Understanding impact of pre-dissolved polymers on dissolution behavior of soluble carbamazepine cocrystal

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Abstract: Cocrystallization is a novel approach for tackling the lower solubility concerns when they can yield solution concentration a lot better than their corresponding parent drug in crystalline form. To get the actual solubility and dissolution gains offered by the cocrystals, phase changes in solution (dissolution) has to be interrupted. In current study, we selected commonly used polymers in order to study their effects on the super saturation of carbamezepine-succinic acid (CBZ-SUC) cocrystal during dissolution studies. To observe solid phase changes during dissolution *in situ* Raman spectroscopy was used. At the completion of each test the solid phase was analyzed by Fourier-transform infrared spectroscopy (FTIR) and powder X-Ray diffractometry. In polymers absence, no dissolution improvement was achieved by the cocrystal owing to its quick transformation to the stable carbamazepine dihydrate (CBZDH). Pre-dissolved PVP at 2% w/v concentration did not inhibit CBZ crystallization as a dihydrate, whereas at 0.025% w/v pre-dissolved hydroxypropyl methyl cellulose acetate succinate (HPMCAS) did stabilize the cocrystal in buffer solution (pH 6.8) for the course of time studied. This cocrystal stabilization resulted in enhanced CBZ solubility (~4fold) caused by cocrystal super saturation state. Seeding of this stable supersaturated state with 1% w/v CBZDH resulted in CBZ crystallization as dihydrate with ultimate loss of solubility advantage.

Keywords: Cocrystals, super saturation, crystallization inhibitor polymers, crystallization.

INTRODUCTION

Solubility improvement of poorly soluble drugs is a key property that endorses the successful development of number of novel drugs. Common approaches adopted to increase the drug(s) solubility include but not limited to development of amorphous solid dispersion (ASDs), salt forms creation, using a metastable polymorph(s) and cocrystal formation. These solubility enhancement approaches improve the drug substance dissolution profile that is thought to be a key feature affecting the drug absorption (McNamara *et al*, 2006; Shiraki *et al*, 2008).

In past decade pharmaceutical cocrystallisation arose as a novel technique that modifies the drug physico-chemical properties principally its solubility, and can effectively be applied to a range of molecules, hence has appealed enormous attention from researchers (Aakeroy *et al*, 2009: Bethun *et al*, 2009: Goods *et al*, 2010). Like amorphous form, cocrystals also undergo 'solution mediated phase transformation' (SMPT) that is crystallization of stable solid phase (metastable phase) as a result of the achieved super saturation in solution or at the dissolving solid surface. The main problem with SMPT is higher drug dissolution rate loss encountered because of stable phase crystallization phenomenon in solution (Greco *et al*, 2012). Thus during dissolution, transformation to a least soluble form is a basic concern taken into account while estimating techniques for improving the solubility of sparingly soluble drugs (Greco *et al*, 2012).

The rate of crystal growth and nucleation is affected by excipients. Thus, appropriate selection of stabilizing agents is considered a vital phase in formulation development for sparingly soluble drugs. Besides screening, it is of significance to differentiate thermodynamic stabilization (to cut the degree of super saturation), from kinetic stabilization (to defer crystal growth ratio/ nucleation) (Vandecruys *et al*, 2007: Janssens *et al*, 2008). The impact of polymers on the solubility/dissolution of low soluble API's is generally limited so their stabilizing action is predominantly kinetic. A number of studies prove that the course of nucleation as well as crystal growth rate are delayed because of Drug–polymer contacts in dissolution medium and by the polymers adsorption on the nucleus or the developing crystals (Raghaven *et al*, 2003: Terayam *et al*, 2004). The polymer complexion and interacting abilities are key for enhancing drug solubility and persistent super saturation. As polymers have typical binding spots that permit Van der Waal's interactions or hydrogen bonding with API's, resulting in its stability in the matrix (Alonzu *et al*, 2010).

