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Characterization of acyclovir-isonicotinamide cocrystal by solvent evaporation method with ethanol and glacial acetic acid

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ABSTRACT

Background: Acyclovir is used as the first-line therapy for genital herpes simplex virus, but acyclovir has low solubility and bioavailability. Acyclovir has a water solubility of 2.5 mg/ml at 37°C and belongs to Class IV according to the bioavailability classification system. Cocrystalization is a technology that can be used to improve the solubility of materials by modifying the physical properties of materials, such as melting points, stability, mechanical properties, and polymorphisms of materials. **Materials and Method:** This study aims to prepare cocrystal of acyclovir with isonicotinamide as coformer using different solvents, namely, ethanol and glacial acetic acid by solvent evaporation method. The crystalline formed was characterized using differential scanning calorimetry, powdered X-ray diffraction, Fourier transform infra-red, and scanning electron microscope. **Result:** The results showed a change in the melting point, the formation of a new peak on the diffractogram, a shift in the IR spectrum, and a change in the shape and surface of the crystal morphology. **Conclusion:** This research shows that acycolvir and isonicotinamide can form cocrystal using ethanol and glacial acetic acid solvents through the method of solvent evaporation.

Keywords: Acyclovir, cocrystal, ethanol, glacial acetic acid, isonicotinamide, solvent evaporation

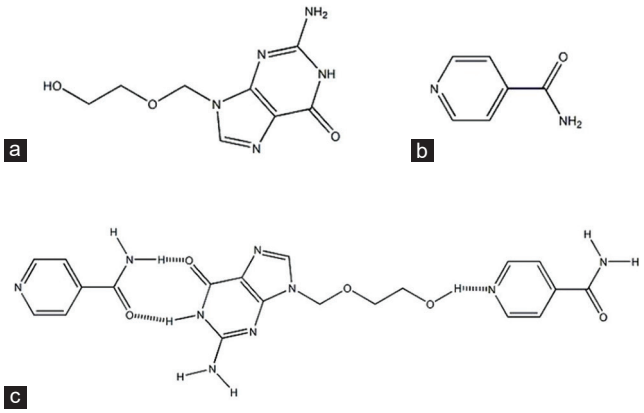
INTRODUCTION

Acyclovir is a drug that is categorized in Class IV according to the bioavailability classification system classification (the solubility in water is 2.5 mg/ml at 37°C) and has low bioavailability.[1] The World Health Organization recommends acyclovir as the first-line therapy for genital herpes simplex virus.[2]

The bioavailability of drugs can be improved by conducting physical engineering to increase the solubility of the material. Cocrystal formation can be used to modify the physical properties of materials, such as melting points, stability, mechanical properties, and polymorphisms of materials, as well as more stable properties compared to the amorphous form Karagianni *et al*.[3] The formation of acyclovir cocrystal is proven in studies conducted by Winantari *et al*. with the formation of acyclovir-succinic acid (1:1) and can increase the solubility of the material in the dissolution test conducted by Winantari *et al.* in acyclovir-nicotinamide (1:1) crystal.[4]

Cocrystal is composed of active ingredient and coformer which held together by supramolecular synthons. In supramolecular synthons approach, steps involved in developing cocrystals are selecting the target molecule (active ingredient), finding the ingredients which are capable of form a hydrogen bond (coformer), and methods of preparation.[5] In addition, cocrystal can be made from non-ionizable drugs which cannot undergo salt formation.[6] Based on the chemical structure, one of the ingredients that can carry out these bonds is isonicotinamide [Figure 1b], due to the N Pyridine group and carboxylate act as acceptors and primary amine groups as acceptors and donors.[7] Acyclovir [Figure 1a] as an active ingredient also has a donor group and an acceptor in its carboxylic group and a primary amine group and a secondary amine group so that hydrogen bonds can be made between the two materials.

