Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(11):291-299

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Characterization, phase solubility studies and molecular modeling of Isoniazid and its β-Cyclodextrin complexes

Shariza Abdul Razak[1], Syed Fariq Fathullah Syed Yaacob[2*], Jafri Malin Abdullah[2] and Rohana Adnan[3]

[1]School of Health Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia
[2]Department of Neurosciences, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia
[3]School of Chemical Sciences, Universiti Sains Malaysia, Minden, Pulau Pinang, Malaysia

---

ABSTRACT

Isoniazid, the first line anti-tuberculosis agent, and its complexes with β-cyclodextrin were investigated. The 1:1 stoichiometry ratio for the complex has been proposed based on Job's plot and A_L diagram from phase solubility studies. These results were further interpreted using PM3 semi-empirical method molecular modeling. The most optimum position of isoniazid was located at the center of β-cyclodextrin cavity with pyridine ring of isoniazid facing towards primary rim of β-cyclodextrin with minimum energy of -2.2990407 Hartree (-1442.67 kcal mol^{-1}). Two intermolecular hydrogen bonds were formed and weak molecular interaction between β-cyclodextrin and isoniazid was detected. In conclusion, the theoretical results were in good agreement with experimental results.

Keywords: Cyclodextrin; Isoniazid; Inclusion complexation; Molecular modeling; Phase solubility studies

---

INTRODUCTION

Over the past decade, tuberculosis (TB) has become the most dangerous contagious chronic bacterial infection around the world. In fact, it caused nearly 3 million deaths annually that make World Health Organization (WHO) declared TB as global emergency [1]. Currently, TB was cured using first line drugs like isoniazid, rifampicin, pyrazinamide and ethambutol. In the first two months, TB will be treated intensively with isoniazid, pyrazinamide and rifampicin. Then, to eliminate any persistence tubercle bacilli, isoniazid and rifampicin will be used for the next four months [2].

Isoniazid, pyridine-4-carboxylic acid hydrazide, is an anti-tuberculosis agent, which is usually used to prevent the development of clinical tuberculosis It is denoted with the formula $C_6H_7N_3O$ with a molecular weight of 137.14 g/mol. Isoniazid is white powder, freely soluble in water (about 1g/10ml), odorless and melts at high temperature (about 170-174 °C) [3-4]. However, the intensive strategy to counter the spread of the disease has been disturbed by some problems. In the case of isoniazid, although it dissolved in water easily, it is also easily degraded by light. Hence, it is important to protect isoniazid against degradation by light. One method to overcome such problem is by including isonazid complexation in cyclodextrin cavity.

Cyclodextrins are cyclic oligosaccharides containing α-, β-, and γ-cyclodextrins that comprised of 6, 7, and 8 glucopyranose units, respectively, and linked by α- (1,4) bonds. Cyclodextrins are produced via intramolecular transglycosylation reaction during degradation of starch by cyclodextrin glycosyltransferase (CGTase) enzyme [5]. Cyclodextrin ring has cylindrical shape or more precisely like a conical cylinder, which is usually illustrated as a doughnut or wreath-shaped truncated cone. Since glucopyranose exists as chair conformation, it makes a surface more hydrophilic because the hydroxyl functional group faces towards the cone exterior with the primary hydroxyl situated at the narrow and wider edge. Then, this surface also provides a hydrophilic environment, which dissolves cyclodextrin in water. Meanwhile, the ethereal oxygen and skeletal carbon of glucopyranose unit form the central cavity which gives cyclodextrin a hydrophobic inner surface that enables it to function as a host to trap several guest molecules [6-9].

From the literatures review, cyclodextrin complexation has been shown as the most effective approach to overcome the problem faced by most drugs in terms of solubility, stability, and bioavailability in the pharmaceutical industry. Moreover, in food industries, cyclodextrin has the potential to act as masking agent to cover the bad taste and odor of food. On the other hand, cyclodextrin can also act as a drug-releasing system to prolong drug release in our bodies [10-13].

Hence, the purpose of this study was to investigate the possibility of complexation between β-cyclodextrin and isoniazid, as the host-guest interaction of this complex has not been explored widely. The complexation isoniazid with β-cyclodextrin was prepared using five different methods: physical mixture, kneading, co-precipitation, co-evaporation and freeze-drying. The ratio of complexes was also determined using phase solubility studies and Job's plot. Fourier Transform Infrared Analysis (FTIR), Thermogravimetric Analysis (TGA) and Different Scanning Calorimetry (DSC) were used to characterize the physiochemical determinations of the complexes. Molecular modeling was also performed to study the molecular interaction between β-cyclodextrin and isoniazid using Gaussion 03W and Hyperchem software.

EXPERIMENTAL SECTION

2.1 Materials

β-cyclodextrin was purchased from Sigma Aldrich, USA and isoniazid was obtained from Sigma Aldrich, India. Both materials were used without any further purification. Other chemical and reagents used were of analytical grade.

