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Fluorescence Resonance Energy Transfer Quenching On Concentration Determination Of Sudan I In Food

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ABSTRACT

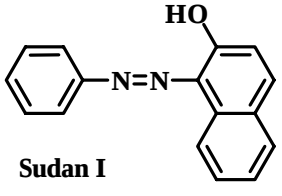
Depending on the isochromatism between the fluorescence of acridine Yellow (AY) and the absorption of Sudan I, at $\lambda_{ex}/\lambda_{em}$ = 280/495 nm, Sudan I absorbed the fluorescence of AY effectively in the medium of ethanol, which made AY quench, so we built the new way to determine the concentration of Sudan I. The range of determining concentration of Sudan I was 5.0×10^{-7} ~ 3.5×10^{-5} mol l^{-1}. The detection limit of the method was up to 1.82×10^{-7} mol l^{-1} The method had applied to the determination of Sudan I in capsicum powder and chili sauce. The relative standard deviation was 0.8 ~ 2.7 % for 6 parallel determination of Sudan I samples and the recovery of Sudan I was 89.5 ~ 109.0 %.

KEYWORDS

Acridine yellow;
Sudan I;
Energy transfer;
Fluorescence;
Quenching.

INTRODUCTION

Sudan I is a kind of dye that belongs to Azocomp ounds. Sudan I (Figure 1) is non ionic fat-soluble dyes used in the gasoline, diesel, lubricating grease and polymer dye production, and as dye for food (chilli) and tattoos. Azocompounds are by far the most widely used synthetic organic colorants[1] and more than 2000 of such substances are listed in the color index (CI)[2]. The genetic toxicity of some azo-



Sudan I

(M.W.248.28)

Figure 1: Chemical structures of Sudan I

dyes has been confirmed[3] and structure-activity relationships have been assessed[4,5]. Among organic colorants most of the azo-dyes are recognized to be carcinogens[6]. Sudan I is not a permitted colour under the colours in Food Regulations 1995[7]. It is considered to be a genotoxic carcinogen[3,8] and its presence is not permitted in foodstuffs for any purpose at any level. So it is of great importance to build a new way to determine the concentration of Sudan I. Fluorescence methods with the advantages of speediness, sensitivity and convenience has not been applied to determine Sudan I. We found that the absorption wavelength of non-fluorescent Sudan I is close to the fluorescence wavelength of the fluorescent substance Acridine Yellow (AY), the difference was only 15 nm. So due to the mechanism of energy transfer, Sudan I could absorb the fluorescence of AY. So we built the way of determination of Sudan I by fluorencence resonance energy transfer (FRET). Through experiments, it was indicated that the type of the quenching was dynamic quenching. The method has been applied to the determination of Sudan I in capsicum powder and chili sauce with good results.

EXPERIMENTAL

Apparatus

All fluorescence spectra were recorded by Shimadzu RF–540 spectrofluorophotometer; Absorption was measured with an UV-vis recording spectrophotometer (UV–265 Shimadzu Japan).

Chemicals

Sudan I(124 mg l^{-1}): Sudan I (produced by ACROS ORGANICS, New Jersey, USA) of 31.0 mg was diluted into 250 ml volumetric flask with ethanol and stored at 4°C. When used, diluted into 12.4 mg l^{-1}.Acridine Yellow (AY) was converted in ethanol solution. The stock solution concentration was 5.0×10^{-4} mol l^{-1}. Petroleum ether (boil range: 30~60 °C); Aluminum oxide neutral for TCL (100~200 meshes) was used for column chromatography after activation dealt. Ethanol (v/v 95%), benzene, 1.2-Dichloroethane and Carbon tetrachloride solutions were all analytical purity grades.

Procedures

Add certain volume 12.4 mg l^{-1} Sudan I and 1.0 ml 5.0×10^{-5} mol l^{-1} of Acridine Yellow (AY) into 10 ml colorimetric cylinder with 95% ethanol solution, shake uniform and lay aside for 5 min. Fluorescence measurement were carried out at 495 nm keeping the excitation wavelength at 280 nm in a 1 cm quartz cell, the excitation and emission slits were both 10 nm and measurement against reagent blank was done at the same time.

RESULTS AND DISSCUSSION

The choice of dye-pair

We measured the absorption spectra of Sudan I diluted with ethanol, 1.2-Dichloroethane, carbon tetrachloride and benzene. And we also measured the fluorescence spectra of an array of dyes of ethanol solution, the results were shown in TABLE 1 (available as SUPPLEMENTARY INFORMATION).

It was found that in medium of ethanol, the isochromatism between fluorescence of Acridine Yellow (AY) and the absorption of Sudan I was better, $\Delta\lambda$ = 15 nm, and the solvency of them in ethanol is good. Furthermore, when Sudan I in 1.2-Dichloroethane, carbon tetrachloride and benzene reacted with Acridine Yellow (AY), organic solvents could make the fluorescence of AY quench and Sudan I couldn't quench AY. Mixture solvents caused the low repeatability and methanol is noxious, so we chose the Acridine Yellow (AY) as a donor and ethanol as a solvent.

The reaction between Sudan I and AY

In the medium of ethanol, Sudan I absorbed the fluorescence of AY effectively and energy transfer could occur, seen from figure 2; Meanwhile, before or after the fluorescence quenching, the fluorescence peak of Acridine Yellow (AY) unchanged, so we deduced the type of quenching was dynamic quenching.

