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Research Article

Label-Free Fluorescent Determination of Sunset Yellow in Soft Drinks Based on an Indicator-Displacement Assay

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This work reported a fluorescence sensing platform for Sunset Yellow (SY) determination based on competitive host-guest interaction between cucurbit[7]uril (CB7) and signal probe/target molecules. Luteolin/epigallocatechin gallate (EGCG) and SY were selected as the probe and target molecules, respectively. When luteolin or EGCG entered the CB7 host, its fluorescence significantly improved. However, upon the presence of SY in the performed luteolin·CB7 or EGCG·CB7 complex, this led to a remarkable decrease in fluorescence. This result was due to the fact that the binding constant of CB7/SY (4.9×10^4 M^{-1}) was greater than that of CB7/luteolin (3.2×10^3 M^{-1}) or CB7/EGCG (4.8×10^3 M^{-1}). The fluorescence intensities of CB7/luteolin and CB7/EGCG complexes decreased linearly with increased SY concentration ranges of 0.5–50.0 and 2.0–50.0 μM. The proposed method had detection limits of 0.12 and 0.45 μM and was successfully used to determine SY samples with good recoveries ranging from 96.3% to 103.8%. This competitive mode provided a promising fluorescence assay strategy for potential applications in food safety.

1. Introduction

Synthetic colorants, a very important class of food additives, have been used to replace natural ones for many years because of their remarkable advantages. However, synthetic colorants must be controlled strictly by the laws because of their potential risks to human health caused by the presence of azo groups (–N=N–) and aromatic ring structures [1]. Sunset Yellow (SY) is a widely used synthetic colorant, which not only can improve the appearance and texture of foods but can also maintain their natural color during process and storage [2]. However, excess intake of SY can cause many adverse health effects, such as allergies, migraines, eczema, anxiety, and childhood hyperactivity, if excessively consumed [1]. Therefore, the use of SY in food products is strictly controlled. In China, the allowable maximum limit of SY addition in soft drinks is 0.1 g/kg (GB2760-2011). Therefore, convenient, rapid, and reliable methods for the determination of SY is extremely required for the assurance of food safety. Until now, various methods such as high performance liquid chromatography (HPLC) [3], HPLC-mass spectrum (HPLC-MS) [4], capillary electrophoresis [5], and chromatography [6] have been developed for the determination of SY. However, these methods are time-consuming because complicated sample preparation procedures are needed and generally require expensive instrumentation obligatorily. Therefore, developing a simple and convenient approach for the determination of SY is highly desirable [3].

The concept of indicator-displacement assay (IDA) has attracted considerable attention with the development of host-guest chemistry, which exploits the potential of artificial receptors, particularly macrocyclic hosts, because of its promising applications in molecular recognition and sensing [7]. IDA has become a popular approach for electrochemical/optical sensing by utilizing noncovalent interactions between a receptor (the host), indicator (the guest), and an analyte (the competitive guest) [1]. The sensing principle of IDA relies on the competition between a test substance and an indicator for the same binding site on the host [8–10]. When an analyte is added to a solution containing a host-indicator complex, the analyte displaces the indicator from the binding site. Upon displacement of the indicator, a change in signal is observed [2]. Cucurbit[*n*]urils (CB[*n*]s), along

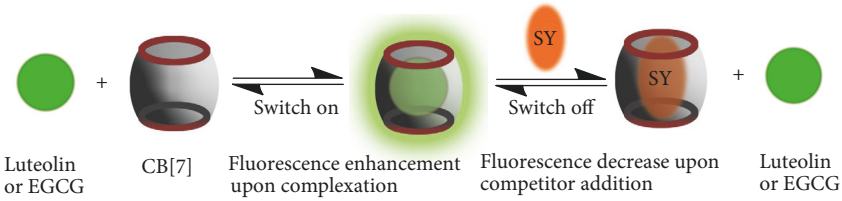


Figure 1: Indicator-displacement assay towards SY using CB7·luteolin or CB7·EGCG as the "reporter pair."

with crown ethers, cyclodextrins, and calixarenes, are the fourth-generation macrocyclic hosts that have played crucial roles in supramolecular chemistry in recent decades [11–15]. CB[*n*] are highly symmetrical macrocyclic hosts composed of glycoluril units linked through methylene bridges, and they possess highly polar carbonyl portals with a hydrophobic cavity [16]. The popularity of CB[*n*] is ascribed to their novel properties in the formation of inclusion complexes with various guest molecules with high selectivity and high binding constant in aqueous solution [17–21]. In recent years, fluorescence enhancement approach with IDA principle based on CB[*n*] host-guest complexation has been used extensively for the determination of pesticides [22, 23], drugs [24], and other compounds [25]. This method, by converting CB[*n*]-indicator complexes into optical sensors, has attracted widespread attention because of their simplicity, rapidity, sensitivity, and selectivity.

