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A Novel of Cocrystalization to Improve Solubility and Dissolution rate of Simvastatin

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Abstract : Simvastatin (SV) is the drug of choice for hypercholesterolemia, which belong to the BCS class II as very troubled with drug solubility. We report the novelty of increased solubility and dissolution rate of simvastatin with cocrystallization methods, using Malic acid (MA) as coformer with molar ratios (1:1). Simulation modeling of molecules against MA and SV has performed with in silico using auto dock 4.2. Synthesis of cocrystal has done with Liquid-Assisted Grinding (LAG). Cocrystal formed subsequently confirmed by tests of saturated solubility, invitro dissolution, Scanning electron microscope (SEM), Powder X-ray Diffraction (PXRD), Fourier Transformations Infrared Spectrophotometry (FTIR), and Differential Scanning Calorimetry (DSC). Insilico evaluation against the interactions between the MA and SV has demonstrated the existence of hydrogen bonding interactions. Tests of saturated solubility and invitro dissolution of Cocrystal SV: MA (1:1) was indicating an increase in the solubility and dissolution rate of the better as a result of the formation of cocrystal SV: MA (1:1). All characterization against cocrystal SV: MA (1:1) has indicated the formation of a new solid crystalline phase, which differs with SV, MA, and physical mixtures (SV: MA). Cocrystalization has can be used as a method to improve the solubility and dissolution rate of SV.

Keywords : cocrystallization, Simvastatin, Solubility, dissolution.

Introduction

Drugs that quite soluble in water will demonstrate good oral absorption and show a good biological availability. Approximately 40% of drugs on the market showed less soluble in water, this causes the drug to be absorbed more slowly so that the levels of which enter the blood lower than should be[1]. In the pharmaceutical industry, the poor nature of biopharmaceutical includes toxicity and lack of efficacy of the drug is 1% of the major cases of a drug on the market[2]. An effectiveness of drug therapy is highly dependent on the levels of a drug in the blood, thus, the most important nature biopharmaceutical drugs have solubility properties[3]. Approximately 70% of drugs nominee had problems with solubility, so this is a challenge in the pharmaceutical manufacturing to develop drugs and preparations for better solubility especially for oral preparations [4]. Based on the solubility and permeability properties in the biopharmaceutical classification system (BCS) is classified into four classes of drugs, including drugs with low solubility is class II, like SV. SV is the drug of choice of

cholesterolemia that included into BCS class II, which is having a problem with solubility[5]. A solubility of SV in water about 30 µg/ml and was believed as a drug that has low solubility and low bioavailability[6].

Several methods have been developed to enhance the solubility rate, dissolution rate and bioavailability of SV such as inclusion complex formation techniques using polymers[7], solid dispersion technique[8], solubilization by surfactant techniques[9] and reduction of particle size with the downsizing of the mill[10]. All methods mentioned above have a weakness such as micronized powder process high energetic surface that indicating deprived flow feature and particle frequently agglomerate[11]. Complexation with cyclodextrin indicates low drug loading, solid dispersion involves the use of a higher amount of polymer and scale up procedure is complicated[12]. Researchers have not been reported knowledge of crystallization applications to improve the solubility of SV. We used a crystallization approach as a simple method in the terms process and procedure as an alternative approach to generating good dissolution profiles of SV as a previous method[13].

Cocrystallization technique has been already a method commonly used in the pharmaceutical field to alter a trait of active pharmaceutical ingredients into a material with the desired properties including solubility 4 and could be applied generally to compound acids, bases and unionized 14. Co-crystal is a complex substance that is solid at room temperature, which is formed by two or more compounds involving a coformer connected by a synthon thus forming supramolecular synthon with certain stoichiometric ratio 15. Relationship of synthon is an interaction noncovalent which include hydrogen bonds, van der walls, electron π-stacking, electrostatic interaction, and halogen bonding 16. SV has a lactone group that contains a carbonyl, hydroxyl, and ether functional groups so it's possible to form hydrogen bonding with proper co-crystal former (coformer) such as malic acid. Interaction of synthon could be predicted by in silico computing as a tool to ensure interaction between active pharmaceutical ingredients (API) and co-former by Gibbs-free energy value and bond distance17, so we use in silico as an effective approach to predict interaction model between SV against MA.

