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Co-crystals of Gliclazide: Formulation and Characterisation

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ABSTRACT

The main focus of the present study was to explore co-crystallization technique to engineer pharmaceutical co-crystals of poorly aqueous soluble drug- Gliclazide (GLZ) using different GRAS listed coformers; benzoic acid (BA) and itaconic acid (IA). The complexation energy of GLZ and coformers were predicted by Quantum Mechanics method. Co-crystals in 1:2 molar ratios were formulated using solvent evaporation technique using acetone, a class III solvent as a medium for drug and coformer interaction at molecular level. The characteristics of GLZ and resultant co-crystals were evaluated in terms of flowability, drug content uniformity, saturation solubility and *in-vitro* dissolution studies. Further Fourier transformation infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), X-ray diffraction (XRD) and scanning electron microscopy (SEM) were used to identify hydrogen bonding interaction, melting point, crystallinity and surface morphological characteristics of prepared co-crystals respectively. The obtained GLZ co-crystals indicated presence of additional peaks as well as band shifts in the infrared spectrum. Additionally DSC thermograph and XRD confirmed change in crystal lattice. The results of *in-vitro* dissolution indicated a significant enhancement in the dissolution profiles of both the co-crystals as compared to GLZ. Thus it could be concluded that the co-crystallization technique could be successfully exploited to develop new formulation of GLZ.

Keywords: Gliclazide, Coformers, Co-crystals, Complexation energy, Quantum mechanics, Solvent evaporation.

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INTRODUCTION

During the development and formulation of any active pharmaceutical ingredient (API) and for its successful delivery, a range of stringent performance parameters such as solubility, dissolution rate, stability, compressibility needs to be carefully considered. Thus in the pharmaceutical industries, poor biopharmaceutical properties is of the one the main reasons why less than one percent of active pharmaceutical ingredients (APIs) finally appear into market place [1]. Amongst the biopharmaceutical properties, solubility remains a key issue, because according to the Biopharmaceutics Classification System (BCS) solubility and permeability are important factors used to describe oral absorption [2]. Drugs with poor solubility have slow dissolution in biological fluids and hence have systemic exposure which is insufficient and inconsistent and consequently lead to sub-optimal efficacy in patients, particularly when administered through oral route [3].

Number of approaches to improve and maximize the dissolution rate of poorly aqueous soluble drugs has been adopted such as micronisation [4], salt formation [5], solubilisation of drugs in co-solvents and micellar solutions [6], complexation with cyclodextrins and the use of polymer drug vehicles for delivery of poorly soluble drugs [7]. Even though these techniques helps in enhancing solubility and consequently oral bioavailability, success of these techniques depends at times on the specific physicochemical characteristics of the molecules being studied [1]. Solubilization techniques such as micellar systems are dependent on acceptable solubility and compatibility of drug molecules in a limited range of pharmaceutically acceptable excipients, while the increasing number of weakly ionisable and neutral molecules entering development limits the opportunities for salt formation as a method for improving solubility. Although micronisation increases rate of dissolution of drugs through increased surface area, it does not increase equilibrium solubility. Also the potential for increased Van der Waals interaction and electrostatic attraction between micronized particles can act to reduce the effective surface area for dissolution and thereby limit improvement in bioavailability [8].

The particle engineering consists of number of approaches ranging from the conventional size reduction approaches to the newer particle technologies, to modify the drugs' solubility characteristics and to develop drugs which have better solubility in water and hence in biological fluids and therefore can be readily formulated into various dosage forms [9]. A type of particle engineering approach is crystal engineering [9], which is emerging as an interesting alternate and fruitful method for modification of drugs having compromised biopharmaceutical properties [8].

Crystal engineering was introduced by Pepinsiky and established by Schmidt in 1955 through the topological reaction of cinnamic acid. This field gained importance from the 1900's with the advent of metal organics, organometallics and organic solids and since then the field of crystal engineering has advanced resulting in greater understanding of how to design viable crystalline forms. Gautam Desiraju, a pioneer in Crystal Engineering field, defined crystal engineering as "*the understanding of intermolecular interactions in the context of crystal packing and in the utilization of such understanding in the design of new solids with desired physical and chemical properties"* [10]. Intermolecular forces, more importantly non covalent interactions which includes hydrogen bonding, hydrophobic forces, Van der Waals forces, electrostatic forces and π-π interactions, which help in crystal packing and self-assembly plays an important role in crystal engineering [11].

Recent advances in crystal engineering have led to the design of co-crystals in which two or more molecular compounds are incorporated within the same crystalline lattices in specific stoichiometric amounts. Synthesis of co-crystals does not involve making/breaking of covalent bonds, and it may therefore be possible to fine-tune physical properties by exercising precise control over the supramolecular assembly, since the crystal structure determines the resulting physical properties of the compound [1].

Crystalline forms of API are used in pharmaceutical industry due to their stability and purity characteristics, but these also have their own sets of complications arising from low aqueous solubility. With the advent in crystal engineering a new platform has been created to improve these problems. A pharmaceutical cocrystal is a cocrystal with one of the cocrystal components as an API and the other components as coformers connected by non-covalent interactions where all the components are solid under ambient condition. Appropriate coformers would be those substances appearing on the GRAS list [8] for example, saccharin, vanillin, nicotinamide, maleic acid, succinic acid, maleic acid, malic acid, benzoic acid and itaconic acid etc. to mention few. The newly emerging class of pharmaceutical co-crystals have been studied in the context of improving the physicochemical properties including modifying the solubility of parent API. In pharmaceutical industry a new API with limited solubility is converted into salt form of the drug based on the ionisable functional group present in it. Though salt formation is an effective tool for improving the properties of drug without affecting its biological properties, the presence of ionisable group makes it a limited approach for neutral molecules [8].

Pharmaceutical co-crystals on the other hand opens door for multiple functional groups, including weakly or non-ionisable, and molecules that possess a broader range of hydrogen bonding properties [12]. Moreover, since pharmaceutical co-crystals have new physical properties, it is

considered as a new compound and hence can be patented [10]. Thus the overall inclination for investigating pharmaceutical co-crystals as an alternative approach in drug development is because it helps in adjusting the physicochemical properties to enhance the overall stability and efficacy of dosage form [8]. Physicochemical properties extensively studied include melting point, solubility, dissolution and stability [11]. Considering these properties of co-crystals, cocrystallization technique was applied to manipulate the physicochemical properties of poorly-water soluble drug gliclazide.

