J Fluoresc (2006) 16:487–493
DOI 10.1007/s10895-006-0106-8

ORIGINAL PAPER

Fluorescence Enhancement of Carbendazim Fungicide in Cucurbit[6]uril

Na'il Saleh · Nathir A. F. Al-Rawashdeh

Received: 6 April 2006 / Accepted: 9 June 2006 / Published online: 29 June 2006
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Abstract The potential increase in fluorescence of a benzimidazole-type fungicide (carbendazim) due to complexation with cucurbit[6]uril is reported. The fluorescence of the probe carbendazim in aqueous Na_2SO_4 solution (pH = 7.61) at room temperature is found to increase by a maximum factor of ~10.0 and blue-shifted up to ~11 ± 1 nm with the increase in cucurbit[6]uril concentration up to ~5 mM. This fluorescence enhancement is the result of formation of a 1:1 guest-host inclusion complex, in which the guest carbendazim is incorporated inside the hydrophobic cavity of the host curbit[6]uril through the amido-ester part. Such mode of inclusion is supported by NMR spectral measurements, in which upon encapsulation, the resonance of the methyl-protons of the amido-ester moiety is shifted significantly to upfield in the ^{1}H NMR spectrum. Also, to assess the formation of inclusion complex, solid samples prepared by co-evaporation have been studied, using differential scanning calorimetry (DSC). Measurement of the enhancement as a function of cucurbit[6]uril concentrations yielded a value of the equilibrium constant (K_a) of 271 ± 10 M^{-1} at 25°C. From the temperature dependence of the equilibrium constants, ΔH and ΔS values have been negative in sign, indicating the dipole-dipole interactions and the steric factors associated with the formation of this inclusion complex. It might be proposed that the spectral changes due to the inclusion of carbendazim are the result of decrease in the polarity of the surrounded media rather than the loss of carbendazim rotational mobility.

Keywords Host–guest chemistry · Fluorescence · Cucurbituril · Thermodynamics · NMR

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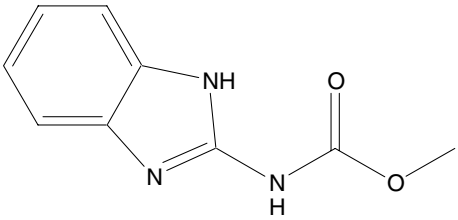
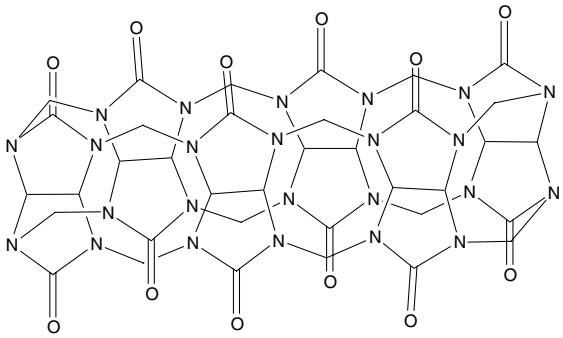
Introduction

For the past 20 years there has been continued growth in the applications of fluorescence spectroscopy in physical and biological sciences. Because of the sensitivity of fluorescence detection, the fluorometeric method has been one of the selected techniques to determine compounds at low concentrations [1]. Examples of such compounds are agrochemicals (pesticides, fungicides, insecticides, etc), which have attracted the attention worldwide due to their usage in agriculture. These chemicals and their degradation products remain as toxic contaminants in environmental samples, such as soil, agricultural water, and also in food supply [2–4]. Thus, it is of great interest to investigate a process leading to their elimination even if they exist at trace level.

Carbendazim (MBC = methyl 2-benzimidazolecarbamate) (see, Fig. 1) is a well-known fungicide, which prevents plant diseases caused by various fungi [5]. Recently, some works [4, 6, 7] demonstrated the use of fluorimetric technique to quantitatively determine the amounts carbamates pesticides in water using cyclodextrins macrocyclic species. The authors argued that the inclusion complex of carbamate pesticides with cyclodextrin host is expected to give rise to a



Carbendazim (methyl-2-benzimidazole) (MBC)



Cucurbit[6]uril (Q6)

Fig. 1 Molecular structure of the fluorescent guest molecule carbendazim (MBC) and the host molecule cucurbit[6]uril (Q6)

significant increase in the relatively weak fluorescence signal of MBC.