2.2 Molecular modeling studies

Molecular recognition and molecular discrimination of β-cyclodextrin over isoniazid were done through molecular modeling technique, using software such as Gaussian [14] and Hyperchem [15]. The geometry structure of β-cyclodextrin was obtained from the Protein Data Bank (PDB) in crystal β-cyclodextrin complex (PDB ID-3CGT) and the geometry of isoniazid was also obtained from PDB (PDB ID-1ZID). The method and strategy used to build and design the host-guest complex of β-cyclodextrin and isoniazid were based on coordinate system shown in Figure 1. Similar method was used by other researchers with some modifications to suit the guest molecule [16, 17]. The calculation system to optimize all geometries was done at the Parameterized Model number 3 (PM3) level using quantum mechanics (QM). Calculations were carried out in the gas phase, with the salvation effects excluded from the calculation. The shape of complexes, energy, preferred binding orientation, and selectivity are typically computed. Using PM3 semi empirical system, the orientation of the drug in cyclodextrin cavity, the depth of penetration and the rotational orientation of the drug inside cyclodextrin cavity were evaluated in order to get as close as possible to the global minimum.

2.3 Phase solubility studies

The stability constant for inclusion complex between β-cyclodextrin and isoniazid were calculated using phase solubility studies method described by Higuchi and Connors [18]. Excess amount of isoniazid was added to 10 mL β-cyclodextrin aqueous solution with increasing concentrations (1×10^{-3} to 1×10^{-2} M). The flasks were sealed with aluminum foil and stirred with magnetic bar using magnetic stirrer at 100 rpm at room temperature for 48 hours until it reached equilibrium. After 48 hours, the stirrer was turned off and the temperature was maintained for 6 hours to avoid forced solubility during stirring. The solution was filtered using 0.2 μm Whatman™ microfiber filter paper to remove undissolved compounds. The solutions were analyzed spectrophotometrically using UV-VIS spectrophotometer (Perkin Elmer 25 UV-Vis lambda) at 262 nm. These steps were repeated in triplicates.

The stability constant (K_a) of the complex was calculated using the slope and the y-intercept of the straight line from the phase solubility diagram based on equation below:

K_a= slope/S_0 (1-slope) (1)

where S_0 was the equilibrium solubility of isoniazid in water.

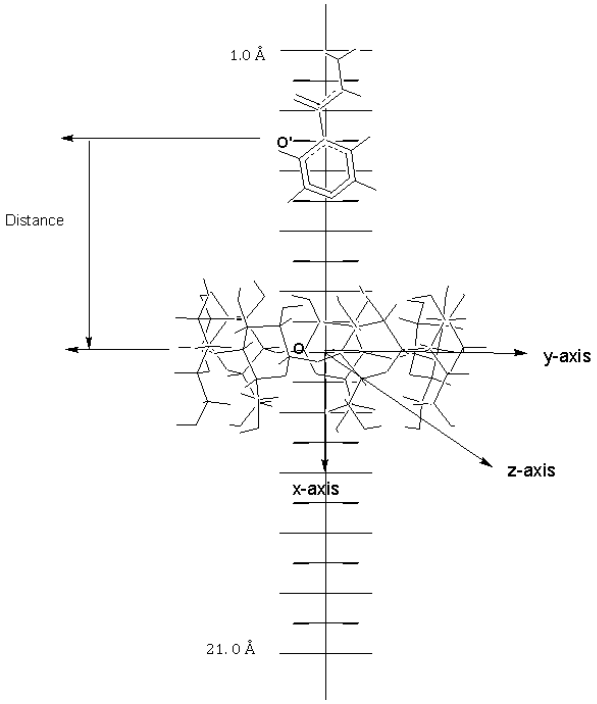

Figure 1: The scheme of movement of isoniazid at position 4Å and 0°

2.4 Continuous Variation Method (Job's Plot)

Job's plot was carried out according to Job [19]. Equimolar 2.25 x 10^{-4} M solution of β-cyclodextrin and isoniazid were mixed to a fixed volume and the concentrations of both solutions were kept constant. All solutions were stirred for about 24 hours at room temperature at 100 rpm. The solutions were analyzed using UV-Vis spectrophotometer (Perkin Elmer 25 UV-Vis lambda) at 262 nm. These steps were also done in triplicates. The graph $\Delta A = A - A_{o,}$ which shows the difference in absorbance in the present and absent of β-cyclodextrin against R, where R was R = [INH]/ {[INH] + [β -CD]} was plotted.

2.5 Preparation and characterization of host-guest complexes

2.5.1 Physical Mixture

The mixtures were prepared by pulverization using mortar and pestle for 30 minutes. Then, the mixture was collected and kept in desiccators for further experiment.

2.5.2 Kneading Method

An equal molar of β-cyclodextrin and isoniazid were mixed simultaneously in mortar with a pestle for 10 minutes. 1 ml of a 1:1 ratio mixed ethanol-water (v/v) was added to the mixture, and the paste was further kneaded for another 1 hour. Kneading steps were done until the product was dried at the wall of the mortar. The product was collected and dried in the oven at 60°C for 24 hours. Then, it was pulverized to a fine powder and kept in desiccators for further evaluation.

2.5.3 Co-Precipitation

An equimolar of β-cyclodextrin and isoniazid were prepared separately and stirred using magnetic bar. After these solutions reached equilibrium, β-cyclodextrin aqueous solution was added slowly, drop by drop, into the aqueous

solution containing isoniazid. Then, the mixture was stirred for 48 hours at 100 rpm at room temperature. After it reached equilibrium, the solution was filtered using 0.2 μm Whatman™ microfiber filter paper to remove undissolved compound. The residue was collected and dried in the oven at 60°C for 24 hours. The product was pulverized to a fine powder and kept in desiccators for further experiment.

