PREPARATION OF ACYCLOVIR-NICOTINAMIDE COCRYSTAL BY SOLVENT EVAPORATION TECHNIQUE WITH VARIATION OF SOLVENT

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ABSTRACT

Objective: This research aims to prepare cocrystal of acyclovir (ACV)-nicotinamide (NCT) by solvent evaporation with a variation of solvent (ethanol, glacial acetic acid, and HCl 0.1 N) to improve the bioavailability of ACV as an antiviral drug.

Methods: Cocrystal were developed by solvent evaporation with 1:1 molar fraction, using variation of solvent such as ethanol, glacial acetic acid, and HCl 0.1 N. Further, the prepared ACV-NCT cocrystal were characterized for differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD), Fourier transform infrared spectroscopy (FT-IR), scanning electron microscope (SEM), and in vitro dissolution.

Results: DSC thermogram showed that ACV-NCT cocrystal in ethanol and glacial acetic acid exhibited new endothermic peak at 221.16°C and 216.40°C, whereas no peaks were found for HCl 0.1 N. PXRD diffractogram showed that ACV-NCT cocrystal in ethanol exhibited new diffraction peaks at 2θ 5.9°; 9.2°; dan 13.3°, whereas no peaks were found for glacial acetic acid and HCl 0.1 N. FT-IR characterization of ACV-NCT cocrystal in ethanol showed disappearance of transmission peaks at 3373/cm indicating the loss of NH bands of NCT. Furthermore, G=0 of ACV and NCT were observed at 1693/cm, and 1666/cm indicated the formation of hydrogen bonding between ACV and NCT. SEM micrographs showed that cocrystals have a different shape compared to ACV and NCT. DE₅₀ showed that there was a significant increase of ACV-NCT cocrystal dissolution rate in ethanol compared to the physical mixture and ACV.

Conclusion: The study concludes that ACV-NCT cocrystal in ethanol were successfully formed and the dissolution rate of ACV can increase significantly (α=0.05).

Keywords: Cocrystallization, Solvent, Acyclovir, Nicotinamide, Solvent evaporation.
evaporation with ethanol, glacial acetic acid, and HCl 0.1 N to increase the solubility of ACV. Physicochemical characterization was performed by differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD), Fourier transform infrared spectroscopy (FT-IR) spectroscopy, scanning electron microscope (SEM), and also in vitro dissolution.

METHODS

Materials

ACV and NCT were purchased from Sigma-Aldrich®, China. Ethanol was purchased from Emsure®, Merck KGaA, Germany. HCl was purchased from Smart-Lab®, PT. Smart Lab Indonesia. Glacial acetic acid was purchased from Mallinkrodt AR®, France. Aquadest was purchased from PT. Widatra Bhakti, Indonesia.

Methods

Preparation of phase diagram of binary system

ACV and NCT were sifted and weighed to obtain particle size in a similar range. The obtained physical mixtures were obtained by simply mixing ACV with NCT at different %w/w as follows: (9:1), (8:2), (7:3), (6:4), (5:5), (4:6), (3:7), (2:8), (1:9), and (0:10), respectively. The mixtures were gently mixed in a mortar. The melting point of physical mixtures of ACV-NCT was determined by DSC. Endothermic peak was plotted against the molar fraction of the mixture to obtain the phase diagram of ACV-NCT.

Preparation of ACV-NCT physical mixture

ACV and NCT (equimolar) carefully weighed, 500.0 mg and 271.1 mg, respectively. Both powders were homogeneously mixed in a mortar.

Preparation of cocrystal using solvent evaporation technique with ethanol

ACV and NCT (equimolar) carefully weighed as much as 100.0 mg and 54.2 mg, respectively. Each compound was dissolved in ethanol separately. ACV was dissolved in approximately 500 ml of ethanol to form a clear solution. NCT was dissolved in approximately 5 ml of ethanol. The two solutions were mixed and stirred for a few minutes. Equimolar solution of both components was evaporated at room temperature for 48 hrs. The obtained cocrystal solids were stored in a desiccator under vacuum.

Preparation of cocrystal using solvent evaporation technique with glacial acetic acid

ACV and NCT (equimolar) carefully weighed as much as 1.0 g and 542.2 g, respectively. Each compound was dissolved in glacial acetic acid separately. ACV was dissolved in approximately 170 ml of glacial acetic acid to form a clear solution. NCT was dissolved in approximately 30 ml of glacial acetic acid. The two solutions were mixed and stirred for a few minutes. Equimolar solution of both components was evaporated at room temperature for 48 hrs. The obtained cocrystal solids were stored in a desiccator under vacuum.

