Chapter III

Results

3.1 Determination of spectrophotometric properties of pesticides

3.1.1 The maximum absorption of pesticides and pesticide-βCD complexes

The UV spectra of free pesticides and pesticide-βCD complexes were compared. The samples were prepared in deionized water and analyzed over the absorption spectrum in the range of 200-400 nm. Spectrum of βCD was determined as a control. βCD should give no UV spectra due to its structure, but when 5 mM solution was scanned, very small peak of contaminants at 359 and 384 nm were observed (Figure 25, Appendix 1). For carbaryl, the maximum absorption was observed at 276 nm, with a minor peak at around 380 nm. Carbaryl 85WP showed similar spectrum to carbaryl with the addition of peaks of interferences around 350-400 nm (Figure 26, Appendix 1). The spectra of carbendazim and carbendazim 50WP exhibited maximum absorption at the same λ of 284 nm (Figure 27-28, Appendix 1). When methidathion was examined, the peak at 210 nm was observed (Figure 29, Appendix 1). No spectral shift of all pesticides studied were observed when pesticides were incubated in the presence of 5 mM βCD. However, the increase in absorption at λmax was found in the case of carbaryl, carbaryl 85WP and methidathion.

3.1.2 Calibration curve of pesticides

The calibration curves of pesticides were plotted as shown in Appendix 2. Slope and coefficient of determination (R^2) were calculated from linear regression analysis.

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

Solubility study was used to compare the ability of complex formation between pesticides and CDs. The phase solubility diagrams were examined with three derivatives of β-CDs in water at 30°C, shaking speed of 200 rpm for 24 hours for carbaryl, carbaryl 85 WP, carbendazim, carbendazim 50 WP and methidathion. The method was as described in Methods section 2. The data were analyzed by plotting the molarity of pesticide found in solution against the molarity of CD added.

3.2.1 Phase solubility of carbaryl

Phase solubility diagrams of pure carbaryl in the presence of CDs are shown in Figures 5 and 6. In the absence of CDs, the concentration of carbaryl found in solution was in the range of 0.25-0.38 mmol/l, which is the solubility of carbaryl in water at 30 °C.

The solubility of carbaryl increased as increasing concentrations of G_2-βCD and methyl-β-CD (Figure 5b and c). An average of 14 fold increase in solubility was observed at 100 mM concentration of βCD derivatives. The pattern of phase solubility diagram obtained was an A_L type which indicates that the complex formed was soluble and did not form a precipitate regardless of the amount of CDs added.

In contrast, βCD showed an A_N type solubility diagram (Figure 5a). In this pattern, βCD increased the total solubility of carbaryl to the maximum concentration, which reached the solubility limit of the complex formed at about 2.34 mmol/l when concentration of βCD used was at 30 mM or above. Thus, the solubility of carbaryl was increased 6.2 folds over free carbaryl. Upon the initial addition of βCD, the solubility of carbaryl rose linearly owing to complexation. At the plateau region, the

system was saturated with respect to the complex and to the carbaryl itself. Further addition of βCD resulted in the continuous formation of the complex which then precipitated out from the saturated solution. At this point, the system still contained excess solid carbaryl, therefore, total solubility of carbaryl in solution remained constant.

The formation constant (K_c) of 1:1 complex was calculated from the initial linear portion of the phase solubility diagram according to the equation of Higuchi and Connors, 1985 as mentioned in Methods. The slope of the initial straight line, the intercept, the formation constant (K_c) and type of solubility diagram were summarized in Table 5. The observed rate constant of the formation of the complex (K_c) calculated for βCD, G_2-βCD and methyl-βCD were 210.53, 163.45 and 205.87 M^{-1}, respectively. From the data obtained, it can be concluded that CDs increased carbaryl solubility in aqueous solution. The ability of βCD and methyl-βCD was about the same for solubility enhancement.

When carbaryl 85 WP was used in place of standard carbaryl, the result was shown in Figures 7 and 8. In the absence of CDs, concentration of carbaryl 85 WP in water was 0.54-0.58 mmol/l. All complexes between carbaryl 85 WP and βCD derivatives displayed the A_L type solubility diagram. The apparent stability constant of the complexes at 30°C (Table 6) were calculated to be 120.92,130.79 and 223.18 M^{-1} when formed by adding βCD, G_2-βCD and methyl-βCD, respectively. Methyl-βCD at 100 mM concentration increased solubility by 20 folds and was thus the best type of βCDs for complexing with carbaryl 85 WP. When compared the result with pure carbaryl in the same conditions, carbaryl 85 WP was more soluble than pure carbaryl in each various CDs. For example, a 100 mM methyl-βCD solution

increased the aqueous solubility of pure carbaryl about 12.9-fold, while 18.4-fold increase was observed with carbaryl 85 WP.

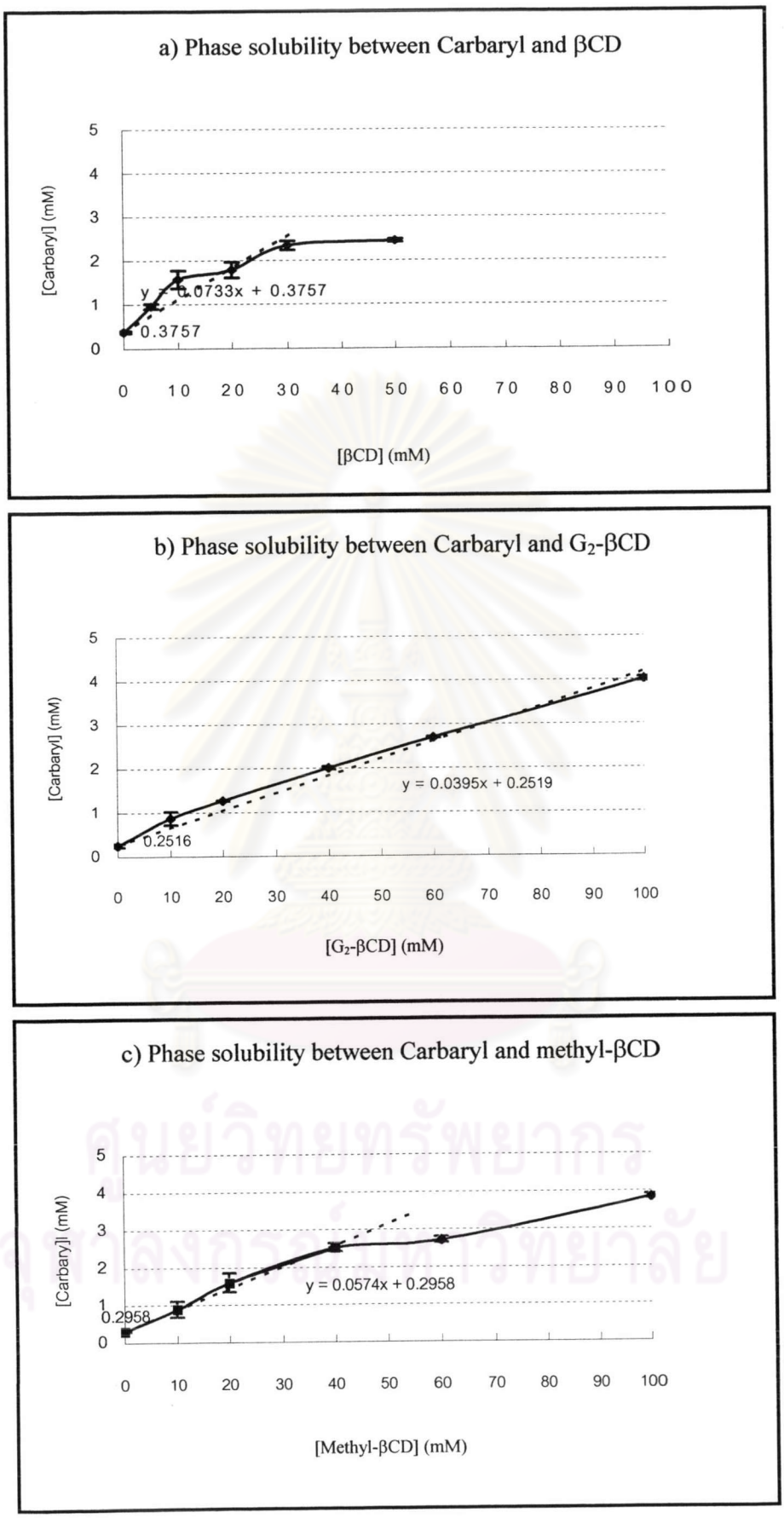

Figure 5. Phase solubility diagrams between carbaryl and CDs in water at 30°C

a) carbaryl and βCD, b) carbaryl and G_2-βCD and c) carbaryl and methyl-βCD

* Dotted line was estimated from linear regression performed by Excel program

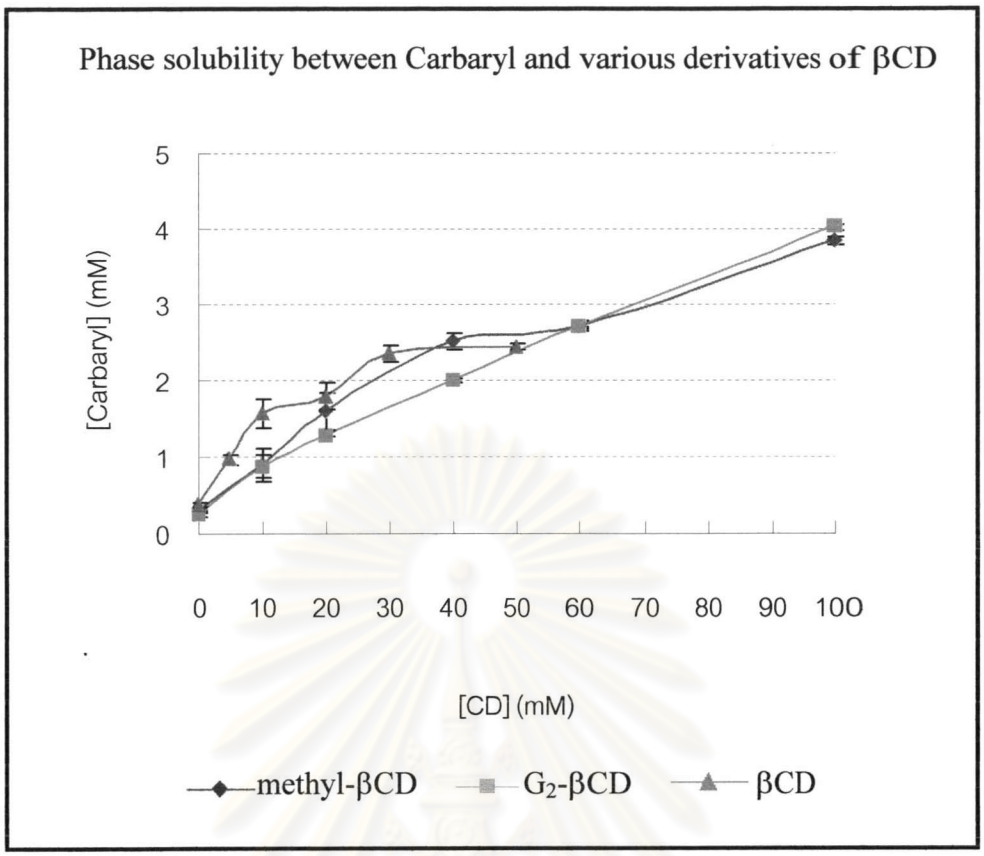

Figure 6. Comparison of phase solubility diagrams between carbaryl and CDs in water at 30°C

