNZ in these systems due to one or more molecular interactions between PNZ and co-formers to form distinct chemical species which may be called complexes. In this light, the type curve indicates the formation of soluble complexes between PNZ and co-formers, thereby increasing the total amount of PNZ in the solution. Graphical data from phase solubility studies show the linear correlation coefficient R^2=0.986 with slope (m) of 0.145 and R^2=0.9951 with slope (m) of 0.1625 suggesting the stoichiometry of a 1:1 molecular complexes formation. Thus, it can be concluded that coformers were suitable to form stable CCRs in both cases [15].

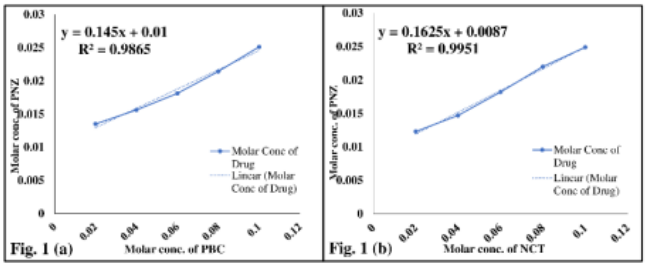


Figure 1: Fig. 1 (a): Phase solubility diagram of PNZ in the presence of PBC & Fig. 1 (b): Phase solubility diagram of PNZ in the presence of NCT

4.2 Saturation solubility study:

Pure PNZ shows 2.62± 0.16 mg/ml aqueous solubility. CCRs and CCAs showed significant improvement in the aqueous solubility of PNZ due to the formation of the crystal form of the drug which causes the improvement in the wetting time of the drug (Table 2 & Fig. 2). Solubility as a function of the wettability of the drug remarkably changes with changes in the crystallinity and crystal size. Due to enhancement in surface area and wettability improved aqueous solubility of PNZ was observed by the formation of CCRs and CCA [31]. pH was measured during the final sampling of saturated solution and was found to be on the higher side for CCAs as compared to CCRs. This indicates that alkaline substrate is only weakly bound in agglomerates whereas co-formers are chemically bound in the case of co-crystals. More importantly, solubility wasn't found to be influenced by the change in pH but it was increased because of the formation of new chemical entities as in the case of CCRs. This also negates the possibility of solubility enhancement by raised pH as evident from the higher values of pH and lower values of solubility in the case of CCAs.

Table 2: Saturation solubility of PNZ and its crystal forms

Sr. No	Crystal Types	Formulation code	Saturation Solubility (mg/ml)	pH of the solution at sampling	Folds Increase in Solubility
1		Pure drug	2.62 ± 0.16	--	--
2	CCRs	A-1 (PNZ-PBC)	13.58 ± 0.5	6.8 ± 0.01	5.18
3		A-2 (PNZ-NCT)	12.51 ± 0.37	6.2 ± 0.01	4.77
4	CCAs	B-1 (PNZ-PBC)	7.13 ± 0.22	8.2 ± 0.01	2.72
5		B-2 (PNZ-DCP)	4.49 ± 0.53	7.4 ± 0.01	1.71

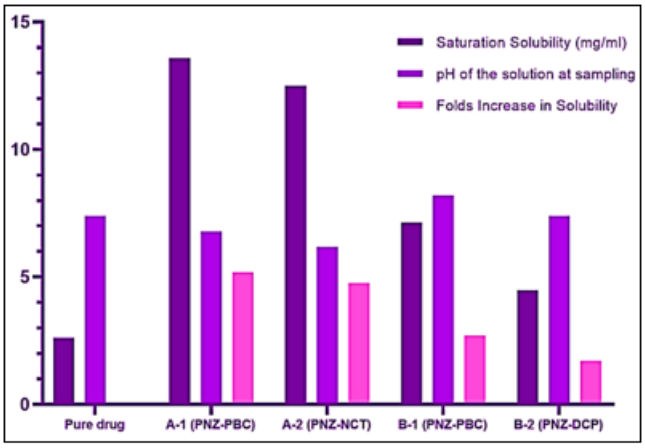


Figure 2: Saturation solubility of PNZ and its crystal forms

4.2 Micromeritic Properties Study

Results of micromeritic properties of pure drugs, CCRs and CCAs have been tabulated in Table 3. The angle of repose in the range of 23.05^0 - 37.17^0 for crystals is indicative of improved flow ability as compared to the pure drug. Higher values of the angle of repose for pure drug, PNZ, may be because of its fluffy nature. Lower values of Hausner's ratio and Carr's index suggested the improved compressibility of the drug when converted to crystals. The improved flowability and compressibility of crystal forms can be attributed to the increase in the sphericity of crystals [32].

