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Self-assembled Conjugated Polymer/Carboxymethyl Chitosan Graft Poly (p-dioxanone) Nanomicelles and Their Functionalized Indicator Paper for Fast and Visual Detection of Banned Food Dye

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ABSTRACT: Novel sensing colloidal nanoprobe and indicator paper on the basis of fluorescent carboxymethyl chitosan graft poly(p-dioxanone) (CMCs-g-PPDO) copolymer nanomicelles encapsulating hydrophobic conjugated polymer-poly(9,9-dioctylfluorene) (PFO) were developed for the fast and sensitive determination of banned food dye Sudan I. The PFO/ CMCs-g-PPDO colloidal solution can selectively detect Sudan I in aqueous media among the common appearing natural pigments: β-carotene, Monascorubrin and Lycopene. The sensing constant (K_{sv}) for the PFO/CMCs-g-PPDO aqueous solution toward Sudan I was 1.74×10^7 M^{-1}, which was over 400 times larger than that of single PFO in toluene solution due to the maximized interaction between

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sensing material and analyte within the chitosan-based nanomicelles. The fluorescent indicator paper functionalized with PFO/CMCs-g-PPDO also showed outstanding selectivity for Sudan I. By using this flexible and portable indicator paper, Sudan I dye could be immediately and visually discriminated from the natural pigments. This material may be used for the real-time and on-site food safety testing.

KEYWORDS: micelle, chitosan derivative, conjugated polymers, pigments, fluorescent detection

INTRODUCTION

Sudan dyes (Sudan I, II, III and IV), analogs of lipophilic phenylazo dyes, are industrial synthetic dyes containing conjugated chromophore azo (–N=N–) groups.[1, 2] The dyes are widely used as colorings agents in chemical industries for oils, inks, plastics, waxes, petrol, printing floor polishing, and spirit varnishing, etc.[3, 4, 5] Sudan dyes have also been extensively used in drugs, medical devices, and cosmetics because of their low price and color stability. However, azo dyes have significant carcinogenic properties caused by their metabolites,[6] which can also induce gene mutation through affecting DNA-binding intermediates.[7] Sudan dyes are categorized as class 3 carcinogens by the International Agency for Research on Cancer (IARC) and considered as a possible genotoxic carcinogen and mutagen to human.[4, 5] Due to their potential effect to public health, Sudan dyes are not allowed as additive in foods according to both the Food Standards Agency and the European Union.[8] However, Sudan dyes, especially Sudan I, have still been deliberately added into the food products. The contamination of a batch of Worcester sauce with Sudan I had ever led to the UK's biggest food recall accident in 2005.[9, 10] Sudan I had been largely found in the chilli products and the duck feedstuffs in China, and

caused huge panic among consumers. Thus, the fast and sensitive detection of Sudan I is of growing concern.

Many analytical methods have been developed for determining Sudan dyes, most of which are based on chromatographic methods, including capillary electrophoresis,[11] immunoaffinity chromatography (IAC),[12] high-performance liquid chromatography (HPLC),[13] gas chromatography-mass spectrometry (GC-MS)[14] and liquid chromatography-mass spectrometry (LC-MS).[15] Recently, enzyme linked immune method (ELISA)[16, 17] and molecular imprinting method have also been developed.[18, 19] Although these methods are sensitive and quantitative, they usually rely on expensive instruments and need time-consuming separation procedures, which make them not suitable for real-time and in-situ measurement. For environmental and food safety surveillance, it is often required to determine harmful analytes rapidly and inexpensively. For these reasons, potentially portable sensing technologies like fluorescence sensing are highly desirable.

Conjugated polymers (CPs) have emerged as promising fluorescence sensing materials and applied in a variety of trace detection of biological or chemical analytes in the past two decades.[20, 21] The superior optical properties of CPs are mainly caused by their delocalized electronic structure that allows for efficient electron coupling and energy transfer with the slight disturbance of external agents.[20] However, the application of conjugated polymers in aqueous environment, which is required by most chemical and biomolecules detention, is very limit due to their lipophilic nature. Various methods for improving the water solubility of CPs have been developed. Pendent modification of CPs with water-soluble ionic groups is an effective way. However, the pendent modifications toward CPs are very difficult and usually involve many complex synthesis procedures.[22, 23] In recent years, water-dispersible conjugated polymers

nanoparticles have been prepared by using re-precipitation or mini-emulsion methods, which are promising to be applied in water-phase sensing and bioimaging due to the high sensitivity and good photochemical stability.[24, 25, 26, 27] In our previous work, a type of cellulose-based nanoparticles was employed to encapsulate and protect liposoluble conjugated polymers for sensitive detection of hydrophobic nitroaromatic explosives in aqueous solution, which indicates the potential of inexpensive biomass nanoparticles in improving the fluorescence sensing of CPs in aqueous media.[25] In addition, chitosan relevant system, such as chitosan coated nanoparticles/alginate coated nanoparticles nano-network[28] and alginate-chitosan-PLGA complex[29] was recently reported to be a good cargo of liposoluble substances.

In this work, a novel amphiphilic biomass material carboxymethyl chitosan graft poly (p-dioxanone) (PPDO) with self-associating property is proposed as the cargo of liposoluble conjugated polymers for constructing an aqueous sensing system for Sudan I. Carboxymethyl chitosan is a water-soluble derivative of natural polysaccharide chitosan. Poly (p-dioxanone) (PPDO) is biodegradable aliphatic polyester. Both of carboxymethyl chitosan and PPDO have good biocompatibility and have been widely applied in food and medical field. Thus, the nanoparticles composed by them should be safe to be used in the food safety detection. Moreover, it is well known that indicator paper is the most flexible, easy-handling and cost-efficient measuring tool. Carboxymethyl chitosan (CMCs) that makes up the outer layers of the composite nanoparticles is a good surface sizing agent for papermaking.[30, 31] Taking advantage of the property of CMCs, the functional sensing nanomaterials will be combined with the paper substrate to develop a sensitive indicator paper for fast and sensitive detection of Sudan I.

