Food Science and Nutrition Studies
ISSN 2573-1661 (Print) ISSN 2573-167X (Online)
Vol. 3, No. 4, 2019
www.scholink.org/ojs/index.php/fsns

Original Paper

A Synergistic Sensitized Fluorescent Determination of 2,4-Dichlorophenoxyacetic Acid in Vegetable Samples Based on the Derivatives of Calix[4]arene

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Received: October 24, 2019 Accepted: November 5, 2019 Online Published: November 18, 2019

doi:10.22158/fsns.v3n4p142 URL: http://dx.doi.org/10.22158/fsns.v3n4p142

Abstract

A novel fluorescent determination of 2,4-dichlorophenoxyacetic acid (2,4-D) based on the derivatives of calix[4]arene (SAX) in β-cyclodextrin(β-CD)/ sodium dodecyl sulfate (SDS) synergistic sensitized system was developed. The results were shown that the fluorescence intensity of SAX could be quenched by 2,4-D, and the fluorescence quenching ($\Delta F=F_{SAX}-F_{2,4\text{-}D\text{-}SAX}$) was synergistic sensitized in β-CD/ SDS medium. Under the conditions of $\lambda_{ex/em}$=332/468 nm and pH 7.0, the linear range for 2,4-D were found to be 0.020-4.00 μg/mL. The mechanism of determination was discussed with quenching type analysis, inclusion interaction and sensitizing effect. This method has been applied for the determination of 2,4-D in vegetable samples with satisfactory results.

Keywords

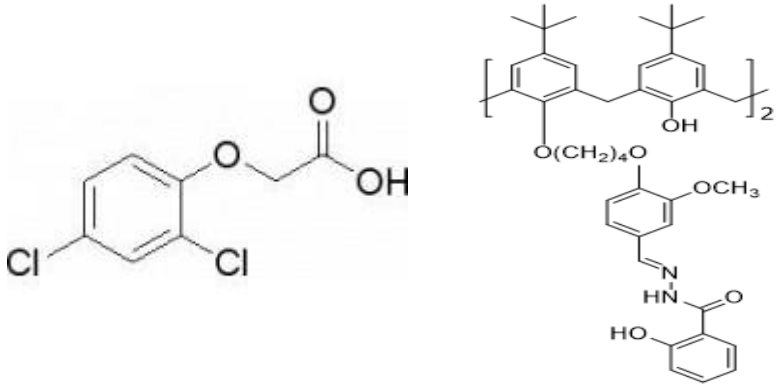
2,4-dichlorophenoxyacetic acid, β-cyclodextrin, SDS, synergistic sensitization, fluorescence quenching

1. Introduction

2,4-dichlorophenoxyacetic acid (2,4-D, Figure 1(a)) belongs to the category of benzoic acid pesticides, which has the biological activity of auxin and can be used as plant growth regulator and preservative for vegetable and fruit (Jiang, Zha, & Tie, 2015). Nevertheless, 2,4-D residues in agricultural products and environment have great harm to human health due to its carcinogenic, mutagenic and estrogenic activity (Garabrant & Philbert, 2002). Up to now the reported techniques for 2,4-D determination have been performed on LC-MS (Jiang, Zha, & Tie, 2015), fluorescence spectroscopy (Wang, Yua, & Wu, 2016;

Boroduleva & Eremin, 2016; Atta, Bera, & Chattopadhyay, 2015), high performance liquid chromatography(HPLC) (Wu, Ee, & Lee, 2005), capillary electrophoresis (CE) (Zhu & Lee, 2001) and gas chromatography(GC) (Rezazadeh, Yamini, Seidi, Tahmasebi, & Rezaei, 2014). Although these techniques have good performance, they are complicated, expensive and time-consuming. So it is necessary to establish a rapid, simple and high selectivity method for 2,4-D detection.

Calixarenes are macrocyclic compounds through a series of phenol connected with the ortho methylene units. In calixarene molecule, the upper edge is composed of para-position substituent of benzene; the lower edge is formed by neatly arranged phenolic hydroxyl group; the middle hydrophobic cavity is composed of benzene rings. Schiff base calix[4]arene (SAX, Figure 1(b)) has been followed with great interests due to the simple structure and high symmetry. The cavity of SAX is composed of four benzene rings, meeting the size and stability required for the inclusion interaction. The analytical method based on host-guest chemistry of calix[4]arene derivatives have been reported (Ma & Zhu, 2012; Yang, Yan, & Zhu, 2014; Wang, Zhu, & Yan, 2013; Yang, Qin, Yan, & Zhu, 2015; Li, X. Y., Li, M., & Chen, 2011; Khan, Shah, & Ahmed, 2016). But the fluorescence quenching methods using schiff base calix[4]arene derivatives as a fluorescent chemosensor for the determination of 2,4-D seems to be lacking.



(a) **(b)**

Figure 1. Chemical Structure of (a)2,4-D and (b)SAX

The sensitivity of spectral analysis could be improved in suitable medium, such as surfactant (Ma & Zhu, 2012; Yang, Yan, & Zhu, 2014; Wang, Zhu, & Yan, 2013; Yang, Qin, Yan, & Zhu, 2015; Li, X. Y., Li, M., & Chen, 2011), β-cyclodextrin (β-CD) (Sanchez, Rubio, & Blanco, 1988; Márquez, Hernández, & García, 1990; Zhang, Liu, & Fan, 2009; Sánchez, Lopez, & Gómez, 1987; Zhu, Sun, Bao, & Guo, 2006; Sun, Zhu, & Wu, 2007) room temperature ionic liquid (Liu & Zhao, 2008; Berton & Martinis, 2009; Martinis & Olsina, 2008; Zhu & Jiang, 2011). In our previous publications, the sensitizing effects of surfactant (Wu, Ee, & Lee, 2005; Zhu & Lee, 2001; Rezazadeh, Yamini, Seidi, Tahmasebi, & Rezaei, 2014; Ma & Zhu, 2012), β-CD and its derivatives (Zhu, Sun, Bao, & Guo, 2006; Sun, Zhu, & Wu, 2007), ionic liquids (ILs) (Zhu & Jiang, 2011) on the ultraviolet spectrometry and spectrofluorimetry

were developed. Further research showed that mixed medium (such as surfactant/ILs, β-CD/surfactant) could synergistically sensitize fluorescence method, which has a better sensitization effect than that single medium (Zhu, Sun, Bao, & Guo, 2006; Zhu & Jiang, 2011; Ren & Zhu, 2016).