Interaction of active ingredient with coformer can occur in the state of dissolved substances and concentrated conditions through the process of evaporation of the solvent,



AQ3 **Figure 1:** Chemical structure of acyclovir (a), isonicotinamide (b), and possible interactions between acyclovir-isonicotinamide (c)

crystallization can occur.[8] Therefore, one of the methods used to produce good quality crystals with high purity that can be used is solvent evaporation method.[3] In this method, both active ingredient and coformer are dissolved stoichiometric in a suitable solvent and allowed to evaporate at room temperature.[9] During evaporation process, there will be hydrogen bonding reaction between molecules.[10] This method requires simple equipment and produces high quality and high purity cocrystals.[3] The solvents that used to dissolve materials are ethanol with a dielectric constant of 24.9 and glacial acetic acid with a dielectric constant of 6.2. The difference in the dielectric constant of a solvent can affect the solvent adsorption on the crystal lattice and affect the nucleation stage and crystal growth.[11] The results of cocrystal formed were characterized using powder X-ray diffraction (PXRD), Fourier transform infrared (FTIR), differential scanning calorimetry (DSC), and scanning electron microscope (SEM).[3]

MATERIALS AND METHODS

Materials

The materials used in this study are acyclovir (Sigma-Aldrich®, US), isonicotinamide (Sigma Aldrich®, US), ethanol, and glacial asetic acid, (Emsure® Merck, Germany).

Method and Procedure

Preparation of binary phase system of acyclovir-isonicotinamide

A binary phase diagram was made to predict the accurate ratio in forming cocrystal. Materials (acyclovir and isonicotinamide) are weighed according to a predetermined molar ratio (10:0; 9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8; 1:9; and 0:10). After each ingredient is weighed then physically mixed in a mortar. The physical mixture formed was analyzed using DSC. Ratio acyclovir-isonicotinamide (1:1) was predicted to form cocrystal based on melting point be showed between two eutectic point.

Preparation of acyclovir-isonicotinamide cocrystal with ethanol and glacial acetic acid by solvent evaporation

Acyclovir and isonicotinamide weighed with a molar ratio (1:1). Two hundred twenty-five milligrams of acyclovir and 112 mg of isonicotinamide are added to the cup and then dissolved using ethanol. The solution is stirred using a magnetic stirrer with room temperature conditions for one night to form crystals. The crystals that have been formed are collected and put into vials then stored at ± 40% relative humidity. The same process is carried out in the manufacture of cocrystal with glacial acetic acid solvent.

Characterization

PXRD

Crystal lattice formed was analyzed using PXRD (Philip X'Pert, Netherland). Target conditions needed in the analysis are Cu, Kα filter, 40 kV voltage, and 15–30 mA carried out at 2θ = 5–40°.

FTIR

Crystal be analyzed using FTIR (Jasco FT-IR/5300, Japan) with KBr (potassium bromide) in a ratio of 1% w/w at a wavelength of 400–4000/cm.

DSC

Crystals to be analyzed were weighed 5–7 mg then analyzed using DSC (Mettler Toledo DSC 1 Star®System, Switzerland) in the temperature range of 30–300°C (heat rate 5°C/min).

SEM

Samples were placed in a container and then coated using aluminum gold with a thickness of 10 nm. Samples were analyzed using SEM (FEI Inspect 550, USA) set at 20 kV and 12 mA.

RESULTS AND DISCUSSION

The phase diagram of acyclovir-isonicotinamide mixture was made using different molar ratio. Figure 2 showed a congruent binary phase formation. The mixture of each material has decreased melting point at 145.06°C in the molar ratio of acyclovir-isonicotinamide 6:4, this point is referred to as the eutectic point 1. Then, the addition of the isonicotinamide showed the melting point of the mixture increases at 184.03°C in the molar ratio of acyclovir-isonicotinamide 5:5 (1:1 molar equilibrium). In this molar ratio, an equilibrium point occurs between solid and liquid.[4] The addition of the isonicotinamide will reduce the melting point of the mixture at 145.23°C in the molar ratio of acyclovir-isonicotinamide 3:7, this point is referred to as the eutectic point 2. Based on these data, the cocrystal formation of acyclovir-isonicotinamide used molar ratio of 1:1.

