## Technical Notes

# Visual Detection of Sudan Dyes Based on the Plasmon Resonance Light Scattering Signals of Silver Nanoparticles

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**A visual light scattering detection method of Sudan dyes is reported in food products based on the formation of silver nanoparticles (NPs) . Sudan dyes including I, II, III and IV have reducibility due to the nitrogen−nitrogen double bond and phenol group in their molecular structure, and a redox reaction could occur with AgNO$_3$. Owing to the formation of silver NPs as a result of the redox reaction, color changes could be observed by eye from the red of Sudan to the brown of silver NPs, resulting in strong plasmon resonance light scattering (PRLS) signals characterized at 452 nm, which could be measured using a common spectrofluorometer. It was found that the PRLS intensities were proportional to the dye concentrations over the range of 0.2−2.4 $\mu$M Sudan I, 0.1−2.4 $\mu$M Sudan II, 0.1−2.4 $\mu$M Sudan III, and 0.2−3.0 $\mu$M Sudan IV, with the corresponding limits of determination (3$\sigma$) of 3.2, 3.0, 3.2, and 2.9 nM, respectively. Using hot chili as a model sample, detection could be made with the recovery of 90.8−103.3% and RSD of 4.0−4.9%, and the results are identical with that of a liquid chromatographic method approved by the European Commission. To make the PRLS method much more practical, we could visually detect the quantity of Sudan dyes based on the PRLS signals using simple devices such as a portable laser pointer (653 nm) and a light emitting diode (458 nm). Mechanism investigations show that the functional group of Sudan oxidized by AgNO$_3$ is the phenol group, not the nitrogen−nitrogen double bond.**

Metal nanoparticles (NPs) have been of interest for centuries, and they have been paid much attention in recent years due to their special optical and electronic properties, which are not present in the bulk metal.[1] One important optical phenomenon is that the particle suspensions display brilliant colors giving rise to plasmon absorption and scattering.[2,3] It is for these particular features that metal NPs have wide and potential applications in many aspects involving the development of optical sensors,[4−8] the detection of DNA hybridization,[9,10] studies of protein folding,[11] and aptamer−protein complexes.[12]

In principle, the plasmon absorption and scattering properties are due to electron oscillations in the metallic particles. The irradiation of light to a small metallic NP would lead to coherent oscillation of the conduction electrons, which is called plasmon resonance of particles.[13] Subsequently, the oscillating electrons radiate electromagnetic waves with the same frequency as that of the incident light beam, and thus, the small metallic NPs are often referred to as plasmon scatterers.[13] The oscillation frequency is determined by the factors of the density of electrons, the effective electron mass, and the shape and size of the charge distribution.[14,15] In general, dipole plasmon resonance occurs for small particles less than the incident wavelength, whereas a quadrupole mode or higher order multipole resonance would be observed as the particle size increases.[16] As an example of metallic particles, silver NPs exhibit characteristic optical properties in the visible range due to the plasmon resonance. In fact, single silver

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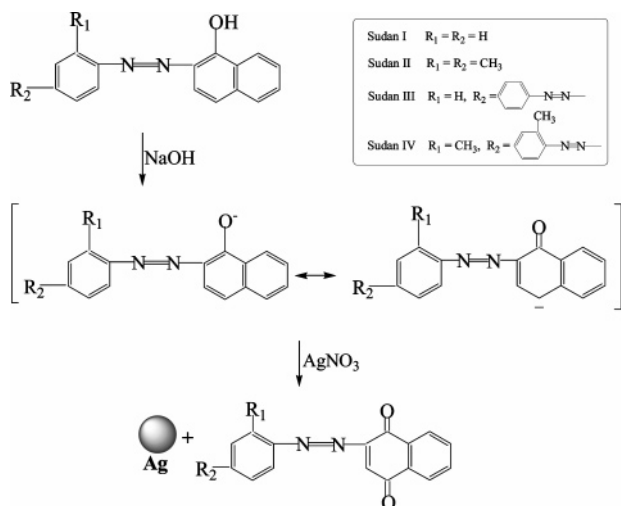
**Figure 1.** Molecular structures of Sudan and the chart of their reactions with AgNO₃.

NPs could interact with a photon more efficiently than any other particle of the same dimension. Such high efficiency together with the optical properties makes silver NPs very attractive for a lot of optical applications.[17] We expect that the light scattering signals resulting from the plasmon scatterer could be detected using a common spectrofluorometer, and we herein propose a visual light scattering detection method of Sudan dyes in food products based on the measured plasmon resonance light scattering (PRLS) signals.

Sudan I, II, III, and IV (Figure 1 shows the molecular structure) are a series of artificial "azo" dyes usually used in oil paint, printing, waxes, plastics, and dyeing[18,19] since their bright and vivid colors could improve the luster of commercial products. Sudan is not permitted to be used in food production as it is classified as a category carcinogen by the International Agency for Research on Cancer (IARC).[20] There is evidence that Sudan I is potentially carcinogenic in rodents and causes damage to genetic material since it can react with a given sequence of DNA in vitro. The major DNA adduct formed in this reaction has been characterized and identified as the 8-(phenylazo)guanine adduct.[21] In addition to microsomal enzymes, Sudan I and its C-hydroxylated metabolites are also oxidized by peroxidases, and as a consequence, DNA, RNA, and protein adducts are formed.[22,23]

However, the family of Sudan dyes, especially Sudan I, has been found in food products containing hot chili in some parts of the world, which is a crucial risk to human health. In May 2003, the French Food Authority (AFFSA) discovered that a number of products containing chili powder imported from India contained Sudan I.[24] In China, Sudan was also found in batches of roast chicken wings and chicken burgers on sale and in some commercial products such as piccalilli and chili sauce.