Gliclazide (Figure 1), selected in the present study is chemically 1-(Hexahydrocyclopenta[c]pyrrol-2(1H)-yl)-3-[(4-methylphenyl) sulphonyl] urea belonging to BCS class II drug used in the treatment and management of Type 2 Diabetes Mellitus when metformin is contraindicated or not tolerated, classified under sulfonyl urea class of drugs [13]. GLZ helps in reducing blood glucose levels in patients with non-insulin dependent diabetes mellitus by correcting both defective insulin secretion and peripheral insulin resistance. GLZ also has extra pancreatic effect which helps in restoring peripheral insulin sensitivity, such as reducing glucose production in liver, and increasing glucose clearance and skeletal muscle glycogen synthesis. GLZ also improves defective haematological activity in patients with type II diabetes. However due to poor and pH dependent solubility of GLZ, it has wide inter and intra patient variability in absorption which subsequently results in its poor bioavailability [13].

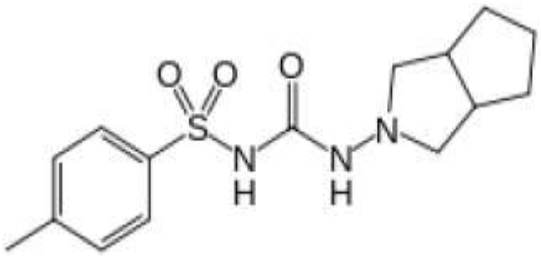

Figure 1: Gliclazide (GLZ)

Numbers of techniques have been employed to address the poor aqueous solubility of GLZ. Researchers have attempted to improve its solubility by complexation with cyclodextrin, but the use of cyclodextrin is associated with the risk of nephrotoxicity [14]. Also solid dispersions of GLZ have been developed extensively. Despite this solid dispersions are not widely used commercially because there is a possibility that during processing or storage the amorphous state may undergo crystallization. Moreover most polymers used in solid dispersions are mostly hygroscopic which may result in phase separation, crystal growth or conversion from the amorphous form to the crystalline state to form a more stable state during storage [15]. Additionally GLZ has been

formulated as nanoparticles and self-emulsifying drug delivery systems for improving its bioavailability [16],[17].

Therefore for safe, stable and efficient delivery of GLZ, it is essential to overcome its bioavailability problem. Literature review of cocrystallization technique indicates that crystal engineering through cocrystallization method is a promising solution for the aforesaid problem. GLZ co-crystals would be a viable and justifiable venture to improve the solubility problem of GLZ hence to increase and improve bioavailability.

Thus an attempt was made to tailor solubility of gliclazide in biological fluids by developing co-crystals of GLZ using GRAS listed coformers, benzoic acid and itaconic acid. The pharmaceutical co-crystals of GLZ were prepared by solvent evaporation process in 1:2 molar quantities of drug to coformers. The prepared co-crystals were characterized by Fourier transformation infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), Differential scanning calorimetry (DSC), flowability, drug content and saturation solubility studies. Co-crystals as well as pure GLZ was further examined for their dissolution performance in phosphate buffer (pH 7.4).

MATERIALS AND METHOD

Materials

GLZ was obtained as a gift sample from M.J. Biopharm Pvt. Ltd., Mumbai, India. Benzoic acid (Extra pure grade) was purchased from Sigma Aldrich Ltd., Mumbai, India and Itaconic acid (Analytical grade) was purchased from Avra Chemicals, Mumabi, India. All other reagents used were of analytical grade. Distilled water was used throughout the experiment.

Methods

Prediction of complexation energy of GLZ-BA and GLZ-IA by Quantum Mechanics method

The tendency of GLZ to interact with benzoic acid and itaconic acid was determined by Quantum mechanical method using Gaussian03 running on windows platform. Individual molecules and the complexes in 1:1 ratio were optimized in gas phase and acetone using hybrid density functional method B3LYP with 6-311++G (d p) basis set. The solvent phase optimization was performed using polarizable Continuum Model (PCM) in Gaussian03.

Table 1: Energy calculations for GLZ-BA complex in 1:1 ratio

Parameter	Value	
	Complex 01	Complex 02
Complexation energy (kcal/mol)	-9.12	-6.58
Gas phase energy (kcal/mol)	-1125468.02	-1125464.66

Solution phase energy (kcal/mol)	-1125493.40	-1125490.86
Solvation energy (kcal/mol)	-25.38	-26.20

Table 2: Energy calculations for GLZ-IA complex in 1:1 ratio

Parameter	Value		
	Complex 1	Complex 2	Complex 3
Complexation energy (kcal/mol)	-6.48	-9.37	-6.67
Gas phase energy (kcal/mol)	-1172064.66	-1172067.65	-1172064.29
Solution phase energy (kcal/mol)	-1172093.51	-1172096.39	-1172093.70
Solvation energy (kcal/mol)	-29.45	-28.74	-29.40

Preparation of co-crystals of GLZ with benzoic acid and itaconic acid

The co-crystals of GLZ were prepared by solvent evaporation method [18]. GLZ and coformers (benzoic acid and itaconic acid) were dissolved in 70 ml of acetone in 1:2 (drug: coformer) molar ratio by stirring. Stirring was continued till a clear solution was obtained. The system was covered with an aluminium foil and about 5-6 fine holes were pierced in the foil. Solvent from the clear solution was allowed to evaporate at a slow constant rate at room temperature with constant stirring. The process was continued till solid co-crystals were obtained.

Solid state characterization

Fourier transformation infrared spectroscopy (FTIR)

FTIR spectra were obtained with the help of Jasco FTIR-4100 Fourier Transform spectrophotometer (Japan) by KBr disc method. Briefly 10 mg of sample was thoroughly mixed with 50 mg of potassium bromide and potassium bromide disks with GLZ were prepared using electrically operated KBr Press Model HP-15 (India) at 10 tons pressure and it was analysed over the range of 400-4000 cm^{-1} with 15 accumulative scans having resolution of 4 cm^{-1}.

Differential Scanning Calorimetry (DSC)

Pyris-6 Perkin Elmer Differential Scanning Calorimetry (USA) was used to perform the melting characteristics of GLZ, coformers and co-crystals. Solid sample, 3-4mg was placed on aluminium pan; the pan was covered with lid and crimped using DSC crimper. The crimped pans containing solid sample were heated against blank crimped pans from 20^{o}C-250^{o}C at the rate of 10^{o}C/min under nitrogen flow of 17 ml/min and endothermic peaks obtained were studied.

