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# Highly sensitive and selective spectrofluorimetric determination of tolnaftate through the formation of ternary inclusion complex of β-naphthol/β-cyclodextrin/anionic surfactant system

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**Abstract**

An indirect spectrofluorimetric method with high sensitivity and selectivity was developed for the determination of antifungal drug: tolnaftate (TNF), depending on the supramolecualr multirecognition interaction among the anionic surfactant sodium laurylsulfate (SLS), β-cyclodextrin (β-CD) and β-naphthol (ROH). The mechanism of the inclusion was studied and discussed by means of fluorescence spectrum, infra-red spectrograms and $^1$HNMR spectroscopy. Results showed that the naphthalene ring of ROH and the hydrophobic hydrocarbon chain of SLS were included into the β-CD's cavity to form a ROH:SLS:β-CD ternary inclusion complex with stoichiometry of 1:1:1 at room temperature, which provided effective protection for the excited state of ROH. At $\lambda_{ex}/\lambda_{em}$ = 273/360 nm, the fluorescence intensity was linear over a tolnaftate concentration range of $2.46 \times 10^{-9}$ to $2.10 \times 10^{-6}$ mol L$^{-1}$. The detection limit and relative standard deviation was $7.50 \times 10^{-10}$ mol L$^{-1}$ and 1.4%, respectively. The interference of 31 foreign substances was slight. The proposed method had been successfully applied to the determination of tolnaftate in artificial mixed samples with almost quantitative recovery.


*Keywords:* Tolnaftate; β-Cyclodextrin; β-Naphthol; Sodium laurylsulfate; Supramolecular multirecognition; Spectrofluorimetric determination

## 1. Introduction

Tolnaftate (*o*-2-naphthyl *N*-methyl-*m*-tolyl thiocarbamate, TNF, Scheme 1), has been widely used as a kind of topical antifungal drug in the treatment of cutaneous diseases [1]. Because the sulfur atom of TNF's thiono ester nucleus contains unbonding electron, so the lowest singlet excited state of TNF is *n*, π* transition and belongs to the spin-forbidden transition [2], which resulted the weak or no fluorescence emission in aqueous solution. Moreover, the trace amount detection of TNF in aqueous phase suffered the restriction of the poor solubility of TNF in water. By now, the determination of TNF was mainly SCF method [3] and spectrophotometric method [4]. Spectrophotofluorimetry has been widely used in the determination of biological samples [5,6] and environmental substances [7–9] because it is sensitive, selective, easily operated and low cost. Khashaba et al. [10] developed an indirect spectrofluorimetric method with the detection limit of 6.1 ng mL$^{-1}$ for the determination of TNF based on the measurement of the induced fluorescence of β-naphtholate or β-naphthol anion (RO$^-$), the alkaline hydrolysis product of TNF in boiling water bath. But in that report, we found that the solution with 60% volume contents of methanol was used to increase the fluorescence quantum yield of RO$^-$ and to improve the sensitivity of the method, which resulted in relatively high toxicity and waste of reagents. Moreover, the fluorescence measurement was performed in a strong alkaline environment with pH higher than 13.0, a hard experimental condition under which not only a lot of metal ions were inclined to hydrolysis to form precipitation interfering the determination, but also the quartz cells was easy to be corroded. So, for routine quality control, the development of a simple, highly sensitive and selective spectrofluorimetric method for the determination of TNF in aqueous solution has important application value.

Cyclodextrins (CDs), the cyclic oligosaccharides consisting of six or more D-(+)-glucopyranose units, are well known to have hollow truncated cone with hydrophobic cavity and hydrophilic wall to form inclusion complexes with guest organic or inorganic

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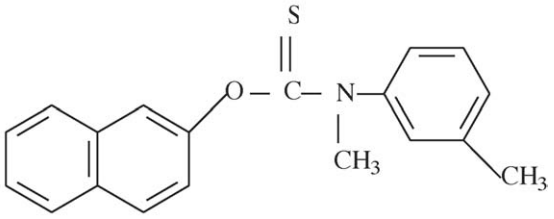


Scheme 1. The chemical structure of tolnaftate.

molecules which possess suitable polarity and dimension. So, as the excellent enzyme model and molecule receptor, CDs has been widely used in a great many of fields in science and technology. The formation of supramolecular complex with CDs can alter the photochemical and photophysical properties of the guest molecules and considerable attentions have been focused on the luminescence application of the CDs [11]. Drug molecules can demonstrate dramatically different physical, chemical and biological properties through the formation of inclusion complexes with CDs [12], such as the enhancement of the solubility, stability and bioavailability [13]. Surfactants can provide a similar microenvironment like the CDs [14], which can enhance solubility, stability, and sensitivity of analytical systems. Some chemical and physical properties of surfactants can be changed when they are included in CDs cavity, so the interactions of surfactants with CDs have attracted much attention recently [15]. Studies on the formation of multirecognition ternary CDs complexes, in which two different guests are compounded in a single CD host cavity, have been performed [16]. In a drug:CD:surfactant multirecognition ternary complex, drug molecules can show remarkably different spectral characteristics from those in a binary drug:CD complex, which leads to very high sensitivity and selectivity of the analytical system and has very important theoretical and applicative value.