Cocrystal preparation could be done in the following manner such as melting by using hot melt extrusion 18, supercritical fluid (SFC)[19], spray drying[20], the formation of a slurry[21], an ultrasound of the pulping processes [22], with the evaporation of the solvent[23], solid state grinding[24] and liquid-assisted grinding (LAG) [25]. A LAG is much less use of chemical solvents, so it is considered environmentally friendly and requires relatively less energy. This method has good repeatability in the process of formation of crystals [26.] The LAG is considered more simple, easy to do and this method involves physical interaction through grinding and chemical interaction with the solvent involved as a catalyst

Material and Methods

Simulation of interaction molecular modeling

The 3D-chemical structures of the compounds were built using Hyperchem 7.0 (Ref) and energy minimization using MM+. Then, the compound conformations were generated using the Discovery Studio 2.5 with CATALYST best conformation module. CHARMM forced field was adopted for energy optimization . The generated compounds which had higher than 20 kcal/mol as compared to the global minimum for conformation l minimum were rejected. The maximum number of conformations was set to 255 [27].

Molecular docking simulations were performed with AutoDock 4.2[28]. The AutoDockTools (ADT) script was used to convert the ligand PDB to the pdbq format by adding Gasteiger charges, checking polar hydrogens and assigning ligand flexibility. In addition, the ADT was also performed to prepare the protein targets for the simulations. Using ADT interface, the Kollman charges were added for the macromolecule and a grid box of 60 x 60 x 60 points, with a spacing of 0.375 Å.

Synthesis of Cocrystal

Preparation of cocrystal was done by weighing SV (Teva, Belgium) >99%, and MA (Merck, Germany) equivalent to a molar ratio (1: 1) and then carried by grinding a mixture of SV and MA assisted methanol pro analisis (Merck, Germany) as solvent, for 10 minutes and then stored in a water bath for 24 h.

Scanning Electron Microscopy (SEM) evaluation

The morphologies of the samples were analyzed using a scanning electron microscope SEM analysis (JSM6360A, JEOL, USA), samples were mounted on a double-faced adhesive tape, sputtered with platinum. Scanning electron photographs were taken at an accelerating voltage of 5kV.

Saturated solubility evaluation

Weighed dried cocrystal equivalently to SV 100mg each for cocrystal with a molar ratio (1:1) of replication input into the three vials reconstituted with 50 ml of distilled water and then shaken for 24 hours using an agitator shaker, then calculate the amount of the SV was dissolved by validated spectrophotometry Uv-Vis method using Spectrophotometry UV-Vis (Analytical Zena, Germany), the same way was done for pure SV, and a physical mixture of SV : MA (1:1).

In vitro dissolution Evaluation

The in vitro release behaviors of the SV and its cocrystals were measured using a dissolution tester (USP type 2 paddle apparatus). A typical experiment equal consisted of 40 mg crystal powders SV in a 900 ml simulated Intestinal Fluid (less enzyme) pH 6, 8, stirred at 100 RPM. Sampling (5mL) was done until 60 minutes of pre-determined 6-time points, and a fresh 5mL solution was added to the system after each sampling. Each sampled solution was filtered through a syringe filter of 0,45 μm pore size and its UV absorbance were measured at 240 NM. SV concentration was calculated using a pre-constructed calibration curve using validated Uv-Vis Spectrophotometry method (Analytical Zena, Germany).