EXPERIMENTAL SECTION

Materials. Chitosan (Mw: 50000) was obtained from Golden-Shell Biochemical CO., Ltd. p-dioxanone(PDO) was purchased from Jiaxing Jlight Chemicals CO., Ltd. Conjugated polymer poly (9,9-dioctylfluorene) (PFO) (M$_w$: 38131, polydispersity: 2.9) was synthesized via Suzuki polymerization according to the literature.[32, 33] Sudan I (1-[(2,4-dimethylphenyl)azo]-2-naphthalenol) was purchased from Tianjin Damao Chemical Reagent Factory. beta-carotene (β-carotene) was obtained from Tokyo Chemical Industry Co., Ltd. Monascorubrin was purchased from Guangdong Jiangmen Kelong biotechnology Co., Ltd. Lycopene was purchased from Shanghai Dyestuffs Research Institute Co., Ltd. The base paper was supplied by the Hangzhou Wohua Base Paper Co., Ltd. All the other chemicals used were of analytical-reagent grade and used without further purification.

Synthesis of Carboxymethyl Chitosan. Carboxymethyl chitosan was synthesized following a literature method with slight modifications,[34] specifically: purified chitosan (5 g) was first dissolved in saturated solution of sodium hydroxide and frozen for one night, then isopropanol (150 mL) was added to the thawed swollen chitosan sample, stirring well. Monochloroacetic acid (5 g) was added to the as-prepared solution in batches under mechanical stirring, and then the mixed solution was reacted for 12 h. Isopropanol was filtered out after the reaction, and then pure water was added to dissolve the product. The pH value was adjusted to neutral using dilute acetic acid, followed by dialysis against pure water (MWCO=3500) for 3 days. Then the resulting solution was freeze dried to give the white colored carboxymethyl chitosan product (yield: 65%) with a carboxymethyl substitution degree of about 1.0 per glucosamine unit calculated from potentiometric titration. IR (KBr): v = 3432 cm^{-1} (s; v_s(OH)); 1591 cm^{-1} (s; v_{as}(COO$^-$); 1410 cm^{-1} (m; v_s(COO$^-$)); 1080 cm^{-1} (s; v_s(OH)); 875 cm^{-1} (w; γ(NH$_2$)). ^{1}H-NMR

(400 MHz, D$_2$O, δ): 4.3 ppm (-O-C**H**$_2$-COOR); 3.6-3.9 ppm (H3-H6, Gn); 2.95 ppm (H2, Gn); 1.96 ppm (-C**H**$_3$-CO).

Synthesis of Carboxymethyl Chitosan Graft Poly(p-dioxanone) Copolymer. Carboxymethyl chitosan (CMCs) (0.2 g) prepared above was added to 10 g [Bmim]Cl in a 25 mL dried three-neck flask. The mixture was vigorously stirred at 100 °C for 3 h with the protection of nitrogen to make a homogeneous solution. Then the monomer p-dioxanone (PDO) (0.388 g) and the catalyst 4-dimethylamino-pyridine (DMAP) (0.128 g) were slowly added into the solution, and the ring opening graft polymerization was carried out at 100 °C under nitrogen atmosphere with vigorous stirring for 12 h. After cooling to room temperature, the resultant copolymer was isolated by precipitating the reaction mixture into 250 mL ethanol and washed for several times. Subsequently, the copolymer was purified in soxhlet extractor using acetone as the extraction solvent at 80 °C for 24 h to remove the homo-PPDO, which could be formed during the grafting reaction. The final product was dried in vacuum for 48 h at 60 °C to obtain carboxymethyl chitosan graft poly(p-dioxanone) copolymer (CMCs-g-PPDO) (yield: 73.4%) with DS of 0.77 determined by ^{1}H-NMR (($I_{a'}$/2)/I_{H2}). IR (KBr): v = 3432 cm^{-1} (s; vs(OH)); 2920 cm^{-1} (s; vas(C-H)), 2875 cm^{-1} (s; vs(C-H)); 1457 cm^{-1} (m; δs(C-H)); 1050 cm^{-1} (m; vs(C-O-C)). ^{1}H-NMR (400 MHz, D$_2$O, δ): 4.3 ppm (-O-C**H**$_2$-COOR); 3.45-4.06 ppm (H3-H6, Gn); 4.22 ppm (-CO-CH$_2$-O-CH$_2$-C**H**$_2$-OH); 4.15 ppm (-CO-C**H**$_2$-O-CH$_2$-CH$_2$-OH); 4.17 ppm (-CO-C**H**$_2$-O-CH$_2$-CH$_2$-O-); 3.71 ppm (-CO-CH$_2$-O-C**H**$_2$-CH$_2$-OH, -CO-CH$_2$-O-C**H**$_2$-CH$_2$-O-); 2.72 ppm (H2, Gn); 1.96 ppm (-C**H**$_3$-CO).

Preparation of Fluorescent Amphiphilic CMCs-g-PPDO Nanomicelles. Conjugated polymer PFO encapsulated CMCs-g-PPDO nanomicelles were prepared by a method similar to the reprecipitation method.[35, 36, 37] In a typical procedure, PFO was firstly dissolved in

tetrahydrofuran (THF) by stirring to obtain a concentration of 0.5 mg/mL. CMCs-g-PPDO copolymer was dissolved in pure water to make a series of nanomicelles aqueous solution with different concentrations. Certain amounts of PFO/THF solutions were slowly added into CMCs-g-PPDO nanomicelles aqueous solution under vigorous stirring, followed by ultrasonic treatment, and then the THF solvent was removed by rotary evaporation from the resulted solution, finally the resultant solution was filtered through a 0.45 μm microfilter to eliminate non-incorporated PFO.

Preparation of Fluorescent Indicator Paper. PFO/CMCs-g-PPDO functionalized fluorescent indicator paper was simply prepared as follows: a number of base paper strips with the same sizes were coated with the same amount of PFO-loaded CMCs-g-PPDO nanomicelles aqueous solution (the mass of PFO was 0.004 mg), and then dried at room temperature.

