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Current Issue

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Preparation Of An Inclusion Complex System Of Ethyl *P*-Methoxycinnamate - Hydroxypropyl-β-Cyclodextrin: Characterization and Solubility Evaluation.

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ABTRACT

Ethyl p-methoxycinnamate (EPMC) isolated from Kaempferia galanga rhizome is a potential compound which shows many biological activities, but it has poor solubility in water and it is difficult to prepare in oral formulation. Therefore, it was formed as an EPMC inclusion complex using hydroxypropyl-β-cyclodextrin (HP-β-CD) to increase its solubility. The objective of this work were preparation of the EPMC/HP-β-CD inclusion complex by kneading method (at 1:1 molar ratio); characterization of its physicochemical properties by FTIR and ROESY ^{1}H-NMR spectroscopic method, differential thermal analysis (DTA), X-Ray diffractometry (XRD) and evaluation of its thermodynamic interaction in water solution. Thermodynamic interaction of the inclusion complex was performed by the formation of inclusion complexes in water containing various concentrations of HP-β-CD and evaluation of their solubility in various temperatures. The result established that inclusion complex of EPMC-HP-β-CD has been generated. Dissolution rate of the EPMC-HP-β-CD inclusion complex is higher than EPMC and its physical mixture. The solubility of EPMC enhanced by the increasing the concentration of HP-β-CD but an increase in temperature causes the complex stability constanta slightly decline. The formation of inclusion complexes between EPMC-HP-β-CD has a negative ΔH, a negative ΔG, and a positive ΔS value.

Keywords: Ethyl p-methoxycinnamate, hydroxypropyl-β-cyclodextrin, inclusion complex,dissolution rate, thermodynamic parameter.

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INTRODUCTION

Kaempferia galanga, Linn. is a tropical plant that grows in various regions in Indonesia, and is known as analgetic, anti-inflammatory, antifungi and insecticide in traditionally medicine [1]. Based on the data of Gas Chromatography and Mass Spectrometry (GC-MS), the largest peak area which correspond to volatile oil of kencur rhizome is ethyl p-methoxycinnamate (EPMS) (31.77%) [2]. Umar et al, (2014) [3] reported the cancer chemopreventive activity of EPMS which is resulted from inhibition of angiogenesis process.

EPMS potentially used in oral dosage form, but it has low solubility in water because its ester moiety. Poor solubility can cause low dissolution rate and bioavailability because of poor absorption in the body [4]. An effort to increase the solubility of compound in water is inclusion complex formation by cyclodextrin (CD) group, where the drug molecule as guest trapped in the cavity of a CD. Interior area of CD consisted of hydrophobic moiety area and the exterior area contained hydrophilic moiety, which facilitated the complex system to soluble in water media [5].

The structure of EPMS consist of an aromatic ring, a vinylic double bond, ether and ester moieties (Fig.1). Ferulic acid (4-hydroxy-3-methoxycinnamic acid), which has structural similarities with EPMS, can form an inclusion complexes with HP-β-CD through hydrophobic interaction between aromatic ring and its conjugated double bond moieties with interior area of HP-β-CD and hydrogen bonding interaction between its carboxylate moiety with exterior area of HP- β-CD [6,7], . Solubility and dissolution rate of ferulic acid increase in the inclusion complex form. Therefore, the EPMS/HP-β-CD inclusion complex will also be produced and will increase its water solubility.

The ability of a molecule to associate and dissociate before reach equilibrium can be observed from the complex stability constants (K). K value is directly related to the free energy (ΔG) of complex formation [8].

Based on the above background, in this study we prepare the inclusion complex between EPMS and HP-β-CD by kneading method and characterized its physicochemical properties, compared with the EPMS starting material and its physical mixture. The formation process of inclusion complex between EPMS with HP-β-CD can be determined from solution of EPMS and HP-β-CD in water by calculated the thermodynamic parameters (ΔG, ΔH and ΔS). Those parameters can be inferred from solubility test of EPMS in various concentrations of HP-β-CD at various temperatures.

