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Observation Of Polymorphic Transformation Of Amorphous Efavirenz During Heating And Grinding Processes Using Raman Spectroscopy.

Yoga Windhu Wardhana[1,2*], Sundani N. Soewandhi[2], Saleh Wikarsa[2], Veinardi Suendo[3]

[1] Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, UNPAD
[2] Pharmaceutics Research Groups , School of Pharmacy, ITB and
[3] Inorganic and Physical Chemistry Division, Faculty of Mathematics and Natural Sciences, ITB

ABSTRACT

Drug amorphization is oftenly used for medication. The reason using an amorphous form is the potential to improve its bioavailability, rather than stable crystalline form. For example, efavirenz as an anti HIV drug is belong to BCS (*Biopharmaceutical Classification System*) class 2 drugs with has a poor water solubility and a good permeability. It have a lot of polymorph forms, including amorphous form. As known well that thermodinamically properties of amorphous form is meta-stable or unstable form, so stability of using amorphous drug in solid dosage form should been studied well. However, the stability studies for amourphous form are limited. Therefore, the aim of this studies was to investigate the stability forms or transformation phenomenone from amorphous to crystalline form by heating and grinding effects. Form I was prepared by recrystallization with acetonitrile, whereas From A was made by quench cooling method. The resulted amorphous (Form A) and crystalline forms (Form I and original) were characterized, including its nature solubility and others properties by Thermogravimetry-Differential Thermal Analysis (TG-DTA), X-Ray Powder Diffraction (XRPD), Fourier-Transform Infra Red (FTIR) and Raman Spectroscopy. Polymorphic transformation in oven with variations of storage temperatures and grinding time were observed by Raman Spectroscopy. The Raman spectrum showed that the transformation of Form A was quickly happened by grinding process after 10 minutes, meanwhile, it occured around 85^{o}C by heating effects. These results suggest that the physicochemical benefit from amorphous or meta-stable polymorph forms should be carefully attended before using in dosage formulation products.

Keywords : Polymorphic Transformation, Amorphization, Amorphous, Efavirenz

Corresponding author

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INTRODUCTION

Efavirenz (EFV) is chemically called as (S)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one. Efavirenz is one of a second-generation non-nucleoside inhibitor of HIV-1 reverse transcriptase and as an important play to changing single-stranded viral RNA into double stranded DNA prior to merge to the genome of the human host [7]. The literature described that efavirenz had polymorphism phenomenone with 23 different polymorph forms include amorphous [1-6; 9-13].

Different solid forms of a compound may vary in physicochemical properties including solubility due to differences in thermodynamic properties of the crystal conformations. Theoretically meta-stable forms will exhibit higher solubility compared to their crystalline counterparts, which makes them potentially useful for delivering poorly soluble drug whose limited bioavailability by their low solubility. The more stable the crystal form, the stronger the bonding forces between the molecules. Amorphous forms being the least stable form of a compound so that oftenly used as an active ingredients [8].

Amorphous solids is able to be earned by cooling of liquids, evaporating of solutions, and condensing of vapors while avoiding crystallization. Amorphous solids formed by cooling of liquids are commonly called as the glasses. In the glass process formation, molecular motions tend to increase slower with cooling at last, at the so called glass transition tempe-rature, T_g, the system can no longer reach internal equilibrium with each decrease of temperature and becomes kinetically frozen. With respect to molecular packing, amorphous solids are usually envisioned as having significant local order (e.g., each molecule having similar number of nearest neighbors), but lacking long-range order that characterizes molecular packing in crystals [14].

Aim of this research was to observe on crystal growth in organic glasses under several energy such as heating and grinding. This study is toward understanding and enhancing the stability of amorphous pharmaceutical solids against crystallization affect by heat and mechanical forces.

MATERIALS AND METHODS

Materials

A pharmaceutical grade of efavirenz (EFV, Batch No. EZ1670711, Hetero Labs Ltd., India) was purchased from PT. Kimia Farma Tbk, Indonesia. The analytical grade of solvents such as methanol and acetonitrile (ex JT. Baker, USA) was purchased from PT. Bratachem, Indonesia.

Methods

Preparation of Polymorph Purification

Original EFV was recrystallized with acetonitrile (50 mL, 75°C) until supersaturation solution achieved, then filtered and kept for crystallization at 28°C (slow recrystallization). The solid phase appears after 20 days from solution of acetonitrile. The solutions were filtered and dried under vacuum. The recrystallization from acetonitrile called as form I (stable form).

Preparation of Amorphous Form (quench cooling)

A desired amount of original EFV was placed onto the aluminum foil and allowed to melt on calibrated hot plate which set at 140°C. The aluminum foil was thereafter quickly removed from the hot plate and subjected to ambient conditions. The melt instantly solidified into a glassy solid. The obtained glass was dubbed Form A.