In this study, the fluorescence intensity of SAX could be quenched by 2,4-D, the fluorescence quenching value ($\Delta F=F_{SAX}-F_{2,4\text{-D-SAX}}$) were enhanced in β-CD/SDS due to the synergistic sensitization, which has a much better quenching effect than that in single β-CD or SDS medium. There was a linear relationship between fluorescence quenching value (ΔF) and concentration of 2,4-D, a novel β-CD/SDS synergistic sensitized fluorescence quenching method for the determination of 2,4-D was successfully developed. The mechanism of determination was also investigated. The proposed method was applied to analyte 2,4-D in real samples with satisfactory results.

2. Experimental Reagents and Instruments

Schiff base calix[4]arene was synthesized according to the published methods (Bi, Sun, & Yan, 2012). 0.01% SAX (M=1328.7 g/mol, $c = 7.5\times10^{-6}$ mol/L) was prepared in ethanol.

100.0 μg/mL stock solution of 2,4-dichlorophenoxyacetic acid (2,4-D) was prepared by dissolving 0.100g 2,4-D in 100 mL volumetric flask and diluting with anhydrous ethanol to scale. The stock solutions were further diluted with anhydrous ethanol to obtain a standard working solution of 10.0 μg/mL for experiment.

1.0% β-CD solution was prepared by dissolving 1.00g of β-CD in 100.0 mL with distilled water. 1.0% SDS solution was prepared by dissolving 1.00g of SDS in 100.0 mL volumetric flask with distilled water. And pH=7.0 CH_3COONH_4 buffer solution was employed.

All the fluorescence measurements were performed on a Hitachi F-4500 spectrofluorimeter (Japan) with excitation and emission slits at 10.0 nm and 5.0 nm, λex=332 nm. The pH was measured on a pH FE20 pH meter (Mettler Toledo). A UV 2501 spectrophotometer (Shimadzu, Japan) was used for all absorption spectral recordings and absorbance measurements .

3. Experiment Method

Fluorescence measurements. In centrifuge tube (5.0 mL), 2.0 mL 0.01% SAX, 1.0 mL CH_3COONH_4 buffer solution (pH = 7.0), 0.5 mL 1.0% β-CD solution, 0.5 mL 1.0% SDS and 0.5 mL reference substance solution of 2,4-D (10.0 μg/mL) were added and then diluted to the scale with distilled water. Then fluorescence spectra was recorded in the range of 300- 650 nm with excitation at 332 nm.

Quenching type analysis (Gong, Zhu, & Hu, 2007). 2.0 mL 0.01% SAX solution, 1.0 mL buffer solution, 0.5 mL 1.0% β-CD, 0.5 mL 1.0% SDS and different amount of 10.0 μg/mL 2,4-D solutions were added into 5.0 mL centrifuge tube, then diluted to the scale with distilled water and mixed completely. The fluorescence intensity of SAX was measured at different temperature (288 K, 298 K and313 K), respectively. Quenching type could be analyzed by Stern-Volmer Eq.(1):

$$\frac{F_0}{F} = 1 + KC_Q = 1 + K_q \tau_0 C_Q$$

F_0 and F were the fluorescence intensities of SAX in the absence and presence of 2,4-D respectively, K was the Stern–Volmer quenching constant, C_Q was the concentration of quencher 2,4-D, Kq was the quenching rate constant, τ_0 was the average lifetime of the SAX without 2,4-D.

If the quenching type is single static or dynamic quenching, the curve of F_0/F versus C_Q (Stern–Volmer curve) would be linear within certain concentration.

Absorption spectrum titrations (Fu, Zeng, & Mu, 2012). The recognition ability of SAX and β-CD to 2,4-D can be evaluated through the change of absorption spectrum. The absorption spectrum titrations of 2,4-D with SAX and β-CD was made in the range of 200.0-600.0 nm. The absorbance of 2,4-D was measured with n_{SAX}: $n_{2,4\text{-}D}$ and $n_{\beta\text{-}CD}$: $n_{2,4\text{-}D.}$

Inclusion interaction. The solution of a certain amount of SAX, 1.0 mL buffer solution and different amount of 10.0 μg/mL 2,4-D solutions were added into 5.0 mL centrifuge tube, then diluted to the mark with distilled water and mixed thoroughly. The fluorescence intensity was measured at 25℃, then the Benes-Hildebrand method (Vimal, Ajay, & Narinder, 2008) (double reciprocal plot) was used to calculate the inclusion constant (K) of SAX-2,4-D and β-CD-2,4-D assuming a 1:1 inclusion model. The Benesi-Hildebrand method is a spectroscopic method to determine the inclusion constants of the host guest complexes (including fluorescence spectroscopy and absorption spectroscopy). The equation is as follows (SAX as an example):

$$1/\Delta F = 1/\left(K \cdot \alpha \cdot \left[SAX\right]_0\right) \cdot 1/\left[2{,}4 - D\right] + 1/\left(\alpha \cdot \left[SAX\right]_0\right)$$

where $[SAX]_0$ was the concentration of SAX, ΔF was the quenching value of fluorescence intensity, α was a constant. Thus, the inclusion constant (K) of the 1:1 inclusion complex could been calculated by dividing the intercept by the slope of the double reciprocal plot.

Determination of critical micelle concentration (cmc). cmc values of the medium were measured by conductivity measurements. The cmc was obtained from the inflection point of the straight lines of before and after micellar concentration range (Kumaraguru & Santhakumar, 2006; Mehta, Bhawna, & Ram, 2010).