Table 3: Micromeritic study of PNZ and its crystal forms

Sr. No.	Crystal Type	Formulation code	Angle of repose (θ°)	Hausner's ratioa	Compressi-bility index
1		Pure drug	44.57 ± 0.11	1.45 ± 0.06	38.03± 0.11
2	CCRs	A-1 (PNZ-PBC)	26.35 ± 0.15	1.13 ± 0.05	16.69± 0.1
3		A-2 (PNZ-NCT)	23.05 ± 0.59	1.15 ± 0.05	18.34± 0.065
4	CCAs	B-1 (PNZ-PBC)	31.26± 0.13	1.2± 0.11	22.02± 0.12
5		B-2 (PNZ-DCP)	37.17 ± 0.1	1.27± 0.13	24.9± 0.15

([a]Mean ±SD, n=3.)

4.3 Compressibility of pure PNZ and PNZ crystal forms

The results of the compressibility study parameters for tablets of pure drug PNZ and its crystals have been given in Table 4. When compressed without directly compressible diluents under constant pressure, tablets prepared from crystals were found to have higher hardness values than the compared drugs. Higher values of hardness are indicative of more densification of the compacts, eventually leading to mechanically stable tablets. This ensures well-handling characteristics and good compressibility of tablets produced from crystals. Tablets prepared from the CCRs were found to have a shiny surface as compared to tablets of CCAs, which appear dull (Fig. 3). Improved hardness of compacts may be attributed to the alteration of crystal face and better packing of crystals than the powder mass. The stacked and repetitious molecular arrangement in crystal lattice is likely to improve the compression properties as observed in the case of crystals compared to pure drugs [33,34].

Table 4: Compressibility study of PNZ and its crystal forms

Sr. No.	Crystal Type	Formulation code	Compression pressure (without directly compressible vehicle) (kN)	Thickness[a] (mm)	Hardness[a] (Kg/cm2)
1		Pure PNZ	4.9-15	1 ± 0.1	1.8± 0.13
2	CCRs	Batch A-1 (PNZ-PBC)	4.9-15	1 ± 0.1	4.1± 0.1
3	CCAs	Batch B-1 (PNZ – PBC)	4.6-15	1 ± 0.1	3.0± 0.05

([a]Mean ±SD, n=5)

Figure 3: Directly compressed tablets (without any compressible vehicle) of pure PNZ (Fig A), CCRs batch A-1(Fig B) and CCAs batch B-1(Fig C)

4.4 Production yield and drug content determination of crystal forms

The findings of production yield and drug content of CCRs and CCAs are shown in Table 5. It was observed that the production yield of both batches of CCA was found markedly greater than that of both batches of CCRs. The drug content of CCRs was found to be higher as compared to CCAs owing to the mere physical involvement of conformers with the drug.

Table 5: Results of % product yield and % drug content of CCRs and CCAs

S. No.	Crystal Type	Formulation Code	Product Yield (%w/w)	Drug Contents (%w/w)
1	CCRs	A-1 (PNZ-PBC)	77.28 ± 1.52	91.00 ±1.6
2		A-2 (PNZ-NCT)	66.21 ± 1.85	89.27 ± 0.44
3	CCAs	B-1 (PNZ-PBC)	83.84 ± 1.56	88.65% ± 1.30
4		B-2 (PNZ-DCP)	94.60 ± 1.71	74.025% ± 1.15

4.5 Fourier transformation infrared spectroscopy (FT-IR)

FT-IR spectra of CCAs and CCRs, along with pure drug PNZ, are shown in Fig. 4 (a) and Fig. 4 (b), respectively**.** CCA being a physical agglomeration, no considerable change was expected and observed in the positions of characteristic absorption bands of various functional groups present in the drug. However, in the case of CCRs, new absorption bands were observed in the formulation A-1 and A-2 at 2345.44 and 2360.87 cm^{-} respectively. The drug and co-former showed intermolecular hydrogen bonding via the formation of the N-H bond in both batches of CCRs [35]. As CCAs are just like coordination compounds, no chemical interactions occurred between drug and co-formers. Agglomeration is a mere physical association or aggregation of the crystals. Whereas, CCRs are mostly formed because of the formation of hydrogen bonds between the drug and co-formers. Both these aspects were proved by the FT-IR study of CCAs and CCRs along with the PNZ.