Carbamazepine is classified as a BCS II drug (low aqueous solubility and high permeability) is widely used

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for treating epilepsy (Kovacevic *et al*, 2008). Not surprisingly, its oral bioavailability is governed by its dissolution rate, which is influenced by the CBZ crystal form (four different crystalline forms) used in the dosage form (Kobayashi *et al*, 2000). In literature over 50 cocrystals have been reported for CBZ and its cocrystal with SUC have greater solublity than pure CBZ but is thermodynamically not stable i.e, transforms to its stable dihydrate when in solution (Qiao, 2014: Ullah *et al*, 2015: Ullah *et al*, 2016). So with the intention of capturing the solubility advantage of CBZ-SUC, it was empirical to stabilize this cocrystal system with common pharmaceutical polymers like HPMCAS and Kollidon (crystallization inhibitors). We were also inspired by recent studies where CBZ/HPMCAS solid dispersion produced CBZ super saturated state with enhanced drug penetration across Caco-2 membrane (Ueda *et al*, 2014). Also relative bioavailability performance of CBZ supersaturtable-SMEDDS formulated with PVP in beagle dogs was compared to its commercially available tablets. The results revealed that addition of even trivial concentration of PVP to unstable SMEDDS, effectively sustained the supersaturated state by delaying crystallization kinetics *in vitro* with 5-fold bioavailability enhancement (Zhang *et al*, 2011).

MATERIALS AND METHODS

Materials

CBZ was acquired from Sigma Aldrich (USA), Kollidon 90-F and Kollidon-30 were obtained from BASF (Germany). HPMCAS (HF) was purchased from *Shin Etu* Chemical Company, Ltd. (Japan). All chemicals were used as provided without any further processing. Buffer pH 6.8 (Potassium phosphate 0.2M) was the dissolution medium used for all dissolution experiments.

The CBZDH was used as reference and was obtained by adding adequate volume of water to CBZ and was left overnight. Transformation to DH form was confirmed by PXRD, IR and Raman spectroscopy.

Preparation and characterization of cocrystal

The preparation as well as characterization of CBZ-SUC 2:1 cocrystal were detailed before (Ullah *et al*, 2015: Ullah *et al*, 2016). In brief 4.725 g (1.99mM) of CBZ and 1.181g (1mM) of SUC were mixed by hand in a mortar for 30 min with constant dilution of methanol.

Method

Cocrystal dissolution studies with and without pre-dissolved PVP (K30/90) and HPMCAS (HF)

The dissolution experiments were performed at room temperature (25 ±0.5ºC) under non-sink conditions with phase solubility method. Approximately 200-250mg CBZ-SUC cocrystals were added to 30mL of buffer in 50mL vials and constantly agitated with magnetic stirrer. After stated time, aliquots were taken, filtered through nylon membrane filter (0.22µm) and diluted appropriately for UV/Vis analysis. The Raman spectra of pure cocrystal, drug, dihydrate and slurred cocrystal in buffer were collected. The IR/Raman instrument had compass 1064-500 laser and aD 425 Ge-Diode detector (Vertex 70/Bruker Optics Inc., MA, USA). Raman *in situ* spectra were collected with a dipping optic connected to an MR probe, laser power was around 400 mW, spectra collected ranged 4000-400nm (wave number) at 4 cm^{-1} (resolution). Data obtained were analyzed by built-in software (OPUS version 8.00, V5.5/Bruker Optics Inc., MA, USA) and were imported and treated in Originlab (V.8; Northamton, MA, USA). At every collection point, remaining suspension was filtered (vacuum) and examined by FTIR/PXRD (as sample decomposed above its MP so DSC was not used). The concentration of CBZ was measured by UV-Vis spectroscopy. All the experimental conditions were retained in the presence of pre-dissolved polymers.

UV spectrometry

The concentrations of drug in medium were determined by UV/Vis spectrometer (DU-530 UV/Vis Spectrophotometer; B-Coulter, MN, USA) at λ = 288 nm.

