

THEOPHYLLINE-NA-SACHARRINE SINGLE CRYSTAL ISOLATION FOR ITS STRUCTURE DETERMINATION

ILMA NUGRAHANI*, EVALIANTY AGIANA PERTIWI, OKKY DWICHANDRA PUTRA

School of Pharmacy ITB, Jl. Ganesha 10 Bandung Indonesia 40132

Email: ilma_nugrahani@fa.itb.ac.id

Received: 25 Jul 2015 Revised and Accepted: 27 Oct 2015

ABSTRACT

Objective: Co-crystal is a crystal form which consists of two or more components in a specific stoichiometric ratio. Theophylline has been reported to form a co-crystal with sodium saccharin, but its single-crystal has not been isolated, so its structure also has not been known yet. This research aimed to isolate the theophylline-sodium saccharin co-crystal with several solvent, studied its single-crystal structure, and observed the stability of the co-crystal formed in a high humidity.

Methods: Theophylline anhydrous powder (TA), sodium saccharin dihydrate (NSD), and the physical mixtures of TA-NSD were characterized using a polarizing microscope, Fourier-Transform Infrared Spectroscopy (FTIR), Powder X-ray Diffractometer (PXRD), and Karl Fischer Titration (KFT); respectively. The single TA-NSD co-crystal formation was done by slow evaporation at ambient temperature (25 °C) and 12 °C, using four types of solvent mixtures, such as: water-isopropanol; water-acetone; ethanol-dichloromethane; and ethanol. The rate formations of co-crystals were observed and characterized using polarizing microscopy, FTIR, PXRD. The isolated co-crystal was analyzed using a Single-crystal X-Ray Diffractometer (SC-XRD). Finally; stability test was applied to TA-NSD co-crystal at an RH 95% chamber.

Results: Single-crystals at most formed in water-isopropanol (1:7), with the size of 0.6 to 1.2 cm (at ambient temperature) and 0.6 to 1 cm (at 12 °C) and solvent of water-acetone 1: 9 with a size of 0.5 to 1.1 cm (at ambient temperature/25 °C). Meanwhile from the other solvents, co-crystal just was produced in the crystalline form; and thin crystals with a size of ≤ 5 mm both in ambient temperature and 12 °C. Visual observations that were supported by polarizing microscope magnification showed TA-NSD co-crystal has rod-shaped and translucent color. FTIR spectra analysis results showed N-H stretching of the co-crystal shift from a wavenumber 3124.12 to 3129.7-3131.83 cm^{-1} . The diffractogram from PXRD analysis indicated that TA-NSD co-crystal has sharp peaks on $2\theta = 5.58, 12.32, 17.48, 22.40, 26.86, 27.4, 28.84,$ and 34.1° . The SC-XRD result showed the TA-NSD co-crystal has monoclinic crystal system, which has not been reported previously. The stability test in high humidity proved that the co-crystal shows unchanged shift N-H strain, which means that TA-NSD co-crystal is stable.

Conclusion: Based on all data, it is concluded that TA-NSD co-crystal has rod-shaped with translucent color, and has a monoclinic crystal system with volume 1583.9 (7) \AA^3 . The single-crystals yielded from the water-isopropanol solvent system at ambient temperature and 12 °C; while from water-acetone 1: 9 was formed at 12 °C. The stability test in an RH 95% chamber showed that TA-NSD co-crystal is stable against the moisture.

Keywords: Co-crystal, Single-crystal, Slow evaporation, Theophylline anhydrous, Sodium saccharin dihydrate

INTRODUCTION

The physicochemical properties of active pharmaceutical ingredients (APIs) are an important thing to be considered in pre-formulation, because it determines the drug solid dosage form's performance. The API's nature will influence its particle's flowability, compressibility, physical stability, solubility, dissolution rate, and bioavailability. Recent, co-crystal arrangement is a procedure which can be taken to improve the physicochemical properties.

Co-crystal is a three-dimensional lattice structure consists of two or more components with a specific stoichiometry. Co-crystal components can be atomic, molecular, or ionic compounds; it formed an intermolecular or supra-molecular with the other component which said as a *co-former* at the site of *synthon*. The interaction involves the kind's interaction such as van der Waals, hydrogen, or coordinate-metal bonding.

Theophylline is an alkaloid compound, a xanthine derivative; which has a nitrogen atom and is generally used as a bronchodilator. Theophylline is hygroscopic, readily absorbs water from the environment or on the manufacturing process, and can form hydrate crystals [1]. Hydrate is a crystal with water molecules incorporated in the crystal lattice beside the main compounds. Moreover, theophylline has been reported to form co-crystals with the assortment co-former with saccharin-base [2], besides the other co-former such as: nicotinamide, urea [3], oxalic acid, malonic acid, maleic acid, and glutaric acid [1]. Synthon types that have been reported involved in saccharin co-crystals are OH-N (-), NH-N (-), NH-O, NH-O, OH-O, NH-N [4]. Theophylline has amine groups,

which can donate protons to hydrogen bond, while sodium saccharin has a group N (-) which is a proton acceptor, so theophylline-sodium saccharin predicted can form a co-crystal at the synthon: NH-N (-).

On the other hand, saccharin also has been reported can be a co-former for many APIs. The research study of the dynamics and the stoichiometric ratio of theophylline-sodium saccharin co-crystal was performed using ball milling had been done previously [5, 6]. Beside as co-former, saccharin base also was produced as a salt form to increase its solubility. However, as a very soluble in water, in general, the salts will have hygroscopic property, that cause physical instability then should be followed by chemical instability [7, 8].

Next, this research purpose was to arrange a co-crystal from theophylline with the saccharin salt, i.e. sodium saccharin, to optimize the physicochemical of theophylline supported by the co-former. This study observed the suitable solvents to produce a single crystal of the co-crystal, which can later be used for its structure determination, and observe interactions that occur with certainty.

Single crystal formation between theophylline with sodium saccharin in a composition of 2.4: 3.6 [5] was performed by slow evaporation method, in which evaporation gradually expected to provide sufficient time for the nucleation to form the desired crystal. In addition, to physical observation using a polarizing microscope, characterization of the co-crystal was also done by observing the spectral shift of strain NH on FTIR spectrogram, and look the PXRD diffraction pattern.

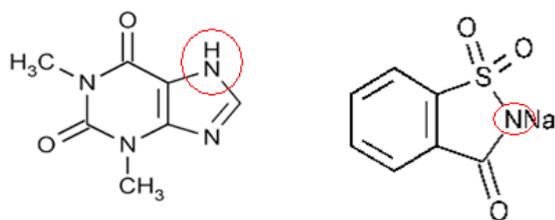


Fig. 1: Structure of the molecule theophylline (left) and sodium saccharin (right)

MATERIALS AND METHODS

Instrument

Electronic scales milligrams (Mettler M3), powder X-ray diffraction Philips (PW 18 350 Xray Diffraction), Fourier transform infra red/FTIR (Jasco-4200 type A, Japan), volumetric Karl Fischer Titrator V20 METLER Toledo, polarization microscope Olympus BX 50, single-crystal x-ray diffraction R-AXIS RAPID (Rigaku, Japan), magnetic stirrer, stirrer-electric stoves, and other glassware used in the laboratory.

