

SOLUBILITY AND DISSOLUTION STUDY OF PHYSICAL MIXTURE OF CARBAMAZEPINE AND AMINO ACIDS

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ABSTRACT

Objective: The aim of this study was to determine the solubility and dissolution of carbamazepine (CBZ) mixed with an amino acid as a prodrug candidate promoeity compounds CBZ-amino acids, namely glycine (GLY), alanine (ALA), and lysine (LYS).

Methods: Physical mixture (PM) of CBZ and amino acids were prepared by mixing CBZ and amino acid equimolar, and then crushed until homogeneous. The content of CBZ in PM determined using UV spectrophotometry at λ 285 nm. PM was evaluated for solubility and dissolution were further characterized by Differential Thermal Analysis (DTA), and Fourier Transform Infrared Spectroscopy (FTIR). Solubility and dissolution were carried out in CO₂ free water distilled at a temperature 37 ± 0.5 °C. Dissolution performed with USP dissolution apparatus II (paddle type) and stirred at a speed of 75 rpm for 60 minutes.

Results: Solubility test of carbamazepine and carbamazepine physical mixture of amino acids CBZ-GLY, CBZ-ALA, and CBZ-LYS respectively for 278.62, 258.77, 266.40 and 301.10 mg / L. The dissolution test results showed % dissolved for 60 min physical mixture (PM) of CBZ-LYS, PM of CBZ-GLY, PM of CBZ-ALA and CBZ alone, respectively for 81.74%, 67.98%, 61.44%, and 56.30%. AUC₆₀ of PM of CBZ-LYS, PM of CBZ-GLY and CBZ-ALA and CBZ alone respectively for 3777.91, 3071.47, 2820.67, and 1743.99.

Conclusion: No significant difference between CBZ solubility of PM compared CBZ solubility. Based on the statistical test with one-way ANOVA obtained AUC₆₀ of PM of CBZ-LYS > PM of CBZ-GLY = PM of CBZ-ALA > CBZ. Physical mixture of CBZ and amino acids significantly improve the dissolution of CBZ. Based on the results of DTA and FTIR CBZ in aqueous media showed that CBZ may have been to transformation form III to dihydrate.

Keywords: Carbamazepine, Glycine, Alanine, Lysine, Physical mixture, Solubility and Dissolution.

INTRODUCTION

Carbamazepine (CBZ) is an anticonvulsive drug used in the treatment of simple and complex seizures, trigeminal neuralgia. CBZ is the first choice in the treatment of epilepsy because of the effectiveness and side effects of the changes in behavior and cognitive lower than other anticonvulsive [1,2]. CBZ including class II in biopharmaceutics classification system (BCS) [3], since it has a low solubility in water (120 mg/L at 25 °C) [4] and high membrane permeability (2.45 partition coefficient in octanol/water) [5].

CBZ has four polymorphic anhydrate forms (I, II, III and IV) and a dihydrate form [1,6,7]. Form III is the most stable at room temperature as thermodynamically and the only commercially available form and used in the formulation of products on the market [7]. The fourth form of anhydrous polymorph carbamazepine undergo conversion to the dihydrate form in aqueous media. Dihydrate form is the most stable form in water. The solubility of dihydrate form is one third of its anhydrous forms [8]. Kobayashi et al. (2000) [9] investigated that the intrinsic dissolution rate of CBZ forms I and III in the media solution pH 1.2 at 37 °C,

were 61.8 and 64.7 $\mu\text{g}/\text{cm}^2/\text{min}$ respectively. According to Kaplan (1972), dissolution of drugs that have intrinsic dissolution rate less than 0.1 $\text{mg}/\text{cm}^2/\text{min}$ is generally rate limiting step of absorption [9]. CBZ has low solubility in water resulting dissolution rate-limited absorption. Consequently CBZ absorption becomes slow when used orally to be erratic bioavailability [10-12].

The dissolution rate is proportional to the saturation solubility of the drug and the surface area of the drug particles under hydrodynamic conditions. Dissolution rate can be increased by decreasing the particle size and increasing the saturation solubility of the drug. Various attempts were made to enhance CBZ solubility, among which is the formation of prodrug with promoeity that easily soluble in water. Prodrug is an inactive molecule that requires a transformation of pharmacological and enzymatic or chemical compounds to release the active form in the body before the therapeutic effect [13,14]. Two prodrug approach aimed at improving the solubility of poorly soluble compounds in water are: (1) lower the melting point of the parent compounds with derivatization and / or (2) adding a polar promoeity /which can be ionized in the parent compound [13].

