

IMPROVEMENT OF CARVEDILOL DISSOLUTION RATE THROUGH FORMATION OF INCLUSION COMPLEX WITH B-CYCLODEXTRIN

JESSIE SOFIA PAMUDJI, RACHMAT MAULUDIN, VISI ANISA LESTARI

School of Pharmacy, Institut Teknologi Bandung.

Email: jessie@fa.itb.ac.id

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ABSTRACT

Objective: Carvedilol is practically water-insoluble drug which has poor dissolution rate. These properties are associated with slow drug absorption leading to inadequate and unpredictable bioavailability. An inclusion complex is one of several methods to increase solubility and dissolution rate of an active pharmaceutical ingredient in water. The aim of this study is to improve dissolution rate of carvedilol by developing an inclusion complex with β -cyclodextrin.

Methods: Preparations of the inclusion complex were performed by kneading, co-precipitation, and freeze drying methods. The variations of molar ratio of 1:1, 1:2, and 1:3 between carvedilol and β -cyclodextrin were carried out. The amount of carvedilol included in β -cyclodextrin was calculated based on the amount of included carvedilol in binary system and free carvedilol in solution. Evaluations of the inclusion complex were performed by infrared spectroscopy, X-ray diffractometry (XRD) and Scanning Electron Microscopy (SEM). Furthermore, dissolution test was performed on pure carvedilol, physical mixture of carvedilol and β -cyclodextrin and all type of inclusion complexes. The amount of dissolved carvedilol in the medium were determined using UV spectrophotometer at $\lambda = 286$ nm.

Results: The highest entrapment efficiency of inclusion complexes (89.68%) was obtained by freeze drying method with a molar ratio of 1:3. Infrared spectrum and SEM characterization revealed there are interaction between carvedilol and β -cyclodextrin. Meanwhile XRD evaluation showed a change on the crystalline status of carvedilol in all inclusion complexes. The results of one way ANOVA and LSD statistical test at 95% confidence level showed that the percentage of dissolved carvedilol at 10th minute and dissolution efficiency of carvedilol after 120 minutes were significantly different on all of those inclusion complexes. However, the obtained inclusion complex by freeze drying method with ratio molar of 1:3 revealed the highest percentage of the dissolved carvedilol within 120 minutes (87.68%).

Conclusion: According to the evaluation on complex formation efficiency, characterization of the inclusion complex, percentage of the dissolved drug, and dissolution efficiency, the best formulation of the inclusion complex has been obtained by freeze drying method with molar ratio of 1:3.

Keywords: Carvedilol, β -cyclodextrin, inclusion complex, kneading method, coprecipitation method, freeze drying method, dissolution rate.

INTRODUCTION

Carvedilol is, a nonselective β -adrenergic blocking agent with α_1 -blocking activity, widely used in clinical practice for the treatment of cardiovascular diseases such as hypertension, congestive heart failure and myocardial infarction. It is a white to off-white crystalline powder, practically insoluble in water, gastric and intestinal fluids. Carvedilol is a weak base (pK_a value is approximately 7.8) and lipophilic drug (log P value is about 3.967). It is belong to class II in Biopharmaceutical Classification System [1]. Drugs belonging to this class have a low solubility and high permeability characteristic, hence the dissolution rate becomes the factor that is governing for its oral bioavailability. These drugs exhibit variable bioavailability and need enhancement in the dissolution rate to improve its bioavailability. Many methods have been used to improve the solubility and dissolution rate of Carvedilol such as solid dispersion [2, 3] microsphere [4], addition of surfactant as solubilizing agent [5]. Meanwhile intensive investigation of Carvedilol by inclusion complexes is not yet obviously carried out. In addition, drug inclusion in β -cyclodextrin is a simple method to improve solubility and dissolution rate. A study has demonstrated improving the dissolution performance of hydrochlorothiazide by its complexation with hydroxypropyl β -cyclodextrin in presence of poloxamer188 using co-evaporation and spray drying technique [6]. The cyclodextrin complexation serves an important tool in current armamentarium of agent for relief of pain and inflammations [7]. β -cyclodextrin is a cyclic oligosaccharide consist of 7 unit D-(+)-glycopyranose with α -(1,4) glycoside binding. This derivate of cyclodextrine is a most type used as complexing agent.

Although it has a relative low solubility compare to other derivate (1.85g/100ml at 25°C), β -cyclodextrin is cheaper than its derivate and available in commercial market. and has an ability to form inclusion complex with a lot important molecules [8]. In inclusion complex, a drug molecule behaves as guest molecule entraped in the hydrophobic inner cavity of cyclodextrin, while the outer part has a hydrophilic properties so that the complex molecule is easily to dissolve in aqueous medium [9].

The aim of this study is to improve dissolution rate by developing a carvedilol inclusion complex with β -cyclodextrin using various methods such as kneading, coprecipitation and freeze drying.

