ORIGINAL PAPER

Rapid fluorescence assay for Sudan dyes using polyethyleneimine-coated copper nanoclusters

Yu Ling · Jia Xing Li · Fei Qu · Nian Bing Li · Hong Qun Luo

Abstract We report that the intensity of the blue fluorescence of copper nanoclusters coated with polyethyleneimine (PEI) is strongly reduced in the presence of the food dyestuffs Sudan I-IV. This finding was exploited in a label-free fluorescence assay for these Sudan dyes both in ethanol and aqueous solutions. The PEI-capped nanoclusters have an average diameter of 1.8 nm and are displaying, under 355 nm excitation, a blue emission at 480 nm that matches the absorption bands of the Sudan dyes. The clusters are stable in solution for at least 1 month. Under optimum conditions, this assay can be applied to the quantification of the dyes Sudan I, II, III, and IV, respectively, in the 0.1−30, 0.1–30, 0.1–25, and 0.1–25 μM concentration ranges, and the detection limits (3σ/slope) are 65, 70, 45, and 50 nM, respectively. The capability of reducing the fluorescence of the PEI-capped copper nanoclusters is directly related to the number of the functional groups in that Sudan III and IV give lower detection limits. This analytical scheme exhibits a remarkably high selectivity for the Sudan dyes over potentially interfering substances. The method was successfully applied to determine Sudan I, II, III, and IV in hot chilli powder.

Y. Ling · J. X. Li · F. Qu · N. B. Li (✉) · H. Q. Luo (✉)
Key Laboratory of Eco-environments in Three Gorges Reservoir Region (Ministry of Education), School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, People's Republic of China
e-mail: linb@swu.edu.cn
e-mail: luohq@swu.edu.cn

F. Qu
Shandong Provincial Key Laboratory of Life-Organic Analysis, College of Chemistry and Chemical Engineering, Qufu Normal University, Qufu, Shandong 273165, People's Republic of China

Introduction

Sudan I-IV, a series of artificial azo dyes, are traditionally used for different industrial and scientific applications such as plastics, textiles, floor polishes, and cosmetics [1]. Because of their low cost and wide availability, Sudan I-IV are also attractive as food colorants. They are used as additives in various foods, such as hot chili powder [2], sausages, chutneys, sauces, and ready meals. However, these dyes are demonstrated through laboratory experiments as potential carcinogens to both human beings and animals and thus are classified as a category carcinogen by the International Agency for Research on Cancer [3]. They are banned for food usage in most countries, including in the European Union. As reviewed [4, 5], many analytical methods have been proposed to detect the presence of Sudan dyes in food. To date, the standard used to detect the Sudan dyes is based on a liquid chromatographic method approved by the European Commission. Then the most extensively used method is high performance liquid chromatography (HPLC) coupled with different detectors such as ultraviolet visible (UV–vis) absorption spectroscopy [6], mass spectrometry [7], photodiode array [8], chemiluminescence [9], and electrochemical detector [10]. Although these methods which need separation with HPLC first are well-proven and widely accepted, they require relatively expensive equipments, high cost, and much more time. Additionally, these time-consuming methods bring about inconvenience to real-time analysis. Hence, developing simple and low-cost detection method is urgent. In addition, a variety of other analytical methods, including

mass spectrometry [2], flow injection chemiluminescence assay [11], electroanalytical technique [12], immunoassay [13], UV–vis spectroscopy [14], Raman spectroscopy [15], and resonance light scattering [16] have also been reported. On the other hand, as one of the most important research communities in analytical chemistry, fluorescent methods are attractive for various analytical purposes because of their high sensitivity, a wide linear range, and low time-consuming. However, just a few fluorescence methods were reported on the detection of Sudan dyes [17].

