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Formation of Inclusion Complexes between Cyclodextrins and Carbaryl and Characterization of the Complexes

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Abstract

The solubility of carbaryl increased with increasing concentrations of β-CD, G_2-β-CD, and M-β-CD. The result suggests the formation of soluble inclusion complex. Solubility increase was highest in M-β-CD-carbaryl, being 18.4 fold higher than that of carbaryl when 100 mM M-β-CD was used. The apparent formation constant for the complex calculated from phase solubility diagram was 223.18 M^{-1}. The preparation of the complex in solid form for characterization was successful by kneading and freeze-drying. The DSC curves for kneading and freeze-drying mixture did not show the endothermic peak characteristic of carbaryl, but a small new endothermic peak was observed. FTIR analysis showed a shift of the major peak of carbonyl group in carbaryl molecule from 1717 to 1744 and 1734 cm^{-1} in kneading and freeze-dried mixtures, respectively. M-β-CD-carbaryl complex demonstrated higher dissolution rate, higher thermal and UV stability but lower toxicity than its parent carbaryl compound.

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides of 6–8 glucose units (α,β,γ-form) with a hydrophilic outer surface and a lipophilic central cavity. The unique property of CD is the ability to form inclusion complex with variety of organic/inorganic guest molecules of suitable size and polarity, leading to a change in physicochemical properties of the guest [1]. Protection against oxidative degradation or destruction by UV light, improvement of the solubility of hydrophobic substances in aqueous solution, stabilization of volatile compounds, alteration of the chemical reactivity, modification of liquid substances to powders, or reduction of undesirable smell or taste in products are among known useful properties of the host CDs. The applications of CDs as emulsifiers, antioxidants, and stabilizing agents have rapidly increased in food, cosmetics, pharmaceutical, agrochemical, and plastic industries.

Several CD derivatives with specific desirable properties have been developed through chemical or enzymatic means. Examples are those with solubility better than parent compounds, e.g., methylated, hydroxypropylated, and maltosyl-CDs. CD-polymers are used as stationary phase in various liquid chromatography systems [2]. These modified CDs, in addition to native ones (the α, β, and γ-) can be chosen according to properties to be used as the suitable host molecules.

Carbaryl (α-naphthyl-N-methylcarbamate), a broad spectrum insecticide, is one of the three most commonly used insecticides in Thailand. Besides inhibiting many enzyme systems in insects, it also has similar effect in fish, bird, and mammal [3]. In this context, heavy dose usage for crop protection and residual left of this pesticide are of concern. The application of CDs and derivatives in changing properties of carbaryl for a better insecticide formulation is our interest. This study aims at investigating the interactions between carbaryl and CDs and their effect on aqueous solubility, dissolution, stability, and toxicity of carbaryl.

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Methods

Studies in solution

Solubility studies

Solubility measurements were carried out according to Higuchi and Connors [4]. Excess amount (10 mg) of Carbaryl 85WP, a commercial grade carbaryl, was added in 10 ml of aqueous solutions of β-CD, methyl-β-CD (M-β-CD) or maltosyl-β-CD (G_2-β-CD) of various concentrations. The concentration of β-CD was 0–50 mM while that of M-β-CD and G_2-β-CD was 0–100 mM. The mixtures were shaken at 30 °C for 24 h. The solution was filtered through 0.45 μm membrane and analyzed for carbaryl spectrophotometrically at 276 nm. The concentration of carbaryl was then determined from carbaryl standard curve. Phase solubility diagram was plotted between carbaryl concentration and CDs concentration. Apparent formation constant (K_c or $K_{1:1}$) was calculated from the plot using the equation:

$$K_c = \frac{[\text{Carbaryl-CD}]}{[\text{Carbaryl}][\text{CD}]} = \frac{\text{slope}}{\text{intercept (1-slope)}} \quad (1)$$

Preparation of carbaryl-M-β-CD solid complexes

The 1:1, 1:2 and 2:1 molar ratios of carbaryl:M-β-CD were mixed by different methods as follows.

By physical mixing

Before mixing, carbaryl and M-β-CD powders were incubated at 40 °C overnight. They then were mixed together (only the 2:1 molar ratio was made) on a small concave glass for 10 min at room temperature. The samples were kept desiccated.

By kneading

The kneaded mixture was prepared by mixing different ratios of carbaryl to M-β-CD. Deionized water, 15% of total weight, was gradually added and kneaded was performed on the concave glass for 10 min to obtain homogeneous paste. The paste was dried at room temperature overnight, then ground and finely screened through a 40 mesh sieve.

