**Introduction**

Synthetic colorants are widely used in the production of foodstuffs for improving their consumer characteristics and appearance due to their many advantages, including easy coloring, high stability, low microbiological contamination and low pollution properties. The molecular structures of sunset yellow (SY) and acridine orange (AO) are shown in Scheme 1.

SY is a common artificial food additive that has been extensively applied in food preparations to make food more attractive and appealing. However, it is vital to note that SY is one of the synthetic colorants most likely present in common food products that may lead to allergies, diarrhea, anxiety migraines and also could damage to the kidney and liver if excessively consumed. Currently, the use of such additives is drawing much attention due to the series of health risks induced by food colorants, because it plays an indispensable part in human public health. Therefore, undoubtedly, the use of synthetic dyes in foodstuffs must be strictly controlled by legislation throughout the world and the detection of SY in a simple and sensitive manner is significant.

It is acknowledged that SY is a vital constituent in the food industry with a concentration in food lower than 0.1 g kg$^{-1}$. The analytical techniques frequently used for the determination of SY because of its potential pathogenicity include spectrophotometry, enzyme-linked immunosorbent assay (ELISA), electrochemical methods, high performance liquid chromatography, thin layer chromatography-high performance liquid chromatography (TLC-HPLC), capillary electrophoresis. Although chromatographic methodology offers sensitive and specific multi-analytical results, it is time consuming and dependent on highly skilled personnel and expensive equipment. Capillary electrophoresis is rapid and effective, yet it lacks reproducibility. The electrochemical technique is used owing to advantages like low-cost, simplicity, sensitivity, selectivity and mechanical stability. However, it may take a long time to fabricate a functionalized electrode, so those methods are not suitable for easy, rapid analysis of large numbers of samples. Thus, a rapid, sensitive and inexpensive technique for detecting SY is urgently needed.

In this approach, AO ($pK_a = 10.4$) mainly existed as a cationic alkaline fluorescence dye in the optimum conditions; SY had almost no fluorescence and it could react with AO to form a ion-association complex in pH 3.4 Britton-Robinson (BR) buffer solution medium. This resulted in the fluorescence quenching of the former and helped to detect the latter with the maximum excitation wavelengths ($\lambda_{ex}$) and emission wavelengths ($\lambda_{em}$) near 490 and 530 nm, respectively. The assay exhibits high sensitivity and selectivity with a detection limit of 0.002 μmol L$^{-1}$ and the remarkable quenching of fluorescence was proportional to the concentration of SY in the range of 0.008 – 9.0 μmol L$^{-1}$. Herein, this finding was utilized to develop a new strategy for simple, rapid, sensitive and selective detection of SY by combining AO based on fluorescence quenching. In addition, the optimum reaction conditions and the effect of foreign substances were studied. The reasons for fluorescence quenching were also investigated, which showed the quenching of fluorescence of AO with SY was a static quenching process. Furthermore, the proposed method was applied in a real sample analysis with satisfactory results.

**Keywords** Acridine orange, sunset yellow, detection, fluorescence quenching

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the contents of each flask was mixed well at room temperature for purification. Doubly distilled water was used throughout the experiment. All reagents were of analytical reagent grade and used without further purification. Complex that decreased the AO fluorescence intensity. That is, the fluorescence of AO could be entirely quenched when forming a complex with SY. Furthermore, the fluorescence quenching was proportional to the concentration of SY in a relatively wide range, so a new spectrofluorometric quenching method was developed for the determination of SY with high sensitivity and good selectivity. Moreover, it has also been applied to determine SY in beverages with favorable results.

Experimental

Instruments

A Hitachi F-2500 spectrofluorophotometer (Tokyo, Japan) was used to record fluorescence spectra and fluorescence intensity with the slits ($E_x/E_m$) set at 10.0/10.0 nm for the fluorescence spectra. A UV-2450 spectrophotometer (Shimadzu, Japan) was employed to obtain absorption spectra. A pH S-3C pH meter (Shanghai Scientific Instrument Company, China) was used for adjusting the pH values.

Reagents

A stock solution of SY (1.0 × 10⁻³ mol L⁻¹) and AO (1.0 × 10⁻³ mol L⁻¹) were prepared and stored at 4°C, and a working solution was freshly drawn up $via$ diluting the stock solution to 1.0 × 10⁻⁴ mol L⁻¹. A Britton–Robinson (BR) buffer solution with different pH were prepared by mixing the mixed acid (composed of 2.71 mL 85% H₃PO₄, 2.36 mL HAc and 2.47 g H₃BO₃) with 0.2 mol L⁻¹ NaOH in different proportions; the buffer solution was used to control the acidity. All reagents were of analytical reagent grade and used without further purification. Doubly distilled water was used throughout the experiment.