X-Ray powder diffraction (XRPD) Analysis

The powder diffraction patterns were recorded on an X-ray diffractometer (XPERT-PRO, PANalytical, Netherlands) with Cu as tube anode. The diffractograms were recorded under following conditions: voltage 40 kV, 30 mA and fixed divergence slit using the configuration; 2Θ range: 5Θ to 45Θ, 0.02 step size, 0.8 s time per step, care was taken to avoid phase transformations during sample preparation.

Thermal behaviour profile

The TG-DTA scans were obtained on Pyris Diamond TGDTA–Perkin Elmer, USA. TGA traces were recorded at heating rates of 10° per minute under a nitrogen purge of 50 mL per minute. Samples with masses between 5-10 mg were analyzed using aluminium pan. Mass loss (%) was calculated based on the mass of the original sample.

DTA curves were obtained under a nitrogen purge of 50 mL per minute at a heating rate of 10° per minute with the temperature range from 50–250°C.

Solubility Study

Calibration Curve Preparation

Standard solutions was made from 10 mg of original EFV which was dissolved in 40 mL of metanol then added slowly aquadest till 100 mL. Dilute the standard solution with 5 different concentrations i.e. 5, 7, 9, 11 and 13 ppm. All of concentrations were measured by spectrophotometer UV–Vis (SPECORD, Analytic Jena) at 248 nm. Absorbance parameter obtained from each standard solutions were plotted to be calibration curve to determine regression linear equation for content calculation standard.

Solubility Test

Amounts of 10 mg samples was dissolved in 100 mL aquadest using mechanical agitator shaker at 120 rpm for 24 and 48 hours under room temperature. Sample solution was filtered using millipore 0.45 µm and suitably diluted prior to measured by spectroscopy UV at 248 nm.

Fourier Transform Infra Red (FTIR) Spectrophotometer

Each EFV polymorph was identified using FTIR multiscope spectrophotometer (Shimadzu, Japan) by sealing the sample between two KBr plates by a hydraulic press under 200 kg/cm^2 for 15 seconds to form a disc. The spectrum for each sample was recorded over the 300–4500 cm^{-1} spectral region with a resolution of 4 cm^{-1}.

Treatment for Polymorphic Transformation

Under Storage Enviroment

Based on DTA information, the temperature of polymorphic transition predicted among 70°–115°C. So that information used for storage the amorphous solids in oven (Memmert UN30, Germany) at 70°, 85°, 100° and 115°C for 60 minute each temperature. All of samples was monitored by Raman spectroscopy.

Under Mechanical Grinding

The amorphous solid was grinded in Retsch RM 100 at 90 rpm for 10, 30, 60, 90 and 120 minutes. Each samples was observed by Raman Spectroscopy.

Raman Spectrophotometer

Raman spectra were obtained on Bruker–Senterra Micro-Raman Spectrometer uses diode laser system (785 nm, 100 mW) as the excitation source for spectrum recording in room temperature in the spectral region at 50–3500 cm^{-1}.

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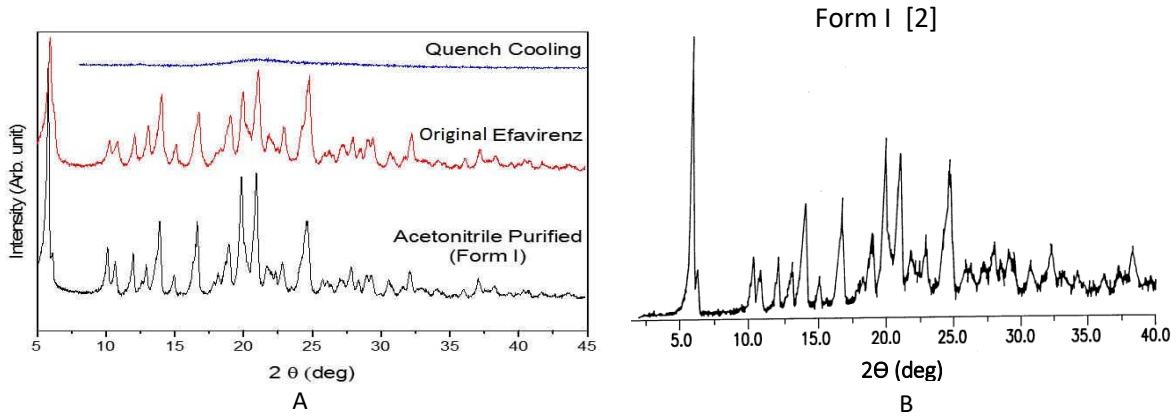
RESULT AND DISCUSSION

Characterization of Polymorphs

Several analytical techniques have been found for polymorphs characterization, such as microscope technique, X-ray diffraction, thermal behaviour analysis, and Raman spectroscopy as commonly techniques for characterizing polymorphs. Each technique has its strengths and limitations with respect to diferentiate between polymorphs. The differentiation among crystalline and amorphous solid should be defined.