Determination of fluorescence quantum yield. Fluorescence quantum yields of SAX were measured using 1.0×10^{-6} g/mL quinine sulfate as reference substance (Zhao & Wei, 2006; Zhu, Gong, & Yu, 2008). Under the same apparatus conditions, the quantum yield of the SAX was calculated.

Kinetics of the reaction. The kinetics of the reaction could be described by the first order kinetic model, the second kinetic model and the Weber Maurice diffusion model (Azizian & Fallah, 2010; Wang, Wei, & Li, 2015). In this study, the first order kinetic fitting of the quenching process was carried out.

Sample preparation. Vegetable samples (green vegetables, Chinese cabbage and chrysanthemum coronarium) were purchased from local market. A certain amount of each vegetable sample was cut up

and homogenized. Then, 20 g of each sample was weighed and placed in a 50 mL centrifuge tube and 50 mL ethanol was added as well. Then tighten up the lid, dipped for 1 hour, shaken thoroughly for 20 min. After centrifugation 10 min (5000 r/min), the test sample solution was prepared after the upper solution was filtered.

In order to reduce the fluorescence background, the sample solution was diluted 15 times and then used as an analytical sample (Farokhcheh & Alizadeh, 2013).

4. Results and Discussion

Choice of medium. The effect of different medium on ΔF ($\Delta F = F_{SAX} - F_{2,4\text{-}D\text{–}SAX}$) was studied. As can be seen in Figure 2 that the sequence of ΔF was $\Delta F_{\beta\text{-}CD\text{-}SDS} > \Delta F_{SDS} > \Delta F_{\beta\text{-}CD} > \Delta F_{H2O}$. The fluorescence quenching value in β-CD/ SDS synergistic sensitized medium was greater than that in single β-CD or SDS medium. So β-CD-SDS medium was selected for further experiment.

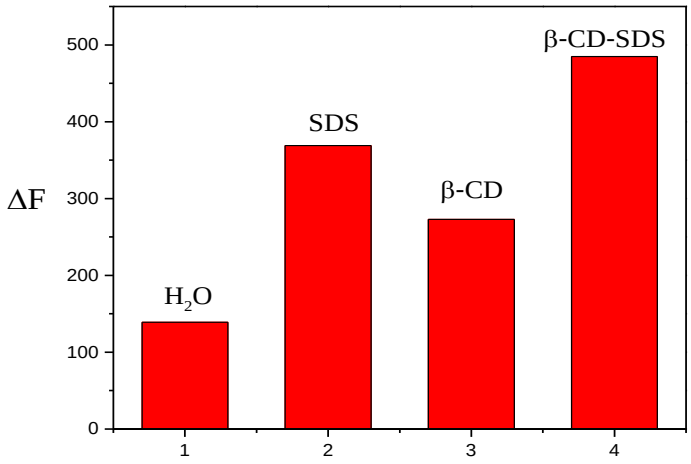


Figure 2. Effect of Different Medium on Fluorescence Intensity

Fluorescence spectra. The fluorescence emission spectrum of SAX (present or absent of 2,4-D) in β-CD-SDS and H_2O medium were shown in Figure 3. It can be seen that (1) the fluorescence intensity of SAX (F_{SAX}) was enhanced in β-CD/SDS medium (curves a and c); (2) the fluorescence intensity of SAX (F_{SAX} or $F_{SAX\text{-}\beta\text{-}CD\text{-}SDS}$) was quenched when 2,4-D was added (curves b and d) and gradually diminished with the increase concentration of 2,4-D (inset Figure 3); (3) $\Delta F = F_{SAX\text{-}\beta\text{-}CD\text{-}SDS} - F_{2,4\text{-}D\text{-}SAX\text{-}\beta\text{-}CD\text{-}SDS}$ was larger than that $\Delta F' = F_{SAX} - F_{2,4\text{-}D\text{-}SAX}$ with the same concentration of 2,4-D, which was the synergistic sensitizing effect in β-CD/SDS.

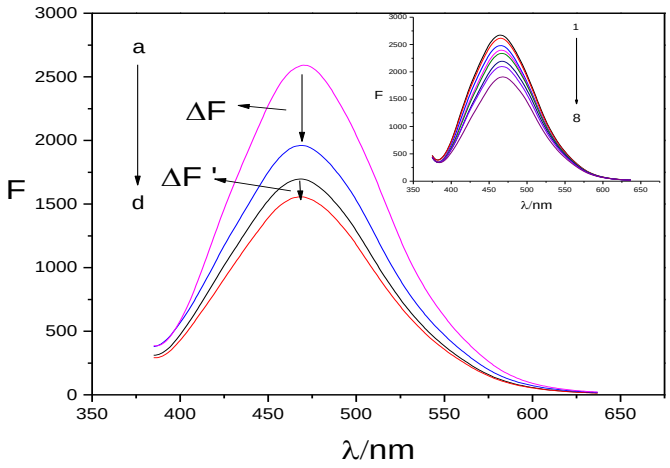


Figure 3. Fluorescence Spectra

(a). SAX-β-CD-SDS (b). 2,4-D-SAX-β-CD-SDS (c). SAX-H_2O (d). 2,4-D-SAX-H_2O

Inset 1-8: 2,4-D-SAX-β-CD-SDS, [2,4-D]: (1) 0 μg/mL, (2) 0.02 μg/mL, (3) 0.08 μg/mL, (4) 0.1 μg/mL, (5)0.2 μg/mL, (6) 0.4 μg/mL, (7) 0.8 μg/mL, (8) 1.0 μg/mL

Effect of pH. The influence of pH on ΔF was investigated. As could be seen in Figure 4, ΔF gradually increased with the increase of pH and reached maximum at pH = 7.0, but it diminished at pH > 7.0. The reason may be related to the formation of SAX-2,4-D inclusion, which will be discussed in section 3.13.2. So 1.0 mL of pH = 7.0 CH_3COONH_4 buffer solution was chosen for the further study.

