CHAPTER IV

DISCUSSION

Cyclodextrins are valuable material that could form inclusion complex with many inorganic/organic compounds resulting in desirable properties which could be utilized in various fields such as industry and agriculture. For agricultural application, CDs are mainly used as solubilizer and stabilizer. Natural CDs are α-, β- and γCD. The solubility in water are 14.5, 1.85 and 23.2 g/100 ml, respectively (Szejti, 1988). The most useful natural CDs is βCD because it has a suitable cavity size to include the majority of guests such as pesticide molecules; moreover it is produced in the large scale with a reasonable price. However, βCD is the least soluble, many derivatives of βCD are more interesting. In this present study, the methyl-βCD and maltosyl-βCD are used in comparison with the natural CDs. For pesticides used, carbaryl, carbendazim, and methidathion were chosen as they are heavily used in Thailand and with the problem of residual left in environment. It is important that the physicochemical properties of pesticide, when it is complexed with CDs, may have different pesticide properties, especially in solubility and stability of UV and heat. FTIR and DSC are usually used for evaluating complex formation and explaining different product obtained.

Selection of the best pesticide-CD system was first performed in this study. Solid complex was then prepared. The methods of preparation were co-precipitation, freeze-drying and kneading method. The methods were simple and easy, moreover this may be used in the manufactures. The next objective was to obtain the formulation that gave the increasing in solubility and stability for developing pesticides powder. Furthermore, to study the influence of different methods, temperature and physicochemical properties of pesticide powders and pesticide-CD complexes were determined.

4.1 The maximum absorption of pesticide and pesticide-βCD complexes

The spectrophotometric property of pesticides, in pure form and commercial formula, and their complexes with βCD were investigated. Absorption maximum wavelengths were observed at 210, 273 and 284 nm for pure methidathion, carbaryl and of carbendazim, respectively. Carbaryl 85WP and carbendazim 50WP also showed absorption peaks at 273 and 284 nm, which confirmed carbaryl and carbendazim as active ingredients. Some other peaks were also observed in these commercial formula which might be due to other added ingredients or contaminants. For βCD solution, no main UV absorbtion peak was found since it contained no UV absorbing functional group. When excess pesticides were incubated in the presence of βCD for 24 hrs., then filtered and the filtrates (which should contain pesticide-βCD complex, free pesticide, and free βCD, were scanned for UV spectra, no shifts in λ maxima of the active pesticides were observed in all cases. However, band broadening were observed. In UV analysis, band broadening has usually been observed in the spectrum of an UV active guest complexed with βCD (Szejtli, 1982). These changes were intensified as the concentrations of βCD increased. For carbaryl and methidathion and their commercial formula, complex formation was suggested by the increase in UV absorbance at λ maxima of pesticides in the presence of βCD. No change in absorbance was observed in carbendazim case which suggested no or little solubility complex formation with βCD occurred.

4.2 Solubility study : Selection of the best type of pesticide in forming soluble complex with CDs

The cyclodextrin solid dispersion of a poorly water-soluble pesticide is usually more hydrophilic than the free pesticide itself (Szeijtli, 1988). The occurrence of an increased solubility can be concluded from a phase solubility diagram. The intrinsic solubility can be determined from the intercept of the ordinate by the phase solubility diagram. This method is a simplified way to determine the solubility.