Analysis using DSC is used to observe the thermodynamic properties of a material, by providing thermal energy.[4] Thermogram of DSC is shown in Figure 3. Acyclovir and isonicotinamide as a single substance have an endothermic peak with temperature and enthalpy values at 243.25°C, −109,76 J/g and 156.28°C, and −199,03 J/g, respectively. Acyclovir-isonicotinamide (1:1) cocrystal using glacial acetic acid showed an endothermic peak at 118.29°C, 152.39°C, 184.85°C, and 217.42°C. Endothermic peak at a temperature of 118.29°C shows the process of hydrate release/solvent desolvation process.[12] At a temperature of 152.39°C is estimated to be a crystal with effect of isonicotinamide because

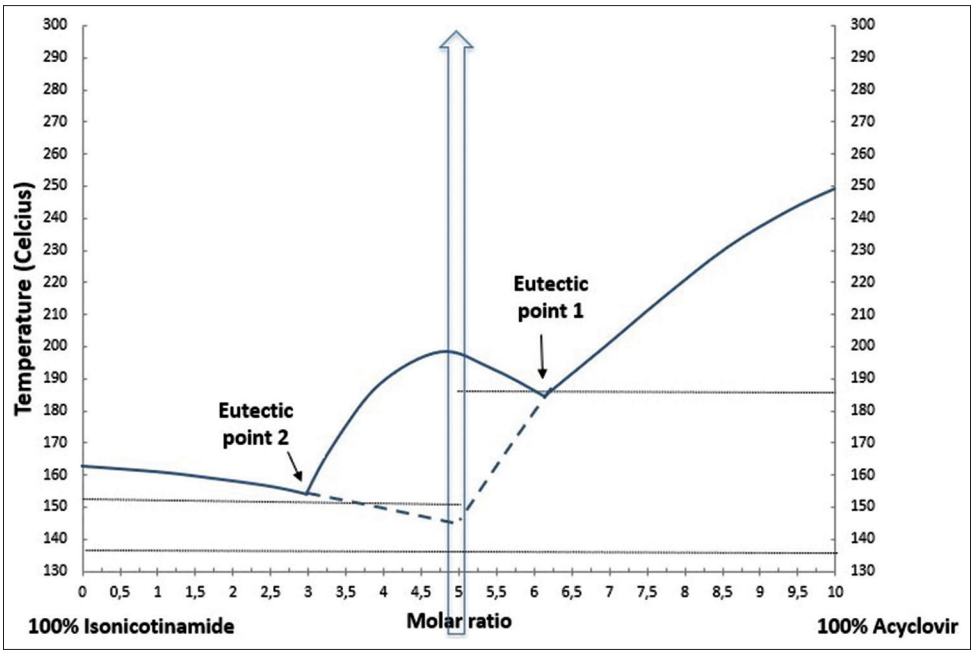


Figure 2: Phase diagram of binary system acyclovir-isonicotinamide with various compositions