MATERIALS AND METHODS

Materials

Carvedilol was procured as a kindly gift from PT Kalbe Farma while β -cyclodextrin was also a gift from PT Kimia Farma. Metanol, DMSO, HCl pro analyze grade and distilled water were used.

Instruments

Analytical balance (AG204), oven (WTB Binder Model ED 115), thermostated shaker (GFL 1092), magnetic stirrer (Thermolyne Cimarec 2), thermometer, pH meter (Mettler Toledo S20), dissolution tester (Erweka DT 6), UV-Visible spectrophotometer (Beckman DU 7500i), infrared spectrophotometer (FTIR-Shimadzu 8501), Scanning Electron Microscopy (JEOL JSM-6360LA), Powder X-ray diffractometer (Diano tipe 2100 E), freeze dryer (Christ.), and common laboratory glassware were used in this research.

Optimization of Carvedilol- β -Cyclodextrin inclusion complex formation

Kneading method: In kneading method, optimization processes were conducted for mixing method and time of mixing in the preparation of inclusion complex. First method, about 250 mg of carvedilol and 1396.1 mg of β -cyclodextrin (ratio molar of 1:2) are mixed in the mortar. Then 4 mL of mixture of water : ethanol (1:1) was added and mixing was continued until it formed paste. For the second method, initially β -cyclodextrin was mixed with 4 mL of mixture of water : ethanol (1:1) until it become a paste and then a powder of carvedilol was added and mixed until it become homogeneous. If needed the mixture of water : ethanol (1:1) can add to the paste. Furthermore the paste was dried in the oven of 50°C until constant weight. Dried powder was washed with methanol to remove a free carvedilol and then dried again. The inclusion complex was sieved with sieve no. 80 (315 μ m). For optimization of period of mixing, the study was carried out for 1x15 minutes and once addition of solvent and also for 3x15 minutes with 3 times addition of 4 mL solvent.

Co-precipitation method: In co-precipitation method, the preparation of inclusion complex was optimized for mixing method and time of mixing and precipitation temperature. The first method, 250 mg of carvedilol was dissolved in 75 mL of methanol and then this solution was added to a solution of β -cyclodextrin (1396.1 mg) in 75 mL of water. The second method was prepared by adding solution of carvedilol in methanol drop by drop into solution of β -cyclodextrin in water and stirred continuously. Optimization of mixing time was carried out in period of 12 and 24 hours using magnetic stirrer at room temperature. Then the mixture was allowed to stand for 24 hours until the inclusion complex has been precipitated. The temperature for precipitation process was performed at room temperature (25°C) and in the refrigerator (4°C) [10]. After that the mixture was filtered and the inclusion complex was vaporized at 50°C until it's have a constant weigh. Then the powder was washed with methanol to remove of free carvedilol and dried again. The obtained inclusion complex was sieved with sieve no. 80 (315 μ m).

Freeze drying method: Optimization process for freeze drying method was carried out for type and amount of the solvent. The amount of 250 mg carvedilol powder and 1396.1 mg of β -cyclodextrin was dissolved in water, the mixture of water-methanol (1:1) or in dimethylsulphoxide (DMSO). The volume of solvent used was 1000 mL, 150 mL, and 60 mL respectively. The temperature of freeze dryer used in this experiment was -79°C. Then the complex powder obtained was washed with methanol to remove of free carvedilol and dried again in the oven of 50°C. After that the inclusion complex powder was sieved with sieve no. 80 (315 μ m)

Preparation of solid inclusion complex of Carvedilol- β -Cyclodextrin

Base on the result from optimization process, the preparation of inclusion complex was performed for the molar ratio of carvedilol and β -cyclodextrin, 1:1, 1:2, and 1:3 respectively for all methods.

Assay of carvedilol in the inclusion complex.

The total concentration of carvedilol was the concentration of free carvedilol in solution and carvedilol in inclusion complex. This concentration was determined by weighing 10 mg of inclusion complex before it was washed with methanol and dissolved in 100 mL of water. The absorbance of this solution was measured using UV spectrophotometer. The similar procedure was also used to determine the concentration of carvedilol in inclusion complex after it was washed with methanol. These data was used to determine entrapment efficiency of inclusion complex [11].

Evaluation of solid inclusion complex of carvedilol- β -cyclodextrin

Evaluation of inclusion complex was carried out utilizing of Infrared spectrophotometer, Powder X-Ray diffractometer and Scanning Electron Microscope.

Infrared Spectrophotometry

Identification of infrared spectrum was performed for carvedilol, β -cyclodextrin, physical mixture of carvedilol- β -cyclodextrin, and inclusion complex that obtained from each preparation method. The infrared spectrum was measured from wave number of 4000-400 cm^{-1} and the result was compared especially at the finger print area.

