

Studies on the Supramolecular Interaction between Dimethomorph and Disulfide Linked β -Cyclodextrin Dimer by Spectrofluorimetry and Its Analytical Application

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ABSTRACT: The supramolecular interaction of disulfide linked β -cyclodextrin (β -CD) dimer and dimethomorph has been studied by spectrofluorimetry. Based on the significant enhancement of the fluorescence intensity of dimethomorph, a new spectrofluorimetric method with high sensitivity and selectivity was developed for the determination of dimethomorph in bulk aqueous in the presence of the disulfide linked β -CD dimer. The inclusion complexation behavior of β -CD dimer with dimethomorph was studied in a $\text{KH}_2\text{PO}_4\text{--H}_3\text{PO}_4$ buffer solution of pH 3.86 at room temperature. The apparent association constant of the complex was 2.25×10^4 L/mol. The linear range was 12–7500 ng/mL with the detection limit 3.70 ng/mL, and the limit of quantification was 12.4 ng/mL. The proposed method had been successfully applied to the determination of dimethomorph residues in vegetables with recoveries of 89.0–115%.

KEYWORDS: disulfide linked β -cyclodextrin dimer, dimethomorph, supramolecular interaction, spectrofluorimetry

INTRODUCTION

Dimethomorph, chemically, 4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)acryloyl]morpholine (Figure 1) is a highly effective fungicide with low toxicity, which is widely used to protect plants from fungi. Dimethomorph has been shown to be effective against many crop diseases caused by peronosporales or phytophthora such as late blight and blue mold.¹ However, the adverse effects of pesticides on both human health and the environment have been a matter of public concern. Thus, both the actual state and the transition of dimethomorph residue in various materials including water, soil, and agricultural products should be extensively monitored. At present, methods for separation and determination of dimethomorph have been reported, such as GC-ECD,² GC-MS,^{3,4} HPLC-MS.⁵ Most of these methods used complicated extraction and cleanup procedures. It would be advantageous to develop a method utilizing the sensitivity and selectivity of spectrofluorimetry to simply and rapidly determine dimethomorph residues.

Cyclodextrins (CDs) are made up of glucose units coupled to form a rigid, conical structure with an interior hydrophobic cavity. CDs have a unique ability to form stable inclusion complexes with a variety of molecules in which target molecules are included in the hydrophobic cavities of CDs through host-guest complexation in aqueous solution.^{6–8} This inclusion capability has led to an important use of cyclodextrins in various fields, such as analytical chemistry, enzymology, pharmaceuticals, pesticides and so on.^{9–16}

As a very important family of cyclodextrin derivatives, CD dimers are known to greatly enhance the original molecular binding ability and selectivity of native CDs through the cooperative binding of a single model substrate by two hydrophobic cavities located in a closely vicinity.¹⁷ Hence, diverse functional groups such as organoseleniums,¹⁸ disulfides,¹⁹ pyridines,^{20,21}

ethylene glycol,²² aromatic diamine²³ and pyrene²⁴ have been used as the linker between two cyclodextrin units. Unexpectedly, their molecular recognition behaviors have not been extensively investigated.

In this paper, we studied the supramolecular interaction between dimethomorph and disulfide linked β -CD dimer (Figure 2). On the basis of the significant enhancement in the fluorescence of dimethomorph produced through complexation with disulfide linked β -CD dimer, the determination of dimethomorph was developed by spectrofluorimetry. Then we applied the supramolecular recognition to the determination of dimethomorph in cucumber and potato with satisfactory results.

MATERIALS AND METHODS

Apparatus. All the spectrofluorimetric measurements were carried out on a Cary Eclipse (Varian, Australia) spectrofluorimeter equipped with a xenon lamp and 1.0 cm quartz cells. pH measurements was made with a pH-3 digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China), with a combined glass-calomel electrode.

Reagents. Dimethomorph standard (99.5%) was provided by Shangdong Institute for the Control of Agrochemicals (China). Its stock solution (1.00 mg/mL) was prepared with methanol. Disulfide linked β -CD dimer was prepared according to our reported procedures.²⁵ Other chemicals used were analytical reagent grade. Double distilled water was used throughout.