Materials

Theophylline anhydrous (PT BRATACO Chemical), sodium saccharin dihydrate (CV Supreme Eternal Tower, Bandung), potassium bromide (MERCK), Karl Fischer reagent (MERCK), distilled water, isopropanol (MERCK), acetone (MERCK), ethanol pro-analysis (MERCK), dichloromethane (MERCK), potassium sulfate (MERCK).

Procedure

All of each raw material and the mixture (2.4:3.6) were characterized by solid analysis of the procedures as follows:

Characterization with differential thermal analysis (DTA)

Approximately 5-10 mg samples were kept in a special aluminum cup for the preparation of the DTA. Subsequently, the sample was heated under a stream of nitrogen gas with heating rate 10 °C/min, from 30 to 350 °C.

Identification and characterization with FTIR

Samples in powder form mixed crystals of potassium bromide that had previously been put into an oven at 100 °C with a weight ratio of 1: 100, then were crushed until homogeneous with agate mortar. Next, the dispersion was loaded into the mold of stainless steel discs measuring with the dimension ± 13 mm, afterward compressed at a pressure of $\pm 7.5 \times 10^{-3}$ mm Hg using hydraulic presser. Finally, the disc was mounted on the holder spectra measured at wave number 4000 to 400 cm^{-1} using FTIR spectroscopy Jasco-4200 type A (Japan).

Characterization by PXRD

Analysis using PXRD done by a number of 200 mg samples, which was prepared at the sample plate. The plate then tested by diffractometer with type: PW 1710 BASED; tube anode: Cu; voltage 40 kV, current of 30 mA, 0.2 inches wide split. Data were collected at a scan speed of 0.8 seconds per step, with scanning distances at $2\theta = 5$ to 45 °.

Analysis of total hydrate with KFT

A total of 50.0 mg of sample was weighed, and the water content were determined by volumetric KFT apparatus METLER Toledo titrator V20, using Karl Fischer reagent standardized.

Co-crystallization with slow evaporation method

TA-NSD mixture weighed according to a molar ratio of 2.4:3.6 [6]. The mixture was then dissolved in four types of solvents with stirring speed of 200 rpm and a temperature of 55 °C for 30 min.

Table 1: Solvent composition for Co-crystallization

Solvent	Composition
Water: Isopropanol	1: 7
Water: Acetone	1: 9
Ethanol	1
Ethanol: Dichlormethane	9: 1

Then the clear solution was filtered by using a filter paper into an erlenmeyer. Erlenmeyer subsequently closed using the plastic wrap that has been hollowed out a little and stored in two different temperatures, namely ambient temperature and a temperature of 12 °C. Solvent allowed to evaporate slowly to form crystals. The crystals that formed were then analyzed using PXRD and FTIR.

Polarization microscopy

A small number of crystals that were formed were placed above the etched of object glass and dropped with liquid paraffin, then closed and dispersed by using a cover glass measuring 2.2 cm x 2.2 cm. Afterwards, the sample on the object glass was observed with a polarizing microscope Olympus BX-50 using a lens, with a certain magnification.

Structure determination using SC-XRD

Data SC-XRD were taken at ambient temperature conditions in ω -scan mode with the R-AXIS RAPID (Rigaku, Japan), using Mo K α sealed tube source with a graphite monochromator. The data have been scaled and integrated empirically corrected absorption effect by using ABSCOR. The initial structure was solved by using the direct method and refined at 97 SHELXS F02 using SHELXL 97. Hydrogen atoms of H1A and H1B placed on the differential Fourier map and refined in anisotropic until all hydrogen atoms were geometrically obtained.

RESULTS AND DISCUSSION

Characterization of raw materials and physical mixture

Characterization of TA, NSD, and a physical mixture of TA-NSD 2.4:3.6 were done with instrument PXRD, DTA, FTIR, and KFT.

Here are the results of the identification using PXRD instrument (fig. 2).

On fig. 3, the DTA thermogram shows two endothermic curves, at the temperatures of 67.6 and 273.2 °C. The curve at a temperature of 67.6 °C shows the discharge point of water, from the hydrate lattice of theophylline. The existence of water in the theophylline lattice also was confirmed by analysis using FTIR and KFT. Then, the endothermic curve at 273.2 °C shows the melting point of theophylline. Meanwhile, DTA thermogram in fig. 3b shows three curves: the first and second at a temperature of 66 and 102 °C, show the releasing hydrates from the lattice of sodium saccharin, and the third at 134 °C, shows sodium saccharin's melting point, which is followed by decomposition.

Further analysis was conducted using a KFT and FTIR to confirm the water on theophylline and sodium saccharin lattice.

The FTIR analysis yielded the results which is shown in fig. 4 as follows:

From FTIR spectra obtained, it can be known that theophylline anhydrous has a peak at the wave number 3424.96 and 3347.82 cm^{-1} , which is the common wave number of the vibration of water molecules in a hydrate. This FTIR spectra confirms the results of previous theophylline DTA analysis. As results, it can be seen a peak at wave number 3120.26 cm^{-1} , which shows the -NH stretching; the wavelength of 2919.7 cm^{-1} and 2842.56 cm^{-1} which show the presence of methyl groups, and wavelength 1666.2 cm^{-1} indicate the presence of C = O amide group. The FTIR spectra of saccharin sodium peak at wave number 3031.55 cm^{-1} indicate the presence of aromatic CH group; next, group C = O amide is indicated by the peak at a wavelength of 1658.46 cm^{-1} ; and 1052.94 cm^{-1} for group S = O. From the physical mixture, obtained FTIR characteristic peaks at wavenumbers 3124.12 cm^{-1} [5].