In the present work, we found that after being acidified and adjusted to the pH at 4.50 by acetic acid–sodium acetate buffer solution, $RO^-$ was protonated to ROH and a ternary inclusion complex with the stoichiometry of 1:1:1 was formed among ROH, β-CD and anionic surfactant SLS when the solutions of β-CD and SLS were added. It was found that the determination of TNF could be well accomplished through the measurement of ROH's fluorescence and the formation of the ternary inclusion complex improved the determination conditions greatly. Compared to Khashaba's method, the pH range was greatly broadened from 0.50 to 12.0 and the volume contents of methanol was decreased to less than 5%. The fluorescence intensity of ROH was increased drastically to effectively improve the sensitivity and selectivity of the method. The mechanism of the inclusion was studied and discussed by means of fluorescence spectrum, infra-red spectrograms and $^1$HNMR spectroscopy. An indirect spectrofluorimetric method with high sensitivity and selectivity for the determination of TNF depending on the supramolecular multirecognition interaction was established.

## 2. Experimental

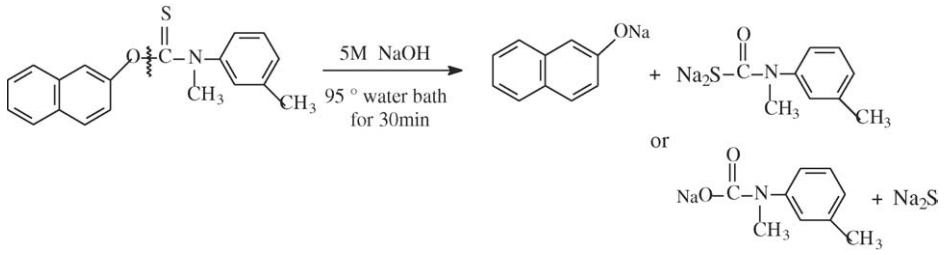
### 2.1. Instruments and chemicals

All fluorescent 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. Infrared spectra were obtained from a PE-983G IR-spectrophotometer (Perkin-Elmer). $^1$HNMR spectra data were recorded at a FX-90Q nuclear magnetic resonance spectrometer (JEOL Company, solvent: $D_2O$, internal standard: TMS, 90 MHz). pH Measurements were made with a pHS-3C digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass–calomel electrode. The sample chamber accommodated a thermostated cuvette holder, controlled to $25 \pm 1\,^\circ$C via a CS-50 constant temperature circulator (Chongqing Experimental Equipment Works, Chongqing, China).

TNF (purchased from Sigma Company, purity >99.5%) was used as received without further purification. Its stock solution ($1.00 \times 10^{-5}$ mol L$^{-1}$) was prepared in methanol and stored in the dark in amber bottle at 4 °C. β-CD (obtained form China Medicine Group Shanghai Chemical Reagent Corporation) was of analytical reagent grade and was purified by twice recrystallization in double-distilled water, followed by vacuum drying at 60 °C for 12 h and used with a concentration of $1.0 \times 10^{-2}$ mol L$^{-1}$ aqueous solution. β-naphthol (purchased from Tianjin Bodi Chemical Engineering Limited Company) for the purpose of spectral contrast was of analytical reagent grade and was used as received without further purification. Its stock solution ($1.00 \times 10^{-5}$ mol L$^{-1}$) was prepared in methanol and stored in dark in amber bottle at 4 °C. SLS (purchased from Tianjin Nankai Chemical Engineering Works) was of chemical reagent grade and was ground in an agate mortar to obtain a fine powder, then the powder was purified by twice washing in absolute ether and twice recrystallization in 95% ethanol, sequentially. 0.05 mol L$^{-1}$ aqueous solution was prepared in double-distilled water. Acetic acid–sodium acetate buffer solution (0.20 mol L$^{-1}$, pH 4.50). Other chemicals used were of analytical reagent or higher grade. Doubly distilled water was used throughout.

### 2.2. Experimental procedure

Into a 10-mL colorimetric tube were added an aliquot of TNF stock solution containing $0.0–2.3 \times 10^{-8}$ mol of TNF, then 0.60 mL of 5.00 mol L$^{-1}$ sodium hydroxide solution was added and mixed well. The solutions were allowed to stand for 30 min in a water bath at 95 °C, cooled to room temperature and added 1.00 mL of 3.00 mol L$^{-1}$ hydrochloric acid solution. After being slightly shaken, 2.50 mL of 0.20 mol L$^{-1}$ acetic acid–sodium acetate buffer solution (pH 4.50), 1.00 mL of $1.00 \times 10^{-2}$ mol L$^{-1}$ β-CD and 2.00 mL of 0.05 mol L$^{-1}$ SLS was added, sequentially. The mixed solution was diluted to 10 mL with doubly distilled water and allowed to equilibrate at $25 \pm 1\,^\circ$C for 10 min. Then the fluorescence intensity was measured at $\lambda_{ex}/\lambda_{em}$ = 273/360 nm against a reagent blank.