Calibration Graph. Into a series of 10 mL colorimetric tubes were added different aliquots of the dimethomorph stock solution containing 0–7.5 $\mu\text{g/mL}$ of dimethomorph, 4.00 mL of 1.00×10^{-3} M disulfide linked β -CD dimer, and 2.00 mL of 0.10 M $\text{KH}_2\text{PO}_4\text{--H}_3\text{PO}_4$

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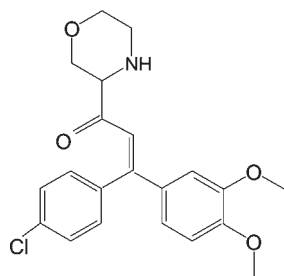


Figure 1. The structure of dimethomorph.

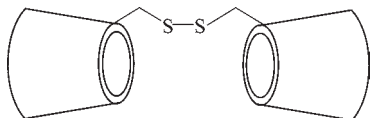


Figure 2. The structure of disulfide linked β -CD dimer.

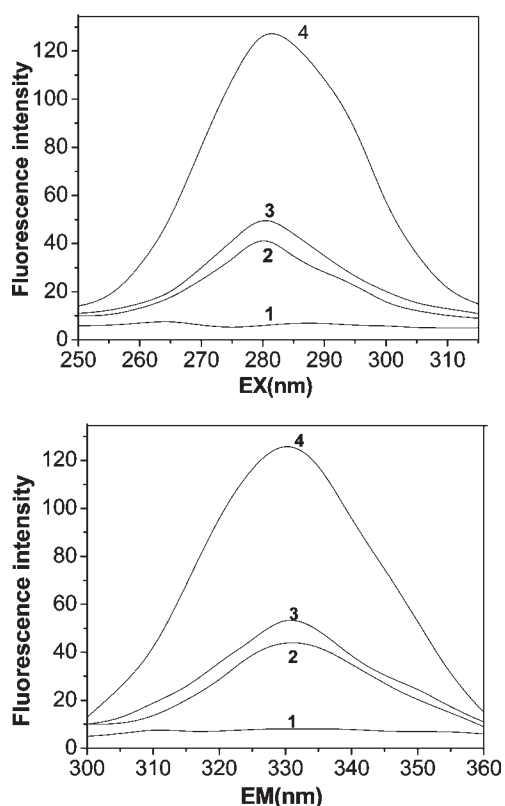


Figure 3. Excitation and emission spectra: (1) reagent blank; (2) dimethomorph solution; (3) dimethomorph + β -CD; (4) dimethomorph + β -CD dimer. $C_{\text{dimethomorph}} = 1.00 \mu\text{g/mL}$, $\text{pH} = 3.86$, $C_{\beta\text{-CD}} = 8.00 \times 10^{-4} \text{ M}$, $C_{\beta\text{-CD dimer}} = 4.00 \times 10^{-4} \text{ M}$.

buffer solution ($\text{pH} = 3.86$) sequentially. The mixture was diluted to the mark with double distilled water, shaken thoroughly and equilibrated at room temperature for 10 min. Then the fluorescent intensity of the solution was measured at $\lambda_{\text{ex/em}} = 280/329 \text{ nm}$ against a reagent blank.

Determination of the Apparent Association Constant.

1.00 mL of 10.0 $\mu\text{g/mL}$ dimethomorph, varied amounts of $1.00 \times 10^{-3} \text{ M}$ disulfide linked β -CD dimer, and 2.00 mL of 0.10 M $\text{KH}_2\text{PO}_4\text{--H}_3\text{PO}_4$ buffer solution ($\text{pH} = 3.86$) sequentially were added

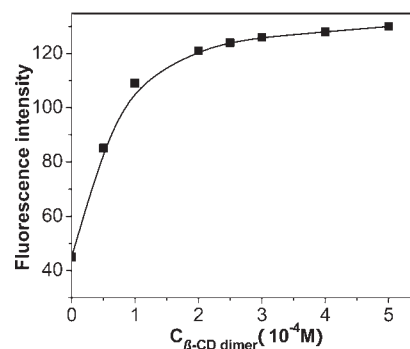


Figure 4. Influence of disulfide linked β -CD dimer concentration on the fluorescence intensity of the complex. $C_{\text{dimethomorph}} = 1.00 \mu\text{g/mL}$, $\text{pH} = 3.86$.

into 10 mL colorimetric tubes and diluted to 10 mL with double distilled water. The mixed solution was shaken thoroughly and equilibrated at room temperature for 10 min. Then the fluorescent intensity of the solution was measured at $\lambda_{\text{ex/em}} = 280/329 \text{ nm}$ against a reagent blank.