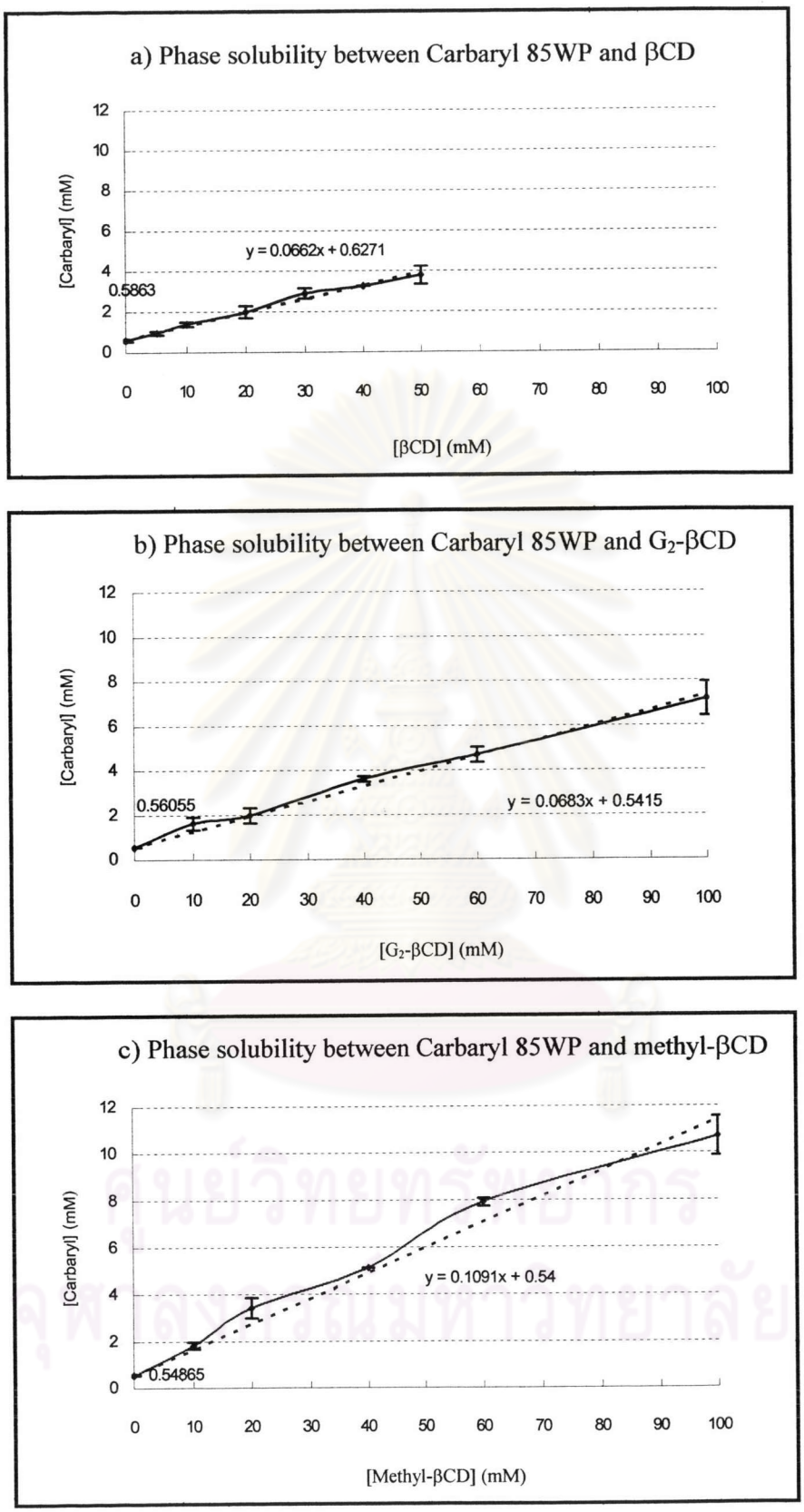

Figure 7. Phase solubility diagrams between carbaryl 85 WP and CDs in water at 30°C

a) carbaryl 85WP and βCD, b) carbaryl 85WP and G_2 βCD and c) carbaryl 85WP and methyl-βCD

* Dotted line was estimated from linear regression performed by Excel program

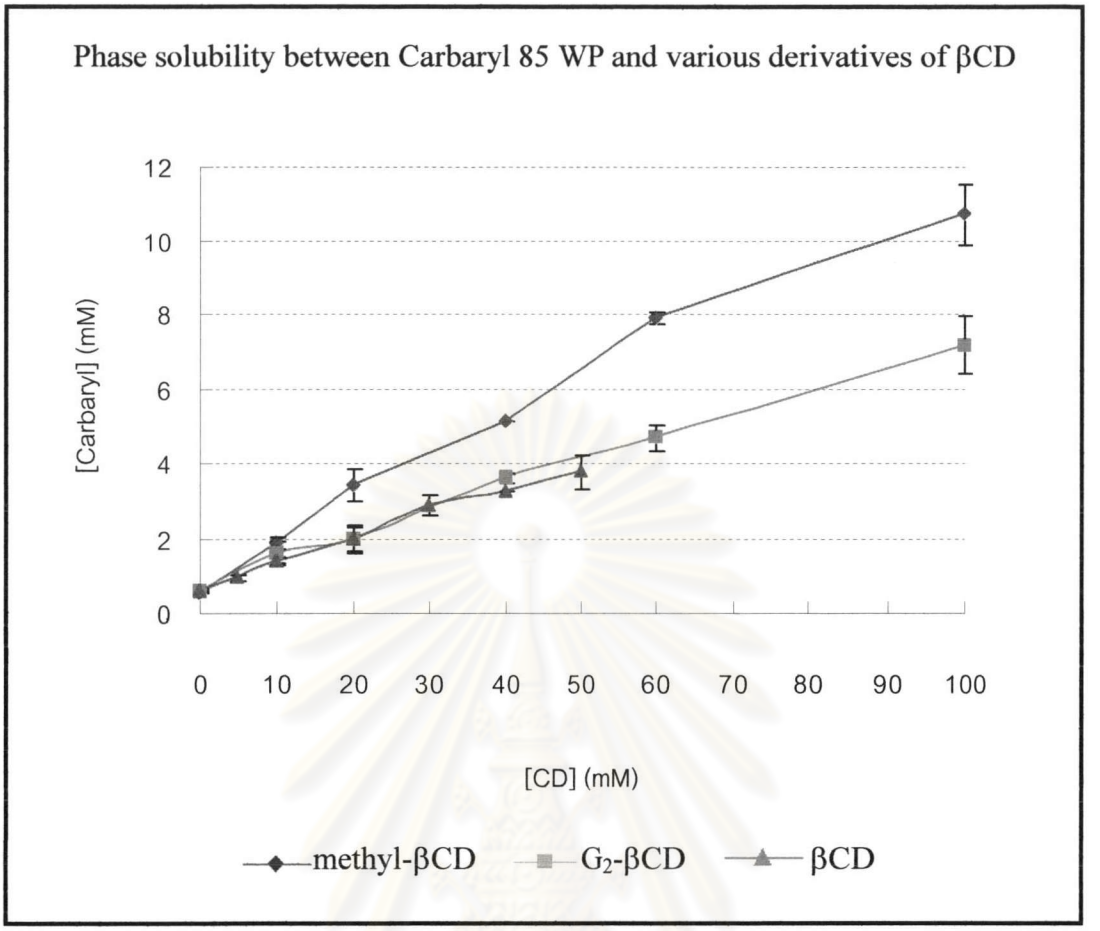

Figure 8. Comparison of phase solubility diagrams between carbaryl 85 WP and CDs in water at 30°C

3.2.2 Phase solubility of carbendazim

In the absence of CD, solubility of carbendazim in aqueous solution at 30°C was in the range of 0.12-0.14 mmol/l (Figure 9). Phase solubility diagrams of pure carbendazim shown in Figures 9 and 10 can be classified as the type A_N when complexed with βCD or G_2-βCD and as type A_L when complexed with methyl-βCD. Solubility increase was the highest with methyl-βCD, being approximately 3 folds at 100 mM concentration. The formation constants between carbendazim-βCD, G_2-βCD and methyl-βCD were 15.35,16.83 and 17.74 M^{-1}, respectively (Table 6). Stability constants were very low and no significant difference among various CDs used. When carbendazim 50 WP was used in place of pure carbendazim, the result was shown in Figures 11 and 12. The solubility of carbendazim 50 WP in various CD solutions did not significantly increase when either of the three CDs was used. This result suggests that complexation between carbendazim 50 WP and CDs in the conditions used was a failure.

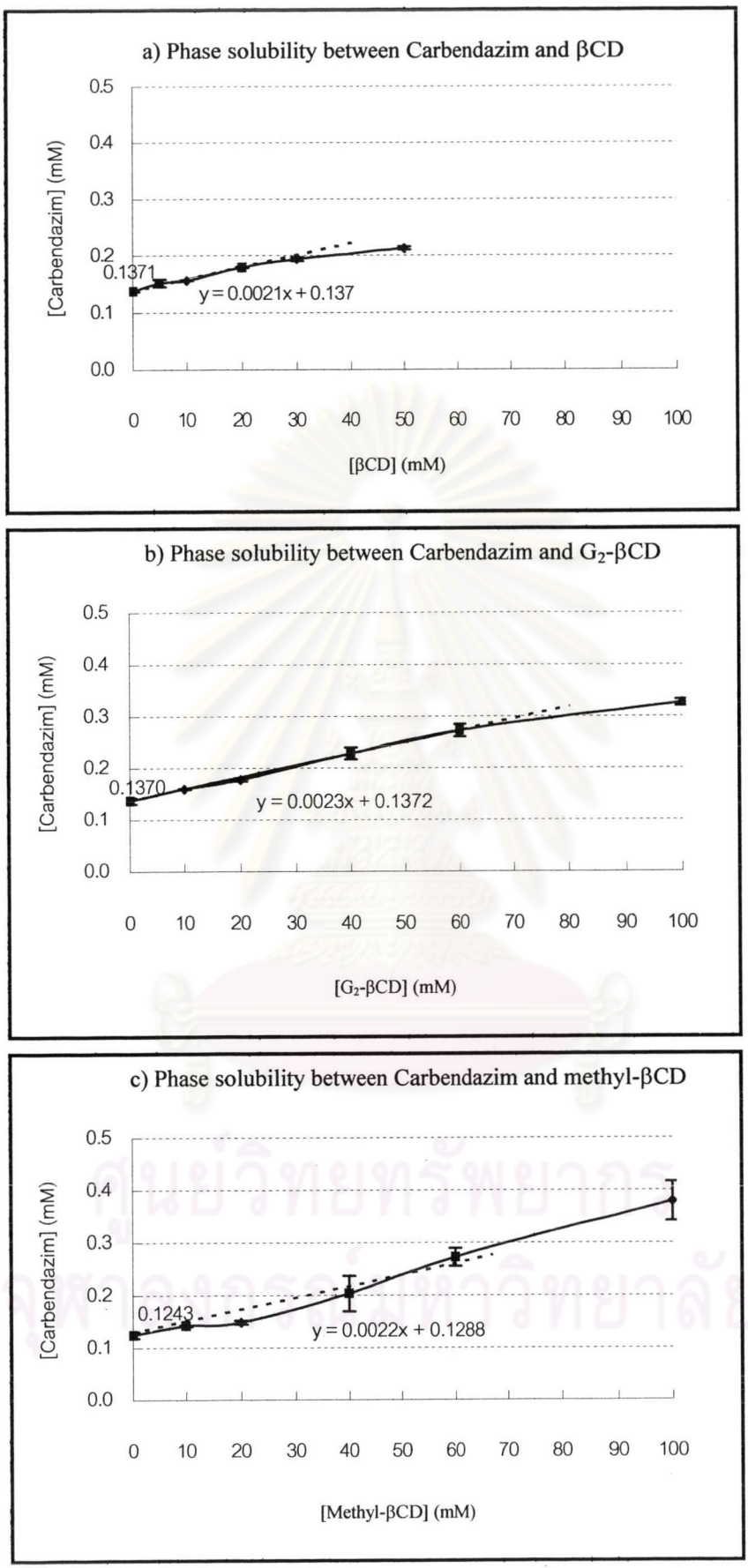

Figure 9. Phase solubility diagrams between carbendazim and CDs in water at 30°C

a) carbendazim and βCD, b) carbendazim and G_2-βCD and c) carbendazim and methyl-βCD

* Dotted line was estimated from linear regression performed by Excel program

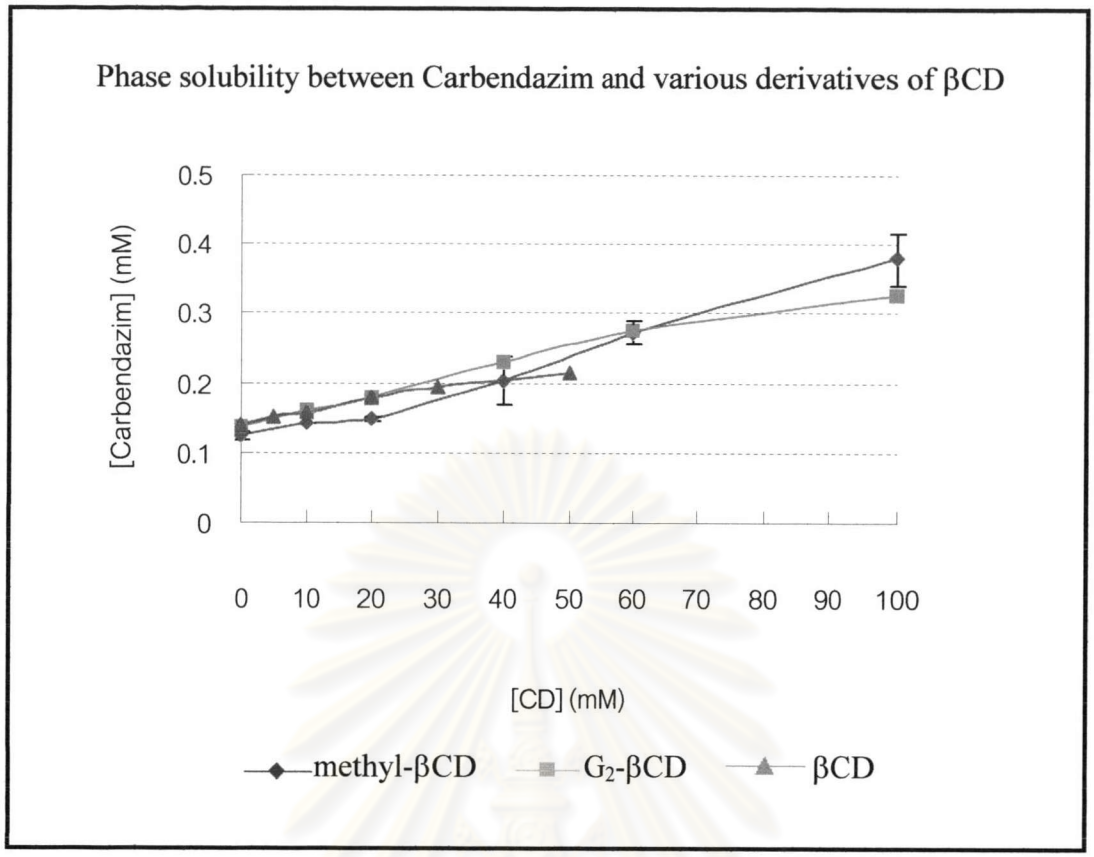

Figure 10. Comparison of phase solubility diagrams between carbendazim and CDs in water at 30°C