Powder X-Ray Diffractometry

Identification of X-Ray diffraction patterns are done for carvedilol, β -cyclodextrin, physical mixture carvedilol- β -cyclodextrin, and the optimum of inclusion complex. The measurement was carried out in the range angle of 2θ between 5-40°, rate of 0.02 ° per 0.8 second and Cu-K α radiation. Then the pattern of X-ray diffraction of each sample was compared.

Scanning Electron Microscope (SEM)

The morphology of the particle was identified using SEM for carvedilol, β -cyclodextrin, physical mixture of carvedilol- β -cyclodextrin, and the optimum of inclusion complex. Sample was covered by gold-palladium, and place into SEM sample holder instrument for electron shooting and photographing of sample morphology.

Dissolution test of inclusion complex

Dissolution test was conducted for carvedilol, physical mixture of carvedilol- β -cyclodextrin, and inclusion complex that obtained from each method with the molar ration of 1:1, 1:2, and 1:3. The amount of inclusion complex powder which was equivalent to 50 mg of carvedilol was weighed and tested for dissolution utilizing paddle method (apparatus type II) with 900 mL of solution of hydrochloric acid pH 1.2 as a medium, at temperature of 37 \pm 0.5°C stirring rate of 50 rpm. Sampling of aliquot was performed at 10, 20, 30, 40, 60, 90, and 120 minutes (USP 23). Then the concentration of carvedilol in each of solution was determined using spectrophotometer.

RESULTS AND DISCUSSION

This study was carried out to improve the dissolution rate of carvedilol through of inclusion complex with β -cyclodextrin. In the inclusion complex, a drug molecule as a guest molecule will entrapped at hydrophobic inner cavity of cyclodextrin, while the outer part molecule of cyclodextrin has a hydrophilic characteristic so that make it easily to dissolve in aqueous medium. Hydrogen bonding, Van der Waals interaction includes of dipole-dipole interaction and London disperse force were the intermolecular forces that play a role in stabilization of inclusion complex [12]. The kneading, co-precipitation and freeze drying methods were utilized in this study.

The study was begun with optimization process of all parameters that governed the formation of inclusion complex of each method. In kneading method, the optimization processes were done on mixing method and duration of mixing. It was found that the optimum inclusion complex was obtained by preparation of β -cyclodextrin paste prior the addition of carvedilol. This process was easier than others to form an inclusion complex because β -cyclodextrin has already wetted by a mixture of solvent (water and methanol in ratio of 1:1). The mixing duration of carvedilol- β -cyclodextrin for 3x15 minutes was better than 1x15 minutes because the contact time between carvedilol and β -cyclodextrin was longer. The recovery efficiency of inclusion complex formation using this method was 68.73%.

In the co-precipitation method, optimization process was performed for mixing method, mixing duration and temperature process. The result showed that the optimum process was an addition of methanol drop by drop to the solution of β -cyclodextrin in water and allowed to stand at room temperature for 24 hours. Otherwise precipitation of inclusion complex at 4°C was yielded higher of inclusion complex recovery than those precipitate at room temperature because the cooling process can promote the precipitation of the formed inclusion complex. The recovery efficiency of the inclusion complex formation using this method was of 76.01%.

In freeze drying method the optimization process was only conducted on choosing of the solvent. The mixture of carvedilol and β -cyclodextrin has a good solubility in the mixture of water: methanol 1:1. The freezing point of methanol was -96°C , while the freeze dryer instrument only can be used only until -79°C , so methanol will not freeze and the final product was still in the liquid form. On the other hand DMSO as a solvent has a freezing point higher than water (19°C), but the result obtained from this solvent was a viscous mass. Therefore the best solvent for this method is water. The recovery efficiency of freeze drying method was of 84.03%.

The next step was the preparation of carvedilol- β -cyclodextrin inclusion complex in the molar ratio of 1:1, 1:2, and 1:3. The aim of this preparation is to explore which composition that will give better recovery efficiency. The concentration of carvedilol in inclusion complex was determined before and after washing with methanol and assayed using UV spectrophotometer. Base on this study, it was found that the more amount of β -cyclodextrin in the formula the higher the percentage of recovery is yielded. Significant difference was revealed from the ratio of 1:1 and 1:2 which were prepared by all three methods. In addition the difference of carvedilol inclusion between ratios of 1:2 and 1:3 was not significant (Table 1).

Table 1: Recovery percentage of Carvedilol in β -cyclodextrin inclusion complex.

Method	Ratio of carvedilol : β -Cyclodextrin	Included of Carvedilol (%)
Kneading	1:1	48.975 \pm 1.46
	1:2	70.471 \pm 1.25
	1:3	77.163 \pm 1.12
Co-precipitation	1:1	50.689 \pm 1.38
	1:2	77.859 \pm 1.29
	1:3	83.941 \pm 1.31
Freeze drying	1:1	67.842 \pm 1.21
	1:2	84.546 \pm 0.97
	1:3	89.683 \pm 1.11

According to those data, it was found that freeze drying method was better than the other method. The recovery percentage of kneading method was smallest. This can be occurred because of in kneading method the formation of inclusion complex only depend on mechanical process and moreover the volume of used solvent is only for wetting the mixture. Therefore both ingredients are in dispersion form not in the dissolved form, so the contact between them is not optimum.