Characterization. The Fourier transform infrared (FT-IR) spectra were recorded on a Bruker TENSOR 27 spectrophotometer using a KBr disc containing 1% finely ground samples at frequencies ranging from 400 cm^{-1} to 4000 cm^{-1}. The average hydrodynamic diameter and size distribution of the as prepared nanomicelles were determined using a Malvern 90 Plus particle size analyzer equipped with a 30 mW semiconductor laser diode (659 nm) with output at a scattering angle of 90°. All measurements were made at 25 °C. The UV-vis absorption spectra were recorded with the use of a HP 8453 spectrophotometer. Photoluminescence (PL) spectra were obtained by a Jobin-Yvon spectrometer. The photographs of Sudan I fluorescent indicator paper were taken by Canon PowerShot A2300 under a WFH-204B portable UV analyzer.

RESULTS AND DISCUSSION

Synthesis of Carboxymethyl Chitosan (CMCs) and Carboxymethyl Chitosan Graft Poly (p-dioxanone) Copolymer (CMCs-g-PPDO). Chitosan is only soluble in dilute acidic solution. Its water-soluble derivative: carboxymethyl chitosan (CMCs) was synthesized from chitosan by an etherification reaction with chloracetic acid. Amphiphilic carboxymethyl chitosan graft poly (p-dioxanone) copolymer (CMCs-g-PPDO) was prepared by homogeneous open-ring polymerization of p-dioxanone onto CMCs in BmimCl ionic liquid with DMAP as catalyst. The FT-IR spectra of chitosan (CS), CMCs and CMCs-g-PPDO copolymers are displayed in Figure 1. Compared with the spectrum of unmodified CS, absorption peaks at 1591 cm^{-1} and 1410 cm^{-1} corresponding to the asymmetric and symmetric stretching vibration of carboxylic group appear in the spectrum of CMCs illustrating the successful introduction of carboxymethyl group. Moreover, the peak associates with the primary hydroxyl group (1030 cm^{-1}) in the spectrum of CS disappear in the spectrum of CMCs, while the absorption band of the secondary hydroxyl group (1080 cm^{-1}) is not changed, suggesting the carboxymethylation primarily occurs at the C6-OH of CS.[38] In the spectrum of CMCs-g-PPDO copolymer, new absorption peaks appear at 1745 cm^{-1} and 1210 cm^{-1}, which are attributed to the carbonyl stretching vibration of ester carbonyl and the ether bond of the grafted branched chain PPDO.[39] The occurrence of the grafted branched chain PPDO is also confirmed by the ^{1}H-NMR spectrum of CMCs-g-PPDO in Figure S1 (Supporting Information), in which new signals representing a-, a′-, b, b′- and c-methylene protons of PPDO graft chain appear at 4.17, 4.15, 3.71, and 4.22 ppm, respectively.[40]
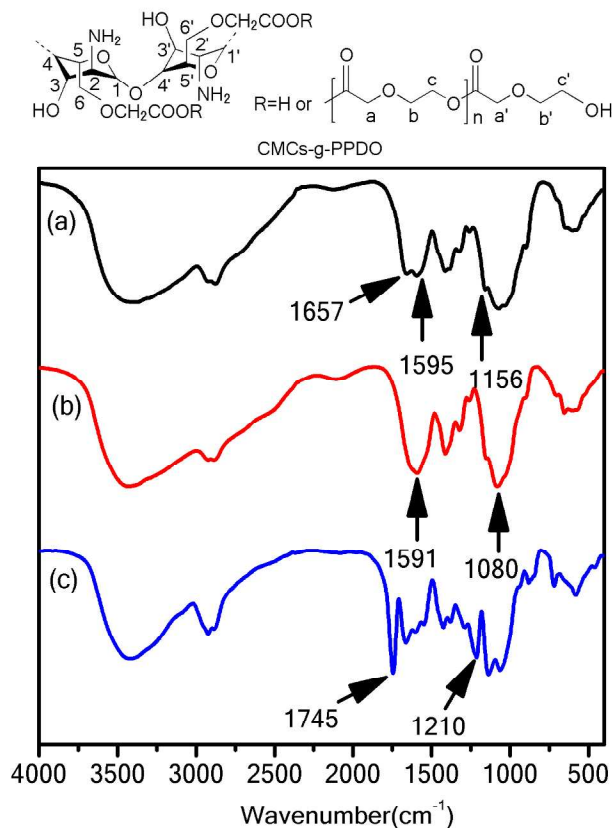
Figure 1. The structure of CMCs-g-PPDO and FT-IR spectra of CS(a), CMCs(b) and CMCs-g-PPDO(c).

The Self-assembly of Amphiphilic CMCs-g-PPDO Nanomicelles and the Loading of PFO.
The CMCs-g-PPDO copolymer contains both hydrophilic CMCs segments and hydrophobic PPDO segments, which makes it able to form polymeric nanomicelles in water. The self-assembled CMCs-g-PPDO nanomicelles were characterized by transmission electron microscope (TEM) and dynamic light scattering (DLS) as shown in Figure 2. The average hydrodynamic diameter of the CMCs-g-PPDO aggregates measured by DLS is 43.59 nm with PDI of 0.440, indicating a narrow distribution. Figure 2 shows that CMCs-g-PPDO could form near spherical particles with diameters in the range of 25-65 nm. The diameter of particles determined by DLS

is a little larger than the TEM results. This phenomenon is reasonable because the hydrophilic