PXRD evaluation

Powder X-Ray Diffractogram (X Philips Analytical PW1710, USA), patterns were collected using Cu Ka radiation (l = 1.54 Å), a tube voltage of 40kV and a tube current of 40 mA. Data were collected from 2 Θ angle 50 to 480 at a continuous scan rate of 40/ minute.

FTIR evaluation

Samples of the powder laced with crystals of potassium bromide with molar ratio (1:10) and crushed until homogeneous, then compressed with a pressure of 20 Psi using a suppressor plate KBr. Spectra analyzed for a range of wavenumbers 4000-400 cm-1 using a spectrophotometer FT-IR.

Thermal Analysis

Differential scanning calorimetry (DSC) Thermal analysis of the samples was performed on a DSC/TGA *apparatus* (Linseis P.T 200, USA) which was calibrated for temperature and cell constants using indium. Samples (1–3mg) crimped in the aluminum pan were analyzed from 50 -300^{0}C with a heating rate of 10^{0}C/min. Samples were continuously purged with nitrogen at 50 ml/ minute.

Result

In silico evaluation of interactions SV against MA (Figure. 1), showed a lowest Gibbs-free energy generated at -2.4 kcal / mol from all configurations. Bond distance between the SV and MA in the amount of 2.4 Å and 2.9 Å and type of interaction was hydrogen bonding.

Synthesis of cocrystal SV-malic acid 1:1 presented on photo SEM (Figure. 2), showed comparing the range of particle size and surface morphology of pure SV with its co-crystal formed, can be observed co-crystal seen forming more complex globules with higher density. Evaluation of co-crystal SV-malic acid 1:1

Saturated solubility test carried out on pure SV and simvastatin co-crystal : MA (1: 1) , the test results show an increased solubility of co-crystal against pure SV significantly worth two- fold.

Dissolution test carried out to pure SV, cocrystal SV-MA 1: 1 (Figure. 4). Results of in vitro dissolution evaluation showed that co-crystal SV - MA (1: 1) increase two-fold compared to pure SV, co-crystal 89% and pure SV 49%, for 60 minutes.

Spectrum of FTIR showed the hydrogen bonding that occurs between the SV and MA, (Figure. 5), Spectrum overlay between SV, MA and co-crystal SV-MA 1:1 exhibit, the tape showed to widen cocrystal absorption band at wavenumber 3600-3200 cm – 1.

Diffractogram of PXRD (figure.6), showed crystallinity of co-crystal SV–MA (1:1) formed as compared with pure SV and its physical mixture, diffractogram peaks generated by each exhibit distinct peak at an angel 2Ɵ: 110, 35-370, 39-410.

Thermogram (Figure. 7) showed the melting point (endotherm phase) of co-crystal SV-MA (1040C) < pure SV (1220C), enthalpy co-crystal SV-MA 1:1 < SV (87 Joule/g > 203,32 Joule/g).

Discussion

Simulation modeling of molecule In silico

The negative Value of the Gibbs-free energy indicates the likelihood of the reaction between SV and MA can occur to form a cocrystal. Insilico approaches can be used to predict the formation of cocrystal rate based on shape and polarity coformer[29], and only the Thermodynamics of liquid phase coformer that can be used in the screening of coformer for cocrystallization[30]. Interactions that occur between SV and MA is the interaction of hydrogen bonding. Bond distances in the hydrogen bridges formed very close approximately 2.4 Å (figure. 1), this shows the hydrogen bonds are formed. SV has a lactone group that contains a carbonyl group and hydroxyl, ether, and MA has a hydroxyl group and a carboxylic acid, therefore these two compounds can act as donors or recipients of protons to form hydrogen bridges. Structure analysis for designing cocrystal is essential for predicting synthon between fire and coformer[31].