To confirmed and identify the presence of hydrates in the crystal lattice theophylline and saccharin sodium hydrate, further analysis

using the KFT was done. Titration results obtained are listed in table 1 as follows:

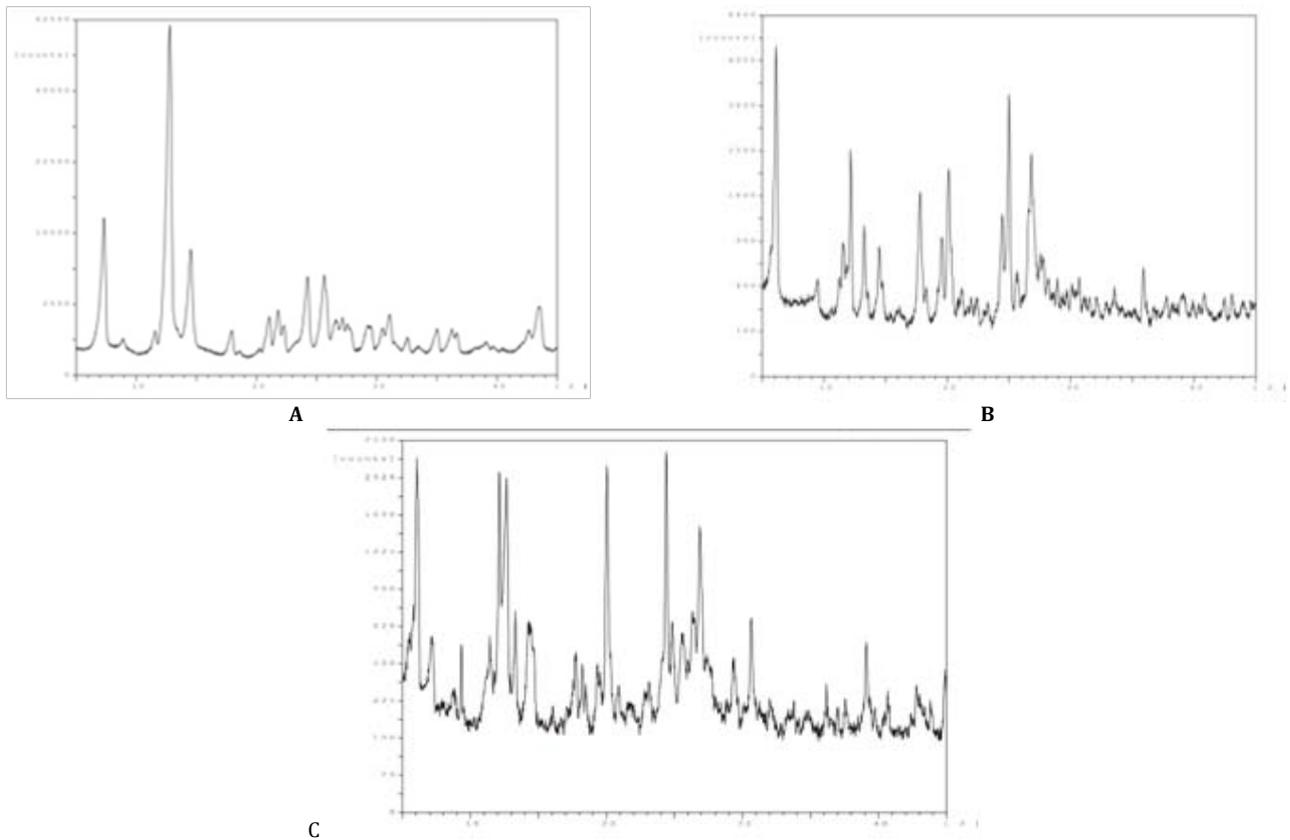


Fig. 2: Diffractogram of (A) TA; (B) NSD; (C) physical mixture TA-NSD

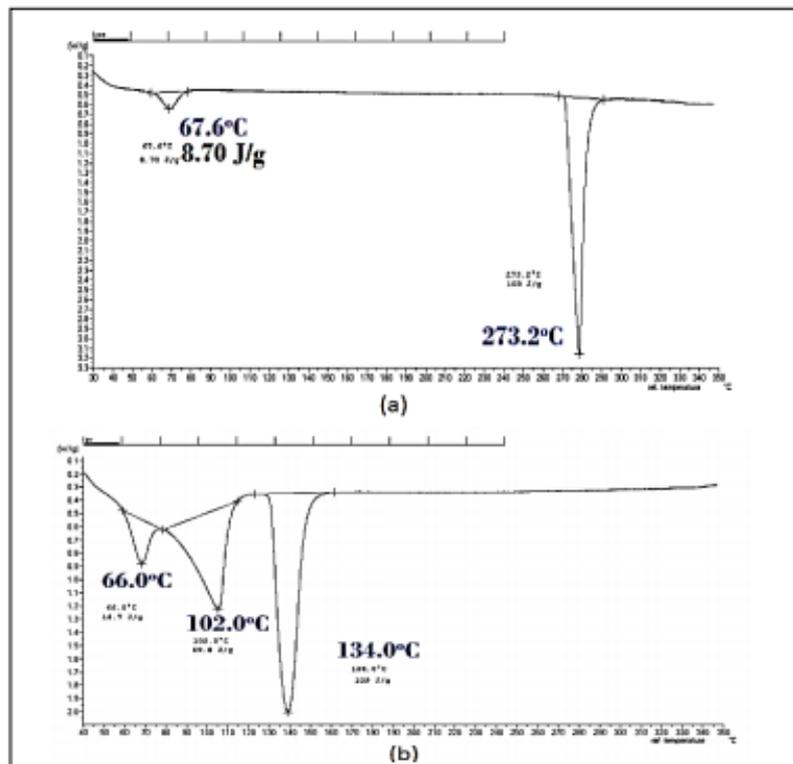
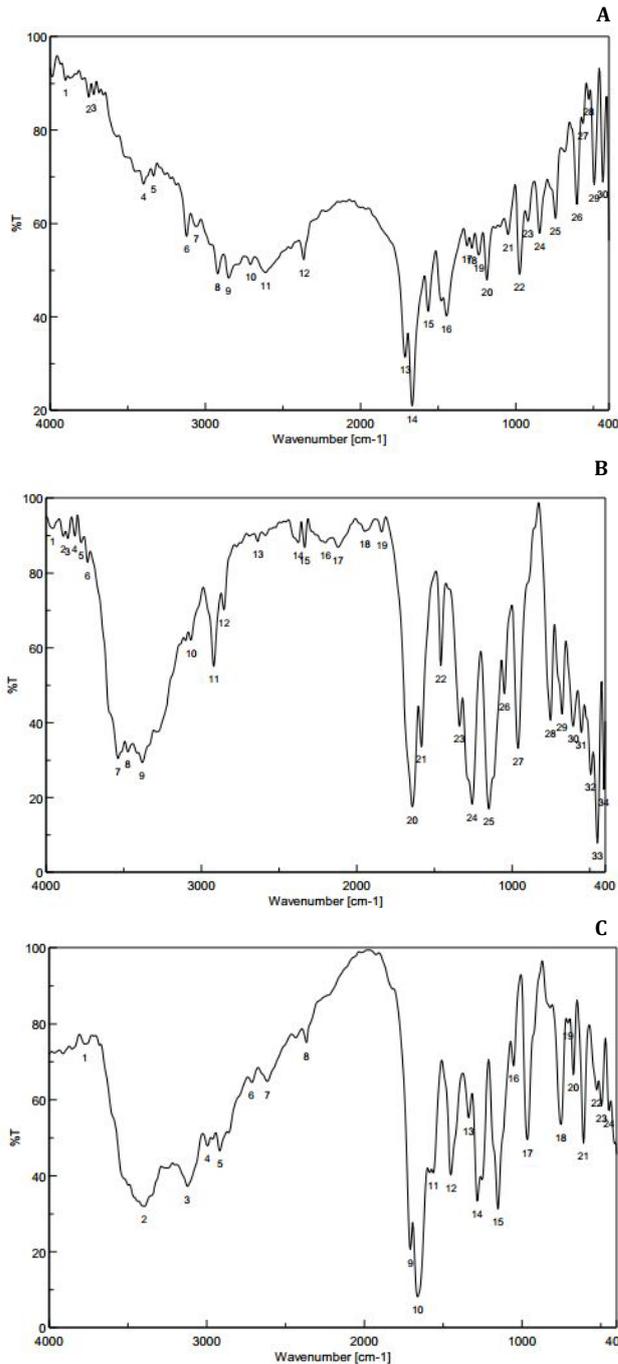


