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Original Research Article

Simvastatin-nicotinamide co-crystal: design, preparation and preliminary characterization

Iyan Sopyan[1,2], Achmad Fudholi[2]*, Muchtaridi Muchtaridi[3] and Ika Puspita Sari[4]
[1]Department of Pharmaceutical and Technology Formulation of University of Gadjah Mada, Yogyakarta, 55281, [2]Department of Pharmaceutical and Technology formulation. Faculty of Pharmacy, University of Padjadjaran, Bandung, Indonesia, [3]Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, University of Padjadjaran, [4]Department of Pharmacology and Clinical Pharmacy, Faculty Pharmacy, University of Gadjah Mada, Yogyakarta, 55281, Indonesia

For correspondence: **Email: i.sopyan@unpad.ac.id; **Tel:** + 62 (022) 7796200; **Fax:** +62 (022)7796200*

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Abstract

Purpose: *To improve the solubility of simvastatin (SV) by co-crystallization using nicotinamide (Nic) as co-crystal agent (co-former).*
Methods: *In silico molecular modeling of Nic counter to SV were investigated using Auto Dock 4.2. Co-crystal of Nic-SV was obtained by solvent evaporation (SE) using an equimolar ratio of Nic and SV. Co-crystal of SV-Nic was evaluated by scanning electron microscopy (SEM), saturated solubility, intrinsic dissolution, x-ray powder diffraction (XRPD), differential scanning calorimetric (DSC), infrared spectrophotometry (FT-IR), binary phase diagram, and for stability at 40 oC and relative humidity (RH) 75% in one month.*
Results: *In silico results showed that the interaction of Nic with SV took place through hydrogen bonding as the synthon agent. The solubility and intrinsic dissolution properties of the co-crystal improved significantly compared to pure SV. Characterization of the co-crystal SV: Nic (1: 1) by SEM, XRPD, DSC, FT-IR, and binary phase diagram indicate the formation of a new solid phase that was different from either SV or Nic. Furthermore, the cocrystal of SV: Nic remained stable for one month.*
Conclusion: *Co-crystallization using Nic has the potential to enhance drug solubility, intrinsic dissolution, and the stability of solution.*

Keywords: *Simvastatin, Co-crystal, Nicotinamide, Solubility, Dissolution*

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INTRODUCTION

The effectiveness of drug delivery to a target organ or system in the body depends on the capability to produce suitable formulation of the drug. Deficiencies in the possessions of the solid pharmaceutical ingredient, such as solubility and bioavailability, have proved to be a major stumbling block in the successful manufacturing of medicines. The bioavailability of an oral preparation depends on its solubility which determines how quickly it is absorbed in the gastrointestinal tract and its permeability over cell membranes [1]. In pharmaceutical practice, many drugs show poor solubility in water, leading to problems with regard to dissolution and bioavailability [2]. Several drugs, including BCS class II and intravenous (IV) drugs like simvastatin (SV), have problems with solubility.

SV is an inhibitor of A (HMG-CoA) reductase; it belongs to the class of statins. Statins are the drugs of choice for the management of hypercholesterolemia due to their recognized

efficacy and safety profile [3]. However, SV has a low solubility at about 30 µg/mL; moreover its bioavailability is only 5 % [4]. Several methods have been developed to improve the solubility of SV, such as solid dispersion [5], reduction of particle size micro emulsion [6], addition of a surfactant [7], but these methods have been somewhat insufficient. They have some shortcomings including, the high energy required of the process. Also, they involve the use of a number of matrices, and furthermore the upscaling process is complicated [4].

Co-crystallization is an emerging crystal engineering techniques used for modulating solid-state properties of the API in order to enhance pharmaceutical performance. This is possible since co-crystallization develops new solid forms with structures that are different from the constituent molecules [8]. Co-crystallization is a relatively recent technology. It has been reported to increase the solubility and dissolution profile of a water insoluble drugs [9] and is consequently considered a potential method for enhancing the bioavailability of drugs with low solubility.