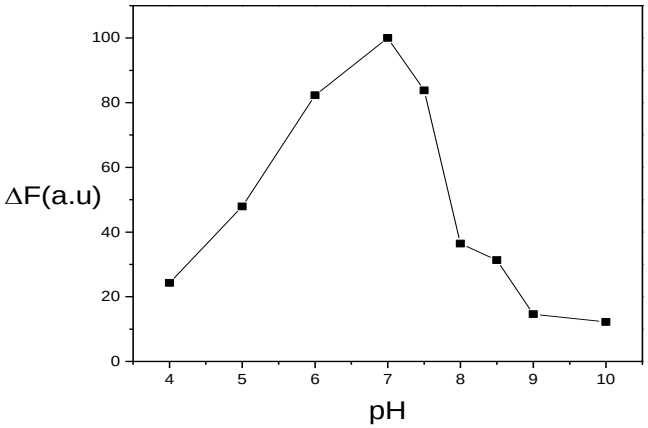


Figure 4. Effect of pH on Fluorescence Quenching Value

Effect of SAX amount. The effect of the amount of SAX was studied in Fig. 5. It was shown that the ΔF was increased and reached a maximum value at a SAX (7.5×10^{-6} mol/L) amount of 2.0 mL, and then decreased (curve 1). This was because that the $F_{SAX\text{-}\beta\text{-}CD\text{-}SDS}$ gradually decreased with the increase of SAX amount due to the self-quenching of SAX at higher concentration (curve 2). Thus, 2.0 mL SAX (7.5×10^{-6} mol/L) was selected for the optimized method.

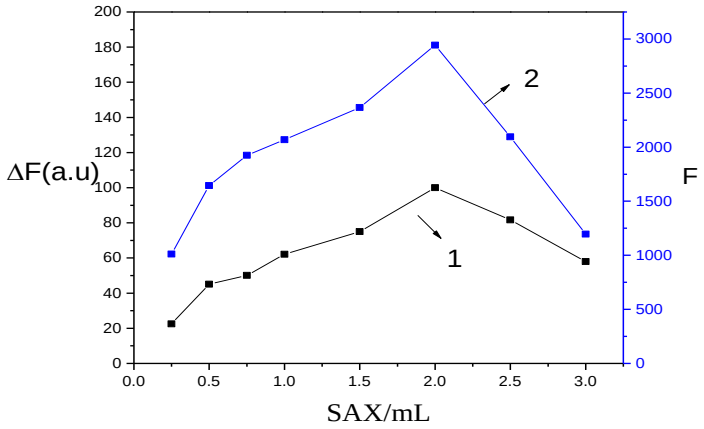


Figure 5. Effect of the Amount of SAX on Fluorescence Quenching Value (Left Axis) and Fluorescence Intensity of SAX (Right Axis)

(1: fluorescence quenching value, 2: fluorescence intensity of SAX)

Effect of β-CD amount. Effect of β-CD was investigated. As is shown in Fig. 6, with the increase of β-CD (1.0%) amount, ΔF gradually increased and reached the maximum when the β-CD amount was 0.50 mL, but decreased when β-CD amount was more than 0.50 mL.

The change trend of ΔF could be explained from the change of $F_{SAX\text{-}\beta\text{-}CD\text{-}SDS}$ and $F_{2,4\text{-}D\text{-}SAX\text{-}\beta\text{-}CD\text{-}SDS}$ with β-CD amount (inset Figure 6). (1) $F_{SAX\text{-}\beta\text{-}CD\text{-}SDS}$ gradually increased with β-CD amount while $F_{2,4\text{-}D\text{-}SAX\text{-}\beta\text{-}CD\text{-}SDS}$ slowly decreased ($V_{\beta\text{-}CD}$ = 0-0.50 mL) ($\Delta F\uparrow = F_{SAX\text{-}\beta\text{-}CD\text{-}SDS}\uparrow - F_{2,4\text{-}D\text{-}SAX\text{-}\beta\text{-}CD\text{-}SDS}\downarrow$); (2) $F_{SAX\text{-}\beta\text{-}CD\text{-}SDS}$ gradually decreased, while $F_{CR\text{-}SAX\text{-}M\text{-}\beta\text{-}CD\text{-}Tx\text{-}100}$ remain unchanged ($V_{\beta\text{-}CD}$ = 0.50-0.80 mL) ($\Delta F\downarrow = F_{SAX\text{-}\beta\text{-}CD\text{-}SDS}\downarrow - F_{2,4\text{-}D\text{-}SAX\text{-}\beta\text{-}CD\text{-}SDS}$). Therefore, 0.50 mL 1.0% β-CD was chosen for the following experiments.

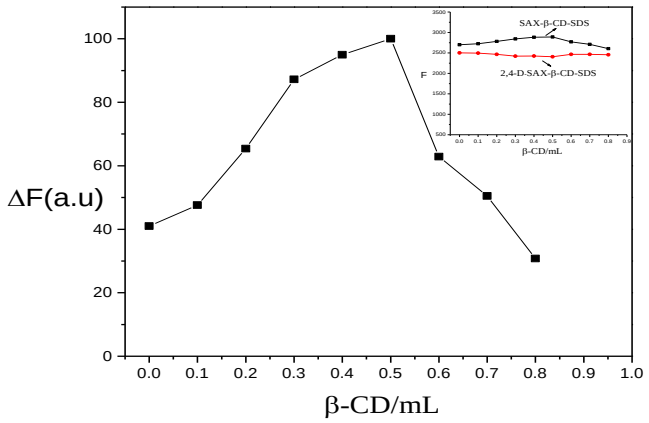


Figure 6. Effect of the Amount of β-CD on Fluorescence Quenching Value

Effect of SDS amount. Effect of SDS was studied. As is shown in Figure 7, ΔF gradually rose up with the increase of SDS (1.0 %) amount and up to the maximum when the SDS amount was 0.50 mL, but ΔF decreased when SDS amount was greater than 0.50 mL.