Sample Treatment. The sample treatment was done according to the following procedures: Into a 125 mL screw-capped tube were added 20.0 g of the chopped vegetables and a certain quantity of dimethomorph. The vegetables were extracted with 30 mL of acetonitrile for 20 min in an ultrasonic bath at room temperature. The supernatant was collected and dried in a rotary evaporator at 40°C . The obtained solids were dissolved with 2.00 mL of methanol and diluted with water to 10 mL, and this solution was used for analytical determination. The dimethomorph content was calculated according to the linear regression equation.

Statistical Analysis. All experiments were repeated three times, and the data were calculated with Microsoft Excel. Statistical significance was established at $p < 0.05$.

RESULTS AND DISCUSSION

Excitation and Emission Spectra. According to the procedure above, the excitation and emission spectra were scanned (Figure 3) on a Cary Eclipse (Varian, Australia) spectrofluorimeter equipped with a xenon lamp and 1.0 cm quartz cells. The wavelengths of maximum excitation and emission were 280 nm and 329 nm respectively. As can be seen, dimethomorph possessed a low fluorescence quantum yield in aqueous solution, and a significant increase of the fluorescence intensity was observed when disulfide linked β -CD dimer was added to the aqueous solution of dimethomorph. Complexation of dimethomorph with disulfide linked β -CD dimer exhibited greatly enhanced fluorescence intensity compared to the complexation with native β -CD. It was due to the cooperative binding of two adjacent hydrophobic cavities with dimethomorph in disulfide linked β -CD dimer.

Influence of Disulfide Linked β -CD Dimer Concentration.

The CD concentration is an essential parameter for the optimization of the fluorescence intensity of the complex. The influence of disulfide linked β -CD dimer concentration is shown in Figure 4. The fluorescence intensity of the complex increased with the increasing concentration of disulfide linked β -CD dimer. The fluorescence intensity reached a maximum over a CD range of $3.00 \times 10^{-4} \text{ M}$ to $5.00 \times 10^{-4} \text{ M}$. Thus $4.00 \times 10^{-4} \text{ M}$ disulfide linked β -CD dimer was selected.

Influence of pH. The pH of the buffer solution is another important parameter affecting the fluorescence intensity of the

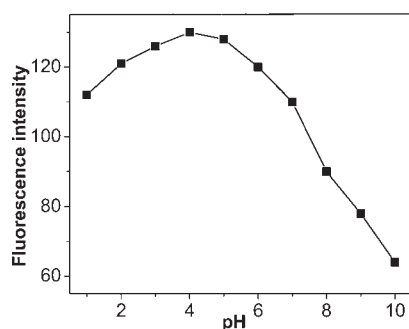


Figure 5. Influence of pH on the fluorescence intensity of the complex. $C_{\text{dimethomorph}} = 1.00 \mu\text{g/mL}$, $C_{\beta\text{-CD dimer}} = 4.00 \times 10^{-4} \text{ M}$.

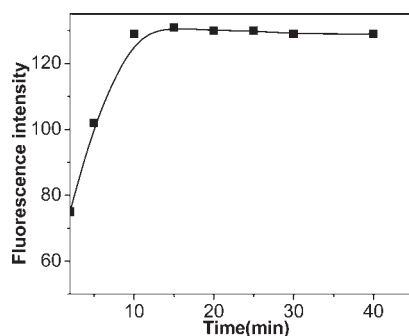


Figure 6. Effect of reaction time on fluorescence intensity of the complex. $C_{\text{dimethomorph}} = 1.00 \mu\text{g/mL}$, $\text{pH} = 3.86$, $C_{\beta\text{-CD dimer}} = 4.00 \times 10^{-4} \text{ M}$.

complex.²⁶ Experiments were performed at different pH values in the range of 1.00–10.0. Figure 5 showed that the optimum pH for dimethomorph measurements was 3.00–5.00. Therefore, a pH of 3.86 was fixed using $\text{KH}_2\text{PO}_4\text{--H}_3\text{PO}_4$ buffer solution.