In co-precipitation method carvedilol and β -cyclodextrin are in the form of soluble solution at the beginning, although in two different solvent. So it is required a time to transfer of carvedilol from methanol to water. A type of used solvent also can influence the formation of inclusion complex because there are some solvent that can make an inclusion complex with β -cyclodextrin such as benzene, n-heptane, cyclohexane, and alcohol [13]. The obtained recovery percentage of this method was higher than kneading method because the volume of the used solvent was also higher, so more of carvedilol and β -cyclodextrin molecules were in contact in the form of a molecular.

Upon freeze drying, carvedilol and β -cyclodextrin were in the form of soluble solution in similar solvent (water), so it makes both of ingredient molecules easily to contact each other to form an inclusion complex. On the other hand an obtained powder from this method was also easily to wet due to the higher rehydration degree of the freeze drying product. The limitation of this method was the quantity of the solvent. In this method requires large amount of a solvent because carvedilol is slightly soluble in water and it take a long time to vaporize in the freeze dryer instrument.

Furthermore the inclusion complexes are characterized using FTIR spectroscopy, X-Ray diffractometry, and SEM methods. Characterization of inclusion complex with using infrared spectroscopy method showed that there was a different in the spectra of carvedilol, β -cyclodextrin, physical mixture, and inclusion complexes. Specific absorbance of carvedilol is in the range of 3500-3000 cm^{-1} for vibration of N-H; 3050-2950 cm^{-1} for vibration of C-H; 1600-1480 cm^{-1} for vibration of aromatic C=C; 1454-1444 cm^{-1} for vibration of C-N; 1403-1256 cm^{-1} for vibration of phenyl ring of C-C and 1040-800 cm^{-1} vibration of substitute benzena [14]. Significant different were observed in the inclusion complex spectra, for instant: N-H vibration at 3343 cm^{-1} is disappear from the spectra of yielded inclusion complex, while in the physical mixture that vibration is still exist. In addition, the intensity of C-H vibration of carvedilol at of 3050-2950 cm^{-1} and C-C aromatic at 1600-1480 cm^{-1} are obviously decreased.

Disappearing and decreasing of vibration intensity in carvedilol spectra, because inclusion complex will disturb the vibration. The higher in decreasing of intensity of vibration was showed by freeze drying method. This can be occurred due to the interaction between carvedilol and β -cyclodextrin in the form of solution in the similar solvent is stronger than in the form of solution with different type of solvent (co-precipitation method) or in the form of particulate dispersion (kneading method). The result is presented in figure 1.

Characterization using X-ray diffractometer showed that there were differences between diffractogram of carvedilol, β -cyclodextrin, physical mixture and inclusion complex crystalline. The crystallinity peak of carvedilol are clearly showed at 2θ of 5.72; 11.53; 12.87; 14.72; 16.36; 17.43; 18.36; 24.13; 26.07; and 29.30. The diffractogram peaks of β -cyclodextrin are clearly showed at 2θ of 9.10; 10.61; 12.45; 12.77; 17.10; 17.98; 18.85; 19.55; 20.85; 22.83; and 27.10. While the diffractogram of physical mixture with ratio molar of 1:2, showed the combination between of peak of carvedilol and β -cyclodextrin. These clearly showed at peak of 2θ of 5.72; 9.10; 12.55; 14.64; 16.36; 17.43; 18.36; 22.83; and 24.50, which were similar to the diffractogram of pure carvedilol and of β -cyclodextrin. The obtained diffractogram of inclusion complex was clearly showed a decreasing in the high of the those peak above and there were also showed an increasing of peak width.

These result indicated, there was a change in the structure of carvedilol in the form of carvedilol- β -cyclodextrin inclusion complex to be an amorphous form. The amorphous form was created through the formation of a new solid phase in the inclusion complex when the molecular dispersion of water solution of both ingredients was freeze dried. The interaction between carvedilol and β -cyclodextrin in the inclusion complex changed the crystal form of carvedilol into an amorphous form and leading to improving of dissolution rate and solubility rather than before.

Evaluation of surface morphology the powder of inclusion complex using Scanning Electron Microscope (SEM) showed the change in the crystallinity of carvedilol in the inclusion complex compared to the other. The microphotograph of the inclusion complex particles showed the combination of carvedilol and β -cyclodextrin and looked likes aggregates with rough surfaces. In contrast the microphotograph of carvedilol, β -cyclodextrin, and the physical mixture showed that particles in the crystalline form with smooth of surfaces. These indicated, the particles in inclusion complex were in the amorphous form. The microphotograph of all of them can be seen at Figure 3.