General procedure

Into a 10.0-mL calibrated flask, the followings were added: 1.0 mL pH 3.4 BR buffer solution, 2.0 mL 1.0 × 10⁻⁴ mol L⁻¹ AO solution and appropriate amounts of SY solution. The mixture was diluted to the mark with doubly distilled water, and the contents of each flask was mixed well at room temperature (15 ± 5)°C. After 10 min, we recorded the fluorescence spectra and measured the fluorescence intensity of reagent blank ($F_0$) and complexes ($F$) at $A_{ex}/A_{em}$ = 490/530 nm. The quenching of fluorescence intensity ($\Delta F$) of the system was presented by $\Delta F = F_0 - F$, where $F_0$ and $F$ were the fluorescence intensities of the reagent blank and the complex, respectively.

Results and Discussion

Fluorescence spectrum

As depicted by the excitation and emission spectra shown in Fig. 1(A), it can be seen that spectra characteristics of AO were maintained almost the same when reacted with SY forming product, which was the essence of the fluorescence intensity quenching. It clearly showed the corresponding tendency of the fluorescence spectra after various concentrations of SY were added to the BR buffer solution containing AO in the optimum conditions. The corresponding emission intensity of AO gradually decreased with the addition of SY, demonstrating that the AO could potentially act as a fluorescent probe to detect SY. Furthermore, the absorption behavior of AO (a) and SY (c) is illustrated in Fig. 1(B) and we note that when they mixed together, the AO-SY (b) absorption spectra had a distinct change with a little blue shift, which primarily proved that AO could interact with SY to form a complex resulting in the changed spectra.

Effect of pH

The influence of pH on the fluorescence intensity of the reaction system was investigated and the results are shown in Fig. 2. It can be seen intuitively in Fig. 2(B) that the fluorescence quenching extent of the system kept relatively stable in the pH range at 3.3 – 3.5 and reached the maximum at pH 3.4 by keeping AO and SY concentrations constant, while the relative intensities ($\Delta F$) decreased remarkably when the pH was out of the range. Therefore, the pH of 3.4 was chosen as the optimum pH value for the analytical system in this work and the appropriate amount of the buffer solution was determined to be 1.0 mL.

Effect of concentration of AO

Studies of the concentration of AO, which could affect the relative fluorescence intensity ($\Delta F$), were conducted and the...
The intensity dramatically dropped with the addition of SY, and it was obvious that fluorescence quenching achieved a maximum concentration of AO for the detection of SY. Fluorescence no change after 10 min. So the reaction was finished completely. Therefore, the concentration of AO at 2.0×10^{-5} mol L^{-1} was selected in this work.

Effect of reaction time
At room temperature (15±5)°C, the response of reaction time on the fluorescence intensity of the system was also researched. When all solutions were added and mixed uniformly, the recording of the intensity of fluorescence spectra showed nearly no change after 10 min. So the reaction was finished completely in 10 min and fluorescence intensity remained constant for at least 3 h in the study. Hence, the measurement was carried out after 10 min.

Selectivity of the method
Under the above-optimized experimental conditions, the effect of some foreign coexisting substances on the determination of SY was investigated in BR buffer solution (pH 3.4). As displayed in Table 1, when the concentration of SY was 5.0 μmol L^{-1}, some other synthetic colorants or dyes caused slight interference, thus we recognized the need to control for this in the scope of light concentration. Whereas the common metal ions, inorganic anions, saccharine and amino acids that were considered had few interferences with the determination of SY; these coexisting compounds caused a relative error of <±5% in the fluorescence intensity change of the system. The method of the study not only possesses good selectivity, but also the basis for quantitatively determining SY.

Table 1 Effects of foreign coexisting substances (C_{SY} = 5.0 μmol L^{-1} = 2.3 μg mL^{-1})

| Species          | Conc./μg mL^{-1} | Relative error, % | Species | Conc./μg mL^{-1} | Relative error, % |
|------------------|------------------|------------------|---------|------------------|------------------|
| KCl              | 149.1            | –1.5             | d(+)–sucrose | 1026            | –1.6             |
| ZnSO_{4}         | 345              | –2.8             | L(+)-Arginine | 253             | –3.4             |
| CuSO_{4}         | 200              | –0.5             | L–Serine    | 262.7           | –1.3             |
| NiSO_{4}         | 210              | –1.6             | Glucose    | 297             | –1.4             |
| CoSO_{4}         | 176.3            | 1.9              | Glycine    | 150             | –3.9             |
| NH_{4}Cl         | 107              | –3.6             | EDTA       | 45              | –4.1             |
| MnSO_{4}         | 101              | 2.5              | Neomycin   | 160             | 3.8              |
| KBr              | 95               | –3.4             | Acridin     | 2.4             | 2.9              |
| CaCl_{2}         | 166              | –0.8             | Methyl violet | 8.2             | –3.7             |
| NaNO_{2}         | 207.5            | 3.8              | Ethyl violet | 9.8             | –4.9             |
| KAl(SO_{4})_{2}  | 380              | –3.7             | Safranine  | 3.5             | –4.6             |
| Vc               | 167              | –0.6             | Tartrazine | 2.7             | –4.5             |