To date, the standard used to detect the Sudan family is based on a liquid chromatographic method approved by the European Commission,[25] and other approaches such as HPLC–UV,[26] HPLC/APCI-MS,[27] and HPLC-DAD detection[28] have also been made. Whichever method is used, separation with HPLC is needed first, which is time-consuming and brings about inconvenience to analysis in real time. Thus, the safety quality control of Sudan dyes in food products is very crucial, and developing simple detection method is urgent. Herein, we propose a new simple method to detect Sudan directly based on the formation of silver NPs.

## EXPERIMENTAL SECTION

**Apparatus.** The PRLS spectrum and intensity were measured with a F-4500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan). The plasmon absorption was measured with a U-3010 spectrophotometer (Hitachi, Tokyo, Japan). A TecNai-10 electron microscope (FEA) was used to measure the TEM images of silver NPs. A vortex mixer QL-901 (Haimen, China) was used to blend the solution. Besides, a LC system (Hitachi, Tokyo, Japan) with diode array detector L-2450, column oven L-2300, and pump L-2130 was applied for sample detection for comparison. A low-speed 800-model centrifuge (Shanghai Operational Instrumental Limit, Shanghai, PRC) was used for the real sample pretreatment. The IR spectrums were obtained with a Spectrumgx spectrophotometer (Perkin-Elmer). To construct a simple and practical procedure, a laser pointer (653 nm, 2.0 mW) and a light emitting diode (LED; 458 nm, 0.5 mW), whose output power has been calibrated with a WL-4 power meter (Laser Institute of Physics, Southwest University, Chongqing, PRC), were used for visual light scattering detection. For safety consideration, special attention should be paid to the safe use of the laser pointer, which is harmful to eyes.

**Reagents.** Sudan I and II were commercially purchased from Chemical Reagent Co. (Shanghai, China), while Sudan III and IV were from Kasei Kogyo Co. Ltd. (Tokyo, Japan). The $1.0 \times 10^{-3}$ M stock solutions of Sudan I–IV were prepared respectively by directly dissolving their commercial products in DMF. The working solution was obtained by diluting the stock solution with DMF to $1.0 \times 10^{-5}$ M.

A 0.1 M stock solution of AgNO₃ was prepared by dissolving solid AgNO₃ in doubly distilled water, and the working solution was obtained by diluting the stock solution to $1.0 \times 10^{-3}$ M with water. The 0.1 M NaOH, 0.4% NH₃·H₂O, and 0.2% TritonX-100 working solutions were used. All the reagents were of analytical grade without further purification. Water used throughout was doubly distilled.

**General Procedure.** A total of 2.25 mL of $1.0 \times 10^{-3}$ M AgNO₃, 0.4 mL of 0.1 M NaOH, and 0.25 mL of 0.4% NH₃·H₂O working solution were added in a 10-mL test tube. The mixture was vortexed thoroughly, and then an appropriate volume of the

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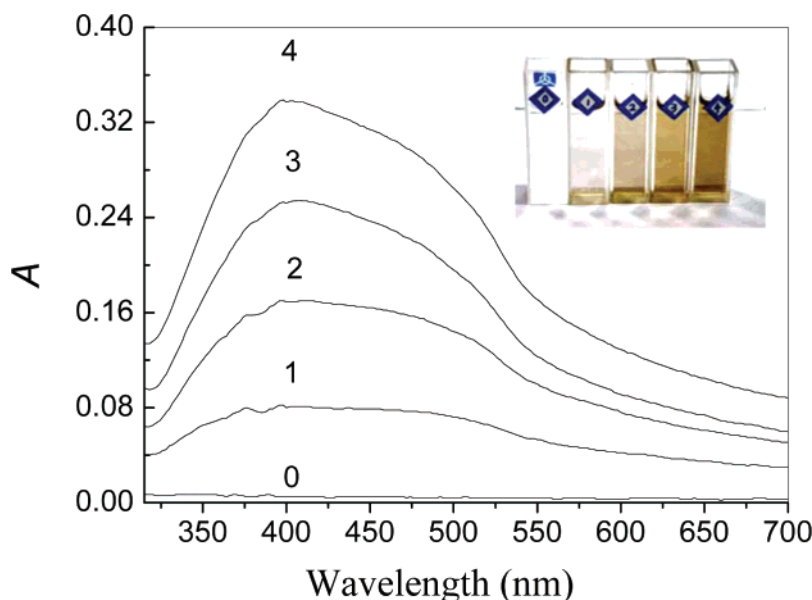
**Figure 2.** Plasmon absorption of silver NPs resulting from the reaction of AgNO$_3$ and Sudan I. (The inset picture shows the color change for the visible sensing detection in response to different quantity of Sudan I.) Concentrations: AgNO$_3$, $4.5 \times 10^{-4}$ M; NaOH, $8.0 \times 10^{-3}$ M; NH$_3$·H$_2$O, 0.02%; Sudan I (curves 0−4, $\mu$M), 0, 1.0, 2.0, 3.0, 4.0; Triton X-100, 0.012%.