Metal nanoparticles have been extensively investigated because of their unique electronic and optical properties that are substantially different from bulk materials. A lot of effort has been, in particular, devoted to the synthesis and characterization of stable dispersions of nanoparticles made of silver, gold, and other noble metals. The use of nanoparticles as fluorescent indicators in biological applications has dramatically increased since the 1990s. The methods using nanoparticle-based fluorescence probes have been used for analysis of metal ions, oligonucleotides, proteins, cancer cells, and other biomolecules [18]. When the particle size of nanoparticles is further reduced and approaches the Fermi wavelength of electrons, the continuous density of states breaks up into discrete energy levels. Consequently, metal nanoclusters with sizes below 2 nm exhibit remarkable optical, electrical, and chemical properties which are significantly different from the metal nanoparticles. These metal nanoclusters have been shown to have potential applications in optical sensing, catalysis, imaging, and other fields [19–21]. Up to now, a large amount of literature was reported on the synthesis and application of Au and Ag nanoclusters because of their lower toxicity than semiconductor quantum dots and better photostability than organic fluorophores. Specially, copper has similar properties to gold and silver, whereas it is much less noble than Ag and Au. Several methods have been successfully developed to synthesize ultrasmall copper nanoclusters [22–26]. Nevertheless, compared to the considerable researches on gold and silver, papers on tiny copper nanoclusters are still deficient primarily because of their susceptibility to oxidation and aggregation, especially in aqueous solutions. It is very interesting to explore new sensing platform which costs much lower and can be applied more widely. Herein, we synthesized stable and highly fluorescent Cu nanoclusters using the polyethyleneimine (PEI) as a template. In this paper, the PEI-capped Cu nanoclusters have been utilized as a fluorescence probe for detection of Sudan I-IV in ethanol solution. Moreover, PEI has been proved to be a biocompatible and efficient gene delivery vehicle both in vitro and in vivo [27]. Therefore, our fluorescence method would be a promising candidate for detection of Sudan I-IV in biotechnology.

Experimental

Synthesis of polyethyleneimine-capped copper nanoclusters

Chemicals and apparatus were given in the Electronic Supplementary Material. The process of synthesis was as follows: Briefly, 50 μL of 0.0940 g mL^{-1} PEI (Aladdin Reagent Co., Shanghai, China, www.aladdin-reagent.com) solution was diluted with water (395 μL), and homogenized under stirring for 2 min. Subsequently, 25 μL of 0.1 M CuSO$_4$ solution was added and the solution was mixed well under vigorous stirring. Then 30 μL of diluted 100-fold hydrazine hydrate was added and the solution was mixed well for 2 min. The final mixture was heated at 95 °C for 12 h. The as-prepared Cu nanoclusters were stored at room temperature for at least 48 h before its further application.

Fluorescence detection of Sudan I-IV

The detection procedure of Sudan I-IV (Tianjin Kermel Chemical Reagents Co., Ltd., Tianjin, China, www.chemreagent.com) was conducted as follows: 40 μL of Cu nanoclusters solution was added to 860 μL of ethanol in a 1.5 mL Eppendorf tube. Subsequently, 100 μL of working solution of Sudan I-IV with different concentrations was added. After 20 min, the fluorescence emission spectrum of the mixture (1 mL) was recorded by a Hitachi F-4500 spectrofluorophotometer (Tokyo, Japan, www.hitachi.com).

The procedure for Sudan I-IV detection in commercial chilli powder samples was as follows: The pretreatment of the sample was preceded by the method of National Standard of the People's Republic of China (2005). Then the final eluate was heated to remove the organic solution. And the resulting residue was diluted to 5.0 mL with ethanol for the determination of Sudan I-IV. For spiked tests, appropriate amounts of Sudan I-IV standards were added to the pretreated chilli powder sample, respectively. Finally, the fluorescence intensities were measured at room temperature (25±1 °C). All of the fluorescence detections were performed under the same conditions.

Results and discussion

Characterization of polyethyleneimine-capped copper nanoclusters

The synthesis of Cu nanoclusters was based on a previous report [28]. PEI is essential to form the ultrasmall Cu