X-Ray Diffraction (XRD)

X-Ray diffraction patterns of co-crystals and drug were analysed on Bruker D8 Discover XRD analyser (Germany) (Cu Kα radiation, voltage of 40 kV and current 40 Ma). About 1.5 g of sample was placed on the sample holder and the sample holder was placed on the rotating sample stage. The sample was placed in the horizontal position and the X-Ray tube and the detector were moved

over the sample simultaneously over the 2θ angular range from 10^o to 70^o with a continuous scan rate of 4^o per minute. The integrated patterns display diffraction intensity as a function of 2θ.

Scanning electron microscopy (SEM)

The morphological characteristics of drug and co-crystals were examined by FEI Quanta 250 (USA). The samples were loaded on aluminium stub with carbon adhesive tape. The samples were scanned at a voltage of 10 kV.

Evaluation of flow property

The flow properties of pure GLZ and co-crystals was acquired by evaluating the solids in terms of bulk density, tapped density, Carr's index, Hausnar's ratio and angle of repose. Hausnar's ratio was calculated as a ratio of tapped density to bulk density. Angle of repose was determined by fixed funnel method and Carr's index was calculated using following formula:

$$Carr's\ Index = \frac{[Tapped\ density - Bulk\ density]}{Tapped\ density} \times 100$$

Determination of drug content in coformers and cocrystals

Drug content was determined by dissolving co-crystals quantity equivalent to 80 mg of GLZ in 50 ml of methanol and the volume was adjusted to 100 ml with methanol AR. The solution was filtered through Whatman filter paper no 41 and 1 ml of the resulting solution was diluted 100 times with methanol AR. Absorbance of the resultant solution was measured at 226 nm using double beam UV spectrophotometer (Jasco 530V, Japan).

Saturation solubility studies

Saturation solubility studies of GLZ and co-crystals of GLZ were performed in phosphate buffer pH 7.4. Briefly, an excess amount of sample was taken in the eppendroffs to which 1 ml of phosphate buffer pH 7.4 was added. Thereafter, the eppendroffs were sealed and shaken for 72 hrs at 37^oC in water bath shaker (Remi, CM 101, Mumbai, India). Subsequently, the solutions were filtered through 0.22 µ filter and the supernatant was suitably diluted and GLZ content was quantified at 226 nm on a Jasco-V-530 UV spectrophotometer, using a validated UV spectrophotometric technique. To eliminate the solvent effect on absorbance, phosphate buffer pH 7.4 was used as blank.

***In vitro* drug release studies**

The *in vitro* drug release studies were performed in 900 ml of phosphate buffer (pH 7.4) at 100 rpm maintained at 37 ± 0.5^oC in USP Dissolution Apparatus II (Electrolab, India). 80 mg of GLZ or its equivalent amount of co-crystals were weighed and placed in hard gelatin capsules size 1. The hard gelatin capsules were clamped using sinkers and placed in dissolution medium and samples

were withdrawn at appropriate time intervals. The withdrawn samples were filtered through Whatman filter, suitably diluted and analysed spectrophotometrically at 226 nm.

RESULTS AND DISCUSSION

Prediction of complexation energy of GLZ-BA and GLZ-IA by Quantum Mechanics method

Quantum mechanics method was applied in order to predict the structure of cocrystal that would be formed after solvent evaporation method. As in co-crystals, drug and coformer are associated via hydrogen bonding, possible hydrogen bond motifs within drug and coformer was identified. Structure of complexes that can be formed was optimized and complexation energy for each complex was calculated.

$$\Delta G_{com} = E_{complex} - (E_{drug} + E_{coformer})$$

Where,

ΔG_{com} is complexation energy; $E_{complex}$, E_{drug} and $E_{coformer}$ is energy of complexation of complex, drug and coformer respectively.

Amongst the complexes predicted, the complex having higher negative value is most likely to be formed and considered to be stable.

In case of GLZ-BA, after identifying H-bond motifs, 2 complexes (Figure 2) were predicted. From the complexation energies of both the complexes of GLZ with BA, it can be seen that complex 1 has higher negative value; therefore the probability of complex 1 forming as a result of solvent evaporation of GLZ and BA is more.

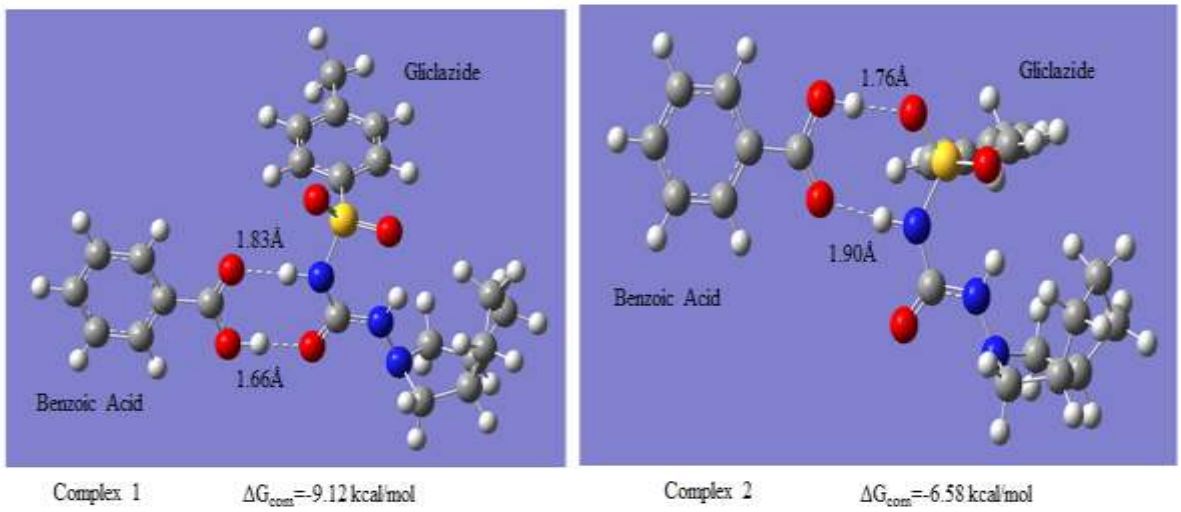

Figure 2 Possible complexes of gliclazide and benzoic acid

In case of GLZ-IA, after identifying H-bond motifs, 3 complexes (Figure 3) were predicted. The complexation energies of the complexes of GLZ with IA clearly indicates that complex 2 has

higher negative value, therefore the probability of complex 2 forming as a result of solvent evaporation of GLZ and IA is more.