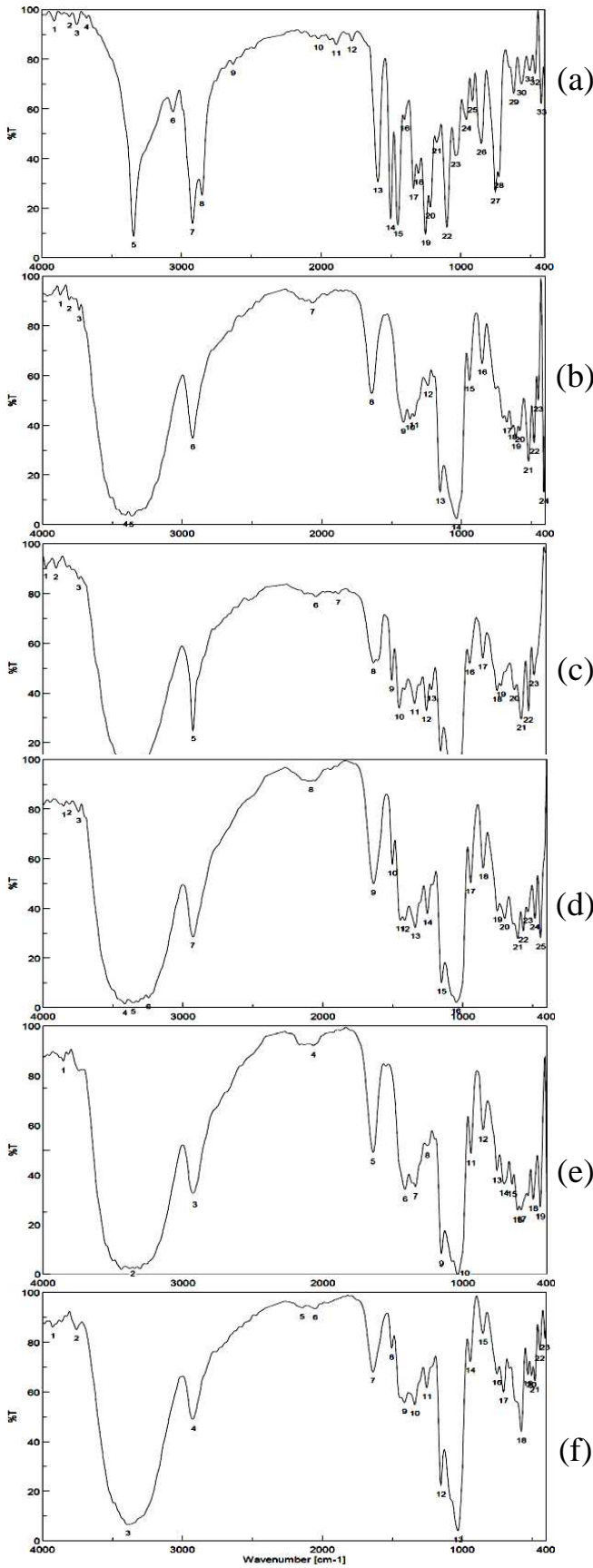


Fig. 1: Infrared spectrum of (a) Carvedilol, (b) β -cyclodextrin, (c) physical mixture, (d) inclusion complex prepared by kneading method, (e) inclusion complex prepared by co-precipitation method, (f) inclusion complex prepared by freeze drying method