backbone of the micelles tends to shrink during the drying process of TEM sample preparation.
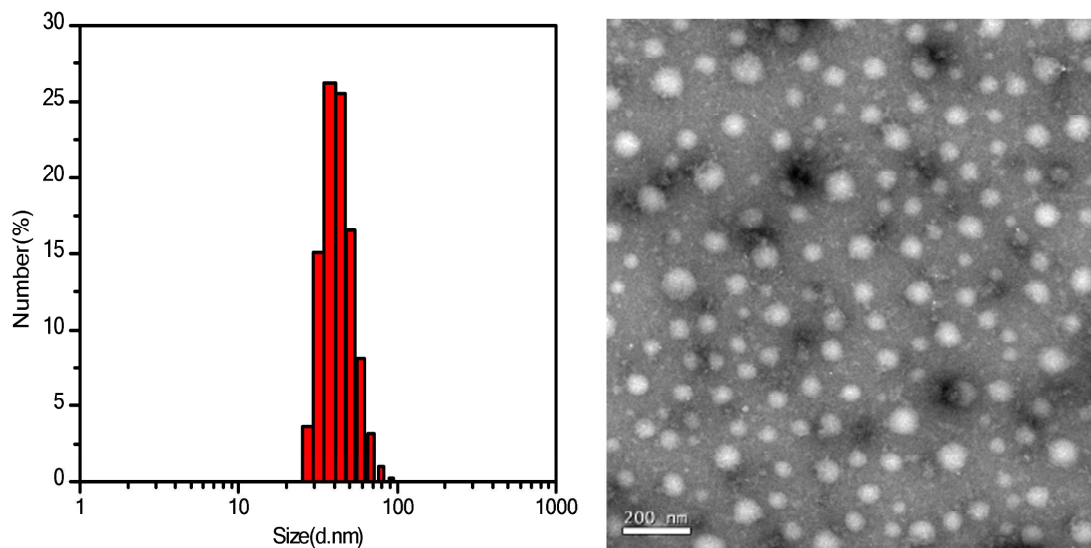
Figure 2. Size distribution of amphiphilic CMCs-g-PPDO aggregates determined by DLS and the TEM image.

The hydrophobic conjugated polymer PFO could be easily entrapped into the core of the self-assembled CMCs-g-PPDO nanomicelles. Conditions affecting the loading efficiency and amount of the PFO in the CMCs-g-PPDO nanomicelles are summarized in Figure 3. PFO encapsulation efficiency (EE, %) was determined by the following equation:

EE (%) = (weight of loaded CPs/weight in feed) ×100%

in which, weight of loaded CPs was the amount of PFO loaded into the micelles determined by the absorption spectra with reference to a calibration curve of PFO in THF.

As shown in Figure 3(a), the CMCs-g-PPDO nanomicelles solution with larger copolymer concentration can achieve higher EE(%), while increased PFO feeding amount results in reduced EE(%) value. These results could be understood as larger copolymer concentration tended to

form more micelles and thus encapsulated more PFO. More PFO feeding amount would accelerate precipitation, and lead to the reduction of EE(%) value. On the other hand, higher feeding amount of PFO and CMCs-g-PPDO nanomicelles concentration could both contribute to an increased loading weight of PFO. After PFO encapsulation, the CMCs-g-PPDO nanomicelles present increased hydrodynamic diameters, which is probably because the encapsulation of PFO makes the hydrophobic core of CMCs-g-PPDO nanomicelles expand, and finally results in the increase in the diameter of composite nanomicelles. It is also noticed that the hydrodynamic diameter of PFO/ CMCs-g-PPDO nanomicelles becomes larger as the loading amount of PFO increases, as shown in Figure 3(b, c).
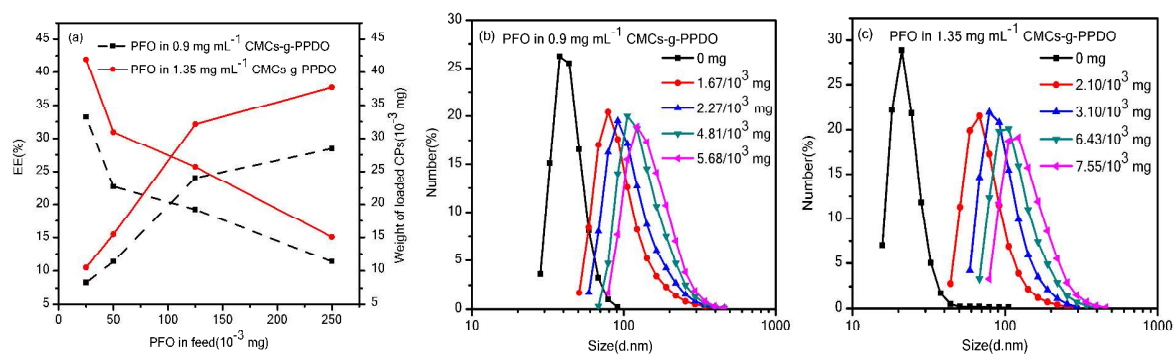
Figure 3. EE(%), weight of loaded PFO in CMCs-g-PPDO nanomicelles (a), and the size and size distribution of blank and PFO-loaded micelles determined by DLS (b, c).

Optical Properties. The photoluminescence (PL) and UV-vis absorption spectra of PFO in organic solvent toluene and in CMCs-g-PPDO nanomicelles aqueous solution are compared in Figure 4. As shown in Figure 4, PFO exhibits an absorbance peak at 389 nm in toluene. In the PFO/CMCs-g-PPDO nanomicelles aqueous solutions, the absorbance peak of PFO shows a little red-shift from 389 nm to 405 nm and there appears a small shoulder absorbance peak at around 436 nm, which is generally attributed to the aggregation or crystalline β-phase formation of polyfluorenes.[41] The corresponding PL emission peaks of PFO in toluene mainly appear at 415

nm, 440 nm and 465 nm, while the PL emission profile of PFO in CMCs-g-PPDO nanomicelles aqueous solution exhibits obviously red-shifted peaks at 439 nm, 466 nm and 497 nm, respectively. These results can be attributed to the change in the chain conformation or increased inter and intra-chain interactions of PFO in the hydrophobic micelle core.[24]
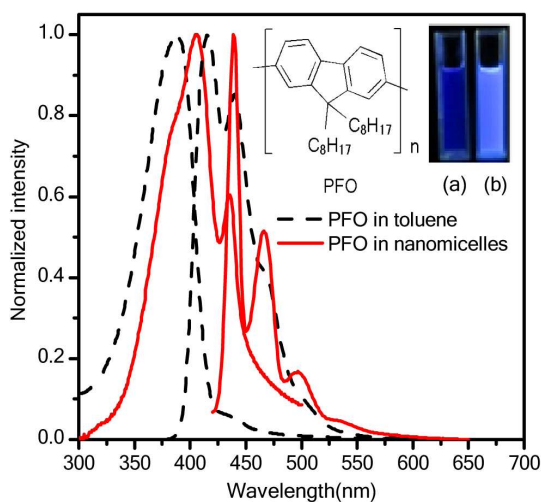
Figure 4. The UV-vis and PL spectra of PFO in toluene (dashed lines) and CMCs-g-PPDO nanomicelles aqueous solution (straight lines). The picture of PFO toluene solution (a) and PFO aqueous solution (b) are shown in the inset.