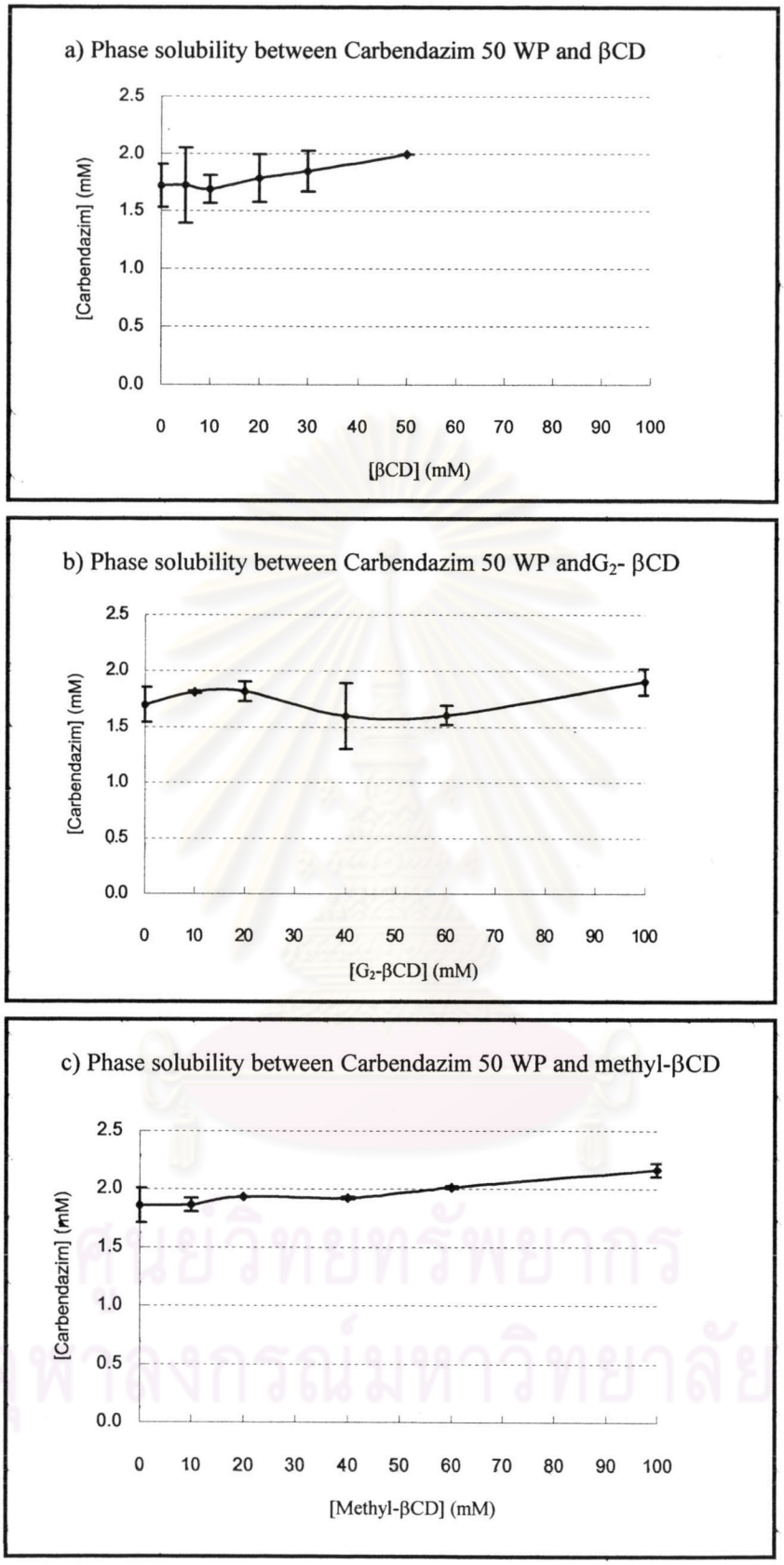

Figure 11. Phase solubility diagrams between carbendazim 50 WP and CDs in water at 30°C

a) carbendazim 50 WP and βCD, b) carbendazim 50 WP and G_2-βCD and c) carbendazim 50 WP and methyl-βCD

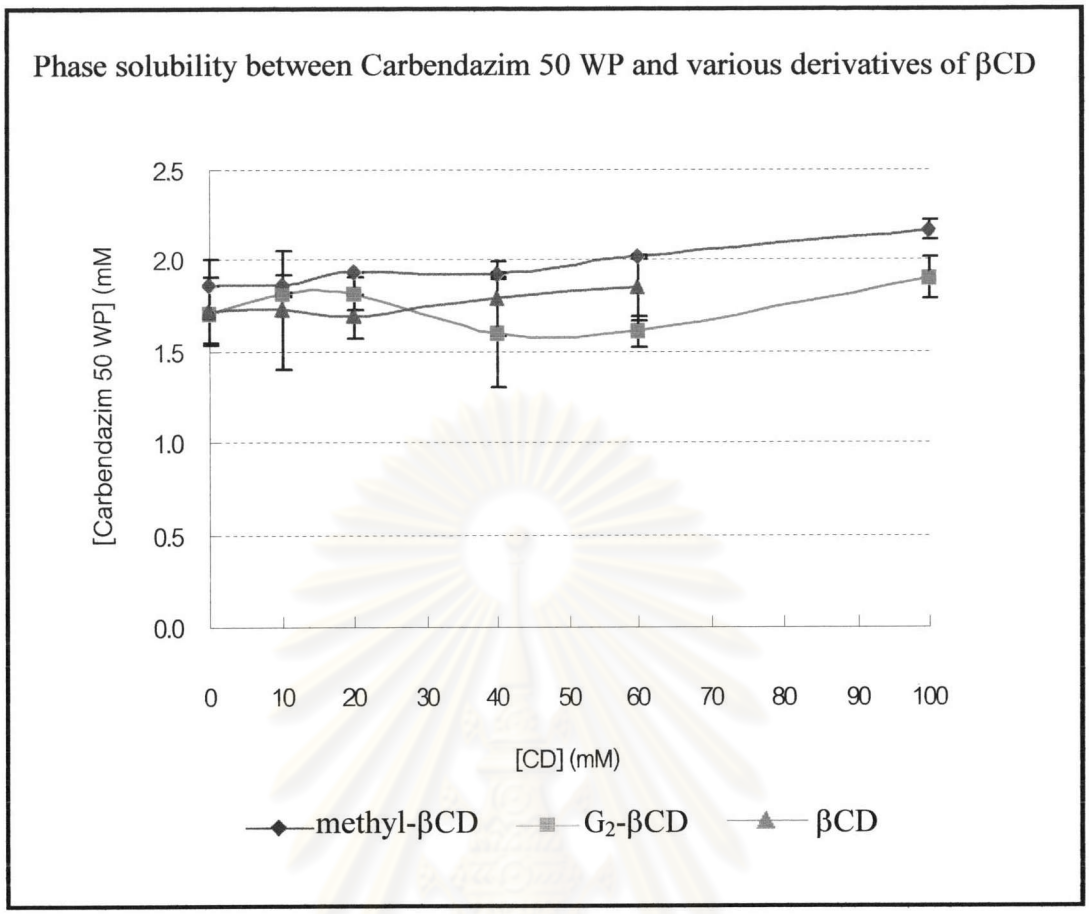

Figure 12. Comparison of phase solubility diagrams between carbendazim 50 WP and CDs in water at 30°C

3.2.3 Phase solubility study of methidathion

Phase solubility diagrams of methidathion in the presence of cyclodextrins are presented in Figures 13 and 14. In the absence of CDs, the concentration of methidathion found in solution was 0.88 mmol/l. When complexing with βCD, an insoluble microcrystalline complex was formed at high βCD concentrations. The pattern in Figure 13a could be classified as type B_S. The diagram shows an initial rise in the range of 0-5 mM βCD and a decrease in methidathion solubility when 5-20 mM of βCD was added which was due to continued formation and precipitation of the complex. Total solubility of methidathion remained constant when βCD was further added. The apparent formation constant (K_c) for such a complex could be determined from the initial straight-line portion. In this case, a K_c value of 157.06 M^{-1} was listed in Table 5.

In the case of methidathion-G_2-βCD (Figure 13b), the diagram was a linear with a curvature increase in the line at high cyclodextrins which indicated the Ap type. The increase in solubility was of higher order in the range of 60-100 mM than 0-60 mM G_2-βCD. If the 1:1 complex formation was assumed, the stability constant was found to be 161.47 M^{-1}.

Methidathion-methyl-βCD complexation could be classified as type A_L (Figure 13c) with a stability constant (K_C) of 200.63 M^{-1}. Solubility increase was about 20 folds at 100 mM methyl-βCD which was the best for all derivatives tested.

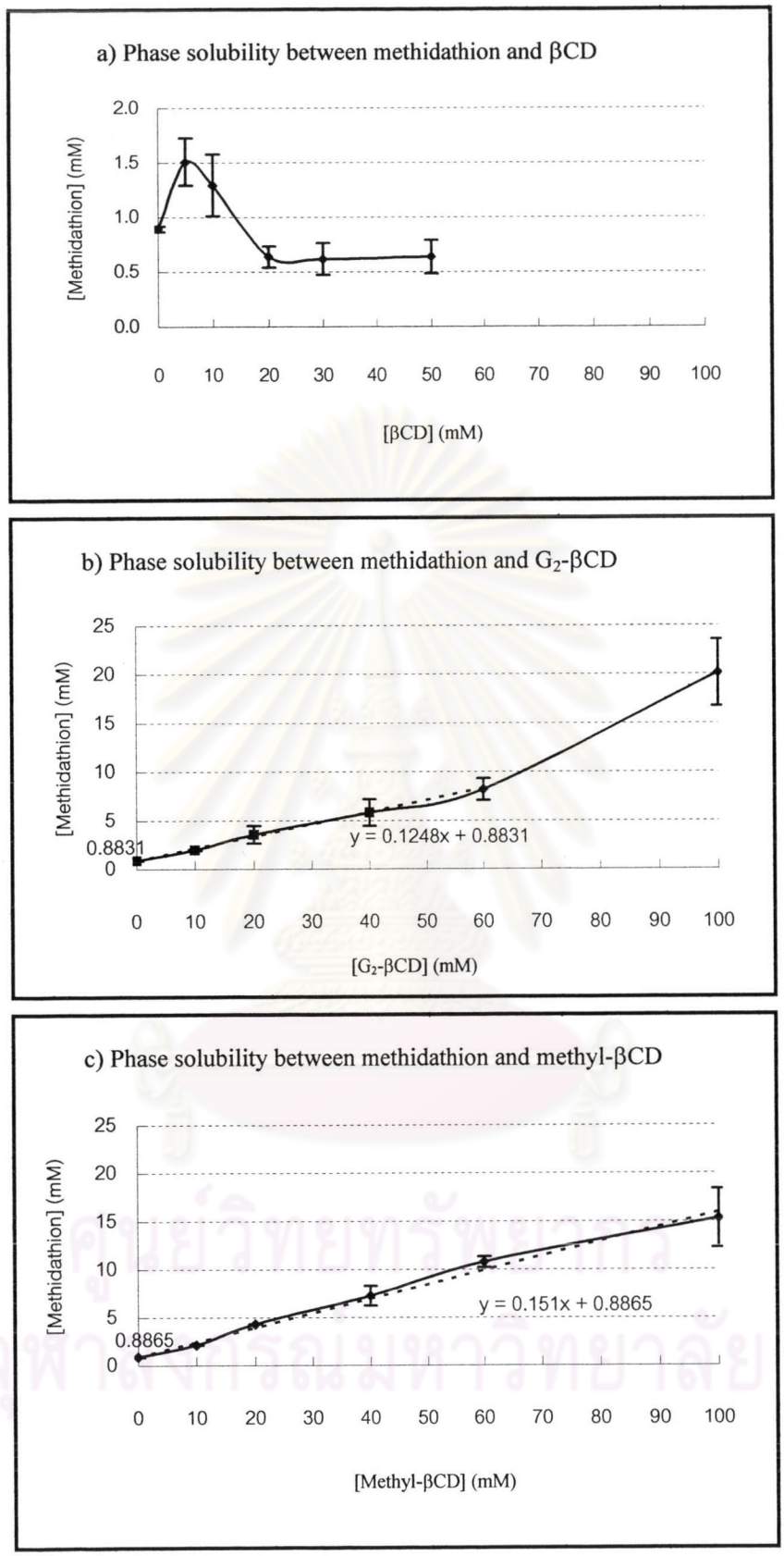

Figure 13. Phase solubility diagrams between methidathion and various CDs in water at 30°C

a) methidathion and βCD, b) methidathion and G_2-βCD and c) methidathion and methyl-βCD