4.2.1 Phase solubility of carbaryl

The inclusion complex stability constants at 30°C for the carbaryl and carbaryl 85WP with various CDs are shown in Table 6 and Figures 5-8. For the utilized concentrations of CDs, all the phase solubility diagrams were of the A_L-type except for the carbaryl-βCD systems, which resulted in an A_N-type. All the A_L-type diagrams had a slope less than 1.0 indicating the probable formation of 1:1 complexes in aqueous solution (Juan and Gines *et al.*, 1996). The formation of 1:1 complexes for carbaryl and carbaryl 85WP resulted in a significant increase in the solubility for the complexes compared to the free carbaryl. Though carbaryl-βCD gave the A_N type, the complex gave a high stability constant (K_C value) since determination was based on the initial slope. The stability constant of carbaryl-βCD was determined to be 210.53 M^{-1}. This was lower than previously reported value of 310 ± 5 M^{-1}, of which the A_L type phase solubility diagram was suggested (Barboto *et al.,* 2000). This could be due to different experimental and analysis methods and the batch of guests and hosts applied. Realistic reproducibility of the inclusion complex stability constants can be varied by the factor of 2 (Connors, 1997). In the study of Barboto *et al.,* (2000), they reported the A_L type because the highest concentration of βCD used was 12 mM while upto 50 mM was used in the present study. When we replaced pure carbaryl with commercial form in the phase solubility study, lower formation constant for β CD-carbaryl was obtained. While the K_C values of the complexes between G_2-βCD or methyl-βCD with carbaryl or carbaryl 85WP were not different. Other ingredients besides the active carbaryl in commercial formula might interfere with complex formation between βCD and carbaryl while had no effect on G_2-βCD or methyl-βCD complex formation. The effect on βCD made K_C lower and the type of solubility diagram changed from A_N to A_L.

Our study was the first to use G_2-βCD and methyl-βCD with carbaryl which are much better than βCD in solubility property. Methyl-βCD was better than G_2-βCD in forming complex with carbaryl since it gave higher K_C value. Commercial carbaryl, carbaryl 85WP, was the practical form used as pesticide and it was much cheaper than pure carbaryl. Carbaryl 85WP-methyl-βCD showed the highest K_C of 223.18 M^{-1}.

Carbaryl is a non-polar compound, the aromatic ring of carbaryl should be easily contained within βCD ring, satisfying the condition for inclusion complex formation. The carboxyl group of carbaryl should be able to form hydrogen bond with one secondary CD hydroxyl. Maltosyl- or methyl- may have the profound effect on β CD structure, thus reflecting the complex formation.

4.2.2 Phase solubility of carbendazim

All the CDs used gave a small increase in carbendazim solubility, while the experiments with carbendazim 50WP were apparently unsuccessful. This result suggests that carbendazim had no or very small potential for complexation with CDs, and other ingredients beside the active carbendazim in commercial carbendazim interfered complex formation. The low K_C values indicated that binding affinity between carbendazim and CDs was fairly weak. The low stability constants gave too weak interaction to improve the solubility (Higuchi and Connors, 1965). Structurally, Carbendazim was unfavorable in forming a stable complex with CDs. Only the benzene ring might be partially penetrated in βCD cavity while the aliphatic part with tertiary amine was easily protonated and the carbonyl group would most probably be situated outside the cavity as suggested by Lee and Lin (1996). This was in agreement with suggestion by Szejtli (1982) that ionizable N-atom containing fragment of molecules was weak as complex-forming partners with CDs. Our result from phase solubility study suggesting difficult complex formation between carbendazim and CD was further supported by the UV absorption spectra in Figure 27 (Appendix 1) which was discussed in 4.1.

4.2.3 Phase solubility study of Methidathion

The methidathion-βCD complex was essentially insoluble in water, the solubility in βCD was linear upto 5 mM for methidathion dissolved was observed, indication of the insoluble complex formed (Higuchi and Connors, 1965). Only a small amount of methidathion remained in the aqueous solution. The diagram of the B_S type was observed (Table 6). G_2 and methyl-βCD did not form precipitation as the aqueous solutions remained clear. Thus βCD showed the lowest solubilising ability among various βCD derivatives used. The formation constant of methyl-βCD was higher than G_2-βCD complex. Enhancement of the solubility of poorly water soluble compounds by G_2 and methyl-βCD was much more marked than by βCD, and that the solubility of the complexes was affected by the side chain. As G_2- and methyl-βCD have almost the same cavity dimension, the K_C values in Table 6 suggested that solubilizing ability and type of phase solubility diagram were affected by the functional groups of side chain. The linear approximation resulted in good estimate of the increased solubility measure of methidathion when complexed with methyl-βCD, whereas both the estimation inclusion complex stability constants for G_2-βCD resulted in a positive deviation from linearity and can there by be classified as A_P-type diagram according to Connors (1987). Although the deviation of the models from the data points beyond 60 mM, could be attributed to higher order complexes. But in the present study, linear approximation was applied to calculate the individual inclusion complex stability constant from the A_P-type diagram by assuming only 1:1 complex was formed.