nanoclusters, because PEI contains many amino groups which exhibits outstanding adsorption ability for Cu^{2+} ions, and its branched structure not only can be used as the template which can form stable Cu nanoclusters, but also prevents further growth of nanoclusters to large nanoparticles. The fluorescence intensities of Cu nanoclusters were increased with increasing molar ratio of PEI to $CuSO_4$, and the maximum fluorescence intensity occurred at a molar ratio of 4:1. When the molar ratio is further increased, the fluorescence intensity was gradually decreased. Obviously, a higher molar ratio leads to a decrease in fluorescence intensity. The reason is maybe that the higher concentration of PEI may help in the delocalization of electron holes produced from amine groups in the excited state [29] and result in decrease in fluorescence intensity. Moreover, when the copper nanoclusters were synthesized, the mixture was heated at 95 °C for 12 h. Under such conditions, the amount of residual hydrazine was tiny. After hydrazine hydrate reduction, the solution of Cu nanoclusters is bluish-green. And the diluted 20 times solution is slight blue under visible light, whereas it emits blue fluorescence under a UV lamp. The PEI-capped Cu nanoclusters exhibited a quantum yield of 3.8 % in ethanol, calculated by using quinine sulfate in 0.1 M H_2SO_4 as a reference [30]. The high resolution transmission electron microscopy image of Cu nanoclusters (Fig. S1, Electronic Supplementary Material, ESM) displays an average diameter of 1.8 nm, which confirms the formation of small copper nanoclusters. The UV–vis absorption spectrum illustrates that the PEI-capped Cu nanoclusters have two absorption bands centered at 268 and 355 nm. The absorption band at 355 nm is attributed to the oligomeric copper species. No characteristic surface plasmon band was observed at around 400–500 nm, which was assigned to larger Cu nanoparticles. The maximum fluorescence excitation and emission wavelengths of PEI-capped Cu nanoclusters are 355 and 480 nm, respectively. The as-prepared copper nanoclusters are stable for at least 1 month at room temperature. Repetitive syntheses ($n=6$) of PEI-Cu nanoclusters were done and a relative standard deviation (RSD) of 4.1 % was obtained, demonstrating a good reproducibility of making the material.

Optimization of the fluorescence sensing system for Sudan I-IV

In order to achieve the sensitive detection of Sudan I-IV, many tests were carried out to optimize the sensing parameters, such as probe concentration, pH, temperature, and reaction time.

Firstly, we tested the fluorescence intensities of PEI-capped Cu nanoclusters in the absence of Sudan I-IV. From the relationship between the fluorescence intensity and the concentration of the PEI-capped Cu nanoclusters as shown in the inset of Fig. S2 (ESM), it can be seen that the fluorescence intensities are proportional to the concentrations of the probe

in the range of $5-50$ μL mL^{-1}, and when the concentration of the diluted PEI-capped Cu nanoclusters is higher than 50 μL mL^{-1}, the fluorescence intensity increases slowly and then there is a trend to saturation. Additionally, Fig. S3 (ESM) displays the relationship between the probe concentration (5–50 μL mL^{-1}) and the fluorescence quenching effect (F_0 and F represent the fluorescence intensity of Cu nanoclusters in the absence and presence of Sudan I-IV, respectively. $(F_0–F)/F_0$ is the fluorescence quenching efficiency by Sudan I-IV). The $(F_0–F)/F_0$ value increases gradually with increasing concentration of the diluted PEI-capped Cu nanoclusters in the range of $5-40$ μL mL^{-1}. And when the concentration of the diluted PEI-capped Cu nanoclusters is higher than 40 μL mL^{-1}, the sensor for Sudan I-IV detection becomes relatively insensitive. Generally, in the presence of quencher of a given concentration, the fluorescence quenching is more efficient at a lower concentration of fluorescence probe, and thus the detection of target has a higher sensitivity. However, on the other hand, the signal-to-noise ratio would be decreased when using too low concentration of fluorophore [31]. Therefore, the concentration of 40 μL mL^{-1} PEI-capped Cu nanoclusters was eventually chosen for the detection of Sudan I-IV.

The pH values of the ethanol solutions were controlled by the addition of H_2SO_4 or NaOH in the experiments. Sudan dyes exist mainly in acidic solution as molecular state, whereas they exist in basic solution as ionized state. Therefore, the changes of fluorescence quenching efficiency in different concentrations of H_2SO_4 and NaOH ethanol solutions were carefully investigated. It is illustrated in Fig. S4 (ESM) that the fluorescence quenching efficiencies nearly keep constant with the change in the concentrations of H_2SO_4 and NaOH. To avoid the complication of the detection, we did not add H_2SO_4 or NaOH in the following experiment.

Next, the effect of temperature was studied. In the range of 15 to 65 °C, the fluorescence intensities of the PEI-capped Cu nanoclusters solutions in the absence and presence of Sudan I-IV have been tested and the results are shown in Fig. S5 (ESM). Considering that the boiling point of ethanol is 78.4 °C, we did not investigate the higher temperature. The fluorescence quenching efficiency of the sensing system achieved a maximum at 25 °C. Consequently, we chose 25 °C as the optimum temperature. Finally, we studied the effect of reaction time. As indicated in Fig. S6 (ESM), the Sudan-induced fluorescence quenching of PEI-capped Cu nanoclusters would be completed within 20 min.