In the present work, a facile fluorescent approach for SY sensing based on a competitive host-guest recognition between CB7 and signal probe/target molecules was developed by selecting luteolin and epigallocatechin gallate (EGCG) as signal probes. Luteolin and EGCG are two very weakly fluorescent molecules that can form inclusion complexes with CB7 to greatly enhance their fluorescence emission. The design principle of the developed fluorescent sensing platform for SY sensing is shown in Figure 1. Luteolin/EGCG and SY were selected as the probe and target molecules, respectively. When luteolin or EGCG entered into the CB7 host, its fluorescence enhanced significantly. However, upon the presence of SY to the formed luteolin·CB7 or EGCG·CB7 complex, the luteolin or EGCG probe molecules were displaced by SY from the host of CB7, leading to a "switch-off" fluorescence response.

2. Materials and Methods

2.1. Chemicals and Materials. SY, luteolin, and EGCG were purchased from Shanghai Adamas Reagent Co., Ltd. (Shanghai, China). CB7 was obtained from the National Engineering and Research Center for Colloid Material in Shandong University. All other reagents were of analytical grade, and all aqueous solutions were prepared with deionized water (DW, 18 MΩ cm).

2.2. Instrument. Fluorescence titration experiments were performed by a fluorescence spectrophotometer (Hitachi F-4500, Tokyo, Japan) at room temperature.

2.3. Stoichiometry Determination. The stoichiometry of the host-guest complexes was measured using the continuous variation of Job's method by fluorescence spectroscopy according to a previous work [31]. The total molar concentration of the guest and host aqueous mixture was kept constant at 40 μM. The fluorescence was recorded at different molar ratios ranging from 0 to 1.

2.4. Fluorescence Titration Experiments. Fluorescence titration experiment was performed according to a previously reported work with minor modification [31]. The luteolin or EGCG stock solution was diluted to a final concentration of 20 μM. CB7 was then gradually added to the guest molecule solution and mixed by vortexing well before the fluorescence was recorded. Then, the required amount of SY was gradually added to the mixture of host-guest complex. The fluorescence was recorded after being mixed well by vortexing.

2.5. Molecular Docking. The crystal structure of host molecule CB7 (number 1513097) was obtained using the Crystallography Open Database. The 3D structures of host (CB7) and guest (SY) were constructed by the UCSF Chimera software. The structures of the host and guest molecules were fully optimized using a UCSF Chimera software. The Dock Prep module was used to add hydrogen atoms. For the molecular docking study, the molecular surface of the CB7 molecule was generated using the DMS tool with a probe radius of 1.4 Å. The sphgen module was applied to generate spheres surrounding the binding site. The Grid file was generated by using Grid module of DOCK6. The flexible docking method was utilized producing 1000 different conformational orientations for the guest molecule. Finally, to retain the best results, clustering analysis with root-mean-square deviation threshold of 2.0 Å was carried out.

2.6. Sample Preparation and Analysis. For real sample analysis, three soft drinks (Xianchengduo, Mangguoduo, and Fenda), which were bought from the local supermarket were chosen in the experiment. A stock solution of SY (500 μM) was prepared in DW and diluted to different concentrations by DW for further use. The three soft drink samples were filtered through 0.45 μm membrane filter, diluted fifty times with DW, and mixed with known amount of SY; this solution was used to detect SY according to the procedure described above.