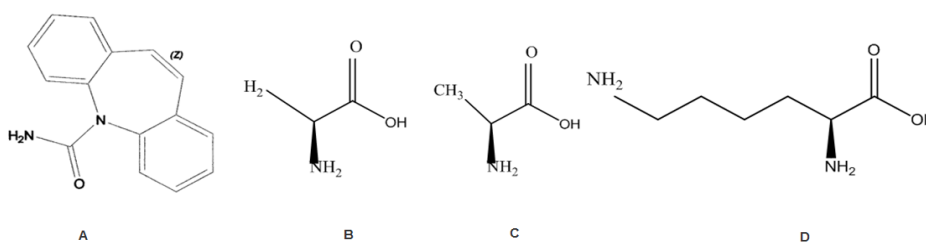


Fig. 1: Molecular structure of carbamazepine (A), glycine (B), alanine (C), lysine (D)

Promoeity amino acid is soluble in water and currently widely used in the manufacture of water-soluble prodrug. In addition to providing good solubility in water, the use of amino acids as

promoeity that can be readily converted by esterase or peptidase in the body and is released naturally as the integrity of the non-toxic when there is a change in body shape [15].

Jalali et al. (2006) [16] studied the correlation of in vitro-in vivo effects of anticonvulsive CBZ after made co-grinding with microcrystalline cellulose. The results showed an increase in bioavailability (C_{max} , t_{max} and AUC) with increasing dissolution of CBZ after co-grinding with microcrystalline cellulose which is a hydrophilic carrier. Before being used as promoeity for CBZ prodrug designed to have better solubility in water, conducted a study of solubility and dissolution of CBZ by mixing CBZ with the amino acids GLY, ALA and LYS. This study aimed to determine the solubility and dissolution of CBZ were mixed with an amino acid as a potential promoeity prodrug compounds, namely GLY, ALA and LYS amino acids with aliphatic chain.

MATERIALS AND METHODS

Carbamazepine (*pharmaceutical grade*) was obtained from PT Mersifarma Tirmaku Mercusana Indonesia (Batch No. 09083044), glycine, alanine, and lysine obtained from PT Ajinomoto Indonesia. For all experiments, methanol p.a (Merck) and CO₂ free distilled water were used.

Preparation of physical mixture carbamazepine with amino acids

Physical mixture of CBZ with amino acids (GLY, ALA and LYS) were prepared with a 1:1 molar ratio. Both ingredients are mixed until homogeneous. Physical mixture obtained is determined for its drug content and tested solubility, dissolution and characterized by Differential Thermal Analysis (DTA) and Fourier Transform Infrared Spectroscopy (FTIR).

Determination of maximum wavelength of carbamazepine and physical mixture

Carbamazepine 50 mg were weighed and dissolved in 10 mL of methanol. CBZ solution was diluted to obtain concentration 10 mg/L. Physical mixture of CBZ and amino acids (GLY, ALA and LYS) equimolar equivalent to carbamazepine 50 mg weighed. Amino acids dissolved in water and CBZ dissolved in methanol, both mixed in a flask. Solution obtained was diluted until a concentration of 10 mg/L. Absorbance CBZ and PM measured UV-Vis spectrophotometer in the wavelength range of 200-400 nm.

Preparation of standard calibration curve

Standard calibration solution with concentration range 1 to 30 mg/L CBZ was prepared. Standard calibration solution absorbance determined by UV-Vis spectrophotometer at maximum wavelength of 285 nm.

Drug content

Physical mixture equivalent 50 mg CBZ were dissolved in methanol, filtered with Millipore membrane filter 0.45 µm. The filtrate was diluted with distilled water and drug content was analyzed by UV spectrophotometer at 285 nm. Each drug content evaluation was performed three times.

Solubility studies

An excess quantity of CBZ or PM (CBZ with amino acids of GLY, ALA and LYS) was added to 5 mL vials containing CO₂ free distilled water. The vials were shaken in shaker waterbath at 200 rpm speed and at a temperature of 37 ± 0.5 °C until the solution becomes saturated. Sample solution withdrawn and filtered through 0.45 µm Millipore membrane filter. The concentration of solution CBZ was determined spectrophotometrically at 285 nm. Each solubility study was performed three times.