RESULTS

To capture the in-depth dissolution behavior together with possible phase change of cocrystal in buffer (pH 6.8) *in situ* Raman spectroscopy was used. The distinctive bands of the CBZDH at 350-400 & 1000-1050 were observed in the initial 5min, as cocrystals with fairly higher differential co-formers solubility transformed to thermodynamically stable parent constituents e.g. CBZDH in this case as shown in fig. S_1, analogous outcomes were also observed by Childs *et al*. 2008 for CBZ-SUC cocrystal (Childs *et al*, 2008). Phase confirmation with off-line IR confirmed Raman spectra results as solid residue (after dissolution experiments) IR spectrum also demonstrated distinctive dihydrate bands in the regions 3200-3500 cm^{-1}. Likewise CBZ sharp peak at 3500 cm^{-1} was absent that confirmed rapid cocrystal conversion in soution and dihydrate crystallization (fig. S_2). Filtrate powder X-Ray diffraction pattern also ratified the dihydrate crystallization, as peaks observed at 8.9°, 12.1° and 18.7° were characteristic dihydrate peaks (Tian *et al.*, 2006).

Primarily, the impact of pre-dissolved PVP K-30 in solution (at conc ranges of 0.25% w/v to 2% w/v) was studied on cocrystal kinetic stability. *In situ* Raman spectroscopy confirmed abrupt crystallization of CBZDH and off-line PXRD/FTIR also validated Raman spectroscopy results as individual dihydrate peaks are clearly visible in SIF_2 & SIF_3. Also, at similar concentrations of pre-dissolved PVP K-90 (higher mol. wt

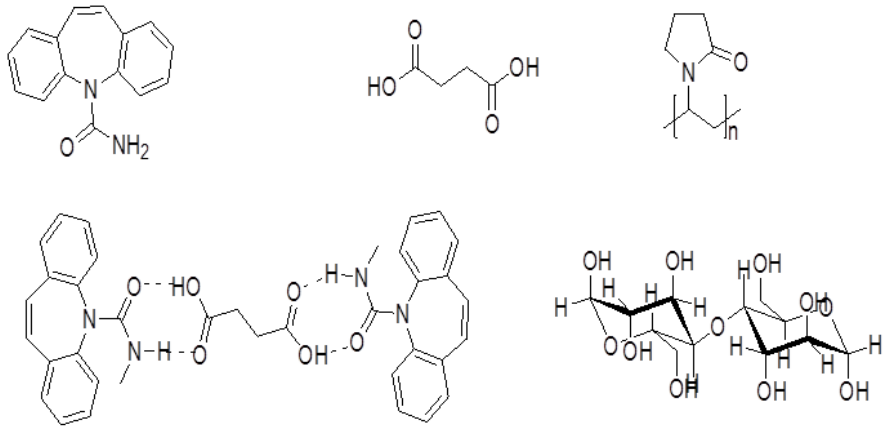

Fig. 1: Structures of Carbamazepine (B) Succinic acid SUC (C) CBZ-SUC Cocrystal (D) PVP (E) HPMCAS