2.5.4 Co-Evaporation

An equimolar of β-cyclodextrin and isoniazid were prepared separately and stirred using magnetic bar. After these solutions reach equilibrium, β-cyclodextrin aqueous solution was added slowly to isoniazid solution. Then, the mixture was stirred for another 48 hours at 100 rpm at room temperature. After it reached equilibrium, the solution was filtered using 0.2 μm Whatman™ microfiber filter paper to remove undissolved compound. The residue was collected and dried at room temperature for another 72 hours. The product was pulverized to a fine powder and kept in desiccators for further experiment.

2.5.5 Freeze-drying

An equimolar of β -cyclodextrin and isoniazid were prepared separately. The aqueous solution of β-cyclodextrin and isoniazid were mixed together and continuously stirred with magnetic bar for 48 hours at room temperature until it reached equilibrium. The resultant solution was then frozen at -20 °C for 24 hours and was lyophilized for another 48 hours in the freeze dryer. The product was collected and kept in the desiccators for further analysis.

2.5.6 Fourier Transform Infrared Analysis (FTIR)

Before analyzing the samples, the individual sample and potassium bromide (KBr) were mixed in a 1:3 ratio using mortar and pestle. Eight tons hydraulic pressure was used to form a pallet mixing sample. The spectra were scanned in the range of 400- 4000 cm^{-1} using Perkin Elmer System 2000 FTIR spectrometer.

2.5.7 Thermogravimetric Analysis (TGA)

About 5-10 mg of the samples was weighed using aluminum crucible. The sample was then placed in a pre-equilibrated furnace at ambient temperature. The sample was heated from 20 to 700 °C at a heating rate of 10 °C / min using TGA, Mettler TGA/SDTA 851e under dynamic argon atmosphere. The change in weight with temperature was recorded for all samples.

2.5.8 Different Scanning Calorimetry (DSC)

5- 10 mg of samples was prepared and placed in aluminum pans. Then, the sample was heated between 0 to 250 °C at a rate of 20 °C / min using DSC, Pyris 1DSC Perkin Elmer under nitrogen flow. The thermograms for all samples were recorded. The empty aluminum pans were used as reference.

RESULTS AND DISCUSSION

3.1 Molecular Modeling Studies

Figure 2 shows the structure of the complex obtained through molecular modeling studies according to the method described in the experimental section. The low energy conformation of β-cyclodextrin/isoniazid complex was found at -2.2990407 Hartree (-1442.67 kcal mol^{-1}) with the isoniazid positioned in the center cavity of β-cyclodextrin and pyridine ring of isoniazid facing towards the primary rim of β-cyclodextrin cavity.

The binding energy of complex $\Delta E_{1:1}$ was -0.020 Hartree (-12.64 kcal mol^{-1}). The binding energy was defined as the difference between the sum of energy of individual host and guest molecule and the energy of the inclusion complex. Negative value indicates stronger interaction. Therefore, the more negative the value is, the stronger is the interaction between molecules. Based on the calculated value, the interaction between β-cyclodextrin and isoniazid was weak [20].

The molecular interaction between O-H---O atom in the complex forms intermolecular hydrogen bonding formation. From Figure 1(b), we can see that an interaction occurred between the oxygen atom at isoniazid with H-O bond at the sixth ring of D-glucopyranose of β-cyclodextrin. The distance of hydrogen bond H---O between hydrogen atom at the sixth ring of β-cyclodextrin and the oxygen atom at isoniazid was 1.80675 Å and its angle was 169.9°. The distance for the second hydrogen bond H----O between the hydrogen atom at the sixth ring of β-cyclodextrin and the oxygen atom at isoniazid was 1.82730 Å and its angle was 173.2°. The distances and angles were possible to be formed in hydrogen bonding interaction.