XRPD pattern on those samples showed that the original and purified EFV were crystalline, while the quench cooling was amorphous. The purified EFV looks have a sharp peak with high intensity than the original one. Its means that crystallinity EFV which purifed by acetonitrile was increased. This pattern has similar with polymorph Form I from the reference found [2].



,Figure 1: Comparison between XRPD of (A) EFV treatments and (B) EFV reference

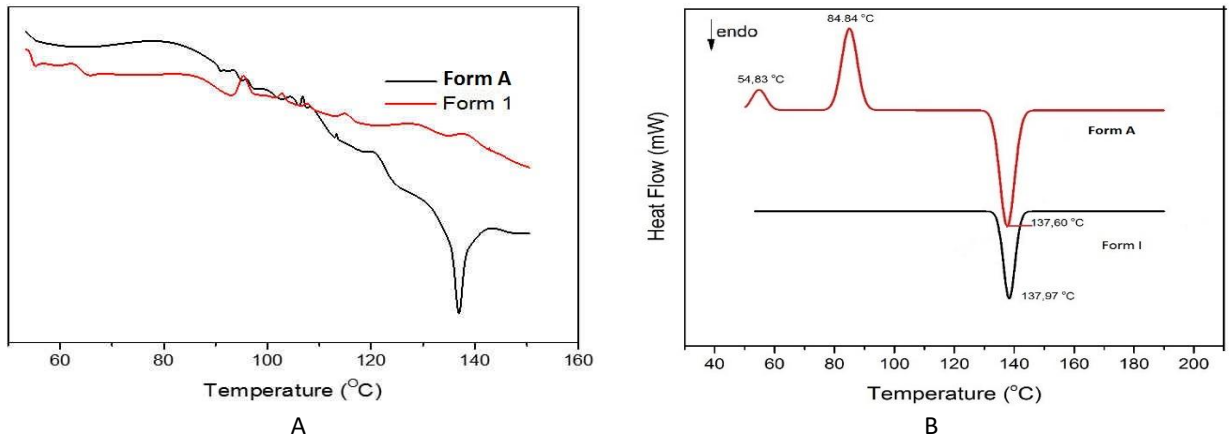


Figure 2: Thermogram from (A) TGA and (B) DTA

From DTA thermogram that is shown in **Figure 2,** Form A had two peaks of exotherm at 54.83° and 84.84°C followed by an endotherm peak at 137.6 °C. It is assumed that Form A instability below 85°C to become the stable form (Form I). TGA thermogram shown shows that Form A had lossing more mass than form I. It is indicated that Form A degraded easily than Form I with low energy effect.

Solubility properties study between original, Form I and Form A was carried out. As shown in **Table 1** that Form A was the most soluble in 24 hours, but after 48 hours the solubility droped excessively rather than others has risen. These results also prove that Form A was unstable form.

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Table 1: Solubility properties of Form I, Amorphous and Original EFV

Samples	Solubility Properties (µg/mL)	
	24 hrs	48 hrs
Original	5.685 + 0.0009	6.798 + 0.0018
Form I	5.492 + 0.0016	6.597 + 0.0017
Form A	7.305 + 0.0079	5.960 + 0.0062

To verify that all samples were the similar chemical compound, FTIR study was carried out. Spectra from FTIR patterns in **Figure 3** looks like resemblance between all samples. It can be assumed that those samples have the same functional groups or in other words those samples are the identical compound.

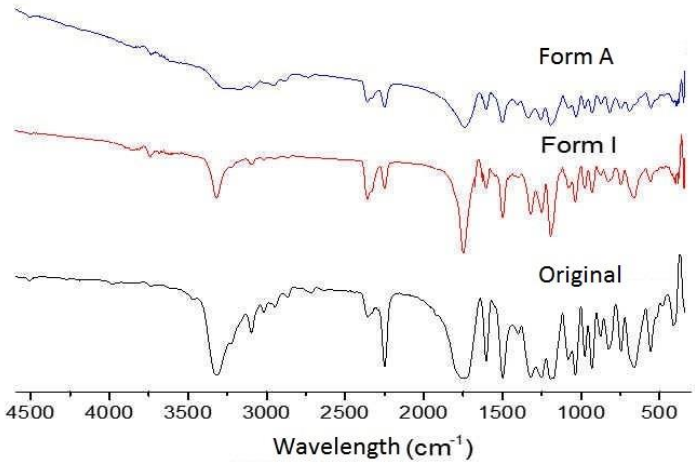

Figure 3: Fourier transform infrared of Form I, Form A and Original Efavirenz

Transformation Polymorphic Evaluation

Based on the thermal profile behaviour and solubility properties information of Form A unstable nature, make question for other factors probably influence. Generally, substances are exposed by heat and mechanical treatment such as grinding in manufacture. So, those enviroment conditions were used to monitor–form changing in this investigation.