* Dotted line was estimated from linear regression performed by Excel program

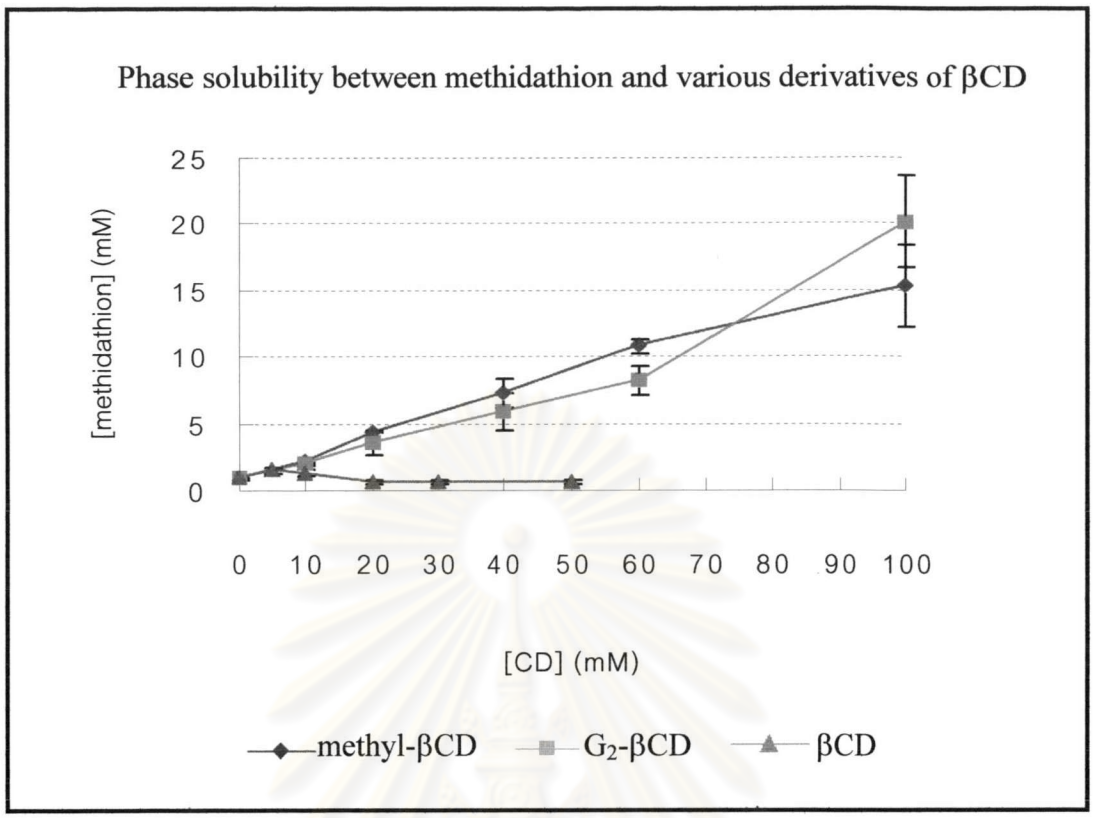

Figure 14. Comparison of phase solubility diagrams between methidathion and various CDs in water at 30°C

3.3 Selection of the best types of pesticide and cyclodextrin in soluble complex formation

The overall result from phase solubility studies suggested that carbaryl 85 WP was the most suitable pesticide among those tested in forming inclusion complex with CDs. All types of βCD gave the same A_L patterns (Figure 7) when complexing with carbaryl 85 WP, while methyl-βCD gave the most favorable effect on aqueous solubility of carbaryl 85 WP. Up to 20 folds increase in solubility was observed at 100 mM concentration of methyl-βCD. The highest Kc value of 223.18 M^{-1} was obtained for carbaryl 85 WP-methyl-βCD as shown in Table 5.

Further experiments then used carbaryl 85WP and methyl-βCD for formation of the complex in solid form and characterization of the complex.

Table 5. The data on phase solubility diagram of various types of pesticide in the presence of βCD, G_2-βCD and methyl-βCD

Type of pesticide	CD	slope	intercept	K_C (M^{-1})	Type of solubility diagram
Carbaryl	βCD	0.0733	0.3757	210.53	A_N
	G_2-βCD	0.0395	0.2516	163.45	A_L
	Methyl-βCD	0.0574	0.2958	205.87	A_L
Carbaryl 85 WP	βCD	0.0662	0.5863	120.92	A_L
	G_2-βCD	0.0683	0.5605	130.79	A_L
	Methyl-βCD	0.1091	0.5487	223.18	A_L
Carbendazim	βCD	0.0021	0.1371	15.35	A_N
	G_2-βCD	0.0023	0.1370	16.83	A_N
	Methyl-βCD	0.0022	0.1243	17.74	A_L
Carbendazim 50 WP	βCD	-	-	-	-
	G_2-βCD	-	-	-	-
	Methyl-βCD	-	-	-	-
Methidathion	βCD	0.123	0.8930	157.06	B_S
	G_2-βCD	0.124	0.8831	161.47	A_P
	Methyl-βCD	0.151	0.8865	200.63	A_L

- , no change in solubility was observed when increasing concentration of CD

3.4 Determination of the amount of active ingredient in commercial form of carbaryl 85WP

This experiment was to determine carbaryl content in carbaryl 85WP, the commercial form of carbaryl used. The HPLC chromatogram of standard carbaryl was performed and the result was shown in Appendix 6. The retention time of the carbaryl peak was around 5.83 minutes. The carbaryl standard curve was plotted between standard carbaryl concentration and peak area. The equation derived for the carbaryl standard curve was shown in Figure 32 in Appendix 6. When HPLC profile from the carbaryl 85WP was determined, the chromatogram of carbaryl 85WP gave a distinct peak at the same retention time as carbaryl (Appendix 7). From the peak area of carbaryl 85WP, carbaryl content could be calculated from the equation derived from standard curve. It was found that carbaryl 85WP had 42% carbaryl content (Appendix 7).

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

The solid complex of carbaryl 85WP-methyl-βCD was formed by various methods. The appearance of carbaryl 85 WP is grey, odorless, fine powder. While methyl-βCD is white crystalline powder. In co-precipitation method, the solid complex was prepared by stirring the solution containing carbaryl guest and methyl-βCD host at 30°C for 3 hours (Method section). The solid complex with 2:1 guest: host ratio was precipitated as grey microcrystalline powder, then it was filtered and dried at room temperature for 1 day. On the contrary, when prepared the solid complex with the ratio of 1:1 and 1:2, there was no precipitate even after 1 day. This result suggests that co-precipitation method was failed for preparation of complexes with the 1:1 and 1:2 ratios. The soluble part of the 1:2 ratio was then subjected to freeze-drying, this product was called "co-precipitation freeze-dried mixture".

For the freeze-drying and kneading, the products were obtained for all three ratios of guest: host. In the case of freeze-drying, the products were white fluffy and less dense. For kneaded mixtures, they were easy to prepare. During preparation of kneaded mixtures, the kneaded products were smooth, grey homogeneous paste. Finally the grey sticky viscous mass product was obtained and then the product was dried and sieved. The kneaded mixture was finer and denser.

In order to confirm solid complex formation between carbaryl 85 WP and methyl-βCD, only the products of 2:1 carbaryl 85WP to methyl-βCD ratio prepared by different methods was subjected to DSC and FTIR analysis. Complexes formed at different guest: host ratios by freeze-dried and kneaded methods were investigated by comparison of dissolution and stability properties to determine the best method and/or condition for complex formation.

3.6 Investigation of the carbaryl 85 WP-methyl-βCD complexes

3.6.1 Differential scanning calorimetry (DSC)

Thermograms of carbaryl 85WP, methyl-β-CD, physical mixture, kneading mixture, co-precipitation mixture and freeze-dried mixture are shown in Figure 15. The thermogram of free carbaryl gave the characteristic melting endothermic peak at 143.3 °C and another broad peak at 202° C which could be referred to decomposition of carbaryl or contaminants in the commercial preparation. Methyl-β-CD had no defined peak for melting point, but formed broad endothermic peak around 87.3 °C which could be referred to the loss of water or dehydration process. When examined the thermograms of the complex, the pattern which gave

different peak(s) from those of free carbaryl and methyl-β-CD was interesting since a new peak indicated a product which should be the complex formed.

When the thermograms of the complexes prepared by different methods were compared, the co-precipitation mixture and the physical mixture yielded different pattern from the others. In the case of co-precipitation (Figure 15 d), a sharp endothermic peak at 141.5°C which was closed to endothermic point of carbaryl was observed together with a large broad peak at 195.7 °C. This technique thus retained a lot of free carbaryl. The second peak at 195.7°C might then be due to decomposed carbaryl. Co-precipitation then could not yield complex formation. FTIR will be further performed to find more information. For thermograms of the physical mixture (Figure 15e), the pattern showed prominent peaks at 74.2 °C which should be of methyl-βCD and the small endothermic peak at 168.8°C. It indicated that small amount of a product occurred while more free methyl-βCD was left in the mixture.

DSC thermograms of the complexes prepared by kneading, freeze-drying and co-precipitation-freeze-drying were similar. A product of an endothermic peak at 188-189°C was observed, together with a higher peak at 86.5-91.6°C which should be free methyl-βCD left. No free carbaryl was left in these preparations. The result of DSC studies suggested that freeze-drying and kneading should be the best method of complex preparation since both gave a peak of significant height at 188-189 °C which should be referred as the inclusion complex formed, while no free carbaryl was left.

