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Sensitized effect of β-cyclodextrin on the fluorescence in the determination of carbaryl

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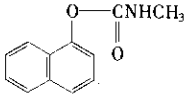
Abstract: Based on the significant enhancement of fluorescence intensity of carbaryl in inclusion complex, a spetrofluorimetric method with high sensitivity was developed for the determination of carbaryl in aqueous solution. Under the optimum conditions, the complex had excitation and emission maxima at 278 nm and 332 nm, respectively. The linear range of the method was 7.0 ng/ml—1500 ng/ml with a detection limit of 1.2 ng/ml. The proposed method was successfully used to determine quantitatively of carbaryl in cottonseeds.

Keywords: carbaryl; β-cycclodextrin; spectrofluorimetry; supramolecular interaction; determination

Introduction

1-naphthyl-N-methylcarbamate (carbaryl, Scheme 1) is a kind of highly efficacious carbamate insecticide which has been widely used in agriculture because it can kill over 100 species of injurious insects. It is also used as a molluscide and an acaricide. Carbaryl remained in crops is harmful to human's health by skin contact, inhalation or ingestion (Fang, 1998). In recent literature, various methods for separation and determination of carbaryl have been proposed, such as thin layer chromatography (TLC) (Rane, 1997), gas chromatography (GC) (Liang, 1999), high performance liquid chromatography (HPLC) (Tena, 1992; Galeano, 1996), enzyme-linked immunosorbent assay (ELISA) (Abad, 1997) and room-temperature phosphorimetry (RTP) (Capitan-Vallvey, 1998). The RTP method has been developed in recent years. But this method has many disadvantages. For example, O_2 must be removed in advance, the determination of carbaryl is influenced by the humidity and complicated operation is needed by this method. Therefore we need to develop a simple, highly sensitive and selective method such as spectrofluorimetry for the determination of carbaryl.



Scheme 1 The structure of carbaryl

Cyclodextrins (CD), the cyclic oligosaccharides consisting of six or more D-(+)-glucopyranose units, are well known to have the property of forming inclusion complexes with guest molecules which possess suitable polarity and dimension. The formation of the inclusion complex can alter the photochemical and photophysical properties of the guest molecules (Tang, 2002).

In this paper, we found that carbaryl exhibited intensive fluorescence signals in organic solvent; however, it possessed a low fluorescence quantum yield in the aqueous solution. When β-CD was added to the aqueous solution of carbaryl, an obvious increase in fluorescence intensity of carbaryl was observed. So the spectrofluorimetric study of the interaction between β-CD and carbaryl was carried out.

Based on the inclusion reaction, carbaryl was spectrofluorimetricly determined with high sensitivity and selectivity. The possible mechanism of the reaction was discussed. The stoichiometry and association constant of the β-CD-carbaryl complex were studied in details. The proposed method was applied to the determination of carbaryl remained in cottonseeds with satisfactory results.

1 Experimental

1.1 Apparatus

All fluorescence measurements were carried out on a Perkin-Elmer (Norwalk, CT, USA) LS-5 spectrofluorimeter, equipped with a xenon lamp, 1.0 cm quartz cells and a Perkin-Elmer Model 561 recorder. All pH measurements were made with a pHs-3C digital pH-meter (Shanghai Leici Device Works, Shanghai, China) with a combined glass-calomel electrode.

1.2 Reagents

Carbryl (obtained from Pesticide Appraisal Board of Agriculture, China, content > 99.8%) was used without further purification. The stock solution (1.0×10^{-3} mol/L) was prepared in absolute ethanol. β-cyclodextrin (purchased from China Medicine Group Shanghai Chemical Reagent Corporation) was purified by twice recrystallization in double-distilled water. After dried in vacuum at 60℃ for 1 h, 1.0×10^{-2} mol/L of β-CD was prepared. An acetic acid-sodium acetate buffer solution (0.10 mol/L, pH = 3.50) was used.

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All chemicals used were of analytical reagent grade. Double-distilled water was used throughout.

1.3 Procedure

1.3.1 Determination of carbaryl

In a 10 ml color comparison tube were placed 2.00 ml of β-CD(0.01 mol/L), 2.00 ml of acetic acid-sodium acetate buffer solution(pH = 3.50) and different amount of carbaryl standard solutions. After dilution to volume with demonized water, the mixture was shaken and equilibrated at room temperature for 30 min, then the fluorescence intensity of the solution was measured at 332 nm with excitation at 278 nm against a reagent blank.