MATERIAL AND METHOD

Material

Kaempferia galanga Linn. was obtained from Purwodadi Botanical Garden, Malang, Indonesia, HP-β-CD was purchased from Sigma-Aldrich. All solvent used were analytical grade reagent.

Methods

Isolation of EPMS:

Dry powders of Kaempferia galanga rhizome (1 kg) was percolated with 1.5 L of ethanol overnight according to our previous study [9]. The percolate was fenced and the percolation was repeated until all of EPMS was extracted. The percolate was concentrated by rotavapor at 40°C, and then stored in a refrigerator to obtain the pale yellow solid of ethyl p-methoxycinnamate (EPMS). The solid was filtered by a Buchner funnel and recrystallized from ethanol to give white needle crystals with melting point of 52.8°C, which is observed by Differential Thermal Analyzer (DTA) Mettler Toledo. The structure characterization of EPMS determined subsequently by spectroscopic methods, i.e. Perkin-Elmer Lambda EZ-201 UV-vis Spectrophotometer, Perkin Elmer Spectrum One Fourier Transform Infra Red (FTIR), JEOL RESONANCE NMR (400MHz) ^{1}H-NMR and ^{13}C-NMR spectrometer. The spectral data are as follow : UV (ethanol, □max.): 310 nm; IR (KBr, cm^{-1}): 3007 (-CH, aromatic ring), 1706 (-C=O, ester), 1630,. 1630 cm^{-1} (unsymmetrical conjugated of -C=C- of alkene), 1586 and 1473 cm^{-1} (-C=C- moiety of aromatic ring), 1276 cm^{-1} (-C-O– vibration of methoxy moiety). ^{1}H-NMR (400 MHz, $CDCl_3$, □H, ppm) : 3.82 (3H, s); 6,29 (1H, d, J = 15.8Hz); 6.87 (2H, d, J = 7.2 Hz); 7.63 (1H, d, J = 15,8 Hz); 7,46 (2H, d, J = 7.2 Hz); 4,24 (2H, m); 1,32 (3H, t, J = 7.2 & 7.2Hz). ^{13}C-NMR (400 MHz, $CDCl_3$, □C, ppm) : 127.3, 129.8,

115.8, 114.4, 161.4, 115.8 (6 C of aromatic ring), 144.3 & 114.4 (2C of –C=C- alkene), 167.4 (1C of –C=O), 60.4 (1C of $-CH_2-$), 55.4 (1C of $-OCH_3$), 14.4 (1C of $-CH_3$). All of data prove that the isolate is EPMS a compound with aromatic ring, vinylic double bond, methoxy and ester moiety (Fig.1), as also reported by Umar et al. [3].

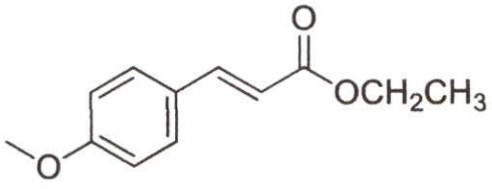

Fig. 1. Ethyl *p*-methoxycinnamate.

Phase solubility study:

The effect of HP-β-CD concentrations (0, 2.5, 5.0, 7.5, 10mM) was evaluated on EPMS solubilities (5.0 mg/L) which was added by each HP-β-CD concentrations. The mixture of EPMS and HP-β-CD in water was shaked for 10 min at 32±0.5°C using ultrasonic apparatus in water bath shaker at 150 rpm until an equilibrium is reached. The solution was filtered using Whatman paper (0.45 μm pore size) and the supernatant was analyzed using a UV-vis spectrophotometer at 310 nm. The EPMS solubility (mg/ml) were plotted against HP-β-CD concentrations. The complexation constant was determined in two temperatures (37±0.5°C and 42±0.5°C). Each sample was assayed in triplicate.