Several techniques have been explored to formulate co-crystals, such as melting extrusion [10], supercritical fluids [11], forming a slurry with ultrasound [12], micronize particles [13], solid grinding [14], spray drying [15], and solvent evaporation (SE) [16]. The SE method is widely used in pharmaceutical manufacturing [17]. In the present work, we developed a simple technique of co-crystallization to improve the solubility and dissolution profile of SV using Nic as coformer.

EXPERIMENTAL

Simvastatin was obtained from Teva (Belgium) with purity > 99 %). Methanol pro analysis was obtained from Merck (Germany), and nicotinamide pro analysis was obtained from Sigma-Aldrich (Germany). Furthermore, potassium dihydrogen phosphate pro analysis was sourced from Merck (Germany).

In silico molecular docking simulation

The 3D-structures of SV and Nic were built using LigandScout 4.1 and energy minimization by MM+. Moreover, the compound conformations were produced using the Discovery Studio 2.5 with CATALYST finest conformation module. Chemistry at harvard macromoleculer mechanic (CHARMM forced field was applied for energy optimization. Molecular Docking simulations of the molecules were done as follow Ikram *et al* [18]. The AutoDockTools (ADT) script was directed to convert the ligand protein data bank (PDB) to the protein bank charge partial (PBQ) format. Also, Gasteiger charges were added and polar hydrogen was inspected.

Co-crystal synthesis by solvent evaporation

SV and Nic, accurately weighed in the molar ratio of 1:1 were mixed and diluted assisted by methanol, shaken for 10 min, and later stored in a water bath at 30 ^{o}C for 24 h for drying. The cocrystal thus obtained was stored in room temperature.

Scanning electron microscopy (SEM)

The external morphology of the samples (co-crystals of SV-Nic) was examined using SEM (JSM6360A, JEOL, USA). Samples were placed on a double-handled sticky tape sputtered by platinum. Scanning photos were reserved at an accelerating step of 5 kV.

Saturated solubility studies

Dried co-crystal equivalently to SV 100 mg of reconstituted with 50 mL of distilled water in a vial. It was shaken for 24 h using an agitator shaker, the concentration of dissolved SV was determined using validated Uv-Vis spectrophotometry (Analytical Zena, Germany). The procedure was repeated for pure SV, and physical mixture of SV: Nic (1:1).

Intrinsic dissolution studies

The intrinsic release behaviors of the SV and its co-crystals were determined using a dissolution tester (USP type two: paddle apparatus). A co-crystal equal to 250 mg SV in powders was compressed into pellet forms with hardness 30 KgF, then put into a 900 mL simulated intestinal fluid (less enzyme) pH 4.5 stirred at 100 rpm. Sampling (5 mL) was done at 10 min interval up to 60 min a fresh 5 mL SIF solution was added into the system to replenish withdrawn samples after each sampling. Each sample withdrawn was filtered through a syringe filter of 0.45 µm pore size, and its UV absorbance was measured at 240 nm (spectrophotometer, Analytical Zena, Germany). SV concentration was calculated using a validated pre-constructed calibration curve. The same procedure was repeated for pure SV dissolution procedure.

Fourier transforms infra-red (FT-IR) spectrophotometry studies

Powdered samples were mixed with potassium bromide crystal at the molar ratio (1: 10), and then compressed at a pressure of 20 psi. The spectra were analyzed over a range of wavenumbers 4000-400 cm^{-1}, using FT-IR (Specord 200, Germany).

X-ray powder diffraction(XPD) analysis

The x-ray powder diffractometer (X Philips Analytical PW1710, Germany) arrays were collected using Cu Ka radiation (l = 1.54 Å), a duct stage of 40 kV and a duct current of 40 mA. Records were collected from 2Θ angle 5^o - 48^o at a constant image rate of 4 ^{o}/min.