Syntesis of cocrystal SV: MA (1:1)

Synthesis of cocrystal SV: MA (1:1) has been made by the LAG. Photos of SEM (Figure 2) has shown the difference between the surface morphology of SV and his cocrystal. Morphology of cocrystal SV: MA (1:1) have formed a more complex structure with high density, the phenomenon is due to hydrogen bonding between SV and MA, due to hydrogen bonds forming very is dominant in cocristal[32]. Cocrystal synthesis has been carried out using the LAG because this method uses a less solvent so that method is more effective and environment-friendly[33]. This method can also be relied upon to discovery new cocrystal, and the presence of a small amount of liquid phase can increase the rate of formation of cocrystal.[34.]

Evaluation of Cocrystal SV-MA (1:1)

An evaluation of the solubility of saturated, have been carried out against SV pure, physical mixture of SV-MA, and cocrystal SV-TA (1:1) (figure. 4). Evaluation of the solubility of saturated cocrystal SV-TA (1:1) has shown increased solubility up to two-fold compared to the SV, and its physical mixture.An increased solubility due to affinity the solvent against SV more intense and lattice energy decreases with formation of cocrystal [35, 36]

Increase in vitro dissolution profiles are against cocryrstal SV: MA (1:1), due to the interaction of the solvent with the SV is more intensive, cocrystal has been able to increase the release of the behaviours by increasing the affinity of the solvent against dissolved substance and reduce the energy of the lattice[37]. The dissolution profile of cocrystal has the same value with the loadingof the SV in macrocellular foam nanoparticles[38], and an evaporation method using matrix[31], release the drug in 60-minutes about 90%, so crystallization SV has can be considered as an alternative approach to increase the dissolution profiles.

Diffractogram (Figure 5) has produced a diffraction peaks differ between cocrystal SV: MA (1:1), SV, and a mixture of physical (SV: MA) at an angle of 2Ɵ: 110, 35-39-370, 410, a difference of peak produced shows different crystal phase formation between cocrystal SV: MA (1:1), SV and physical mixtures (SV: MA), it also allows the distinction between the nature of physiochemistry SV and SV cocrystal : MA (1:1) in terms of solubility.

Infrared spectroscopy can be an optional tool in detecting the formation of cocrystal, especially for carboxylic acid used as a coformer[39], FTIR of cocrystal evaluation focused on the formation of a hydrogen bond

interactions which occur between SV and MA (Figure 6). Spectrum overlay between pure SV,MA and cocrystal SV-MA 1:1 has shown the peak absorption of cocrystal widens in wave numbers 3600-3200 cm^{-1}, widening very specific absorption peak for hydrogen bonds, in fact, peak absorption of OH will appear in wavenumbers 3500 cm^{-1}. The peak absorption characteristics of SV found at 3.545 cm^{-1} (free O-H stretching), 2.970 cm^{-1} (methyl asymmetric C-H stretching), 1.695 cm^{-1} (ester C = O is stretching, associated), 1.265 cm−1 (lactone-C-O-C stretch) [23],at the spectra of cocrystal SV-MA (1:1), The peak of SV still appears, only the intensity of the peak has been reduced.

DSC thermogram (Figure 7) has shown the melting point of cocrystal lower than pure SV, this has can be observed from the endotherm phase of thermogram has shown the melting point compounds both SV and cocrystal. The melting point of cocrystal will fall between the melting point (SV) and coformer (MA) 2, if coformer eutectic < eutectic API, then the Solubility of cocrystal > solubility of FIRE 40. A decrease in melting point will be directly correlated to the level of solubility and dissolution, therefore, the rate of dissolution of co Crystal SV-MA (1:1) is greater than the rate of dissolution of the SV.

Conclusion

Synthesis of co-crystal SV using MA (1: 1) can be made by solvent drops grinding well, the solubility test and characterization of co-crystal exhibit the rate of dissolution cocrystal SV - MA (1: 1) increase two-fold compared to pure SV, and all confirmations against cocrystal exhibit formation the new solid crystalline phase. Based on a result of SV-MA (1:1) co-crystallization we consider that crystallization technique can be use as an alternative approach to increase the dissolution rate of SV.