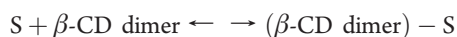
As the volume of the buffer added (from 1.00 to 4.00 mL) had little effect on the fluorescence intensity, 2.00 mL of buffer solution was used in subsequent experiments.

Influence of Temperature and Reaction Time. The higher temperature increased the collision of molecules, which made the collision probability of excited molecules and solvent molecules increase and resulted in the decline of fluorescent intensity.²⁷ Therefore room temperature was finally chosen.

The experimental results (Figure 6) showed that the fluorescence intensity reached a maximum at 10 min and remained constant for at least 40 min. Hence, after inclusive reaction was carried out for 10 min, the subsequent fluorescence measurement was made.

Apparent Association Constant. The apparent association constant of the inclusion complex was calculated by the following method:

Assuming stoichiometry 1:1 complexation, the inclusion complexation of dimethomorph (S) with $\beta\text{-CD}$ dimer is expressed by



The formation constant of the complex (K) is given by

$$K = \frac{[(\beta\text{-CD dimer})\text{-S}]}{[\text{S}][\beta\text{-CD dimer}]}$$

where $[\beta\text{-CD dimer}]$, $[\text{S}]$ and $[(\beta\text{-CD dimer})\text{-S}]$ are equilibrium concentrations. An apparent association constant value for the inclusion complex can be determined through typical double

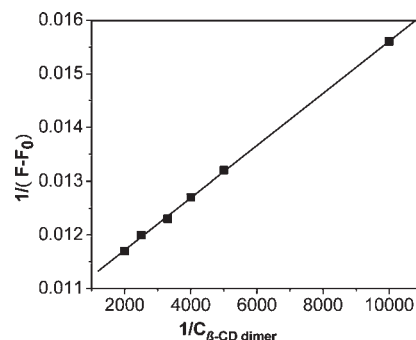


Figure 7. Plot of $1/(F - F_0)$ versus $1/C_{\beta\text{-CD dimer}}$.

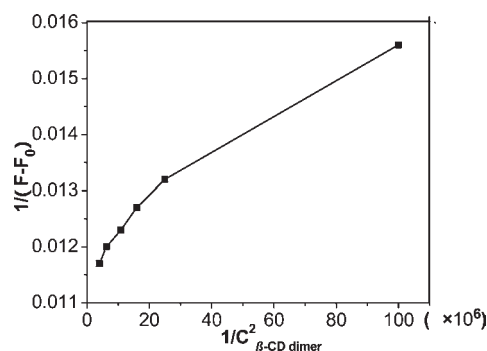


Figure 8. Plot of $1/(F - F_0)$ versus $1/C_{\beta\text{-CD dimer}}^2$.

reciprocal plots:²⁸

$$\frac{1}{(F - F_0)} = \frac{1}{[(F_\infty - F_0)KC_{(\beta\text{-CD dimer})}] + 1/(F_\infty - F_0)} \quad (1)$$

where F is the observed fluorescence intensity of the dimethomorph solution at each disulfide linked $\beta\text{-CD}$ dimer concentration tested, and F_0 and F_∞ are the fluorescence intensity in the absence of the disulfide linked $\beta\text{-CD}$ dimer and when all the dimethomorph molecules are complexed, respectively. Disulfide linked $\beta\text{-CD}$ dimer is in large excess with respect to dimethomorph, where $C_{(\beta\text{-CD dimer})}$ denotes the initial $\beta\text{-CD}$ dimer concentration.

A good linear relationship was obtained when $1/(F - F_0)$ was plotted against $1/C_{(\beta\text{-CD dimer})}$ supporting the existence of a 1:1 complex ($R = 0.9994$, Figure 7). Its apparent association constant was determined to be $2.25 \times 10^4 \text{ L/mol}$.