By freeze-drying

Different molar ratios of carbaryl to M-β-CD were dissolved in 100 ml of deionized water and magnetic stirred at 30 °C for 3 h. The solution was frozen at −80 °C and freeze-dried in a Flexidry μP lyophilized apparatus.

By co-precipitation

Different amounts of M-β-CD were dissolved in 200 ml deionised water, then carbaryl was added to give the desired molar ratio. The preparation was magnetic stirred at 30 °C for 3 h. The precipitate was filtered and dried at room temperature for 1 day.

Analysis of solid complex

To investigate true complex formation, Differential scanning calorimetry (DSC) and Fourier Transform Infrared Spectrometry (FTIR) of each complex formed from 2:1 molar ratio of carbaryl:M-β-CD by different methods were performed in the previous section.

DSC

Samples ranging from 3–7 mg were placed in pierced aluminum pans and scanned at a rate of 10°C per min. DSC measurements were carried out under dry nitrogen (10 ml/min) on a NETZS CH GERATEBAU GMbH Thermal analysis apparatus.

FTIR

Solid samples were prepared by the potassium bromide disc method and scanned for absorbance from 400–4000 cm^{-1}. The spectra were obtained on a Perkin Elmer Model 1760X.

Both DSC and FTIR were performed at the Technological Research Equipment Center of Chulalongkorn University.

Determination of properties of the solid complex

Dissolution studies

Excess amount of samples (200 mg) were added in 100 ml water and shaken at 30 °C for 0–180 min. Samples were withdrawn for analysis of carbaryl at 276 nm. From this experiment, the suitable method of complex formation and appropriate molar ratio between carbaryl and M-β-CD were selected. The criteria of chosen were highest amount of carbaryl dissolved and low quantity of M-β-CD used.

Thermal stability

The complex formed by the most appropriate method chosen above was placed in a hot air oven at 80 °C. At time intervals (0–180 min), the sample was withdrawn for carbaryl analysis at 276 nm.

UV stability

The complex was placed under the 30 W UV lamp at a distance of 22.5 inches. At time intervals (0–180 min), the sample was withdrawn for carbaryl analysis at 276 nm.

Toxicity

Brine Shrimp (*Artemia salina* Linnaeus) cytotoxicity test was performed. Brine shrimp eggs were hatched in artificial seawater prepared from 38 g/l NaCl [5]. After 24 h incubation at room temperature, nauplii were collected with a micropipette after attracting the organisms to one side of the box with a light source. Three different concentrations of free carbaryl and the 2:1 complex were incubated with 100 μl suspension of nauplii (8–12 organisms) for 6 h in each microwell. Six repeats were performed for each concentration. The number of dead (non-motile) nauplii were counted and the data were treated by Probit Analysis [6]. The lethal dose LC_{50} was determined.

Result and discussion

Phase solubility diagram of carbaryl was shown in Figure 1. In the absence of CDs, the concentration of carbaryl found in aqueous solution at 30 °C was in the range of 0.54–0.58 mM. The solubility of carbaryl increased with increasing concentrations of β-CD, G_2-β-CD, and M-β-CD. The result suggests the formation of soluble inclusion complex. Solubility increase was highest in M-β-CD-carbaryl, being 18.4 fold higher than that of carbaryl when 100 mM M-β-CD was used. The solubility diagram was of A_L type for all CDs indicating the complex formation of 1:1 stoichiometry [4]. The apparent formation constants were calculated from Equation (1) and shown in Table 1. The constant for M-β-CD-carbaryl was 223.18 M^{-1} which was nearly twice that of β-CD-carbaryl. This result indicates that M-β-CD is better than β-CD in interaction with carbaryl.

