

CHAPTER IV

DISCUSSION

Cyclodextrins are cyclic molecules produced from starch that have strong impact in industrial applications, because of their ability to form inclusion complexes with a variety of chemical substances. Usually, CDs improve guests' properties, e.g. thermal and chemical stability, the guests thus have an increased resistance to damaging ambient factors such as oxygen, light and heat. In addition, CDs also increase the solubility of substances. Natural CDs compose of α -, β - and γ -CD. The most useful natural CDs for industrial use is β CD because it has a suitable cavity size to include aromatic and heterocyclic compounds, moreover it is produced in large scale with a reasonably low price (Martin, 2004). However, due to the low aqueous solubility of β CD (about 1.8 % w/v, at 25 °C), many derivatives of β CD are more interesting. In this study, hydroxypropyl- β CD and methyl- β CD are used in comparison with β CD. These CDs were chosen because their solubilities were 27-30 times higher than β CD. The guest molecule of our interest is fixolide which is used as fragrance in fabric softener. Owing to the semivolatile nature of fixolide that could be lost during applicable use, therefore, we proposed that complex formation between fixolide and CDs might result in better properties especially in stability for UV and heat. Co-precipitation, kneading and freeze-drying method were used to prepare the inclusion complexes. DSC and FTIR were used for investigation of complex formation. The amount of fixolide included in the complex was determined by solvent extraction and GC analysis. The most effective complex formation system was chosen for study on the stability and solubility both in solid powder and in fabric softener.

4.1 Analysis of fixolide

The determination of fixolide in municipal sewage effluent using gas chromatography was previously reported by Osemwengie *et al.* (2001). Fixolide was quantified using HP-5 capillary column (30m x 0.25 mm. I.D., 0.25 μ m film thickness), and the following temperature program: 90 °C, 0 min hold, 10 °C/min to

300 °C, and 5 min hold with helium carrier gas at a linear velocity of 37 cm/s. This GC condition could detect the amount of fixolide at nanogram level in wastewater. The GC condition used in our study was similar to these conditions, with slight modification as described in Section 2.3.1.2.1. However, for more convenient and less cost, we explore the possibility of using spectrophotometry as another means for quantitation of fixolide. The spectrophotometric property of fixolide and its soluble complex with β CD were investigated. Absorption maximum wavelength was observed at 258 nm of fixolide, whereas λ_{\max} of 216 nm was also found. In this study, absorption peak at 258 nm was chosen for analysis of fixolide since 216 nm was nearly the cut-off point of absorption wavelength of ethanol which was used to dissolve fixolide [λ_{\max} of ethanol was 210 nm, แม้น อมรสิทธิ์ (2534)]. For β CD solution, no UV absorption peak was found since it contained no UV absorbing functional group. When fixolide was mixed with β CD in the approximate mole ratio of 1:2, the soluble complex was expected to be formed (as evidenced by result from phase solubility study). From the scanning for UV spectra, λ_{\max} of the complex was still at 258 nm indicating that there were no shift in λ maxima of fixolide and no interference from β CD upon complex formation.

4.2 Phase solubility study

The phase solubility diagrams of fixolide-CDs system in 50 % (v/v) aqueous ethanolic solution are presented in Figure 13. The solubility in β CD was increased as increasing concentration of β CD, then reached the plateau, followed by the decrease due to limited solubility of the complexes at which the insoluble complex form was observed. This solubility diagram could be classified as B_S type (Higuchi and Connors, 1965). Methyl- and HP- β CD did not form precipitation as the solutions remained clear even at 100 mM concentration. The A-type solubility diagrams were observed. For methyl- β CD, the solubility of fixolide increased linearly as a function of methyl- β CD concentration, the result of which indicates the A_L -type. While for HP- β CD, the A_N -type was suggested, since the plot illustrates negative deviation at higher concentration of the cyclodextrin derivative. It was proposed that self-association of

the guests at high CD concentrations might occur (Martin, 2004). The result also shows that β CD demonstrated the lowest solubilising ability among various β CD derivatives used. Enhancement of the solubility of poorly water soluble compounds by methyl- and HP- β CD was affected by the functional group of side chain. The apparent stability constant (K_c) were calculated from the initial straight line portion of solubility diagram, assuming that a 1:1 complex is initially formed if the slope is smaller than 1. The K_c values obtained suggested that the interactions of fixolide with β CD derivatives were weaker than with β CD. The spatial relationship between host and guest molecules seems to be responsible for the stability constant (K_c) and stoichiometry of the complexes (Uekama, 1983). However, the K_c values calculated in our study were in the range of 15-45 M^{-1} , indicating a low stability complex. Similar low K_c values between 7-65 M^{-1} have been reported for α - and β CD complex with benzaldehyde (Ukema et al., 1983) and famotidine (Hassan et al., 1990), in spite of the low of K_c values, these complexes still exhibited favorable properties e.g. stability, dissolution rate.