Phase solubility diagram of methidathion-G_2-βCD shows A_P type which suggested that in the presence of an excess of CD, traces of a 1:2 (guest: CD) complex could be detected. Higuchi and Connor (1965) suggested that the complexes formed contain more than one molecule of CDs. As the CDs concentration increased the contribution complexes increases thus complexes gave a variable stoichiometry ratio of CDs. Guest molecules were located both within the central cavity and between CD molecules and some CD molecules may contain only water molecules (Szejtli, 1987). However, guests may not be included, but bound by hydrogen bonding to one CD hydroxyl, it was only an outer sphere complex (Szejtli, 1982).

4.3 Selection of the best pesticide-CD system

βCD, G_2- and methyl-βCD showed the same tendency in the complex formation properties, except in carbaryl-CDs system (compound the slops of all cases in Table 6). When comparing the inclusion complex forming properties for the three CDs, methyl-βCD was overall found to yield the highest inclusion complex stability constants, followed by the G_2-βCD and βCD, respectively. The higher stability constant observed for methyl- and G_2-βCD could be due to a more flexible structure obtained by the modification in the C-6-OH position of the glucose units in CDs (Madsen, 2000). Furthermore, from the K_C values obtained, there was no clear evidence that G_2-βCD binds better than βCD (small increasing in K_C values comparing with βCD complexes). This suggests that G_2-βCD with the extra glucose unit outside βCD cavity had no significant influence on inclusion capacity but only on the solubility. In contrast with methyl-βCD, K_C values were increased. A probable explanation of the increasing inclusion properties for the branched βCD compared to βCD could be the presence of the external unit which might result in an alteration of the cavity structure and there by an increase in the accessibility and binding of the guest molecule entering the CD cavity. However, no apparent reason can explain why methyl-βCD should form a relatively more stable complex than G_2-βCD. Overall result can be concluded that branched CDs may show large or small increase in K_C values and solubility property which mainly dependent on the guest molecule.

The K_C value, with in a range of 100-1000^{-1} M, shows the possibility of formation of inclusion complex (1:1) which may contribute to the improvement in bioavailability of poorly water-soluble pesticides (Higuchi and Connors, 1965). From the K_C values obtained, the highest was 223.18 M^{-1} which belonged to carbaryl 85WP-methyl-βCD complex. This pair was chosen for further study.

4.4 Determination of the amount of active ingredients in commercial form of carbaryl 85WP

Carbaryl is an insecticide marketed mainly as wettable powder formulation under the name Servin 85WP. This formulation is labeled as containing 85% carbaryl, besides conventional formulating auxiliary substances. In HPLC analysis, we found that the percentage content of carbaryl in carbaryl 85WP were 42%. The lower than expected value may be due to degradation during storage as the dissipated half-life equals to 12-22 days (ARS pesticide properties, 1995).

4.5 Preparation of carbaryl 85WP-methyl-βCD solid complexes

The preparation of solid complex was carried out between carbaryl and methyl-βCD using 1:1, 1:2 and 2:1 guest: host molar ratios. Physical mixing, kneading, freeze-drying and co-precipitation were different methods employed.

In co-precipitation method, there was no precipitate out of the solution. Alisara (1999) suggested that it is better to work with a solution of low concentration to avoid any risk of precipitation of pure CD or pure pesticide when mixing the two solutions. Therefore, we increased the volume to 200 ml or more (500 ml) and mixing for 3 hrs. Still no precipitation was obtained, even at 24 hrs. of mixing. The result was in agreement with the phase solubility diagram which showed the A_L type. The diagram is a straight line with a slope less than 1, which should be ascribed to the formation of a 1:1 complex in solution. For this reason, the co-precipitation method, is generally employed only for preparation of complexes with a B_S type solubility curve (Higuchi and Connors, 1965).

Freeze-dried method has the advantage of faster preparation time than the co-precipitation method. These is no waste product to process and the yield of powder formed is higher with freeze-drying compared to kneading. It is quite possible to scale up. The main disadvantage is that these methods are expensive. In general an amorphous substance is obtained (Alisara *et al.*, 1999).