Fig. 3: DTA thermogram a) TA b) NSD



Result of Peak Picking

No.	Position	Intensity	No.	Position	Intensity
1	3899.36	90.616	2	3752.8	87.0787
3	3718.08	87.6075	4	3397.96	68.3781
5	3332.39	70.1292	6	3120.26	57.2272
7	3053.55	59.3229	8	2919.7	49.2264
9	2850.27	48.2913	10	2707.57	51.1974
11	2611.14	49.4601	12	2364.3	52.2567
13	1712.48	31.3656	14	1666.2	20.9068
15	1562.06	41.1937	16	1446.35	40.1535
17	1315.21	55.2099	18	1284.36	54.7143
19	1238.08	53.2394	20	1184.08	47.9368
21	1049.09	57.6037	22	975.804	49.0599
23	921.807	60.4419	24	844.669	57.8775
25	744.388	61.011	26	605.539	64.0795
27	566.969	81.3805	28	532.257	86.5552
29	493.688	68.2534	30	439.69	68.8283

Result of Peak Picking

No.	Position	Intensity	No.	Position	Intensity
1	3957.21	91.8554	2	3887.79	89.7619
3	3860.79	89.1796	4	3814.51	89.7832
5	3772.08	88.0541	6	3733.51	82.8271
7	3536.81	30.4065	8	3475.1	32.1294
9	3382.53	29.324	10	3066.26	61.9977
11	2919.7	54.9756	12	2854.13	70.1315
13	2638.14	88.3878	14	2375.87	88.0495
15	2337.3	86.7764	16	2202.31	88.04
17	2121.31	86.8397	18	1947.75	91.0718
19	1839.76	90.9223	20	1643.05	17.4358
21	1585.2	33.5053	22	1457.92	55.2798
23	1338.36	39.0128	24	1257.36	18.2115
25	1149.37	16.9074	26	1049.09	47.6811
27	960.377	33.1219	28	752.102	40.5983
29	678.82	42.2287	30	605.539	39.0268
31	555.398	37.3067	32	493.688	26.0815
33	451.261	7.73507	34	408.835	22.1492

Result of Peak Picking

No.	Position	Intensity	No.	Position	Intensity
1	3772.08	74.6204	2	3397.96	31.9438
3	3124.12	37.197	4	2996.84	47.7833
5	2915.84	46.5222	6	2715.28	64.5027
7	2615	64.778	8	2368.16	74.9683
9	1708.62	20.5117	10	1662.34	8.06137
11	1562.06	40.6935	12	1450.21	40.139
13	1338.36	55.2609	14	1284.36	33.3096
15	1153.22	31.1435	16	1052.94	68.9346
17	964.233	49.4395	18	752.102	53.4557
19	709.676	80.183	20	674.963	66.5853
21	609.396	48.5774	22	524.543	62.5229
23	493.688	58.4431	24	447.404	57.0991

Fig. 4: FTIR spectra of (A) TA; (B) NSD (C) physical mixture of TA-NSD (2.4:3.6)

Table 2: KFT results

Substance	Weight (mg)	Water content (%)		Average (%)
		I	II	
Theophyllin	50	0.326	0.312	0.319
Sodium saccharin	50	14.781	14.666	14.724

The KFT results then were converted to the amount of water molecules, with the yields are shown in table 3.

Table 3: Water molecule amount in the hydrate determination

Substance	Amount of water (mmol)	Number of molecules (mmol)	Water/molecule	Conclusion
Theophyllin	0.009	0.277	0.032/1	1/31 hydrates
Sodium saccharin	0.409	0.208	1.966/1	dihydrate

From the results of KFT can be seen an average moisture content of theophylline after two trials were 0.319% and sodium saccharin were 14.724%. Based on the calculation, the mole ratio of water: theophylline of 0,032: 1 or it can be said there is one molecule of water of 31 theophylline molecules in the crystal lattice.

This is why the analysis of FTIR and DTA previously seen that there hydrate in the raw materials used theophylline anhydrous. As for sodium saccharin can be concluded that the raw materials used are correct sodium saccharin dihydrate, sodium saccharin in

which each molecule binds two water molecules, namely dihydrate. To identify the characteristics of the shape of the TA and NSD particles, as well as a physical mixture of TA-NSD then analyzed using a polarizing microscope, the results obtained were shown in fig. 5 as follows:

Analysis results of co-crystal from solvent slow evaporation

The co-crystal yielded is translucent colour with habit looks a like needle as shown in fig. 6 and 7 below:

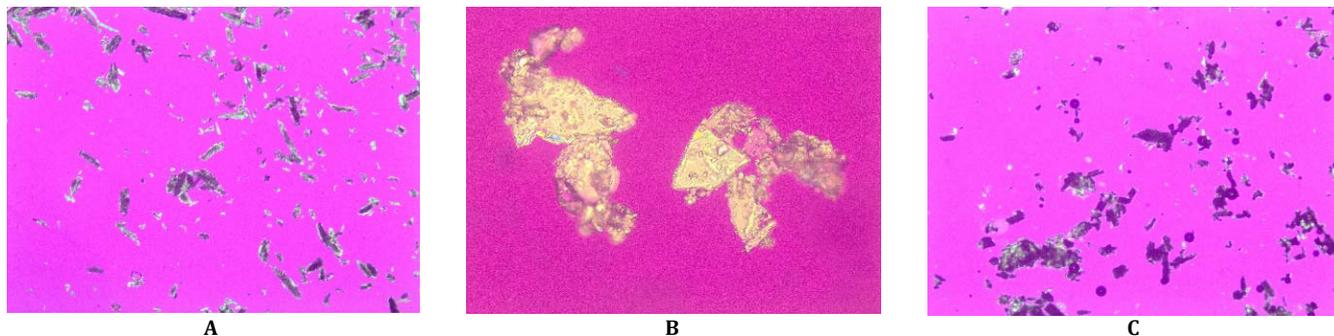


Fig. 5: Results of: polarization microscope (A) TA-40x magnification; (B) NSD-200x magnification; (C) TA-NSD physical mixture-40x magnification