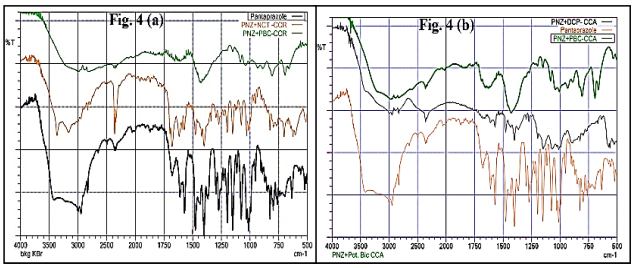


Figure 4 (a): FT- IR spectrum of PNZ and CCRs Fig. 4 (b): FT- IR spectrum of PNZ and CCAs

4.6 Scanning electron microscopy (SEM)

The surface characteristics of the PNZ and its CCRs with PBC and NCT are shown in Fig.5, 6 & 7, respectively. Drug PNZ appeared as irregularly shaped particles, which were found to be tightly bound with no signs of porosity. Cohesive forces were evident, which might be the cause of poor flow property. Particles were widely distributed in terms of their size, which ranges between 10 µm to 100 µm. This could be one of the possible reasons for the excellent flowability of the crystals. In the case of CCRs, larger particle sizes were observed that exhibit tubular and platy crystal habit. While CCAs show crystallinity and irregularly shaped agglomerates with no defined habit (Fig. 8 and Fig. 9). However, CCAs were devoid of porosity, which could be attributed to lower solubility and dissolution compared to CCRs. These findings suggested improved flow properties of PNZ crystals attributable to the

conversion of partially crystallized form to crystalline form, increased particle size and higher porosity as compared to pure PNZ.