Based on the heat exposed, Form A was stored in the oven with various temperature i.e. 70°, 85°, 100° and 115°C at the same time exposure for 60 minutes storage. All various temperatures were observed by Raman Spectroscopy, the spectra shown in **Figure 4**, the transformation had started from 85°C and completely changed at 100°C.

The grinding processes give closer approximation with enviroment manufacture condition. After treatment by grinder with 90 rpm for 10, 30, 60, 90, and 120 minutes the spectra exhibits the transformation of Form A at 10–30 minutes during grinding. Raman spectra polymorphic transformation were shown at shifting wavelength at 865 to 863 cm^{-1} and 927 to 930 cm^{-1}, corresponding to stretching mode of ν(C≡C) [7]. The instability of Form A more clearly describe if it used as active ingredient. From this research found that Form A have a better solubility but must be taken hardly careful to keep in amorphous form.

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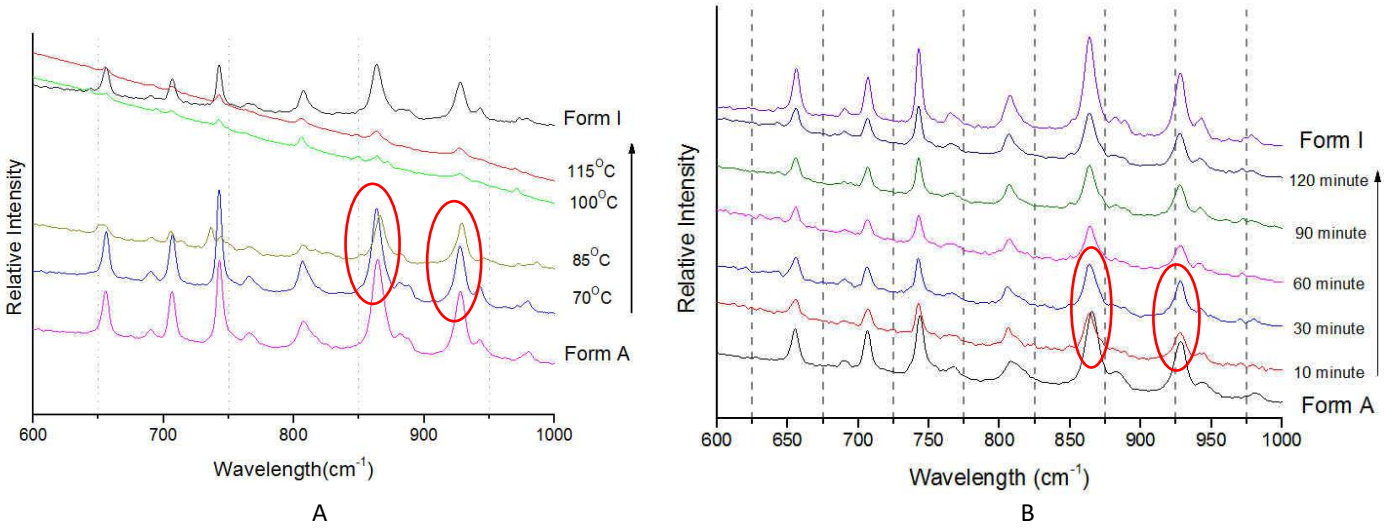

Figure 4: Raman Spectra from (A) heat exposure in oven and (B) grinding process at 90 rpm in the region 600–1500 cm^{-1}

CONCLUSION

Amorphization of efavirenz by quench cooling method was successfully done to result an amorphous solid form. There was no different chemical compound but had different physical properties. Amorphous form has better soluble (24 hours) than stable crystalline form, but very impermanent. Thermogram DTA information showed that around 50°–80°C transition was occured. Meanwhile, the Raman spectrum for transformation shows started in the heat enviroment exposured occured after 85°C at 60 minutes storage, The same effect appear with grinding at 90 rpm for 10–30 minutes process. These results suggest that before using efavirenz in amorphous form should be carefully handed, due to its unstable nature and environmental exposure.

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