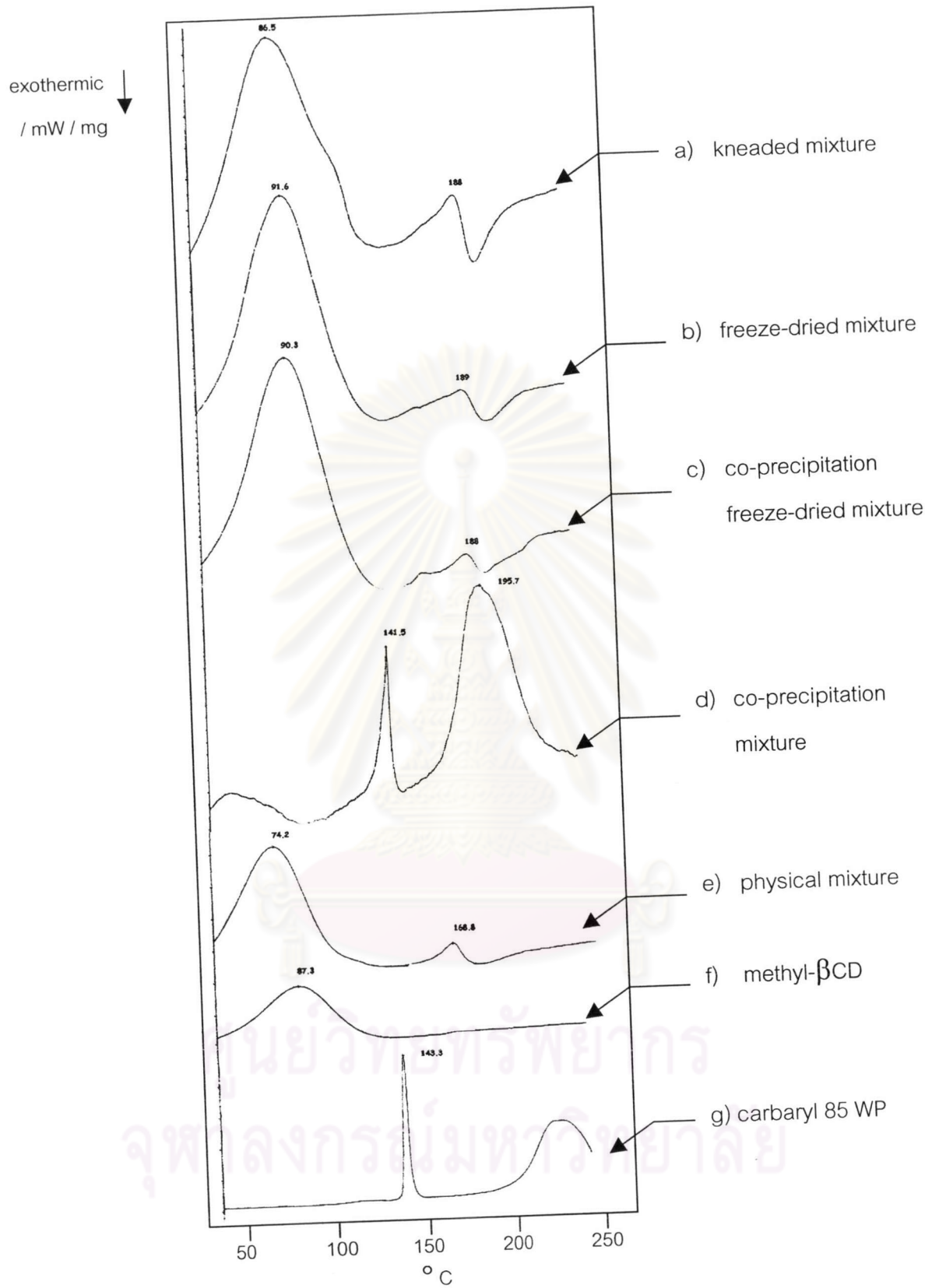

Figure 15. DSC thermograms of carbaryl and carbaryl complex: a) kneaded mixture, b) freeze-dried mixture, c) co-precipitation freeze-dried mixture, d) co-precipitation mixture, e) physical mixture, f) methyl-βCD and g) carbaryl 85 WP. All mixtures were of the 2:1 molar ratio of carbaryl 85 WP: MβCD.

3.6.2 Fourier Transform Infrared Spectrometry (FTIR)

Inclusion compounds of carbaryl with methyl-β-CD were characterized by FTIR to obtain the information on the vibrational variation of functional groups. The IR spectra of free carbaryl 85 WP, free methyl-β-CD and the mixtures obtained by different methods of preparation are shown in Figures 16 and 17.

Methyl-βCD contained many hydroxyl groups, the FTIR spectrum showed strong broad peak of OH stretching about 3300-3600 cm^{-1} and OH bending at 1637 cm^{-1}. Other characteristics were shown in Table 6. The IR spectrum of carbaryl 85 WP showed the major peak at 1717 cm^{-1} indicated the C---O stretching of the carbonyl group. The peaks at 1542 and 1507 cm^{-1} were referred to phenyl group and the peak at 1651 cm^{-1} was due to C---C stretching of aromatic ring. Broad peaks at 3430, 3322 and 3063 cm^{-1} were resulted from NH stretching (Table 7).

IR spectra showed a shift of the major peak of carbaryl from 1717 to 1734 and 1733 cm^{-1} in freeze-dried mixture and co-precipitation freeze-dried mixture but to 1744 cm^{-1} in physical mixture and kneading mixture (Figure 17). The result suggested a modification of electronic environment of C=O group which means inclusion complexes could be formed in solid state when prepared by these methods. In the case of co-precipitation mixture, the C---O stretching peak was nearly the same as that of free carbaryl (1717 cm^{-1}). The lack of C=O shift of carbaryl in the spectrum of the co-precipitation mixture confirmed that inclusion complex was not formed in this system.

From spectra of the mixture prepared by different methods as shown in Figure 17, the assignment of each peak in each spectrum was suggested and summarized in Tables 8-12. Only the spectra of the co-precipitation mixture showed no involvement of functional groups of cyclodextrin (Table 10) which confirmed no complex formation was achieved by this method of preparation.

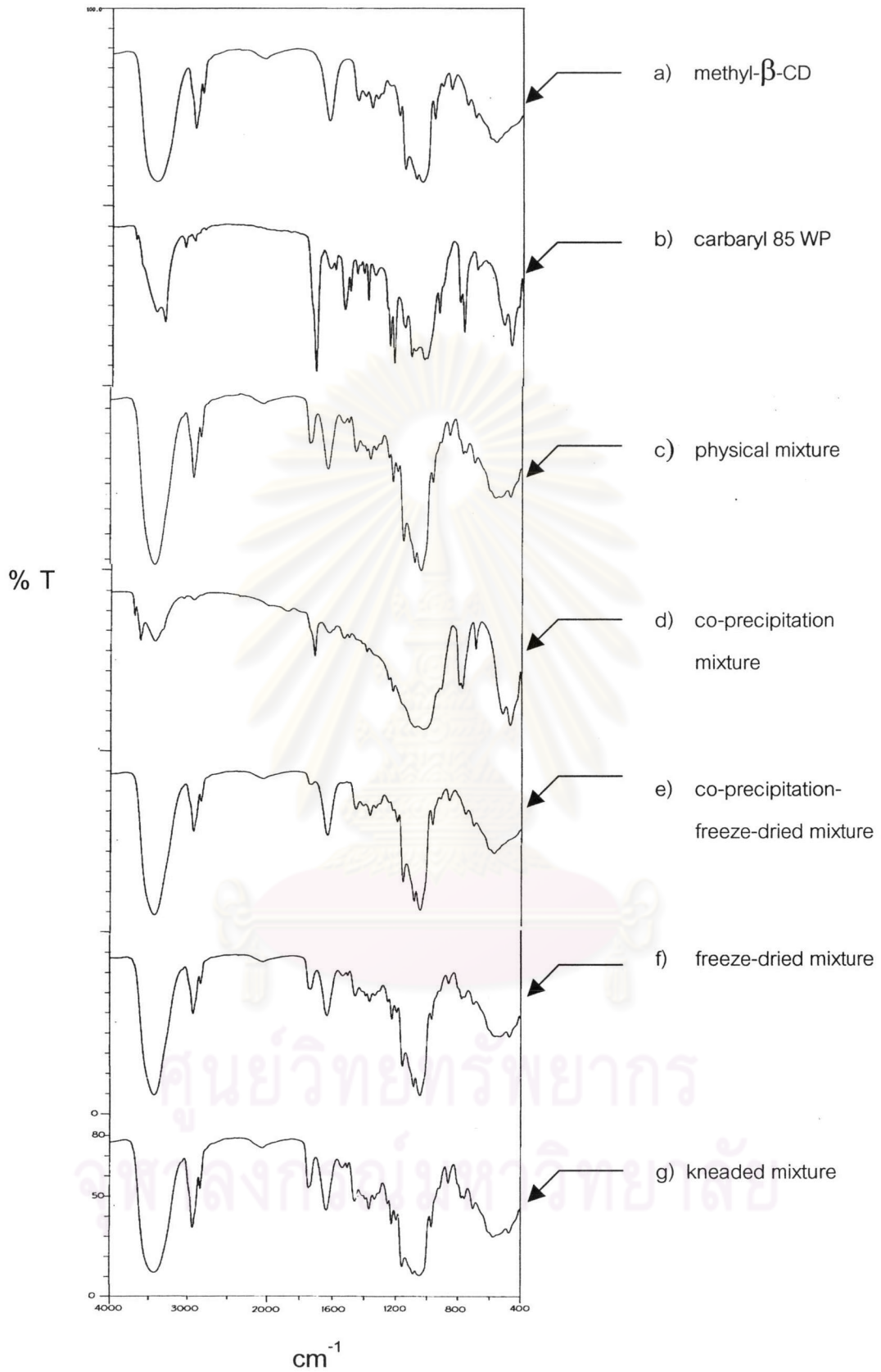

Figure 16. The FTIR spectra of carbaryl and carbaryl complex: a) methyl-βCD, b) carbaryl 85 WP, c) physical-mixture, d) co-precipitation mixture, e) co-precipitation freeze-dried mixture, f) freeze-dried mixture and g) kneaded mixture in the range of 400-4000 cm^{-1}. All mixture were of the 2:1 molar ratio of carbaryl 85 WP: MβCD.

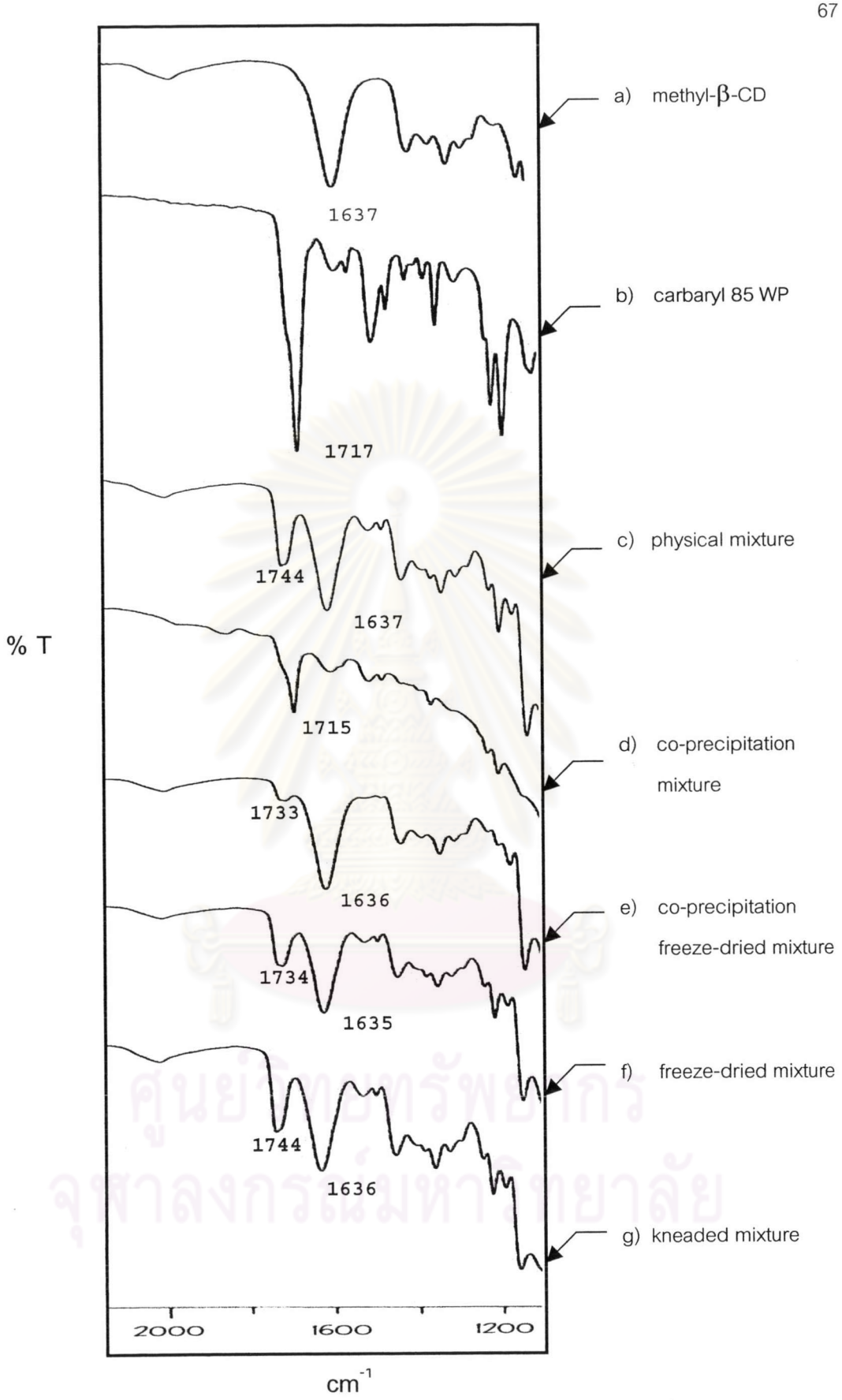

Figure 17. The FTIR spectra of carbaryl and carbaryl complex: a) methyl-βCD, b) carbaryl 85 WP, c) physical-mixture, d) co-precipitation mixture, e) co-precipitation freeze-dried mixture, f) freeze-dried mixture and g) kneaded mixture in the range of 1100-2400 cm^{-1}. All mixture were of the 2:1 molar ratio of carbaryl 85 WP: MβCD.