Dissolution studies

Samples CBZ or PM (CBZ with amino acids GLY, ALA and LYS) equivalent to 50 mg of CBZ were placed in 900 mL of CO₂ free distilled water as the dissolution medium, stirred at 75 rpm at a temperature 37 ± 0,5 °C using dissolution apparatus II. Five ml aliquots were withdrawn at predetermined time intervals of 5, 10, 15, 30, 45 and 60 min and the same volume distilled water was added to the medium to compensate for each sample taken. Samples were filtered through 0.45 µm Millipore membrane filter and the dissolved drug was assayed spectrophotometrically at 285 nm. Each dissolution study was performed in triplicate.

Differential Thermal Analysis (DTA)

Weighed sample of about 5 mg, inserted into an aluminum sample pan, then placed in a DTA instrument. The heating rate is set at 10 °C per minute, with the heating temperature range of 50-300 °C. The resulting thermogram profile is then analyzed.

Fourier Transform Infrared Spectroscopy (FTIR)

Each sample was weighed as much as 2 mg, then added with KBr powder pro spectrophotometry that has been dried as much as 300 mg. Then crushed in a mortar mix until homogeneous, put in a KBr disc creation tools, compressed by hydraulic pressure to obtain a transparent disc. Then the disc is placed in the sample holder and recorded. Samples were observed in the absorption band or wave number 4000 - 450 cm⁻¹.

RESULTS AND DISCUSSION

The physical mixture of CBZ and amino acids prepared by mixing both until homogenous and then its drug content evaluated. The drug content in various PM was estimated spectrophotometrically at the maximum absorbance of 285 nm (Figure 2).

The linearity of the assay method was evaluated by eight point standard curves (Figure 3), with concentration range selection of 1 - 30 mg/L. The linear regression of the calibration curve produced an equation of ($y = 0.0526 x + 0.0100$), with a correlation coefficient of 0.9998. The carbamazepine drug content in all PM were found to be in the range of 99.35 to 100.84%. The result can be seen at Table 1.

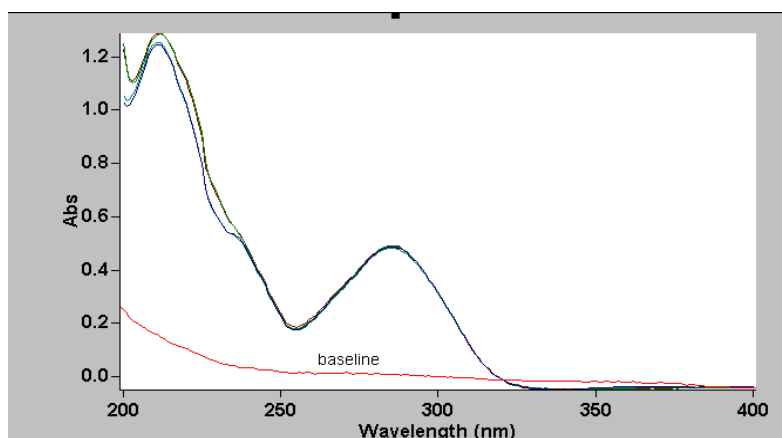


Fig. 2: UV spectrum of 10 µg/L solution of CBZ, PM of CBZ-GLY, PM of CBZ-ALA, and PM of CBZ-LYS depicting maximum wavelength at 285 nm

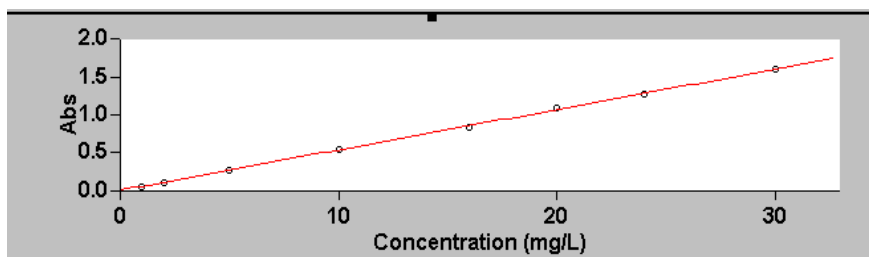


Fig. 3: Standard calibration curve of carbamazepin measured at 1-30 mg/L concentration