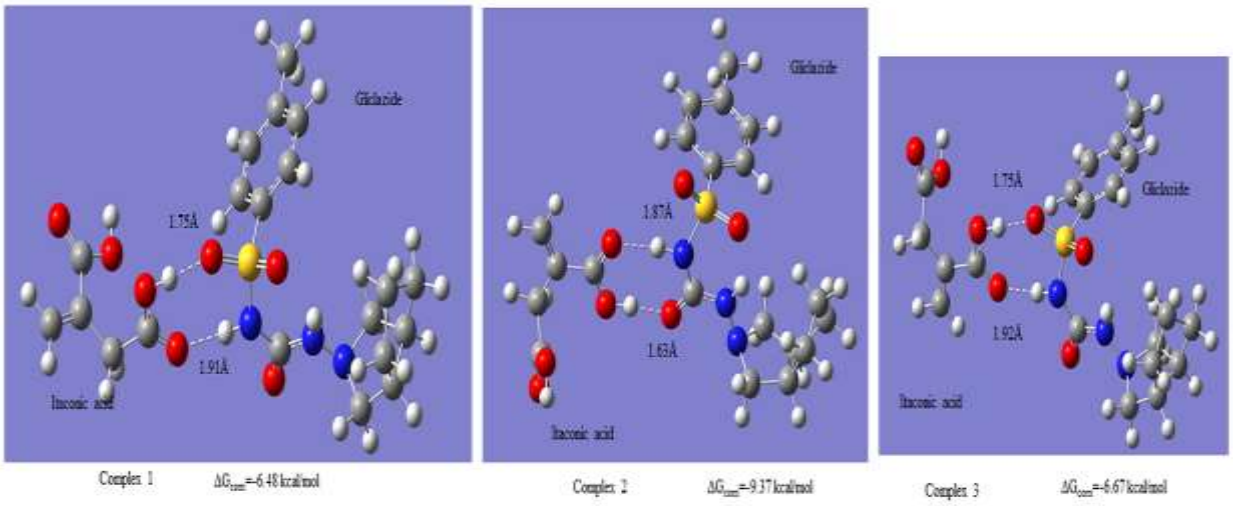

Figure 3 Possible complexes of gliclazide and itaconic acid

Solid state characterization

Figure 4 shows the FTIR spectra of GLZ, benzoic acid, itaconic acid and their corresponding co-crystals. IR spectrum of GLZ is characterised by principal absorption peaks at 3273 cm^{-1} (N-H stretch), 1709 cm^{-1} (C=O stretch), 1596 cm^{-1} (N-H deformation band), 1350 cm^{-1} (S=O symmetrical vibration band) and 1164 cm^{-1} (S=O asymmetrical vibration band). Benzoic acid exhibited weak IR band at 3460 cm^{-1} (O-H carboxylic) due to intermolecular hydrogen bonding in solid state. However, it produced strong bands at 3071 cm^{-1} (C-H aromatic), 1680 cm^{-1} (C=O carboxylic), 1582 cm^{-1} (C=C aromatic), 1072 cm^{-1} (C-O) and 707 cm^{-1} (monosubstituted benzene). Itaconic acid exhibited bands at 1700-1630 cm^{-1} (C=O carboxylic), 1440-1390 cm^{-1} (O-H vibrations), 1308 cm^{-1} (C-O) and 900-880 cm^{-1} (germinal disubstitution).