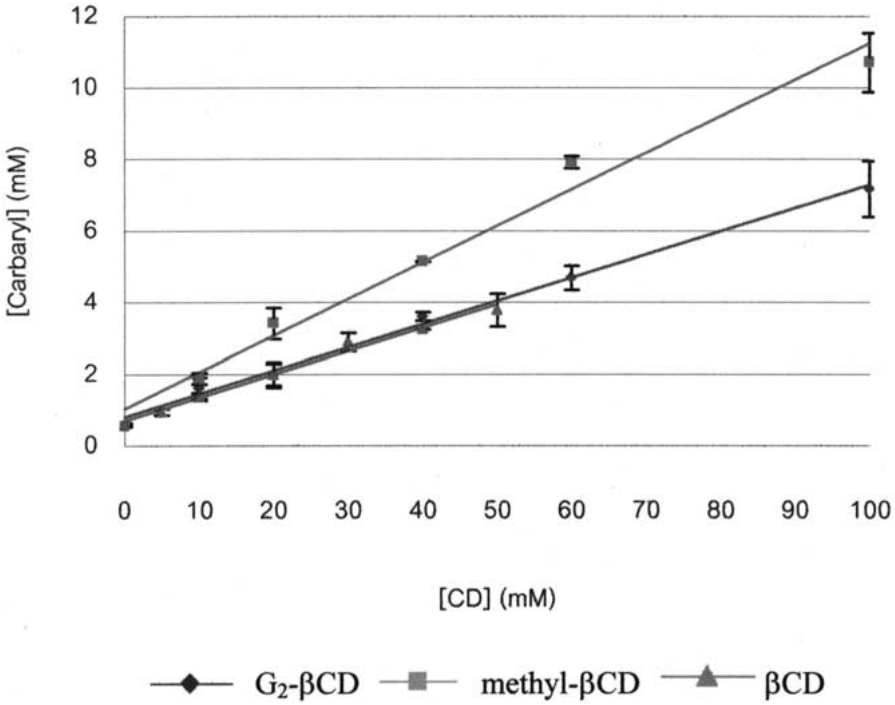

Figure 1. Comparison of phase solubility diagrams between carbaryl and CDs at 30 °C.

Table 1. Apparent formation constants of carbaryl-CD complexes and the type of solubility diagram

Type of agriculture toxic substance	CD	Slope	Intercept	K_c (M^{-1})	Type of solubility diagram
Carbaryl	β-CD	0.0662	0.5863	120.92	A_L
	G_2-β-CD	0.0683	0.5605	130.79	A_L
	Methyl-β-CD	0.1091	0.5487	223.18	A_L

The effect of β-CD on carbaryl solubility was previously reported by Barbato *et al.* [7]. The A_L type phase solubility diagram was also found but with higher apparent formation constant. The lower value in our study might be due to commercial grade carbaryl used without extraction of carbaryl as performed in the previous report. When we replaced commercial with pure carbaryl in the phase solubility study, higher formation constant for β-CD-carbaryl was obtained. Thus our work is in agreement with previous data on β-CD-carbaryl, whereas for G_2-β-CD and M-β-CD no previous study was performed.

The preparation of solid complex was carried out between M-β-CD and carbaryl using 1:1, 1:2 and 2:1 molar ratios. Physical mixing, kneading, freeze-drying, and co-precipitation were different methods employed. The complex mixture was then analyzed by DSC and FTIR to check for method which resulted in true complex formation. In DSC and FTIR analysis, only the 2:1 molar ratio was analyzed since no precipitation was formed from the 1:1 and 1:2 by co-precipitation method. In addition, the 2:1 complex demonstrated better dissolution characteristics. DSC thermograms (Figure 2) of different mixtures were compared with that of free carbaryl and M-β-CD. The thermogram of free carbaryl gave the characteristic melting endothermic at 143.3 °C and another broad peak at 202 °C which was due to decomposition of carbaryl [7]. M-β-CD had no defined peak but formed broad endothermic around 87.3 °C which could be referred to the loss of water of dehydration process [8]. When thermograms of the mixtures were examined, co-precipitation yielded a sharp endothermic peak at 141.5 °C which was closed to that of carbaryl and a large broad peak at 195.7 °C which might be decomposed carbaryl. This technique thus retained a lot of free carbaryl and could not lead to complex formation. Thermograms of the kneading and freeze-drying mixtures were similar, an endothermic peak at 188–189 °C which was not present in free carbaryl and free M-β-CD profiles and should be referred as the complex product was observed. No free carbaryl was left while a high peak at 86.5–91.6 °C which should be free M-β-CD retained was found in both preparations. Surprisingly, physical mixing resulted in some interactions between M-β-CD and carbaryl since no free carbaryl was left and small new peak at 168.8 °C was observed. The result suggests that