Scheme 2. The alkaline hydrolysis of tolnaftate.

## 3. Results and discussion

### 3.1. Fluorescence spectra

The alkaline hydrolysis reaction of TNF followed Scheme 2 [10]. The hydrolytic product with maximum fluorescence emission at 418 nm was sodium β-naphtholate ($RO^-$, Fig. 1a), whose relative fluorescence quantum yield $¢_f$ was lower than that of ROH [17]. With increasement of the solution acidity, $RO^-$ was gradually protonated and converted to its conjugate acid ROH, whose fluorescence peak was at 353 nm. With the decreasing of pH, the concentration of ROH increased and the concentration of $RO^-$ decreased, which resulted in the decreasing of the fluorescence intensity at 418 nm and the increasing of the fluorescence intensity at 353 nm (Fig. 1c–e and h). It was known that in water, ROH was more acidic in the excited state than in the ground state. The ground state p$K_a$ of ROH was 9.5, whereas p$K_a$ was 2.8 for the excited state. This could be explained by the faster proton-transfer reaction rate of ROH in the excited state than the decay process rate of ROH, or in other words, the proton-transfer in the excited state was fast enough to allow complete dissociation of ROH in its excited lifetime [18]. So the conjugate pair of ROH and $RO^-$ coexisted in the solution in the pH range of 2.8–9.5 [19]. When they are excited and the partial spectral overlap occurred. Apparently, the sensitivity and selectivity of the determination was decreased, as a result of spectral overlap, which was a serious drawback from the analytical point of view. Undoubtedly, the solution pH must be limited to lower than 2.8 to obtain satisfactory sensitivity and selectivity and to eliminate the spectral overlap (Fig. 1g). But when the solution pH was lower than 1.0, occurrence of the proton-induced quenching reaction [17] of ROH was observed and the fluorescence intensity was decreased (Fig. 1h). Hence, methods that could both inhibit the proton-transfer reaction and proton-induced quenching reaction of excited state ROH to provide higher sensitivity and more convenience of determination was in urgent need. Spectral contrast was performed using standard β-naphthol solution under the completely same experimental condition to show that no fluorescence emission of other hydrolytic product besides $RO^-$ and ROH in the present method (Fig. 1b and f, dotted line).

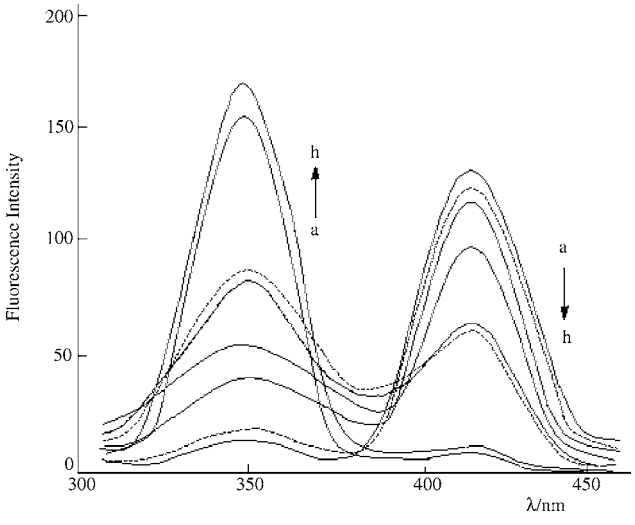



Fig. 1. Effect of pH on hydrolytic product of $5.00 \times 10^{-7}$ mol $L^{-1}$ tolnaftate (solid line) and $5.00 \times 10^{-7}$ mol $L^{-1}$ standard β-naphthol solution (broken line) from (a) to (h), the pH is 14.0, 13.0, 12.0, 10.0, 4.50, 4.50, 1.50 and 0.50, the methanol contents in the solvent (v/v) is 5%.

Effects of β-CD concentration on the fluorescence spectra was studied owning to the formation of a 1:1 inclusion complex between ROH and β-CD [20]. With increasing of β-CD concentration, the intensity of fluorescence peak at 418 and 353 nm gradually decreased and increased respectively, but the emission of $RO^-$ at 353 nm was still relatively strong (Fig. 2a, b and d). This was due to the weak stability of the binary inclusion complex with the formation constant of $590 \pm 50$ $mol^{-1}$ L [20] and the poor solubility of β-CD in water at room temperature [21], which led to insufficient protection for the excited state ROH and insufficient decreasement of the ROH's relatively strong acidity with the p$K_a$ of 3.0 [20]. The determining conditions for ROH was partially optimised by Djoufac-Woumfo et al. [22] using SLS micellar solutions to inhibit the proton-transfer and proton-induced quenching reaction of ROH (Fig. 2e–g). But the pH was limited to lower than 8.0 because the proton-induced reaction was strengthened in the pH range higher than 8.0, which was resulted from changes of the micellar interior structure (Fig. 2e). Spectral comparison was performed using standard β-naphthol solution under the same experimental conditions, which shows that no fluorescence spectral changes produced in the other hydrolysis products of tolnaftate through the addition of SLS or β-CD except $RO^-$ and ROH in the present method (Fig. 2c and f, dotted line).