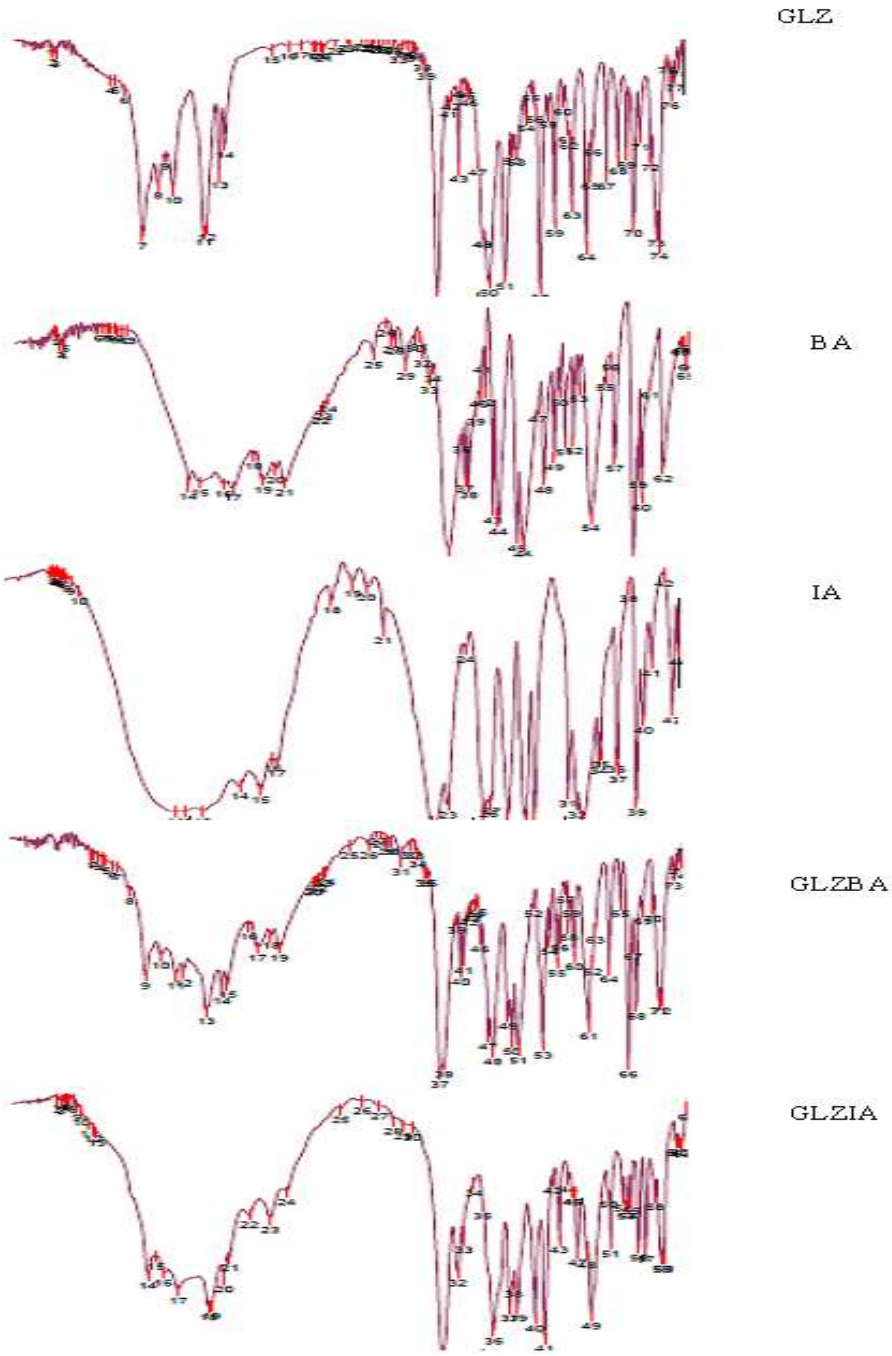

Figure 4 FTIR spectra of pure gliclazide, coformers and co-crystals. GLZ: Gliclazide; BA: Benzoic acid; IA: Itaconic acid; GLZBA: gliclazide-benzoic acid co-crystal; GLZIA: gliclazide-itaconic acid co-crystal.

The GLZBA co-crystals exhibited decrease in intensities and changes in peak positions of SO_2 stretch of GLZ, from 1164 and 1350 cm^{-1} to 1163 and 1348 cm^{-1} respectively. Moreover, the IR band at 3460 cm^{-1} (O-H carboxylic) of benzoic acid was not observed in the IR spectrum of GLZBA co-crystals, inferring involvement of these functional groups in intermolecular hydrogen

bonding between GLZ and BA in GLZBA co-crystals [19]. Furthermore, GLZBA co-crystals exhibited shifts in N-H stretch and N-H deformation band of GLZ from 3273 cm^{-1} and 1596 cm^{-1} to 3271 cm^{-1} and 1598 cm^{-1} with reduction in their intensities.

The GLZIA co-crystals exhibited shifts in N-H stretch of GLZ from 3273 cm^{-1} to 3272 cm^{-1} with reduction in its intensity. Also, intensities of S=O vibration band of GLZ at 1163cm^{-1} and 1350cm^{-1} were changed. The disappearance of IR band at 1408 cm^{-1}, characteristic for O-H carboxylic of IA from the GLZIA spectrum infers involvement of these functional groups in formation of intermolecular hydrogen bond between GLZ and IA. Also few additional peaks, peaks different from GLZ, BA and coformer IA were observed in the IR spectrum of GLZBA and GLZIA co-crystals, confirming the formation of a new cocrystalline compound.

Figure 5 shows the DSC thermograms of GLZ, benzoic acid, itaconic acid and their corresponding co-crystals. The thermogram of GLZ exhibited endotherm at 171.79^{o}C. Similarly, BA and IA showed sharp melting endothermic peaks at 126.97^{o}C and 173.58^{o}C. The GLZ-BA and GLZ-IA co-crystals prepared by solvent evaporation exhibited sharp melting peaks at 106.10^{o}C and 110.20^{o}C respectively. The peaks observed for GLZ-BA and GLZ-IA co-crystals were found to be considerably different from the melting endothermic peaks of GLZ, BA and IA, which may be attributed to the changes in the crystalline structure of GLZ indicating formation of new cocrystalline compound. The difference in melting points in cocrystals as compared to plain drug and coformers might be attributed to the hydrogen bond formation between functional groups of API and coformer which may further lead to moderate or complete alterations in molecular arrangement of cocrystal formed, thereby resulting in the formation of new crystalline substance having altered physicochemical properties [19]. Also shift in endotherm position to the lower side with respect to drug and coformer is hypothesized to indicate improvement in solubility of prepared GLZ cocrystals. Bak et al and Aakeroy et al hypothesised that the melting point and aqueous solubility of an API may be able to be tailored finely by cocrystallising that API with the coformers [20], [21]. According to data compiled by Schultheiss and Newman, in order to determine if any correlation exists between the melting point of the cocrystal with that of the melting points of API and coformers, it was observed that the cocrystals having higher melting points than that of its API and coformer, results in poorly soluble cocrystal. They also reported an analysis which documented that 51% cocrystals had melting points between the API and coformer, 39% were lower either than API or coformer and 4% had the same melting point as either the API or coformer [22]. In the present study melting point of GLZ cocrystals was lower than GLZ and BA and IA coformers; it can be thus concluded that cocrystals can be utilized as a vital technique in

altering the physicochemical properties of GLZ, thereby resulting in formation of a new GLZ crystal form with superior physicochemical characteristics.