Calculation of enthalpi of solution (ΔH)

Calculation of ΔH can be done by determining the relationship between the logarithm of the complex stability constant (K) versus 1/T (K^{-1}). Furthermore, the ΔH value can be calculated from Eq.1

$$\log K = \frac{\Delta H}{-2{,}303R} \cdot \frac{1}{T} + C \quad \text{(Eq. 1).}$$

ΔH positive indicated that the dissolving process of EPMS is an endothermic process so its solubility will increases with the increasing temperature. If ΔH value is negative, it is suggested that dissolving process of EPMS is exothermic so its solubility decreases with increasing temperatures.

Calculation of Free Energy (ΔG)

The free energy (ΔG) value can be calculated by the equation : (ΔG = -2.303 RT log Ka) (Eq. 2). Negative ΔG value indicates a spontaneous reaction, zero ΔG value indicates that the reaction is in equilibrium, and positive ΔG value indicates that the reaction is not spontan.

Calculating of Entropy (ΔS)

The entropy (ΔS) value was obtained by entering the value of ΔH and ΔG into the equation 3, i.e. (ΔG = ΔH – TΔS). If ΔS is positive, the occurrence of events which include the leaching process of mixing two or more components, indicating the degree of disorder of the system, but if ΔS value is negative indicating that the system is more regular.

EPMS-HP-β-CD inclusion complex (KI) preparation

EPMS-HP-β-CD inclusion complexes (at molar ratio of 1:1) were formed using EPMS and HP-β-CD by kneading method. The mixture of EPMS and HP-β-CD 0.010 mol respectively was suspended with suitable quantity of ethanol-water (1:1). Then it was grinded continuously at a room temperature until dry precipitate obtained. The dry precipitate was grinded and sieved, then stored at the exiccator in relative humidity 40-50% for 48 h.

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EPMS-HP-β-CD physical mixture (CF) preparation

EPMS and HP-β-CD (at molar ratio 1:1) were mixed in the mortar for 30 min and were collected in the room temperature (25° ± 2.0°) C at the same moisture conditions as the related inclusion complex form.

Inclusion complex characterization

The inclusion complexes formation were confirmed by DTA, FTIR and XRD analyzer. For inspection with all of the methods, the inclusion complexes were compared with EPMS itself, physical mixture EPMS/HP-β-CD and HP-β-CD. The change in chemical shifts and the interaction of guest and host each other, and the formation of EPMS-HP-β-CD inclusion complex (KI) have also been investigated using ^{1}H- NMR. The spectra were recorded on Bruker Ultra shield 600 MHz (^{1}H) spectrometer at 26.85ºC. Chemical shifts were measured using TMS as external standard and the resonance was set as 0 ppm. Penetration of EPMS in the EPMS/HP-β-CD inclusion complex (KI) was explained through ROESY ^{1}H-NMR experiment using D_2O as solvent.

Dissolution study

The EPMS releasing profile was determined from the dissolution study of its inclusion complexes. The dissolution tests were applied to EPMS, CF and KI. Dissolution analysis was performed using buffer system pH 6.8 as dissolution medium. The sample equivalent to 50.0 mg EPMS was weighed and put into 900 ml of CO2-free water as dissolution medium which was maintained at pH of 6.8±0.05. The temperature was set at 37±0.05°C and the paddle-type stirrer was rotated at 50 rpm. The sample of aliquot (5 ml) were taken at 5,10,15,30 and 60 min periodically, filtered by 0.45μm filter paper, and diluted suitably with dissolution medium. The concentration of EPMS was determined using UV spectrophotometer at 310 nm. The dissolution test was performed triplicate.

RESULTS AND DISCUSSION

Solubility Study of EPMS

Study of solubility revealed that solubility of EPMS in various temperatures (i.e. 32, 37 and 42°C) is $3.11.10^{-4}$±0.09M, $3.95.10^{-4}$±0.06 M, $3.94.10^{-4}$±0.2 M respectively. The temperature increased solubility of EPMS. The relationship between concentration of HP-β-CD and EPMS dissolved in various temperatures are presented in Fig.2. The EPMS solubility enhanced as increasing of HP-β-CD concentration. There were linear curve with slopes between 0 and 1 which indicate A_L-type of phase solubility according to Higuchi and Connors concepts [10].