Sudan solution and 0.3 mL of Triton X-100 were added. At last, the mixture was diluted to 5 mL with doubly distilled water and mixed thoroughly again. Twenty minutes later, the PRLS spectra and the intensities were measured against the reagent blank solution treated in the same way without Sudan.

The PRLS spectrum was obtained by scanning simultaneously the excitation and emission monochromators of the F-4500 spectrofluorometer from 320 to 700 nm (namely, $\Delta\lambda = 0$ nm), and all PRLS measurements were made with a 10.0-nm slit width of the excitation and the emission of the spectrofluorometer. The PRLS intensity was measured at the maximum PRLS peak.

**Preparation of Quinone Product.** To understand the reaction mechanism, the products of the redox reaction were separated and characterized by using the following procedures. A total of 10.0 mL of 0.1 M AgNO$_3$, 10.0 mL of 1.0 M NaOH, and 1.8 mL of 28% ammonia were at first placed into a 200-mL conical flask. After the mixture was vortexed thoroughly, 100 mL of $1.0 \times 10^{-3}$ M Sudan I solution was added dropwise. The reaction mixture was then stirred for 30 min at room temperature, filtered, and extracted with CHCl$_3$ (30 mL × 3). The combined organic phase was dried by anhydrous sodium sulfate and evaporated under reduced pressure to give an oily residue. Last, the residue was purified by silica gel column using acetic ether/petroleum ether (distillate of 60−90 °C; 1:50 v/v), evaporated, and dried in a vacuum, giving a yellow product.

**Pretreatment of Samples.** Real samples including cayenne oil, chili sauce, and redeye were commercially purchased from a supermarket (Beibei Chongbai, Chongqing, PRC). A 1.0 g aliquot of the real samples were placed respectively into a 10-mL volumetric flask and then dissolved to 10 mL with DMF. The mixture was vortexed thoroughly, and centrifugal sedimentation was carried out for 2 min at 3600 rpm. After 10 min, the top pellucid liquid was transferred for detection according to the general procedure. For comparison, standard liquid chromatographic detection for Sudan proposed by the European Commission was made.[25] The real samples for liquid chromatographic

detection were prepared by dissolving 20 g of cayenne oil or chili sauce in 100 mL of acetonitrile. The mixture was vortexed thoroughly and filtrated 1 h later, and the filtrate was used directly for sample injection.

## RESULTS AND DISCUSSION

**Spectral Features of the Redox Reactions of Sudan with AgNO$_3$.** Sudan I, II, III, or IV alone in the alkaline state has different color and absorption spectra (shown in Figure 1S in Supporting Information). The characteristic absorptions are located at 376 and 486 nm for Sudan I, 396 and 502 nm for Sudan II, 344 and 524 nm for Sudan III, and 374 and 518 nm for Sudan IV. The absorption features, however, only characterized at 398.0 nm, are greatly changed after reacting with AgNO$_3$ (shown in Figure 2). The inset picture in Figure 2 shows the colorimetric response of the sensing detection of Sudan I. The color change reactions of Sudan II, III, or IV with AgNO$_3$ are the same as Sudan I.

The four Sudan dyes possess two reducible groups, which are a nitrogen−nitrogen double bond and a phenol group. More precisely, the latter turns into a phenate ion at a high enough pH and becomes reducible (Figure 1) and, thus, could be oxidized by AgNO$_3$. As a result, AgNO$_3$ is deoxidized and present as brown silver NPs.[29,30] Figure 3 shows the TEM images of silver NPs formed in the presence of 0.4 (A), 1.2 (B), and 2.0 $\mu$M Sudan I (C), respectively. These silver NPs are nearly of the same size. The average dimensions are about $17.9 \pm 2.0$ (A), $17.2 \pm 2.0$ (B), and $17.5 \pm 1.5$ nm (C), which are calculated by averaging all the observed particles of the picture in Figure 3 (3 for A, 16 for B, and 22 for C). However, the number of silver NPs generated at a high concentration of Sudan I is more than that at a low concentration. Namely, the concentration of silver NPs is in-