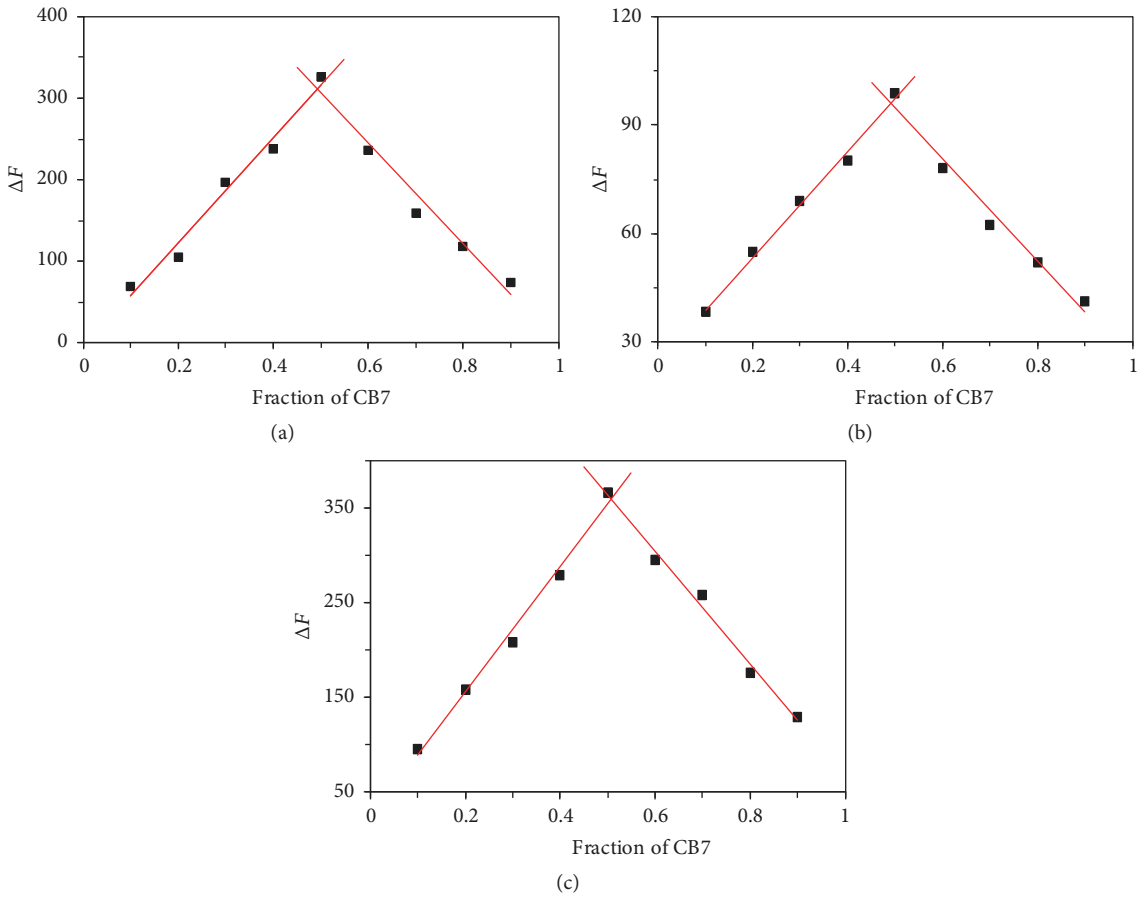


Figure 2: Job's continuous variation plot of the CB7/SY (a), CB7/luteolin (b), and CB7/EGCG (c) complexes.

3. Results and Discussion

3.1. Stoichiometries of CB7/SY, CB7/Luteolin, and CB7/EGCG. The stoichiometry for the inclusion complexation of CB7 with SY was determined using Job's experiments by fluorescence spectroscopy. As shown in Figure 2(a), the plot maximum point appears at a CB7 molar fraction of 0.5, which obviously indicates that a 1 : 1 inclusion complex was formed between CB7 and SY. A similar result was obtained in the case of the inclusion complex of CB7/luteolin and CB7/EGCG (Figures 2(b) and 2(c)).

3.2. Fluorescence Titration. The fluorescence spectra of luteolin and EGCG in the presence of various concentrations of CB7 were investigated. Figure 3(a) shows the fluorescence titrations of luteolin (20 μM, λex = 285 nm) upon successive addition of CB7 (up to 50 μM) in aqueous solution. The fluorescence intensity of luteolin enhanced significantly upon the complexation with CB7. This finding can be attributed to the inclusion of luteolin into CB7 and the change in its structure or conformation to produce the fluorescent complex. The fluorescence titrations of EGCG (20 μM, λex = 260 nm) were also obtained (Figure 3(c)). The addition of CB7 also caused a remarkable enhancement of the fluorescence intensity of EGCG. Herein, a slight hypsochromic shift was observed in the inclusion process of CB7/luteolin and CB7/EGCG. This blue shift suggested that luteolin and EGCG were located in a more hydrophobic environment. The results demonstrated that the luteolin and EGCG molecules were inserted into the hydrophobic cavity of CB7, and resulting hydrophobic interaction led to the hypsochromic shift and the fluorescence enhancement. Interestingly, the addition of SY to the CB7 and luteolin mixture led to a successive reversion of the fluorescence changes originally caused by the addition of CB7 (Figure 3(b)) and SY to the CB7/EGCG complex, which reverted the fluorescence of EGCG (Figure 3(d)). This finding was attributed to the displacement of luteolin or EGCG by SY from the CB7 host.