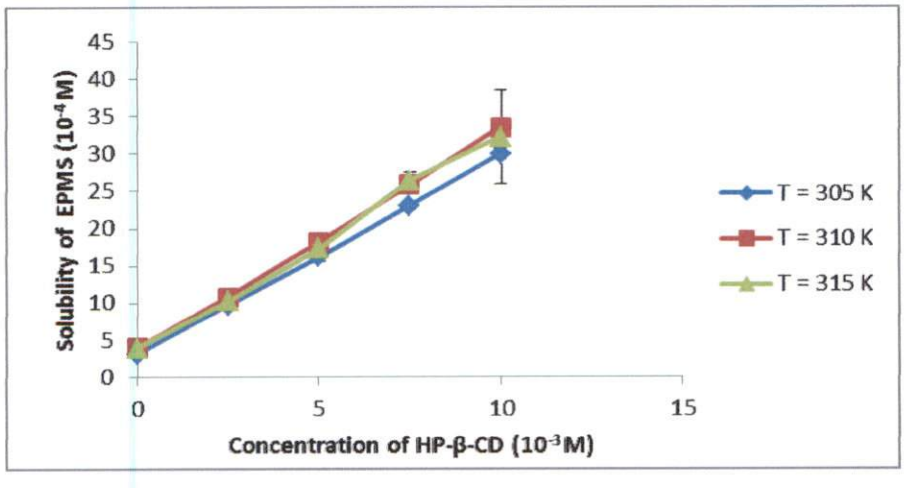

Fig.2. The curve relationship between concentrations of HP-β-CD (10^{-3}M) and EPMS dissolved (10^{-4}M) at various temperatures (305,310 and 315 K). The values are mean ±SD of triplicate.

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The regression equations at various temperatures (°K) respectively are: .

$Y = 0.2679X + (3.004.10^{-4})$, r = 0.99992 (eq.4) at 305 K
$Y = 0.2963X + (3.632.10^{-4})$, r = 0.99976 (eq.5) at 310 K
$Y = 0.2903X + (3.512.10^{-4})$, r = 0.99784 (eq.6) at 315 K
X = concentrations of HP-β-CD (10^{-3}M); Y = EPMS dissolved (10^{-4}M)

The regression curve of EPMS dissolved versus HP-β-CD concentration in various temperatures are displayed in Fig. 3.

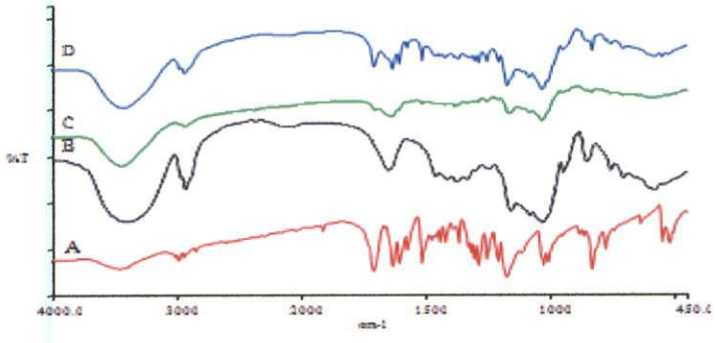

Fig. 3. FTIR spectra of EPMS (A), HP-β-CD (B), KI (C), CF (D)

From the eq.4, eq.5, and eq.6 we obtain association constant (K_a) for each temperatures using Higuchi & Connors equation [10].

$$K_{a:b} = \frac{slope}{So(1-slope)} \qquad \text{(Eq.7)}$$

So = intrinsic solubility of the drug in certain circumstances

The K_a value for each temperatures were 1176.63 (at 305 K), 1065.97 (at 310 K), 1050.02 (at 315 K). The results showed that K_a value decrease as the temperature increase, due to the stability of ester moiety in EPMS. The Plot of log K_a (M^{-1}) versus 1/T (K^{-1}) give the linier regression equation: Y= 445.055X + 1.6039; r = 0.9265...(Eq. 8), where X= 1/T and Y= log K_a. Eq.8 was used to calculate the thermodynamic parameters, i.e. ΔG, ΔH and ΔS using the equations as follow : Eq. 1, Eq. 2, and Eq. 3; where R= 1.987 cal/mol, T = temperature (K), K = stability constant of inclusion complex. The results of these equations obtained the ΔG, ΔH and ΔS value as described in Table 1.