Standard curve and detection limit
A calibration curve was manufactured under the optimum conditions by conducting similar experiments at a series of concentrations of SY and the respective results are depicted in Fig. 4. As shown in Fig. 4(A), it is clear that the chart was linear of the SY concentration with a correlation coefficient, R^2 = 0.9997, and the detection/quantitation limits were determined according to the IUPAC definition (detection limit = 3σ/k, quantitation limit = 10σ/k, where σ is the standard deviation of the black signal, k is the slope of the linear equation). Thus the fluorescence intensity of the system was linearly proportional to SY over the concentration ranges of 0.008 – 9.0 μmol L^{-1}, and the limit of detection was 0.002 μmol L^{-1}. In addition, another calibration curve in the range of 1 – 10 nM is depicted in Fig. 4(B); it does not show a good linear relationship apart from the far right point (>0.8 nM) for the concentration range of SY (0.008 – 9.0 μmol L^{-1}) demonstrated in this work. Moreover, Table 2 presents the sensitivity of the approach and shows it was lower than those of the published methods.

The fluorescence quenching effect
Fluorescence quenching behaviors are important for understanding the system and exploiting them in novel applications. The main forms of fluorescence quenching include static and dynamic quenching.27 As is known, both static and dynamic quenching require molecular contact between the fluorophore and the quencher. In this section, the quenching mechanism was analyzed using the Stern-Volmer equation:28

Fig. 3  The fluorescence intensity of different concentrations of AO in pH 3.4 BR buffer. C_{AO} = (0.5, 1.0, 1.5, 2.0, 3.0, 4.0)×10^{-4} mol L^{-1}, C_{SY} = 0 (a), C_{SY} = 0.6×10^{-4} mol L^{-1} (b).

Fig. 4  Linear correlations of fluorescent intensity toward SY concentrations, C_{SY} = 2.0×10^{-5} mol L^{-1}. (A) C_{SY} = (0.0008, 0.001, 0.005, 0.01, 0.05, 0.1, 0.15, 0.3, 0.45, 0.6, 0.75, 0.9)×10^{-4} mol L^{-1}. (B) C_{SY} = (1, 2, 4, 6, 8, 10)nM.
\[ F_0/F = 1 + K_{SV}[Q] = k_q\tau_0[Q] \]

Where \( F_0 \) and \( F \) are the fluorescence intensities in the absence and presence of a quencher, respectively; \( K_{SV} \) is the Stern–Volmer quenching constant; \([Q]\) is the concentration of the quencher; \( k_q \) is the quenching rate constant and \( \tau_0 \) is the fluorescence lifetime in the absence of a quencher.\(^{29}\) Figure 5 depicts the Stern–Volmer plots of \( F_0/F \) versus \([Q]\) at different temperatures. The quenching type of the system could be distinguished by the differing response depending on temperature. It was quite clear that the quenching constant \( K_{SV} \) decreased distinctly when the temperature rose in this experiment, which was an obvious characteristic of static quenching.

Analytical application

To test the practical application of this study, the method was used to detect SY concentration in soft drink (orange juice), which was obtained from the local market. Prior to detection, the orange juice was diluted from 1.0 to 10.0 mL with doubly distilled water under the optimum conditions. Results showed that in the orange juice the concentration of SY was approximately 0.018 mg mL\(^{-1}\) (39.1 μmol L\(^{-1}\)) RSD <5%. The illustrated consequence obtained by the standard addition method displayed in Table 3 shows the average recoveries of SY were between 98.0 and 99.4% for different samples and the RSD was lower than 2.8%, indicating that the determination of SY was reliable and feasible, which presented good applicability and can be efficiently conducted for the detection of SY in drink products.

Conclusions

In summary, a simple, rapid and highly efficient method using AO as a fluorescence quenching sensor has been successfully performed for detection of SY. In virtue of these advantages, the proposed strategy suggests the potential of combining the AO based fluorescence quenching with SY. The decrease in AO fluorescence intensity was proportional to the concentration of SY in the range of 0.008 – 9.0 μmol L\(^{-1}\). More importantly, the experimental results revealed a good linear relationship and the limit of detection was quite low. Overall, our strategy could be developed into a significant method for SY detection and promising application for practical sample analysis of food and drinks.

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