3.3. Mechanism of Competitive Host-Guest Interaction. To clarify the mechanism of the competitive host-guest interaction, the plots of fluorescence intensity of 20 μM luteolin (Figure 4(a)) and 20 μM EGCG (Figure 4(c)) versus CB7 concentration and the double reciprocal plots of $1/(F_0 - F)$ versus 1/[CB7] for luteolin (Figure 4(b)) and EGCG (Figure 4(d)) to CB7 were obtained, indicating the existence of the 1 : 1 complex. From the plots the binding constants (K) for the 1 : 1 luteolin/CB7 and EGCG/CB7 complexes were calculated to be 3.2×10^3 and 4.8×10^3 M^{-1}, respectively.

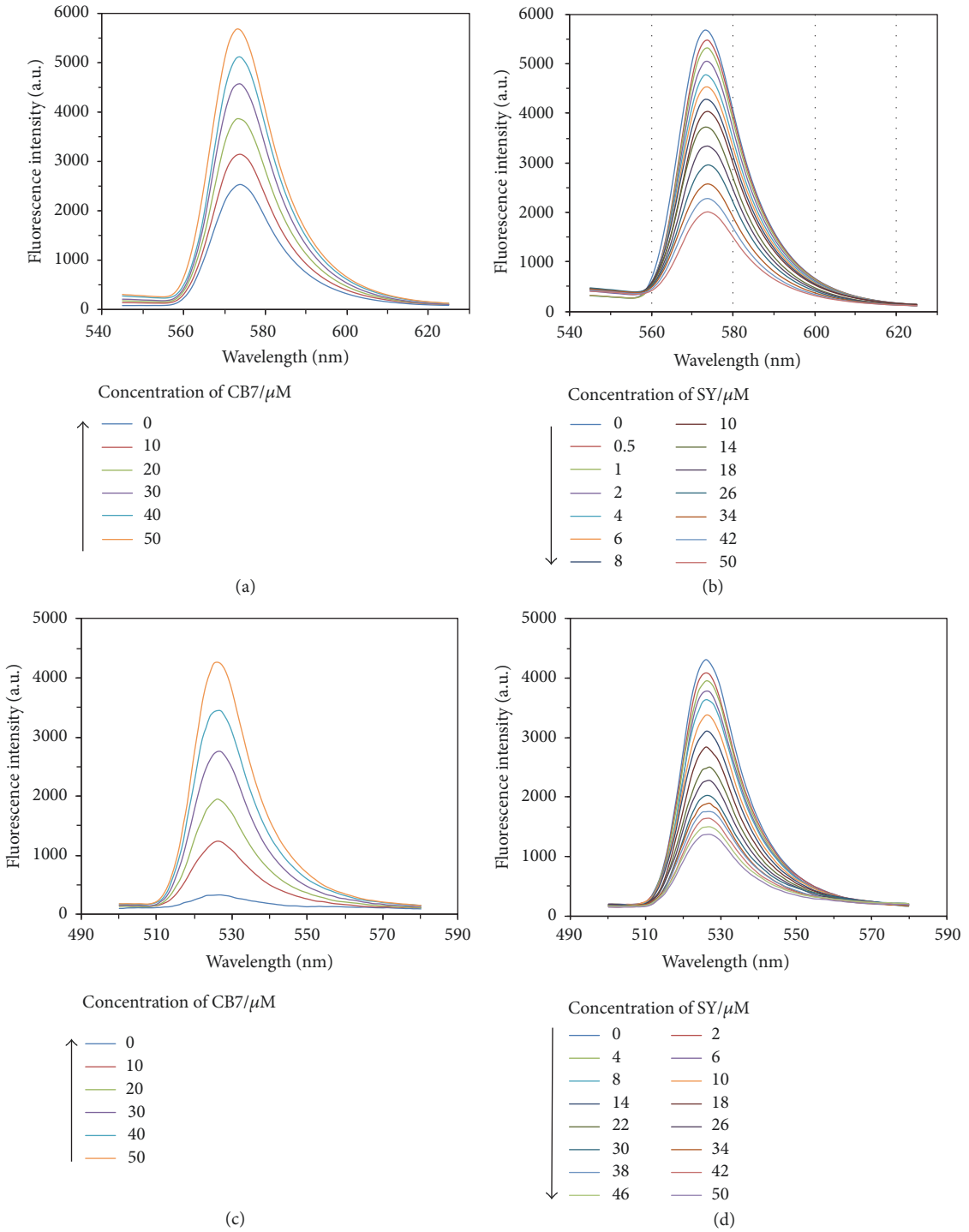


Figure 3: Fluorescence titrations of luteolin ((a) 20 μM, λex = 285 nm) and EGCG ((c) 20 μM, λex = 260 nm) upon successive addition of CB7 (up to 50 μM) in aqueous solution. Fluorescence titration for the competitive displacement of luteolin ((b) 20 μM) and EGCG ((d) 20 μM) from CB7 (50 μM) by SY (up to 50 μM) in aqueous solution. The combined solution was mixed by vortexing well for 5 min and then tested.

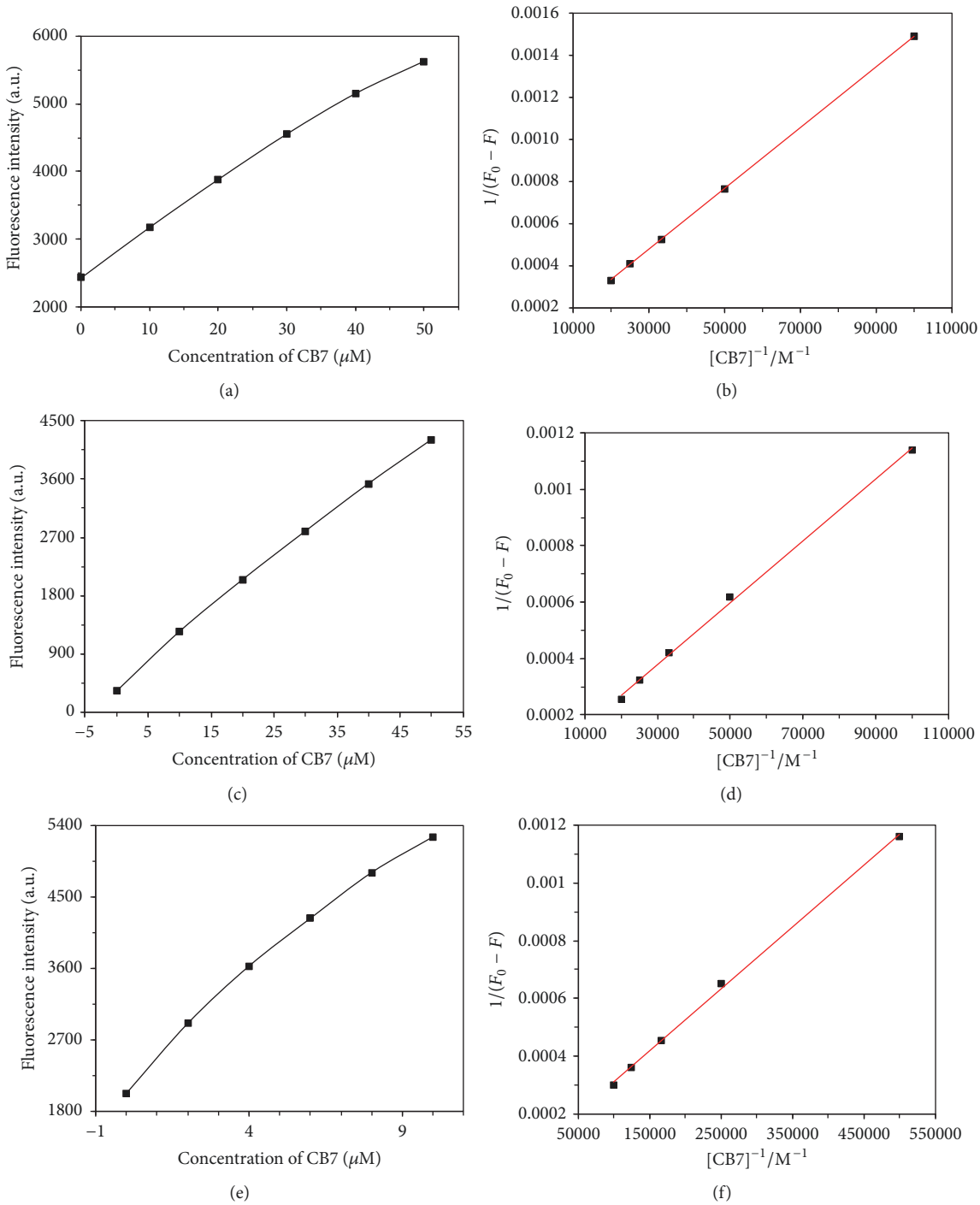
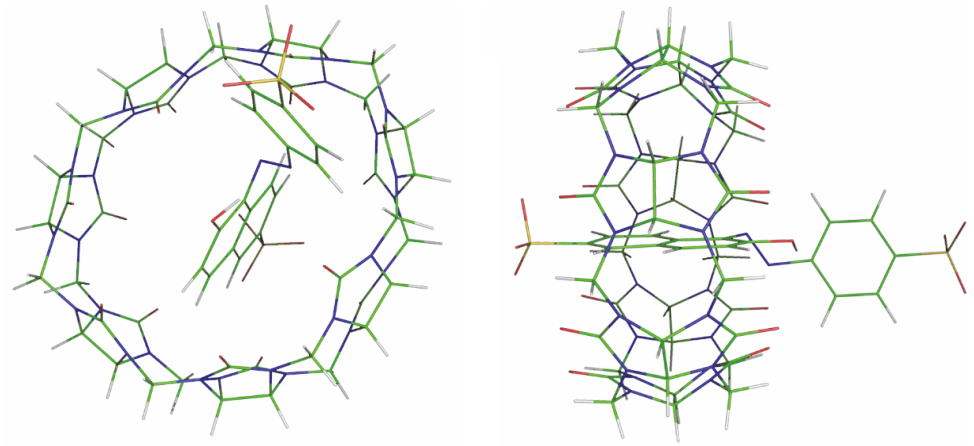