Table 6. FTIR data of methyl-βCD

Wave number (cm^{-1})	Assignment
3300-3600	Broad –OH stretching
2933	-CH stretching
1637	-OH bending
1457,1410	$-CH_2$
1368,1333	$-CH_3$
1196,1159	C-O stretching

Table 7. FTIR data of carbaryl

Wave number (cm^{-1})	Assignment
3696	Ph-OH
3430,3322,3063	-NH
2945	ph-H,CH
2945,2814,1419	COOH
1717	C---O
1717,1631	RCONHR
1651	C----C (cyclic)
1542,1507	Phenyl
1462,1419,1391,1346	CH_2,CH_3

Table 8. FTIR data of methyl-βCD-carbaryl inclusion compound prepared by physical mixing method

Wave number (cm^{-1})	Assignment
3300-3600	Broad –OH stretching (CD)
2933	-CH stretching (CD)
1744 *	C---O
1744,1631	C---O , RCONHR
1637	-OH bending (CD)
1539,1507	Phenyl

Table 9. FTIR data of methyl-βCD-carbaryl inclusion compound prepared by co-precipitation method

Wave number (cm^{-1})	Assignment
3696,3620*	Ph-OH
3429	NH, CH (Ph-H)
2941	Ph-H,CH
1878*	C---O,C-H (phenyl)
1715 *	C---O
1629	RCONHR
1537,1506	Phenyl

Table 10. FTIR data of methyl-βCD-carbaryl inclusion compound prepared by co-precipitation freeze-dried method

Wave number (cm^{-1})	Assignment
3300-3600	Broad –OH stretching (CD)
2934,2839,2057	-CH stretching (CD)
1733 *	C---O
1733,1636	C---O , RCONHR
1636	-OH bending (CD)
1457,1410	$-CH_2$ (CD)
1368,1333	$-CH_3$ (CD)
1196,1150	C-O stretching (CD)

Table 11. FTIR data of methyl-βCD-carbaryl inclusion compound prepared by freeze dried method

Wave number (cm^{-1})	Assignment
3300-3600	Broad –OH stretching (CD)
2933,2838,2055	-CH stretching (CD)
1734 *	C---O
1734,1635,1540,	C---O , RCONHR
1508	Phenyl
1460,1392	$-CH_2$ (CD)
1367,1334	$-CH_3$ (CD)
1251,1227*	-C-O-C
1196,1158	C-O stretching (CD)

Table 12. FTIR data of methyl-βCD-carbaryl inclusion compound prepared by kneading method

Wave number (cm^{-1})	Assignment
3300-3600	Broad –OH stretching (CD)
2933,2839,2056	-CH stretching (CD)
1744 *	C---O
1744,1636,1539,	C---O , RCONHR
1507	Phenyl
1459,1391	$-CH_2$ (CD)
1367,1333	$-CH_3$ (CD)
1251,1226*	-C-O-C
1195,1158	C-O stretching (CD)

3.7 Characterization of solid state inclusion complexes

3.7.1 Dissolution study of inclusion complex

3.7.1.1 Dissolution study of inclusion complex formed by freeze-drying method

The solubility data and solubility profiles of freeze-dried mixtures are presented in Appendix 8 and Figure 18. The dissolution profile of freeze-dried mixtures prepared by stirring at 20°C, 30°C, 40°C, 50°C and 60°C for 3 hours before freeze-drying process, at the 1:1, 1:2, and 2:1 molar ratios of carbaryl 85WP: methyl-βCD were studied. For control, the 3:1 molar ratio of carbaryl 85WP: dextrin mixtures and free carbaryl 85WP were also compared. The method was as described in section 2.3.7. The results are presented in Figure 18. The dissolution profiles are plotted as carbaryl dissolved (mg/l) against time. It was found that methyl-βCD could increase the amount of carbaryl dissolved from the freeze-dried products. And the 2:1 ratio gave the highest solubility in all cases, except for the freeze-dried mixture obtained after stirring at 60°C prior to freeze-dried process (Figure 18e). While the 1:1 ratio was mostly better than the 1:2 ratio. It was noted that, the solubility of carbaryl profiles did not increase as the content of methyl-βCD increased. Carbaryl dissolution increased with the increase in preparation temperature. By comparing solubility data, the 1:1 ratio gave the highest dissolution (154.11 mg/l) at complex preparation temperature of 60°C followed by the 2:1 ratio (149.56 mg/l) prepared at 40°C.

It was also observed that carbaryl-dextrin is not as good as carbaryl-methyl-βCD in the dissolution properties especially at higher temperature of complex formation. When rate of dissolution was concerned, the result from all conditions in

Figure 18 demonstrated the faster rate of complex dissolution than free carbaryl dissolution. For the complex, the concentration of carbaryl dissolved reached the equilibrium level after shaking for 10 minutes while for free carbaryl, equilibrium was reached at around 15 minutes.

3.7.1.2 Dissolution study of inclusion complex formed by kneading method

The solubility profiles of kneaded mixtures are presented in Figure 18f. Slight increase in solubility profiles of carbaryl against time was observed in all complexes of different ratios. The dissolution of carbaryl 85WP did not increase as the weight fraction of methyl-βCD increased. From the dissolution profiles of the kneading mixtures, the solubility of all products was higher than that of pure carbaryl 85WP. Complete solubilization of samples was achieved within 10 minute, while the free carbaryl 85WP needed upto 15 minutes. The kneading mixtures at molar ratio of 2:1 exhibited the highest solubilization, followed by the 1:1 and 1:2. Carbaryl 85WP-dextrin (at 3:1 molar ratio) gave similar values as the 1:2 carbaryl: methyl-βCD but higher than that of free carbaryl. When compared with the freeze-dried products, the dissolution of kneaded mixtures gave nearly the same dissolution profile as the freeze-dried mixtures prepared at 30°C prior the freeze-dried process.

The order of dissolution was as follows :

60°C > 50°C > 40°C > kneaded > 30°C > 20°C for 1:1, 1:2 carbaryl 85WP-methyl-βCD and carbaryl 85WP-dextrin

40°C > 50°C > 60°C > kneaded > 30°C > 20°C for 2:1 carbaryl 85WP-methyl-βCD

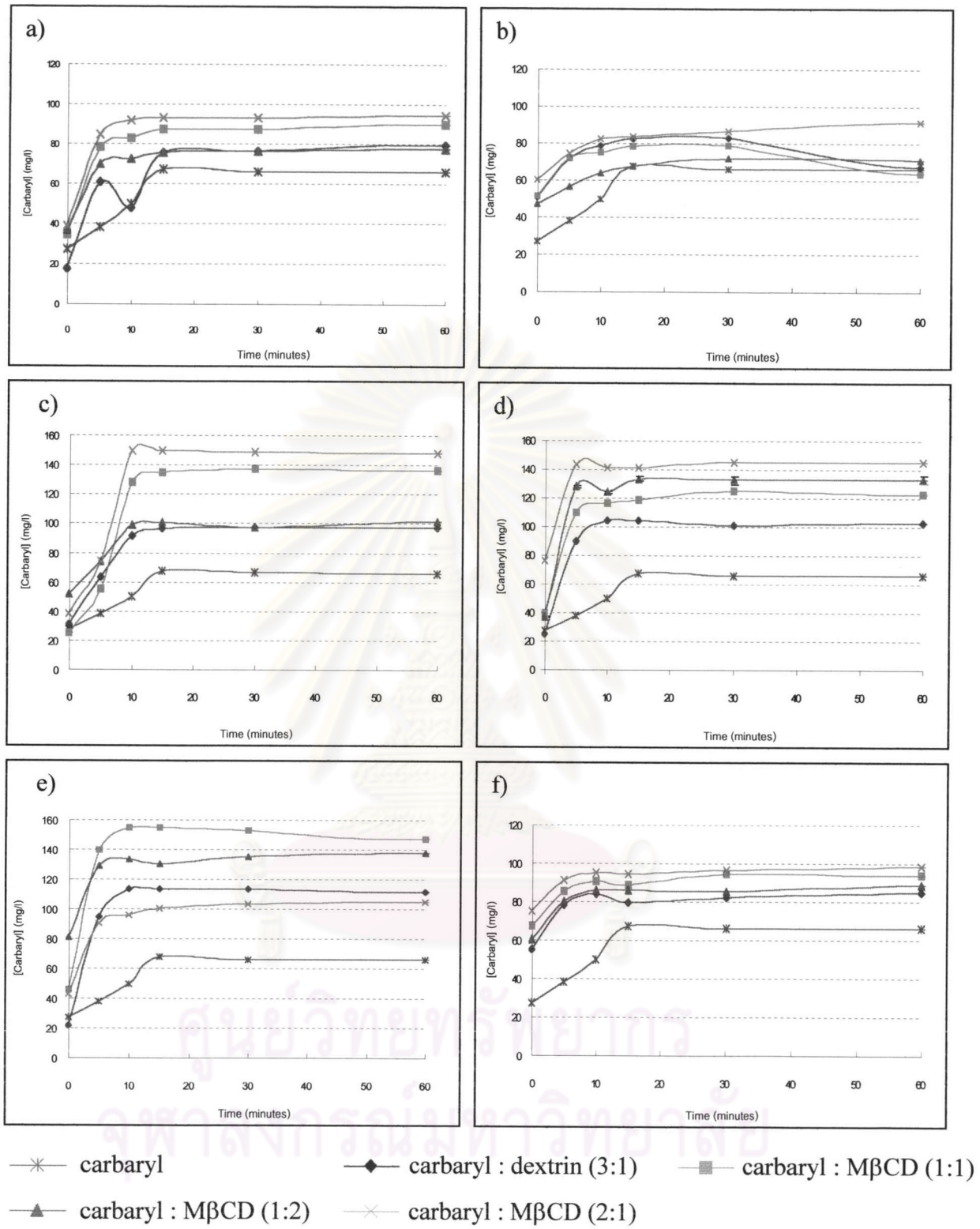

Figure 18. Dissolution study a-e) freeze-dried mixtures, prepared at 20, 30, 40, 50 and 60°C, respectively, before freeze-drying; f) kneaded mixture.

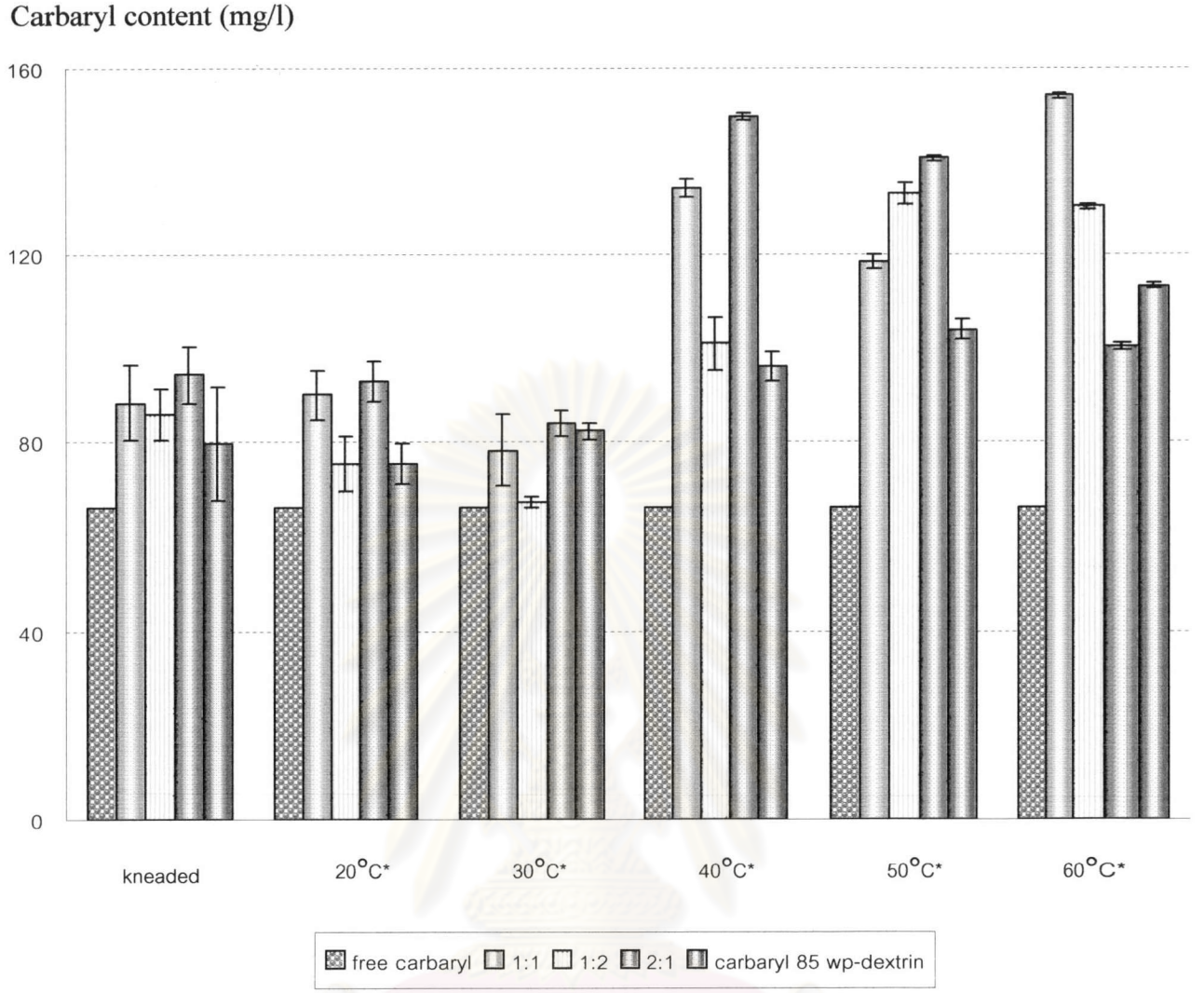

Figure 19. The solubility of carbaryl complex in related to free carbaryl

* = temperature used to prepare the complex before freeze-dried process

3.7.2 Thermal stability of inclusion complex

Carbaryl 85WP , the freeze-dried and kneaded complexes made with methyl-βCD and or dextrin were incubated for 3 hrs at 80°C in hot air oven. Time-course of remaining carbaryl in the solid state was plotted as shown in Figures 20 and 21. Thermodegradation rate was faster in the first 30 minutes for all samples. The three formulations with methyl-βCD in the present study were prepared from the 1:1, 1:2 and 2:1 (carbaryl 85WP: methyl-βCD) molar ratios. Free carbaryl was lost about 40% after heat treatment at 80°C for 40 minutes. And about 20-25% reduction in the loss of carbaryl was observed when complexed with methyl-βCD. It was found that methyl-βCD gave the same stabilizing ability in formulations made from different ratios of carbaryl to methyl-βCD while dextrin could not stabilize (Figure 20 b).