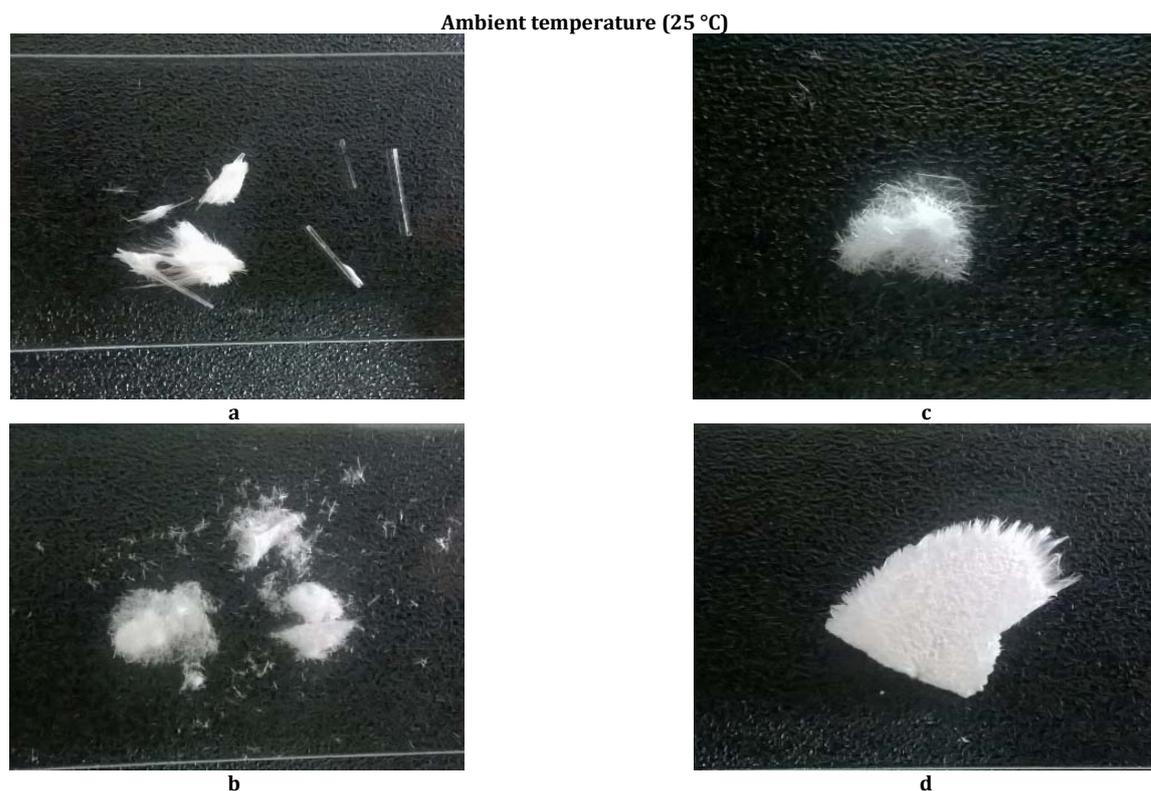


Fig. 6: TA-NSD co-crystals yielded from solvent: (a) Water-isopropanol 1:7; (b) Ethanol-dichloromethane 9:1; (c) Ethanol; (d) Water-acetone 1:9 at ambient temperature and 12 °C

Fig. 6 and 7 show that TA-NSD co-crystal has the different habit and particle sizes from its constituent components. TA-NSD co-crystal shows rod-shaped crystal habit (rod-shaped habit) with translucent color. Single crystal TA-NSD was formed using water-isopropanol solvent 1:7 (at ambient temperature and a temperature of 12 °C) with a size of 0.6 to 1.2 cm and a water-acetone solvent 1:9 (at temperature 12 °C) with 0.5-1.1 cm size.

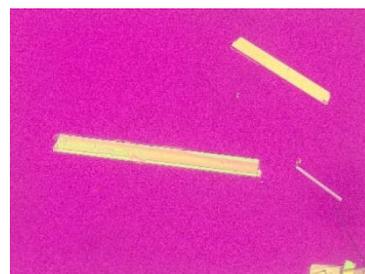
The solvents used and co-crystallization technique will affect the rate of crystal growth, which will influence on the habit formed and its particle size distribution.

Rendermen of co-crystal formation with slow evaporation method was observed for any type of solvent used and the temperature of storage with such results in table 4 below:

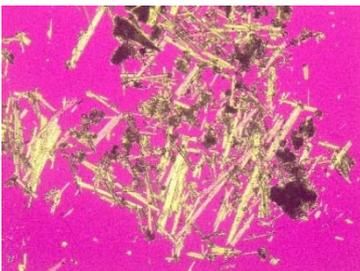
Ambient temperature (25 °C)



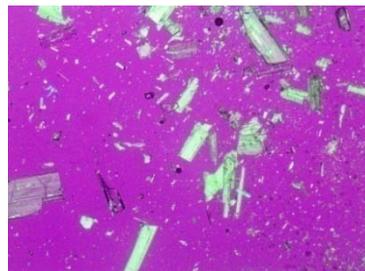
a) Magnification 40x



c) Magnification 40x

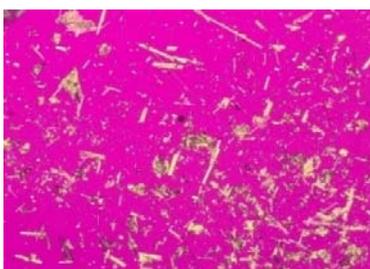


b) Magnification 40x

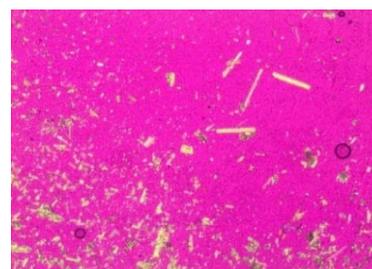


d) Magnification 40x

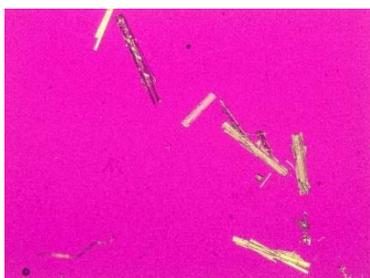
Temperature: 12 °C



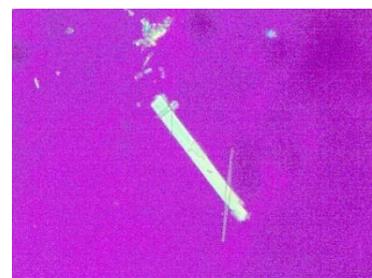
a) Magnification 40x



c) Magnification 40x



b) Magnification 40x



d) Magnification 40x

Fig. 7: TA-NSD co-crystals yielded from solvent: (a) water-isopropanol 1:7; (b) ethanol-dichloromethane 9:1; (c) ethanol; (d) water-acetone 1:9 at ambient temperature and 12 °C using a polarization microscope