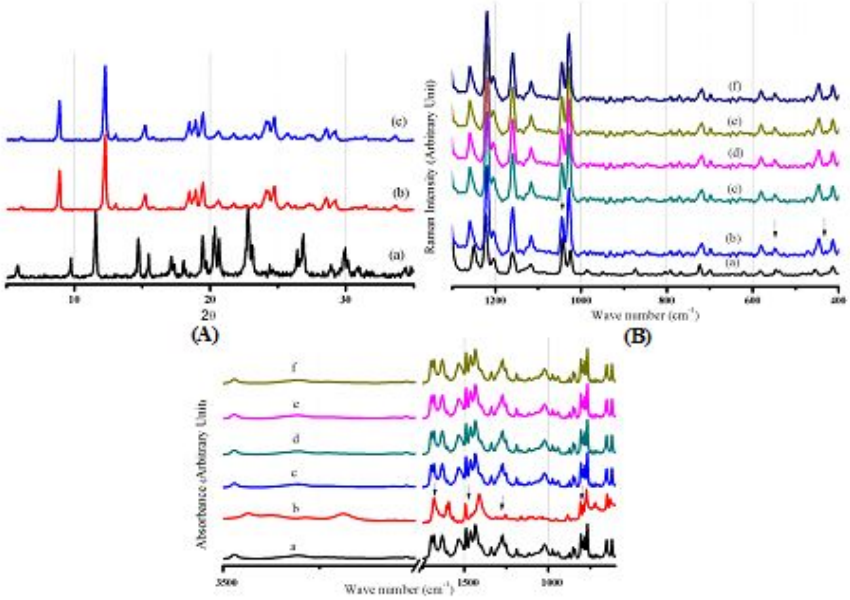

Fig. 2: A) XRD spectra of (a) Phase stable CBZ-SUC in HPMCAS (b,c) Cocrystal crystallized as CBZDH in pre-dissolved PVP-K30/90 B) Raman spectra of (a) CBZDH (reference) (b-f) Phase stable cocrystal in pre-dissolved HPMCAS after 15, 30, 60, 120, 300 min C) FTIR spectra of stable cocrystal till 300 min.

PVP), the CBZ crystallization also occurred as shown by Raman and IR spectral graphs in figs. S_5 & S_6 respectively. Substituting PVP with pre-dissolved HPMCAS in buffer solution, the polymer at concentrations of 0.025% w/v (250 µg.mL^{-1}), stabilized the cocrystals (maintained cocrytal super saturation), for time course studied and gave almost 3-4fold higher CBZ concentrations than its solution stable CBZDH form. Seeding with 1% w/v of dihydrate of HPMCAS stabilized cocrystal in solution was done to assess crystal growth, the metastable system was transformed to dihydrate within 5 min and the solubility gain was gone.

DISCUSSION

As cocrystals phase purity is critical during dissolution experiments, characterization with solid-state and thermal techniques ratified both its formation and phase purity in accordance with published data (Childs *et al*, 2008: Qiao, 2014). The higher aqueous solubility of CBZ-SUC cocrystal than CBZDH has earlier been demonstrated but this corystal endures prompt transformation to its dihydrate when in solution at higher pH (Qiao, 2014).

In order to capture the offered elevated solubility advantage of cocrystal different polymers were selected. At all pre-dissolved concentrations that ranged 0.05 to 2% w/v PVP K-30 & PVP K 90 did not prevent the phase conversion of CBZ-SUC to its dihydrate thus were incapable to upheld CBZ super saturation as shown in Raman and IR spectra (SIF figs. S_3, S_4, S_5 & S_6). Massik *et al*, have shown that PVP did stabilize CBZ against crystallization as dihydrate at 0.1% w/v concentration for 30 min in plain water, though, even at 3% w/v

concentrations the polymer didn't sustain the inhibition after 1 h (El-Massek *et al*, 2006). In another study, pre-dissolved PVP inhibited the transformation of CBZ to the dihydrate form, by delaying the nucleation / crystal growth rate at 1% w/v concentration (Tian *et al*, 2006). Our results didn't comply with these studies, this also suggests that the usefulness of PVP to hinder CBZ phase change in solution is pH dependent. We also confirmed this by experimentation in distilled water (data not shown here).

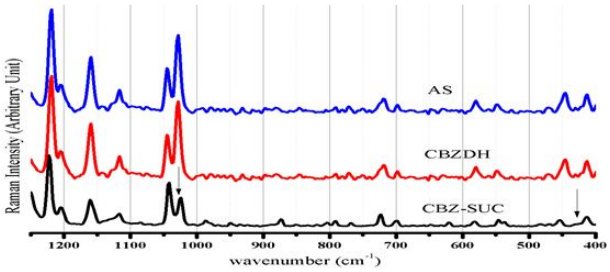

Fig. S_1: *In situ* Raman characterization of CBZ-SUC phase conversion in PB without polymers, Raman spectra of CBZ-SUC and CBZDH are given as reference while AS is added sample of CBZ-SUC to PB.