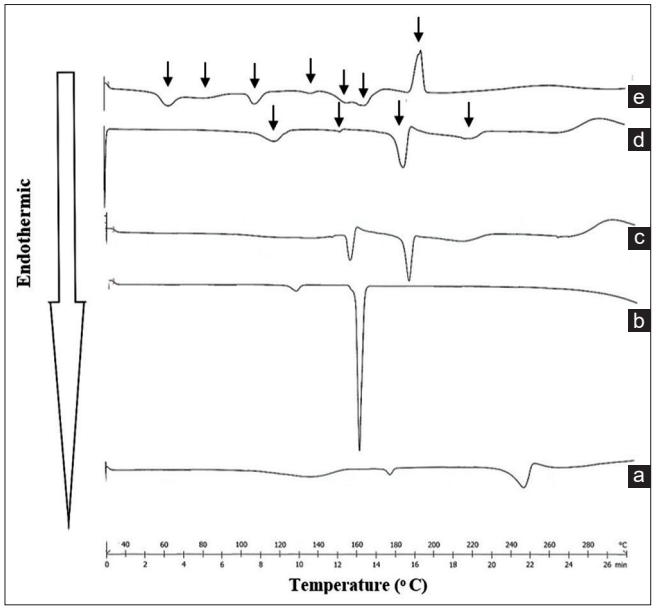


Figure 3: Thermogram of acyclovir (a), isonicotinamide (b), physical mixture of acyclovir-isonicotinamide (1:1) (c), cocrystal of acyclovir-isonicotinamide (1:1) with glacial acetic acid (d), and cocrystal of acyclovir-isonicotinamide (1:1) with ethanol (e)

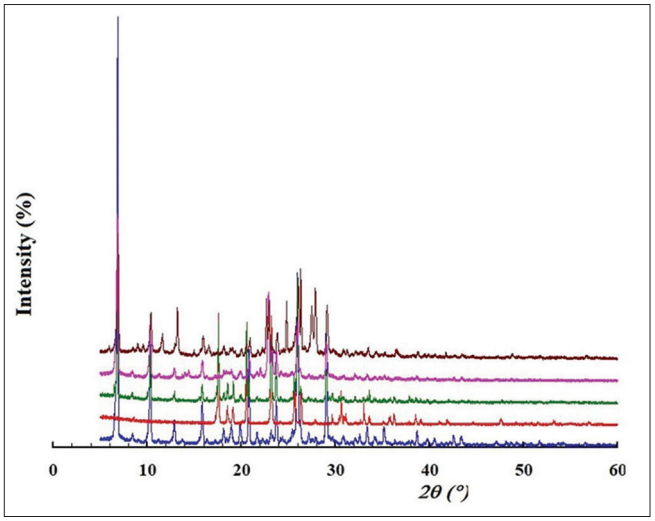


Figure 4: Diffractogram of acyclovir (a), isonicotinamide (b), physical mixture of acyclovir-isonicotinamide (1:1) (c), cocrystal of acyclovir-isonicotinamide (1:1) with glacial acetic acid (d), and cocrystal of acyclovir-isonicotinamide (1:1) with ethanol €

it has a melting temperature similar to isonicotinamide, a temperature of 184.85°C is estimated as cocrystal, identically with the temperature estimate in a physical mixture (1:1)[4] and lies between the melting points of acyclovir and isonicotinamide. Acyclovir-isonicotinamide (1:1) cocrystal using ethanol give an endothermic peak at 62.80°C, 83.02°C, 107.76°C, 136.70°C, 154.24°C, and 164.85°C and the exothermic peak at 194.52°C. The temperature of 83.02oC explains the change in from acyclovir form (3:2 hydrate) to form 3.[13] Temperature of 136.70°C indicates the existence of new crystalline pseudopolymorphs, while a temperature of 164.85°C is estimated to be a cocrystal formed. From these results, it is estimated that the manufacture of acyclovir-isonicotinamide (1:1) cocrystal using glacial acetic acid and ethanol can provide new thermodynamic properties. In addition, the enthalpy values of acyclovir and isonicotinamide peaks in acyclovir-isonicotinamide (1:1) using glacial acetic acid thermogram were lower than their constituents to −95.95 J/g and −1.82 J/g. This was also the same for ethanol that the enthalpy values decreased to −5.72 J/g. This estimated that acyclovir-isonicotinamide (1:1) using glacial acetic acid and ethanol will provide a better solubility than the constituent because crystals with higher enthalpy are less

soluble in water than the pure materials.[14] The information about enthalpy of active ingredients and coformer allows predicting the potential cocrytals with obviously higher active ingredients solubility values than the pure ingredients.[15]

Analysis using PXRD can showed changes in the crystal lattice. Each material has a different and specific diffraction pattern character, changes in intensity or the formation of a new peak indicate the existence of a new solid form.[16,17] Figure 4 and Table 1 showed that acyclovir-isonicotinamide cocrystal resulted a new peak at an angle of 2Θ = 14.00°, 14.43°, and 22.04° in glacial acetic acid and at an angle of 2Θ = 5.97°, 9.53°, and 13.19° in ethanol. The formation of these new diffraction peaks can be concluded that it is possible for a new solid form (cocrystal). In addition, the new diffraction peaks of acyclovir-isonicotinamide cocrystal with ethanol showed a higher intensity than glacial acetic acid.