Table 4: Co-crystal TA-NSD Rendemen

Ambient temperature (25 °C)	
Solvent	Rendemen (%)
Water-Isopropanol 1:7	92.29
Ethanol-Dichloromethane 9:1	96.74
Ethanol	95.76
Water-Acetone 1:9	86.94
Temperature: 12 °C	
Solvent	Rendemen (%)
Water-Isopropanol 1:7	95.55
Ethanol-Dichloromethane 9:1	87.78
Ethanol	87.12
Water-Acetone 1:9	90.93

Factor that influences the size of crystals is nucleation and crystal growth rate. Nucleation is the process whereby new crystal nuclei formed. Nucleation and crystal growth rate itself is influenced by several things such as supersaturation and temperature conditions. Super-saturation is a condition in which the concentration of solute exceeds the saturation concentration. This supersaturated condition can be achieved by a decrease in temperature, evaporation of the solvents, salting out, and the adiabatic evaporation [7]. When the solvent evaporates, the solute concentration increases and reaches supersaturation conditions. Super-saturation condition with decreasing of temperature is explained by the theory of Miers as follows:

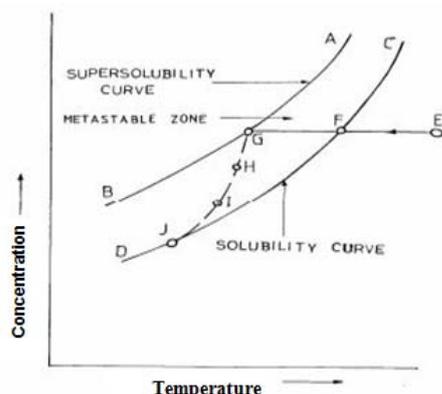


Fig. 8: Miers's solubility curve [7]

On this theory, the assumption is that the solution is saturated and free of impurities. At point E solution at a temperature T cooled to point F and G. Crystallization would only start after the point F or the line CD. When crystallization began around the point G in the meta-stable zone, nucleation occurs and decreases the concentration of solute indicated by point GHJ. After the nucleation process, the next process is the growth of crystals. Basically, this process is the phenomenon of the mass transfer from the liquid phase (solution) to the solid phase (crystals) of the previously formed nucleus. These theories can explain the relationship of the crystal formation temperature and rate of solvent evaporation.

The faster the solvent can be evaporated, the opportunity for the occurrence of nucleation and crystal growth completely. Water-isopropanol solvent 1:7 is the most difficult solvent evaporates, so the crystal formation needs longer time than from the other system. Isopropanol also has a greater viscosity among other solvents, which amounted to 2.1 cP at ambient temperature, and its viscosity will be increased when placed in a temperature of 12 °C. The higher viscosity of the solvent, the crystal growth rate decreases because the viscosity is lowering the rate of diffusion of solute to the surface of the crystal. Although the rate of crystal formation was slower, but the single crystal could be formed clearly with a large enough size. However, the constant dielectricum of the used solvents are almost semi-polar-polar, as explained in table 5. The constant dielectricum is important thing to be known as solubility of one material determined by the proper of this solvent polarity, which must be near to the solute.

Tabel 5: Solvent's dielectricum constante

Solvent	Composition	Dielectric constante
Water: Isopropanol	1: 7	25.68
Water: Acetone	1: 9	26.64
Ethanol	1	24.50
Ethanol: Dichloromethane	9: 1	22.94

To verify the interaction between the TA-NSD, then was performed an analysis using FTIR, PXRD, and analysis of single crystals with SC-XRD.

The FTIR data is shown in table 6.

Table 6: TA-NSD Co-crystal's specific FTIR spectra data

In ambient temperature (25 °C)	
Solvent system	Wave lenght (cm ⁻¹)
Water-Isopropanol 1:7	3127.97
Ethanol-Dichloromethane 9:1	3127.97
Ethanol	3127.97
Water-Acetone 1:9	3131.83
T: 12 °C	
Solvent system	Wave lenght (cm ⁻¹)
Water-Isopropanol 1:7	3127.97
Ethanol-Dichloromethane 9:1	3127.97
Ethanol	3127.97
Water Acetone 1:9	3127.97

FTIR spectra data show that co-crystal formation signed by spectra shifting of synthon group in its wavelength area.

The material characterization data showed that the group NH on theophylline provides a peak at 3120 cm⁻¹ meanwhile the TA-NSD physical mixtures show the peak at wave number 3124 cm⁻¹. It has been reported that TA-NSD co-crystal shifted from 3127 to 3131.83 cm⁻¹ [6]. FTIR spectra obtained as listed in table 6, explain that all co-crystal formed show the shifting of the wave number, which from 3127 to 3131.83 cm⁻¹. To further confirmation, PXRD analysis was performed and compared with the co-crystal milling resulted, whichever reported by Nugrahani *et al.* (2014) [5].

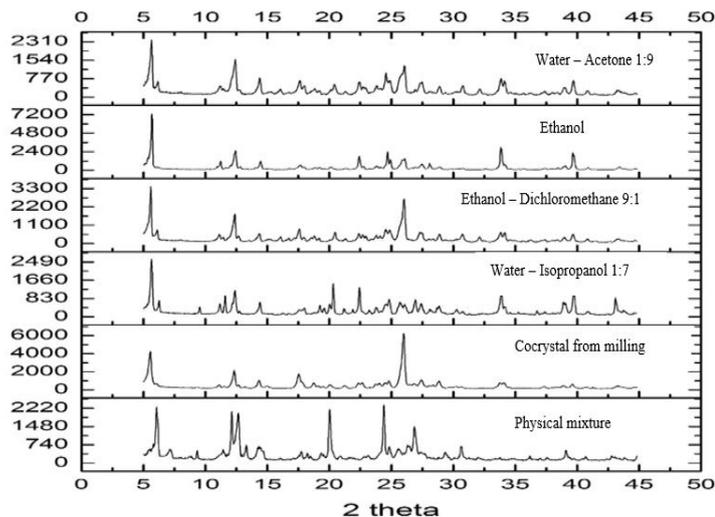
PXRD analysis is a method that can be used to characterize the interaction between solids/solid-state interaction [8]. The interaction between theophylline and sodium saccharin leads to changes in the crystal lattice spacing. X-ray analysis in general has been used to measure the distance of the average crystal lattice [9]. The crystal lattice spacing changes will cause the changes of diffractogram pattern, which characterized by the appearance or loss of the peaks, compared to its physical mixture. It has been reported that co-crystal theophylline and sodium saccharin will form the new peaks at 2θ : 5.58, 12.32, 17.48, 26.86, 27.4, and 28.84 ° [5]; as well as at 2θ : 24.45 ° [6]. Diffractogram data in fig. 9 shows that co-crystal resulted from several solvents at different storage temperatures provide the same peak of the 2θ , coupled with two peaks were detected at around 2θ : 22.4 and 34.1 °. The spectra data is reviewed in table 8.