Thermal analysis

Thermal analysis of the samples was carried out on a DSC/TGA apparatus (Linseis PTA ST 1600, Germany) which was adjusted for heat and cell constants using indium. Samples (2 – 3 mg) crumpled in the aluminium pan were examined from 50-300 ^{o}C at a heating rate of 10 ^{o}C/min. Samples were constantly purged with nitrogen at 50 ml/min.

Preparation of binary phase diagram

The binary phase diagram is made by making the mixture with SV: Nic with mole fraction comparison among SV-Nic (0:10) until (10:0), then each mixture was measured at its melting point, afterward, it was plotted in the charts form.

Stability studies

Profile of co-crystal stability SV: Nic (1:1) was examined thru observing a melting point of the co-crystal that were kept in a storage condition 40 ^{o}C and RH 75 % for one month.

RESULTS

In silico studies (Figure 1) revealed that the lowest Gibbs-free energy of molecule conformation was -2.5 kcal/ mole and distance of the bonding was 2.168 Å.

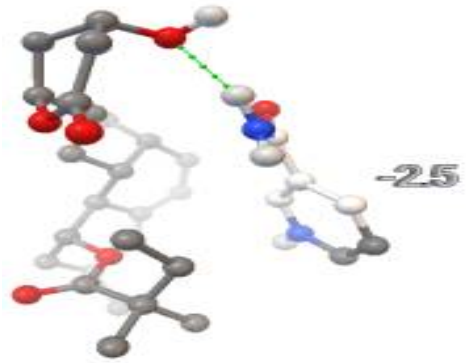

Figure 1: *In silico* molecular interaction model of SV and Nic

SEM of co-crystal of SV-Nic (1:1)

SEM (Figure 2) show particle size and surface morphology of pure SV and co-crystal. Co-crystal of SV: Nic (1:1) presented a more compact structure with a higher density.

Saturation solubility

Result of the saturated solubility of co-crystal show a threefold increase compared to SV and the PM. Cocrystal SV-Nic 1:1 reached 30 µg/mL and SV was 9 µg/mL.

Intrinsic dissolution

The dissolution value of the co-crystal SV-Nic (1:1) shows an increase by approximately four times (400 %) compared to the value of pure SV over a 60 min period (Figure 3)

FT-IR spectra

The spectrum overlay of pure SV, Nic and co-crystal SV-Nic (1:1) show the widening of the co-crystal absorption at wavenumber 3600 - 3200 cm^{-1} (Figure 5).

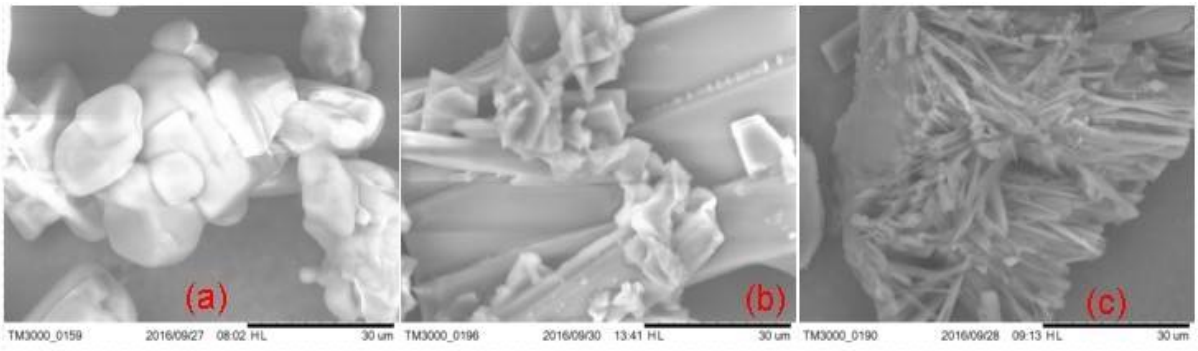

Figure 2: SEM of Nic (a), SV (b), Co-crystal SV-Nic (c)