In this paper, we describe the fluorescence enhancement of carbendazim (MBC) in the presence of another macrocyclic host molecule, cucurbit[6]uril (Q6), in aqueous solution. Though the cucurbit[6]uril ligand was first synthesized in 1905, its chemical structure was reported at least 76 years later [8, 9]. Cucurbit[6]uril is a rigid molecule and possesses a hydrophobic cavity. At each entrance to the cavity, six polar carbonyl groups are located. The cavity has a diameter of ∼5.5 Å with two opposing portals of diameter ∼4.0 Å lined with carbonyl groups. The height is ∼6 Å in length, see Figure 1. The presence of this internal cavity makes cucurbit[6]uril a potential host molecule for encapsulation of smaller guest molecules. This potential for forming supramolecular host–guest inclusion complexes has now been well demonstrated [10–17].

This work presents the thermodynamics parameter associated with the inclusion processes of carbendazim (MBC) in the cavity of the host molecule cucurbit[6]uril (Q6) using steady fluorescence spectroscopy. The NMR spectroscopy is used to complement the fluorescence work to give further insights on the exact mechanism that governs the inclusion process. A third evidence for the mode of inclusion is obtained from differential scanning calorimetry (DSC) measurements of the solid samples prepared by co-evaporation. Moreover, the paper presents a brief comparison between the host abilities for MBC of α- and β-CDs [4, 6, 7] to the host abilities of Q6.

Experimental

Materials

Cucurbit[6]uril (Q6) (purity >99.9%) was purchased from Merck Scientific and used as received. The carbendazim (MBC) (99% pure) was purchased from Aldrich and used as received. The water used was doubly distilled prior to measurements. 1.5×10^{-5} M MBC solutions were prepared in the presence of different concentrations of Q6, see results and discussion section. All samples were made in 0.20 M aqueous Na_2SO_4 solutions (necessary for significant Q6 solubility). The calculated pH value for a 0.2 M solution of $SO4^{-2}$ base ($K_b = 8.3 \times 10^{-13}$) is pH = 7.61, as expected from solving a typical weak base equilibrium problem.

Fluorescence spectroscopy

All absorption and fluorescence measurements were performed on solutions in 1 cm^2 quartz cuvettes. Absorption spectra were measured on a Labomed UV–vis spectrophotometer, while fluorescence spectra were measured on Edinberg luminescence spectrometer with excitation and emission band width of 4 nm. At the excitation wavelength used (285 nm), there was a small absorption by the host cucurbit[6]uril, increasing the absorbance from 0.1937 in the absence of Q6 to 0.4210 at the highest [Q6] = 4.182 mM. Blank solutions containing Q6 only were found to exhibit emission. For this reason, the fluorescence of these blank solutions was measured at the same [Q6] and at the same temperatures as for the carbendazim solutions, and the measured integrated fluorescence spectra were subtracted from that of the solutions containing guest.

NMR spectroscopy

All NMR spectra were performed on a Bruker AMX 400 MHz spectrometer in D_2O with KD_2PO_4/K_2DPO_4 buffer (pH = 6.80, 0.023 M). All ^1H NMR spectra are referenced in ppm with respect to a TMS standard.

Differential scanning calorimetery (DSC)

DSC measurements were performed using a Shimadzu DSC-50. DSC was used as a qualitative measure to characterize the formation of a true inclusion complex. Pure MBC, Q6, the physical mixture (1:1 molar ratio) and the equimolar complex were desiccated over phosphorous pentoxide for 2 days prior to assay to remove surface absorbed water.