Fluorescence Quenching Studies with Food Dyes in Solution. The fluorescence quenching of PFO in CMCs-g-PPDO nanomicelles aqueous solutions and in toluene solution as a function of the successive addition of pigments was investigated. The pigments studied in this work are Sudan I and several other natural pigments: β-carotene, Monascorubrin and Lycopene. β-carotene is a well-known red-orange pigment abundant in plants and fruits. Lycopene is a bright red carotene pigment from tomatoes and other vegetables. Monascorubrin is an orange Monascus pigment isolated from the fungus. β-carotene, Lycopene and Monascorubrin are all insoluble in water and widely used for coloring food stuff. They are the mostly likely occurring interferences

of Sudan dyes in food safety testing. The molecular structures of the dyes are depicted in Figure

S2 (Supporting Information).
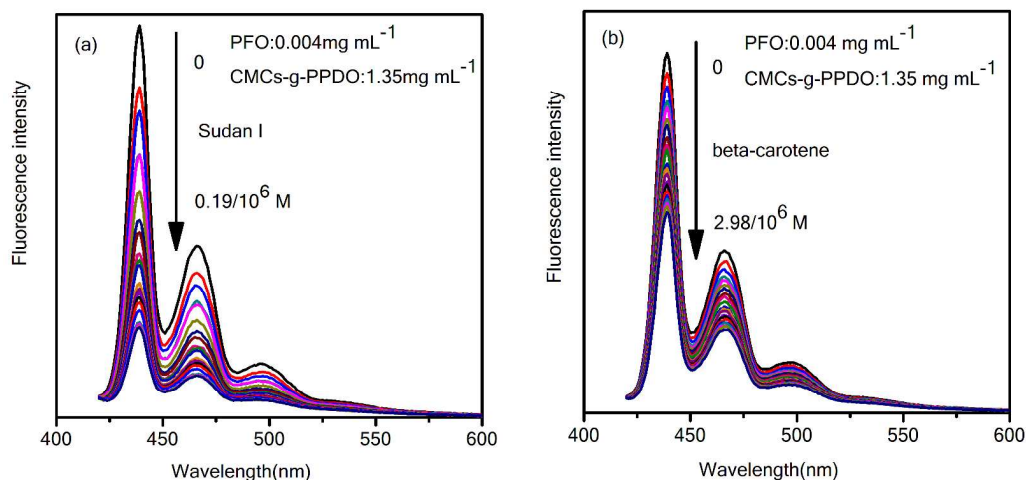
Figure 5. Fluorescence emission spectra of PFO in nanomicelles solution in the presence of

different concentrations of Sudan I and β-carotene (λ_{ex}= 340nm).

   As illustrated in Figure 5, the fluorescence was almost completely quenched by Sudan I when

0.19 μmol Sudan I was added in the PFO/CMCs-g-PPDO nanomicelles aqueous solution. In

contrast, the addition of over 15 times more quantity of β-carotene can only quench about 1/3

fluorescence intensity of the PFO/CMCs-g-PPDO, while Monascorubrin and Lycopene can not

quench the fluorescence of PFO/CMCs-g-PPDO nanomicelles aqueous solution at all even with

so much addition in Figure S3 (Supporting Information). These results indicate that the PFO-

containing CMCs-g-PPDO nanomicelles aqueous solutions can selectively detect Sudan I. For

the PFO in organic solvent toluene, the fluorescence intensity shows slight reduction in respond

to the added Sudan I and β-carotene(Figure 6), while the addition of Monascorubrin and

Lycopene has almost no influence to the fluorescence intensity of PFO toluene solution in Figure

S4 (Supporting Information). These results indicate that CMCs-g-PPDO nanomicelles aqueous

solutions can significantly enhance the sensitivity of PFO with the analytes. The selective quenching of PFO/CMCs-g-PPDO nanomicelles aqueous solution by Sudan I may be explained by its strong electron accepting capability. The electron-withdrawing azo group on the aromatic rings of Sudan I lowers the energy of the empty π^* orbital, thereby making the compound good electron acceptor that can efficiently quench fluorescence by photoinduced electron transfer.[42] Conjugated polymer PFO is a good electron donor. Efficient electron transfer from CPs to Sudan I will occur when they are in close spatial proximity. This explains why the PFO entrapped in CMCs-g-PPDO nanomicelles shows significantly improved quenching sensitivity toward Sudan I in compare with the PFO in toluene solution.[25]
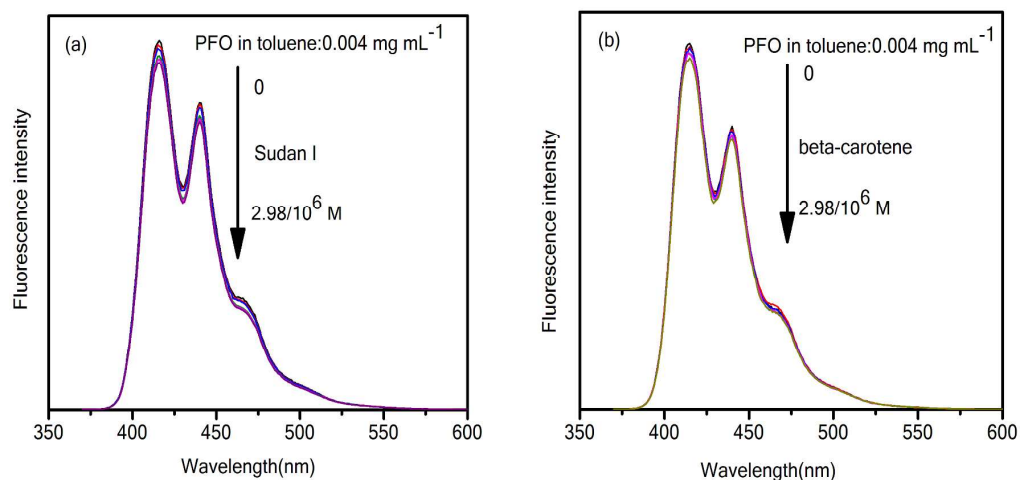
Figure 6. Fluorescence emission spectra of PFO in toluene in the presence of different concentrations of Sudan I and β-carotene (λ_{ex}= 340nm).