The stability of the complex prepared from freeze-dried method at 2:1 molar ratio was further studied for long storage at 40°C for 14 days. It was found that free carbaryl was decomposed about 40% in comparison with methyl-βCD-carbaryl 85WP complexes which was less than 20% decomposed after 14 days. And methyl-βCD was better than dextrin in supporting complex stability (Figure 22).

3.7.3 UV stability of inclusion complex

In this study, the result showed that inclusion of carbaryl 85WP into methyl-βCD improved its UV stability (Figures 23 and 24) except in the case of 2:1 molar ratio of kneaded mixture. The rate of photodegradation of carbaryl in the presence of methyl-βCD was slower than dextrin and free carbaryl 85WP. The photodegradation rate of carbaryl was rapid in the initial phase, but at 30 minutes later, the rate was significantly decreased. When the carbaryl-methyl-βCD was exposed to UV light, more than 70% of carbaryl remained after 3 hrs incubation. The freeze-dried mixtures

at the molar ratio of 1:1 exhibited the highest stabilizing effect of photodegradation, followed by the 1:2 and 2:1 mixture ratios, respectively. However, the difference between different ratios was not much. Interestingly, dextrin did not show power of stabilizing carbaryl against photodegradation.

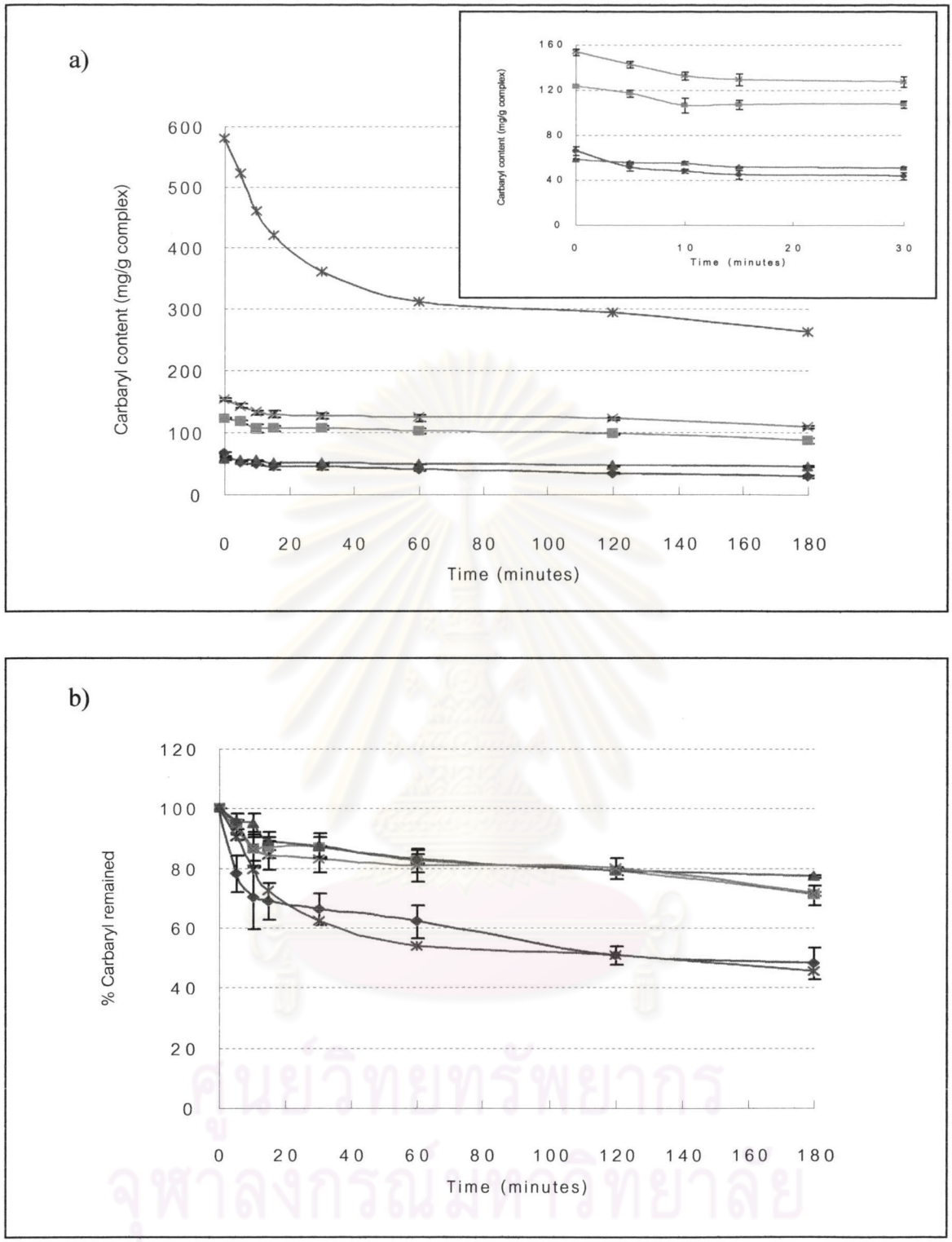

Figure 20. Thermal stability at 80°C of carbaryl 85WP, carbaryl 85WP-methyl-βCD complexes, and carbaryl 85 WP-dextrin complexes prepared by freeze-dried method.
a) carbaryl content versus time, b) % carbaryl remained versus time

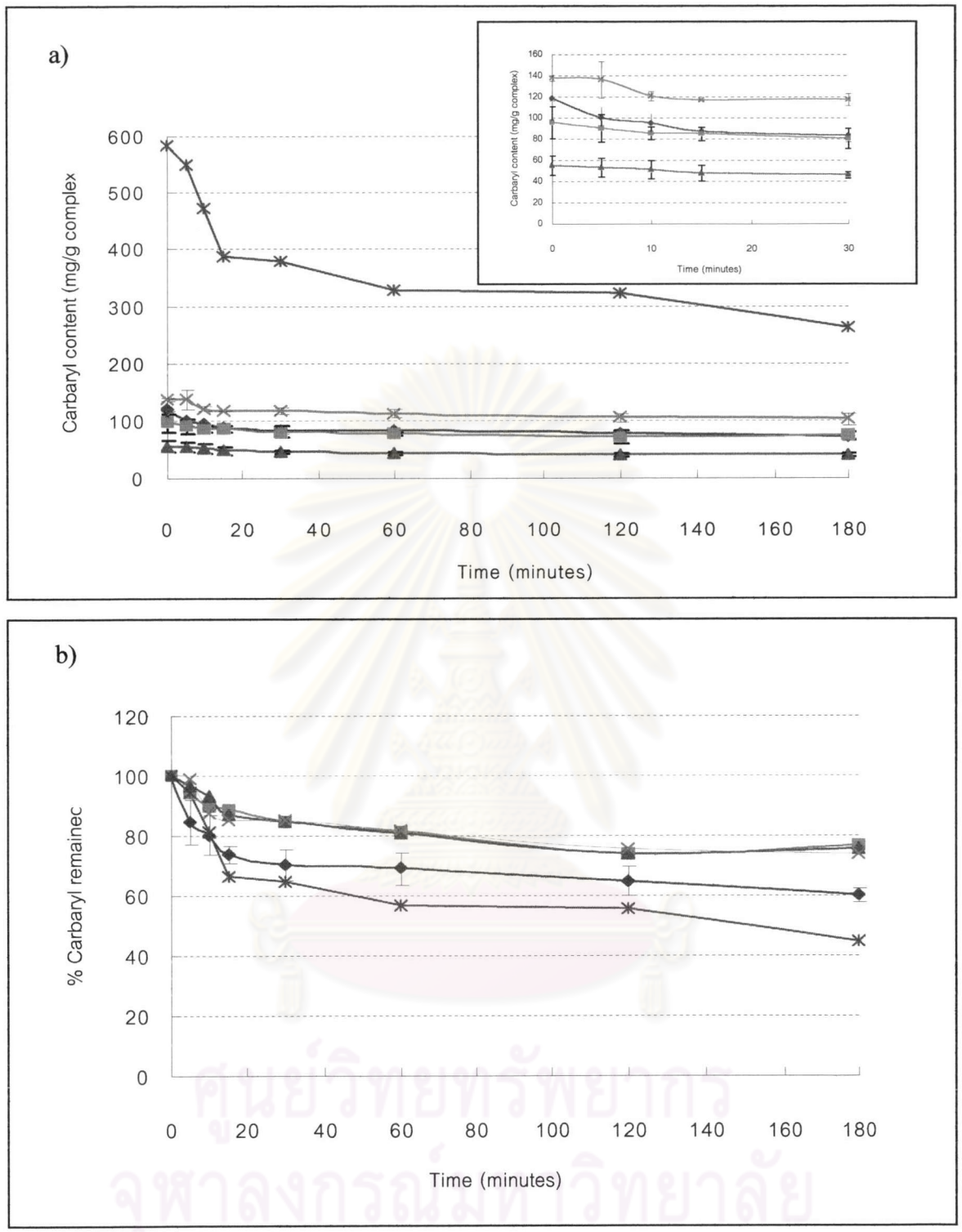

Figure 21. Thermal stability at 80°C of carbaryl 85WP, carbaryl 85WP-methyl-βCD complexes, and carbaryl 85 WP-dextrin complexes prepared by kneaded method.