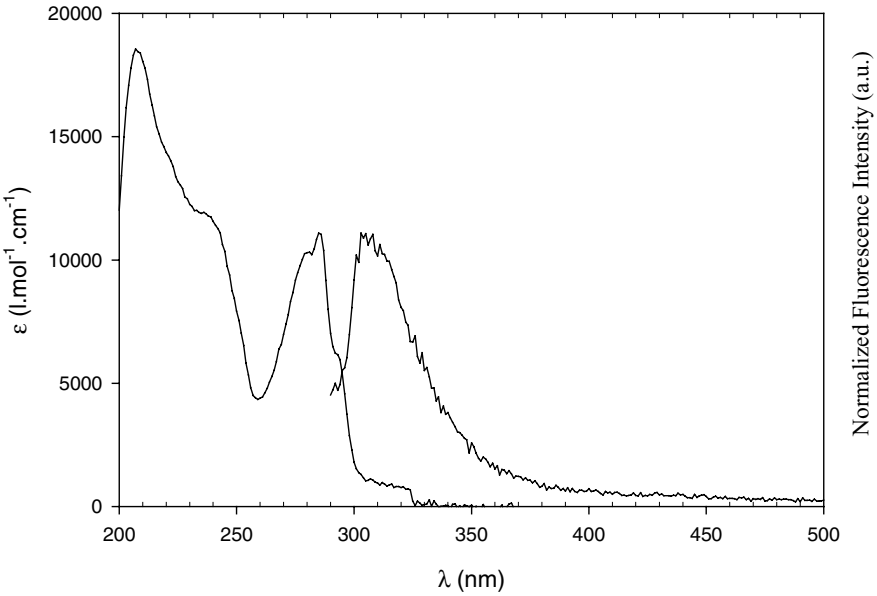


Fig. 2 Absorption and fluorescence spectra of 1.5×10^{-5} M MBC in 0.2 M Na_2SO_4 aqueous solution (pH = 7.61) at 298 K. Intensity of the emission spectrum (excitation at 285 nm) has been normalized for comparison purpose

Samples (10–15 mg) were placed into pierced aluminum pans with a perforated lid under static air and scanned over the temperature of 50–500°C at a heating rate of 5°C/min.

Preparation of solid samples (for DSC measurements)

The solid complex of MBC with Q6 in 1:1 molar ratio was prepared by co-evaporation. MBC (3 mg) was dissolved in ten milliliters of 2 wt% acetic acid (at 80°C) and stirred at 250 rpm for 30 min to achieve the solution of the drug, the hot solution was filtered with a 0.45-m filter, then Q6 powder (15.6 mg) was slowly added to the solution. The resulting mixture was stirred for 10 h at 80°C and was dried to obtain a powder. The powder was washed three times with absolute ethanol and the solvent was eliminated by vacuum evaporation at 80°C. The final product was pulverized and sieved (75–150 μm).

The physical mixture of MBC and Q6 in 1:1 molar ratio was obtained by mixing 3 mg of MBC with 15.6 mg Q6, that had previously been sieved (75–150 μm), together with a mortar and spatula for 10 min.

Results and discussion

Figure 2 shows the absorption and fluorescence spectra ($\lambda_{exc} = 285$ nm) of 1.5×10^{-5} M carbendazim (MBC) in 0.2 M Na_2SO_4 aqueous solution (pH = 7.61). When solid cucurbit[6]uril (Q6) was added to the aqueous solution of MBC, the fluorescence of MBC was found to increase significantly, as shown in Fig. 3. It should be noted that the spectra in Fig. 3 have been corrected for the emission of the Q6 reference solutions. Moreover, a blue shift of the spectrum was also observed with a fluorescence maximum of 305 nm in the absence of Q6 and 294 nm in the presence of approximately 4 mM Q6. The absorption and fluorescence peaks in the presence of different concentrations of Q6 are tabulated in Table 1. It should be said that addition of Q6 has small influence on the absorption spectral features of MBC.