The calibration graph and detection limit

Under the optimum condition defined, the relatively fluorescence intensity ΔF of various concen-

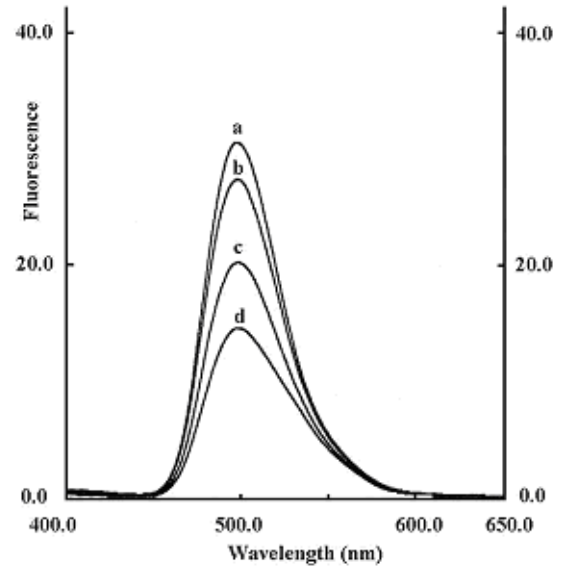


Figure 2: The fluorescence spectra: (a) Acridine Yellow (AY) (b) AY+ Sudan I (1.24 mg l^{-1}) (c) AY+ Sudan I(3.72 mg l^{-1}) (d) AY+ Sudan I(7.44 mg l^{-1}) AY: 5.0×10^{-6} mol l^{-1}

tration of Sudan I standard solution has been detected and drawn up calibration curve. The equation of linear regression is: $\Delta F=1.136\times10^{6}C(\text{mol } l^{-1}) + 3.376$, correlation coefficient r = 0.9956. The range of Sudan I concentration is $5\times10^{-7}\sim3.5\times10^{-5}$ mol l^{-1} that obeys the linear. The detection limit of the proposed method, based on the results of 11 parallel blank tests and the $3\sigma/K$ criterion, is 1.82×10^{-7} mol l^{-1}.

ANALYTICAL APPLICATION

Samples dealt

Measure 10 g chili sauce sample (or 2~3 g capsicum powder) by rule and line, add 10 ml petroleum ether into it, then sonic extract three times with 3 min per time, and put the extract together and drying, finally add 10 ml petroleum ether to dissolve the residues and prepare for column chromatography.

Column chromatography

Put certain quantity aluminum oxide neutral load with petroleum ether into the chromatography column of 1cm×20cm, and keep that the height of Aluminum oxide neutral column was 3 cm (may heighten 1cm to chili sauce sample), cover the Aluminum oxide neutral column with anhydrous Na_2SO_4 whose height is 1 cm; Control the flow rate of petroleum ether at 2 d/s, wait for the petroleum ether reaching to the surface of anhydrous Na_2SO_4, put the sample into the chromatography column; use 5 ml petroleum ether to wash the container of sample several times, put the wash liquid into the chromatography column, then pour with petroleum ether into the chromatography column until that the spectra line of Sudan I in orange was eluted, finally get together the elution of Sudan I and drying.

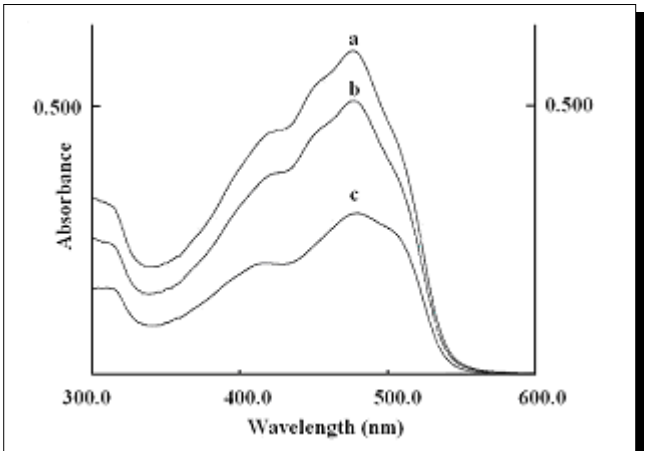


Figure3: The absorption spectra of Sudan I and chili sauce sample: (a) Sudan I in capsicum powder (b) Sudan I in chili sauce (c) Sudan I standard solution

The identification of elution

We also studied the identification of elution. We scanned the absorption spectra of the Sudan I in chili sauce and capsicum powder after dealt as the way introduced above, seen from figure 3. It was indicated that the elution was Sudan I[9-12].

Determination of actual sample

Put the elution of Sudan I together and drying, the residuals were diluted to 10 ml with ethanol. Measure the fluorescence intensity and do the blank experiment at $\lambda_{ex}/\lambda_{em}$ = 280/495 nm. The results were shown in TABLE 2 (available as SUPPLEMENTARY INFORMATION). The results were indicated that there were no Sudan I measured in samples.

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CONCLUSION

In our paper, we introduced the new way on determination of Sudan I by fluorescence quenching for the first time. We extracted the Sudan I in samples with petroleum ether, which made the interfere of soluble dyes decreased greatly; Connected with the way of column chromatography, we erased the interfere of organic materials. So this method to determine the concentration of Sudan I fluorescence methods has advantages of speediness, sensitivity and selectivity and convenience which has applied to determine Sudan I in capsicum powder and chili sauce.

SUPPLEMENTARY INFORMATION AVAILABLE STATEMENT

Following information is available as SUPPLEMENTARY INFORMATION.

- TABLE 1: The fluorescence of different kinds of dyes and the absorption spectra of Sudan I
- TABLE 2: Analytical results of samples and recovery (n=6)

ACKNOWLEDGMENT

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