Sensitivity for Sudan I-IV detection

As shown in Fig. S7 (ESM), the fluorescence intensities of the PEI-capped Cu nanoclusters probe in methanol, ethanol, and aqueous solution in 7 days were in the range of $551.9-564.2$, $579.1-586.1$, $578.6-589.3$, respectively, illustrating that the fluorescence intensities of the probe in these three solvents
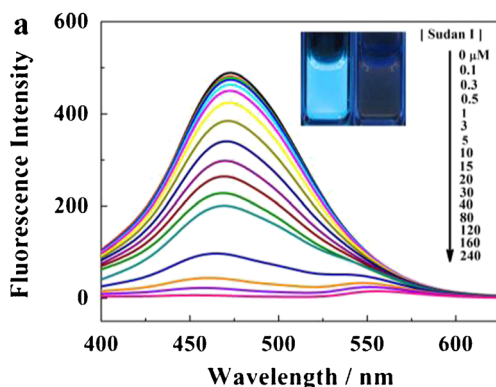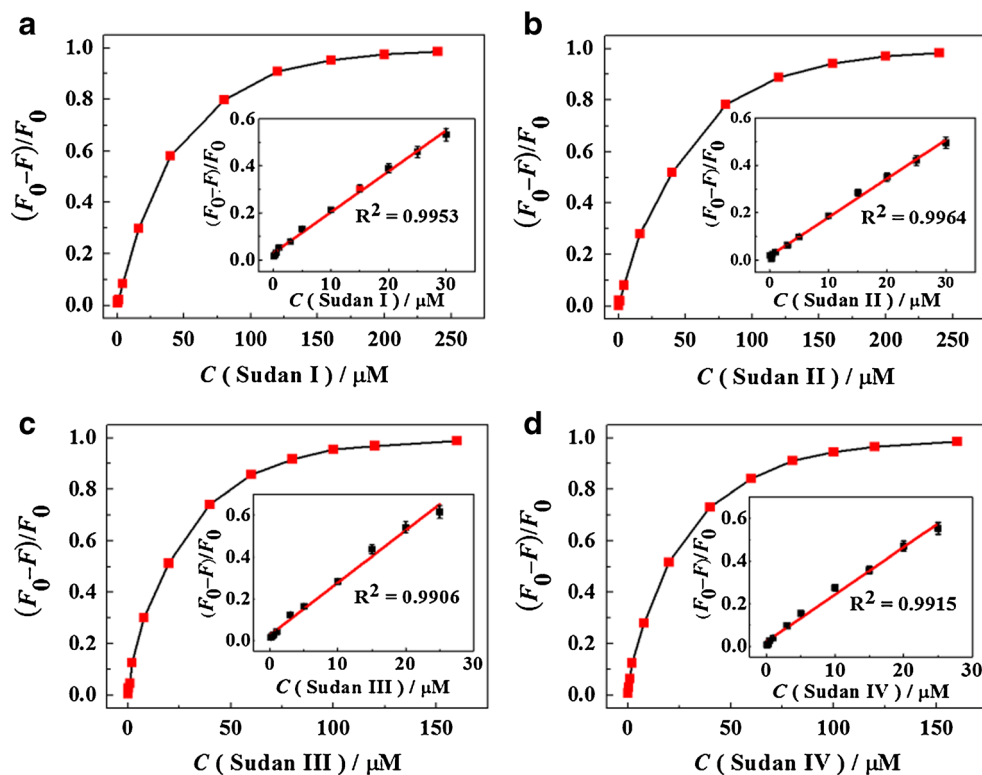
Fig. 1 Fluorescence emission spectra of PEI-capped Cu nanoclusters in ethanol solution and different concentrations of Sudan I under optimum conditions. Inset: the photographs for PEI-Cu nanoclusters (40 μL mL^{-1}) in the absence and presence of Sudan I (40 μM) under the UV-lamp

concentration range of 0.1−30 μM for Sudan I, 0.1−30 μM for Sudan II, 0.1−25 μM for Sudan III, and 0.1−25 μM for Sudan IV, respectively. The detection limits for Sudan I-IV were as low as 65, 70, 45, and 50 nM, respectively. The results indicated that the present method could meet the requirement of food detection. As illustrated in Fig. S9 (ESM), in aqueous solution, the linear ranges for Sudan I-IV are all 1 to 40 μM, and the detection limits for Sudan I-IV are 20, 5, 20, and 5 μM, respectively. Therefore, the sensitivity of the PEI-capped Cu nanoclusters probe for detection of Sudan dyes is higher in ethanol solution than in aqueous solution. In addition, we have compared this method with other optical detection methods for Sudan I (Table S1, ESM)). The proposed method was comparable to or better than others.