When a certain quantitative amounts of SLS and β-CD were added to the solution simultaneously, the fluorescence peak of $RO^-$ at 418 nm disappeared completely, and the fluorescence peak of ROH at 353 nm red-shifted to 360 nm with the fluorescence intensity increased by 100 and 40% compared to that in the

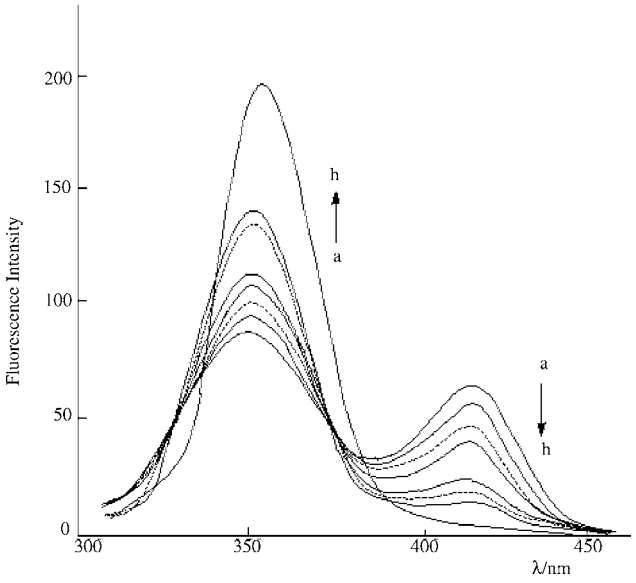



Fig. 2. Effect of addition of SLS or β-CD on hydrolytic product of $5.00 \times 10^{-7}$ mol $L^{-1}$ tolnaftate (solid line) and $5.00 \times \times 10^{-7}$ mol $L^{-1}$ standard β-naphthol solution (broken line) from (a) to (d), β-CD concentration is 0, $5.0 \times 10^{-4}$, $5.0 \times 10^{-3}$ and $1.6 \times 10^{-2}$ mol $L^{-1}$, pH is 4.50; (e) SLS concentration is 0.01 mol $L^{-1}$, pH is 9.00; (f and g) SLS concentration is 0.01 mol $L^{-1}$, pH is 4.50; (h) β-CD concentration is $1.0 \times \times 10^{-3}$ mol $L^{-1}$, SLS concentration is 0.01 mol/L, pH is 4.50. The methanol contents in the solvent (v/v) is 5%.

β-CD or SLS solution, respectively (Fig. 2h). The pH range was greatly broadened from 0.50 to 12.0. Therefore, it was concluded that the microenvironment experienced by ROH was drastically changed and the excited singlet state ROH received the most effective protection, from which the proton-transfer reaction and the proton-induced quenching reaction were inhibited completely. The obviously different phenomenon in the changes of fluorescence spectra of ROH in the β-CD/SLS/ROH system and the fact of the formation of inclusion complex between β-CD and ROH [21], SLS [23] in the aqueous solution made us believe that the ternary inclusion complex was formed among β-CD, β-CD and SLS and ROH.

### *3.2. The supramolecular multirecognition interaction among β-CD, SLS and ROH*

#### *3.2.1. Synthesis and characterization of β-CD-SLS inclusion complex*

According to the reaction routine reported in the reference [24], the calculated amount of SLS (0.01 mol) to be complexation was dissolved in double-distilled water at room temperature and then added dropwise into the solution of β-CD in DMF at 60 °C under continuous soft stirring (the molar ratio of β-CD to SLS was 1:1). The mixed solution was refluxed with agitation at 70 °C for about 6 h, then the majority of the solvent was removed by reduced pressure distillation. After the mixture was cooled to room temperature, it was allowed to be centrifugalized and filtered on a sintered glass filter. The product was obtained and washed twice with doubly distilled water, then filtered, dried in a vacuum oven at an elevated temperature (60–65 °C). White crystalline (6.78 g) was obtained at 47.6% yield.

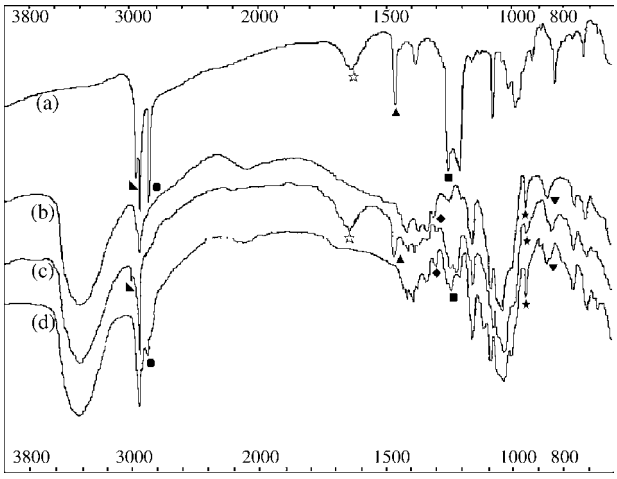



Fig. 3. The infra-red spectra of SLS (a), *β*-CD (b), the physical mixture of SLS and *β*-CD (c) and SLS-*β*-CD inclusion complex (d).