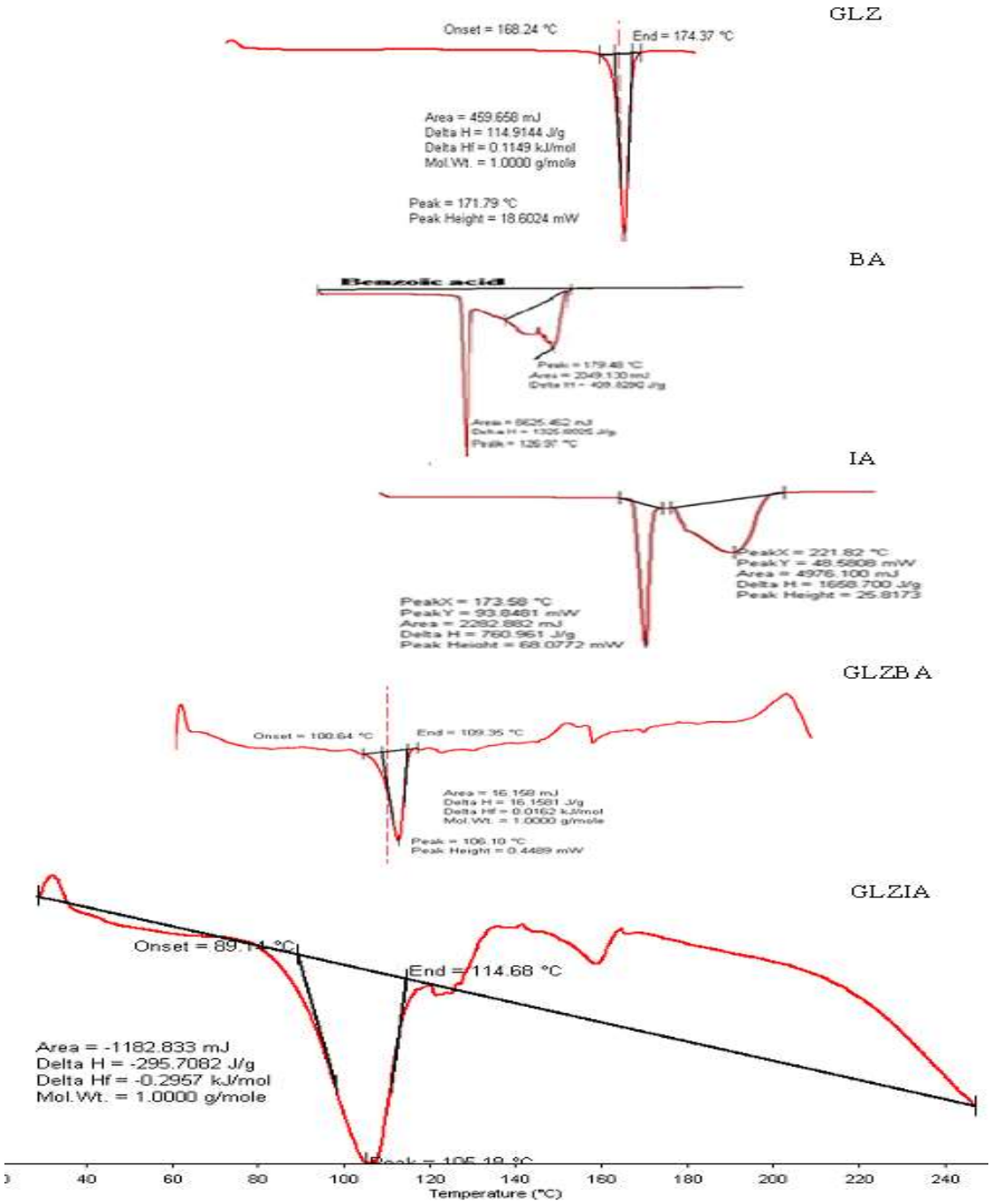

Figure 5: DSC thermograms of pure gliclazide, coformers and co-crystals. GLZ: Gliclazide; BA: Benzoic acid; IA: Itaconic acid; GLZBA: gliclazide-benzoic acid co-crystal; GLZIA: gliclazide-itaconic acid co-crystal.

X-ray diffraction patterns of pure GLZ, coformers and co-crystal, confirmed generation of co-crystalline material. X-Ray Diffraction is a useful technique which helps in determining the

generation of new crystal form during cocrystallization process. As shown in Figure 6, unique XRD patterns distinguishable from the drug and coformer were observed. Table 3 represents the peak intensities of drug, coformers and cocrystals at various diffraction angles ($2\Theta^{o}$). It can be observed that cocrystals exhibited spectra with different peak positions from their respective drug and coformers possessing different internal structure with significant crystal habit modification. Furthermore, the relative intensities of cocrystal peaks were modified which could be due to the formation of a different crystal habit and arrangements of molecule indicating formation of new crystal forms. Melting endotherms observed for the cocrystals also strongly supported the formation of new crystal form as the peaks observed were completely different from their respective drug and coformers, as discussed earlier.

Table 3: Peak intensities of pure gliclazide, benzoic acid, itaconic acid and cocrystals at various diffraction angles ($2\Theta^{o}$)

GLZ		**BA**		**IA**		**GLZBA**		**GLZIA**	
2Θ(degrees)	Intensity	2Θ(degrees)	Intensity	2Θ(degrees)	Intensity	2Θ(degrees)	Intensity	2Θ(degrees)	Intensity
10.82	162	24.12	524	19.4	408	10.62	169	18.14	111
10.86	160	26.06	656	19.42	455	10.64	167	18.24	100
18.22	159	26.08	2166	19.44	418	17.19	150	18.26	100
18.24	170	26.1	2796	20.57	518	17.21	155	18.28	139
18.26	153	26.12	1075	20.29	514	17.23	166	18.3	109
21.15	155	26.14	956	20.63	514	17.25	205	18.32	112
21.17	161	26.16	1518	30.09	403	17.27	170	20.48	107
21.41.	155	26.18	842	30.11	378	17.29	184	20.93	131
21.43	152			30.13	376	17.31	166	20.95	102
21.45	163			32.09	381	18.26	179	20.99	101
21.47	161			32.11	376	18.28	163	21.01	102
						20.33	157	21.23	117
						20.95	223	21.25	104
								21.29	108

GLZ- Gliclazide, BA- Benzoic acid, IA- Itaconic acid, GLZBA- Gliclazide-Benzoic acid co-crystals and GLZIA- Gliclazide-Itaconic acid co-crystals