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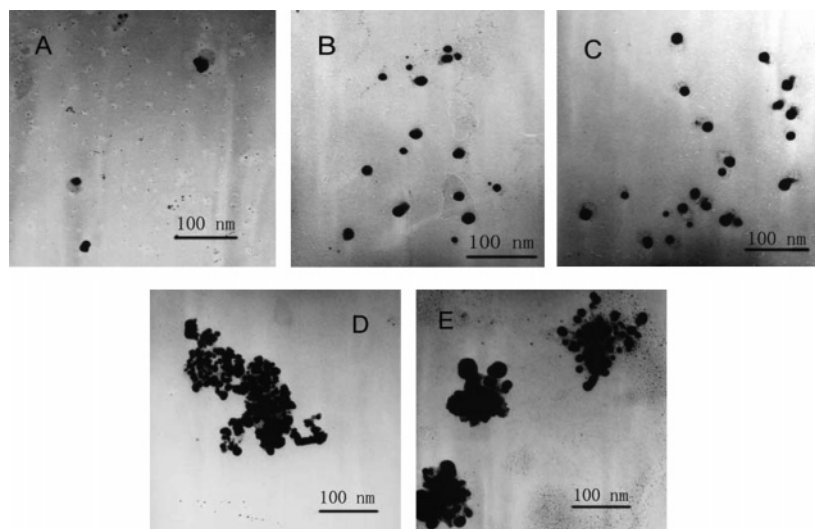
**Figure 3.** TEM images of silver NPs resulting from the reaction of AgNO$_3$ and Sudan I. The average dimensions are about 17.9 ± 2.0 (A), 17.2 ± 2.0 (B), and 17.5 ± 1.5 nm (C), which are calculated by averaging all the observed particles of the picture in Figure 3 (3 for A, 16 for B, and 22 for C). Concentrations: AgNO$_3$, 4.5 × 10$^{-4}$ M; NaOH, 8.0 × 10$^{-3}$ M; NH$_3$·H$_2$O, 0.02%; Sudan I, 0.4 (A), 1.2 (B), 2.0 (C), 5.0 $\mu$M (D), and 8.0 $\mu$M (E); Triton X-100, 0.012%.
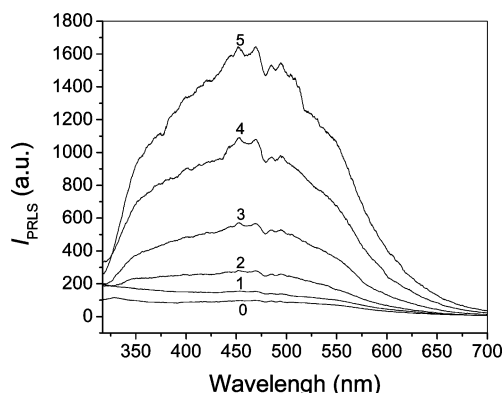


**Figure 4.** PRLS spectra of silver NPs resulting from the reaction of AgNO$_3$ and Sudan I. Concentrations: AgNO$_3$, 4.5 × 10$^{-4}$ M; NaOH, 8.0 × 10$^{-3}$ M except curve 0; NH$_3$·H$_2$O, 0.02%; Sudan I (from curves 0 to 5, $\mu$M), 0, 1.0 (without AgNO$_3$), 0.2, 0.5, 1.0, 1.5; Triton X-100, 0.012%.

creased with increasing Sudan. Thus, the absorption band of the formed silver NPs over the range of 350−525 nm (Figure 2), characterized at 398 nm, should be ascribed to the plasmon absorption of silver NPs according to refs 30−32. Since the size of the formed silver NPs is much less than the wavelength, this plasmon absorption should be mainly ascribed to the dipole plasmon resonance.[16]

The light scattering signals of Sudan or AgNO$_3$ alone detected using a common spectrofluorometer are very weak. When silver NPs are formed from the mixture of Sudan and AgNO$_3$, the light scattering signals get enhanced and increase with increasing Sudan concentration (shown in Figure 4). The light scattering signals with the characteristic peak being in the region of absorption band should be ascribed to the "resonance light scattering".[33−34] It is no doubt that the characteristic light scattering signals displayed at 452 nm (Figure 4), over the 350−525-nm plasmon absorption range of silver NPs (Figure 2) should be ascribed to RLS ones.[17] Thus, the light scattering signals measured using the common spectrofluorometer should indeed be ascribed to the PRLS.[13] In general, the intensity of light scattering depends on the volume and number of the species, the wavelength of incident light, and the real and imaginary parts of the scatterer's polarizability.[33−35] In this experiment, the more Sudan added, the more silver NPs were formed, whereas the size or the volume stayed nearly the same when the concentration of Sudan I was relatively low (Figure 3A−C). So, the enhanced PRLS signals could be mainly due to the increasing number of silver NPs, whereas the silver NPs begin to aggregate if the concentration of Sudan I becomes higher (Figure 3D and E).

**Optimal Conditions for the Redox Reactions.** We carried out the reaction in the alkaline condition since it was observed that silver NPs could not be formed under neutral or acidic pH conditions. Inspired by Tollens' reagent, we employed silver ammonia ion as an oxidant. It is pity that no silver NPs were formed and no color changes were observed. Considering that the formation of silver NPs is dependent on the ionization of the phenol group at a high pH,[30] we added NaOH solution in order to provide high enough alkalinity.[36,37] The addition of NaOH, although providing high alkalinity for the ionization of the phenol group, makes silver cation deposit and greatly weaken the oxidation capacity of AgNO$_3$. Therefore, the synergistic effect of ammonia and NaOH should be detected. Panels a and b in Figure 5 show the dependence of the PRLS intensity on the concentra-