Figure :

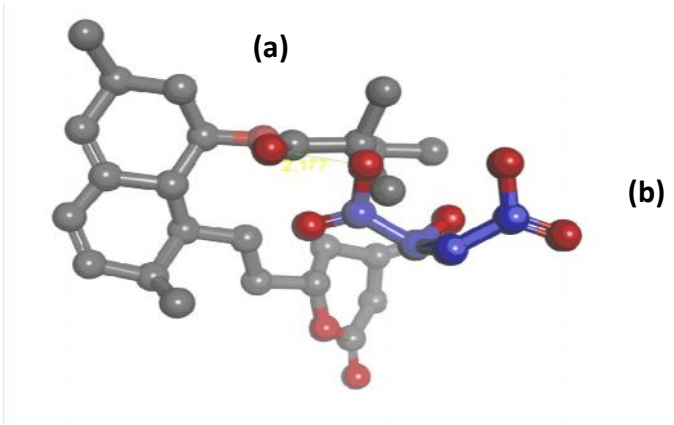

Figure 1. In silico Interaction Molecular Modeling of Co-crystal SV (a)- MA (b) using Molar Ratio (1:1)

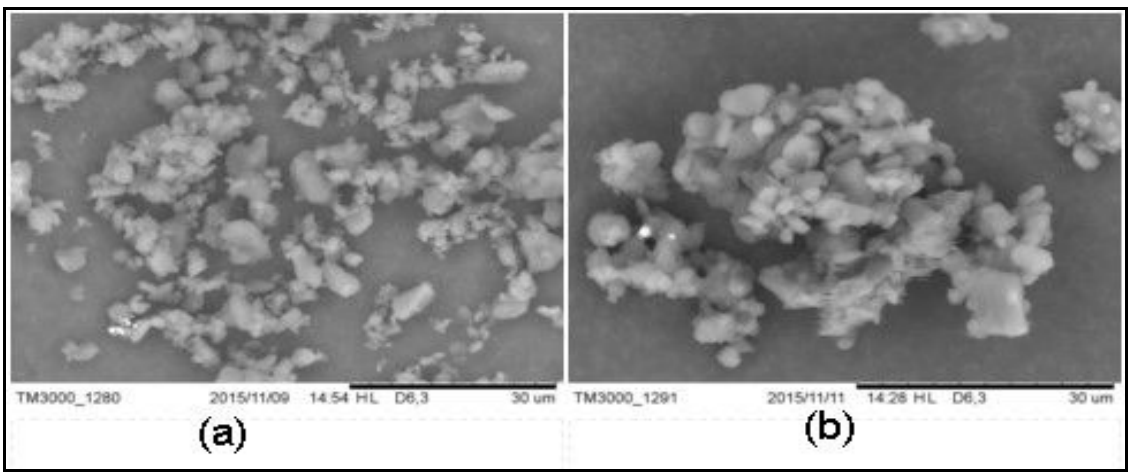

Figure 2. Photo of SEM SV (a), cocrystal simv-malic acid 1:1 (b)