Table 1: Percentage of drug content in physical mixture of CBZ and amino acids (n=3)

Substance	Drug Content (%)
PM of CBZ-GLY	100.84 ± 1.24
PM of CBZ-ALA	99.63 ± 0.20
PM of CBZ-LYS	99.35 ± 0.17

Initial saturated solubility studies were performed for CBZ drug by using CO₂ free distilled water in shaker waterbath at a temperature of 37 ± 0.5 °C. The solubility studies indicated that saturated solubility reached after 5 hours shake. Solubility studies results of CBZ and PM of CBZ and amino acids (GLY, ALA and LYS) can be seen in Figure 4. Based on the results of one-way ANOVA test on the

degree of confidence α = 0.05 concluded there is no difference between the solubility of CBZ and PM of CBZ and amino acids. This showed that even though the amino acid is a water-soluble compound in the solution, there is no interaction between CBZ with amino acids, and therefore no increase in the solubility of CBZ occurs.

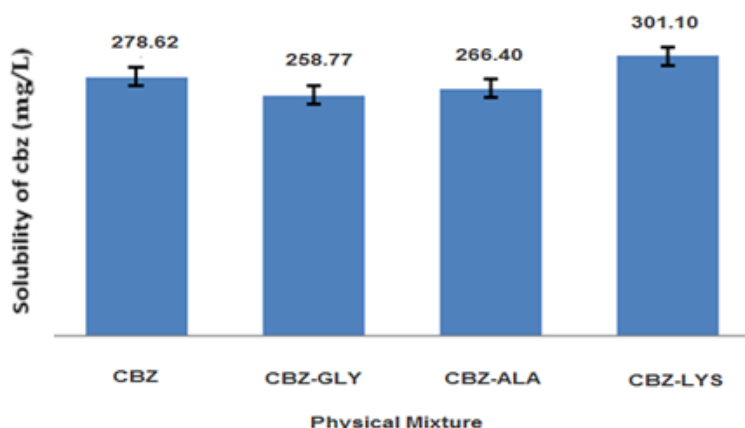


Fig. 4: Histogram solubility of CBZ and physical mixture of CBZ and amino acids (mg/L) in distilled water at a temperature of 37 °C ± 0.5 (n=3)

Profile dissolution of CBZ and physical mixture can be seen in Figure 5, obtained % dissolved CBZ in physical mixture was found to be greater, when it is compared to CBZ. Table 2 was shown percentage drug release during 60 minutes and area under the curve (AUC) for 60 minutes.

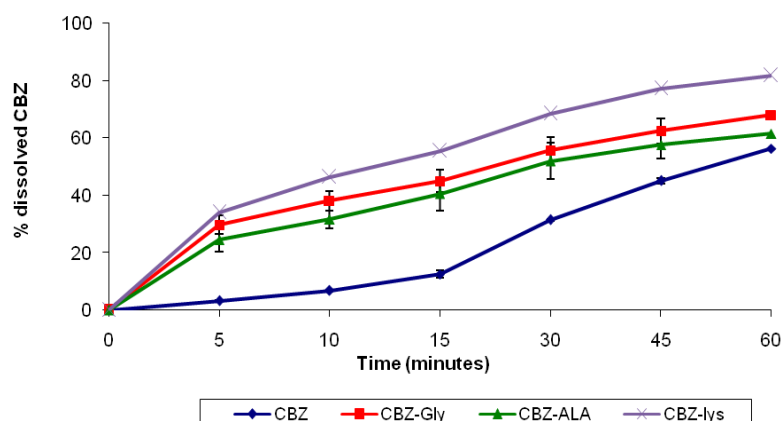


Fig. 5: Dissolution profiles of CBZ and physical mixtures of CBZ and amino acids in the distilled water at a temperature of 37 ± 0.5 °C (n = 3)