Figure 4: Fluorescence intensity of 20 μM luteolin (a), 20 μM EGCG (c), and 20 μM SY (e) versus CB7 concentration and plots of $1/(F_0 - F)$ versus 1/[CB7] for luteolin (b), EGCG (d), and SY (f) (λex = 285 used for luteolin; λex = 260 nm used for EGCG; λex = 282 nm used for SY).

The plot of fluorescence intensity of 20 μM SY (Figure 4(e)) versus CB7 concentration and the double reciprocal plot of $1/(F_0 - F)$ versus 1/[CB7] for SY (Figure 4(f)) to CB7 were also obtained. The binding constant for the 1 : 1 SY/CB7 complex was calculated to be $4.9 \times 10^4\ M^{-1}$. The K value of SY/CB7 complex was more than 10 times higher than that of luteolin/CB7 or EGCG/CB7, which demonstrated the stronger binding of SY with CB7 than that with luteolin or EGCG.

3.4. Molecular Docking. Molecular docking was performed to study the CB7/SY inclusion complex in order to gain an insight into the binding mode. The binding model of CB7/SY was simulated using the DOCK6 program. Initially,



(a) (b)

Figure 5: The typical conformation of the CB7/SY complex by molecular docking ((a) is the top view; (b) is the side view).

Table 1: Molecular docking scores for CB7/SY inclusion complex.

Complex	Pose	Grid_score (kcal·mol^{-1})	Grid_vdw (kcal·mol^{-1})	Grid_es (kcal·mol^{-1})	Int_en (kcal·mol^{-1})
CB7/SY	1	−43.1004	−47.0535	3.9531	5.5096
	2	−42.9977	−47.1133	4.1156	14.5849
	3	−39.7064	−46.9512	7.2448	4.2503

vdw: van der Waals force; es: electrostatic force; Int_en: intramolecular energy.

the docking scores of CB7/SY were obtained and provided in Table 1. Generally, a more negative binding energy means a stronger host-guest interaction. As shown in Figure 5, the lowest energy docked conformation for 1 : 1 complex of CB7/SY was obtained, which indicated that the naphthol part of the SY molecule was inserted into the cavity of the CB7 host molecule, while the benzene sulfonic acid group of the SY molecule was located at the outside of the CB7 host. The very high van der Waals contribution (approximately −47 kcal·mol^{-1}) indicated that strong hydrophobic interactions formed between the SY and the CB7 molecule.