Kneading method is applicable to guests with poorly water soluble active ingredient. The method was to prepare a paste of guest and CD. The solid dispersion is dried and screened to get a fine powder. This method is very attractive due to many advantages such as the process time is short, simple, inexpensive and solvent free. A low temperature level of water was used which may be useful in our study to avoid hydrolysis of carbaryl 85WP and no waste was produced. It is easy to scale up although the solubility of the product was less than freeze-drying product.

4.6 Investigation of the carbaryl 85 WP-Methyl-βCD complexes

According to 4.4, DSC and FTIR were analyzed only for the 2:1 molar ratio since no precipitation was formed from the 1:1 and 1:2 by co-precipitation method.

4.6.1 Differential scanning calorimetry (DSC)

In some instances, DSC can verify the formation of CDs inclusion complex (Szejtli, 1982). In present study, DSC thermograms of different mixtures were compared with that of free carbaryl and methyl-βCD. The DSC curve for carbaryl exhibits a characteristic endothermic peak at 143.3°C and another broad peak at 202° C which was due to decomposition of carbaryl (Barboto *et al.*, 2000). The methyl-β CD displays a wide endothermic effect in the 60-120°C interval, which is ascribed to the CD hydration (Gines *et al.*, 1999). When thermograms of the mixtures were examined, co-precipitation yield a sharp endothermic peak at 141.5°C close to that of free carbaryl and a large broad peak at 195.7°C which might be decomposed carbaryl or contaminant. This technique thus retained a lot of free carbaryl and could not lead to complex formation. However, thermograms of the kneaded and freeze-dried mixtures were similar, DSC profile was dominated by an endothermic portion at 188-189°C and a small exothermic peak which was not present in free carbaryl and free methyl-βCD profiles which should be referred as the complex product was. The carbaryl peak completely disappeared in these two mixing products. These results could be interpreted on the basis of a solid interaction between both components that reduced the crystallinity of pesticide by complex formation while the sample was prepared [Barboto *et al.* (2000), Gines *et al.* (1999)]. A high peak at 86.5-91.6°C which should be free methyl-βCD retained was also found in both preparations.

Surprisingly, physical mixing resulted in some interactions between methyl-βCD and carbaryl since no free carbaryl was left and a small new peak at 168.8°C was observed. It is quite difficult to explain the absence of endothermic effect on the physical mixture. It is possible that the sample suffered an amorphyzation process during the mixture (Szejtli, 1982), or possibly a complexation of the sample on the DSC container during the heating process (Erden and Gelebi, 1988). The result indicates that kneading, freeze-drying, and perhaps physical mixing leads to posible complex formation between methyl-βCD and carbaryl but other techniques have to be applied to confirm this.

4.6.2 Fourier Transform Infrared Spectrometry (FTIR)

When FTIR was analyzed, the major peak at 1717 cm^{-1} of the C=O stretching of the carbonyl groups was the important characteristics of carbaryl. Methyl-βCD spectrum showed the significant OH bonding at 1637 cm^{-1}. A shift of the carbaryl characteristic peak from 1717 to 1734 cm^{-1} was observed in the freeze-drying mixture, while a shift to 1744 cm^{-1} was detected in both kneading and physical mixtures. The result suggests a modification of electronic environment of C=O group which means inclusion complexes could be formed in solid state when prepared by these methods. However, no shift of this carbonyl peak was found in the co-precipitation mixture. The result from FTIR thus confirmed the observation from DSC.

When compared a shift of the carbonyl peak of carbaryl from FTIR spectra, electronic environment of C=O was more modified by methyl-βCD than by βCD. We reported here the shift from 1717 to 1734 cm^{-1} when free carbaryl was compared with freeze-dried MβCD-carbaryl. Barbato *et al.* (2000) reported the smaller shift from 1713 to 1717 cm^{-1} in the case of freeze-dried βCD carbaryl spectrum. Carbonyl characteristic peak of the two studies was 4 cm^{-1} different which may be attributed to purity of carbaryl compound.

Therefore, in the carbaryl-methyl-βCD inclusion complexes the shift could be ascribed to the interaction between the carbonyl group of the carbaryl molecule and the hydroxyl group of the methyl-βCD. On the basis of these results, it can be concluded that carbaryl could be present in the solid state as an inclusion complex with methyl-βCD even when in physical mixture.