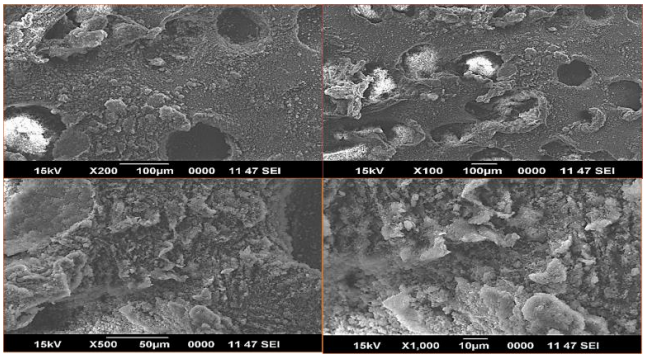


Figure 5: SEM micrographs of pure drug, PNZ

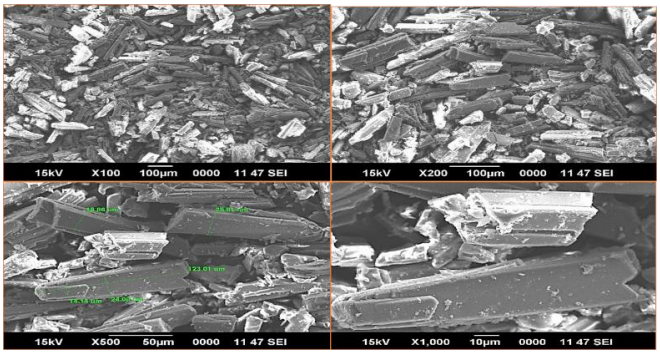


Figure 6: SEM micrographs of formulation A-1 (PNZ-PBC) CCRs

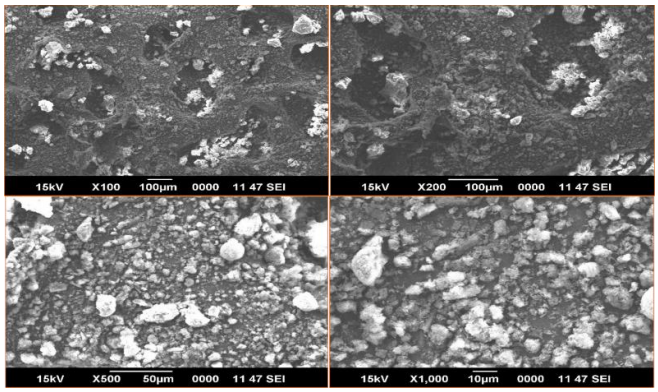


Figure 7: SEM micrographs of formulation A-2 (PNZ-NCT) CCRs

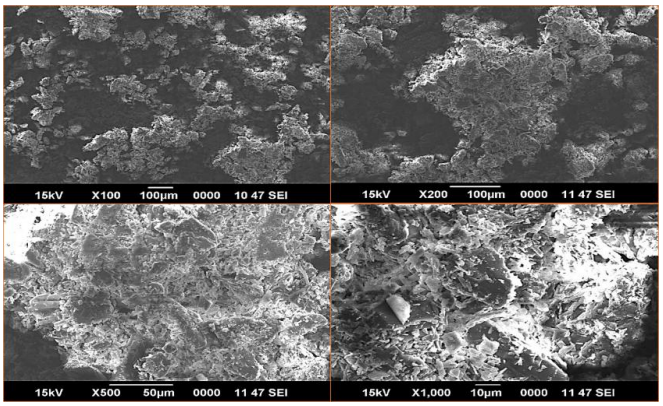


Figure 8: SEM micrographs of formulation B-1(PNZ-PBC) CCAs

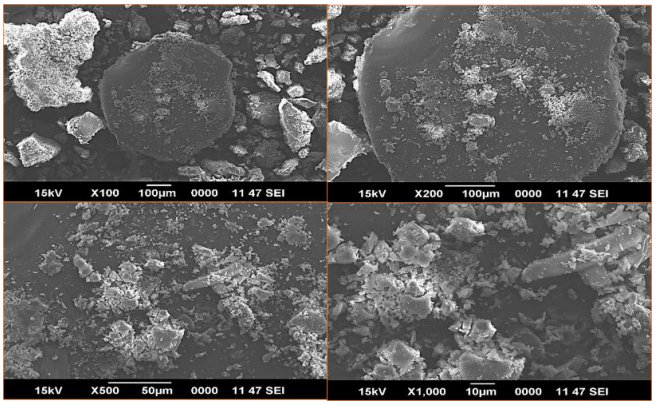


Figure 9: SEM micrographs of formulation B-2 (PNZ-DCP) CCAs

4.7 Differential scanning calorimetry (DSC)

DSC has been used for the determination of crystal forms of pharmaceutical powders. In this study, DSC was performed to determine the formation of CCRs and CCAs of PNZ, by measuring the temperature and heat flow associated with phase transitions in the material. DSC thermograms of the drug, co-formers and crystal forms are shown in Fig. 10. The pure drug, PNZ shows an endothermic peak at 138.16°C corresponding to its melting point. Thermograms of both batches of CCRs show two polymorphic forms, termed I and II. Form-I crystals show the endothermic peak at about 164.71°C and 115.92°C for PBC and NCT, respectively. Form-II crystals show the exothermic peak at about 201.87°C and 174.46°C for PBC and NCT CCRs, respectively [34]. DSC data for CCA shows that the melting endotherm at 122.01°C and 154.40°C for the CCAs of PBC and DCP, respectively. It shows an interesting change in thermograms with decreased enthalpy change as compared to the pure drug, which revealed that there is little crystallisation of PNZ in CCAs.

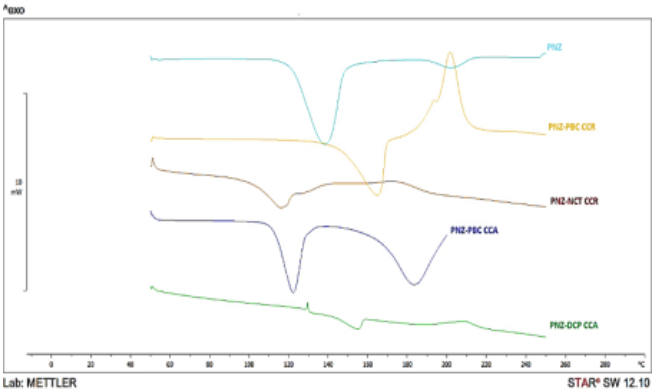


Figure 10: DSC chromatogram of PNZ and its crystal forms

4.8 X-ray powder diffractometry (XRPD):

XRPD patterns of CCRs and CCAs batches produced at 20^0C-80°C show intense crystal peaks as compared to the peaks of pure PNZ, indicating a significant improvement in the crystal content (Fig. 11). X-ray diffractogram of PNZ-PBC CCRs shows more prominent characteristic peaks of PNZ as compared to other crystal forms. Improved crystallinity provides conclusive evidence that the particles crystallized in the presence of a co-former. However, in the case of CCAs, some characteristic peaks of pure PNZ have been significantly suppressed and some newly formed peaks can be