The CBZ apparent solubility from the cocrystal at variable concentrations of pre-dissolved PVP K-30 & K-90 was marginally improved in buffer, and this increase was noticeable at higher polymer concentrations, though the cocrystal was not stable in solution (figs. 3 A,B). This was caused by the rate changes that existed between the cocrystal dissolution and formation of a soluble CBZ-PVP complex in solution. The cocrystal of CBZ-SUC displayed greater solubility and prompt dissolution rate, consequently, throughout dissolution higher CBZ super saturation was achieved in solution. Even though the soluble CBZ-PVP complex might have stabilized the phase conversion of CBZ, however the ratio of CBZ incoming into the solution from the dissolved CBZ-SUC cocrystal was faster than the ratio of creation of CBZ-PVP complex and thus resulted in CBZDH crystallization.

As pre-dissolved HPMCAS kinetically stabilized the cocrystal and this retained super saturation is of immense significance to evade inconsistent drug absorption as shown in fig. 3 (C) & figs. 2 (A,B,C) . Ueda *et al*. have recently shown that the CBZ crystallization inhibition with HPMCAS was caused by the hydrophobic interactions between the two at molecular levels. Meanwhile, it was also reported that HPMCAS prevented CBZ crystallization at higher pH, caused by the polymer hydrophilization resulting from succinoyl ionization (Ueda *et al*, 2014). From formulation standpoint for cocrystals these outcomes are very promising as carbamazepine polymorphs and its dihydrate differ in their pharmacokinetics *in vivo* (Kobayashi *et al*, 2000). In design of formulation approach for CBZ oral products, control over the polymorphic form(s) is thus critical to accomplish the required biopharmaceutical performance. In our recent published paper utilizing this approach in design of stable CBZ-SUC cocrystal suspension we were able to yield significant *in vivo* performance of cocrystals in suspension than unformulated cocrystal (Ullah *et al*, 2017). The critical 'seeding' experiments indorsed that this system was truly supersaturated and deceptively preserved as 'cocrystal' and still transformed to dihydrate as nucleation was probably inhibited. This explained that this wasn't a thermodynamically stable system, but somewhat a "metastable" system 'that upheld an artificial higher drug concentrations, greater than its solubility (thermodynamic) levels if the system was actually at equilibrium'.

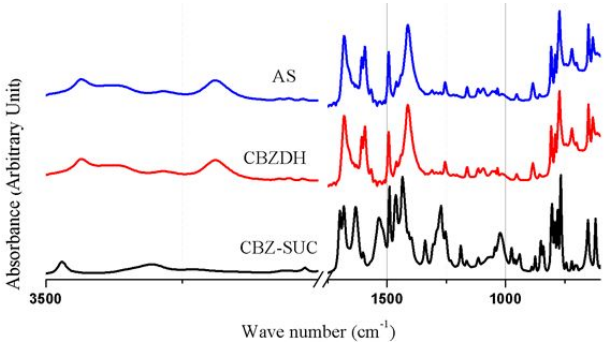

Fig. S_2: IR characterization of filtrate in PB without polymers, IR spectra of CBZ-SUC and CBZDH are given as reference while AS is added sample of CBZ-SUC to PB. Offline IR spectra also confirmed phase conversion of cocrystal in PB.

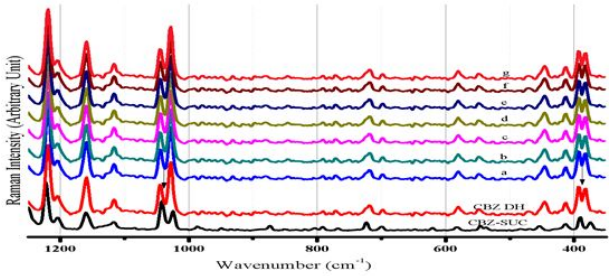

Fig. S_3: *In situ* Raman spectra of cocrystal in varying concentrations of PVP k-30, Raman spectra of CBZ-SUC and CBZDH are given as reference a) 0% b) 0.05% w/v c) 0.1% w/v d) 0.2% w/v e) 0.5% w/v f) 1% g) 2% w/v. At all pre-dissolved polymer concentrations cocrystal converted to DH form.