Inclusion complex formation might be proved by IR spectrometry because bands due to the included part of the guest molecule are generally shifted or their intensities altered [25]. By comparison of IR spectra of SLS (Fig. 3a), β-CD (Fig. 3b), the physical mixture of SLS and β-CD (Fig. 3c) and the inclusion complex (Fig. 3d), it could be seen that the spectrum of c was essentially the combination of a and b, which indicated that physical mixture can not lead to inclusion; there were apparent differences between the spectra of c and d and some characteristic IR absorption peaks of SLS and β-CD changed obviously in the inclusion complex: the 951 $cm^{-1}$ absorption peak (⋆) due to the characteristic α-1,4- bond skeleton vibration of β-CD [26] red-shifted to 945 $cm^{-1}$ in the spectra of inclusion complex; the 1337 $cm^{-1}$ absorption peak (♦) assigned to the C–H bending vibration in *φ*CCH*φ*COH*φ*HCH (*φ* referred to bending mode) of β-CD [27] and the 856 $cm^{-1}$ absorption peak (▼) assigned to the C-1 anomeric carbon vibration in *φ*CCH*r*CO*r*CC (*r* referred to stretching mode) of β-CD [27] red-shifted and blue-shifted to 1333 and 862 $cm^{-1}$, respectively, in the complex spectra. The $-CH_2-$ stretching vibration absorption peak of SLS at 2850 $cm^{-1}$ (●) and the S=O stretching vibration absorption peak of SLS at 1250 $cm^{-1}$ (■) appeared blue-shifted and red-shifted to 2855 and 1247 $cm^{-1}$, respectively. Moreover, the disappearance of the following absorption peaks of SLS in the complex spectra provided additional evidence to the formation of SLS-β-CD supramolecualr complex: 1470 $cm^{-1}$ absorption peak (▲) assigned to the deformation vibration of $-CH_2$, 2955 $cm^{-1}$ absorption peak (◣) assigned to the stretching vibration of $-CH_3$, 1631 $cm^{-1}$ absorption peak (☆) assigned to the stretching vibration of C–O.

According to the partial $^1$HNMR results of SLS, β-CD and the inclusion complex (Table 1), apparent changes in chemical shifts of different protons could be observed: unlike the apparent upfield shifts of β-CD's interior H-5 and H-3 protons, which was resulted from the shielding effect exerted by SLS's hydrocarbon chain included into β-CD's cavity, the shifts of β-CD's outside H-2 and H-4 protons was relatively small.

Table 1
Chemical shifts $\delta$ of protons in β-CD, SLS and SLS-β-CD inclusion complex

| | β-CD | | | | | | SLS | | |
|---|---|---|---|---|---|---|---|---|---|
| | $H_1$ | $H_2$ | $H_3$ | $H_4$ | $H_5$ | $H_6$ | $-(CH_2)_{10}-$ | $-CH_3$ | α-H |
| $\delta_{\beta\text{-CD}}$ ($\delta_{SLS}$) | 5.07 | 3.66 | 3.89 | 3.60 | 3.88 | 3.89 | 1.30 | 0.89 | 3.94 |
| $\delta_{\beta\text{-CD-SLS}}$ | 4.99 | 3.62 | 3.85 | 3.56 | 3.73 | 3.82 | 1.24 | 0.93 | 4.10 |
| $\Delta\delta$ [a] | 0.08 | −0.04 | −0.04 | −0.04 | −0.15 | −0.07 | −0.06 | 0.04 | 0.16 |

[a] $\Delta\delta = \delta_{\beta\text{-CD-SLS}} - \delta_{\beta\text{-CD}}$ ($\delta_{SLS}$).

The dramatical shift changes (0.15 ppm) of β-CD's H-5 indicated that the guest molecule entered β-CD's cavity along its narrow rim which possessed primary hydroxyls [27]. The deshielding effect of β-CD's electron-rich cavity on SLS's α-H linked to $SO_4^-$ resulted in dramatically downfield shifts (0.16 ppm) of α-H. Because of the polarity β-CD's cavity, we could conclude that hydrophobic hydrocarbon chain with an apolar end group $-CH_3$ of SLS was included into the cavity. Through the comparison of the spatial size between β-CD's cavity (internal diameter ∼0.78 nm, depth ∼0.78 nm, total volume ∼0.346 $nm^3$ [28]) and SLS's hydrocarbon chain (length ∼1.54 nm, volume ∼0.346 $nm^3$ [29]), we knew that the whole hydrocarbon chain was not entirely included into the cavity and only six methylene groups (length ∼0.783 nm, volume ∼0.161 $nm^3$) could be included into the cavity [30]. The rest part of SLS molecule stretched out and located outside the cavity, among which partial hydrocarbon chain located to the wide rim and coiled, the $SO_4^-$ group located to the narrow rim and formed hydrogen bond with β-CD's primary hydroxyls.