3.5. Analytical Performance. On account of the competitive host-guest interaction, the proposed fluorescence method was used for quantitative detection of SY. As shown in Figure 6(a), with increasing SY concentration, the fluorescence intensities of CB7·luteolin linearly decreased at the ranges of 0.5–10.0 μM and 10.0–50.0 μM. The linear regression equations were $\Delta F/F_0 = 0.203C$ (μM) + 0.010 and $\Delta F/F_0 = 0.054C$ (μM) + 0.025 with correlation coefficients of 0.998 and 0.994, respectively. The detection limit was 0.12 μM at 3σ. As shown in Figure 6(b), with increasing SY concentration, the fluorescence intensities of CB7·EGCG also linearly decreased at ranges of 2.0–30.0 and 30.0–50.0 μM. The linear regression equations were $\Delta F/F_0 = 0.320C$ (μM) + 0.007 and $\Delta F/F_0 = 0.016C$ (μM) + 0.018 with correlation coefficients of 0.998 and 0.996, respectively, and a detection limit of 0.45 μM at 3σ. The proposed method had higher sensitivity and relatively lower detection limit among other methods used for the detection of SY, as shown in Table 2.

3.6. Selectivity and Analytical Application. The selectivity of the proposed method was studied with the same concentration of other colorants including Tartrazine, New Coccine, Amaranth, Allura Red, and Brilliant Blue. Common molecules such as glucose, sucrose, $MgCl_2$, NaCl, and KCl were also tested at interference concentrations 20-fold that of the colorants. The changes in the fluorescence ratio $(F_0-F)/F_0$ of the CB7·luteolin complex upon addition of a particular competitive binding analyte were displayed in Figure 6(c). Upon interaction with the competitive binding analytes, the fluorescence of the CB7·luteolin complex was increased selectively by addition of SY, while addition of other competitive binding analytes caused nonsignificant fluorescence changes. The proposed method was applied to the determination of SY using standard addition methods in three soft drinks (Xianchengduo, Mangguoduo, and Fenda). The results for the determination of SY in these samples with the proposed fluorescent method are listed in Table 3. The recoveries were in the range of 96.25%–103.83% and RSDs were in the range of 2.5%–4.2%. As can be seen, the precision and accuracy of the proposed method were satisfactory, indicating that this method can be extended for SY detection in soft drinks and food samples.

4. Conclusions

This work established a convenient method for SY determination based on the competitive host-guest interaction between CB7 and probe/target molecule. The formation of CB7/luteolin and CB7/EGCG complexes greatly enhanced