were all stable. However, the sensitivity of the probe for detection of Sudan dyes is different in different solvents. In ethanol solution, the fluorescence emission spectra of PEI-capped Cu nanoclusters upon addition of different concentrations of Sudan I are shown in Fig. 1 and those of Sudan II-IV are shown in Fig. S8 (ESM). It can be seen from the photographs in the inset of Fig. 1 that the blue fluorescence emission of PEI-Cu nanoclusters is strongly reduced upon addition of Sudan I. As shown in Fig. 2, under optimum conditions, the good linear relationships between the fluorescence quenching efficiencies of PEI-capped Cu nanoclusters in ethanol solution and the concentrations of Sudan I-IV were found over the

Selectivity for Sudan I-IV detection

To investigate whether this sensing system is specific for Sudan I-IV, the influences of potentially interfering substances in hot chilli powder such as saccharides, amino acid, and metal ions were tested under optimum conditions. In addition, the influence of other azo dyes, quinone dye, red food additives, and potentially interfering substances such as methyl orange, methyl red, alizarin red, congo red, sunset yellow, acid red, eosin Y, rhodamine B, and orcein have been tested. The tested substances are shown in Fig. 3: alizarin red, congo red,
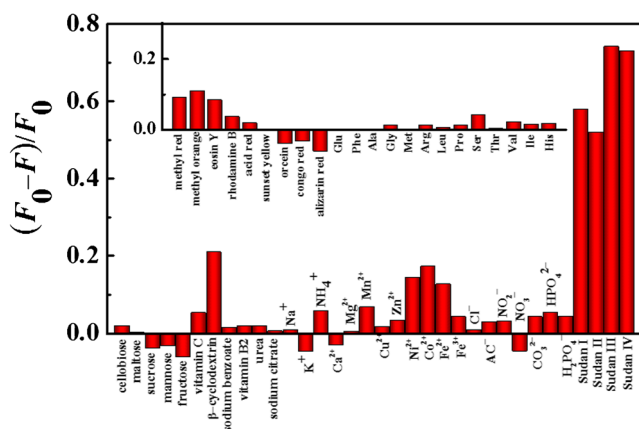
Fig. 2 The relationships between the fluorescence quenching efficiencies of PEI-capped Cu nanoclusters in ethanol solution and different concentrations of Sudan I-IV under optimum conditions. Inset: the linear ranges for Sudan I-IV are 0.1−30, 0.1−30, 0.1−25, and 0.1−25 μM, respectively
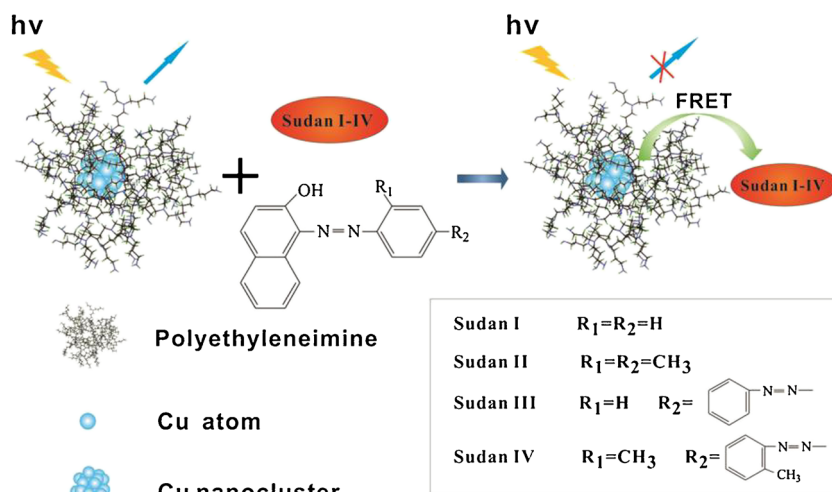
Fig. 3 Selectivity of PEI-capped Cu nanoclusters for the detection of Sudan I-IV over other potentially interfering substances: the concentrations of Sudan I-IV are 40 μM, respectively; the tested substances are as follows: alizarin red, congo red, sunset yellow, sodium citrate, β-cyclodextrin, Cu^{2+}, Co^{2+}, Fe^{2+}, and Fe^{3+} (40 μM); HPO_4^{2-} and $H_2PO_4^-$ (100 μM); Zn^{2+} and Ni^{2+} (200 μM); and other interfering substances (400 μM)