4.7 Characterization of inclusion complex in solid state

Properties of solid inclusion complex between carbaryl 85WP and methyl-β CD were determined. The complexes were prepared by stirring at 20°C, 30°C, 40°C, 50°C and 60°C for 3 hrs prior to freeze-dried and kneaded at 1:1, 1:2 and 2:1 guest: host molar ratio. Properties were compared with free carbaryl 85WP and carbaryl 85WP-dextrin at 3:1 molar ratio. Dissolution, stability, and toxicity were those properties studied.

4.7.1 Dissolution study of inclusion complex

Carbaryl 85WP powder exhibited poor dissolution owing to its hydrophobicity and occurrence of clumping together of the carbaryl particles in the aqueous (International Program on Chemical Safety, 2002). The freeze-dried and kneaded of carbaryl 85WP-methylβCD inclusion complex and carbaryl 85WP-dextrin mixtures exhibited higher and faster dissolution than free carbaryl 85WP. This result can be explained by a local solubilization action of methyl-βCD operating in the microenvironment or the hydrodynamic layer surrounding the carbaryl particles in the early stages of dissolution process. The methyl-βCD dissolved in a short time thus improving the wettability and hence dissolution of the carbaryl particles (Hassan *et al*., 1990). Moreover, during the dissolution tests, the powders of carbaryl 85WP and kneaded mixture were poorly wetted because they floated on the aqueous surface thus hindering dissolution. This poor wettability resulted in low dissolution of free carbaryl 85WP and kneaded mixture. For the carbaryl 85WP-methyl-βCD freeze-dried mixture, the more particles sank to the bottom of the flask and rapidly dissolved in the dissolution aqueous, which indicated that methyl-βCD might reduce the surface tension of the aqueous and facilitated the wetting. These results led to a higher dissolution even in the dextrin. Similar result was reported by Hirayama *et al*.(1988) that DIMEB can decrease surface tension in issosorbride dinitrate. It was evident that the dextrin mixture showed a slight improvement in the solubility rate with respect to the free carbaryl 85WP, due to the dextrin, a linear chain of 23-26 residues of glucose, solubility effect. The dextrin structure is helical which may entrap carbaryl and form a loose complex. However, complex formation with methyl-βCD, a cyclic oligosaccharides of 7 glucose units, was much more effective than with dextrin judging from the significant increase in dissolution property.

A comparison of the freeze-dried products at molar ratio 1:1, 1:2 and 2:1 showed that the average solubility of 2:1 molar ratio of carbaryl 85WP: methyl-βCD was more than the solubility of 1:1 and 1:2. The lower average solubility of 1:2 may be due to the excess amount of methyl-βCD. If one assumed that there was as inclusion formation, when the inclusion formation was completed the excess methyl-β CD which had the higher solubility than carbaryl 85WP will be dissolved and compete with the solubility of carbaryl 85WP inclusion product. Thus reduction of the solubility of carbaryl 85WP was shown. Instead of helping to dissolve carbaryl 85WP initially, methyl-βCD acts as if it formed coating of carbaryl 85WP and prevented carbaryl 85WP from coming into contact with water during the early stage, resulting in the slower rate of dissolution. Also, Figure 19 shows that dissolution value of the 1:1 mixture was higher than the 1:2 in all conditions of preparation except in stirring at 60°C before freeze-dried process. This Figure 19 also shows that carbaryl dissolution increase with the increase in preparation temperature. From the results obtained, factors which determined dissolution properties were: method and condition for preparation type and quantity of CD used.

The solubility profiles of kneaded mixtures were higher and faster than those of the control, free carbaryl 85 WP and dextrin-carbaryl kneaded mixture. It could be stated that kneading method for guest-methyl-βCD complex preparation helps improvement of solubilization characteristics of the guest. Similar results with other guests were previously reported by many researchers [Lin *et al.* (1991), Nozawa and Yamamoto (1989), Uekama *et al.* (1987)]. Overall result was mainly due to improvement of wettability of pesticides powder by changing the surface characteristic. This effect was enhancing in the hydrophilicity and solubility of pesticide.