The association constant (K_a) assuming the formation of 1:1 guest-host complex can be calculated by the use of the following modified Benesi-Hildebrand equation using the fluorescence data.

$$\frac{1}{F - F_0} = \frac{1}{[Q6]K_a\alpha} + \frac{1}{\alpha} \tag{1}$$

where [Q6] represents the concentration of cucurbit[6]uril, F_o and F are the fluorescence intensities in the absence and presence of cucurbit[6]uril, respectively, and α is constant. The K_a value was obtained from the slope and intercept of the double reciprocal plot (inset of Fig. 3). The plot was linear ($R = 0.995$) confirming 1:1 guest-host complexation. From the plot, K_a is evaluated as 271 ± 10 M^{-1} at 297 K. The Benesi-Hilderbrand plots assuming 1:2 stoichiometry can be represented by Eq. (2):

$$\frac{1}{F - F_0} = \frac{1}{[Q6]^2K_{a2}\alpha} + \frac{1}{\alpha} \tag{2}$$

where K_{a2} represents the association constant of 1:2 guest-host complex. The double-reciprocal plot is curved, indicating that MBC/Q6 complex has a 1:1 stoichiometry.

Thermodynamics Enthalpy, Entropy, and Gibbs free energy values were obtained for the inclusion reaction by measuring the fluorescence spectra of MBC in presence of Q6 at different temperatures. Figure 4 gives the double

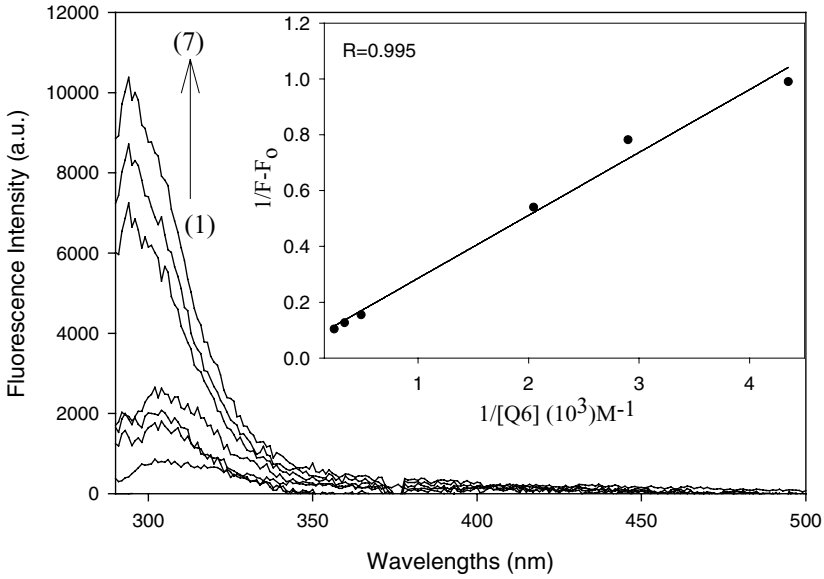


Fig. 3 Fluorescence spectra of MBC in the presence of: (1) 0 mM; (2) 0.2298 mM; (3) 0.3446 mM; (4) 0.4890 mM; (5) 2.068 mM; (6) 2.987 mM; and (7) 4.182 mM of Q6. The inset shows Benesi-Hildebrand plots for 1:1 stoichiometry. All emission spectra were obtained in 0.2 M Na_2SO_4 solution (pH = 7.61) of 1.5×10^{-5} M MBC at 297 K with an excitation at 285 nm. The calculated association constant (K_a) for the 1:1 MBC:Q6 inclusion complex is 270 M^{-1}

reciprocal plot at various temperatures. The obtained K_a values at various temperatures are as follows: $K_a = 367$ (281 K), 327 (289 K), 271 (297 K), 215 (307 K) and 175 M^{-1} (317 K). The association constants were found to decrease with increasing temperatures. The K_a values were used to calculate the thermodynamic parameters by classical method of plotting ln K_a against 1/T (i.e., the Van't Hoff method). In this case, the corresponding enthalpy ΔH° and entropy ΔS° are obtained from the slope and intercept of the graph (R = 0.994). We have obtained $\Delta H^\circ = -15.455$ kJ $mole^{-1}$ and $\Delta S^\circ = -5.66$ J $mol^{-1}K^{-1}$. The results indicate that the formation of complex with Q6 is favored by enthalpic factors. In the cavity of cucurbit[6]uril, three water molecules are enclosed and additional water molecules are located at the carbonyl groups of the ligand. Therefore, the release of water molecules from the cavity only results in small enthalpic contributions. The main contribution to the reaction enthalpies results from dipole-dipole interactions between the amido-ester group of the MBC and the carbonyl groups of Q6 and additional solvent molecules are released during the complex formation. Similar results were obtained by Buchmann *et al.* [13] who indicated that the ion-dipole interactions between the protonated amino groups of amino acids and the carbonyl groups of Q6 are the important factor in the formations of the corresponding host-guest inclusion complexes. The negative ΔS° value is expected due to the restriction in the motion of the encapsulated MBC. The value of the relevant free energy change for this system at 298 is $\Delta G^\circ = -13.77$ kJ mol^{-1} indicating an energetically favored reaction.