The change of $F_{SAX\text{-}\beta\text{-}CD\text{-}SDS}$ and $F_{2,4\text{-}D\text{-}SAX\text{-}\beta\text{-}CD\text{-}SDS}$ with SDS amount (inset Figure 7) could declare the change of ΔF with SDS. (1) $F_{SAX\text{-}\beta\text{-}CD\text{-}SDS}$ obviously increased with SDS amount was larger than $F_{2,4\text{-}D\text{-}SAX\text{-}\beta\text{-}CD\text{-}SDS}$ (V_{SDS} = 0-0.30 mL)($\Delta F\uparrow = F_{SAX\text{-}\beta\text{-}CD\text{-}SDS}\uparrow\uparrow - F_{2,4\text{-}D\text{-}SAX\text{-}\beta\text{-}CD\text{-}SDS}\uparrow$); (2) $F_{SAX\text{-}\beta\text{-}CD\text{-}SDS}$ continuously increased, while $F_{2,4\text{-}D\text{-}SAX\text{-}\beta\text{-}CD\text{-}SDS}$ gradually decreased (V_{SDS}= 0.30–0.50 mL) ($\Delta F\uparrow = F_{SAX\text{-}\beta\text{-}CD\text{-}SDS}\uparrow - F_{2,4\text{-}D\text{-}SAX\text{-}\beta\text{-}CD\text{-}SDS}\downarrow$); (3) $F_{SAX\text{-}\beta\text{-}CD\text{-}SDS}$ gradually decreased, while $F_{2,4\text{-}D\text{-}SAX\text{-}\beta\text{-}CD\text{-}SDS}$ changed rarely (V_{SDS}= 0.50–0.80 mL) ($\Delta F\downarrow = F_{SAX\text{-}\beta\text{-}CD\text{-}SDS}\downarrow - F_{2,4\text{-}D\text{-}SAX\text{-}\beta\text{-}CD\text{-}SDS}$). Therefore, 0.50 mL 1.0 % SDS was chosen for the further study.

In conclusion, the ΔF was biggest when mass ratio or molar ratio of β-CD and SDS are 1:1 and 1:4, respectively, which illustrated that β-CD and SDS showed the synergistic sensitized effect on ΔF. Either excess β-CD or SDS will weaken the synergistic sensitized effect, resulting the decrease of ΔF.

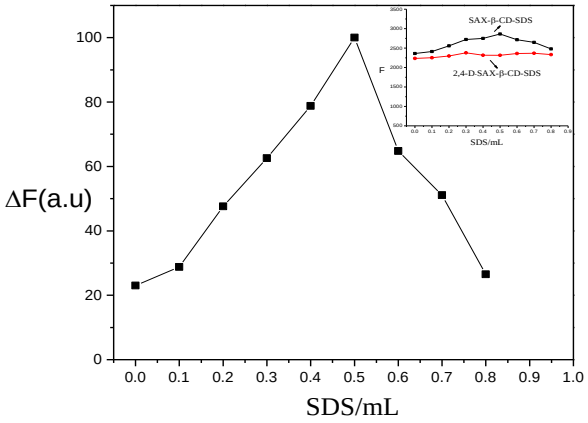


Figure 7. Effect of the Amount of SDS on Fluorescence Quenching Value

Effect of reaction time. The effect of reaction time on ΔF was tested. It was found from Figure 8 that ΔF obviously increased from 10 min to 30 min, then ΔF remained unchanged after 30 min. Therefore, 30 min of reaction time was chosen for the following experiments.

According to the data ΔF-t, the fluorescence quenching reaction kinetics could be discussed. In this study, the first order kinetic fitting of the quenching process was carried out. As shown in inset Fig.8, the curve of $\ln C_{2,4\text{-}D}$ versus t was linear and R^2 was 0.9960. The reaction rate constant was 1.14×10^{-1} min^{-1}. The results show that the fluorescence quenching process followed the first order kinetic model. The reason may be that the amount of SAX is excess in this system, so the rate of fluorescence quenching reaction is only in relation to the concentration of 2,4-D.

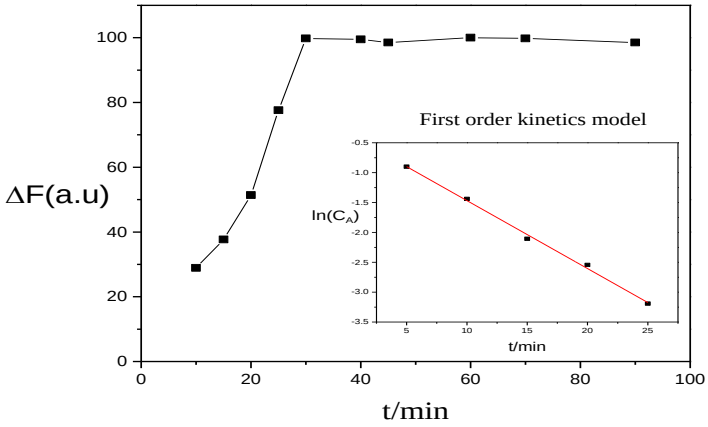


Figure 8. Effect of Reaction Time on Fluorescence Quenching Value

Effect of temperature. The effect of temperature (5-50℃) on ΔF was investigated. As can been seen from Figure 9 that ΔF was increased from 5 to 25℃ then it decreased with the increase of temperature. Thus, the suitable temperature of 25℃ was chosen for the study.

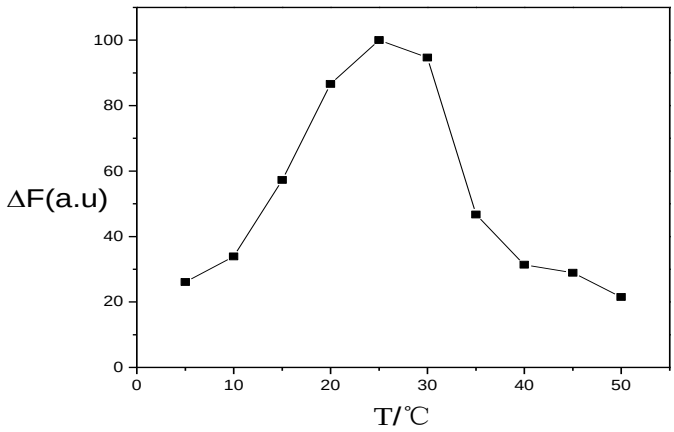


Figure 9. Effect of Temperature on Fluorescence Quenching Value

Effect of foreign substances. The effect of foreign substrates was discussed for the determination of 1.0 μg/mL 2,4-D. With a relative error of less than ±5%, the majority of these substances showed no remarkable interference in the determination of 2,4-D (Table 1).