observed over the extended range. This may be attributed to the change in the crystallinity due to the molecular interactions of conformers with pure drug forming new crystal forms and habits, as evident from SEM images [35].

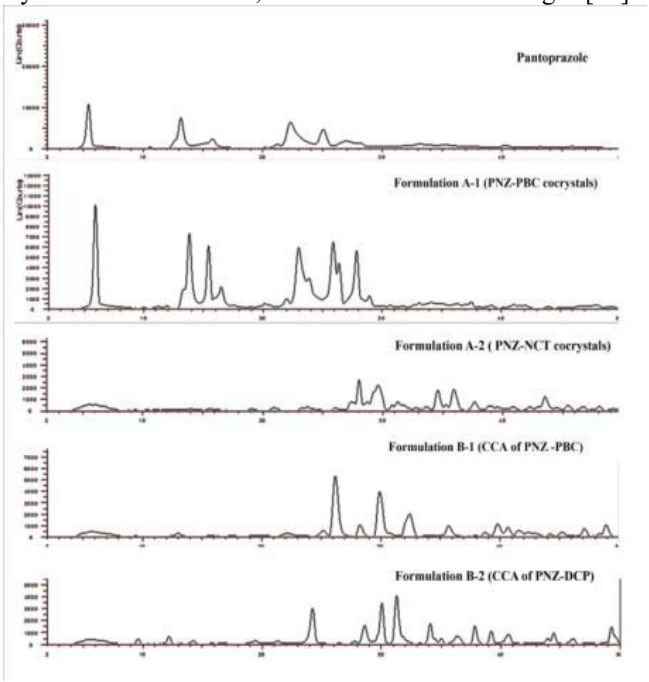


Figure 11: XRPD patterns of PNZ and its crystal forms

4.9 Dissolution study of pure PNZ and PNZ crystal forms

The dissolution profile of pure PNZ and its crystal forms in phosphate buffer pH6.8 are shown in Fig. 12. It was evident from the dissolution curves that, the PNZ crystals have improved the dissolution extent to 80%-95% as compared to pure PNZ showing only 65.23% for the period of 2 hr. This may be attributed to the change in the crystallinity pattern of the CCRs and CCAs which eventually leads to improved wettability and solubility of crystals in dissolution media. The pure drug was found to have less porosity and its fluffy nature might have hindered the wettability which could be responsible for the lower dissolution values [36].

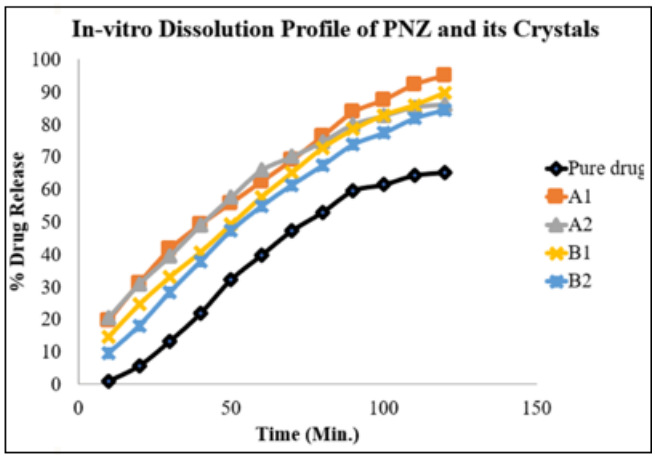
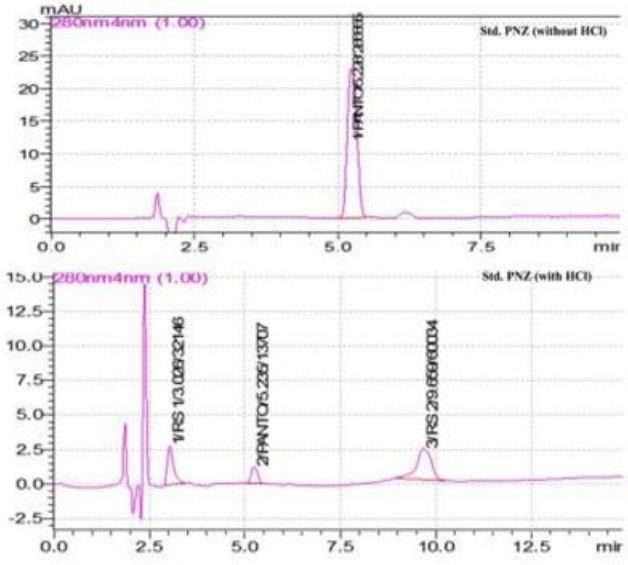