The peak intensity of diffractogram shows crystallinity of a substance being analyzed and the number of crystals in a material [10]. Linearity of the relationship between intensity and concentration have been derived theoretically [11]. All of co-crystal yielded using slow evaporation method, which formed at a temperature of 12 °C with a water-acetone solvent 1: 9, showed the highest crystallinity, but still lower than co-crystal resulted from ball milling process. However, from slow evaporation methods, it can be collected the single crystals, which isolated for structural analysis using the SC-XRD. TA-NSD single crystals obtained from water-isopropanol 1:7 produced at ambient temperature, and a water-acetone 1:9 with storage temperature of 12 °C; were analyzed using the SC-XRD to determine the co-crystal structure completely. Results obtained were described in fig. 10 as follows.

Furthermore, the stability test to TA-NSD co-crystal formed against humidity was performed in RH>90%. The interaction between theophylline with sodium saccharin is greater than its interaction with water molecules, caused the decreasing of theophylline hygroscopicity. It had been reported previously that TA-NSD co-crystal which be prepared by milling methods can decrease the hygroscopicity of theophylline [6]. Moreover, this test was intended to look at the stability of the single crystal resulted using slow evaporation methods, afterward evaluated it by using FTIR.

From FTIR analysis, it was proven that co-crystal typical peak wave number from 3127 to 3131.83 cm⁻¹ showed unchanged after storage in 90% RH, as listed in table 9. These results suggest that the interaction between theophylline and sodium saccharin as co-crystal is stable.

Temperature ambient (25 °C)



T: 12 °C

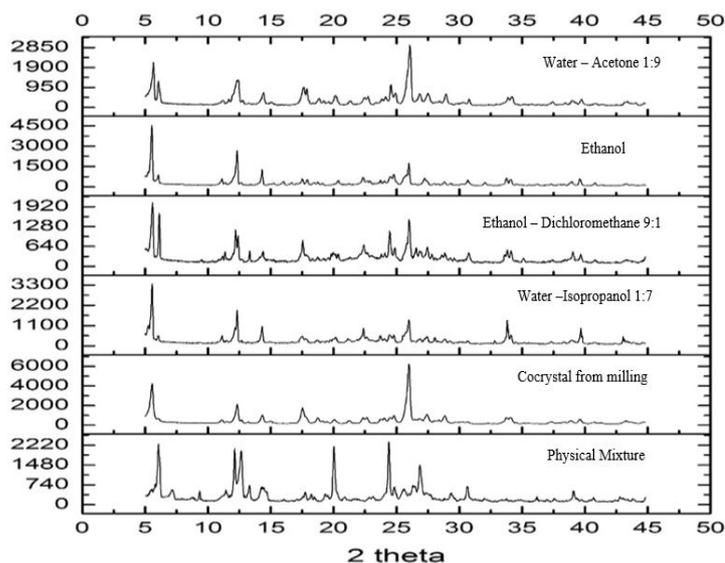


Fig. 9: PXRD of TA-NSD co-crystal

Table 7: TA-NSD Co-crystal diffractogram peak intensity

Ambient temperature (25 °C)									
Co-crystal from milling method		Water-isopropanol 1:7		Ethanol-dichloromethane 9:1		Ethanol		Water-acetone 1:9	
2θ	I	2θ	I	2θ	I	2θ	I	2θ	I
5.58	4045	5.66	2632	5.60	3434	5.68	7310	5.66	2391
12.32	2052	12.38	1197	12.40	1640	12.44	2550	12.42	1592
17.48	1560	17.56	328	17.56	795	17.54	557	17.64	697
26.86	676	26.94	784	26.56	228	26.94	428	26.96	328
27.40	1069	27.44	605	27.42	645	27.3	471	27.46	625
28.84	936	28.88	493	28.90	571	28.84	408	28.86	449
22,36	767	22.40	1318	22.42	571	22.42	1840	22.44	650
34,08	795	34.14	458	34.12	630	34.14	734	34.14	686
T: 12 °C									
Co-crystal from milling method		Water-isopropanol 1:7		Ethanol-dichloromethane 9:1		Ethanol		Water-acetone 1:9	
2θ	I	2θ	I	2θ	I	2θ	I	2θ	I
5.58	4045	5.56	3376	5.58	2061	5.54	4556	5.68	2116
12.32	2052	12.32	1945	12.40	992	12.32	2683	12.40	1310
17.48	1560	17.52	534	17.54	847	17.50	605	17.64	961
26.86	676	26.88	400	26.84	497	26.90	222	26.84	660
27.40	1069	27.44	520	27.48	620	27.44	471	27.44	605
28.84	936	28.82	376	28.86	441	28.84	404	28.96	640
22,36	767	22.28	342	22.40	692	22.32	713	22.50	480
34,08	795	34.10	581	34.14	493	34.20	180	34.18	524