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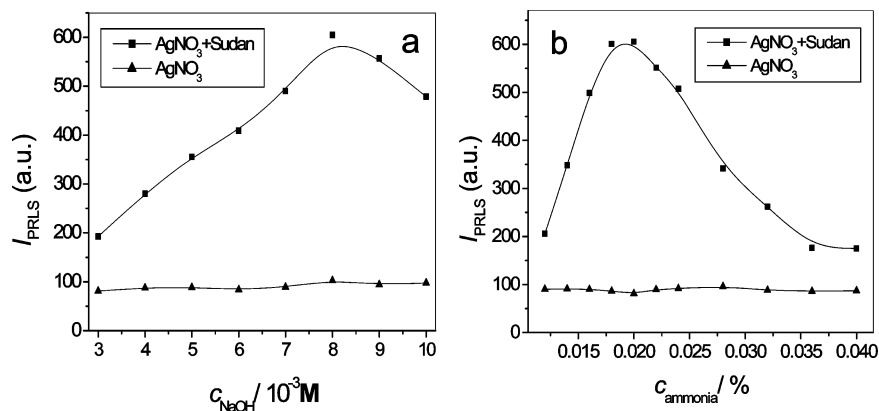(34) Pasternack, R. F.; Collings, P. J. *Science* **1995**, *269*, 935−939.
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**Figure 5.** Dependence of the PRLS intensity on the concentrations of NaOH (a) and ammonia (b). $\lambda$, 452 nm. Concentrations: (a), AgNO$_3$, $4.5 \times 10^{-4}$ M; NH$_3$·H$_2$O, 0.02%; Sudan I, 0.6 $\mu$M; Triton X-100, 0.012%; (b), AgNO$_3$, $4.5 \times 10^{-4}$ M; NaOH, $8.0 \times 10^{-3}$ M; Sudan I, 0.6 $\mu$M; Triton X-100, 0.012%.
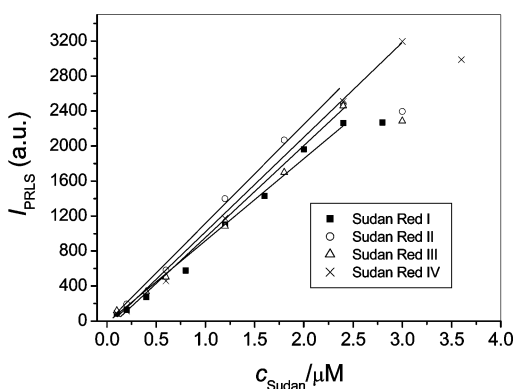


**Figure 6.** Calibration curves for Sudan dyes. Linear regression equation ($c$, $\mu$M), $\Delta I = -130.4 + 1005.5c$, $\Delta I = 1.74 + 1082.6c$, $\Delta I = -67.9 + 1016.7c$, and $\Delta I = -108.9 + 1092.9c$ for Sudan I, II, III, and IV over the corresponding range of 0.2–2.4, 0.1–2.4, 0.1–2.4, and 0.2–3.0 $\mu$M with the correlation coefficients of 0.9973, 0.9958, 0.9969, and 0.9990, respectively. Limits of determination ($3\sigma$) are 3.2, 3.0, 3.2, and 2.9 nM, respectively (corresponding to $7.9 \times 10^{-4}$, $8.3 \times 10^{-4}$, $1.1 \times 10^{-3}$, and $1.1 \times 10^{-3}$ mg/mL, respectively). Concentrations: AgNO$_3$, $4.5 \times 10^{-4}$ M; NaOH, $8.0 \times 10^{-3}$ M; NH$_3$·H$_2$O, 0.02%; Triton X-100, 0.012%. $\lambda$, 452 nm.

tions of NaOH and ammonia. As NaOH and ammonia increase until $8.0 \times 10^{-3}$ M and 0.02%, respectively, the PRLS intensity gets increased correspondingly.

It has been reported that the dispersions of NPs could be much improved by the addition of surfactants,[38,39] and the presence of a physically adsorbed layer of nonionic surfactant on the surface of colloidal gold could prevent irreversible aggregation of gold NPs.[40] To make the silver NPs more stable, surfactants, especially nonionic surfactants, are used as stabilizer in aqueous medium.[41] Our experiments showed that the addition of Triton X-100 could indeed improve the stability of formed silver NPs and would not influence the PRLS intensity of the system.

**Detection of Sudan in Samples.** According to the above general procedures, calibration curves of Sudan I, II, III, and IV

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were constructed (Figure 6). The correlations of PRLS intensity with all Sudan dyes were obtained at 452 nm. There are linear relationships between the PRLS intensities and the concentrations of Sudan I, II, III, and IV over the range of 0.2–2.4, 0.1–2.4, 0.1–2.4, and 0.2–3.0 $\mu$M with the correlation coefficients, 0.9973, 0.9958, 0.9969 and 0.9990, respectively. The limits of determination ($3\sigma$, LOD) are $7.9 \times 10^{-4}$ $\mu$g/mL for Sudan I, $8.3 \times 10^{-4}$ $\mu$g/mL for Sudan II, $1.1 \times 10^{-3}$ $\mu$g/mL for Sudan III, and $1.1 \times 10^{-3}$ $\mu$g/mL for Sudan IV. Compared to the LOD of Sudan I of the standard liquid chromatographic method ($1.3 \times 10^{-2}$ $\mu$g/mL) approved by the European Commission,[25] the present method is much more sensitive.