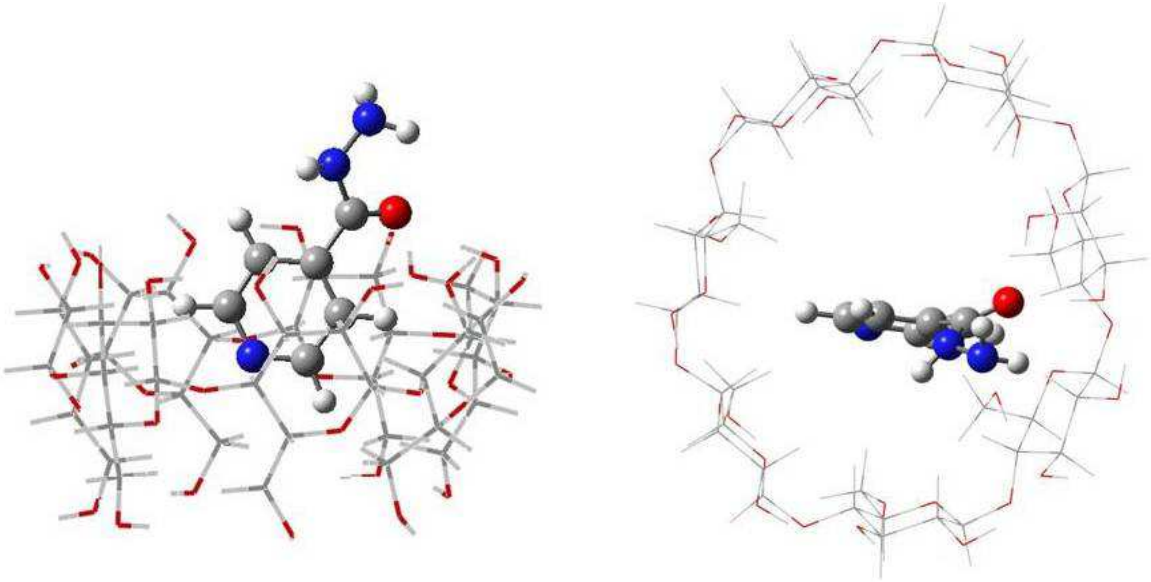

Figure 2: The structure of the complex from side view (a) and top view (b)

3.2 Phase Solubility Studies

Figure 3 shows the phase solubility study for the complex formed between β-cyclodextrin and isoniazid based on Higuchi and Conners study. The plot shows that the aqueous solubility of isoniazid increased linearly in tandem with increasing β-cyclodextrin concentration. Based on the graph, it was suggested that the formation between β-cyclodextrin and isoniazid was in a 1:1 ratio since the linear host-guest correlation slope was less than 1, and it was an A_L type graph with respect to β-cyclodextrin concentration. The stability constant, K_a value calculated using the slope obtained from equation 1 was 5623.7 M^{-1}. The larger K_a indicated that the complex in a 1:1 ratio was a stable complex. Moreover, if the stability constant is too weak, there will be little improvement in terms of drugs solubility. But, if the stability constant is too strong or greater than 10 000 M^{-1}, the complex is unstable and cannot dissociate easily. Hence, this present study shows that the complex was stable and suitable for improving isoniazid stability based on the stability constant.

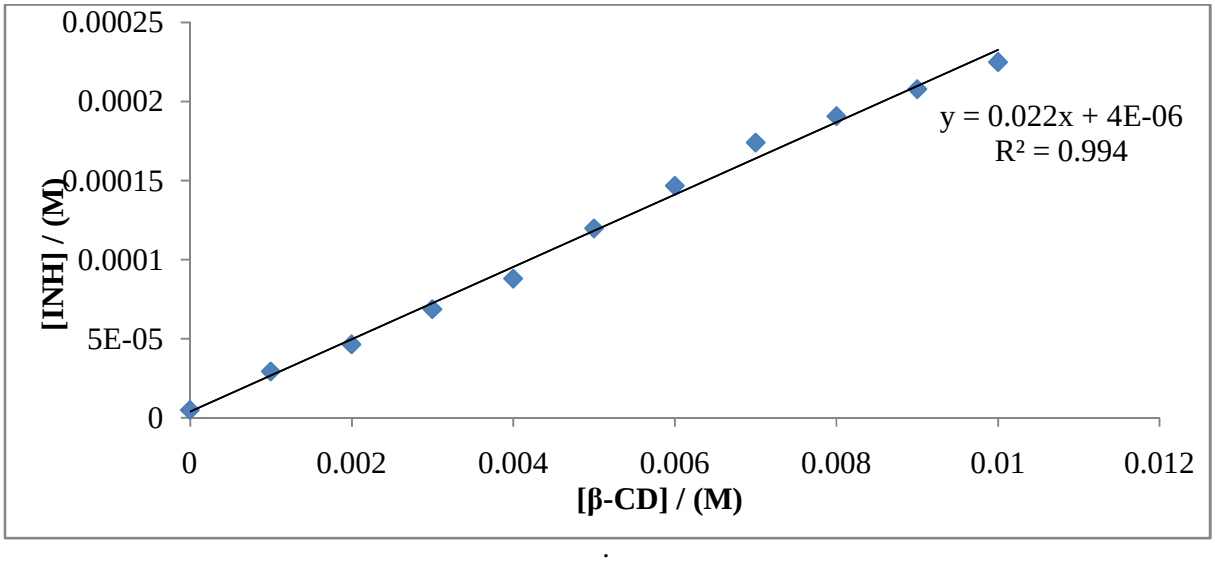

Figure 3: Phase solubility studies profile of isoniazid/β-cyclodextrin complex at 25 °C

3.3 Continuous Variation Method (Job's Plot)

The interaction between β-cyclodextrin and isoniazid was further studied by carrying out continuous variation method (Job's plot) to determine the stoichiometry of the complex. According to Job's plot method, a physical parameter directly related to the concentration of the complex can be measured for a set of sample with continuously varying molar fraction of its components [21]. In the sample, the maximum concentration of the complex occurred when the molar ratio, R corresponded to the complexation stoichiometry. Based on Figure 34, the maximum absorbance variation for isoniazid in β-cyclodextrin was observed at R= 0.5. This result shows that the main stoichiometry of isoniazid and β-cyclodextrin complex was a 1:1 ratio and this indicates that it was in agreement with the phase solubility studies result, which suggested the same molar ratio.