The fluorescence quenching efficiency can be calculated using the Stern-Volmer equation:

$$I_0/I = 1+ K_{sv} [Q]$$

in which I_0 is the initial fluorescence intensity of PFO without the analyte, I is the fluorescence intensity with the added analyte of concentration [Q], and K_{sv} is the Stern–Volmer constant.[20]

Figure 7 exhibited the Stern-Volmer plots of PFO in CMCs-g-PPDO nanoamicelles aqueous solution and in organic solvent for Sudan I and three other natural pigments: β-carotene, Monascorubrin and Lycopene. Linear Stern-Volmer relationship was observed in all cases, indicating that the quenching process is dominated by either a static or dynamic quenching mechanism instead of the synergistic effect of the two mechanisms.[20] Since the sensing chromophore and analyte are confined in the inner core of CMCs-g-PPDO nanomicelles, the quenching process is more likely dominated by the static mechanism via the formation of a chromophore-analyte complex. The PL quenching efficiencies, as indicated by the values of K_{sv}, were determined from the slopes of steady-state Stern-Volmer plots. And the PL quenching efficiencies are summarized in table S1 (Supporting Information). The K_{sv} values for PFO in organic solvent toluene with Sudan I, β-carotene, Monascorubrin and Lycopene are calculated to be $4.31 \times 10^4 \, M^{-1}$, $3.08 \times 10^4 \, M^{-1}$, $5.60 \times 10^3 \, M^{-1}$ and $2.00 \times 10^3 \, M^{-1}$ respectively. In contrast, the K_{sv} values for PFO in CMCs-g-PPDO nanomicelles aqueous solution with Sudan I, β-carotene, Monascorubrin and Lycopene are calculated to be $1.74 \times 10^7 \, M^{-1}$, $1.80 \times 10^5 \, M^{-1}$, $3.61 \times 10^4 \, M^{-1}$ and $1.83 \times 10^4 \, M^{-1}$ respectively. It is noteworthy that the quenching constants of PFO in CMCs-g-PPDO nanomicelles aqueous solution are much higher than those in organic solvent toluene. Especially for Sudan I, the K_{sv} value of PFO in CMCs-g-PPDO nanomicelles aqueous solution is over 400 times larger than that of PFO in toluene solution. This phenomenon is probably due to the interaction between conjugated polymers and the analyte is maximized when they are both confined within the limited space of chitosan-based nanomicelles. The micro-phase extraction effect of the chitosan nanomicelles may also contribute to the significantly improved sensitivity, through which the hydrophobic analytes tend to be enriched in the micelle core and thus the sensor-analyte binding interaction is strengthened.
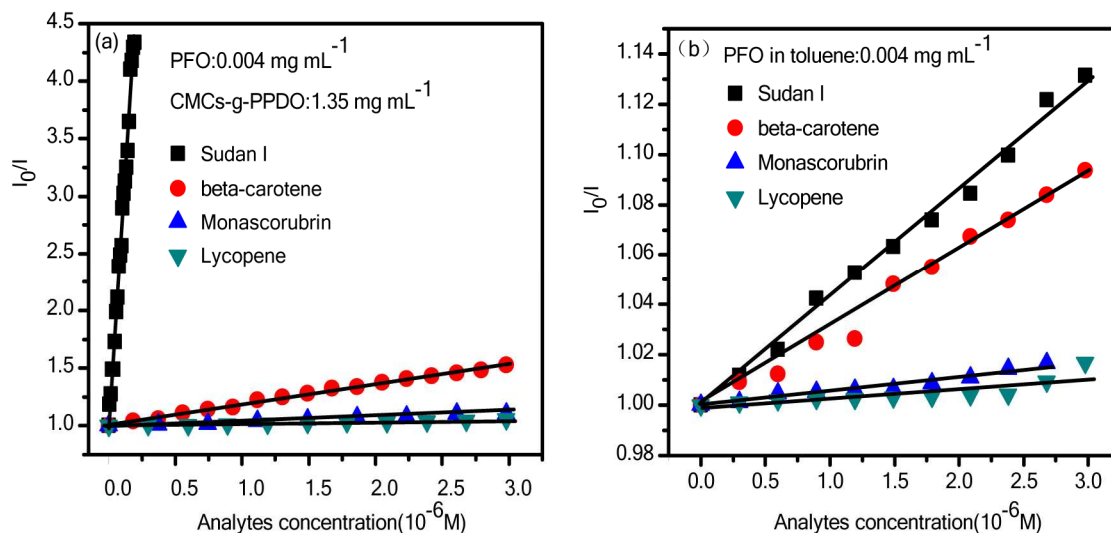
Figure 7. Stern-Volmer plots for PFO in nanomicelles (a) and toluene solution (b) with the analytes Sudan I, β-carotene, Monascorubrin and Lycopene (quenching versus analytes concentration).
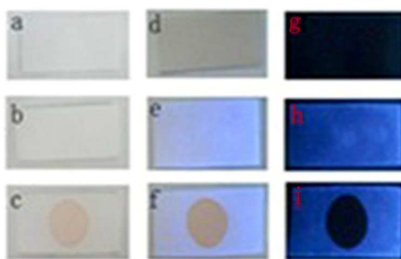
Identification of Sudan I Using the PFO/CMCs-g-PPDO Functionalized Fluorescent Indicator Paper. The PFO/CMCs-g-PPDO functionalized fluorescent indicator paper was conveniently obtained through coating the base paper with PFO/CMCs-g-PPDO aqueous solution by a coating roll, as shown in Scheme 1. The procedure could be easily scaled up by using paper coating machines. CMCs is a good paper coating agent that is in favor of increasing water retention value and the solid content of coating, and also helps carry the brightener and pigments. However, CMCs cannot carry hydrophobic materials. In this work, CMCs-g-PPDO takes the similar function of CMCs as the carrier of hydrophobic PFO.
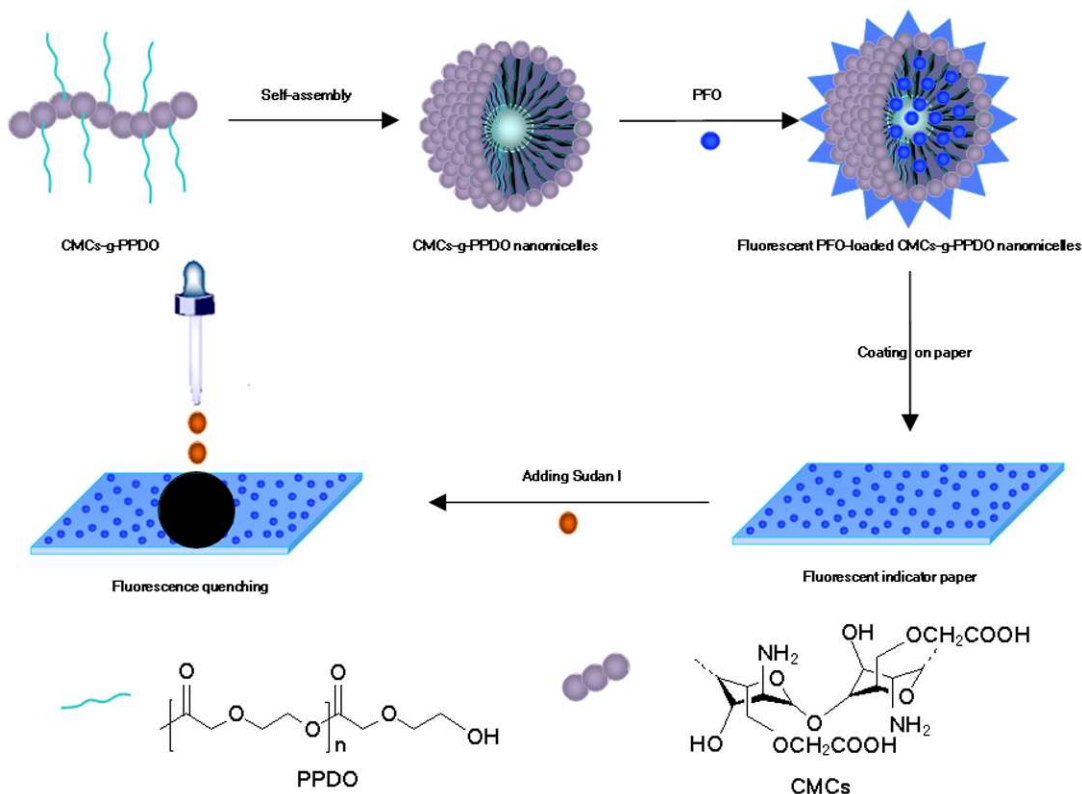
Scheme 1. Schematic illustration of proposed probe for detection Sudan I.