Figure 12: Comparative dissolution profile of PNZ and its crystal forms

4.10 Acid stability study of PNZ and PNZ crystals by HPLC method

The stability of the PNZ in the acid medium was studied by the HPLC method. The area of pure PNZ observed for the retention time of about 5.228 was 265855 after 90 min without treatment of HCl. So, it was considered as a 100% stability drug which was considered as a reference for further determinations of % drug stability and degradation of PNZ in 0.1 N HCl after 90 min. The HPLC chromatograms obtained from pure PNZ and different crystals are shown in Fig.13 - 14. The chromatogram of the pure PNZ shows an area of 265855 at a retention time of 5.228 min for 90 min. without acid degradation. While acid-degraded samples of pure PNZ in the presence of 0.1N HCl show the area 13707 at a retention time of 5.235. It revealed that PNZ degradation is up to 94.85 % in 0.1 N HCl after 90 min. Samples of both batches of CCRs indicated that the % degradation of PNZ at about 19-34%. This indicates the less degradation of CCRs as compared to Std PNZ in 0.1N HCl. The CCRs of batch A-1 (PNZ-PBC) show the highest stability of the drug in HCl, i.e., up to 80%. The formulations of batch B-1 and B-2 showed the acid degradation of the drug up to 95% with an area of about 12937-13249 which is not significant for the aim of the proposed hypothesis, hence CCA technique fails to protect the drug from acid degradation [37]. The chemical reactivity of crystal forms has been dependent on the orientation of molecules and H-bonds in the crystal lattice. Moreover, easy accessibility of the reactive groups in the molecule at the crystal face can be responsible for the higher reactivity of that crystal form. If such reactive groups are bonded with H- bond and particularly shielded in a hydrophobic cage, that crystal forms less reactive [38]. In the case of pantoprazole, the sulfinyl group and nitrogen in pyridine were found to be more reactive. It was evident from the FT-IR spectrum that the drug and co-former showed intermolecular hydrogen bonding via the formation of N-H bond in both batches of CCRs. In the case of CCRs new absorption bands were observed in the formulation A-1 and A-2 at 2345.44 and 2360.87 cm^{-} respectively. By the formation of H-bonds, these reactive groups might shielded in a hydrophobic cage. This could be the probable reason for less reactivity and more acid stability of PNZ when converted to the co-crystal form.