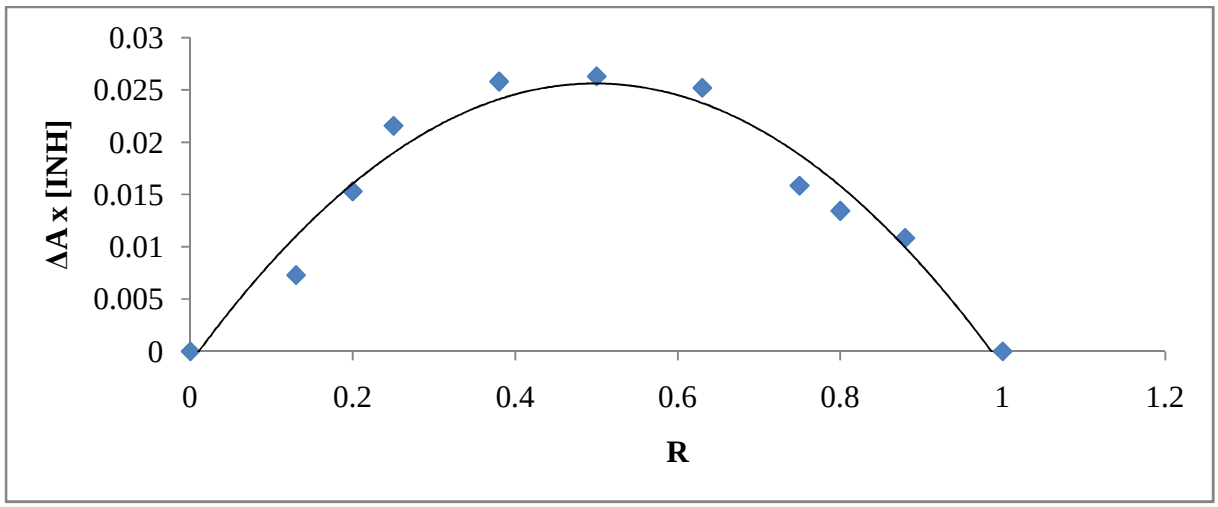

Figure 4: Continuous variation method (Job's plot) for isoniazid/β-cyclodextrin system from absorbance measurements

3.4 Fourier Transform Infrared (FTIR) analysis

FTIR analysis method is the most important technique to estimate the interaction between host and guest in solid state. FTIR spectra for β-cyclodextrin, single isoniazid and complexes from five different methods are shown in Figure 5. Upon complexation, the absorption vibrations of complexes were dominated by β-cyclodextrin bands since β-cyclodextrin structure consisted of seven repeated D-glucopyranose unit. Hence, the recorded FTIR spectra of complexes were quite similar with FTIR spectra of β-cyclodextrin [22, 23]. These phenomena occurred because of two factors. Firstly, the host-guest molecule accidentally had the absorption vibration value due to the same chemical bonding. Secondly, β-cyclodextrin, which had polar functional groups, C-O and O-H, resulted in a strong absorption vibration. There were three absorption bands that showed the formation of host-guest complexation. Firstly, absorption band at ~ 2924 cm^{-1}, which showed stretching of C-H bond in all complex and β-cyclodextrin molecule, but was absent in pure isoniazid. Secondly, absorption band at ~1556 cm^{-1} represented as N-N-H bending both in isoniazid and all complexes, but was absent in β-cyclodextrin. Lastly, absorption band at~ 1334 cm^{-1}showed C-N stretching in isoniazid and all complex spectra, but was absent in β-cyclodextrin. Hence, these observations can be considered as a confirmation for the host-guest formation between β-cyclodextrin and isoniazid [24, 25].

3.5 Thermogravimetric Analysis (TGA)

Figure 6 shows the TGA profile for β-cyclodextrin molecule in two stages of weight loss. The first stage decomposition occurred approximately at 100 °C indicated the loss of water molecules by about 13.74 %. The second stage decomposition occurred between 200-900 °C showed major decomposition of β-cyclodextrin structure of about 80.38 % and the residue was 5.89 % indicates loose material. As for isoniazid molecule, the water decomposition was about 82.9 % at 100 °C. The second phase decomposition occurred between 320-900 °C indicated the decomposition of isoniazid molecule (18.42 %) and the remaining residue of 1.33 % indicated the presence of loose material in the molecule.