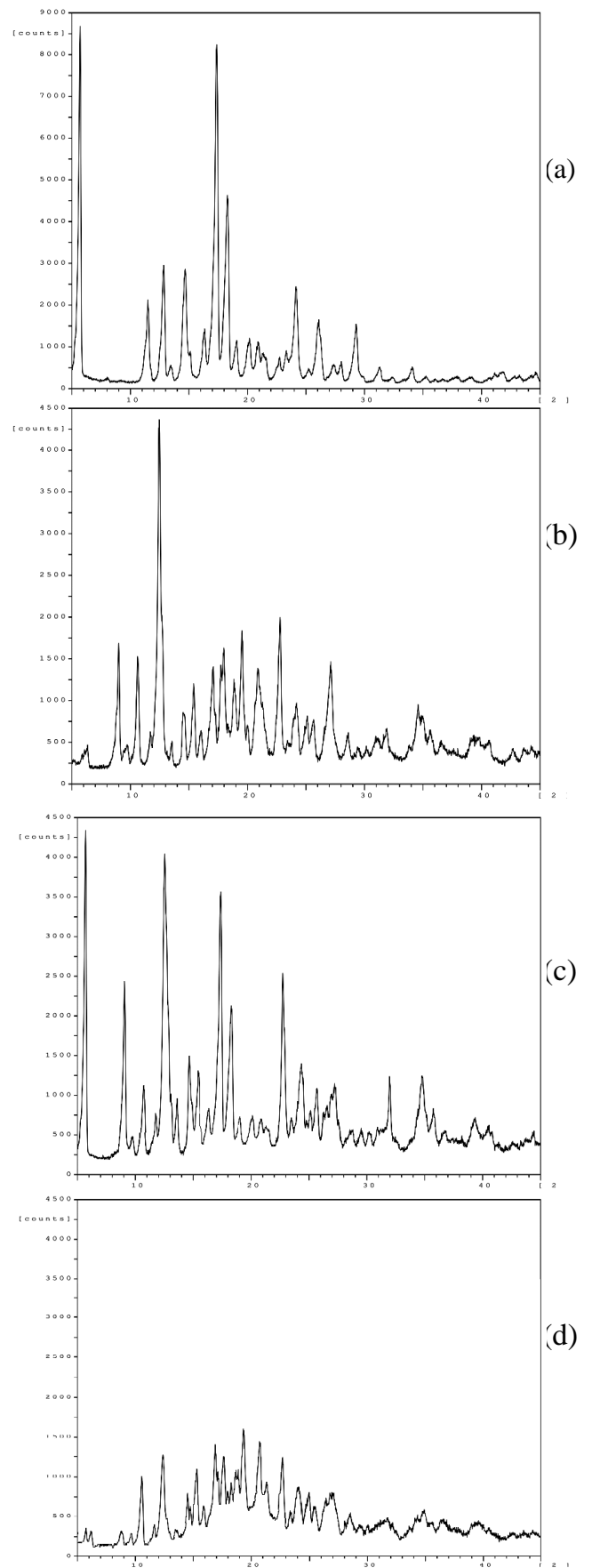


Fig. 2: X-ray diffractogram of (a) Carvedilol, (b) β -cyclodextrin, (c) physical mixture, (d) inclusion complex prepared by freeze drying method

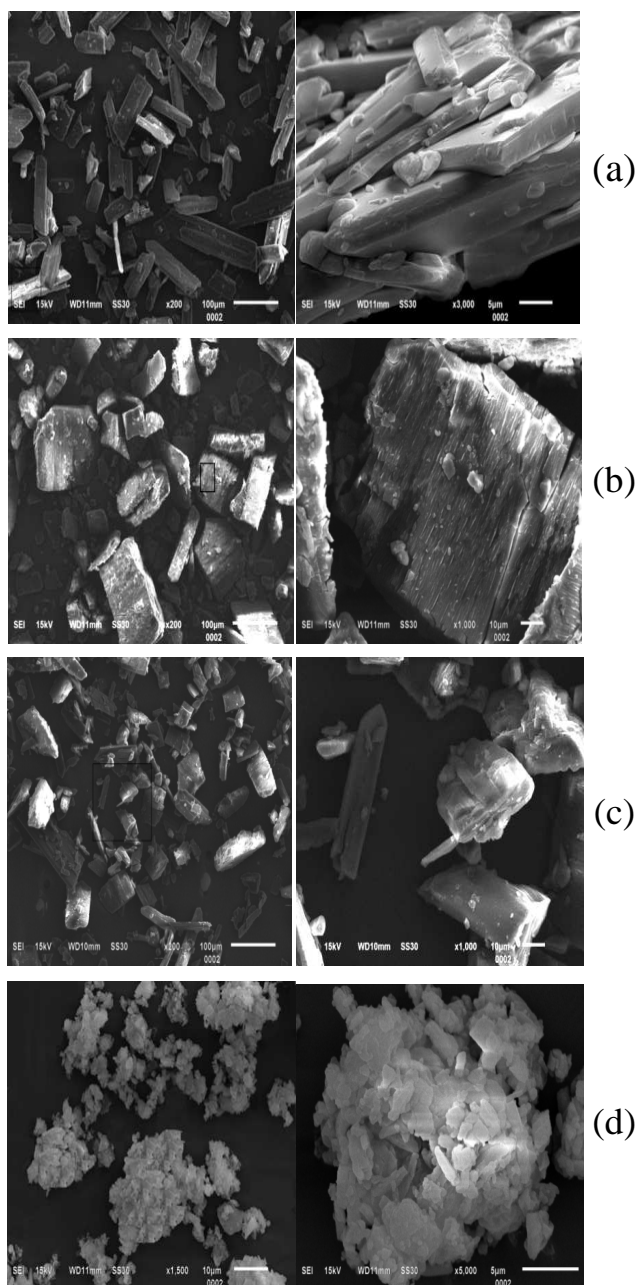


Fig. 3: SEM microphotograph of (a) Carvedilol, (b) β -cyclodextrin, (c) its physical mixture, (d) inclusion complex made by freeze drying method.