Table 1. Thermodynamic parameters of complex inclusion of EPMS-HP-β-CD.

Temperature (K)	ΔH (cal/mol)	ΔG (cal/mol)	ΔS(cal/mol)
305	-2036.60	-4285.69	7.37
310	-2036.60	-4295.10	7.29
315	-2036.60	-4354.94	7.36

Characterization of KI and CF of EPMS

According to the reference [11], the IR spectra of HP-β-CD shows -OH stretching vibration at 3300-3500 cm^{-1} and 2800-3000 cm^{-1} of -CH and -CH_2 groups. IR spectra of the physical mixture (CF) (Fig. 3D) and KI (Fig. 3C) don't show the aromatic ring, alkene and carbonyl moieties of EPMS. The different IR pattern of CF and KI from EPMS indicated an appearance of host-guest interaction. These patterns suggest the possible formation of hydrophobic attraction between aromatic ring and alkene moiety of EPMS with –C-H- moieties of host cavities as reported by [12] in the interaction the hydrophobic cavities of cyclodextrin with pullunase.

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DTA analysis

The endothermic peak of EPMS (Fig.4) (A) appears as a sharp peak at 52.8°C means melting point of crystalline form of EPMS. The endothermic peak of B at 141.9°C appears as a short wide peak showing amorphous form of HP-β-CD. DTA curve of C showed two peaks at 49.1 ° C and 150.0 ° C, while the curve D showed peak at 49.4 ° C with very low intensity and short wide peak at 146.7°C which indicated the possibility that the inclusion complex has been formed. DTA analysis showed decline in melting point of KI form compare to the single compound and different peak size between EPMS and KI. These changes occur due to changes crystalline form of EPMS into amorphous form.

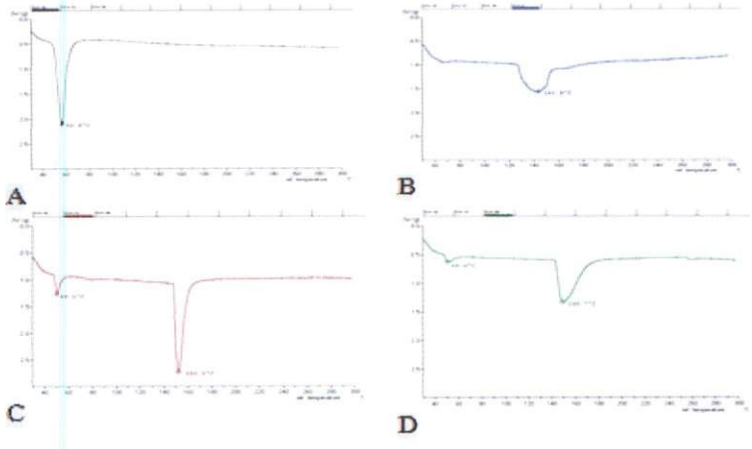

Fig. 4. Thermogram spectra of EPMS (A), HP-β-CD (B), CF (C), KI (D).