4.3 Preparation of fixolide : CD (β CD, Methyl- β CD and HP- β CD) solid complexes

The preparation of solid complex was carried out between fixolide and CDs (β CD, methyl- β CD and HP- β CD) using 1:1, 1:2 and 1:3 guest : host mole ratios. Coprecipitation, kneading and freeze-drying were different methods employed in this study.

The most popular method is to form inclusion complex in a solution of CD. In this method, the guest compound is dissolved into an aqueous solution of CD to form the inclusion complex in a crystalline form. However, various compounds have a low solubility in water, making complexation either very slow or impossible. In such case, the use of an organic solvent to dissolve the guest is desirable. The solvent should be water-miscible organic solvent and should not complex well with CD. (Yoshii, 1999;

Martin, 2004). Therefore, ethanol was appropriate for good complexation in this study.

In co-precipitation method, when fixolide complexes with β CD, there was precipitate out of the solution in all mole ratios. For complexation with methyl- β CD and HP- β CD, no precipitate was formed in the solution even though the stirring time was increased to 48 hours. The result agrees well with high aqueous solubility of methyl- β CD and HP- β CD (more than 50 g/100 ml at 25 °C), thus a soluble complex with the A-type phase solubility diagram was formed. For β CD which has limited aqueous solubility, the complex formation with guests having limited solubility frequently gives rise to B-type solubility diagrams as defined by Higuchi and Connors (1965). Generally, the co-precipitation employed only for preparation of inclusion complexes with a B-type diagrams (Veiga, 1996).

Kneading method was successfully used in our study for the preparation of the inclusion complex of fixolide with CDs. Fixolide has a limited aqueous solubility (only 1.25 mg/L), and this method is appropriate for the guests having poor water solubility. Similar result was reported by Yoshii *et al.*, (1998) that the molecular inclusion powder of d-limonene (liquid flavor) in β CD was prepared by using a twin-screw kneader comparing with the inclusion complex obtained by the micro-aqueous method. The inclusion fraction of d-limonene in the complex powder was much higher than that made by the micro-aqueous method.

Freeze-drying method can also be used to prepare the inclusion complex. This method has the faster preparation time than co-precipitation. In particular, it is appropriate for the sensitive compounds. This advantage agrees with the result of study by Minemoto *et al* (1997) who investigated that methyl linoleate encapsulated by freeze-drying was more slowly oxidized than that encapsulated by hot-air-drying. The high temperature during the traditional spray-drying microencapsulation process leads to an increased oxidation of fish oil. Heinzelmann and Franke (1999) demonstrated the application of a freeze-drying technique with respect to the

production of dried microencapsulated fish oil with high quality and oxidation stability.

4.4 Detection of Fixolide : CD (β CD, Methyl- β CD and HP- β CD) solid complexes

4.4.1 Differential Scanning Calorimetry (DSC)

It is not guaranteed that the powders obtained by different complexation methods are true, homogeneous inclusion complexes. In many cases the product is a mixture of complex, uncomplexed guest, and empty hydrated CD. DSC is widely used to characterize CDs and their inclusion complexes. It is considered to be the proof of inclusion complex formation when the melting peak of the guest does not appear in the DSC thermogram (Orgoványi *et al.*, 2005).