Table 1. Effect of Interfering Substances

Foreign substances	Foreign substances / 2,4-D(w/w)	Foreign substances	Foreign substances / 2,4-D(w/w)
K^+	500	Al^{3+}	20
Na^+	1200	Mn^{2+}	2
Ca^{2+}	1000	Cl^-	1800

Mg^{2+}	300	NO_3^-	700
Ba^{2+}	100	SO_4^{2-}	300
Zn^{2+}	200	Glucose	500
Fe^{3+}	2	Sucrose	500
Cu^{2+}	2	Glycine	200

Analytical performance. Under the optimum conditions, the linear regression equations were determined to be: $\Delta F = 10.14 + 2672.1c$ (μg/mL) in the range of 0.020~0.10 μg/mL, R = 0.9972, the detection limit estimated (S/N = 3) was 0.23 ng/mL; $\Delta F = 331.08 + 290.87c$ (μg/mL) in the range of 0.10μg/mL ~ 4.0 μg/mL, R = 0.9918, the detection limit estimated was 2.8 ng/mL. The relative standard deviation (RSD) was 0.96% (n = 3, c = 1.0μg/mL).

Sample analysis. The proposed method was successfully applied for the determination the amount of 2,4-D in vegetable samples. The data were listed in Table 2. The recovery ratio ranged from 95.0% ~ 105.5% which was satisfactory.

Table 2. Determination Results of 2,4-D

Samples	Added (μg/g)	Found（μg/g）	Recovery (%)
Chinese cabbage	0.0	ND	-
	0.50	0.53	105.5
	1.0	0.95	95.5
	2.0	1.9	98.2
green vegetables	0.0	ND	-
	0.50	0.48	95.0
	1.0	2.0	95.7
	2.0	2.1	102.0
chrysanthemum coronarium	0.0	ND	-
	0.50	0.52	104.9
	1.0	0.96	96.5
	2.0	2.1	103.4

Comparison of different methods. The results obtained from this experiment were compared with those previously reported methods for 2,4-D determination (Table 3). The advantages of the proposed method are: easy operation, high sensitivity, low detection limit and high recovery rate.

Table 3. Comparison with Previously Reported Methods

Methods	Linearity range	LOD	Recovery	Reference
HPLC-MS	0.025-1.0 mg/L	0.005 mg/kg	88.3-95.4%	(Jiang, Zha, & Tie, 2015)
SHPLC	1.0-500 ng/mL	0.3 ng/mL	105-116 %	(Wu, Ee, & Lee, 2005)
CE	3.0-500 ng/mL	0.02 ng/mL	-	(Zhu & Lee, 2001)
GC	10-500 ng/mL	5.0 ng/mL	-	(Rezazadeh, Yamini, Seidi, Tahmasebi, & Rezaei, 2014)
Fluorescence quenching calix[4]arene methods	0.020-4.0 μg/mL	2.8 ng/mL	95.0-105.5%	This method

5. Discussion of Mechanism

In this paper, the discussion of mechanism was included quenching type analysis, inclusion interaction and sensitizing effect.

Quenching type. Quenching types can be divided into static quenching and dynamic quenching. The static quenching is caused by the formation of non or weak fluorescent compound (Gong, Zhu, & Hu, 2007). The dynamic quenching is initiated from the collision of fluorescence substance and quencher, resulting in the decrease of fluorescence intensity and quantum yield.

Quenching type could be discussed with K_{sv} and K_q. With the increasing temperature, K_{sv} would be decreased for static quenching, while K_{sv} would be increased for dynamic quenching (Gong, Zhu, & Hu, 2007). The Stern-Volmer plots of SAX with 2,4-D at different temperature (288 K, 298 K and 313 K) was shown in Figure 10. The order of K_{sv} at different temperature were $K_{sv}^{288K} = 3.90 \times 10^4 > K_{sv}^{298K} = 3.45 \times 10^4 > K_{sv}^{313K} = 3.04 \times 10^4$ L/mol. It demonstrated that the fluorescence quenching mechanism of SAX by 2,4-D was a static quenching procedure and a complex was formed between SAX and 2,4-D.

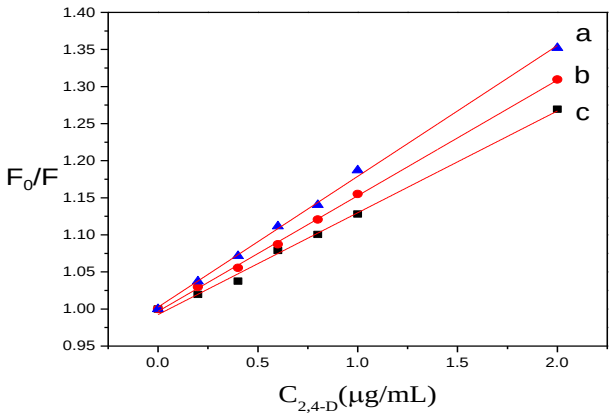


Figure 10. The Stern-Volmer Curves for the Binding of 2,4-D with SAX at 288 K (a), 298 K (b) and 313 K (c)

Inclusion interaction. From the previous discussion, the type of fluorescence quenching was static quenching, which may be caused by the formation of non or weak fluorescent compound. SAX and β-CD have a cavity structure, both of them are likely to form inclusion interaction with 2,4-D.