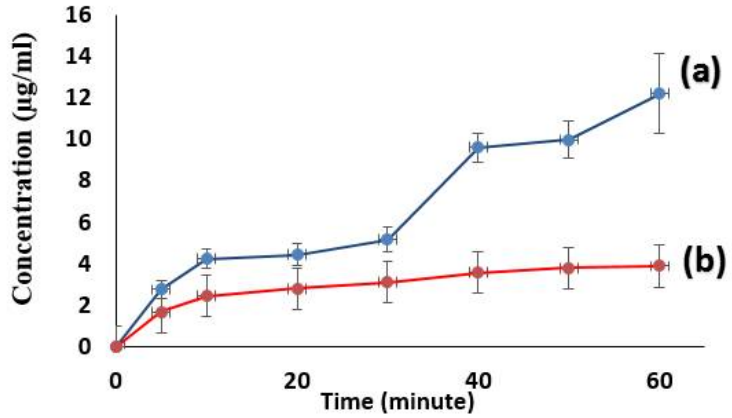

Figure 3: Intrinsic dissolution profile of co-crystal SV-Nic 1:1 (a) and pure SV (b) (n= 6)

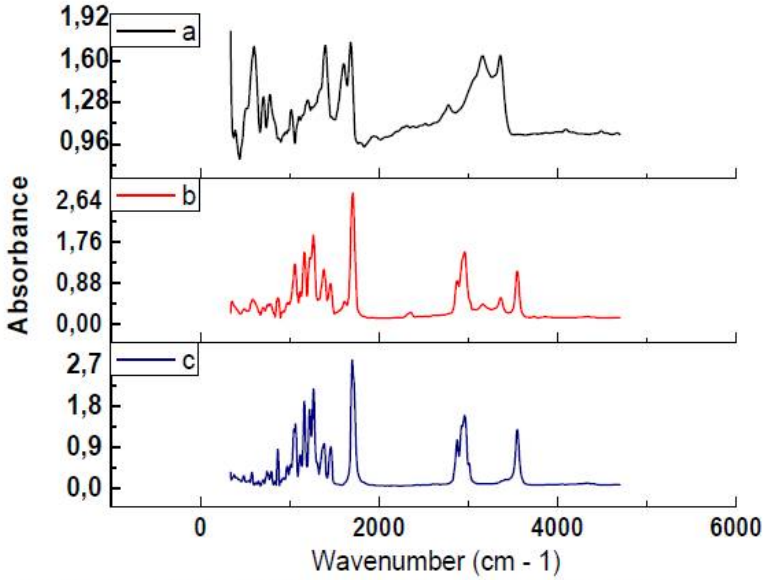

Figure 4: Spectrum of Nic (a), Co-crystal SV-Nic (b) and SV (c)

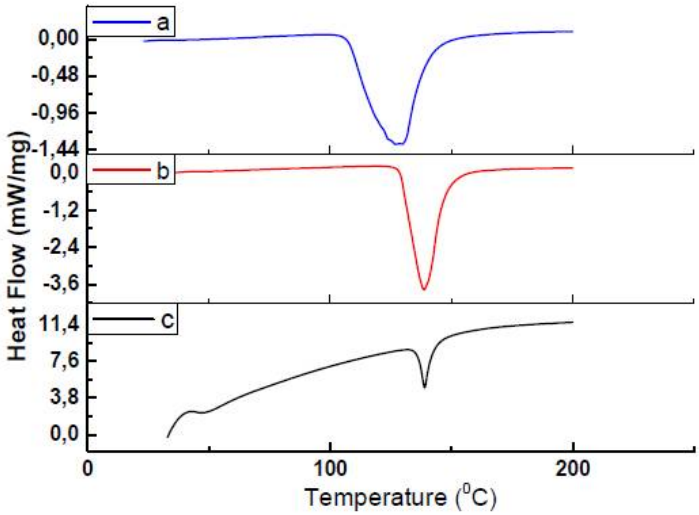

Figure 5: DSC thermogram of co-crystal SV: Nic 1:1 (a), Nic (b), and SV (c)

Thermal properties

The thermogram (Figure 5) reveal that the melting point (endotherm phase) of co-crystal SV-Nic (105.7 °C) **<** pure SV (135.8 °C). The enthalpy of SV (-74.4 Joule/g) **>** co-crystal SV-Nic 1:1 (-80.68 Joule/g).