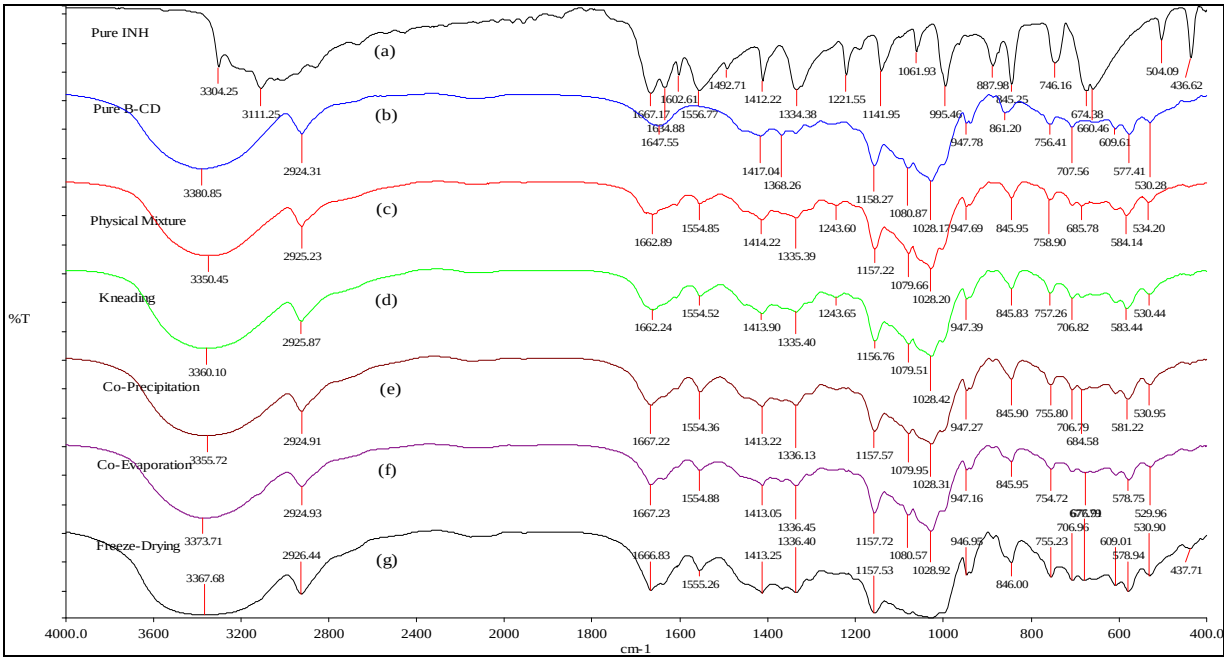

Figure 5: FTIR spectra for isoniazid (a), β-cyclodextrin (b), physical mixture complex (c), kneading complex (d), Co-precipitation complex (e), Co-evaporation complex (f), and freeze-drying complex (g) from 400-4000 cm^{-1}

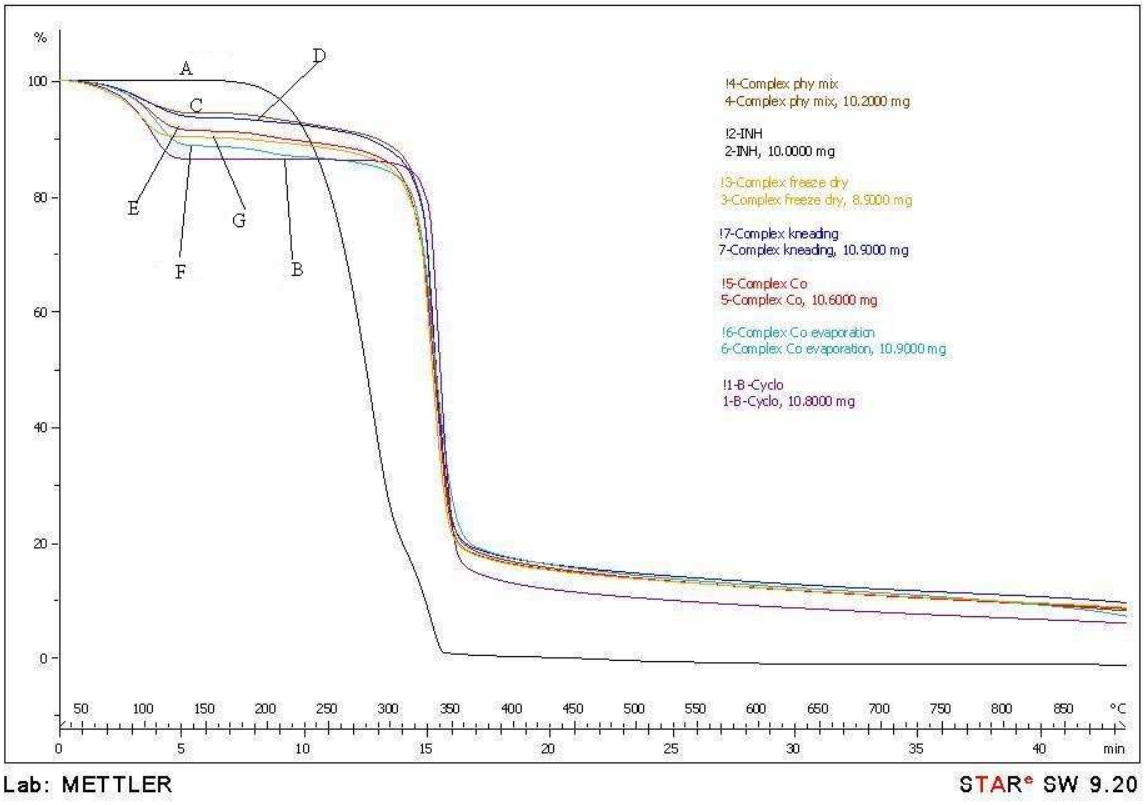

Figure 6: TGA thermogram for isoniazid (A), β-cyclodextrin (B), physical mixture (C), kneading (D), co-precipitation (E), co-evaporation (F) and freeze-drying (G) system