Assuming that dimethomorph and $\beta\text{-CD}$ dimer form a 1:2 complex, it can be written as



where S denotes dimethomorph; the formation constant of the complex (K') is given by

$$K' = \frac{[(\beta\text{-CD dimer})_2\text{-S}]}{[(\beta\text{-CD dimer})]^2[\text{S}]}$$

If $[\beta\text{-CD dimer}] \gg [(\beta\text{-CD dimer})_2\text{-S}] \gg [(\beta\text{-CD dimer})\text{-S}]$, then the following expression is obtained:

$$\frac{1}{(F - F_0)} = \frac{1}{[(F_\infty - F_0)k'C_{\beta\text{-CD dimer}}^2 + 1/(F_\infty - F_0)]}$$

When making a plot of $1/(F - F_0)$ against $1/C_{\beta\text{-CD dimer}}^2$, no linear relationship can be observed (Figure 8), which indicates that the composition of the complex is not 1:2.

Table 1. Influence of Foreign Substances on the Dimethomorph of 1.00 $\mu\text{g}/\text{mL}$ Dimethomorph (Tolerance Error $\pm 5.0\%$)

tolerance ratio (m/m)	interference
3000	K^+ , Na^+ , Cl^- , glucose, sucrose
2000	NO_3^- , CO_3^{2-} , PO_4^{3-}
1500	starch
1000	Ca^{2+} , Ba^{2+} , Mg^{2+} , Sr^{2+} , Mn^{2+} , Zn^{2+}
500	Fe^{3+} , NH_4^+ , Cu^{2+} , Pb^{2+}
100	ascorbic acid
50	folic acid

Table 2. Determination of Dimethomorph in Vegetables ($n = 6$)

dimethomorph added ($\mu\text{g}/\text{g}$)	recovery (%)		GC-ECD method	
	recovery (%)	RSD (%)	recovery (%)	RSD (%)
Cucumber				
0.02	115	6.8	96.5	6.2
0.05	98.0	6.4	104	5.7
0.50	106	5.8	102	4.9
1.00	97.0	3.6	93.5	4.0
Potato				
0.02	89.0	7.3	92.0	6.9
0.05	101	6.5	98.5	5.9
0.50	112	6.2	96.0	5.2
1.00	103	4.7	88.5	3.7

Analytical Characteristics. Under the optimized experimental conditions, the calibration graph was linear in the concentration of the dimethomorph range of 12–7500 ng/mL. The regression equation was $\Delta F = 22.14 + 0.087[\text{dimethomorph}]$ (ng/mL), and the correlation coefficient was 0.9992. The limit of detection (LOD) was determined to be 3.70 ng/mL according to formula $C = KS_0/S$, where the value of K was taken as 3, the standard deviation was 0.108 obtained from a series of 11 reagent blanks, and S was the slope of the standard curve. The limit of quantification (LOQ) was 12.4 ng/mL, which was calculated according to the formula of $C = 10S_0/S$. The relative standard deviation (RSD) was 2.6% for 11 determinations of 1.00 $\mu\text{g}/\text{mL}$ of dimethomorph.

Influence of Interference. To evaluate the potential effect of foreign species on the determination of dimethomorph at 1.00 $\mu\text{g}/\text{mL}$, a systematic study was carried out. A 3000-fold mass excess of each substance over dimethomorph was tested first, and if interference occurred, the ratio was reduced gradually until the interference ceased. The tolerance limit was defined as the ratio of the concentration of the foreign species to that of dimethomorph which resulted in an error of less than 5%. The results (Table 1) showed that this method had high selectivity.

Application. The proposed method was applied to the determination of dimethomorph residues in cucumber and potato. The accuracy, RSD, of the method was assessed by the determination of six replicate recoveries at each fortified value. The results obtained by the proposed method were compared to the GC-ECD method.² The results are shown in Table 2. As can be seen, the precision and accuracy of the proposed method were satisfactory, and there was no significant difference between the two methods.

In conclusion, a spectrofluorimetric method based on inclusion reaction for the determination of dimethomorph in bulk aqueous solution was developed. The results showed that disulfide linked β -CD dimer reacted with dimethomorph to form a 1:1 (host:guest) complex. The method is simple, highly sensitive and selective and has been successfully applied to the determination of dimethomorph in vegetables.

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