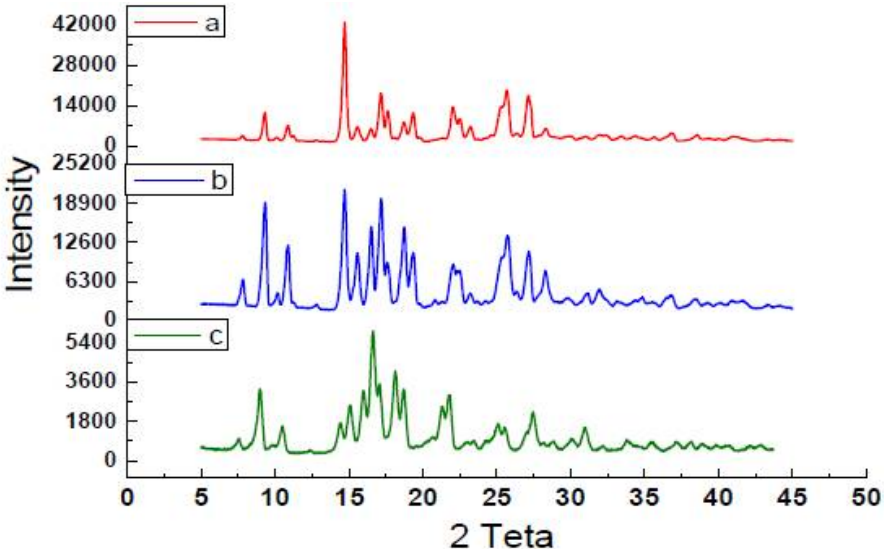

Figure 6: Diffractogram of SV (a), co-crystal SV: Nic 1:1 (b), and PM of SV: Nic (c)

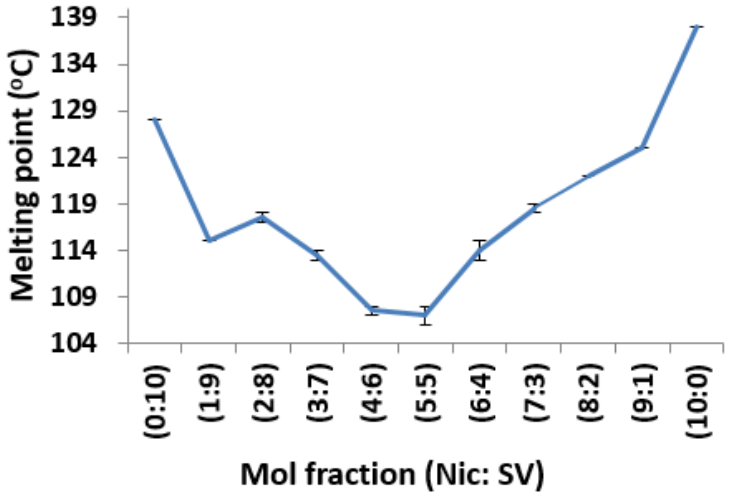

Figure 7: Binary phase diagram of (Nic: SV)

XPD

The XRPD verify the formation of co-crystal of SV: Nic (1:1) as compared to SV and its PM. The diffractogram show distinct peaks and intensity at 17 - 20°, and 20 - 30°. (Figure 6).

Binary phase diagram

The binary phase diagram (Figure 7) showed distinct eutectics points at the mole fractions of (4:6), (5:5) and (6:4). At (5:5) point, the melting point reached the lowest value.