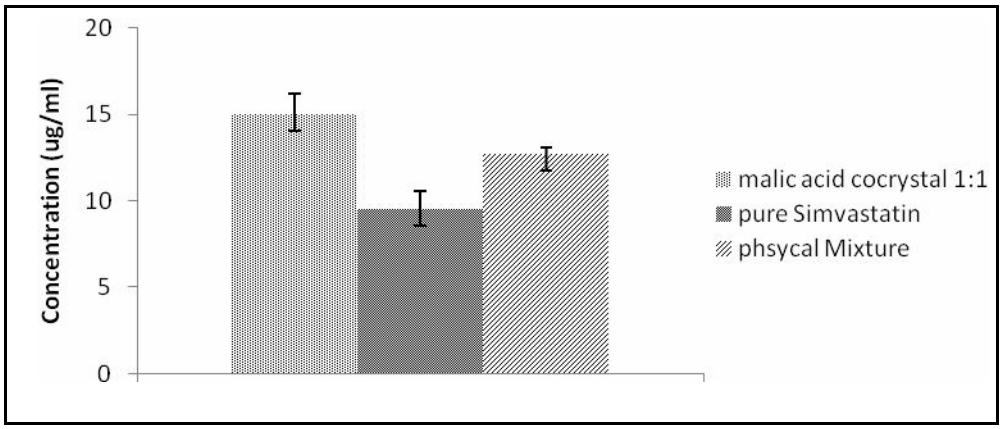

Figure 3. Solubilty profile of cocrystal SV: MA (1:1), Pure SV, and Phsycal Mixture (SV:MA)

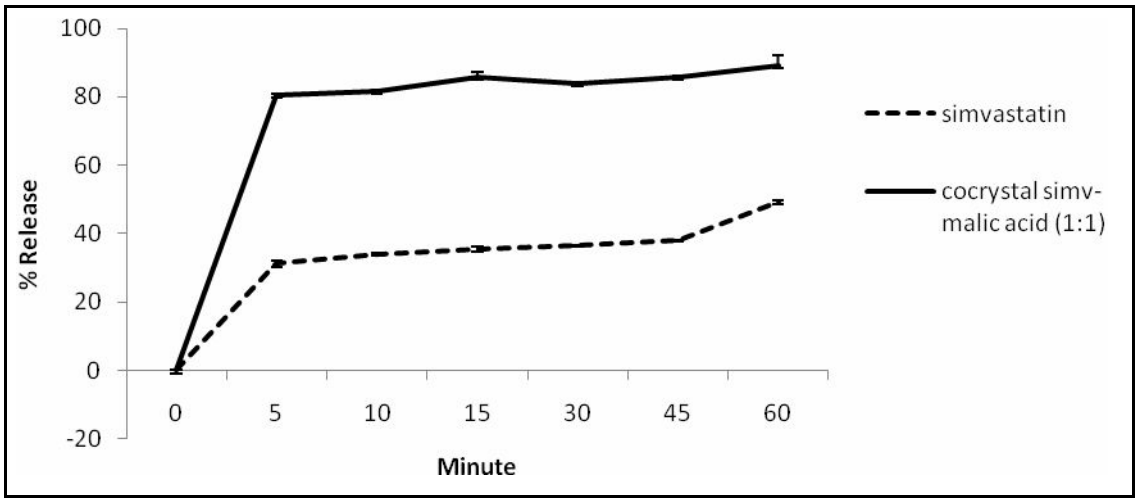

Figure 4. Dissolution Profile of Pure SV(-----),Cocrystal SV-Malic acid 1:1 () ___