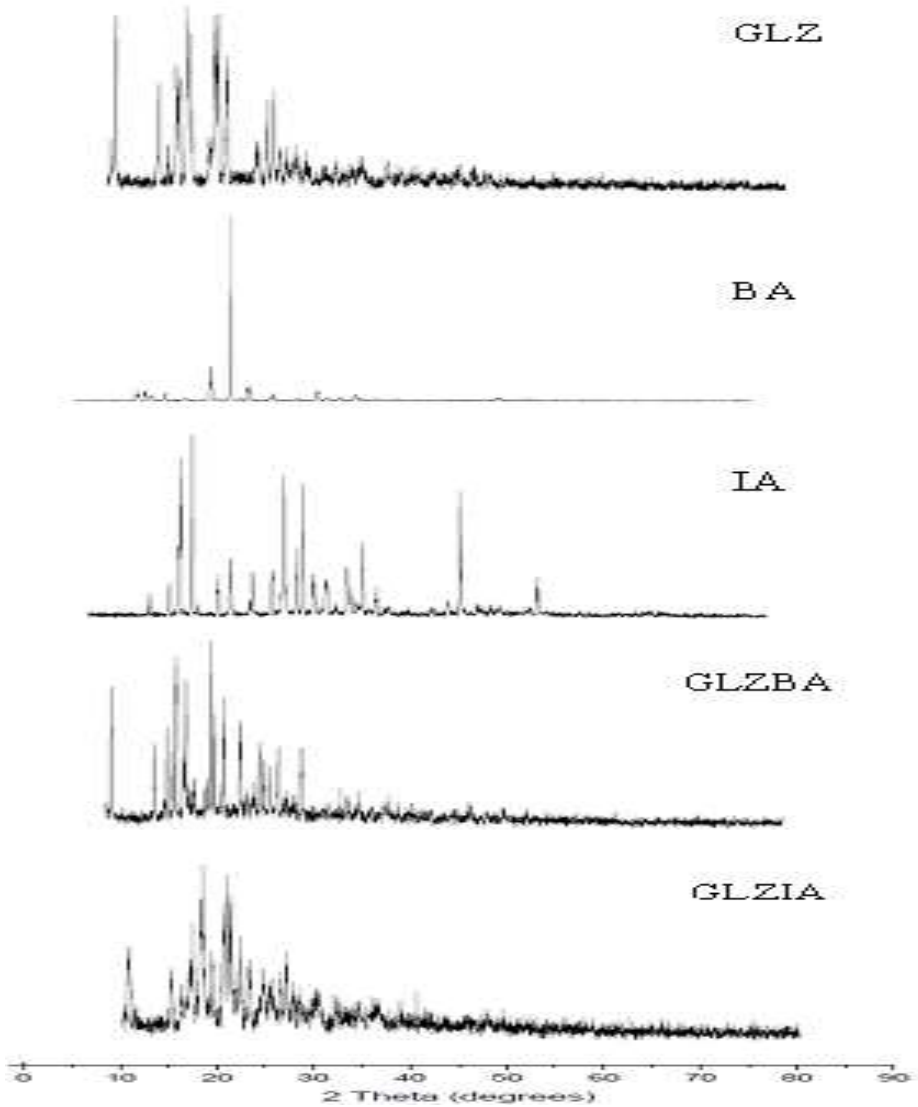

Figure 6 XRD patterns of pure gliclazide, coformers and co-crystals. GLZ: Gliclazide; BA: Benzoic acid; IA: Itaconic acid; GLZBA: gliclazide-benzoic acid co-crystal; GLZIA: gliclazide-itaconic acid co-crystal.

The surface morphological properties of the original drug and cocrystals are shown in Figure 7. It is evident from the microphotographs that the cocrystals of GLZ exhibited larger particle size (185 nm to 1 µm). Pure GLZ appeared as irregular prismatic shaped crystals with the particle size of 121 to 155 nm. The cocrystals formed as a result of solvent evaporation method had larger size (185 nm to 1 µm) but had similar irregularly shaped prism like crystals as that of pure drug.

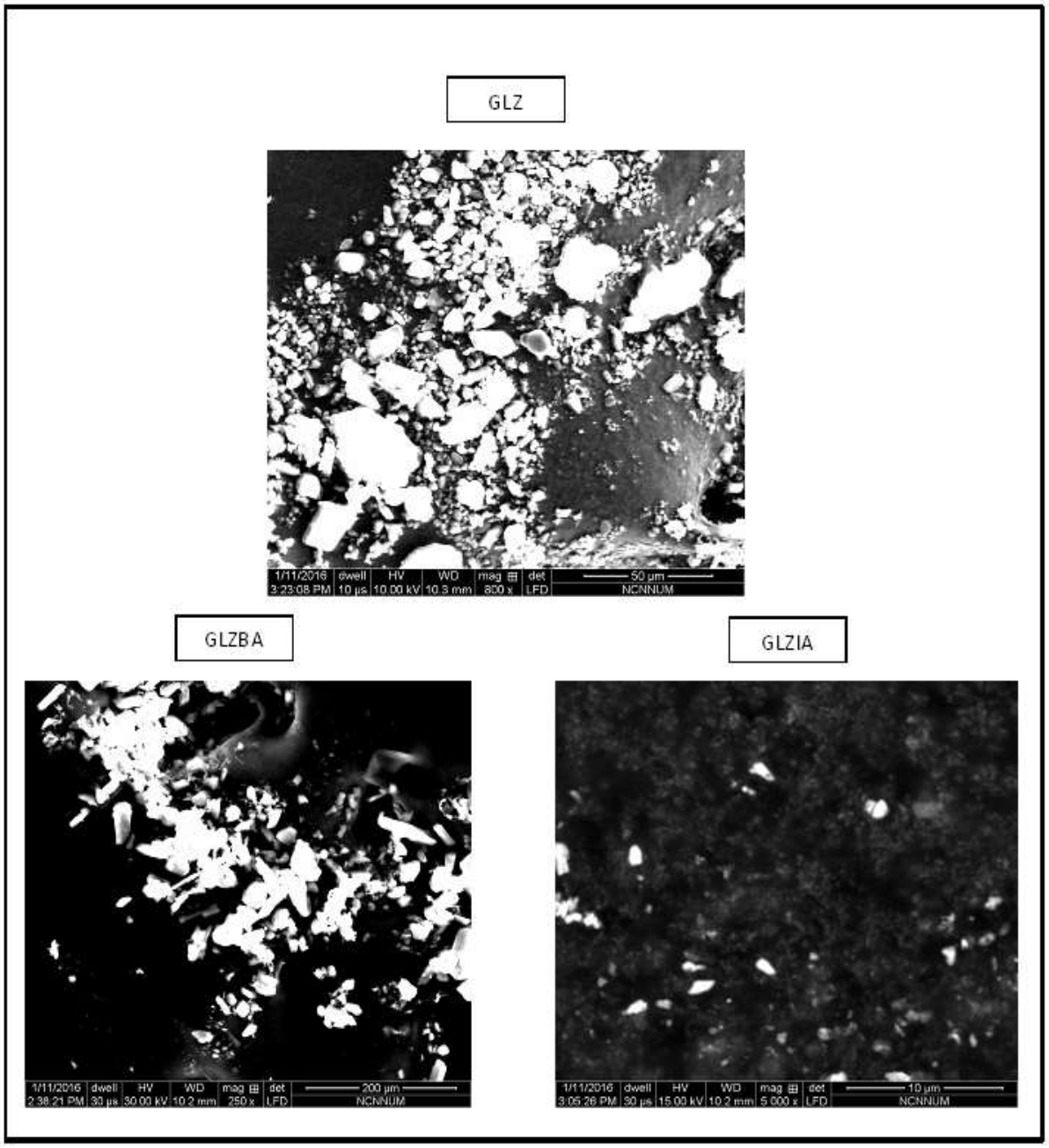

Figure 7: SEM microphotographs of pure gliclazide, coformers and co-crystals. GLZ: Gliclazide; BA: Benzoic acid; IA: Itaconic acid; GLZBA: gliclazide-benzoic acid co-crystal; GLZIA: gliclazide-itaconic acid co-crystal.