Figure 6 also showed TGA thermogram for the complexes. TGA thermogram showed two decomposition phases for all complexes, except co-evaporation. All complexes experienced the same level of water molecules decomposition, but had different percentage of weight loss in the first period of 100 °C. 5.60 % decomposition of water molecules occurred for physical mixture, 6.49 % for kneading, 8.79 % for co-precipitation, 11.42 % for co-evaporation and 9.83 % for freeze-drying. The second phase of decomposition occurred between 140-900 °C (except for co-evaporation) showed the decomposition of entire complexes structure involving β-cyclodextrin and isoniazid. The percentages of decomposition were 85.98 % for physical mixture, 84.04 % for kneading, 83.19 % for co-precipitation, and 81.52 % for freeze-drying. As for co-evaporation, the percentage of decomposition for the second and third phases were 1.90 % and 79.54 % respectively, showed the decomposition of complex in crystal form and decomposition of the entire complex structure. These findings show that water loss percentage in complexes was lower compared to water loss percentage in β-cyclodextrin molecule, which proved that some water molecules in host-guest complexes were displaced to allow guest molecules enter to β-cyclodextrin cavity [26].

3.6 Differential Scanning Calorimetry (DSC) Analysis

The DSC thermogram for β-cyclodextrin, isoniazid and complexes assayed are presented in Figure 7. The DSC curve for isoniazid displayed a sharp endothermic peak at 176.09 °C, which was considered as its melting point. The DSC thermogram for β-cyclodextrin displayed broad endothermic bands about 70-130 °C due to water molecule released. Co-evaporation and freeze-drying showed formation of broad endothermic bands similar to that of β-cyclodextrin, but the sharp endothermic band of isoniazid had disappeared. On the contrary, DSC thermogram for physical mixture, kneading and co-precipitation showed total disappearance of both sharp endothermic bands of isoniazid and broad endothermic band of β-cyclodextrin, indicating the formation of host-guest inclusion complex or an amorphous or both [27].

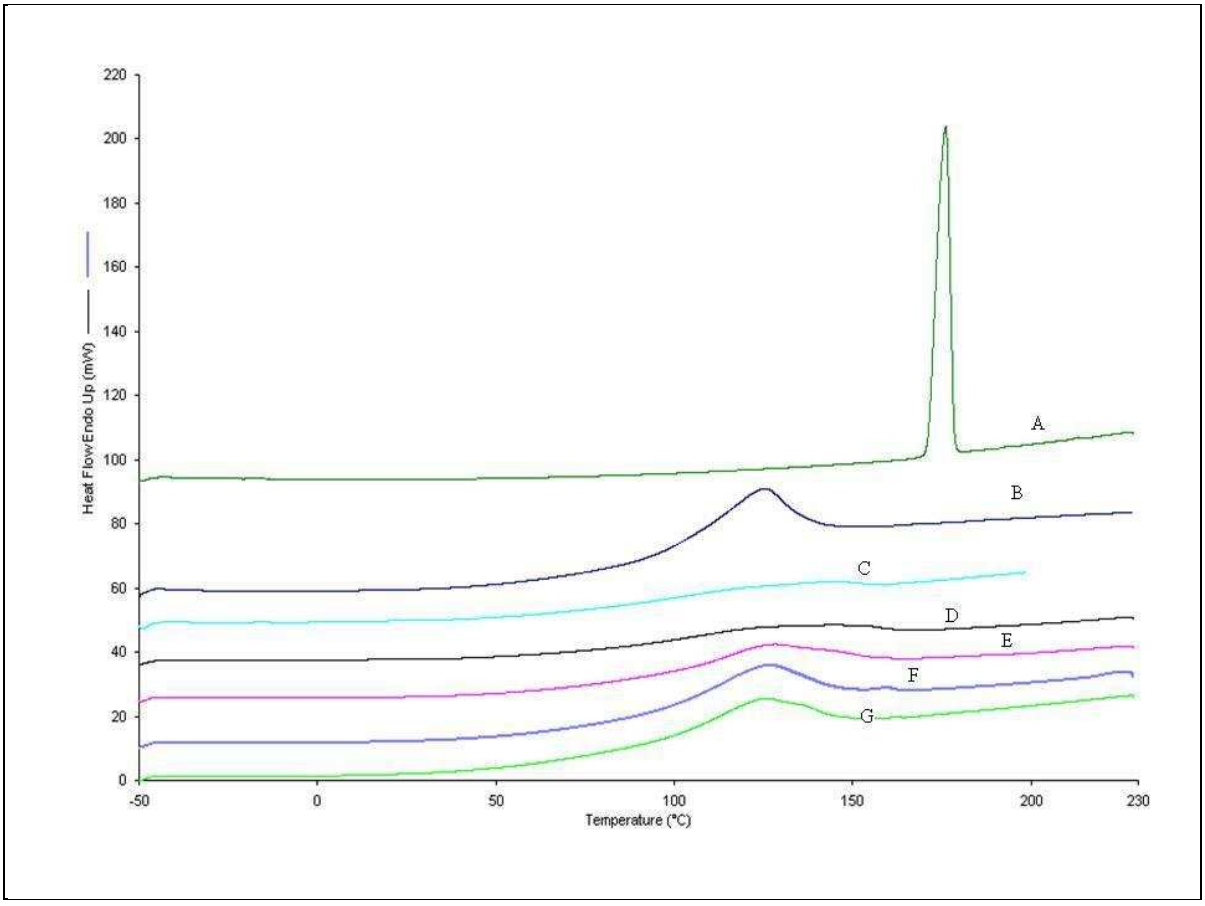

Figure 7: DSC thermogram for isoniazid (A), β-cyclodextrin (B), physical mixture (C), kneading (D), co-precipitation (E), co-evaporation (F) and freeze-drying (G) system