Infrared spectrum analysis is shown in Figure 5. Acyclovir has a primary and secondary amine peaks at 3441.35 cm^{-1} and 3313.11 cm^{-1} and NH bend for primary amines at 1633.41 cm^{-1} and 1612.2 cm^{-1} and secondary amine at 1584.24 cm^{-1}, while isonicotinamide has peaks of primary and secondary amine at 3370.00 cm^{-1}, 3302.50 cm^{-1}, and 3188.72 cm^{-1} and NH bend at 1624.73 cm^{-1} and 1595.81 cm^{-1}. The physical mixture showed that the peak of NH group only at 3440.39 cm^{-1}, 3189.68 cm^{-1}, and 1629.55 cm^{-1} implies that amine group was participating

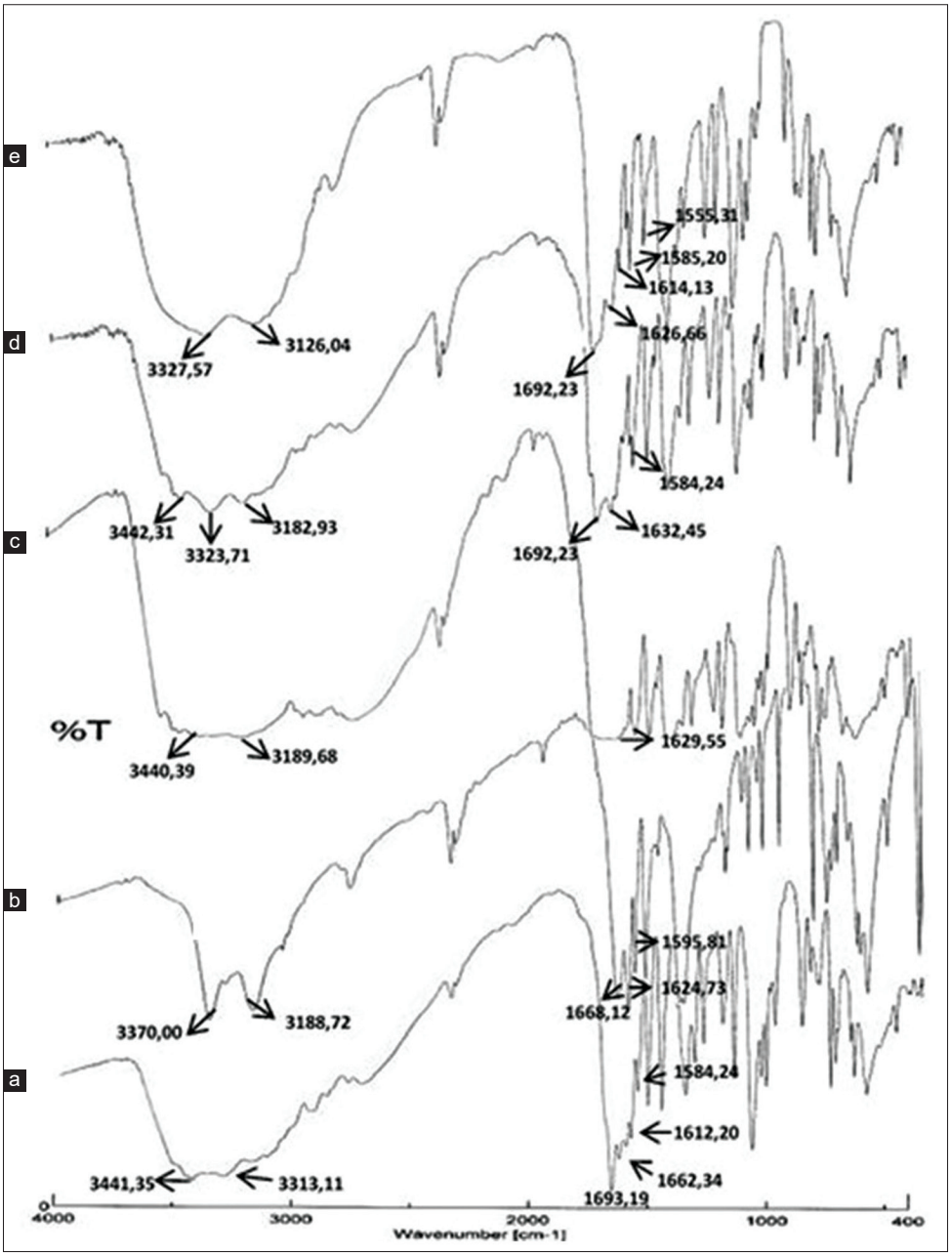


Figure 5: Infrared spectrum of acyclovir (a), isonicotinamide (b), physical mixture of acyclovir-isonicotinamide (1:1) (c), cocrystal of acyclovir-isonicotinamide (1:1) with glacial acetic acid (d), and cocrystal of acyclovir-isonicotinamide (1:1) with ethanol (e)

in hydrogen bonds. There was a peak shift of acyclovir-isonicotinamide (1:1) cocrystal using glacial acetic acid to 3442.31 cm^{-1}, 3323.71 cm^{-1}, 3182.93 cm^{-1}, 1632.45 cm^{-1}, and 1584.24 cm^{-1}. At the peak of 1584.24 cm^{-1}, it was not expected to form a hydrogen bond in the acyclovir secondary amine but in the acyclovir primary amine because the primary amine bend changed the peak shift. Acyclovir-isonicotinamide (1:1) cocrystal using ethanol showed a change at 3327.57 cm^{-1}, 3126.04 cm^{-1}, 1626.66 cm^{-1}, 1614.13 cm^{-1}, 1585.20 cm^{-1}, and 1555.31 