The results from IR spectrometry and $^1$HNMR spectra made us believe that inclusion complex was formed between SLS and β-CD.

### 3.2.2. *Determination of apparent equilibrium constant of ternary inclusion complex*

Equilibrium shifting method [31] was used to evaluate the stoichiometry of the β-CD:SLS:ROH ternary complex, As shown in Fig. 4, the slope of the two plots was 1.07 and 1.13, respectively, suggesting the formation of a 1:1:1 ternary complex.

The formation of the ternary inclusion complex could be described as follows:

$$\mathrm{ROH} + \beta\text{-CD} + \mathrm{SLS} \overset{K}{\rightleftharpoons} \mathrm{ROH : \beta\text{-CD} : SLS} \quad \text{(i)}$$

The 1:1:1 stoichiometry of the β-CD:ROH:SLS system was also confirmed by the Benesi–Hildebrand equation [32]:

$$\frac{1}{\Delta I_p} = \frac{1}{\alpha K}[\mathrm{SLS}][\beta\text{-CD}] + \frac{1}{\alpha} \quad \text{(ii)}$$

where $K$ was the apparent equilibrium constant and $\Delta I_p$ was the difference in the fluorescence intensity of ROH in the presence and absence of β-CD. [SLS] and [β-CD] was the equilibrium concentration of SLS and β-CD, respectively. $\alpha$ Was a combined instrumental constant. When the initial concentration of β-CD, $C_{\beta\text{-CD}}$, was much higher than that of the inclusion complex, [β-CD] could be replaced by $C_{\beta\text{-CD}}$. $1/\Delta I_p$ versus $1/[\beta\text{-CD}]$ for the β-CD:ROH:SLS system was a representative double-reciprocal plot. A good linear relationship was observed with a correlation coefficient of 0.9923, providing additional evidence for the 1:1:1 stoichiometry of a ternary inclusion complex in aqueous solution. The evaluated $K$ from the ratio of the intercept to slope was $(5.48 \pm 0.13) \times 10^3$ $L^2\,mol^{-2}$.

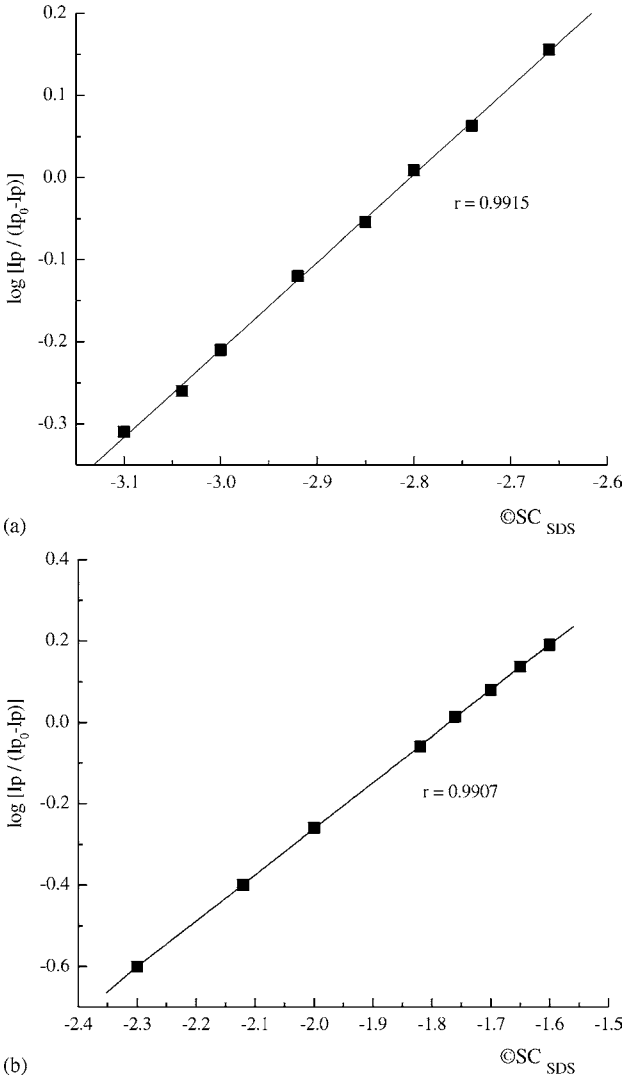



Fig. 4. Equilibrium shifting method for the determination of formation ratio of the ternary inclusion complex $C_{ROH} = 5.00 \times 10^{-7}$ mol $L^{-1}$, pH 4.50, (a) $C_{SLS} = 1.0 \times 10^{-2}$ mol $L^{-1}$, (b) $C_{\beta\text{-CD}} = 1.0 \times 10^{-3}$ mol $L^{-1}$.