CONCLUSION

CBZ−SUC cocrystal has been known to yield higher CBZ solubility (metastable supersaturated state), but this enhancement has not yet been captured with suitable crystallization inhibiting polymers because of prompt transformation of the cocrystal to individual coformers. Deferring or deterring this super saturated state of CBZ-SUC can get better dissolution of drug both *in vitro* as

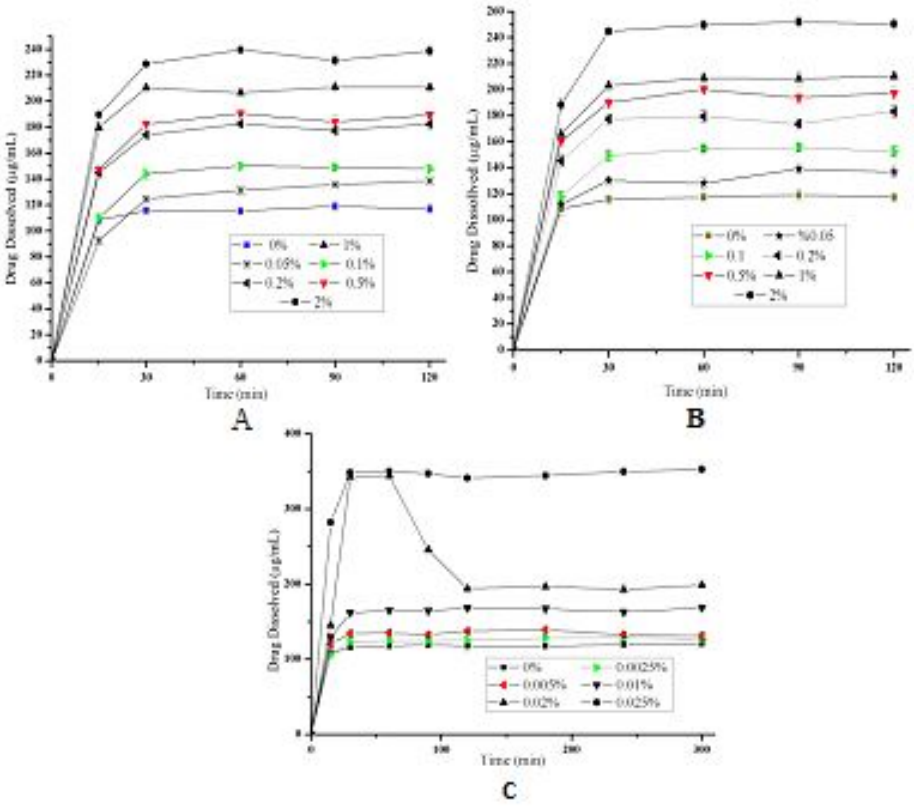

Fig. 3: A). Cocrystal dissolution in pre-dissolved PVP K-30 B). Cocrystal dissolution in pre-dissolved PVP K-90 C). Cocrystal dissolution in pre-dissolved HPMCAS

well as *in vivo* with enabling formulations. We efficiently stabilized this highly unstable system for course of time studied i.e. 5 h, with HPMCAS (HF) having almost 4-fold solubility than the stable CBZDH. The study revealed that with sagacious polymers selection the cocrystal super saturation state can be over extended for a reasonable period for absorption to occur. Seeding of the kinetically stable supersaturated system with 1% w/v dihydrate indorsed that the cocrystal was in 'metastable state' rather than a true thermodynamically stable system.