sunset yellow, sodium citrate, β-cyclodextrin, Cu^{2+}, Co^{2+}, Fe^{2+}, and Fe^{3+} (40 μM); HPO_4^{2-} and $H_2PO_4^-$ (100 μM); Zn^{2+} and Ni^{2+} (200 μM); and other interfering substances (400 μM). The addition of 40 μM Sudan I-IV to the PEI-capped Cu nanoclusters solution caused a much more obvious change in the value of $(F_0–F)/F_0$. Among the interfering substances, cyclodextrin which is not a colored species caused a relatively large effect. Cyclodextrin, with lipophilic inner cavities and hydrophilic outer surfaces, is capable of interacting with a large variety of guest molecules to form noncovalent inclusion complexes [32]. It seems that cyclodextrin interacts with Cu nanoclusters to reduce the fluorescence intensity. Therefore, the present method has a brilliant future for the further applications.

Mechanism exploration of fluorescent detection of Sudan dyes

To understand the mechanism, the absorption and the fluorescence emission spectra of PEI-capped Cu nanoclusters and Sudan I-IV were investigated. Because new absorption peak did not appear when the Sudan dye was added to the PEI-capped Cu nanoclusters solution, static quenching is excluded. The overlap of the fluorescence emission spectrum of PEI-capped Cu nanoclusters and the absorption spectra of Sudan I-IV (Fig. S10, ESM) suggests that fluorescence decrease may arise from the fluorescence resonance energy transfer (FRET) [33], or fluorescence inner filter effect (IFE). IFE would occur effectively only if the absorption spectrum of the absorber possesses complementary overlap with the excitation or emission spectrum of the fluorophore [34]. Although IFE is usually considered as a source of errors in fluorometry, it can convert the analytical absorption signals into fluorescence signals and obtain an enhanced sensitivity and decreased detection limits. The sensors based on IFE did not require any covalent linking between the absorber and the fluorophore, which offers considerable flexibility and simplicity [35]. Quenching is highly temperature dependent, while IFE is not. Dynamic quenching increases with temperature due to an increase in fluorophore-quencher collision. On the other hand, Static quenching decreases with temperature because higher temperature reduces stability of ground state complexes between fluorophore and quencher. Therefore, the fact that temperature has little effect on the fluorescence decrease further suggested that the fluorescence of PEI-Cu nanoclusters should be mainly decreased by the IFE rather than other possible processes. This conclusion could be confirmed from the marginally small change of fluorescence decay time from 3.59 to 3.48 ns before and after addition of Sudan I (Fig. S11, ESM), which is not much more than the experimental error. In addition, the shape of the emission spectrum of PEI-capped Cu nanoclusters changed

Fig. 4 Mechanism scheme for the fluorescence quenching of PEI-capped Cu nanoclusters by Sudan I-IV

by adding enough Sudan dyes (Fig. 1). This phenomenon can be ascribed to the inner filter effects [34].

As shown in the inset of Fig. 4, Sudan I and II have one nitrogen–nitrogen double bond, whereas Sudan III and IV have two nitrogen–nitrogen double bonds. Therefore, upon addition of the same concentration of Sudan I and II, respectively, the fluorescence quenching efficiencies of PEI-capped Cu nanoclusters are almost the same. Furthermore, when the concentration of Sudan I or II is two times larger than that of Sudan III or IV, the fluorescence quenching efficiencies of PEI-capped Cu nanoclusters are similar. Herein, we could deduce that the functional group was the nitrogen-nitrogen double bond.

Analytical applications in chilli powder sample

The chilli powder sample contains many components, including carbohydrate, protein, oil, vitamin C, and so on. So some separation and purification should be introduced for real sample detection. The pretreatment procedure was described in the experiment section. The concentration of Sudan dyes in commercial chilli powder sample after pretreatment has been tested, and no Sudan dye was found in the chilli powder sample by the assay. Furthermore, the standard addition method was used for the analysis of the prepared samples. The experimental results including the relative standard deviation (RSD) of parallel determination for five times and the recovery are shown in Table S2 (ESM. The recoveries of Sudan I-IV ranged between 95.3 and 110.3 % with the RSD below 5 %. These results reveal that this method is potentially applicable for the determination of Sudan I-IV in real samples.