The dissolution test of carvedilol, physical mixture of carvedilol- β -cyclodextrin, and the inclusion complex showed a significant different among dissolution rate of the samples. All inclusion complexes made by kneading, co-precipitation, and freeze drying method in molar ratio of 1:1, 1:2, dan 1:3 have a higher dissolution rate rather than pure carvedilol or its physical mixtures. The percentage of pure carvedilol dissolved in the solution of hydrochloric acid pH of 1.2 was only 26.98% within 120 minutes. The highest dissolution rate of carvedilol inclusion complex was showed by freeze drying method in molar ratio of 1:3 that of 87.68% after 120 minutes. While the lowest dissolution rate was showed by the inclusion complex in molar ratio of 1:1 by kneading method, that of 50.93% within 120 minutes. The physical mixture of carvedilol with β -cyclodextrin was also showed significant improvement on dissolution rate due to the interaction between both ingredients during the dissolution process. It can promote the formation of inclusion complex and increase the rate of dissolution.

Statistical analysis using one way ANOVA followed by *Post Hoc Test* type LSD at 95% confidence level were performed to investigated the differences in the percentage of carvedilol dissolved from pure carvedilol, the physical mixture of carvedilol with β -cyclodextrin and inclusion complex made by three method in all molar ratio composition. It was obtained the value of F calculation (440,650.6) was higher than the value of F from table (2.24) after 10 minutes of dissolution. This result is indicated a significance different on the percentage of dissolved carvedilol in the medium, and the result from LSD statistical analysis also showed a significance different among pure carvedilol and all inclusion complexes. The highest percentage of carvedilol dissolved after 10 minutes was an inclusion complex prepared by freeze drying method with molar ratio of 1:3 (76.84%). The overall dissolution tests are presented in figure 4, 5 and 6.

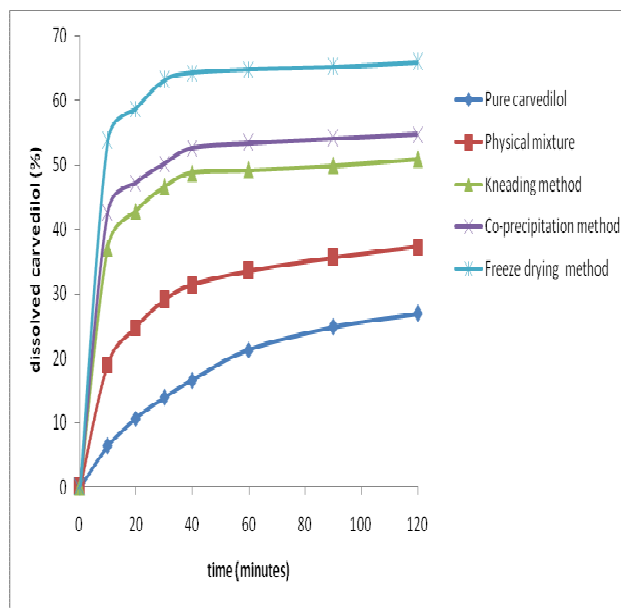


Fig. 4: Dissolution profile of carvedilol from pure carvedilol, physical mixture and β -cyclodextrin inclusion complex at molar ratio of 1:1

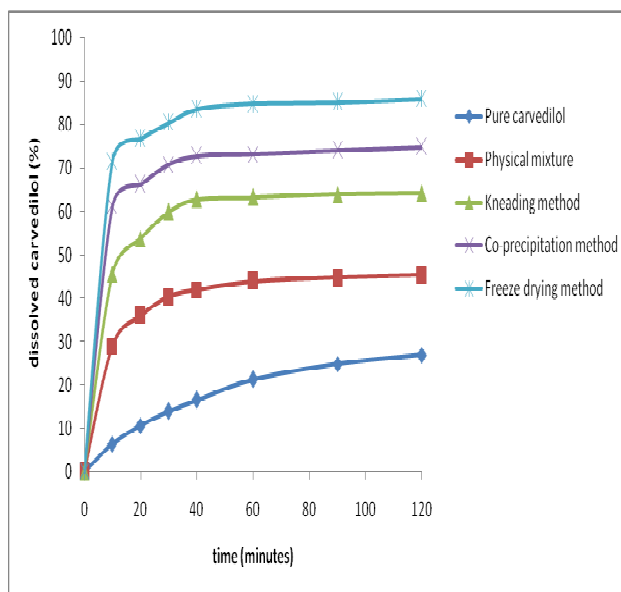


Fig. 5: Dissolution profile of carvedilol from pure carvedilol, physical mixture with β -cyclodextrin inclusion complex at molar ratio of 1:2