cm^{-1}. This peak shift indicates hydrogen bonds. Acyclovir-isonicotinamide (1:1) cocrystal using glacial acetic acid also showed a peak shift of the C=O group at 1692.23 cm^{-1}. Other than that, peak at 1662.34 cm^{-1} and 1668.12 cm^{-1} was disappeared. In acyclovir-isonicotinamide (1:1) cocrystal using ethanol, the same thing was found that there was a peak at 1692.23 cm^{-1} and peak at 1662.34 cm^{-1} and 1668.12 cm^{-1} was disappeared. It was estimated that there is a good intermolecular bond in physical mixture acyclovir-isonicotinamide (1:1) and each acyclovir-isonicotinamide (1:1) cocrystal both with glacial acetic acid and ethanol.[4]

SEM is the instrument used to observe the surface morphology of the material by relying on the reaction between electrons and the surface of the material.[18] Morphological observations in Figure 6 showed the formation of new crystalline forms in acyclovir-isonicotinamide (1:1) crystals (glacial acetic acid) with long needle-like shapes categorized as acicular.[19] The result of cocrystal using ethanol shows a picture or morphology of a rectangular and flat crystal so that it is categorized as a flake and thin particles which are categorized as lath/blades.[19] The two cocrystals have different shapes with acyclovir (long and thick particles) and isonicotinamide (irregularly shaped particles). It is

Table 1: The 2θ (°) angle and intensity (%) on the diffractogram profile of acyclovir-isonicotinamide