The recognition ability of SAX and β-CD to 2,4-D can be evaluated through the change of absorption spectrum. The results of an absorption titration at λ = 281.0 nm (characteristic absorption peak of 2,4-D) (Figure 11) was shown that the absorbance of 2,4-D was gradually increased with the increase of SAX or β-CD and was unchanged when $n_{SAX}:n_{2,4\text{-}D}$ = 1:1 or $n_{\beta\text{-}CD}:n_{2,4\text{-}D}$ = 1:1, which implied a 1:1 stoichiometry for binding between SAX and 2,4-D or β-CD and 2,4-D.

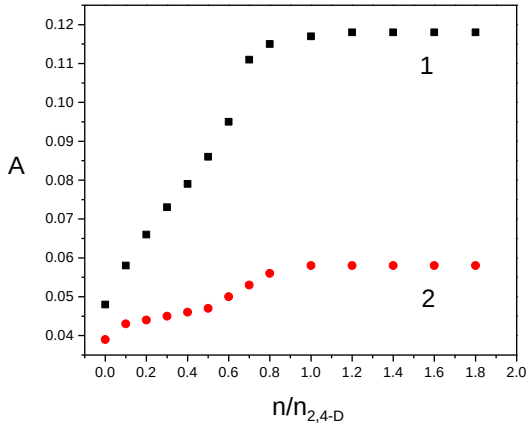


Figure 11. Absorption Spectrum Titrations of 2,4-D with SAX and β-CD

(1: 2,4-D-SAX, 2: 2,4-D-β-CD)

The inclusion constant (K) of SAX-2,4-D and β-CD-2,4-D could be obtained by Benesi-Hildebrand method (Vimal, Ajay, & Narinder, 2008). The larger the value of K, the more steady the inclusion complex. As is shown in Figure 12, double reciprocal plots of SAX-2,4-D and β-CD-2,4-D have good linear relationship, which supports the formation of a 1:1 complex. The calculated inclusion constant (K) was listed in Table 4.

As can be seen in Table 4: (1)$K_{SAX\text{-}2,4\text{-}D} >> K_{\beta\text{-}CD\text{-}2,4\text{-}D}$, which suggested that inclusion complex of SAX-2,4-D is more stable than β-CD-2,4-D. SAX plays a major role in inclusion interaction with 2,4-D, which bringing about static fluorescence quenching. (2) $K_{SAX\text{-}2,4\text{-}D} > K_{SAX\text{-}2,4\text{-}D\text{-}\beta\text{-}CD}$, the inclusion constant of SAX-2,4-D was decreased in presence of β-CD, which is caused by the formation of β-CD-2,4-D complex; (3) $K_{SAX\text{-}2,4\text{-}D,\ pH\ 7.0} > K_{SAX\text{-}2,4\text{-}D,\ pH\ 4.5} > K_{SAX\text{-}2,4\text{-}D,\ pH\ 10.0}$, which indicated that pH had a significant effect on the formation SAX-2,4-D and the inclusion complex of SAX-2,4-D is more stable at pH 7.0 than in acidic or basic solution; (4) the larger the K, the greater the ΔF, the largest fluorescence quenching value ΔF was at pH=7.0, which is in accordance with the discussion about effect of pH on ΔF in section 3.3.

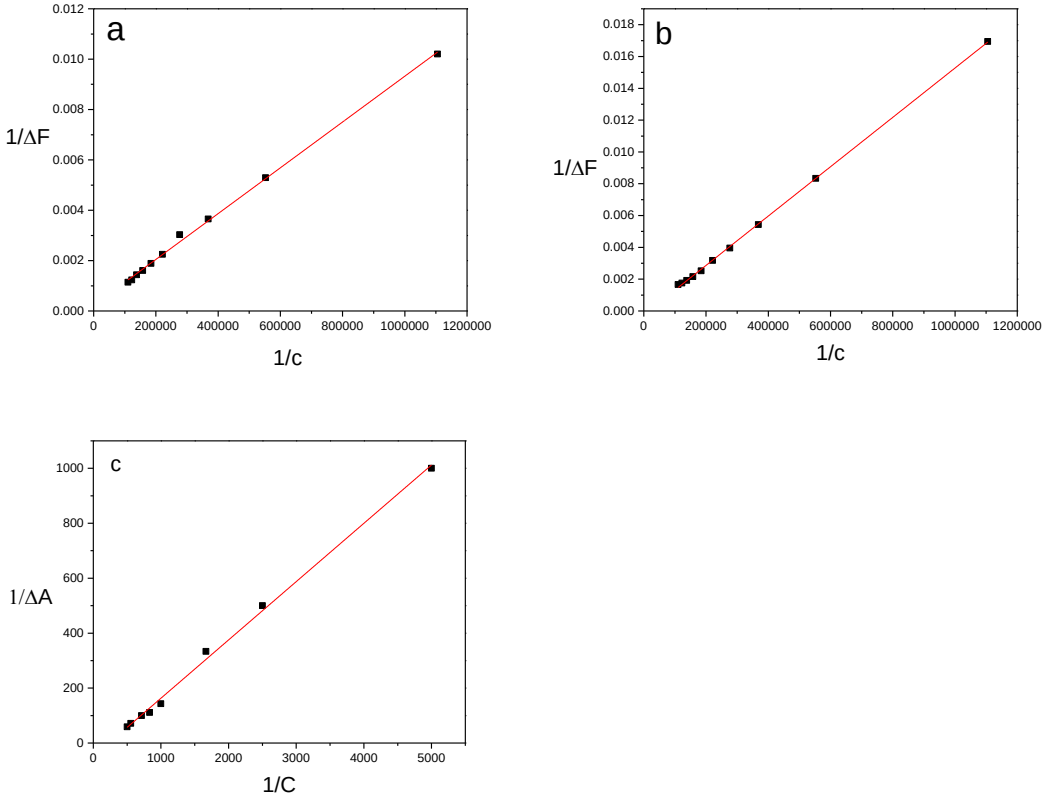


Figure 12. Benesi-Hildebrand Plot

(a: SAX-2,4-D, b: SAX-2,4-D-β-CD, c: β-CD-2,4-D)

Table 4. Results of Inclusion Constant

System	Inclusion constant K（L/mol）		
	pH 4.5	pH 7.0	pH 10.0
SAX-2,4-D	5.71×10^{4}	5.55×10^{10}	2.00×10^{4}
SAX-2,4-D-β-CD		2.21×10^{9}	
β-CD-2,4-D		262.47	

Sensitizing effect. The discussion of sensitizing effect was included the formation of the ordered molecular assembly and fluorescence quantum yield.