XRD analysis

XRD study was displayed in Fig. 5, where Fig. 5A showed intensive and sharp peaks of EPMS that confirm the crystalline nature of the compound while Fig. 5B displayed a wide peak which indicate amorphous material of HP-β-CD. The XRD pattern of CF (Fig. 5C) and KI (Fig. 5D) displayed distinctive peak compared to EPMS. The peak corresponding to 14.0′ (2θ) of EPMS is not shown at XRD pattern of CF and KI. Whereas, the 23-26′ (2θ) peak of EPMS appear less intense on CF and this peak has not shown at KI. The XRD pattern of CF showed sharp peaks which indicated the retention of crystalline structure of EPMS molecule during mixing process (Fig. 5C). Nan et al. (2014) [16] reported similar results in the physical mixture of HP-β-CD and myrcetin. However, the XRD pattern of KI (Fig. 5D) showed a quite similarity with HP-β-CD (Fig. 4B) and did not demonstrate the characteristic peaks of EPMS. The XRD outcomes confirm the confidential transformation at crystal lattice level during infiltration of the two substances by kneading method.

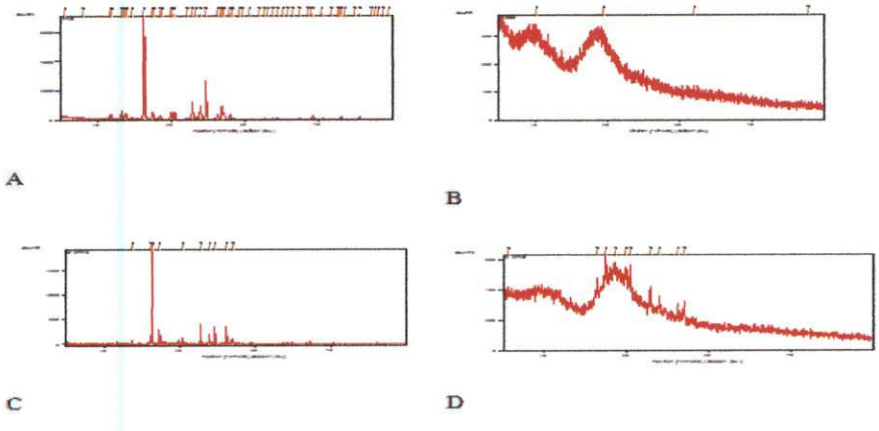

Fig. 5. Powder XRD analysis of EPMS (A), HP-β-CD (B), CF (C), KI (D).

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^{1}H-NMR studies

The 2D ^{1}H-NMR (ROESY) was performed to explain the location of guest proton inside host [13] in the inclusion complex form. There were some weak cross-peaks between protons of vinylic double bond and aromatic ring of EPMS with protons of HP-β-CD (Fig.6) It clarified that the protons of vinylic double bond conjugated with ring aromatic moiety of EPMS were inside the HP-β-CD cavity. No cross-peaks were discovered between the methoxy protons of EPMS and the HP-β-CD protons. The methylene proton of ester moeity is noticed inside the proton HP-β-CD, but methyl proton of ester is not. These results supported the FTIR spectra that aromatic ring, vinylic double bond, and methylene moiety of ester were entrapped in the host cavity (Fig.3). The interpretation of ^{1}H-NMR ROESY experiment proved that the EPMS-HP-β-CD inclusion complex has been formed by kneading method.

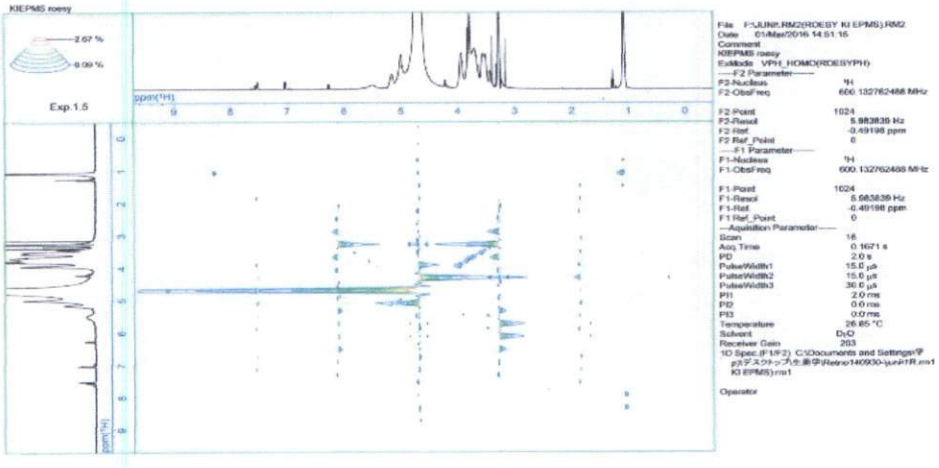

Fig.6. ROESY spectrum of solution containing the 1:1 molar ratio of EPMS-HP-β-CD inclusion complex (D2O).