Conclusions

In this paper, PEI-capped copper nanoclusters exhibiting blue fluorescence emission have been used for the label-free fluorescence detection of Sudan I-IV in both ethanol and aqueous solutions. The synthesis of PEI-capped Cu nanoclusters shows good reproducibility and the probe possesses high stability. The quenching mechanism is based on the inner filter effect. This sensing system exhibits good linear ranges, low detection limits, and high selectivity for Sudan I-IV. Compared to traditional methods, our method is simple, reliable, and sensitive. With traditional pretreatment of the commercial chilli powder sample, the application of the sensor to chilli powder shows satisfactory results. Moreover, with some new sample extraction methods, such as liquid phase microextraction, we hope to extend the application of this sensor in other food samples.

References

1. An Y, Jiang LP, Cao J, Geng CY, Zhong LF (2007) Sudan I induces genotoxic effects and oxidative DNA damage in HepG2 cells. Mutat Res 627:164–170
2. Donna LD, Maiuolo L, Mazzotti F, Luca DD, Sindona G (2004) Assay of Sudan I contamination of foodstuff by atmospheric pressure chemical ionization tandem mass spectrometry and isotope dilution. Anal Chem 76:5104–5108
3. Stiborova M, Martinek V, Rydlova H, Hodek P, Frei E (2002) Sudan I is a potential carcinogen for humans: evidence for its metabolic activation and detoxication by human recombinant cytochrome P450 1A1 and liver microsomes. Cancer Res 62:5678–5684
4. Rebane R, Leito I, Yurchenko S, Herodes K (2010) A review of analytical techniques for determination of Sudan I-IV dyes in food matrixes. J Chromatogr A 1217:2747–2757
5. Valdés MG, Valdés González AC, García Calzón JA, Diaz-García ME (2009) Analytical nanotechnology for food analysis. Microchim Acta 166:1–19
6. He L, Su YJ, Fang BH, Shen XG, Zeng ZL, Liu YH (2007) Determination of Sudan dye residues in eggs by liquid chromatography and gas chromatography–mass spectrometry. Anal Chim Acta 594:139–146
7. Murty M, Chary N, Prabhakar S, Raju N, Vairamani M (2009) Simultaneous quantitative determination of Sudan dyes using liquid chromatography–atmospheric pressure photoionization tandem mass spectrometry. Food Chem 115:1556–1562
8. Cornet V, Govaert Y, Moens G, Loco JV, Degroodt JM (2006) Development of a fast analytical method for the determination of Sudan dyes in chili- and curry-containing foodstuffs by high-performance liquid chromatography–photodiode array detection. J Agric Food Chem 54:639–644
9. Zhang YT, Zhang ZJ, Sun YH (2006) Development and optimization of an analytical method for the determination of Sudan dyes in hot chilli pepper by high-performance liquid chromatography with on-line electrogenerated BrO⁻-luminol chemiluminescence detection. J Chromatogr A 1129:34–40
10. Chailapakul O, Wonsawat W, Siangproh W, Grudpan K, Zhao Y, Zhu Z (2008) Analysis of Sudan I, Sudan II, Sudan III, and Sudan IV in food by HPLC with electrochemical detection: comparison of glassy carbon electrode with carbon nanotube-ionic liquid gel modified electrode. Food Chem 109:876–882
11. Liu YH, Song ZH, Dong FX, Zhang L (2007) Flow injection chemiluminescence determination of Sudan I in hot chilli sauce. J Agric Food Chem 55:614–617
12. Ensafi A, Rezaei B, Amini M, Heydari-Bafrooei E (2012) A novel sensitive DNA–biosensor for detection of a carcinogen, Sudan II, using electrochemically treated pencil graphite electrode by voltammetric methods. Talanta 88:244–251
13. Qi YH, Shan WC, Liu YZ, Zhang YJ, Wang JP (2012) Production of the polyclonal antibody against Sudan 3 and immunoassay of Sudan dyes in food samples. J Agric Food Chem 60:2116–2122
14. Anibal CVD, Odena M, Ruisanchez I, Callao MP (2009) Determining the adulteration of spices with Sudan I-II-II-IV dyes by UV–visible spectroscopy and multivariate classification techniques. Talanta 79:887–892
15. Cheung W, Shadi IT, Xu Y, Goodacre R (2010) Quantitative analysis of the banned food dye Sudan-1 using surface enhanced Raman scattering with multivariate chemometrics. J Phys Chem C 114:7285–7290