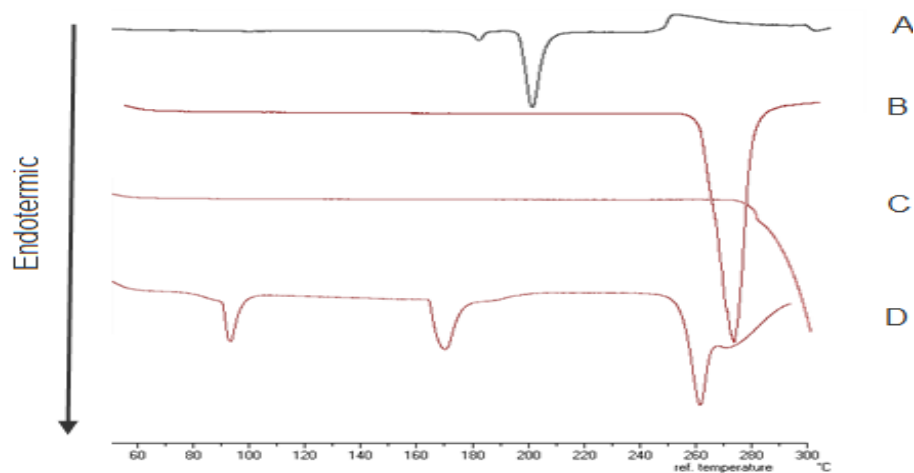
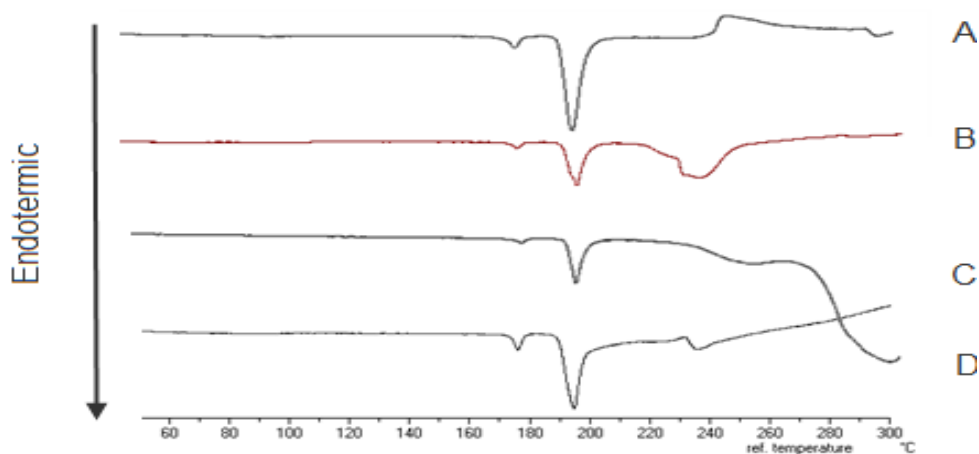
Table 2: Dissolution study in distilled water at a temperature 37 ± 0.5 °C

Substance	% Release on 60 minutes	AUC ₆₀
CBZ	56.30	1743.99
PM of CBZ-GLY	67.98	3071.47
PM of CBZ-ALA	61.44	2820.67
PM of CBZ-LYS	81.74	3777.91

Based on one-way ANOVA test for $\alpha = 0.05$ level value of AUC₆₀ differences between AUC₆₀ treatments. HSD test results showed that AUC₆₀ of CBZ differ significantly with PM of CBZ-GLY, PM of CBZ-ALA, and PM of CBZ-LYS. While AUC₆₀ of PM of CBZ-GLY did not differ significantly with AUC₆₀ of PM of CBZ-ALA. AUC₆₀ of PM of CBZ-LYS is greatest among existing treatments. Among the amino acids used in this study, lysine has a water solubility greater than glycine and alanine. Therefore lysine has the ability to CBZ solubilization greater than glycine and alanine. CBZ ability wetted by amino acids in aqueous media allows carbamazepine dissolution process to increase, due to the amino acid compound that dissolves in water will reduce the contact angle between carbamazepine with water as solvent, which allows carbamazepine having a greater dissolving process. Amino acids, which has greater solubility in water, will provide a decrease in contact angle greater than amino acids with lower solubility.