Flowability studies

The micromeritic properties such as flowability of prepared co-crystals are represented in Table 4. The flowability represented in terms of the bulk density, tapped density, angle of repose, Carr's index and Hausner's ratio were not significantly improved compared to that of the original drug,

this might be attributed due to similar shape of the co-crystals formed as a result of solvent evaporation and GLZ, as reflected from SEM photomicrographs.

Table 4: Flow properties of pure drug GLZ and its co-crystals

Systems	Bulk density (g/ml)	Tapped density (g/ml)	Hausner's ratio	Carr's Index (%)	Angle of repose (degrees)
Gliclazide	0.3850	0.4450	1.15	13.48	33.86
GLZBA co-crystals	0.3921	0.4500	1.13	11.76	28.60
GLZIA co-crystals	0.3889	0.4667	1.20	16.67	19.39

Determination of drug content in coformers and cocrystals

Percentage drug contents of prepared cocrystals were found in the range of 91.53±1.05 w/w to 95.00±1.00 w/w.

Saturation solubility studies

The co-crystals exhibited enhanced solubility in phosphate buffer pH 7.4 as compared to pure drug alone, Table 5. There was 5 and 6 fold increase found in the solubility from the co-crystals with itaconic acid and benzoic acid respectively. Further benzoic acid was found to be better coformer than itaconic acid in improving the solubility. This could be due to existence of correlation between melting point of cocrystal and its solubility. Similar results were reported by Bak et al in the study of AMG 517 wherein cocrystals with high melting points results in lower solubility [20]. Also as per CADD, two complexes of GLZ-BA were possible whereas for itaconic acid there were three possible complexes out of which only one complex had higher negative complexation energy, as discussed in section 3.1. Less improvement in dissolution of itaconic acid coformer could be due to low proportion of complex with higher negative energy.

Table 5: Saturation solubility of pure gliclazide and co-crystals

System	Saturation solubility (mg/ml)
Gliclazide (GLZ)	1.24±0.11
GLZBA co-crystals	7.38±0.13
GLZIA co-crystals	6.50±0.14

***In vitro* drug release studies**

The dissolution curves of pure drug (GLZ) alone and cocrystals in phosphate buffer pH 7.4 are shown in Figure 8. From the graphs it is evident that the cocrystals have improved the dissolution rate of GLZ significantly compared to the pure drug. The dissolution profile depicted in Figure 8 indicated 3.3 fold enhancement in dissolution rate of GLZ from itaconic acid co-crystals whereas it was 5.2 fold from benzoic acid co-crystals within 5 minutes. Percent cumulative release of 53.26% and 37.86% were obtained from benzoic acid and itaconic acid co-crystals respectively within 60

minutes. However, the percent cumulative release of pure drug within the same time was observed to be 27.64%.

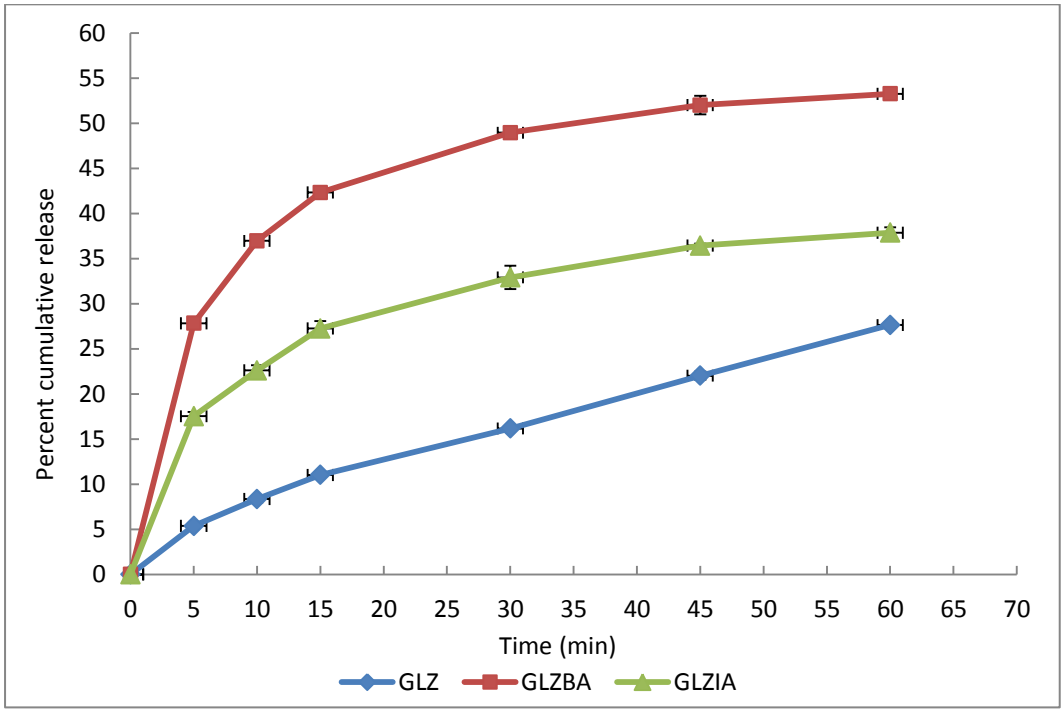

Figure 8 *In-vitro* release profiles of pure gliclazide, coformers and co-crystals. GLZ: Gliclazide; BA: Benzoic acid; IA: Itaconic acid; GLZBA: gliclazide-benzoic acid co-crystal; GLZIA: gliclazide-itaconic acid co-crystal.

CONCLUSION

In the present study, benzoic acid and itaconic acid were used as coformers for improvement of physicochemical properties successfully via co-crystallization technique. The altered solid state characteristics of co-crystals indicated modification in the crystal habit which might be responsible for improvement of solubility and dissolution properties of GLZ from both the co-crystals developed. In the present study, benzoic acid was found to be a better coformer for the selected API as the dissolution rate of GLZ was found to be twice from GLZBA cocrystal than that of GLZIA cocrystal. Thus, it can be concluded that developed cocrystallization approach can be successfully employed to improve solubility of Gliclazide.

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