### 3.3. Effect of varying reaction conditions

#### 3.3.1. Effect of pH

Effect of solution pH on the fluorescence intensity was studied. Solution pH was adjusted by dilute hydrochloric acid solution in acid range and sodium hydroxide solution in alkaline range. The experimental results showed that the fluorescence intensity reached its maximum and remained constant in the pH range 0.5–12.0. The optimum pH range was 0.50–12.0, so 0.20 mol $L^{-1}$ acetic acid–sodium acetate buffer solution was used to adjust pH at 4.50. It had been established that the ROH proton-induced quenching mechanism occurred via protonation leading to proton exchange and the intramolecular charge-transfer structure in the excited state was responsible for the quenching. The migration of the electron from the electron-donating hydroxyl group to the aromatic ring caused formation of the intramolecular exciplex and made one of the carbon atoms of the aromatic ring electronegative, which resulted in protonation mentioned above [33]. In this process, some of the excited electron energy was delivered to the solvent in the form of vibration energy resulting in the fluorescence quenching. The lower polarity and the greater microviscosity experienced by ROH molecules inside the hydrophobic micellar interior layers partially inhibited the formation of intermolecular exciplex and energy delivery. The ROH fluorescence intensity was apparently increased in the ternary complex system compared with that in the micellar solution indicating the obvious changes of the microenvironment around ROH. We found that the synergistic protection of SLS and β-CD could produce lower polarity and greater microviscosity for the excited state of ROH compared with that in the micelles alone, and the hydrogen bonding formation between ROH's hydroxyl group and β-CD's secondary hydroxyl groups could effectively make it hard for the intramolecular charge-transfer process from ROH's hydroxyl to its aromatic ring. The proton-induced reaction in the single micelle solution was strengthened in the pH range higher than 8.0, which was resulted from some changes of micellar interior structure to lose the inhibition effect on the ROH's proton transfer reaction. The dissociation of the ternary inclusion complex resulted from the dissociation of β-CD's hydroxyl at pH higher than 12.0 caused the fluorescence intensity to decrease, which suggested that the synergistic protection or shielding effect of β-CD and SLS was indispensable for the excitation ROH, especially in the pH range of 8.0–12.0.

#### 3.3.2. Effect of β-CD concentration

Studies on the dependence of fluorescence intensity on the β-CD concentration was performed. In the β-CD concentration range of $8.0 \times 10^{-4}$ to $1.5 \times 10^{-3}$ mol $L^{-1}$, the fluorescence intensity was the highest and remained constant. So $1.0 \times 10^{-3}$ mol $L^{-1}$β-CD was used.

#### 3.3.3. Effect of SLS concentration

The influence of SLS concentration was studied. It was found that in the SLS concentration range of $9.5 \times 10^{-3}$ to $1.2 \times 10^{-2}$ mol $L^{-1}$, fluorescence intensity was the highest and remained constant. When the SLS concentration exceeded $1.2 \times 10^{-2}$ mol $L^{-1}$, fluorescence intensity began to gradually decrease, so $1.0 \times 10^{-2}$ mol $L^{-1}$ SLS was fixed. When the SLS concentration was higher than a certain value ($1.2 \times 10^{-2}$ mol $L^{-1}$ in this reaction condition), more and more SLS molecules were driven by the hydrophobic force to compete with ROH for β-CD's cavity. The final results were that the ROH molecules were expelled from β-CD's cavity and were dissolved in a mixed solution with a great deal of SLS and β-CD, which was quite different from the microenvironment in the ternary inclusion complex. Thus the shielding protection for the excited single state of ROH was removed and the intermolecular collision was reinforced, along with the rate of nonradiative decay process increased, which led to drastic lowering of the fluorescence quantum yield and the occurrence of fluorescence quenchment

### 3.4. Analytical parameters

The enhancement of the fluorescence intensity of ROH produced through the formation of the ternary inclusion complex might be very useful from an analytical point of view. Therefore, an indirect spectrofluorimetric method for the determination of TNF in bulk aqueous solution in the presence of β-CD and SLS was developed.

Into a series of 10 mL colorimetric tubes were added TNF standard solutions. Under optimum conditions, the fluorescence intensity was measured. The results showed that the fluorescence intensity was linear over a TNF concentration range of $2.46 \times 10^{-9}$ to $2.10 \times 10^{-6}$ mol $L^{-1}$. The linear regression equation was $I_F = 3.38 \times 10^8 c$ (mol $L^{-1}$) + 29.5 ($r = 0.9908$). The relative standard deviation (R.S.D.) was 1.4% obtained from a series of 11 standards each contained $5.00 \times 10^{-7}$ mol $L^{-1}$ TNF. Based on the definition by IUPAC [34], $C_L = KS_0/S$, where $C_L$ was limit of detection, $K$ was a constant combined with the confidence level, $S_0$ (0.084) was the standard deviation obtained from a series of 11 blank solutions, $S$ was the slope of the standard curve. When the confidence level was 90%, the limit of detection of the proposed method was determined to be $7.50 \times 10^{-10}$ mol $L^{-1}$. The proposed method was compared to the previous reported method. The results showed that the proposed method has high sensitivity and selectivity.