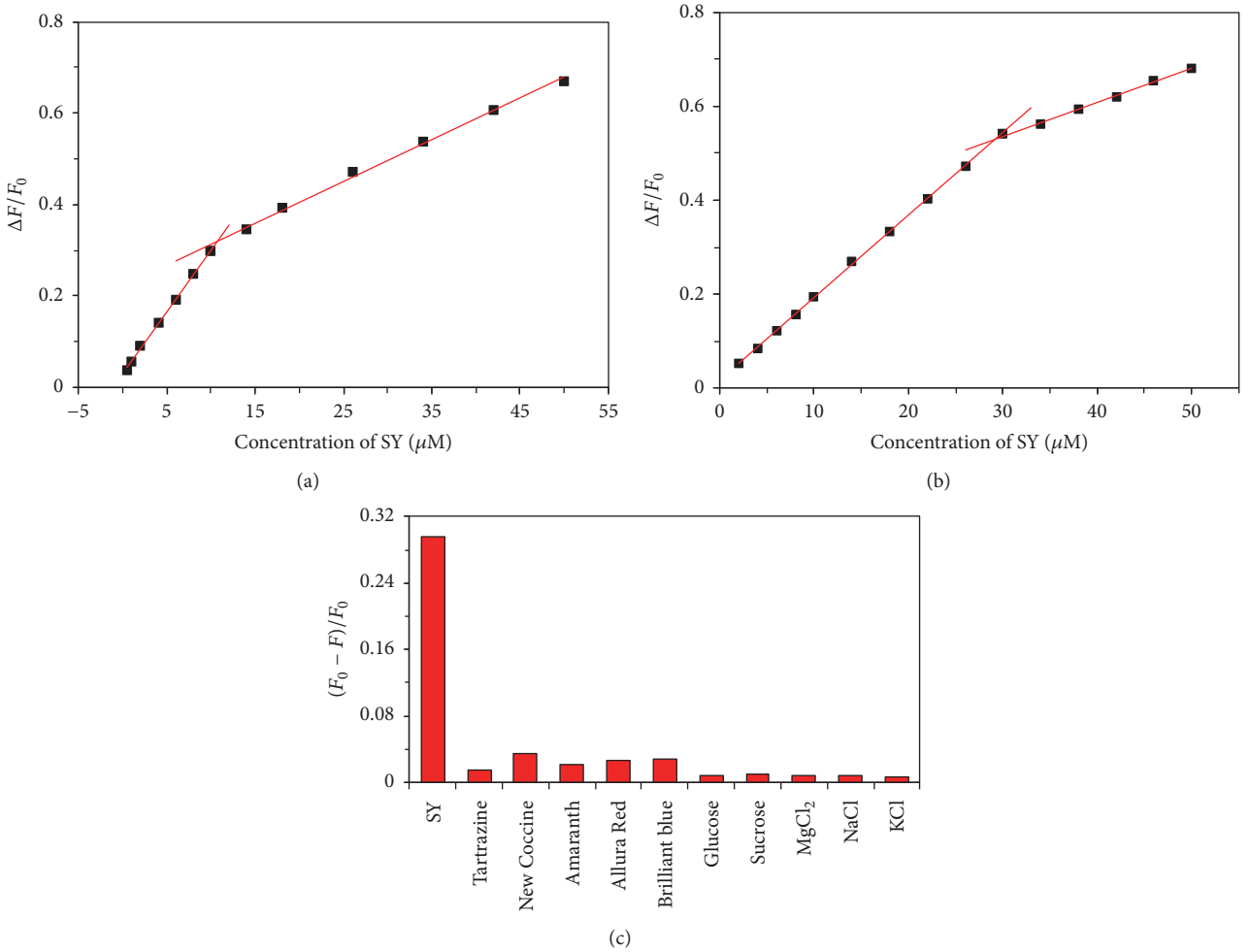


Figure 6: Calibration curves of fluorescence intensity of CB7·luteolin (a) and CB7·EGCG (b) versus SY concentration. (c) Relative fluorescence intensity is calculated by $(F_0 - F)/F_0$, where F_0 and F are the fluorescence intensity without and with the presence of SY (10 μM), Tartrazine (10 μM), New Coccine (10 μM), Amaranth (10 μM), Allura Red (10 μM), Brilliant Blue (10 μM), glucose (200 μM), sucrose (200 μM), $MgCl_2$ (200 μM), NaCl (200 μM), and KCl (200 μM), respectively.

Table 2: Comparison of some methods used for determination of SY.

Methods	Probe	Linear range (μM)	Detection limit (μM)	Ref.
DPV	—	0.1–15	0.07	[26]
DPV	—	0.40–14	0.04	[27]
DPV	—	1–271	0.8	[28]
DPV	—	0.4–110	0.1	[29]
DPV	—	1–50	0.8	[30]
Fluorescence	Luteolin	0.5–50.0	0.12	This work
Fluorescence	EGCG	2.0–50.0	0.45	This work

the fluorescence emission of luteolin and EGCG. However, the presence of SY in the formed CB7/luteolin or CB7/EGCG complex enabled the replacement of luteolin or EGCG in the CB7 by SY because CB7/SY complex possessed a higher binding constant than CB7/luteolin or CB7/EGCG complex, leading to a "switch-off" fluorescence emission response. On account of the IDA principle, this fluorescence method for SY determination showed high sensitivity and good selectivity. The method was successfully used to analyze SY in the soft drinks. The CB7/SY inclusion complex was further studied by molecular modeling calculations, and results indicated that the naphthol part of the SY molecule was included into

Table 3: Determination of SY in soft drink samples ($n = 6$).

Sample	Added (μM)	Founded (μM)	RSD (%)	Recovery (%)
Xianchengduo	0	3.72	3.8	—
	2	5.51	3.1	96.33
	6	9.91	2.8	101.95
Mangguoduo	0	2.35	3.9	—
	2	4.48	4.2	102.98
	6	8.67	3.6	103.83
Fenda	0	2.53	3.9	—
	2	4.36	2.5	96.25
	6	8.43	4.1	98.83

the CB7 cavity. Binding-mode analysis demonstrated that the hydrophobic interaction contributed to the formation of the inclusion complex.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Shilian Wu and Yanqiong Zhang contributed equally to this work.

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