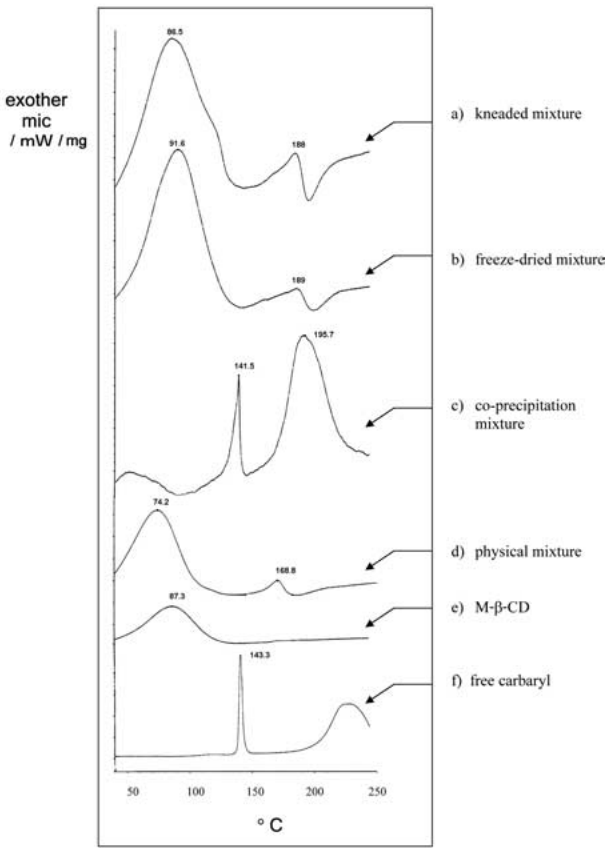

Figure 2. DSC thermograms of (a) kneaded mixture, (b) freeze-dried mixture, (c) co-precipitation mixture, (d) physical mixture, (e) M-β-CD, and (f) free carbaryl. The mixtures are 2:1 molar ratio of carbaryl: M-β-CD.

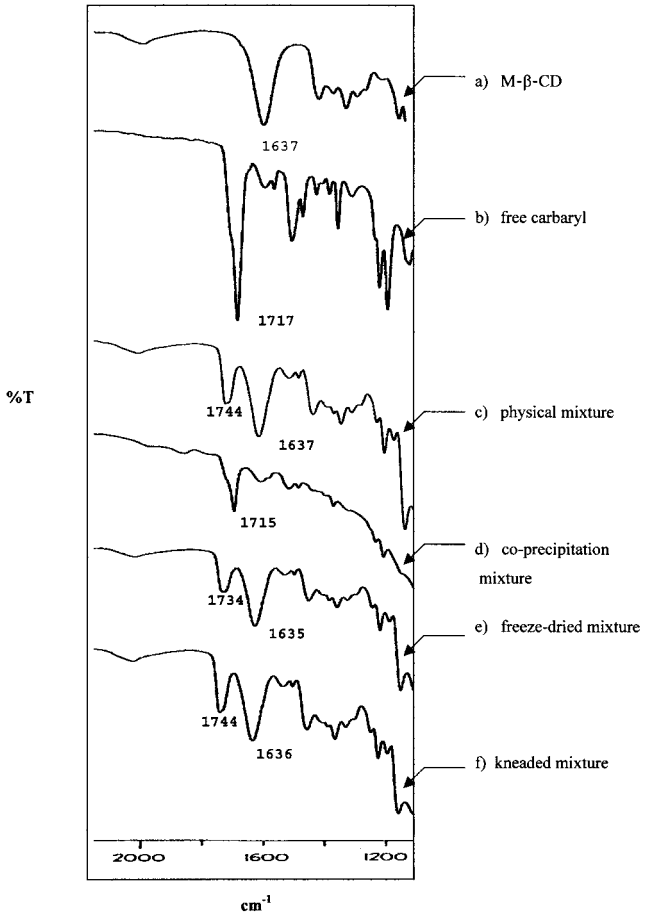

Figure 3. FTIR spectra of (a) M-β-CD, (b), free carbaryl, (c) physical mixture, (d) co-precipitation mixture, (e) freeze-dried mixture and (f) kneaded mixture. The mixtures are 2:1 molar ratio of carbaryl: M-β-CD.

kneading, freeze-drying, and perhaps physical mixing leads to true complex formation between M-β-CD and carbaryl.

When FTIR was analyzed (Figure 3), the major peak at 1717 cm^{-1} of the C=O stretching of the carbonyl groups was the important characteristics of carbaryl. M-β-CD 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 M-β-CD than by β-CD. We reported here the shift from 1717 to 1734 cm^{-1} when free carbaryl was compared with freeze-dried M-β-CD-carbaryl. Barbato *et al.* [7] reported the smaller shift from 1713 to 1717 cm^{-1} in the case of freeze-dried β-CD carbaryl spectrum. Carbonyl characteristic peak of the two studies was 4 cm^{-1} different which may be attributed to purity of carbaryl compound.