Preparation of cocrystal using solvent evaporation technique with HCl 0.1 N

ACV and NCT (equimolar) carefully weighed as much as 1.0 g and 542.2 g, respectively. Each compound was dissolved in HCl 0.1 N separately. ACV was dissolved in approximately 150 ml of HCl 0.1 N to form a clear solution. NCT was dissolved in approximately 10 ml of HCl 0.1 N. The two solutions were mixed and stirred for a few minutes. Equimolar solution of both components was evaporated at room temperature for 8 days. The obtained cocrystal solids were stored in a desiccator under vacuum.

Characterization using DSC

DSC (DSC, Mettler Toledo, US) was used to analyze the thermal properties. A certain amount of samples, that is, 5-7 mg samples were placed in a sealed aluminum pan. The analysis was performed in a temperature range of 30-300°C with a heating rate of 5°C/minute.

Characterization using PXRD

PXRD (Philips X’Pert diffractometer) analysis was performed at room temperature. The condition of measurement was set as follows: Cu metal target, Kα filter, voltage of 40 kV, 15-30 mA. The analysis was performed on the range of 2θ of 5-40°. Sample was placed on the sample holder and flatted to prevent particle orientation during preparation.

Characterization using FT-IR

Approximately 1%w/w dispersion of sample powder in potassium bromide (KBr) was prepared by mixing the sample powder with KBr. The infrared spectrum was obtained using infrared spectrophotometer (Perkin-Elmer Instrument, Jasco FT-IR/5300) in wavelength range of 400-4000/cm.

Characterization using SEM

The sample was placed on the sample holder and coated with gold aluminum with a thickness of 10 nm. The sample was then observed at various magnification using SEM instrument (Jeol-JSM-6360LA, Japan) with voltage was set at 20 kV and 12 mA.

In vitro dissolution

The in vitro dissolution studies of prepared ACV-NCT cocrystal were carried out using USP Type II (paddle) dissolution test apparatus. Cocrystal containing equivalent to 100 mg of ACV were introduced into 900 ml dissolution medium of phosphate buffer pH 6.8 for 45 minutes at 37±0.5°C at a rotation speed of 50 rpm. A volume of 5 ml of the aliquots was withdrawn through a filter (0.45 μ) at the regular interval of 5, 10, 15, 20, 30, 45 minutes and replaced with an equal volume of fresh phosphate buffer pH 6.8. The samples were analyzed with simulant equation at 252.04 nm and 262.07 nm for ACV content using a UV spectrophotometer. The ACV release experiments were carried out in three replicate.

RESULTS AND DISCUSSION

Analysis of phase diagram of binary system

The phase diagram of ACV-NCT mixture was made using different %w/w (i.e., 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:10)%.

The results show that when a physical mixture of molar ratio 1:1 is heated, all of component NCT and part of component ACV melt at the metastable eutectic temperature, forming cocrystal which then melts at 191.94-231.83°C [16]. The molar ratio of 5:5 showed the optimal melting point of forming a cocrystal as shown in Fig. 1.

![Phase diagram of binary system acyclovir:nicotinamide (ACV:NCT) with various compositions, T1: Melting point of NCT, T2: Melting point of cocrystal, T3: Melting point of ACV](image-url)
Characterization study

Based on phase diagram data, the ACV-NCT cocrystal used molar ratio of 5:5 or 1:1. ACV-NCT cocrystal and their physical mixture were then characterized using DSC as shown in Fig. 2. The result showed ACV-NCT cocrystal in ethanol has a new endothermic peak at 221.16°C and \(\Delta H = -68.41 \text{ J/g}\). While the ACV-NCT cocrystal in glacial acetic acid has a new endothermic peak at 216.40°C and \(\Delta H = -64.29 \text{ J/g}\). These melting point is different with constituent materials ACV and NCT. Hence, this is indicate that ACV-NCT cocrystal with ethanol and glacial acetic acid was successfully formed [17]. Whereas, no peaks were found for HCl 0.1 N.