In present study, DSC thermograms of different mixtures were compared with free fixolide and CDs (β CD, methyl- β CD and HP- β CD) (Figure 14-20). The DSC curve of fixolide exhibited a characteristic endothermic peak at 57.5 °C. The β CD, methyl- β CD and HP- β CD displayed a broad endothermic peak in the range of 70-130 °C corresponding to the release of water molecules. The temperature range depends on the structure of the compounds and on experimental conditions (Mura *et al.*, 1998). For the fixolide- β CD systems of co-precipitated at 1:1 mole ratio, kneaded at 1:1 mole ratio and freeze-dried mixtures at 1:2 mole ratio, the thermograms displayed a broad endothermic band between 70-125 °C due to the dehydration of the complex. It was observed that the small peak near 57.5 °C still existed which reflects the presence of small amount of free fixolide in the preparation. For the fixolide-methyl- β CD system of kneaded mixture at 1:1 mole ratio and fixolide-HP- β CD system of kneaded mixtures at 1:1 and 1:2 mole ratios and freeze-dried mixture at 1:2 mole ratio, the DSC curves obtained were similar to the fixolide- β CD system. However, thermal effect appeared more broadened and reduced in intensity of fixolide peak, which suggests some fixolide-CDs interaction. This pattern of thermograms led to a further

indication of the existence of inclusion complexes. However, these complexes did not exhibit complete encapsulation, and fixolide was dispersed in free state in the midst of inclusion complexes (Francisco *et al.*, 2001).

A complete disappearance of the melting endotherm of fixolide was observed in co-precipitated with β CD at 1:2 and 1:3 mole ratios, kneaded dispersions with β CD, methyl- β CD at 1:2 and 1:3 mole ratios, and HP- β CD at 1:3 mole ratio, and also in freeze-dried with β CD, HP- β CD at 1:3 mole ratio and methyl- β CD at 1:2 and 1:3 mole ratios, an indicative of true inclusion complex formation in these conditions. These modifications of the DSC melting peak can be assumed as a proof of interactions between the components in the respective binary system (Mura *et al.*, 1998).

DSC thermograms of all physical mixtures were composed of the endothermic peaks of fixolide and CDs. However, the reduction in intensity of fixolide peak observed suggests that physical mixing method could also lead to possible incomplete complex formation. The reasonable complex formation was supported from heats of fusion of physical mixed fixolide with CDs, the value of which was much smaller than that of fixolide without CD. The enthalpy values were 41.66, 16.86, and 29.09 J/g for fixolide mixed with β CD, methyl- β CD, and HP- β CD, respectively, compare with 72.19 J/g for the corresponding free fixolide. The enthalpy values were calculated from the area of endothermic peak divide by the heating rate of the DSC experiment. The area of the peak and the enthalpy can be expressed by equation (3) and (4), respectively (<http://www.pslc.ws/mactest/dsc.htm>).

$$\text{area} = \frac{\text{heat} \times \text{temperature}}{\text{time} \times \text{mass}} = \frac{\text{J K}}{\text{s g}} \quad (3)$$

$$\text{enthalpy } (\Delta H) = \frac{\text{area}}{\text{heating rate}} = \frac{\frac{\text{J K}}{\text{s g}}}{\frac{\text{K}}{\text{s}}} = \frac{\text{J}}{\text{g}} \quad (4)$$

Heat reduced or the reduction in enthalpy values indicates that these products were not simple physical mixtures (Bayomi *et al.*, 2002).

4.4.2 Fourier Transform Infrared Spectrometry (FTIR)

When FTIR was analyzed, the major peak at 1683 cm^{-1} of the C=O stretching of the carbonyl group was important characteristic of fixolide. β CD, methyl- β CD and HP- β CD displayed the significant OH bending at 1645, 1647 and 1655 cm^{-1} , respectively. FTIR spectrum of only 1:2 mole ratio of solid complexes were analyzed. The characteristic of carbonyl group of the fixolide was shifted from 1683 to 1677 cm^{-1} in co-precipitated and freeze-dried mixtures and was shifted to 1672 cm^{-1} in kneaded mixture with β CD. A shifted of the fixolide characteristic peak from 1683 to 1679 and 1678 cm^{-1} were observed in the methyl- β CD complexes formed by kneading and freeze-drying methods, respectively. However, all fixolide-HP- β CD complexes showed unchanged fixolide carbonyl peak. This is in agreement with DSC analyzed. These results suggest that the steric hindrance of CD by hydroxypropyl group ($\text{CH}_2\text{CHOHCH}_3$) might be occurred, therefore stoichiometric ratio of fixolide : HP- β CD (1:2) was not appropriate for good guest-host complexation.