Stability

The thermograms (Figure 8) showed no change in melting point at 105.5 °C Co-crystal for 1 month in storage conditions of 40 °C and RH 75 %

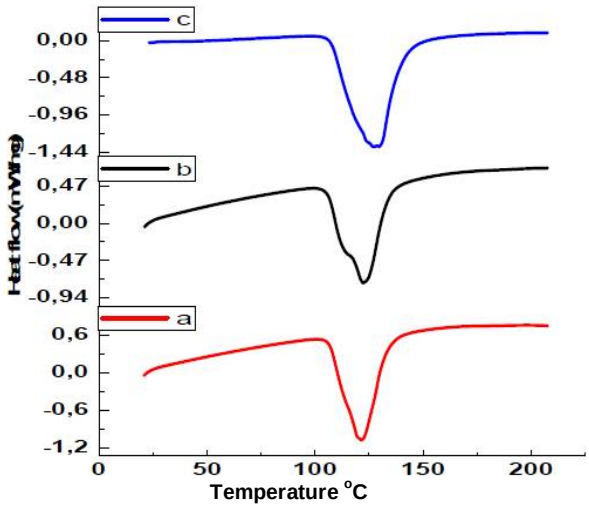

Figure 8: Thermogram overlay of co-crystal SV:Nic (1:1) for 10 day (a), 20 day (b), and 30 day (c)

DISCUSSION

In silico studies by molecular modeling have been performed as a simple tool to study the hydrogen bonding of the SV and Nic [19]. The negative value of Gibbs-free energy obtained through *in silico* simulation modeling indicates that the interaction of SV and Nic occurred spontaneously. The interaction of SV and Nic takes place through hydrogen bonds that have been categorized as the hetero-synthon for this co-crystal. SV and Nic shows very close bonding and also allows the existence of the hydrogen bonds. SE method was used because it yielded co-crystals of a high degree of purity using co-crystal constituents in an equimolar ratio, and the solvent also served as the catalyst [1].

SEM showed that the co-crystals had a more compact structure with higher density. This was due to the hydrogen bonding interaction of the SV and Nic in co-crystal form. Previous studies have also indicated that a hydrogen bonding has an important part in the molecular interaction of co-crystallization [20]. Nic was chosen as the co-former for considerations such as polarity, availability of synthon and stability. The increase in the solubility and dissolution rate was caused by the intensified affinity of the solvent to SV. It was initiated by the existence of the co-former, apart from the decrease in the energy of the crystal lattice via co-crystal formation [12].

FT-IR is a general spectroscopic approach that is very effective to determine co-crystal, since the formation of a co-crystal involves hydrogen bonds [21]. The spectrum has shown a broadening peak as a result of the intermolecular hydrogen bonding, and this is especially applicable to the peak at 3.545 cm^{-1}. It is specifically related to intermolecular hydrogen bonding formation.
A decrease in the melting point and heat content of the co-crystal will directly correlate to increased solubility of the API in the co-crystal. The melting point of the co-crystal would fall between the melting points of the API and its co-former [22].

X-ray powder diffraction is a specific technique to confirm whether the new solid state and the pharmaceutical co-crystal are prone to forming isostructural phases [23]. The intensity of the emissions from the co-crystal showed a decreasing tendency in intensity due to changes in crystal habits [24]. Consequently, a number of distinct co-crystal peaks were obtained as compared to the PM, thus indicating a different structure.

The binary phase diagram exhibited three lowest points forming a W pattern. This may be interpreted as the pattern of co-crystal habits [25]. DSC was used to observe the stability of the co-crystal, as studying the temperature and relative humidity is the most established method to assess stability in solid state [26]. The melting point and XRPD pattern on days 10, 20 and 30 respectively showed constant values. It was confirmed that the co-crystal remained stable for a term of one month under storage conditions of 40 ^{0}C and RH 75 %.

CONCLUSION

Preparation of the co-crystal of SV with co-former Nic (1:1) has been successfully carried out using solvent evaporation method. Evaluation of the saturated solubility and intrinsic dissolution of SV: Nic (1:1) co-crystal indicate that the solubility rate and release behaviour of SV-Nic co-crystal (1: 1) is significantly than the pure SV.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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