It is important to rationalize physically the fluorescence enhancement and the blue shift of MBC upon the addition of solid Q6. The photophysical behavior of MBC excited state is well documented in literature [18, 19]. The absorption and fluorescence spectra of MBC are mirror image with usual stokes' shift (20 nm, see Fig. 2). The sole emission band at 305 nm was assigned to $S_1 \rightarrow S_0$ transition, which is a characteristic of benzimidazole group (i.e., local monomer excited state). No interactions such excimer or exciplex (planar or twisted electron transfer) were indicated in literature. We have confirmed this result by observing similar spectral features of MBC emission spectra (Fig. 2) upon

Table 1 The absorption and fluorescence peak positions of 1.5×10^{-5} M MBC in 0.2 M Na_2SO_4 aqueous solutions (pH = 7.61) in the absence and presence of different concentrations of Q6 and at different temperatures in Kelvin (K)

Concentration of Q6 mM	Absorption maximum λ_a nm (log ε)		Fluorescence maximum λ_f nm (Excitation wavelength = 285 nm) 297 K, 307 K and 317 K	280 K and 289 K
0	284 (4.11)		305	294
0.2298 (~ one quarter)			305	294
0.3446 (~ one third)			305	294
0.4890 (~ half)			305	294
2.068 (~ double)			294	294
2.987 (~ triple)			294	294
4.182 (~ 4 times)	282 (4.45)	276 (4.51)	294	294

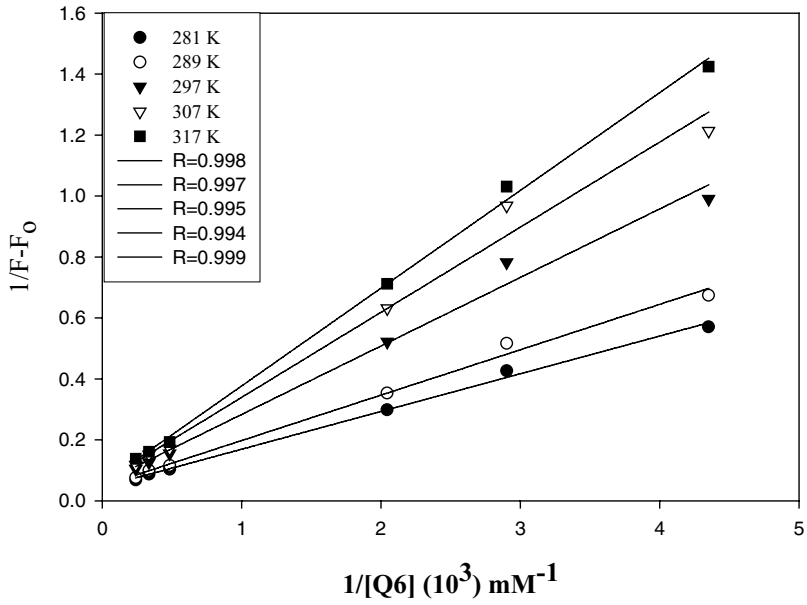


Fig. 4 Benesi-Hilderbrand plot for 1:1 MBC/Q6 inclusion complex at various temperatures