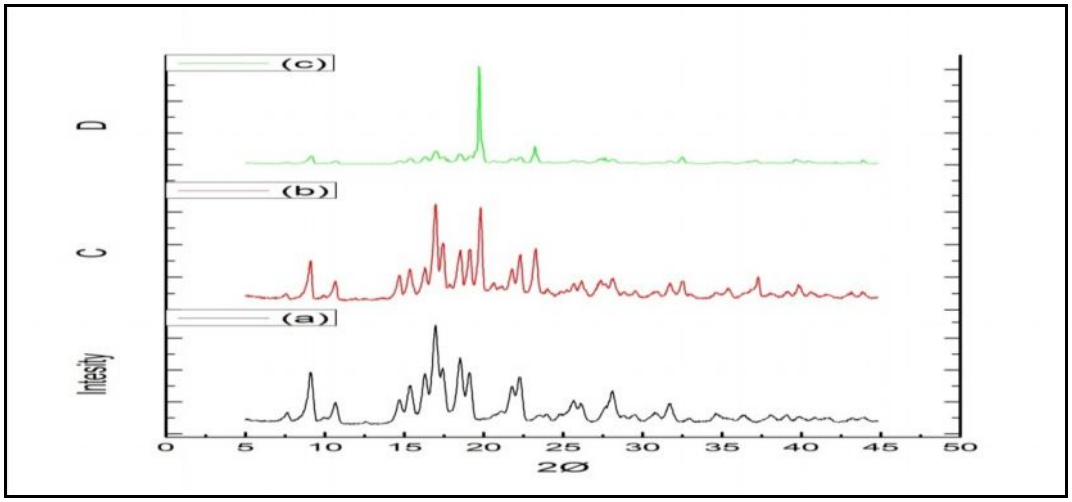

Figure 5 Difractogram of simvastatain (a), Cocrystal SV-MA 1:1(b), Physical mixture of SV-MA (1:1) (c)

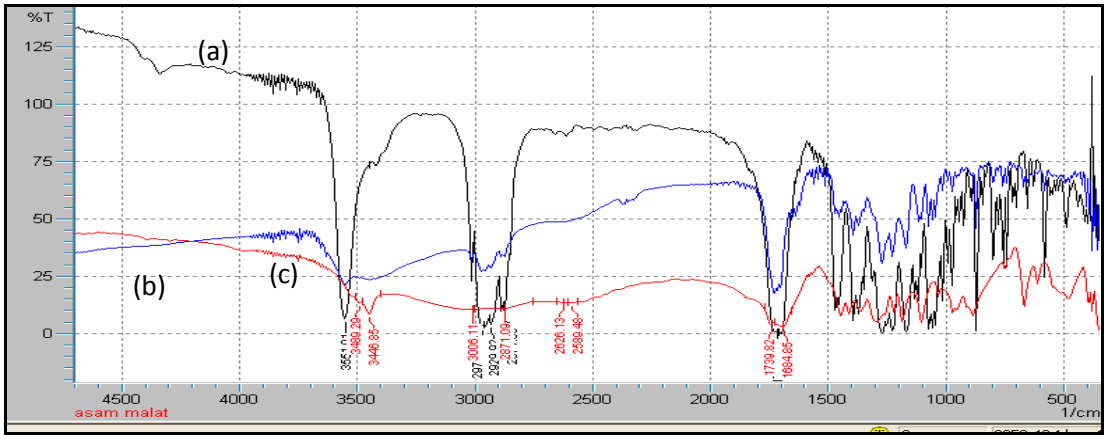

Figure 6. Spectrum Overlay of SV (a), malic acid (b), and cocrystal SV: MA 1:1 (c)

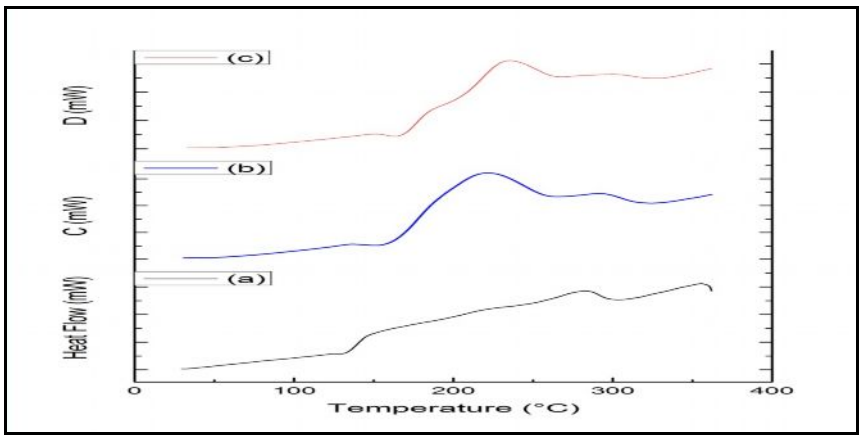

Figure 7. Thermogram (a) cocrystal SV-MA (1:1), (b) Physical mixture cocrystal SV-MA (1:1), (c) Pure SV

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