The formation of co-crystals is primarily characterized by PXRD. If the resulting PXRD pattern of the solid product is different from the reactants, it can be concluded that the new solid phase was formed [18]. Fig. 3 shows the PXRD diffractogram of ACV, NCT, ACV-NCT physical mixture, and ACV-NCT cocrystal. ACV has aspecific diffractogram at 2θ=6.9°; 10.4°; 16.0°; 20.9°; 26.1°; and 29.2°. NCT has a specific diffractogram at 2θ=14.7°; 22.1°; 25.8°; and 27.3°. Diffractogram of ACV-NCT cocrystal in ethanol exhibited new diffraction peaks at 2θ 5.9°; 9.2°; dan 13.3°, whereas no peaks were found for glacial acetic acid and HCl 0.1 N. Diffractogram of the physical mixture was a superposition of two constituent materials. It is widely accepted that PXRD is a very reliable method to provide information of solid systems in terms of interaction between materials. Such interactions may produce new diffraction peaks as compared to the constituent materials [1,2,17].

FT-IR spectroscopy is widely used to study the chemical and physical structure changes in the molecular structure of substance. The FT-IR spectrum of co-crystal showed relevant changes in the absorption frequencies of the typical functional groups of the pure

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**Fig. 2:** Differential scanning calorimetry thermogram, (a) acyclovir (ACV), (b) nicotinamide (NCT), (c) ACV-NCT physical mixture, (d) ACV-NCT cocrystal with ethanol, (e) ACV-NCT cocrystal with glacial acetic acid, (f) ACV-NCT cocrystal with HCl 0.1 N

**Fig. 3:** Powder X-ray diffractogram, (a) acyclovir (ACV), (b) nicotinamide (NCT), (c) ACV-NCT physical mixture, (d) ACV-NCT cocrystal with ethanol, (e) ACV-NCT cocrystal with glacial acetic acid, (f) ACV-NCT cocrystal with HCl 0.1 N

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substances [1]. Fig. 4 shows that ACV-NCT cocrystal in ethanol showed the disappearance of transmission peaks at 3373/cm indicating a loss of NH bands of NCT. Furthermore, NH of ACV were observed at 3440/cm ACV to 3436/cm in cocrystals implies that primary amine group is participating in hydrogen bond. While C=O of ACV and NCT were observed at 1693/cm and 1666/cm indicated a formation of hydrogen bonding between ACV and NCT ACV-NCT cocrystal in glacial acetic acid showed the mixture of ACV and NCT separately at 3441/cm and 3374/cm. This suggests that ACV and NCT molecules in glacial acetic acid are not present in the new phase. ACV-NCT cocrystal in HCl 0.1 N showed the OH bending and stretching frequency were observed at 1660/cm and 3151/cm. It is suspected that hydrogen bonding interaction with water molecules [19]. This suggested that the formation of ACV-NCT cocrystal in ethanol produced more hydrogen bonding than the ACV-NCT cocrystal in glacial acetic acid and HCl 0.1 N.

Observations using SEM provide visual information about the differences of cocrystal morphology compared with the constituent materials [1]. The results showed that the ACV-NCT cocrystal in ethanol (Fig. 5c) are made of aggregates of rod shape particles with regular shape and size in which the original morphology of both components (Fig. 5a and b) has disappeared. This indicate that ACV-NCT cocrystal was successfully formed. While ACV-NCT cocrystal in glacial acetic acid (Fig. 5d) are made of aggregates and appears of ACV crystal (Fig. 5a) and in HCl, 0.1 N (Fig. 5e) are look like needle shape with irregular size.

**Analysis of in vitro dissolution**

Fig. 6 shows that the dissolution tests performed on the samples in powder in phosphate buffer pH 6.8 at 37°C. It is evident that dissolution rate at 5 minutes of ACV is very slow, just about 51.00±2.60 (Fig. 6a), whereas ACV-NCT cocrystal in ethanol dissolve quite rapidly in about 90.93±2.48 (Fig. 6c). While, ACV-NCT physical mixture dissolve in about 72.50±4.26 (Fig. 6b). ACV-NCT cocrystal in glacial acetic acid dissolve in about 61.13%±5.09 (Fig. 6d). ACV-NCT cocrystal in HCl 0.1 N dissolve in about 58.92±5.34 (Fig. 6e) and to be stagnate at 10 minutes until 45 minutes in about 63-66%. This indicates that HCl cannot increase the dissolution rate of ACV.

The statistic analysis of dissolution efficiency at 15 minutes (DE) with Post-hoc LSD test (α=0.05) showed that there was a significant increase of ACV-NCT cocrystal dissolution rate in ethanol (79.32±3.04) compared to ACV-NCT cocrystal in glacial acetic acid and HCl 0.1 N.