The influences of foreign coexisting substances such as proteins, glucide, amino acid, surfactant, and metal ions were tested (Data were shown in Table 1S in Supporting Information,). Of these tested substances, Al$^{3+}$, Ca$^{2+}$, Cu$^{2+}$, Ba$^{2+}$, Mg$^{2+}$, Ni$^{2+}$, K$^+$, glucide, SDBS, amylose, and formaldehyde could be allowed to be higher than $1.0 \times 10^{-5}$ M given the tolerance level of 10%. Amino acids except lysine could be allowed to be higher than $1.0 \times 10^{-3}$ M. The tolerances of ethanol, acetone, BSA, and HSA are relatively high. On the contrary, heavy metal ions including Pb$^{2+}$, Cr$^{3+}$, Fe$^{3+}$, surfactants including CTMAB, Tween-80, and vitamin C could be allowed lower. For a real sample, however, it showed that the interferences created by the latter were not significant.

As the four Sudan dyes have the same redox reactions with AgNO$_3$, it is difficult to separately detect one of them in a mixture. However, it is possible to detect the total quantity of Sudan in a real sample because the four calibration curves in Figure 6 have similar slopes. The problem is that errors will take place resulting from their own slight different responses whichever one is chosen as the standard. Table 1 lists the detection error of Sudan IV with increasing Sudan I. It can be seen that all the detection errors are less than $\pm 10\%$; thus, it is feasible that the approximate calculation of total quantity of Sudan can be made using one of them as the standard.

To test the present method, three real samples including cayenne oil, chili sauce, and redeye from a supermarket were determined according to the general procedures, and the detection results for cayenne oil and chili sauce are given in Table 2. No Sudan was detected in the redeye sample. As Table 2 shows, all

**Table 1. Detection Error of Sudan IV with Increasing Sudan I[a]**

| | added ($\mu$M) | found ($\mu$M) | |
|---|---|---|---|
| no. | Sudan I | Sudan I + Sudan IV | DR (%) |
| 1 | 0.2 | 1.22 | 1.7 |
| 2 | 0.4 | 1.35 | −3.6 |
| 3 | 0.6 | 1.55 | −3.1 |
| 4 | 0.8 | 1.63 | −9.4 |
| 5 | 1.0 | 1.91 | −4.5 |
| 6 | 1.2 | 2.11 | −4.1 |
| 7 | 1.4 | 2.27 | −5.4 |
| 8 | 1.6 | 2.41 | −7.3 |
| 9 | 1.8 | 2.61 | −6.8 |
| 10 | 2.0 | 2.89 | −3.7 |

[a] Data were obtained by using Sudan IV as the standard. Quantity of Sudan IV in sample is 1.0 $\mu$M. Concentrations: AgNO$_3$, 4.5 × 10$^{-4}$ M; NaOH, 8.0 × 10$^{-3}$ M; NH$_3$·H$_2$O, 0.02%; Triton X-100, 0.012%. $\lambda$, 452 nm.

**Table 2. Total Quantity of Sudan in Real Samples ($n = 5$)[a]**

| sample | found ($\mu$M) | added ($\mu$M) | total found ($\mu$M) | recovery (%) | RSD (%) |
|---|---|---|---|---|---|
| cayenne oil | 0.43 | 0.80 | 1.16 | 90.8−102.5 | 4.0 |
| chili sauce | 0.20 | 0.30 | 0.49 | 93.3−103.3 | 4.9 |

[a] All the values were the average of five measurements obtained using Sudan IV as standard. Each sample was prepared by dissolving 1.0 g to 10 mL with DMF and then diluted 10-fold to detect. Concentrations: AgNO$_3$, 4.5 × 10$^{-4}$ M; NaOH, 8.0 × 10$^{-3}$ M; NH$_3$·H$_2$O, 0.02%; Triton X-100, 0.012%. $\lambda$, 452 nm.

of the determinations could be made at a recovery of 90.8−103.3% and RSD of 4.0−4.9%.

To further identify the results presented above, we performed liquid chromatography using the standard method approved by the European Commission[18] for comparison. Figure 7 shows that the quantity of Sudan IV in cayenne oil could be 1.78 × 10$^{-5}$ M, i.e., 3.38 × 10$^{-2}$ mg/g, close to the result of 1.64 × 10$^{-2}$ mg/g using the present PRLS detection method. Thus, the reliability of the present PRLS method could be proved further by the similar results. The liquid chromatographic detection of chili sauce shows that there are other peaks coexisting with the relative little peaks of Sudan I and IV, leading to difficulty in fixing the total quantity of Sudan. However, it proves that there is Sudan in chili sauce indeed.