Properties of solid inclusion complex prepared by mixing carbaryl with M-β-CD at different molar ratios at 40 °C for 3 h prior to freeze-drying and kneading were compared with free carbaryl and carbaryl-dextrin. Dissolution, stability, and toxicity were those properties studied. Figure 4 showed the result from dissolution test. M-β-CD could increase the amount of carbaryl dissolved from the freeze-dried product, while maximum carbaryl dissolved (150 mg/l) was obtained in the complex with 2:1 molar ratio. The rate of dissolution in the presence of M-β-CD was also significantly higher than in its absence. Dextrin, a linear chain of 23–26 residues of glucose, might be able to form loose complex with carbaryl as evidenced by similar dissolution rate with 1:1 carbaryl-M-β-CD complex obtained. The freeze-dried method was better than kneading when dissolution property was concerned.

Thermal and UV stabilities were performed and the results are shown in Figures 5 and 6. M-β-CD could help stabilize carbaryl against decomposition by heat at 80 °C or by UV light while dextrin could not.

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 [5]. The results in Tables 2 and 3 suggest that for acute cytotoxic effect (6 h incubation time), carbaryl-M-β-CD complex exerted a little less toxicity than the parent compound, carbaryl. LC_{50} for carbaryl and the complex

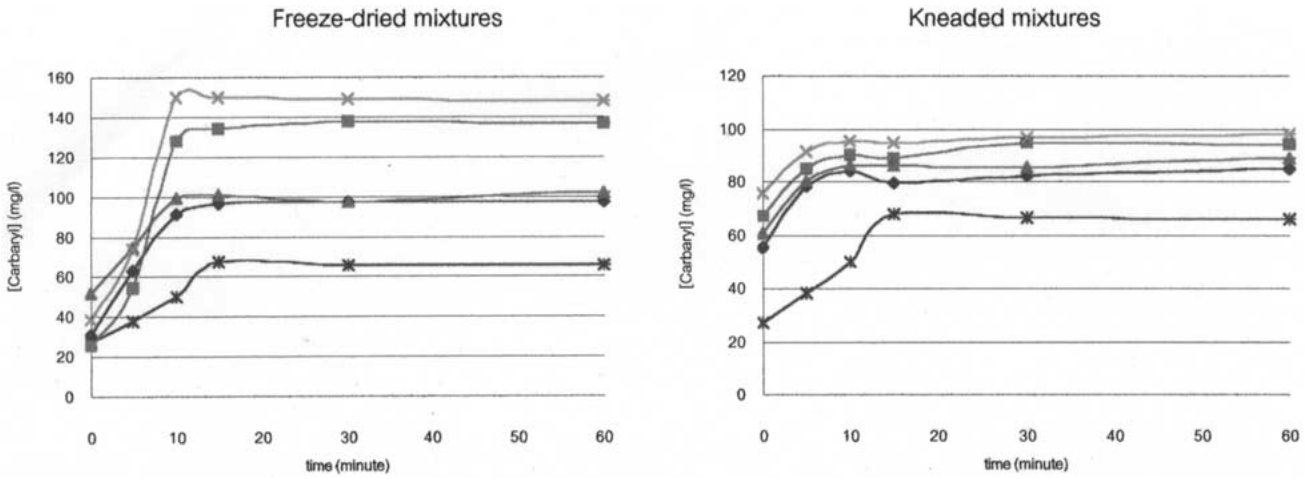

Figure 4. Dissolution profile of free carbaryl (—∗—), freeze-dried or kneaded products of carbaryl: M-β-CD with 1:1 (—■—). 1:2 (—▲—), 2:1 (—×—) molar ratio, and freeze-dried or kneaded products of 3:1 carbaryl: dextrin (—♦—).