In the spectrum which showed a shift of C=O peak, suggesting a modification of electronic environment of C=O group which means inclusion complexes could be formed. However, the spectrum of all fixolide-CD did not show new peaks, indicating no chemical bonds created in the products formed. The spectral shift to a lower frequency of 1683 cm^{-1} observed could be attributed to the formation of intermolecular hydrogen bonding between fixolide and CDs (Lin and Koa, 1989). On the other hand, the C=C band at 1544 cm^{-1} of aromatic ring was not shifted and this could suggest that the aromatic ring was not included into the cavity of CDs (Bayomi *et al.*, 2002).

The IR spectrum of physical mixtures of fixolide with β CD, methyl- β CD and HP- β CD did not show any change of the characteristic peak of C=O group of the fixolide. The result from FTIR confirmed the observation from DSC.

4.5 Determination of fixolide in solid complexes

This experiment was set to determine which method of complex formation and which type of CD were the most appropriate. The inclusion complexes of fixolide with CDs were subjected to solvent extraction and quantity of the fixolide content in CDs was determined by GC analysis. Extractions method was modified and carried out according to Reineccius (2004). The solvent used was a mixture of 30 ml water and 4 ml n-hexane. β CD has a low solubility (1.8 g/100 ml, 25 °C), therefore the fixolide- β CD complex (100 mg) which contained 81-93 mg of β CD could be dissolved in 30 ml of water used for extraction without using high temperature. The fixolide has a low aqueous solubility (1.25 mg/L at 25 °C), thus it should be separated from water and easily dissolved in 4 ml of n-hexane. From preliminary study, it was found that only one extraction could recover the amount of fixolide upto 95.86 %. Recovery from β CD host was also determined by adding known amounts of fixolide and β CD in the 1:1, 1:2 and 1:3 mole ratios, then extracted and analyzed. The extraction and analysis procedures were shown to be very efficient and accurate with the percent recovery from 82-101 %. The internal standard (naphthalene) was used during this assay to correct for the extraction accuracy. From the result, it was found that the complex of fixolide : β CD at 1:2 mole ratio by co-precipitation method at the stirring time of 24 hours gave the highest fixolide content with the complex yield of 74 % and showed complete complex formation as determined by DSC and FTIR. Nevertheless, it was observed that the complexes at the mole ratio of 1:1 of all formulations gave higher amount of fixolide than the co-precipitated complex from 1:2 mole ratio, this was owing to the excess free fixolide as evidenced by peaks shown in DSC and FTIR profiles (Figure 14-20). In addition, the co-precipitation method at the stirring time of 3 hrs showed higher amount of fixolide than the stirring time of 6, 12, and 18 hrs, this was also due to the existence of free fixolide in the complex

evidenced by DSC. In solid state, guest molecules can be enclosed within the cavity of CD or may be aggregated to the outside of the CD molecule (Bekers *et al*, 1999). It is possible that the polycyclic nature of fixolide structure fits well with 1:2 mole ratio. Hara *et al*, 2002 had demonstrated that the 1:1 to 1:3 mole ratios of different types of CD were appropriate for shorter to longer alkyl chain length unsaturated aldehyde odors. For aromatic guests smaller than fixolide such as menthol, vanillin, and carbaryl, guest : host ratios of 1:1 or 2:1 might be better (Reineccius, 2004 ; Saikosin, 2002).

4.6 Determination of properties of inclusion complexes in solid state

4.6.1 The thermal stability and photostability test

The application of cyclodextrin is expected that it will help provide promising extended shelf-life of fragrance. Inclusion complex of fixolide and CD had been formed in order to decrease degradation of fixolide in solid state. The stability of fixolide was studied by heat at 80, 50 °C and by exposure to UV light. These samples were treated under accelerated conditions with a short time. Stabilization during long-term storage (4 months) at 35 °C was also checked. The degradation of fixolide was compared with fixolide- β CD complex formed by co-precipitation method. The rate constants for the degradation of fixolide and fixolide- β CD complex were determined. The results showed that the degradation rate constants of fixolide alone were faster than fixolide inclusion complex with β CD. Therefore CD made dramatic decreases in degradation rate constants of fixolide. It was observed that the amount of fixolide was less lost by heat or UV treatment when it was complexed with β CD. The loss was less than 10 %, while about 15-30 % of the free fixolide was lost. The effect of inclusion complexation of fixolide with β CD on the thermal stability and photostability exhibited acceptable stability in the solid state, thus effectively benefits utilization of fragrance and in particular good for long-term storage and exposure to stress conditions.