To make detection much easier, we could directly detect the quantity of Sudan via observing intuitively the phenomenon of scattered light. Using a laser pointer [*Caution! Laser is harmful to people's eyes. It should be handled with extreme care.*] and an LED to irradiate solutions, respectively (Figure 8), we could see that scattered light signals are enhanced gradually as the concentration of Sudan IV increases, and thus detection could roughly be made using such simple devices, which could be much more practicable. Although the wavelength of the laser pointer (653 nm) is located at the right edge of the PRLS spectra (Figure 4), it could be used for these PRLS signals based on visual detection since its output power (2.0 mW) is much higher than that of the LED (0.5 mW). The two devices thus could complement each other in terms of the wavelength and the output power. In addition, visible light scattering detection using the laser pointer
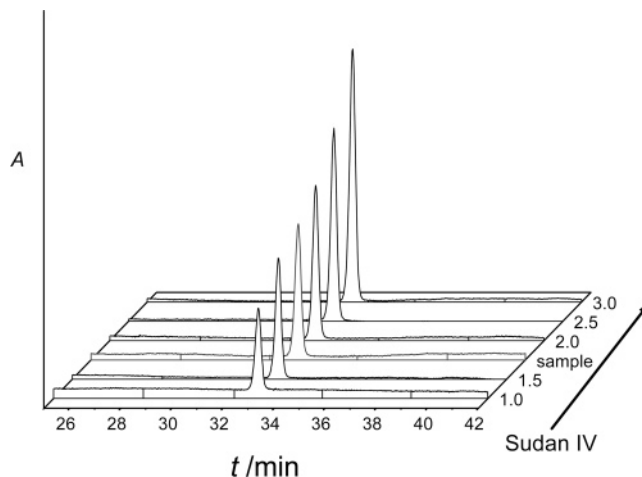
**Figure 7.** Liquid chromatographic detection of Sudan in cayenne oil sample. Liquid chromatographic conditions: mobile phases, reagent A, acidic solution (165 mL of acetic acid dissolved in 1000 mL of water); reagent B, acetonitrile; gradient program, 0−20 (eluent B composition, 70−95%), 20−30 (B, 95−100%), and 30−42 min (B, 95−100%); flow rate, 0.7 mL/min; the wavelength of detection, 520 nm; column temperature, 25 °C. Concentration of standard, Sudan IV (from front to back, ×10$^{-5}$ M): 1.0, 1.5, 2.0, 2.5, and 3.0. Linear regression, $A = 3.46 \times 10^4 + 4.06 \times 10^5 c$ (where $A$ is the peak area, $c$ is the concentration of Sudan IV). The correlation coefficient is 0.9992. From the standard curve and linear regression, the quantity of Sudan IV in cayenne oil is 1.78 × 10$^{-5}$ M (i.e., 3.38 × 10$^{-2}$ mg/g). RSD is 3.6%.

could determine a much lower quantity of Sudan, although the color change of solutions with relatively high concentrations could be directly observed. Namely, the sensitivity is improved further for light scattering sensing detection. The concentration of a cayenne oil sample, the one in the last cell in Figure 8, could be easily detected to be close to 0.4 $\mu$M, which is consistent with the result in Table 2.

**Mechanism Investigations.** The principle of the present method is to detect the PRLS signals of silver NPs resulting from redox reaction. Sudan I, II, III, and IV have reducibility for the functional groups of nitrogen−nitrogen double bond and the phenol group; thus, AgNO$_3$ can be reduced to silver NPs, resulting in the color change of the mixture to brown and displaying large PRLS signals. From Figure 6, we can see that the slopes of four calibration curves are quite close to each other, indicating that these reactions concerning Sudan I, II, III, and IV could produce a similar response. In other words, the reaction mechanism is the same. As Figure 1 shows, there is only one phenol group in each molecule of Sudan, whereas the number of nitrogen−nitrogen double bonds in Sudan III and IV is twice as many as in Sudan I and II. Thus, it is deduced that the functional group oxidized by AgNO$_3$ is the phenol instead of the nitrogen−nitrogen double bond. Suppose that the functional group was the nitrogen−nitrogen double bond; then the number of silver NPs produced by Sudan III and IV would be much more than that by Sudan I and II for a given Sudan concentration, resulting in a different PRLS response and an inconsistent slope. However, the similar slope values indicate no such dependence. Thus, we could deduce that the functional group oxidized by AgNO$_3$ is the phenol group, not the nitrogen−nitrogen double bond.