Acyclovir		Isonicotinamide		Acyclovir-isonicotinamide cocrystal (1:1) using glacial acetic acid		Acyclovir-isonicotinamide cocrystal (1:1) using ethanol	
2θ (°)	**Intensity (%)**	**2θ (°)**	**Intensity (%)**	**2θ (°)**	**Intensity (%)**	**2θ (°)**	**Intensity (%)**
-	-	-	-	-	-	5.97	10.16
6.86	100.00	-	-	-	-	-	-
9.06	0.43	-	-	-	-	-	-
-	-	-	-	-	-	9.53	11.17
-	-	-	-	-	-	13.19	41.11
-	-	-	-	14.00	3.26	-	-
-	-	-	-	14.43	4.23	-	-
-	-	17.56	95.56	-	-	-	-
-	-	-	-	22.04	7.10	-	-
-	-	47.57	4.91	-	-	-	-
-	-	58.31	0.50	-	-	-	-

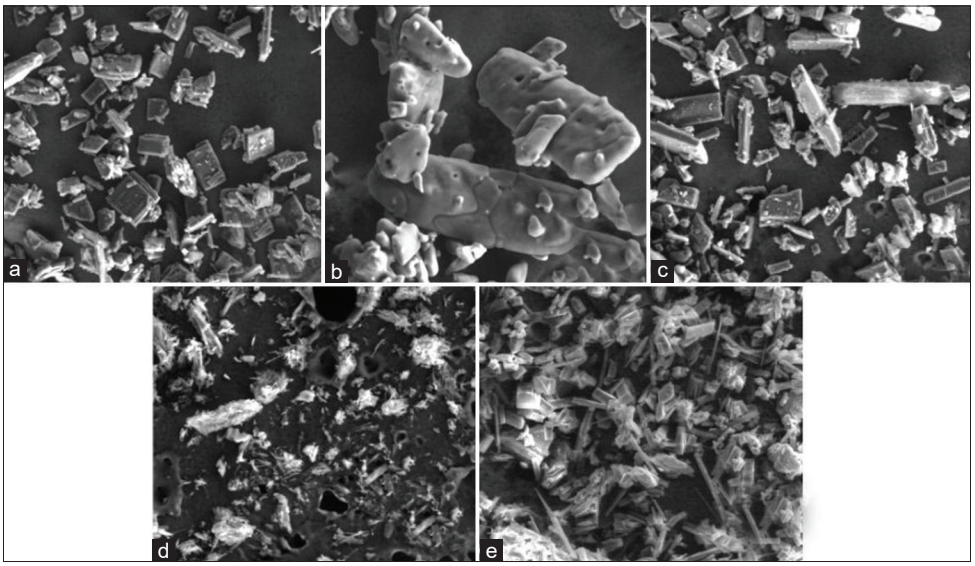


Figure 6: Photomicrograph of acyclovir (a), isonicotinamide (b), physical mixture of acyclovir-isonicotinamide (1:1) (c) cocrystal of acyclovir-isonicotinamide (1:1) with glacial acetic acid (d), and cocrystal of acyclovir-isonicotinamide (1:1) with ethanol (e). ×750

estimated that acyclovir-isonicotinamide (1:1) cocrystal with glacial acetic acid and ethanol (solvent evaporation method) gives a new form of crystal morphology. The morphologies of acyclovir-isonicotinamide (1:1) cocrystal with ethanol [Figure 6e] and glacial acetic acid [Figure 6d] showed more porous than constituent materials. It can be assumed that this cocrystal form can absorb more water and is expected to provide good solubility in the future. In addition, the acyclovir-isonicotinamide (1:1) cocrystal with ethanol produces more homogenous aggregate mixture of crystalline compared with the cocrystal with glacial acetic acid.

CONCLUSION

It can be concluded that acyclovir-isonicotinamide (1:1) cocrystal can be formed using glacial acetic acid and ethanol solvents by solvent evaporation method. This can be proved through their characterization using PXRD, FTIR, DSC, and SEM. The cocrystal formed of acyclovir-isonicotinamide (1:1) using glacial acetic acid and ethanol showed the different physicochemical characteristic compared to the constituent materials. Acyclovir-isonicotinamide (1:1) with ethanol gives a higher intensity of new peaks and more homogenous aggregate mixture of crystalline than glacial acetic acid. For further research, it is possible to carry out solubility and dissolution test to show that the cocrystal formed has a good bioavailability.

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