In this study, the order of degradation reaction was also determined. Linear and non-linear models were used to illustrate the kinetic behaviour of the fixolide molecule in the presence and absence of CD. It appeared that the degradation of fixolide by heat and light followed first-order reaction in all formulations. Similar result was reported by Emara *et al.* (2000) that the degradation of daunomycin at ambient temperature and under day light was appeared to be first-order. Moreover, the activation energy (E_a) values obtained for the degradation of fixolide in the absence and presence of β CD over the temperature range of 35-80 °C from Arrhenius plot were 10.64 and 10.73 Kcal/mole, respectively. E_a of fixolide complexed with β CD was higher than free fixolide suggesting that β CD might give some protection against the influence of heat degradation (Waree, 2001). Furthermore, the Arrhenius plot in the presence of β CD was parallel to that in its absence, indicating that β CD did not affect the mechanism of the degradation of fixolide. This result is consistent with the studies by Dotsikas *et al.* (2002), in which the mechanism of degradation of insulin in presence and absence of methyl- β CD against three temperatures (50, 55, and 60 °C) remained the same since the Arrhenius plot in both formulations were parallel.

4.6.2 Dissolution study of inclusion complexes

Fixolide exhibited poor dissolution owing to its hydrophobicity. The co-precipitated complex formed by stirring for 24 hours with β CD and kneaded complex with methyl- β CD at the molar ratio of 1:2 displayed higher and faster dissolution rate than free fixolide. The significant enhancement in dissolution rate of these complexes might be due to an increase in solubility and marked reduction in crystallinity. The increase of dissolution rate can be attributed, besides an increase in solubility, to the surfactant-like properties of cyclodextrins which can reduce the interfacial tension between fixolide and the dissolution medium, leading to a higher dissolution rate (Veiga *et al.*, 1996). As for the effect of the type of the carrier, it can be observed that the inclusion complex with methyl- β CD showed a dissolution rate higher than the inclusion complex with β CD. The best effectiveness of methyl- β CD can be explained

on the basis of its greater hydrosolubility and higher amorphizing, wetting, and solubilizing (Mura *et al.*, 1999).

4.7 Application of the Inclusion Complex in Fabric Softener

The applications of fragrance on personal care and household products (e.g. fabric softener, soap, cosmetic) were not highly effective due to its unstability against heat, oxygen and light. Fixolide is a main fragrance used in fabric softener, thus the experiment on stability of fixolide or fixolide included in cyclodextrin in fabric softener was performed.

The fabric softener was prepared and fixolide or corresponding cyclodextrin complex was added in formulation and then these formulations were investigated under accelerated conditions by heat at 80 °C for 7 days, 50 °C for 14 days and exposure to UV light for 24 hours. Stabilization of fixolide alone and fixolide complexed with β CD in solution were compared. The loss of free fixolide in fabric softener was higher than fixolide complexed with β CD (17-53 % compare to 8-13 %). For all stability tests, the degradation rate constants of fixolide alone were higher than fixolide in the complex form with β CD. Therefore, complex formation with β CD could effectively stabilize fixolide in fabric softener when treated under stress conditions. The chemical degradation of fixolide inclusion complex or its free form at 80, 50 °C and under UV light was concluded to be first-order reaction. The order of reaction of fixolide degradation in the presence and absence of β CD under accelerated conditions were not different.

However, the degradation rate constants of fixolide in softener solution were about 2-3 times higher than fixolide in solid state (compare values in Tables 16 and 20). This may be attributed by the presence of dipropylene glycol as a solvent for fixolide in softener formulation which can have destabilizing effect on the substance when stimulated by heat or light (Tønnesen, 2001).

The overall results indicated that molecular inclusion of fixolide with β CD using the co-precipitation technique led to formation of solid complex with favorable properties. The inclusion complex formed can efficiently retard the thermal and photodegradation of fixolide both in solid form and in fabric softener upon exposure to heat and UV light. This effect would improve stabilization of the fixolide fragrance for personal care and household products. Higher stability ensured less decomposition and increased the long-lasting odor in the formulation products. In addition, β CD and methyl- β CD could significantly increase solubility of fixolide in solution thus no need to use any solubility enhancer.