CONCLUSION

As a conclusion, it was found that co-precipitation has a potential to be the best method to prepare the complex between β-cyclodextrin and isoniazid as it enhanced the stability of isoniazid in aqueous solution. Co-precipitation showed the optimum results for all stability tests subjected to room light and UV light exposure in different conditions. Based on these results, the suitable method to be used to enhance the stability of isoniazid according the following order: co-precipitation > freeze drying > co-evaporation. In terms of storage, free drugs easily degraded by light especially UV light and also at higher temperature. Furthermore, it is suitable to be kept in room temperature and protected from light. Additionally, acidic solution is the suitable buffer to be used if formulation in liquid phase.

Acknowledgments
We would like to acknowledge to Ministery of Higher Education for the financial support through MyBrain15 MyMaster scheme, Universiti Sains Malaysia for funding this research under grant 1001/PPSP/8123035 and graduate assistance scheme (GA) for financial support.

REFERENCES

[1] S Ranjita; AS Loaye; M Khalil, *J. Pharm Pharmaceut Sci*., **2011**, 14, 100-116.
[2] M Asif. *Int, J. Pharm Chem*., **2012**, 4, 110-120.
[3] AG Brewer. Isoniazid, Analytical Profiles of Drug Substances. Academic press, New York, 1977.
[4] A Carlin; N Gregory; J Simmons, *J. Pharm Bio Anal*., **1998**, 17, 885-890.
[5] J Szejtli, Cyclodextrin in Pharmacy. Kluwer Academic Publisher, Dordrecht, The Netherlands, 1994.
[6] J Szejtli, *Chem Rev*., **1998**, 98, 1743-1753.
[7] J Szejtli. *Pure Appl Chem*., **2004**, 76(10), 1825-1845.
[8] HJ Schneider; F Hacket; V Rudiger, *Chem Rev*., **1998**, 98, 1755-1785.
[9] L Lawtrakul; H Vierstein; P Wolschann, *Int J. Pharm*., **2003**, 256, 33-41.
[10] J Valentino Stella.; A Roger Rajewski *Pharm Res*., **1997**, 14(5), 556-567.
[11] L Szente; J Szejtli, *Trends. Food Sci Tech*., **2004**, 15, 137-142.
[12] R Challa; A Ahuja; J Ali; RK Khar, *AAPS Pharm Sci Tech*., **2005**, 6(2), 329-357.
[13] L Szente; J Szejtli, *Eur J. Pharm Biopharma*., **2005**, 61, 115-125.
[14] E Frisch; RD Dennington II; TA Keith; AB Nielsen; AJ Holder, GaussView. Gaussian, Inc., Pittsburgh, 2003.
[15] HyperChem Release 7.5 for Windows, HyperCube, Inc. USA, 2003.
[16] KS Song; CH Hou; L Liu; XS Li; QX Guo, *J. Photochem Photobiol A.*, **2001**, 139, 105-109.
[17] EC Yang; XJ Zhao; F Hua; JK Hao, *J. Mol Struct (Theochem).*, **2004**, 712, 75-79.
[18] T Higuchi; KA Connors, *Adv Anal Chem Inst*., **1965**, 4, 117-212.
[19] Job, *Ann Chim*., **1928**, 9, 113.
[20] IV Terekhova; RS Kumeev, *Russ J. Phys Chem A.*, **2008**, 84, 1-6.
[21] C Jullian; MM Javier; ZT Gerald; B Aguilera; J Rodriguez; V Aran; OA Claudio, *J. Bioorg Med Chem*., **2008**, 16, 5078–5084.
[22] EJ Wang; ZX Lian; J Cai, *Carbohydr Res*., **2007**, 342, 767-771.
[23] SS Braga; MPM Marques; JB Sousa; M Pilinger; JJC Teixeira-Dias; IS Goncalves, *J. Organomet Chem*., **2005**, 690, 2905-2912.
[24] V Crupi; R Ficarra; M Guardo; D Majolino; R Stancanelli; V Venuti, *J. Pharm Biomedic Anal*., **2007**, 44, 110-117.
[25] AZ Haiyee; N Saim; M Said; MD Illias; AWM Mustapha; O Hassan, *Food Chem*., **2009**, 114, 459-465.
[26] YL Shen; SH Yang; LM Wu; XY Ma, *Spectrochim Acta Mol Biomol Spectros*., **2005**, 61, 1025-1028.
[27] A Ghuzlaan; MM Al Omari; KA Al-Sou'od, *J. Sol Chem*., **2009**, 38, 83-94.