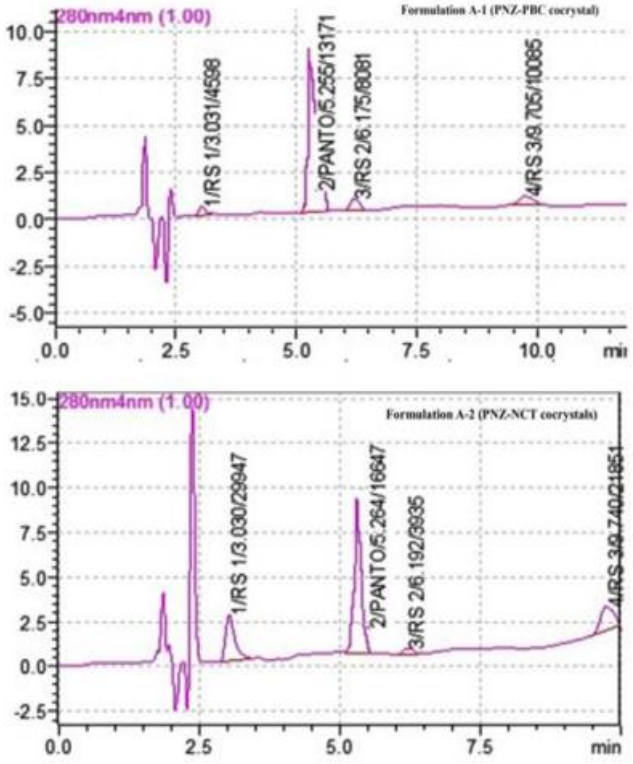


Figure 13: HPLC chromatogram of (a) PNZ; (b) PNZ degraded in 0.1 N HCl; (c) PNZ- PBC CCRs; (d) PNZ – NCT CCRs

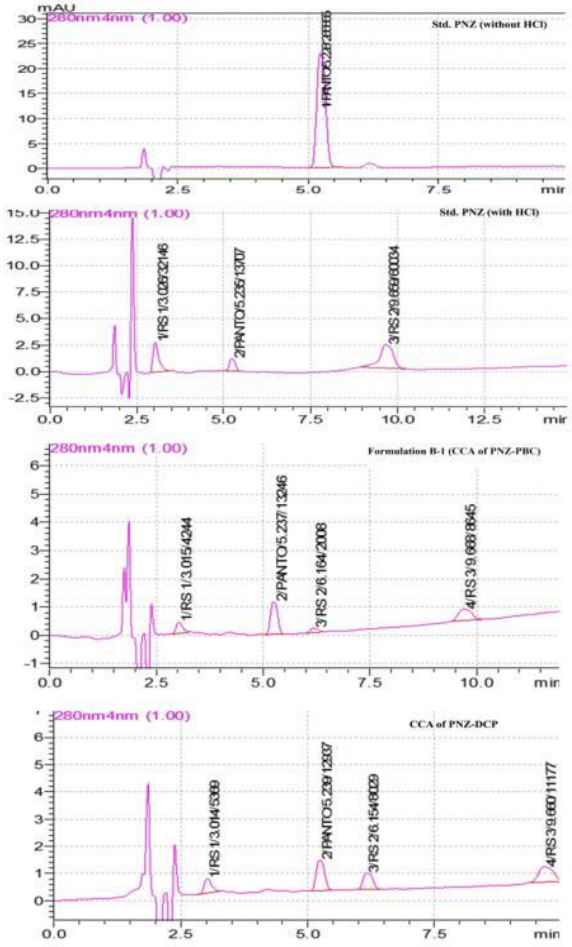


Figure 14: HPLC chromatogram of (a) PNZ; (b) PNZ degraded in 0.1 N HCl; (c) PNZ- PBC CCAs; (d) PNZ – DCP CCAs

5. Conclusion

In the present work prepared PNZ crystals exhibited excellent physicochemical properties (solubility and dissolution) and micrometric properties when compared with pure drugs. From the conducted study, it was concluded that both CCRs and CCAs showed an improvement in the solubility, extent of dissolution and flowability. In the case of the acid stability study, CCRs were more effective in resisting the PNZ degradation as compared to CCAs. FT-IR spectrum indicates the formation of the hydrogen bond between PNZ and co-formers in the case of CCRs. SEM showed the formation of platy crystals and enlarged particle size with signs of porosity in the case of CCRs, whereas agglomerates were observed in the case of CCAs. DSC thermogram indicates the formation of two crystal forms of CCRs whereas CCAs show an interesting change in thermograms with decreased enthalpy change as compared to the pure drug which revealed that there is little crystallisation of PNZ. XRPD reveals improved crystallinity which provides conclusive evidence that the particles crystallised in the presence of a co-former. Enhanced particle size and shape in the case of crystal forms leads to the significant improvement in flowability, solubility and dissolution of PNZ. The acid stability study by HPLC suggested that co-crystallisation is a fruitful technique for improvement in the acid stability of PNZ as compared to crystallo-co-agglomerates. By improved acid stability, an immediate-release dosage form can be prepared by using such CCRs, which could be the best alternative for the prompt delivery of PNZ as compared to its enteric products available in the market.

Author Contributions

Dishank Purandare- Conceptualization; Formal analysis; Methodology; Writing – review and editing

Manasi Palande - Writing – original draft

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