16. Wu LP, Li YF, Huang CZ, Zhang Q (2006) Visual detection of Sudan dyes based on the plasmon resonance light scattering signals of silver nanoparticles. Anal Chem 78:5570–5577

17. Huang ST, Yang LF, Li NB, Luo HQ (2013) An ultrasensitive and selective fluorescence assay for Sudan I and III against the influence of Sudan II and IV. Biosens Bioelectron 42:136–140

18. Chen W (2008) Nanoparticle fluorescence based technology for biological applications. J Nanosci Nanotechno 8:1019–1051

19. Tanaka SI, Miyazaki J, Tiwari DK, Jin T, Inouye Y (2011) Fluorescent platinum nanoclusters: synthesis, purification, characterization and application to bioimaging. Angew Chem Int Ed 50:431–435

20. Gross E, Liu JH, Alayoglu S, Marcus MA, Fakra SC, Toste FD, Somorjai GA (2013) Asymmetric catalysis at the mesoscale: gold nanoclusters embedded in chiral self-assembled monolayer as heterogeneous catalyst for asymmetric reactions. J Am Chem Soc 135:3881–3886

21. Yu JH, Choi S, Dickson RM (2009) Shuttle-based fluorogenic silver-cluster biolabels. Angew Chem 121:324–326

22. Zhao MQ, Sun L, Crooks RM (1998) Preparation of Cu nanoclusters within dendrimer Templates. J Am Chem Soc 120:4877–4878

23. Vazquez-Vazquez C, Banobre-Lopez M, Mitra A, Lopez-Quintela MA, Rivas J (2009) Synthesis of small atomic copper clusters in microemulsions. Langmuir 25:8208–8216

24. Vilar-Vidal N, Blanco MC, Lopez-Quintela MA, Rivas J, Serra C (2010) Electrochemical synthesis of very stable photoluminescent copper clusters. J Phys Chem C 114:15924–15930

25. Wei WT, Lu YZ, Chen W, Chen SW (2011) One-pot synthesis, photoluminescence, and electrocatalytic properties of subnanometer-sized copper clusters. J Am Chem Soc 133:2060–2063

26. Rotaru A, Dutta S, Jentzsch E, Gothelf K, Mokhir A (2010) Selective dsDNA-templated formation of copper nanoparticles in solution. Angew Chem Int Ed 49:5665–5667

27. Godbey WT, Wu KK, Hirasaki GJ, Mikos AG (1996) Improved packing of poly(ethylenimine)/DNA complexes increases transfection efficiency. Gene Ther 6:1380–1388

28. Ling Y, Zhang N, Qu F, Wen T, Gao ZF, Li NB, Luo HQ (2014) Fluorescent detection of hydrogen peroxide and glucose with polyethyleneimine-templated Cu nanoclusters. Spectrochim Acta Part A: Mol Biomol Spectrosc 118:315–320

29. Pastor-Pérez L, Chen Y, Shen Z, Lahoz A, Stiriba SE (2007) Unprecedented blue intrinsic photoluminescence from hyperbranched and linear polyethylenimines: polymer architectures and pH-effects. Macromol Rapid Comm 28:1404–1409

30. Fletcher AN (1969) Quinine sulfate as a florescence quantum yield standard. Photochem Photobiol 9:439–444

31. Qu F, Li NB, Luo HQ (2012) Polyethyleneimine-templated Ag nanoclusters: a new fluorescent and colorimetric platform for sensitive and selective sensing halide ions and high disturbance tolerant recognitions of iodide and bromide in coexistence with chloride under condition of high ionic strength. Anal Chem 84:6220–6224

32. Ghosh P, Maity A, Das T, Dash J, Purkayasth P (2011) Modulation of small molecule induced architecture of cyclodextrin aggregation by guest structure and host size. J Phys Chem C 115:20970–20977

33. Lakowicz JR (2006) Principles of fluorescence spectroscopy. Springer Science+Business Media, LLC, New York

34. Parker CA (1968) Photoluminescence of solutions. Elsevier, New York

35. Shang L, Dong SJ (2009) Design of fluorescent assays for cyanide and hydrogen peroxide based on the inner filter effect of metal nanoparticles. Anal Chem 81:1465–1470