1.3.2 Sample analysis

20.00 g of cottonseed were weighed precisely. After added 40 ml of water, the sample was triturated and dipped into ethyl acetate for 24 h. Then, the solution was put in an ultrasonator for 30 min. After filtrated, the filtrate was dealt with ultrasonication again. At last, the filtrate was collected and added 40 ml of water. After organic solvent was removed with vacuum distillation in 60℃ water bath, the sample was dried with N_2. A standard addition method was used to determine amount of carbaryl in cottonseed sample.

2 Results and discussion

2.1 Excitation and emission spectra

The spectral characteristics of carbaryl were studied and the result showed that the wavelengths of maximum excitation and emission of carbaryl at pH 3.5 were 278 nm and 332 nm, respectively(Fig. 1). When β-CD was added into the carbaryl solution, the wavelength of maximum of emission did not change but the fluorescence intensity dramatically increased. The possible reason is as follows: Carbaryl can enter the hydrophobic cavity of β-CD under the affection of non-covalent bond including van der Waals bond and hydrogen bond. In the cavity, the degree of motion freedom of carbaryl molecule reduced, so that the probability of radiationless transition decreased. At the same time, the cavity can shield the excited signal of carbaryl from quenching by quencher in the aqueous solution. So the fluorescence intensity increased when the β-CD-carbaryl inclusion complex was produced. Based on the inclusion reaction, carbaryl was determined at 332 nm with excitation at 278 nm.

2.2 Optimization of experimental variables

2.2.1 Influence of pH

Because of the instability of cyclodextrin at very low pH, the strongly acidic buffer solution was not used. But carbaryl could be hydrolyzed to form naphtholate in an alkaline medium(Sancenon, 1989) which was not easy to enter the cavity of β-CD, so that the inclusion complex was unstable and the fluorescence intensity decreased with increase of time. Because carbaryl was stable in weakly acidic buffer solution, the pH dependence of the system was

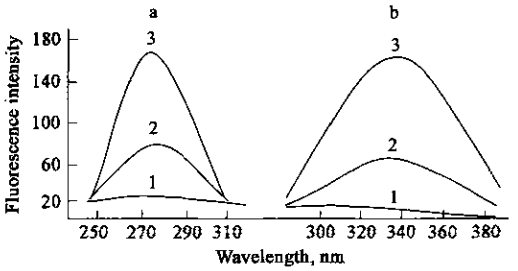

Fig. 1 Excitation(a) and emission(b) spectrum
1. reagent blank; 2. carbaryl solution; 3. β-CD-carbaryl inclusion complex; $C_{carbaryl}$ = 0.60 μg/ml; $C_{\beta\text{-}CD}$ = 2.0 × 10^{-3} mol/L

studied over the ranged 1.00 - 6.50. The experimental results(Fig. 2) showed that the fluorescence intensity was high and almost remained constant over the pH range between 1.50 and 4.80. Therefore, a pH of 3.50 was fixed with the use of acetic acid-sodium acetate buffer solution.

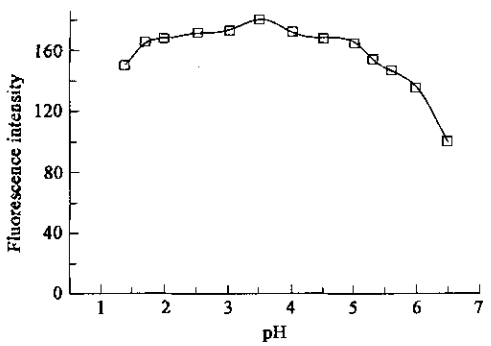

Fig. 2 Influence of pH on the fluorescence intensity of β-CD-carbaryl complex
$C_{carbaryl}$ = 0.60 μg/ml, $C_{\beta\text{-}CD}$ = 2.0 × 10^{-3} mol/L

2.2.2 Effect of the volume of buffer solution and reaction time

In a 10 ml color comparison tube, as the volume of the buffer added(from 1.00 to 3.00 ml) had little effect on the fluorescence intensity, 2.00 ml of buffer solution was used in subsequent experiments.

The effect of interaction time was studied. The result showed that the fluorescence intensity reached a maximum after the reagents had been added for 30 min and remained constant for at least 1 h. Hence, after inclusive reaction was carried out for 30 min, the subsequent fluorescence measurements were made at room temperature within 1 h.

2.2.3 Influence of organic solvent

The influence of organic solvent was discussed. The results in Table 1 showed that most of organic solvents had quenching effect on the reaction system.

Acetone and dimethyl formamide made seriously fluorescence quenching. Methanol and acetonitrile made slightly fluorescence quenching. But ethanol had no obvious influence on fluorescence intensity of carbaryl. So ethanol was used as organic solvent to dissolve carbaryl in the experiments.