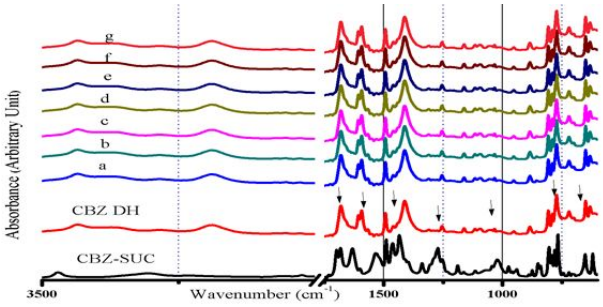

Fig. S_4: IR spectra of cocrystal filtrate after dissolution studies in varying conc of pre-dissolved PVP K-30, CBZ-SUC and CBZDH spectra are given as reference a) 0% w/v b) 0.05% w/v c) 0.1% w/v d) 0.2% w/v e) 0.5% w/v f) 1% w/v g) 2% w/v. IR spectra confirms *in situ* Raman results.

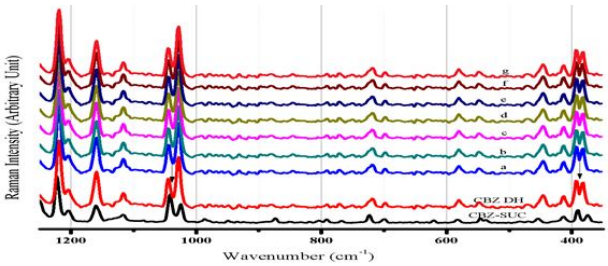

Fig. S_5: *In situ* Raman spectra of cocrystal in varying concentrations of PVP K-90, Raman spectra of CBZ-SUC and CBZDH are given as reference a) 0% b), 0.05% w/v, c) 0.1% w/v d), 0.2% w/v, e) 0.5% w/v, f) 1% w/v g) 2% w/v. At all pre-dissolved polymer concentrations cocrystal converted to DH form.

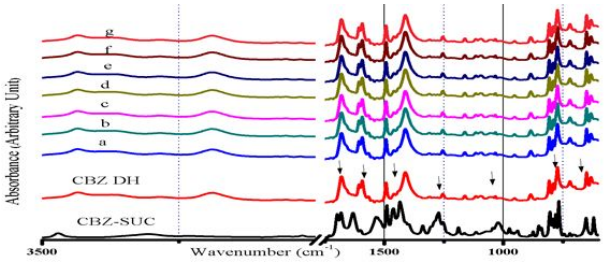

Fig. S_6: IR spectra of cocrystal filtrate after dissolution studies in varying conc of pre-dissolved PVP K-90, CBZ-

SUC and CBZDH spectra are given as reference a) 0% w/v b) 0.05% w/v c) 0.1% w/v d) 0.2% w/v e) 0.5% w/v f) 1% w/v g) 2% w/v. IR spectra confirmed *in situ* Raman results.

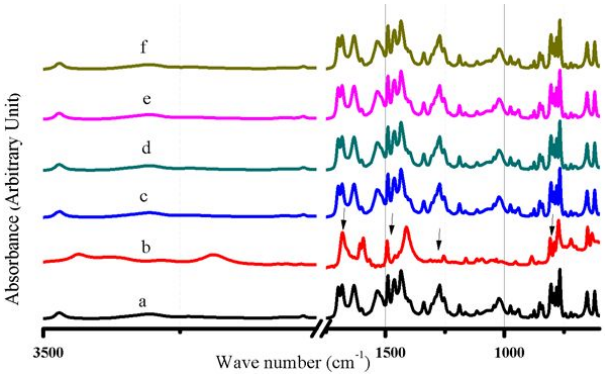

Fig. S$_7$: IR spectra of (a) CBZDH as a reference (b) CBZ-SUC as a reference (c) IR spectra of filterate after 30 min, (d) IR spectra of filterate after 60 min, (e) IR spectra of filterate after 120 min, (f) IR spectra of filterate after 300 min. No phase change observed in HPMCAS till end of experiment

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