### 3.5. Effect of foreign interference

In the light of the method was applied in Section 2.2. A systematic study was carried out on the effects of foreign interferences on the determination of $5.00 \times 10^{-7}$ mol $L^{-1}$ of TNF. A 6000-fold mass excess of each interference over TNF was tested first, if interferences occurred, the ratio was reduced gradually until the interferences ceased. The criterion for interference was fixed at a ±5.0% variation of the average fluorescence intensity calculated for the established level of TNF. The results were shown in Table 2. It was obvious that the proposed method had excellent selectivity.

Table 2
Comparison with other methods for the determination of tolnaftate

| Methods | Detection limits ($ng\,mL^{-1}$) | Linear range | References |
|---|---|---|---|
| SCF | 100 | 0.20–10.00 $\mu g\,mL^{-1}$ | [3] |
| Spectrofluorimetry | 26 | 3.0–7.0 $\mu g\,mL^{-1}$ | [4] |
| Spectrofluorimetry | 6.1 | 20.4–400 $ng\,mL^{-1}$ | [10] |
| This work | 0.23 | 0.76–0.65 $\mu g\,mL^{-1}$ | |

Table 3
Effect of foreign substances on the determination of $5.00 \times 10^{-7}$ mol $L^{-1}$ tolnaftate

| Tolerance ratio in mass | Foreign ions |
|---|---|
| 5000 | $K^+$, $Na^+$, $Ca^{2+}$, $Ba^{2+}$, $Co^{2+}$, $Be^{2+}$, $Zn^{2+}$, $Cl^-$, $AC^-$, $NO_2^-$, $CN^-$, $SO_4^{2-}$ |
| 4000 | $NO_3^-$, $Br^-$, $CO_3^{2-}$ |
| 2500 | $Cu^{2+}$, $Mn^{2+}$, $Ni^{2+}$, ethanol, sodium acetate |
| 500 | $Pb^{2+}$, $Fe^{2+}$, $Fe^{3+}$, $Al^{3+}$, $H_2PO_4^-$, $MnO_4^-$, $PO_4^{3-}$ |
| 80 | $NH_4^+$, $Cd^{2+}$, $Cr_2O_7^{2-}$, acetate acid |

Table 4
Determination of tolnaftate in mixed samples ($n = 5$)

| Sample number | Tolnaftate added ($10^{-7}$ mol $L^{-1}$) | Found total ($10^{-7}$ mol $L^{-1}$) | Recovery (%) | R.S.D. (%) |
|---|---|---|---|---|
| 1 | 1.00 | $0.97 \pm 0.05$ | 97 | 2.1 |
| 2 | 1.50 | $1.48 \pm 0.06$ | 99 | 2.8 |
| 3 | 1.00 | $1.02 \pm 0.02$ | 102 | 1.6 |

### 3.6. Application

To evaluate the accuracy of the proposed method, a standard adding method was performed to detect trace amounts of TNF in artificial mixed samples. The results were shown in Table 3. As could be seen, the precision and accuracy of the proposed method was satisfactory (Table 4).

(1) Polyethylene glycol 4000 ($3.00 \times 10^{-6}$ mol $L^{-1}$); sodium carboxymethyl-cellulose ($2.50 \times 10^{-5}$ mol $L^{-1}$); sucrose ($2.50 \times 10^{-5}$ mol $L^{-1}$); sorbitol ($3.00 \times 10^{-5}$ mol $L^{-1}$).
(2) Methyl cellulose ($3.00 \times 10^{-5}$ mol $L^{-1}$); lactose ($4.50 \times 10^{-5}$ mol $L^{-1}$); mannitol ($4.50 \times 10^{-5}$ mol $L^{-1}$); starch ($6.00 \times 10^{-5}$ mol $L^{-1}$).
(3) NaCl ($5.00 \times 10^{-5}$ mol $L^{-1}$); glucose ($3.00 \times 10^{-5}$ mol $L^{-1}$); gum acacia power ($4.50 \times 10^{-5}$ mol $L^{-1}$); boracic acid ($4.50 \times 10^{-5}$ mol $L^{-1}$).

## 4. Conclusions

Hydrolysis of tolnaftate in 5.0 mol $L^{-1}$ sodium hydroxide we obtained β-naphtholate. The formation of the ternary inclusion complex among anionic surfactant sodium laurylsulfate, β-cyclodextrin and β-naphthol with stoichiometry of 1:1:1 was observed at pH 4.5. The mechanism of the supramolecular interaction was studied. It was found that the formation of the inclusion complex protected the ROH fluorescence against varying quenching factors, and inhibited the proton-transfer reaction and proton-induced quenching reaction of excited state ROH. Comparared to Khashaba's method, the experimental conditions were drastically changed and optimized: the pH range was greatly broadened and the volume contents of methanol were decreased. At $\lambda_{ex}/\lambda_{em} = 273/360$ nm, the fluorescence intensity was linear over a tolnaftate concentration range of $2.46 \times 10^{-9}$ to $2.10 \times 10^{-6}$ mol $L^{-1}$. The detection limit and relative standard deviation was $7.50 \times 10^{-10}$ mol $L^{-1}$ and 1.4%, respectively. The interference of 31 foreign substances was slight. The proposed method had been successfully applied to the determination of tolnaftate in artificial mixed samples with almost quantitative recovery.

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