Dissolution phenomena are different from the facts solubility. During the process of solubility studies in water no interaction

between CBZ and amino acids observed. Solubility studies shaking lasted for 5 hours in order to form saturated solubility whereas dissolution studies carried out in sink condition. Kobayashi et al. (2000) who examined the comparative intrinsic dissolution rate of I, III, and dihydrate forms for 24 hours, indicating that the dissolution form III increased up to two hours and after that the dissolution decreased due to a change in the form of carbamazepine polymorph III into dihydrate form [9]. In sink conditions carbamazepine form III transformed into dihydrate form likely minimal due to the amount of water available to dissolve form III of carbamazepine [8]. Water environment and process solubility studies that lasted more than 2 hours resulted in carbamazepine polymorphs changes shape of form III to form the dihydrate. Changes CBZ form III into dihydrate form causes a decrease in its solubility [8]. The transformation polymorph form III into dihydrate form in this study supported by the results of DTA and IR by comparing between carbamazepine alone, physical mixture of carbamazepine and amino acids, and its mixture in solution (Figure 6 to 9).

**Fig. 6: DTA thermogram of CBZ (A), GLY (B), ALA (C), and LYS (D)****Fig. 7: DTA thermogram of CBZ (A), PM of CBZ-GLY (B), PM of CBZ-ALA (C), and PM of CBZ-LYS (D)**

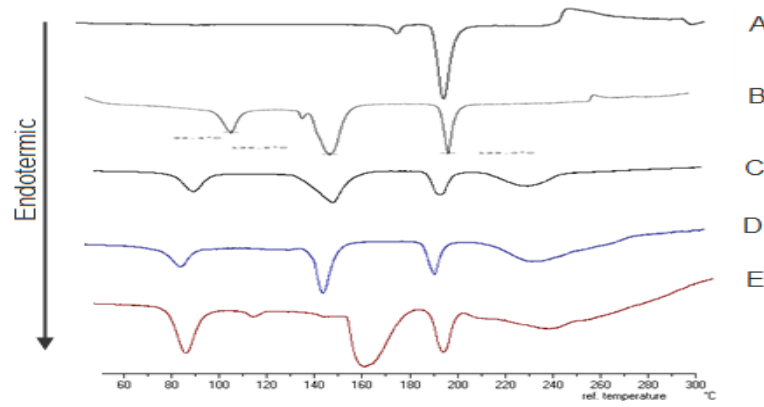


Fig. 8: DTA thermogram of CBZ (A), CBZ in solution (B), CBZ-GLY, CBZ-ALA, and CBZ-LYS in solution (C), (D), (E)

In Figure 6, can be seen DTA thermogram of CBZ showed an endothermic peak at a temperature of 175.5 °C and a sharp peak at a temperature 192.3 °C which is characteristic of the polymorph form III [7]. The results of DTA thermogram of PM of CBZ-GLY, PM of CBZ-ALA and PM of CBZ-LYS showed carbamazepine endothermic peak superimpose with the endothermic peak of each amino acid. CBZ in aqueous media showed an endothermic peak shift from 192.3 °C to 189.0 °C and from 175.5 °C to 136.8 °C, and seen a new peak at a temperature of 93.4 °C. DTA thermogram PM of CBZ and amino acids

(GLY, ALA and LYS) in the solution gave identical to the endothermic peak of CBZ in solution, with endothermic peak at temperatures above 200 °C from amino acids. Kobayashi et al. study shows DTA thermogram of dihydrate CBZ has a board endothermic of 50-75 °C and a sharp endothermic at 190 °C [9]. The phenomenon suggests that in the solution the possibility CBZ form III transformed into dihydrate form exists. There is a peak at 136.8 °C in this study contrasts with the results obtained by Kobayashi. This needs to be investigated further.

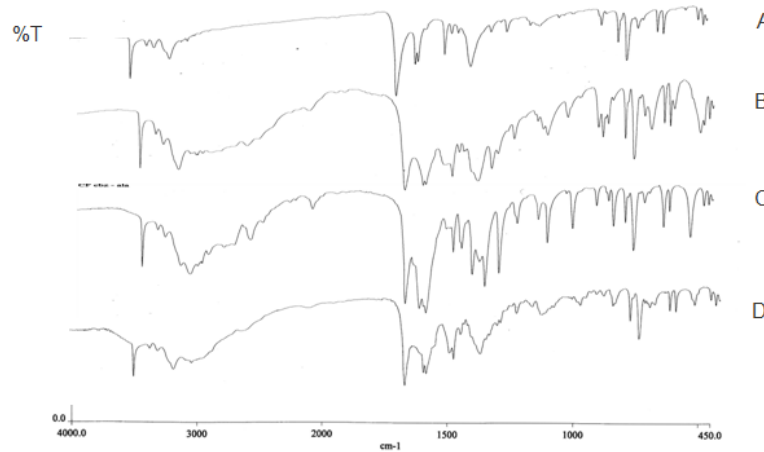


Fig. 9: FTIR spectrum of CBZ (A), PM CBZ-GLY (B), PM CBZ-ALA (C), and PM CBZ-LYS (D)