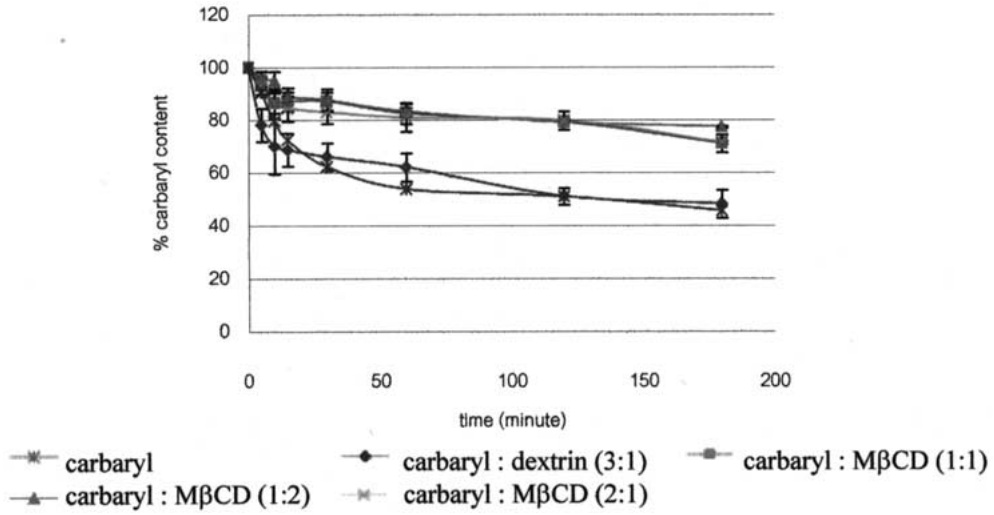

Figure 5. Thermal stability test at 80 °C.

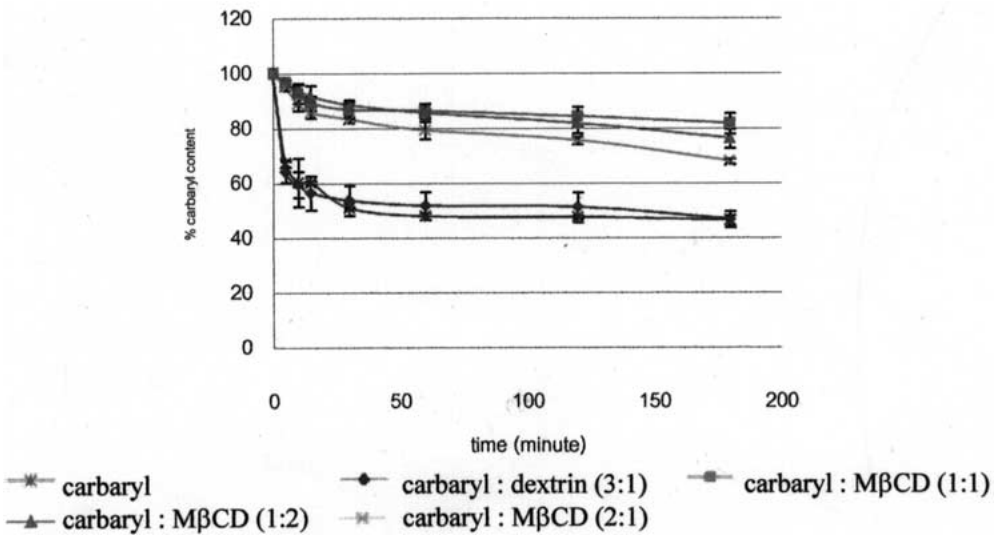

Figure 6. UV stability test.

Table 2. Percent mortality of *Artemis salina* at various concentrations of carbaryl

Concentration of carbaryl (mg/l)	Number of brine shrimp	Percent mortality at 6 hours	
		No. of deaths	Deaths (%)
Control	60	3	5
1	60	7	11.66
5	60	33	55.00
10	62	61	98.38

Table 3. Percent mortality of *Artemia salina* at various concentrations of carbaryl-methyl-β-CD at 2:1 molar ratio

Concentration of carbaryl-M-β-CD (mg/l)	Number of brine shrimp	Percent mortality at 6 hours	
		No. of deaths	Deaths (%)
Control	60	2	3.33
1	64	5	7.81
5	57	29	50.87
10	63	60	95.23

were 4.48 and 5.05 mg/l, respectively. 95% confidence limits of the two samples were in the range of 3.88–5.16 and 4.42–5.76 mg/l, respectively.

The overall results indicate that molecular inclusion of carbaryl with M-β-CD both in solution and in solid form prepared by the freeze-drying technique led to formation of complex with favorable properties. M-β-CD could significantly increase solubility of carbaryl in solution thus no need to use any solubility enhancer. In solid form, the dissolution of carbaryl was enhanced, this effect would improve bioavailability of the insecticide for the insect [1]. Improvement of hydrophilicity could also favorably affect the environmental distribution of the pesticide. 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.

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