Photographs of the PFO/CMCs-g-PPDO functionalized fluorescent indicator paper and the paper in contact with Sudan I under both of daylight lamp and UV lamp are displayed in Figure 8. In Figure 8, a, d and g are pictures of a piece of base paper taken under daylight lamp, daylight lamp and UV lamp, UV lamp respectively; b, e and h are pictures of PFO/CMCs-g-PPDO functionalized fluorescent indicator paper taken under daylight lamp, daylight lamp and UV lamp, UV lamp respectively; c, f and i are pictures of PFO/CMCs-g-PPDO functionalized fluorescent indicator paper in contact with Sudan I taken under daylight lamp, daylight lamp and UV lamp, UV lamp respectively. As shown in Figure 8, pictures of PFO/CMCs-g-PPDO functionalized fluorescent indicator paper emit blue light under UV lamp. When a drop of Sudan I is added onto the indicator paper, the region in contact with Sudan I becomes dark. As contrast,

the addition of the natural pigments: β-carotene, Monascorubrin and Lycopene does not result in obvious change to the fluorescence of the indicator paper in Figure S5 (Supporting Information), indicating the good selectivity of the fluorescent paper to Sudan I. These results suggest the PFO/CMCs-g-PPDO nanomicelles solution can not only be used to detect trace amount of Sudan I pigment in aqueous media, but also be utilized as paper coating agent to prepare qualitative fluorescent indicator paper of Sudan I. The fluorescent indicator paper holds great potential in portable on-site and real-time food safety surveillance.

Figure 8. Photographs of the PFO/CMCs-g-PPDO functionalized fluorescent indicator paper in contact with Sudan I (a, b, c: pictures under daylight lamp; d, e, f: pictures under daylight lamp and UV lamp; g, h, i: pictures under UV lamp)

CONCLUSIONS

In summary, an effective fluorescent sensing material PFO/CMCs-g-PPDO was developed by encapsulating conjugated polymer PFO in the amphiphilic carboxymethyl chitosan graft poly (p-dioxanone) copolymer nanomicelles. PFO/CMCs-g-PPDO can sensitively and selectively detect Sudan I in aqueous media with the sensing constant (K_{sv}) of 1.74×10^7 M^{-1}. The value is over 400 times larger than the K_{sv} of PFO in toluene solution. The natural pigments with similar color with Sudan I that may be simultaneously appear in the food samples cannot quench the fluorescence of PFO/CMCs-g-PPDO. Fluorescent indicator paper functionalized with

PFO/CMCs-g-PPDO was also developed and applied in Sudan I detection. The results show that the indicator paper can selectively identify Sudan I, and could be conveniently used in qualitative determination of Sudan I. This work provides another point of view in prohibited food additive detection. The sensing material and indicator paper can be potentially handled by the common customers and be good to the public food safety.

ASSOCIATED CONTENT

Supporting Information

^{1}H-NMR spectrum of CMCs-g-PPDO copolymer, structures of testing dyes, fluorescence emission spectra of PFO in nanomicelles and toluene in the presence of Monascorubrin and Lycopene, the Stern-Volmer constants (K_{sv}) value for the fluorescence quenching of PFO by the testing dyes and photographs of the PFO/CMCs-g-PPDO functionalized fluorescent indicator paper in contact with β-carotene, Monascorubrin and Lycopene are included. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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REFERENCES

(1) J. Y. Hong, N. H. Park, K. H. Yoo and J. Hong, *J. Chromatogr. A*, 2013, 1297, 186-195.