changing MBC concentrations, solutions temperatures, and excitation wavelengths. In short, no geometrical rearrangements or excited-state reactions are expected for MBC's excited state. The S_1 emission was enhanced and blue shifted in Q6 because the less polar interior cavity of Q6 destabilized the energy of S_1 state, which in turns should decrease the non-radiative deactivation pathways. It might be proposed that the enhancement is not a result of decreased rotational freedom of MBC, but instead a result of decreased polarity of its media. This proposal is supported by the fact that the blue shift observed for MBC in Q6 is much larger than that observed for naphthalene-6-sulfonic acid (2,6-ANS) (less than 3 nm) upon inclusion of the phenyl ring by Q6 [12]. The enhancement in the latter was due to loss in rotational mobility of the phenyl group relative to the naphthyl fluorophore. This argument, however, needs more real support. Direct evidence for inclusion, as well as specific mode of inclusion, could be obtained from X-ray crystallography, if single crystals of the MBC: Q6 complex could be obtained. We are currently working on this approach to conclusively determine the structure of the MBC: Q6 complex.

It is interesting to compare our results to those reported for MBC in α-cyclodextrin (α-CD), which has a slightly larger cavity size (5.7 vs. 5.5 Å), but with a much larger opening (5.7 vs. 4.0 Å). A 1:1 inclusion complex of MBC by α–CD was reported with a lower value for the association constant; the fit value was 14.2 M^{-1} in α –CD, [6] compared with the value of 271 M^{-1}in Q6. The association constant in Q6 can also be compared with those values in β-CD, which has a much larger cavity size (7.8 vs. 5.5 Å), and a much larger opening (7.8 vs. 4.0 Å). The association constant of the 1:1 inclusion complex of MBC by β –CD was reported to be only 22.7 M^{-1} in β-CD, [6] compared with the value of 271 M^{-1}in Q6. Thus MBC forms a much stronger complex with Q6 than with the larger-cavity β-CD. It is proposed that the dipole-dipole interactions of the polar carbonyl-portal in Q6, which interacts strongly with the polar amido-ester end of MBC, seem to enhance the encapsulation process of the probe MBC.

MBC readily forms a stable 1:1 host-guest complex with Q6 in water as evidenced by ^{1}H NMR, which supports the proposed inclusion structure. Figure 5 shows the ^{1}H NMR spectra of MBC as a function of Q6 concentration. In the presence of Q6, the MBC resonances due to the methyl moiety of the amido-ester is initially broaden (Fig. 5B; ○ , ●) and is significantly shifted to higher field after the complex formation (Fig. 5C), while those signals attributed to the benzene moiety of benzimidazole (Fig. 5, ▼) are essentially unchanged. The observed upfield shift of methyl protons (Fig. 5C; ●) and the unchanged of the resonance of aromatic benzimidazole protons as a function of Q6 concentrations is characteristic of molecules encapsulated in the Q6 cavity and indicates that the MBC complexes with Q6 via encapsulation of the methyl group of amido-ester, and the benzimidazole group of MBC is located outside the cavity of Q6.

Solid samples prepared by co-evaporation (in 2 wt% acetic acid solution) have been studied, using differential scanning calorimetry to assess the formation of the inclusion complex. Figure 6 shows the DSC curves for Q6, MBC, physical mixture of Q6 and MBC, and the solid complex of MBC with Q6 in 1:1 molar ratio. The curve of Q6 (Fig. 6A) displays a wide broad (from 100 to 150°C) and corresponds to the dehydration of Q6 ($T_{max} = 120.8$°C). The second endothermic effect, with a maximum at 430°C, is the main step of the Q6 decomposition. The MBC thermal curve (Fig. 6B) is typical of crystalline anhydrous substances and is