The formation of the ordered molecular assembly

The synergistic sensitized effect of β-CD/SDS may be from the formation of the ordered molecular assembly. The interaction of SDS and β-CD can change the critical micelle concentration (cmc). By measuring the cmc of a single and mixed medium, it can be determined whether the β-CD/SDS has formed a new ordered molecular assembly (Zhu & Jiang, 2011). The value of cmc can be obtained from the inflection point of the straight lines of before and after micellar concentration range (Kumaraguru &

Santhakumar, 2006). The results were summarized in Figure 13 and Table 5.

As is shown in Figure 13, the cmc of β-CD and SDS/β-CD were 3.0-5.0 mmoL/L and 5.0-6.0 mmoL/L, respectively. The cmc of the mixed medium model could be calculate by the clint model based on the assumption of ideal mixture behavior. As is shown in Table 5, the cmc of SDS is 8.4 mmoL/L (Mehta, Bhawna, & Ram, 2010), the molar ratio of SDS and β-CD was 4:1. Thus, the cmc of the mixed solution should between 7.8-8.1 mmoL/L. There was a difference between the cmc of the mixed medium (β-CD/SDS) in determined value and the clint model calculated value. This result illustrated that the β-CD/SDS mixed medium formed a new ordered molecular assembly. What's more, the sensitivity of the determination could be enhanced by the new ordered molecular assembly.

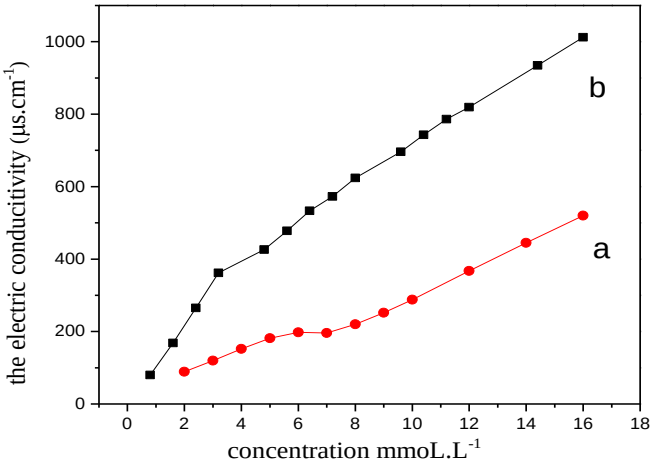


Figure 13. Electrical Conductivity vs. the Concentration of β-CD

(a: Electrical conductivity vs. the concentration of β-CD, b: Electrical conductivity vs. the concentration of SDS/β-CD)

Table 5. cmc of the Different Medium

Medium	cmc (mmoL/L)	
	Determination value	Clint model value
SDS	8.4[a]	
β-CD	3.0-5.0	
SDS/β-CD (4:1)	5.0-6.0	7.8-8.1[b]

a: the literature (Mehta, Bhawna, & Ram, 2010); b: calculate by Clint model

Fluorescence quantum yield. The fluorescence quantum yield represents the ability of translation of absorption energy to fluorescence. It is one of the basic and significant parameters for fluorescence substances. What's more, $\Delta F = F_{SAX} - F_{2,4\text{-}D\text{-}SAX}$, where ΔF must rise up with the increase of fluorescence quantum yield of SAX. The fluorescence quantum yield of SAX (Y) in the medium of

H_2O , β-CD , SDS and β-CD-SDS were listed in Table 6. As can be seen in Table 6, the order of fluorescence quantum yield of SAX in different medium is $Y_{\beta\text{-CD-SDS}} > Y_{SDS} > Y_{\beta\text{-CD}} > Y_{H2O.}$ It is in accord with the sensitivities in different medium. The results could be considered with internal and external environment. The fluorescence quantum yield of SAX in β-CD-SDS medium was increased obviously. It was because that the external environment provided by β-CD/SDS mixed medium is more favorable on solubilization than that in single medium (β-CD or SDS). Moreover, the microenvironment which was formed by the new ordered molecular assembly β-CD/SDS could provide the protective environment for the singlet excited state and could reduce the non-radiation of the fluorescent substance SAX. In other words, both self fluorescence quenching of SAX and fluorescence quenching of external quenchers can be decreased in the medium of β-CD/SDS. As a result, the fluorescence quantum yield of SAX in the mixed medium is largest due to the synergistic sensitizing effect of β-CD-SDS.

Table 6. The Fluorescence Quantum Yield of 2,4-D

Medium	Y
H_2O	0.011
β-CD	0.038
SDS	0.094
β-CD-SDS	0.108

In summary, the mechanism of this method was (1) the fluorescence quenching of SAX is due to the inclusion interaction of SAX-2,4-D; (2)the sensitizing effect is that SDS/β-CD medium could form a new ordered molecular assembly, in which fluorescence quantum yield of SAX increased obviously, resulting synergistic sensitizing effect on fluorescence quenching value.

6. Conclusion

In this study, a novel fluorescence quenching method for the determination of 2,4-D has been developed. The fluorescence intensity of SAX was quenched due to inclusion interaction between SAX and 2,4-D, and the fluorescence quenching value (ΔF) was increased in β-CD-SDS medium. The proposed method has been applied for the determination of 2,4-D in vegetable samples with satisfactory results.

Funding

The National Natural Science Foundation of China (21375117) and the Priority Academic Program Development of Jiangsu Higher Education Institutions are acknowledged for funding this research.

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