To validate the deduced mechanism, we measured the reaction between AgNO$_3$ with other azo dyes including 4-[(5-chloro-2-
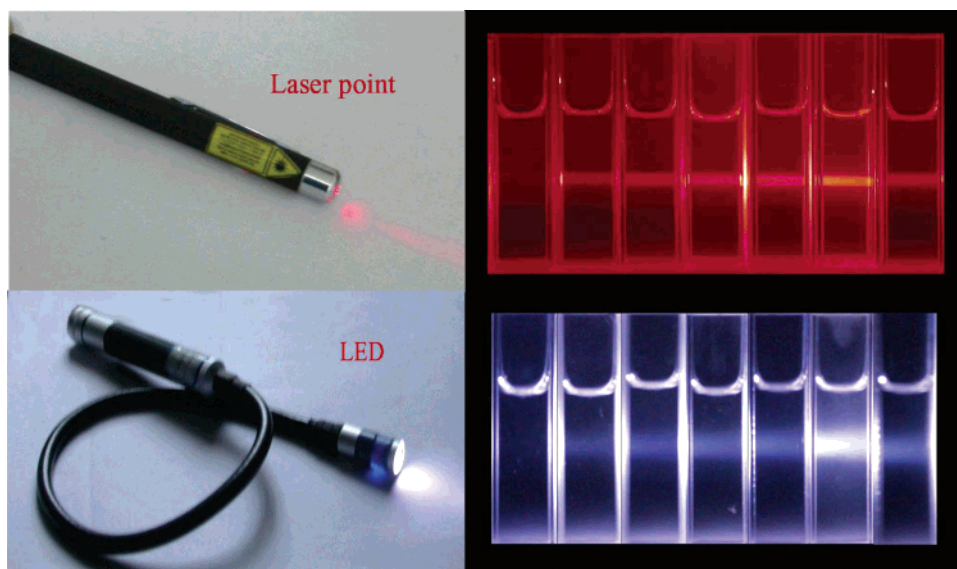
**Figure 8.** PRLS signals based on visual detection of Sudan IV using a portable laser pointer (653 nm, 2.0 mW, up) and an LED (458 nm, 0.5 mW, down) as irradiated light sources. Concentrations: AgNO$_3$, 4.5 × 10$^{-4}$ M; NaOH, 8.0 × 10$^{-3}$ M; NH$_3$·H$_2$O, 0.02%; Sudan IV (from left to right, $\mu$M), 0, 0.1, 0.2, 0.4, 0.6, 1.2, and a real sample of cayenne oil consistent with the one in Table 2. Triton X-100, 0.012%.
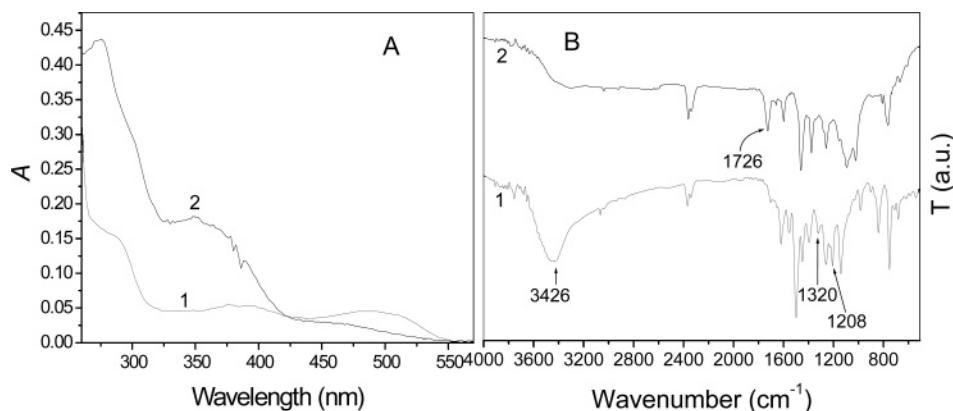


**Figure 9.** (A) UV−visible absorption spectra of Sudan I (curve 1) and the quinone product synthesized and separated via organic approach (curve 2). (B) IR spectra of Sudan I (curve 1) and the quinone product (curve 2).

pyridyl)azo]-1,3-diaminobenzene and Methyl Orange, which contain the functional groups of nitrogen−nitrogen double bond without a phenol group. The results showed that the two dyes do not react with AgNO$_3$ under the experimental conditions above. More exactly, the functional group of Sudan is not the nitrogen−nitrogen double bond.

To confirm the reaction mechanism, the products of the redox reaction were separated and characterized (shown in Figure 9). Sudan I has the maximum absorptions at 376 and 486 nm, which are changed after reacting with AgNO$_3$. The maximum absorptions of the product, obtained via organic synthesis and separation, locate at 276 and 348 nm (Figure 9A), which are in great part due to the produced quinone structure.[42,43] By comparing the IR features of pure Sudan I (Figure 9B, curve 1) and the separated product (Figure 9B, curve 2), we could find that the phenolic stretching vibration ($v_{OH}$) at 3426 cm$^{-1}$,

(42) Tsuda, A.; Fukumoto, C.; Oshima, T. *J. Am. Chem. Soc.* **2003**, *125*, 5811−5822.
(43) Balakrishnan, G.; Mohandas, P.; Umapathy, S. *J. Phys. Chem.* **1996**, *100*, 16472−16478.

the phenolic bending vibration ($\delta_{OH}$) at 1320 cm$^{-1}$, and the C−O stretching vibration ($v_{C-O}$) at 1208 cm$^{-1}$ in Sudan I disappear and the carbonyl stretching vibration ($v_{C=O}$) at 1726 cm$^{-1}$ come into being in the synthetic product, validating the formation of quinone (Figure 1). Whereas, Ar−H stretching and bending vibration, the benzene ring stretching vibration, and N=N and Ar−N stretching vibration still exist (shown in Figure 9) in the synthetic product (For further details of IR features, please see Supporting Information). Thus, we could draw the reaction principle shown in Figure 1. The phenol group, at the high pH, first changes into a phenate group and then is oxidized into a quinonic structure.

## CONCLUSION

In this contribution, we propose a PRLS signals-based sensing detection of Sudan dyes, avoiding the pretreatments of separation. The determination for real samples of cayenne oil and chili sauce shows that our method is sensitive, effective, simple, reliable, and potential to be put into practice. The detection for solid samples including sausages and cayenne powder is ongoing, and we hope

to extend to farther applicable fields. We are trying our best to seek much simpler and easier devices such as a test paper slip to detect one of them in real samples. Besides, this reaction may supply a new idea to synthesize stable silver NPs, when theappropriate condition is under control. This method may also be a valuable approach for the development of detection of other food pigments.

## SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.