Table 1 Effect of organic solvents on fluorescence intensity

Organic solvent	0	Methanol	Ethanol	Acetone	Acetonitrile	Dimethyl formamide
Relative fluorescence intensity	164	160	173	3.3	150	8.4

2.2.4 Influence of β-CD concentration

The effect of β-CD concentration is shown in Fig. 3, which demonstrated that the fluorescence intensity of carbaryl increased with increasing concentration of β-CD. When the volume of β-CD(1.0×10^{-2} mol/L) added from 1.80 ml to 2.20 ml in a 10 ml color comparison tube, the fluorescence intensity is relatively high and almost remained constant, so 2.00 ml was used in subsequent experiments.

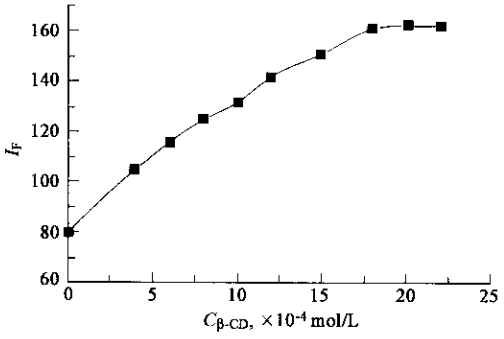

Fig. 3 Influence of β-CD concentration
$C_{carbaryl}$ = 0.60 μg/ml, pH = 3.50, λ_{ex} = 278 nm

2.3 Analytical characteristics

Under the optimum experimental conditions, there was a linear relationship between the fluorescence intensity and the concentration of carbaryl in the range of 7.0—1500 ng/ml with a correlation coefficient of 0.9991. The linear regression equation was $I_F = 0.2822C(\text{ng/ml}) + 6.990$. The standard deviation of the fluorescence measurements was 0.112 obtained from a series of 11 blank solutions. The limits of detection($k = 3$) and of determination($k = 10$) of the method were established according to the IUPAC definitions ($C_1 = kS_0/S$, C_1 is the limit of detection, S_0 is the standard deviation of the blank determination, S is the slope of calibration graph and k is the constant related to the confidence interval) and the values found were 1.2 ng/ml and 7.0 ng/ml, respectively. Relative standard deviation obtained from a series of 9 standards each containing 0.36 μg/ml of carbaryl was 2.2%.

2.4 Effect of interferences

A systematic study of interferences by foreign substances in the determination of 1.00 μg/ml of carbaryl was carried out. A 2000-fold mass excess of each substance over carbaryl was tested first. If interference occurred, the ratio was reduced gradually until the interference ceased. The criterion for interference was fixed at $a \pm 5\%$ variation of the average fluorescence intensity calculated for the established level of carbaryl. The result given in Table 2 showed that this method had high selectivity.

2.5 Application

The proposed method was applied to the determination of carbaryl in commercial cottonseed. To 1.00 ml of the prior separated sample solution were added various amounts of standard carbaryl solutions and the carbaryl content in cottonseed was determined by the proposed method. The standard addition method was used in the analysis procedure. The results are given in Table 3.

Table 2 Influence of exotic substances on the determination of 1.00 μg/ml carbaryl (tolerance error ± 5%)

Tolerance ratio, m/m	Interferences
2000	Glucose, sucrose, lactose, sorbitol, mannitol, sodium chloride, sodium carboxymethyl-cellulose
1500	NH_4^+, Ca^{2+}, Ba^{2+}, Mg^{2+}, Pb^{2+}, Sr^{2+}, Mn^{2+}, NO_3^-, gum acacia powder
1000	Starch, benzene, phenol
500	Cu^{2+}, CO_3^{2-}, gelatin, naphthol
4	Naphthalene

Table 3 Determination of carbaryl in cottonseed ($P = 0.95$)

Sample No.	Sample content, μg/ml	Carbaryl added, μg/ml	Carbaryl found, μg/ml, $n = 5$	RSD, %	Recovery, %
1	0.58	0.40	0.97	1.9	98
2	0.66	0.36	1.02	2.0	100
3	0.78	0.24	1.02	2.1	100
4	0.45	0.45	0.91	2.3	102
5	0.80	0.35	1.15	1.8	100

3 Conclusions

Under the supramolecular interaction, carbaryl can react with β-CD to form inclusion complex which gave off strong fluorescence. Based on the inclusive reaction, a spectrofluorimetric method for carbaryl determination was developed in this work. The proposed method is simple, sensitive and specific in supramolecular recognition for carbaryl. It was applied successfully to determination of carbaryl remained in cottonseeds.

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