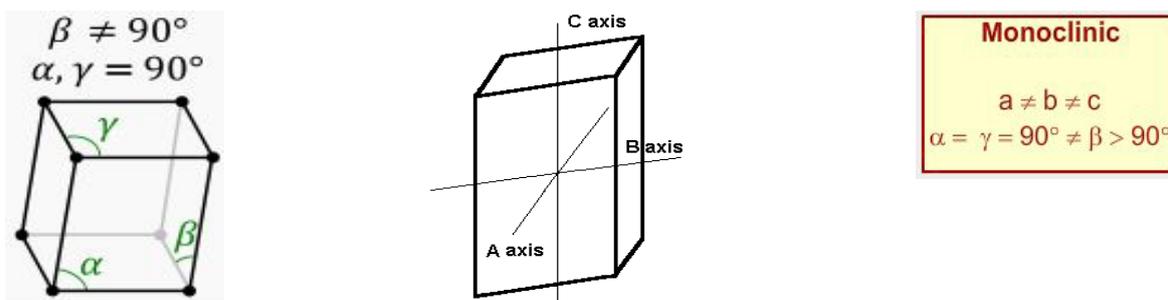
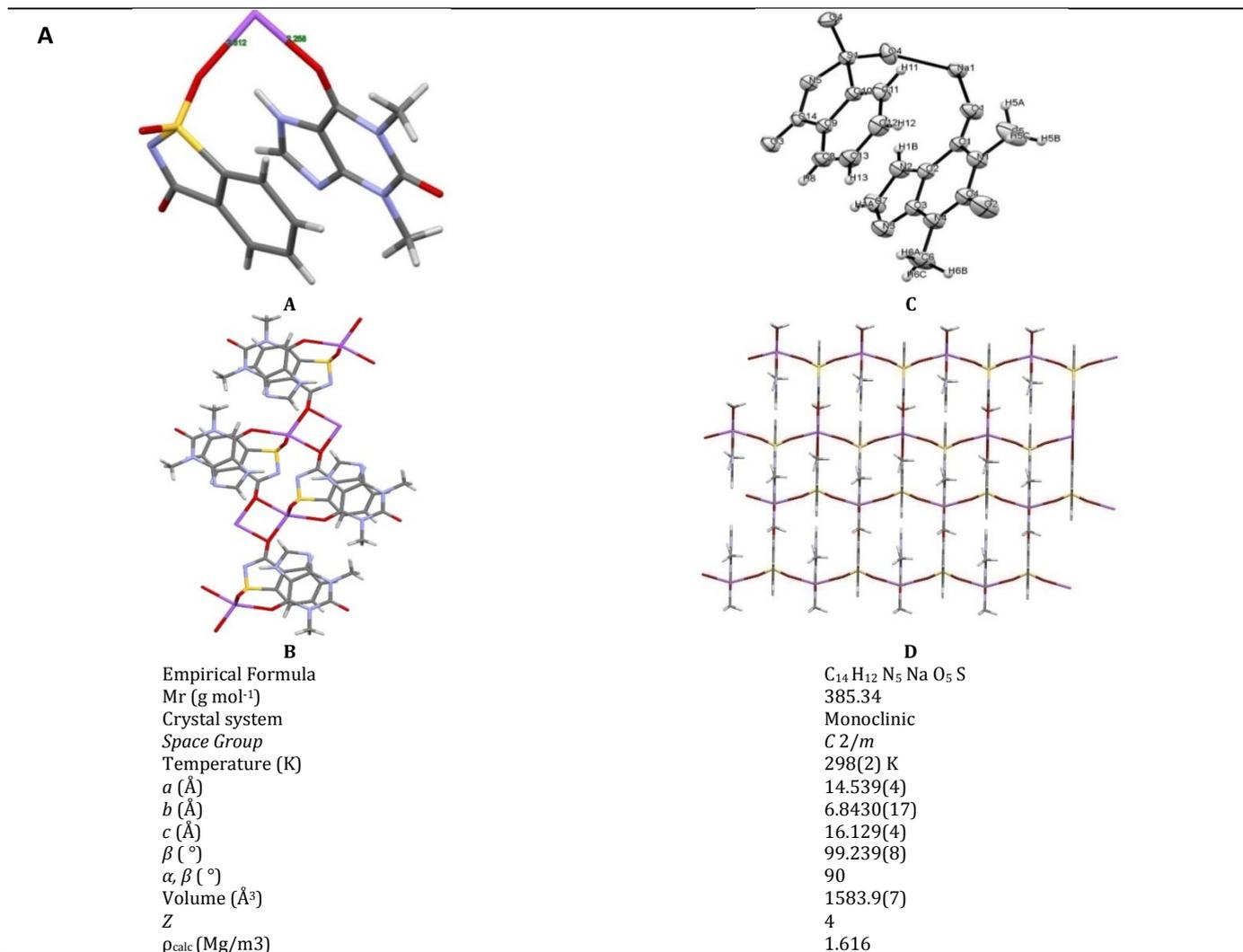


Fig. 10: Monoclinic system of TA-NSD Co-crystal

Table 8: TA-NSD Co-crystal FTIR spectra data from stability test in humidity

Ambient temperature (25 °C)		
Solvent	Wave length (cm ⁻¹) before tested	Wave length (cm ⁻¹) after tested
Water-Isopropanol 1:7	3127.97	3127.97
Ethanol-Dichloromethane 9:1	3127.97	3127.97
Ethanol	3127.97	3127.97
Water Acetone 1:9	3131.83	3131.83
T: 12 °C		
Solvent	Wave length (cm ⁻¹) before tested	Wave length (cm ⁻¹) after tested
Water-Isopropanol 1:7	3127.97	3127.97
Ethanol-Dichloromethane 9:1	3127.97	3127.97
Ethanol	3127.97	3127.97
Water-Acetone 1:9	3127.97	3127.97

CONCLUSION

From all the observations and analysis, it is concluded that TA-NSD co-crystal in single crystal form can be produced by solvent evaporation. The single crystals can be yielded at ambient temperature from a water-isopropanol solvent 1:7; and at a temperature of 12 °C from isopropanol 1:7, as well as water-acetone 1:9. The single crystal yielded is colored translucent with a needle-shaped form, and has a monoclinic system in a volume of 1583.9 Å³. The stability test showed that the TA-NSD co-crystal was stable against moisture.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Trask AV, Motherwell WD, Sam W, Jones W. Physical stability enhancement of theophylline via co-crystallization. *Int J Pharm* 2006;320:114-23.
2. Ronco MPF, Kluge J, Mazzotti M. High-pressure homogenization as a novel approach for the preparation of co-crystals. *Cryst Growth Des* 2013;13:2013-24.
3. Amjad A. Theophylline co-crystals prepared by spray drying: physicochemical properties and aerosolization performance. *AAPS Pharm Sci Tech* 2013;41:265-76.
4. Banerjee R, Bhatt PM, Ravindra NV, Desiraju GR. Saccharin salts of active pharmaceutical ingredients their crystal structures and increased water solubilities. *Cryst Growth Des* 2005;5:2298-09.
5. Nugrahani I, Bahari MU. The dynamic study of co-crystal formation between anhydrous and monohydrate theophylline with sodium saccharine dihydrate by FTIR. *J Chem Biochem* 2014;2:117-37.
6. Syahbani MM, Nugrahani I. Determination stoichiometry of theophylline and sodium saccharine co-crystal and hygroscopicity test. Undergraduate Thesis. School of Pharmacy-ITB, Bandung; 2014.
7. Khanam J. Crystallization. Jadavpur University. Kolkatta; 2007. p. 8-11.
8. Trask AV, Jones W. Crystal engineering of organic co-crystal by the solid state grinding approach. *Top Curr Chem* 2005;254:41-70.
9. Salih AI. Effect of carbon wt % on the iron X-ray diffraction pattern and the volume of iron unit cell. *Sci Studies* 2010;6:155-65.
10. Zhishen MB, Hao Y, Dan-Ni Z. The degree of crystallinity of multicomponent Polymers by WAXD. *Chin J Polym Sci* 1994;12:296-301.
11. Chung FH, Scott RW. A new approach to the determination of crystallinity of polymers by X-ray diffraction. *J Appl Cryst* 1973;6:225-30.