Dissolution Rate analysis

The dissolution rate profiles of EPMS, CF and KI were displayed at Fig.7. The physical mixture (CF) of EPMS-HP-β-CD has faster dissolution rate than EPMS because the compound is dispersed in hydrophilic matrix of host and it will be carried passively into dissolution medium when the matrix liquefies so that its dissolution rate increase. HP-β-CD has ability as in situ host of inclusion complex form in the dissolution medium so that the dissolution rate of drugs increase [14]. The dissolution rate profile of KI is the highest compared with CF and pure sample of EPMS. The dissolution rates of CF and KI showed significant differences with EPMS ($p<0.05$), as well as dissolution rates of CF is different from KI ($p<0.05$). One of the parameters to compare the dissolution profile is dissolution efficiency [15,16] .

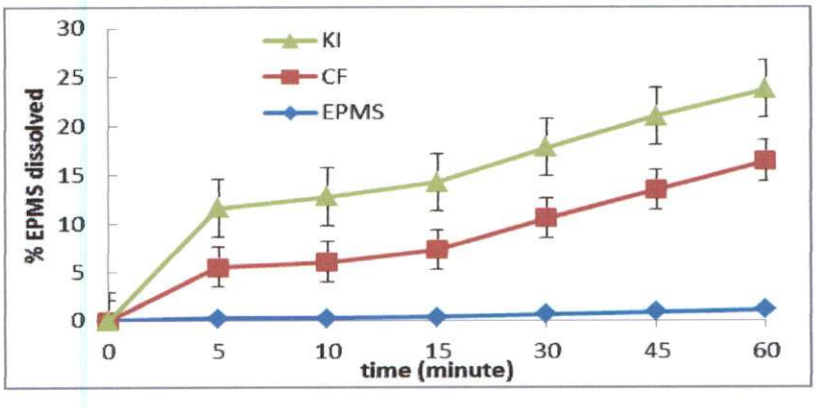

Fig.7. Dissolution profiles of EPMS, CF, KI. Each values is mean ± SD of triplicate.

The dissolution efficiency at 60 minutes (DE60) of KI is the highest and significantly different ($p<0.05$) from CF and EPMS. The DE60 value of EPMS, CF, KI are 17.9743, 292.683, 329.0097, respectively. In CF form, the intermolecular forces involved between EPMS and HP-β-CD only the weakest van der Waals force, namely London dispersion, which causes the contact between two surfaces are not optimum and weak. Shown in Fig.7, that until the 60th minute the solubility of EPMS in CF form increases only up to 17%. Meanwhile in KI form, the solubility of EPMS increases up to 26%.

There is no strong connection between the host and guest in the physical mixture form and they persisted to exist in their original form, whereas in solution as kneading method, they created close association so that the solubility of KI is higher than CF form [17]. The dissolution profile of drug can used to predict its bioequivalent [18].

EPMS is ester compound and not soluble in water, but soluble in ethanol. By kneading method, EPMS and HP-β-CD are dissolved in ethanol-water, then dried until molecular interaction appeared and the contact between them are optimum. Intermolecular force possibly involved between EPMS and HP-β-CD in KI is dipole-induced dipole, one of the van der Waals interactions which is stronger than London dispersion in CF. These results also indicated that there was change in the crystalline form of EPMS into amorphous form which can increase its solubility and dissolution rate in water.

CONCLUSIONS

The inclusion complex of EPMS-HP-β-CD can be prepared by kneading method and it showed different physicochemical properties with EPMS, including the solubility and dissolution rate increase significantly.

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