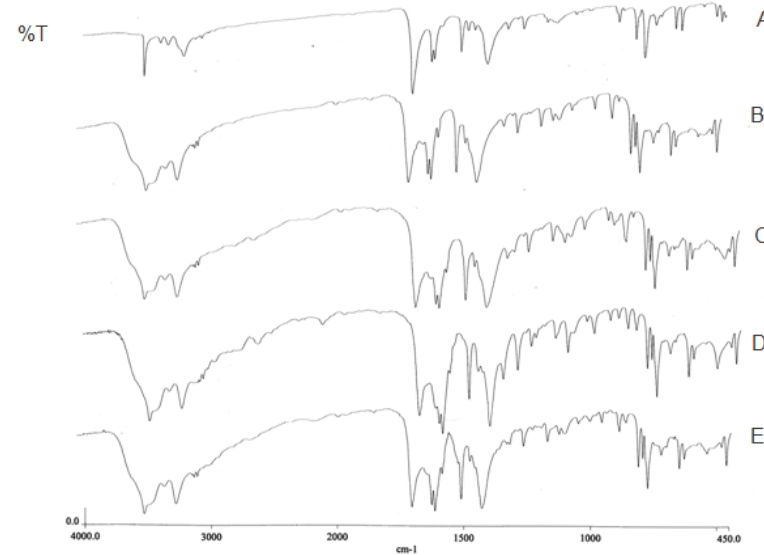


Fig. 10: FTIR spectrum of CBZ (A), CBZ in solution (B), CBZ-GLY, CBZ-ALA and CBZ-LYS in solution (C), (D), (E)

Figure 9 shown, FTIR of CBZ spectra give characteristic absorption bands form III of CBZ at wave number 3465 cm^{-1} (-NH stretching), 1677 cm^{-1} (-C = O stretching), 1605 and 1594 cm^{-1} (range of -C = C - and -C = O vibration and -NH deformation), and 1384 cm^{-1} (-C = N bond). The results are similar to the results obtained Ali et al. (2013), Grzesiak (2003) and Prajapati et al. (2007) [1, 7, 17]. Figure 9 can be seen, the FTIR spectra of the physical mixture gave peaks that indicate the presence of functional groups CBZ and amino acids.

Figure 10 shows, the FTIR spectra of CBZ in the solution. The boardening peak at wave number 3436 cm^{-1} indicates a possible interaction between the amide hydrogen of CBZ and the oxygen of water would occur. The same thing is also observed to CBZ in the presence of amino acids in solution. Based on the identification of the DTA and IR, CBZ in the presence of amino acids in solution, it may indicate a change of form III CBZ to form the dihydrate.

The result shows that it can be considered a formation of amino acids carbamazepine prodrug as an inhibitor to the transformation of polymorphs carbamazepin, where this transformation causes a decrease in its solubility. Furthermore, promoeity glycine, alanine, and lysine can be considered as promoeity group to develop carbamazepine prodrug. The next stage of research is the synthesizing of prodrug carbamazepine using promoeity amino acids glycine, alanine and lysine; examining its physicochemical properties and the bioavailability; and publishing the results.

CONCLUSION

From the research can be concluded that the amino acids in a physical mixture with CBZ did not increase the solubility of CBZ. This is important, because in aqueous media, carbamazepine polymorph form III transformed into dihydrate. This behavior is supported by data from the DTA and FTIR characterization. However, the physical mixture of CBZ and amino acids can increase the dissolution of carbamazepine, PM of CBZ-LYS > PM of CBZ-ALA = PM of CBZ-GLY > CBZ. Therefore, amino acids glycine, alanine, and lysine can be considered to be used as promoeity group in carbamazepine amino acid prodrug formation.

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