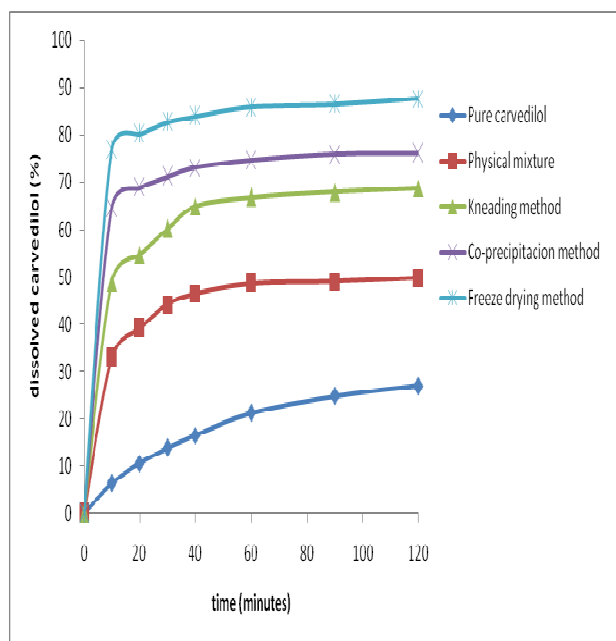


Fig. 6: Dissolution profile of carvedilol, physical mixture with β -cyclodextrin inclusion complex at molar ratio of 1:3.

The similar result was also revealed on dissolution testing after 120 minutes. There were significant different in the percentage of dissolved carvedilol in the medium for all treatment compared to pure carvedilol except on the physical mixture in the molar ratio 1:1. According to statistical analysis data, the fastest dissolution rate is showed by inclusion complex carvedilol prepared using freeze drying method in molar ratio of 1:3.

CONCLUSIONS

The best method of the inclusion complex preparation was showed by freeze drying method in molar ratio of 1:3. The highest dissolution percentage of the inclusion complex carvedilol in HCl solution pH of 1.2 was 87.68% within 120 minutes. The formation of carvedilol inclusion complex can improve the dissolution rate of carvedilol significantly.

REFERENCES

1. Raju V and K.V.R. Murthy. Development and validation of new discriminative dissolution method for carvedilol tablets, Indian J.Pharm Sci 2001; 73(5), 527-536
2. Sharma A, and C.P Jain. Preparation and Characterization of Solid Dispersion of Carvedilol with PVP K30, Res Pharm Sci 2010; 5(1): 49-56
3. Kovačič B, F. Vreder and O Planinšek. Solid dispersion of Carvedilol with porous silica; Chem Pharm Bull 2011; 59(4),427-433
4. Wei L., Sun P, Nie S, Pan W. Preparation and Evaluation of SEEDS and SMEDDS Containing Carvedilol, Drug Dev Ind Pharm 2005; 31: 785-794
5. Kadam V.D. and S.G.Gattani. Dissolution enchantment of carvedilol by using surfactant as a coating material, Asian J.Pharm Clin Res 2009; 2(3), 39-43
6. Pallavi Dhekale, Anita Kulkarni and Mohan Sanodiya. Binary and Ternary Inclusion Complexes of Hydrochlorothiazide in Hp-B-Cd and Poloxamer188, Preparation and Characterization, Int J Pharm Pharm Sci 2013; 5 (4), 718-722
7. Farzana S. Bandarkar and Pradeep R. Vavia. Physico-Chemical Characterization and *In Vivo* Pharmacodynamic Evaluation of Lyophilized Meloxicam: β -Cyclodextrin Inclusion Complexes, Int J Pharm Pharm Sci 2013; 5 (3), 159-165
8. Rowe. R.C. Handbook of Pharmaceutical Excipients. 5th ed. The Pharmaceutical Press. London. 2006
9. Loftsson. T. and Brewster. Pharmaceutical Application of Cyclodextrins 1. Drug Solubilization and Stabilization. J. Pharm. Sci. 1996; 85(10). 1017-1024.
10. Singh. R., Bharti N., Madan J, Hiremath S.N. Characterization of Cyclodextrin Inclusion Complexes – A Review. Drug Dev. Ind. Pharm. 2010; 2 (3) : 171-183.
11. Theivarasu. C., Ghosh S., Indumathi T. UV Spectrophotometric Determination of Carvedilol in Pharmaceutical Formulations. Asian J. Pharm. 2010; 3(4). 64-68.
12. Bekers. O., Uijtendaal E.V. Beijnen J.H., Bult A., and Underberg. W.J.M. Cyclodextrin in The Pharmaceutical Field. Drug Dev. Ind. Pharm. 1991; 17 (11). 1503-1549.
13. Maria G. A.. Studi Kompleks Inklusi Indometasin dengan β -Cyclodextrin, Thesis magister 2002, Sekolah Farmasi-ITB. Bandung. 52-53.
14. Jagannathan. L., Meenakshi R. Gunasekaran S., Srinivasan S. FT-IR, FT-Raman and UV-VIS Spectra and Quantum Chemical Investigation of Carvedilol. NRC Cnd. Sci. Lib. 2010; 36 (4) : 283-290.