a) carbaryl content versus time, b) % carbaryl remained versus time

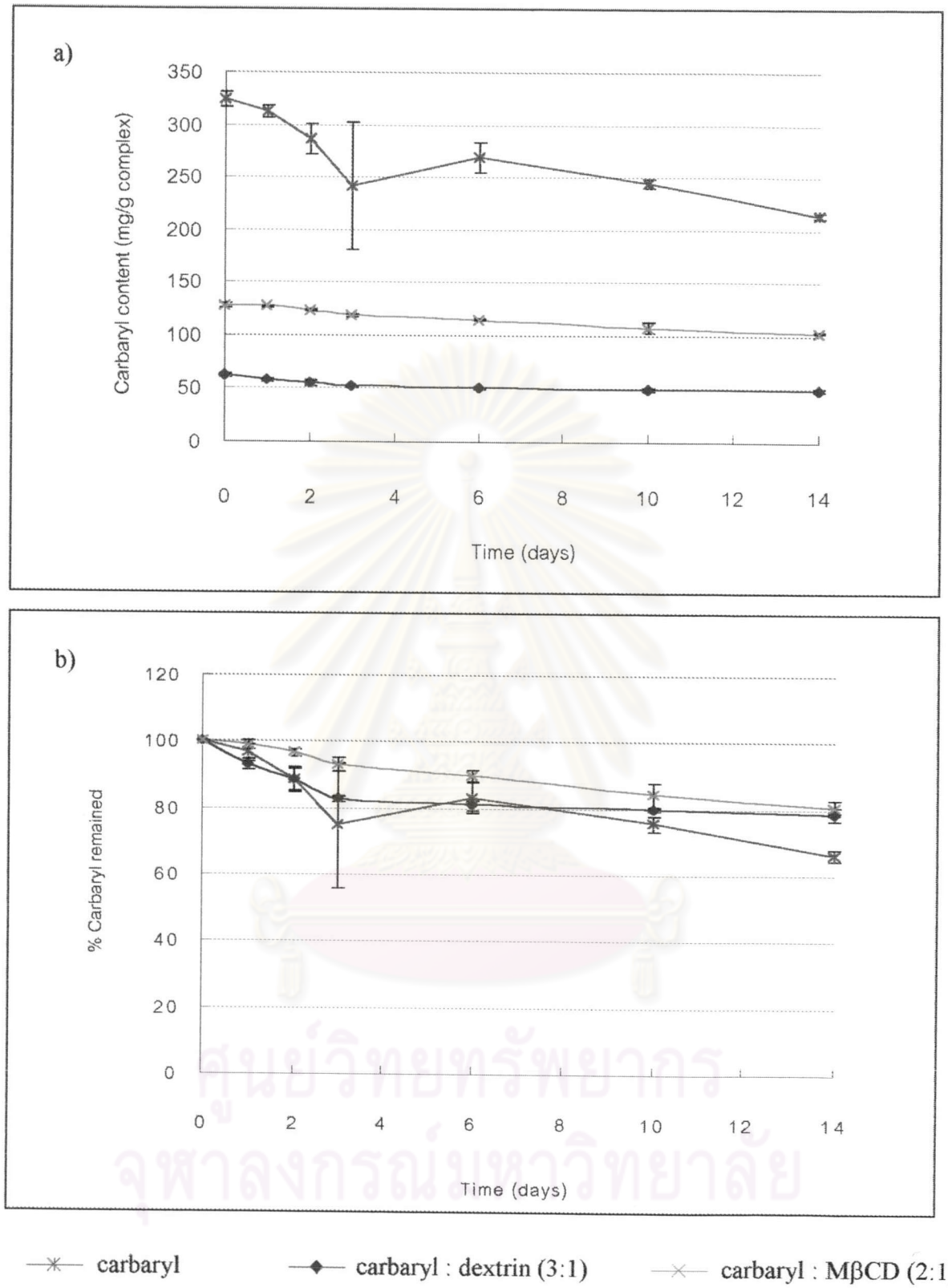

Figure 22. Thermal stability at 40°C of carbaryl 85WP, carbaryl 85WP-methyl-βCD complexes, and carbaryl 85 WP-dextrin complexes prepared by freeze-dried method.

a) carbaryl content versus time, b) % carbaryl remained versus time

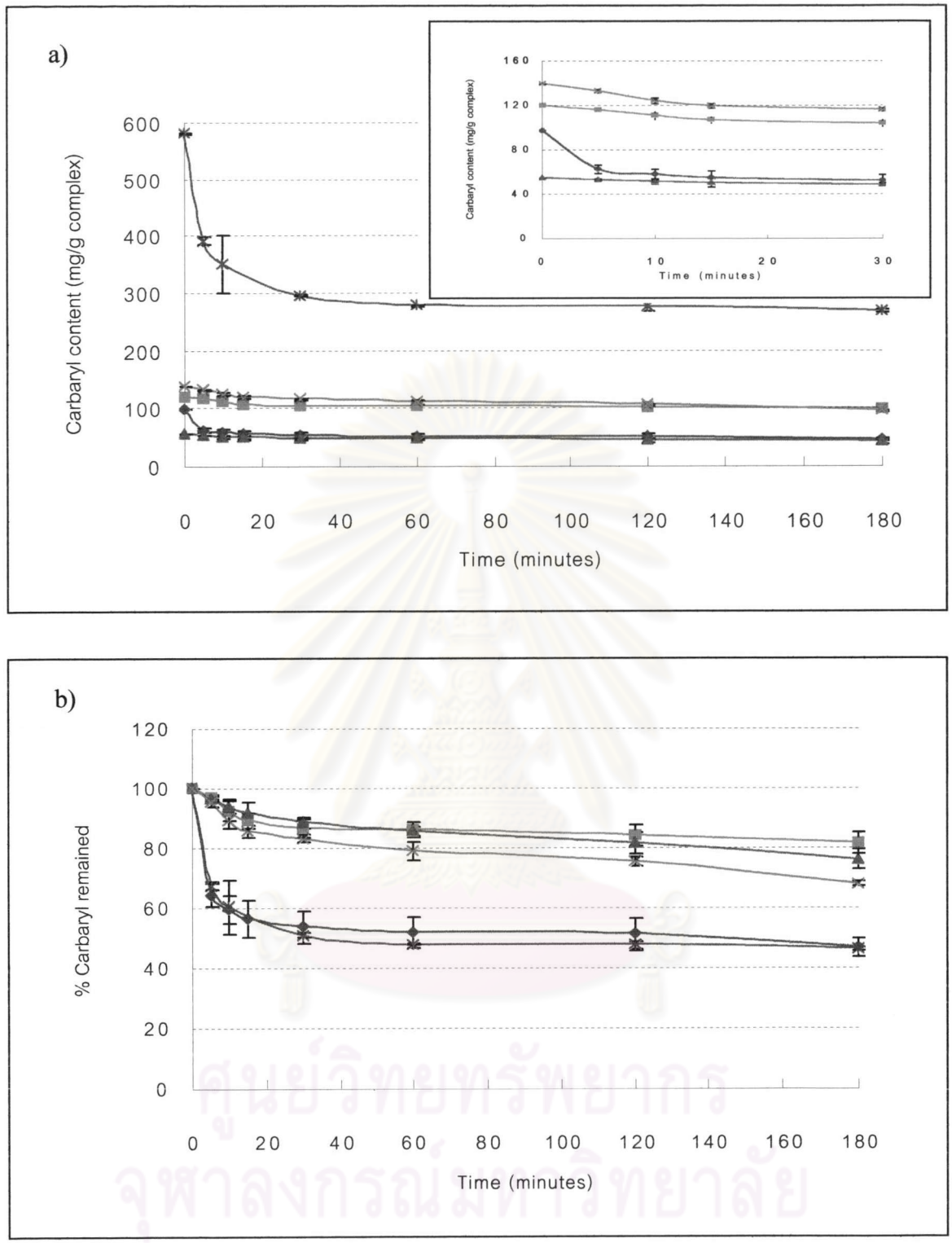

Figure 23. UV stability of carbaryl 85WP, carbaryl 85WP-methyl-βCD complexes, and carbaryl 85 WP-dextrin complexes prepared by freeze-dried method.

a) carbaryl content versus time, b) % carbaryl remained versus time

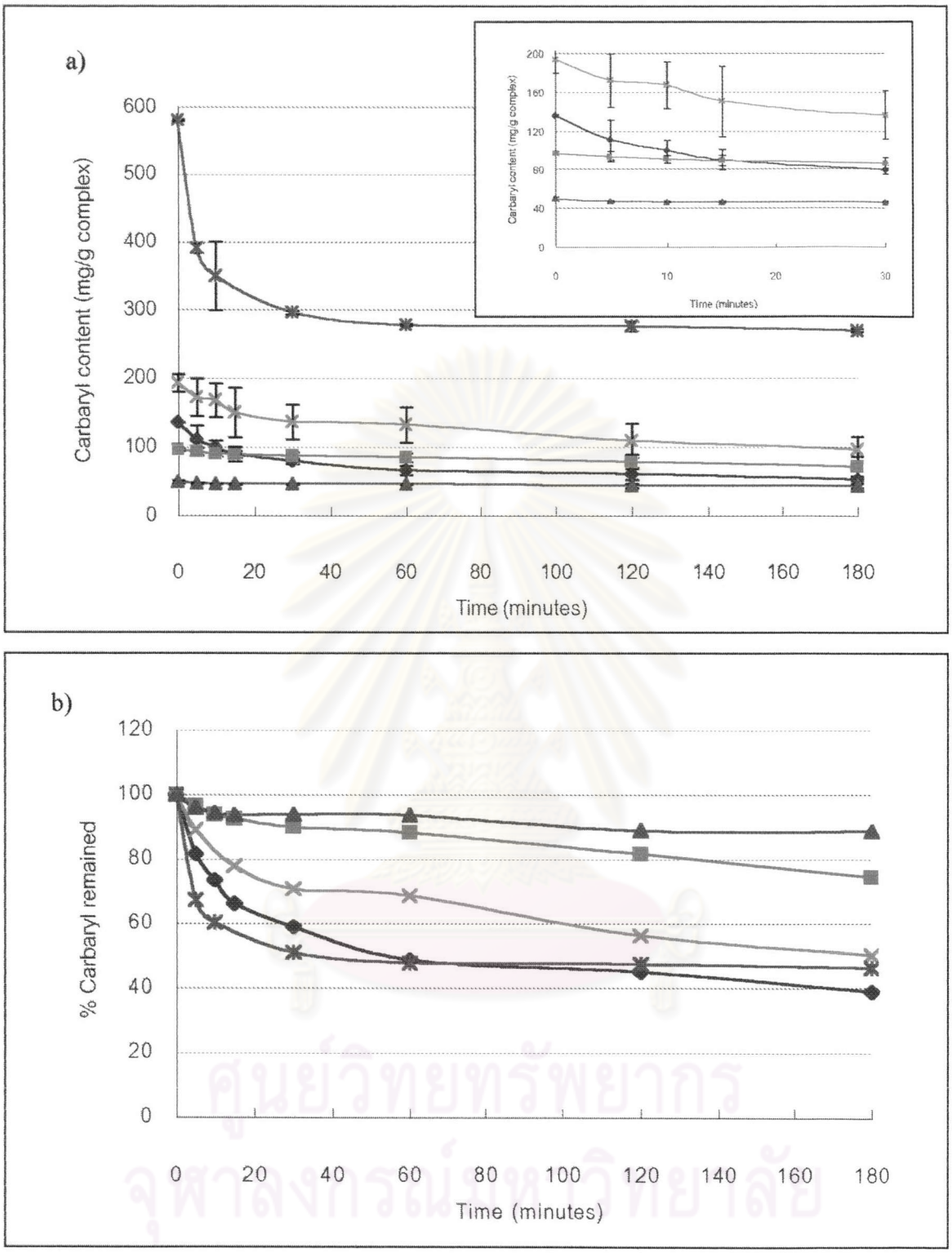

Figure 24. UV stability of carbaryl 85WP, carbaryl 85WP-methyl-βCD complexes, and carbaryl 85 WP-dextrin complexes prepared by kneaded method. a) carbaryl content versus time, b) % carbaryl remained versus time

3.7.4 Acute toxicity test of free carbaryl 85WP and carbaryl 85WP-methyl-β CD complex on Brine shrimp (Artemia salina)

Percent mortality of Brine shrimp exposed to concentrations at 0.1, 1, 5 and 10 mg/l of carbaryl 85WP and its complex of 2:1 molar ratio of carbaryl 85WP: methyl-βCD for 6 hrs were estimated. Exposure to carbaryl and its complex at the concentration of 0.1 mg/l was unable to kill the Brine shrimp within 6 hrs. But mortality could be observed at higher concentrations. After 6 hrs of exposure to 1, 5 and 10 mg/l of carbaryl 85WP, the percent of death were 11.7, 55.0 and 98.4, respectively (Table 13). In the case of 2:1 molar ratio of carbaryl 85WP-methyl-βCD complex, the percent of death was 7.8, 50.9 and 95.2, respectively (Table 14). Percent mortality of the control groups were in the range of 3.3 to 5.0 for both groups (Table 13 and 14). The LC_{50} values of free carbaryl and the complex were 4.48 and 5.05 mg/l (Table 15). Carbaryl-methyl-βCD complex thus exerted a little less toxicity than the free carbaryl. LC values were calculated by Probit program (Finney, 1971) and summarized in Appendix 9 and 10.

Table 13. Percent mortality of *Artemia salina* at various concentrations of carbaryl 85 WP

Concentration of carbaryl 85WP (mg/l)	Number of Brine Shrimp	Percent mortality at 6 hours	
		No. of death	Death (%)
Control	60	3	5.0
1	60	7	11.7
5	60	33	55.0
10	62	61	98.4

Table 14. Percent mortality of *Artemia salina* at various concentrations of carbaryl 85 WP-methyl-βCD at 2:1 molar ratio

Concentration of carbaryl 85WP-methyl-βCD (mg/l)	Number of Brine Shrimp	Percent mortality at 6 hours	
		No. of death	Death (%)
Control	60	2	3.3
1	64	5	7.8
5	57	29	50.9
10	63	60	95.2

Table 15. Calculated LC_{50} values (mg/l) of free carbaryl 85 WP and carbaryl 85 WP-methyl-βCD using Probit analysis.

Sample	LC_{50} (mg/l)	95 % confidence limits (mg/l)
Carbaryl 85WP	4.48	3.88-5.16
Carbaryl 85 WP-methyl-βCD complexes	5.05	4.42-5.76