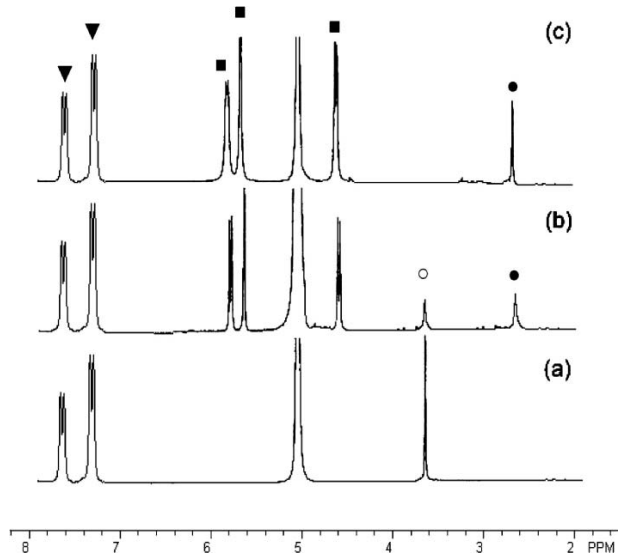


Fig. 5 1H NMR spectra of MBC in the absence of Q6 (A); after the addition of 0.5 equivalents of Q6 (B); and after the addition of 1 equivalent of Q6 (C). ■ , Q6; •, included MBC; ○ , free MBC

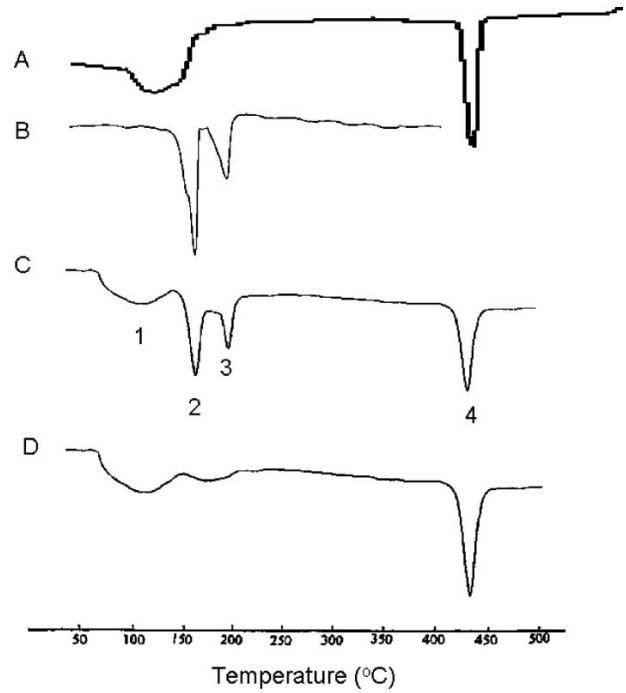


Fig. 6 DSC curves for: (A) Q6; (B) MBC; (C) physical mixture; (D) complex

characterized by a sharp endothermic effect (peaks temperature at 165 and 200°C); these endotherms are assigned to likely unstable forms of the anhydrous MBC. The thermogram of the physical mixture (Fig. 6C) shows the broadband endothermic effect due to the Q6 dehydration process (1) and Q6 decomposition (4). The MBC melting peaks (2 and 3) was observed at the same temperature in absence of Q6. The thermal curve of the complex (Fig. 6D) is similar to that of Q6 and dissimilar to the physical mixture. These observations indicated the formation of inclusion complex between MBC and Q6 in 1:1 molar ratio.

Conclusion

Fluorescence enhancement of the fungicide carbendazim by cucurbit[6]uril has been observed in solution due to formation of a host–guest inclusion complex. The enhancement of the MBC fluorescence (maximum factor of 10) is accompanied by a significant blue shift in the spectrum (11 nm). The proposed structure of the 1:1 inclusion complex would reduce the polarity of benzimidazole fluorophore by the hydrophobic interaction with Q6 interior wall, as well as, by shielding it from water molecules. The reduction in the polarity of MBC leads to an increase in the energy gap between the excited and ground state, which result in emission enhancement.

In comparison to α-CD, the cyclodextrin most similar in cavity size, cucurbituril was found to bind carbendazim more strongly. The association constant in Q6 is even large than that obtained in β-CD, the cyclodextrin with larger cavity size. These results indicate the important role of the carbonyl portals of Q6 in the inclusion process.

The ^{1}H NMR, and DSC results support the formation of 1:1 MBC-Q6 complex, in which the amido-ester group rather than the benzimidazole group is incorporated inside the cavity of Q6.

In general, this work demonstrates the potential usefulness of cucurbit[6]uril in fluorometric analysis of fungicide for agricultural and environmental applications.

Acknowledgements N. S would like to acknowledge Yarmouk University for their financial support (grant no. 2003/26).

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