**CONCLUSION**

ACV-NCT cocrystal was successfully formed using solvent evaporation technique with ethanol as solvent. While cocrystallization of ACV and NCT in glacial acetic acid only produces the eutectic mixture. Moreover, ACV-NCT cocrystal in HCl 0.1 N was not successfully formed. This can be proved through their characterization using DSC, PXRD, FT-IR, and SEM. The formed ACV-NCT cocrystal in ethanol exhibits different physicochemical characteristics compared to the constituent materials.

Analysis of DE, (α=0.05) showed that there was a significant increase of ACV-NCT cocrystal dissolution rate in ethanol compared to ACV-NCT cocrystal in glacial acetic acid and HCl 0.1 N.
REFERENCES

1. Bruni G, Maietta M, Maggi L, Mustarelli P, Ferrara C, Berbenni V, et al. Preparation and physicochemical characterization of acyclovir cocrystals with improved dissolution properties. J Pharm Sci 2013;102(11):4079-86.
2. Sarkar A, Rohani S. Cocrystals of acyclovir with promising physicochemical properties. J Pharm Sci 2015;104(1):98-105.
3. Yan Y, Chen JM, Lu TB. Simultaneously enhancing the solubility and permeability of acyclovir by crystal engineering approach. CrystEngComm 2013;15:6457-60.
4. Zaini E, Halim A, Soewandhi SN, Setyawan D. Peningkatan laju pelarutan trimetoprim melalui metode ko-kristalisasi dengan nikotinamida. J Farmasi Indones 2011;5(4):205-12.
5. Yadav S, Gupta PC, Sharma N, Kunnar J. Cocrystals: An alternative approach to modify physicochemical properties of drugs. Int J Pharm Chem Biol Sci 2015;5(2):427-36.
6. Zaitte AG, Saudagar RB. Advanced techniques in preparation of cocrystals. Int J Sci Prog Res 2015;12(1):32-5.
7. Kotak U, Prajapati V, Solanki H, Jani G, Jha P. Co-crystallization technique: a brief overview and recent development. World J Pharm Pharm Sci 2015;4(4):484-508.
8. Fukte SR, Wagh MP, Rawat S. Coformer selection: An important tool in cocrystal formation. Int J Pharm Pharm Sci 2014;6(7):9-14.
9. Setyawan D, Sari R, Yusuf H, Primaharinsititi R. Preparation and characterization of artesunate-nicotinamide cocrystal by solvent evaporation and slurry method. Asian J Pharm Clin Res 2014;7 Suppl 1:62-5.
10. Soares FL, Carneiro RL. Green synthesis of ibuprofen-nicotinamide cocrystals and in-line evaluation by Raman spectroscopy. Cryst Growth Des 2013;13(4):1510-7.
11. Shayanfar A, Velaga S, Jouyban A. Solubility of carbamazepine, nicotinamide, and carbamazepine-nicotinamide cocrystal in ethanol-water mixtures. Fluid Phase Equilib 2014;363:97-105.
12. Rager T, Hilfiker R. Application of phase diagrams in co-crystal search and preparation. In: Johan W, Luc Q, editors. Pharmaceutical Salts and Co-Crystals. 1st ed. Cambridge: The Royal Society of Chemistry; 2012. p. 110-27.
13. Holan J, Stepánek F, Billot P, Ridvan L. The construction, prediction and measurement of co-crystal ternary phase diagrams as a tool for solvent selection. Eur J Pharm Sci 2014;63:124-31.
14. Grodowska K, Parczewski A. Organic solvents in the pharmaceutical industry. Acta Pol Pharm 2010;67(1):3-12.
15. The United States Pharmacopoeial Convention, Inc. USP 30 NF 25. Vol. 1. Twinbrook Parkway: The United States Pharmacopoeial Convention, Inc.; 2007.
16. Yamashita H, Hirakura Y, Yuda M, Teramura T, Terada K. Detection of cocrystal formation based on binary phase diagrams using thermal analysis. Pharm Res 2012;30(1):1-11.
17. Masuda T, Yoshishashi Y, Yonemochi E, Fujii K, Uekusa H, Terada K. Cocrystallization and amorphization induced by drug-excipient interaction improves the physical properties of acyclovir. Int J Pharm 2012;422(1-2):160-9.
18. Alatas F, Soewandhi SN, Sasongko L, Ismunandar, Uekusa H. Cocrystal formation between didanosine and two aromatic acids. Int J Pharm Pharm Sci 2013;5 Suppl 3:275-80.
19. Gerakines PA, Schutte WA, Greenberg JM, van Disheoeck EF. The infrared band strengths of H2O, CO, and CO2 in laboratory simulations of astrophysical ice mixtures. Astron Astrophys 1995;296:1-17.