However, it is not always necessary to prepare true inclusion complexes to improve dissolution of carbaryl 85WP. Because carbaryl-dextrin in this study showed higher solubility and faster dissolution rates than carbaryl 85WP alone, though the increase was less than that of methyl-βCD complex. Thus it is worthy to note that the improvement of carbaryl dissolution by simply kneaded the carbaryl with dextrin, It can be considered as a very interesting tool to moderate the activity and environmental fate of the pesticide, mostly in view of industrial application.

4.7.2 The thermal stability and photostability of inclusion complex

The molecular entrapment of short half-life pesticides had been performed to effectively decrease degradation of carbaryl in solid complexes from under a stress stability study. The stabilization was illustrated by heat at 80°C or exposure to UV light for 3 hrs. Stabilization for along storage (2 weeks) at 40°C was also checked. The comparison of the free, dextrin and methyl-βCD complexed forms of the carbaryl 85WP was performed to investigate and the extent of stabilization achieved by molecular entrapment.

The result demonstrated that methyl-βCD could help stabilize carbaryl against decomposition by heat at 80°C and by UV light while dextrin could not (Figure 20 and 23). Not much difference was found when the complex at different molar ratios were used. The result that methyl-βCD could effectively stabilize carbaryl in long storage time at 40°C was promising. The carbaryl content of samples was followed as a function of time. The accelerated storage test clearly proved the powerful stabilizing effect of molecular encapsulation. As the loss of carbaryl from the methyl-βCD complexes was found to be less than 20-25% in all cases (exposure to heat at 80°C or UV light for 3 hrs. and storage at 40°C for 14 days) these formulations may be considered as basis for suggested carbaryl formulations of extended self life.

As the thermostability and photostability indicate the methyl-βCD complexation of carbaryl 85WP results in increased physical stability. This enables the development of slow-release, long-term storage and high stability pesticide formulations which will be more acceptable formulations in particular for agriculture uses.

4.7.3 Acute toxicity test on Brine shrimp (Artemia salina)

When toxicity was concerned, Brine shrimp (*Artemia Salina* Linnaeus) cytotoxicity test was performed. This test was often used to screen biological activity of active substances since the Brine shrimp has similar enzyme responsive systems to mammals (Solis *et al*., 1993). The results suggest that carbaryl-methyl-βCD complex, at the 2:1 molar ratio exerted a little less cytotoxicity than the parent compound, carbaryl.

The advantage of carbaryl 85WP-methyl-βCD complex does not only involve the environment issue, but also involves the ecological effects to nontarget insects including bees and beneficial insects. When carbaryl is used to kill insects that cause agricultural problems, other beneficial insects are also killed by exposure to carbaryl which was absorbed though skin. The toxicity arose by contact with carbaryl 85WP-methyl-βCD are considerably decreased, because the complex is not lipophilic and it is not absorbed by the lipophilic regions on the surface of the insect body. These complexes can thus be toxic against only herbivorous insects which destroyed plants. In certain case, following cyclodextrin complexation, a partial or total loss of toxicity had been reported (Yamamoto and Katsuda, 1980). Furthermore, the methyl-βCD complexes of carbaryl should be much safer to handle and to use.

The overall results indicated that molecular inclusion of carbaryl with methyl-βCD using the freeze-drying technique led to formation of solid complex with favorable properties. The dissolution of carbaryl was enhanced, this effect would improve bioavailability of the insecticide for the insect (Szejtli, 1988). Improvement of hydrophilicity could reduce residuals left on plants. However, the complex will be easily washed by rain, thus increases the environmental distribution of the pesticide. But the half life of carbaryl in water is two times shorter than that in the field (i.e. 6-7 days in water, while 12-22 days in field) (International Program on Chemical Safety, 2002). Higher stability ensured less decomposition and reduced the amount used in the formulation and also the dose in plant treatment. A small percentage decrease in toxicity following cyclodextrin complexation observed in this work could also be considered as environmental benefits. The present study, however, cannot pinpoint the percentage of complex formation in solid powder. The complex product may not be worth economic, because the price of 1 kg methyl-βCD already today is higher than the price of 1 kg synthetic carbaryl active ingredient and in the future this will be changed in favor of the CD. Nowadays, the commercial βCD or non-cyclic dextrin resulted in more rational application for economic purpose.