(2) Y. Wang, Y. Sun, Y. Wang, C. Jiang, X. Yu, Y. Gao, H. Zhang and D. Song, *Anal. Methods*, 2013, 5, 1399-1406.

(3) M. R. V. S. Murty, N. Sridhara Chary, S. Prabhakar, N. Prasada Raju and M. Vairamani, *Food Chem.*, 2009, 115, 1556-1562.

(4) R. Rebane, I. Leito, S. Yurchenko and K. Herodes, *J. Chromatogr. A*, 2010, 1217, 2747-2757.

(5) H. Xu, T. M. Heinze, D. D. Paine, C. E. Cerniglia and H. Chen, *Anaerobe*, 2010, 16, 114-119.

(6) R. L. Stingley, W. Zou, T. M. Heinze, H. Chen and C. E. Cerniglia, *J. Med. Microbiol.*, 2010, 59, 108-114.

(7) M. Stiborová, V. Martínek, M. Semanská, P. Hodek, M. Dračínský, J. Cvačka, H. Schmeiser and E. Frei, *Interdiscip. Toxicol.*, 2009, 2, 195-200.

(8) M. Stiborová, V. Martínek, H. Rýdlová, P. Hodek and E. Frei, *Cancer Res.*, 2002, 62, 5678-5684.

(9) FSA. (2005a) Action taken to remove illegal dye found in wide range of foods on sale in UK. http://www.food.gov.uk/news/newsarchive/2005/feb/worcester

(10) FSA. (2005b) Sudan I consolidated product list. http://multimedia.food.gov.uk/multimedia/pdfs/sudanlistno.pdf

(11) E. Mejia, Y. Ding, M. F. Mora and C. D. Garcia, *Food Chem.*, 2007, 102, 1027-1033.

(12) Y. Li, Y. Wang, H. Yang, Y. Gao, H. Zhao and A. Deng, *J. Chromatogr. A*, 2010, 1217, 7840-7847.

(13) C. Long, Z. Mai, X. Yang, B. Zhu, X. Xu, X. Huang and X. Zou, *Food Chem.*, 2011, 126, 1324-1329.

(14) L. He, Y. Su, B. fang, X. Shen, Z. Zeng and Y. Liu, *Anal. Chim. Acta*, 2007, 594, 139-146.

(15) M. Ma, X. Luo, B. Chen, S. Su and S. Yao, *J. Chromatogr. A*, 2006, 1103, 170-176.

(16) J. Wang, K. Wei, H. Li, Q. X. Li, J. Li and T. Xu, *Analyst*, 2012, 137, 2136-2142.

(17) D. Han, M. Yu, D. Knopp, R. Niessner, M. Wu and A. Deng, *J. Agric. Food Chem.*, 2007, 55, 6424-6430.

(18) H. Yan, J. Qiao, Y. Pei, T. Long, W. Ding and K. Xie, *Food Chem.*, 2012, 132, 649-654.

(19) M.-M. Zheng, J.-H. Wu, Y.-Q. Feng and F.-H. Huang, *Anal. Methods*, 2011, 3, 1851-1858.

(20) S. W. Thomas, G. D. Joly and T. M. Swager, *Chem. Rev.*, 2007, 107, 1339-1386.

(21) X. Duan, L. Liu, F. Feng and S. Acc. Wang, *Chem. Res.*, 2009, 43, 260-270.

(22) C. Zhu, L. Liu, Q. Yang, F. Lv and S. Wang, *Chem. Rev.*, 2012, 112, 4687-4735.

(23) B. Liu and G. C. Bazan, *J. Am. Chem. Soc.*, 2006, 128, 1188-1196.

(24) Z. Tian, J. Yu, C. Wu, C. Szymanski and J. McNeill, *Nanoscale*, 2010, 2, 1999-2011.

(25) X. Wang, Y. Guo, D. Li, H. Chen, R.-c. Sun, *Chem. Commun.*, 2012, 48, 5569-5571.

(26) K. Li, J. Pan, S.-S. Feng, A. W. Wu, K.-Y. Pu, Y. Liu and B. Liu, *Adv. Funct. Mater.*, 2009, 19, 3535-3542.

(27) G. He, N. Yan, J. Yang, H. Wang, L. Ding, S. Yin and Y. Fang, *Macromolecules*, 2011, 44, 4759-4766.

(28) Z. Gu, A. A. Aimetti, Q. Wang, T. T. Dang, Y. Zhang, O. Veiseh, H. Cheng, R.S. Langer

and D. G. Anderson, *ACS Nano*, 2013, 7(5), 4194-4201.

(29) B. Büyüktimkin, Q. Wang, P. Kiptoo, J. M. Stewart, C. Berkland and T. J. Siahaan, *Mol. Pharm.*, 2012, 9, 979-985.

(30) S.C.M. Fernandes, C. S. R. Frelre, A. J. D. Silvestre, J. Desbrieres, A. Gandini and C. P. Neto, *Ind. Eng. Chem. Res.*, 2010, 49(14), 6432-6438.

(31) E. Ardelean, R. Nicu(Parpalea), D. Asandei and E. Bobu, *Eur. J. Sci. Theol.*, 2009, 5(4), 67-75.

(32) Q. Hou, Y. Xu, W. Yang, M. Yuan, J. Peng and Y. Cao, *J. Mater. Chem.*, 2002, 12, 2887-2892.

(33) S. Liu, C. Zhong, J. Zhang, C. Duan, X. Wang and F. Huang, *Sci. China Chem.*, 2011, 54, 1745-1749.

(34) X. Wang, Y. Li and Y. Du, *J. Nanosci. Nanotechno.*, 2009, 9(12):6866-6875.

(35) C. Wu, C. Szymanski and J. McNeill, *Langmuir.*, 2006, 22, 2956-2960.

(36) C. Wu and J. McNeill, *Langmuir.*, 2008, 24, 5855-5861.

(37) C. Wu, Y. Zheng, C. Szymanski and J. McNeill, *J. Phys. Chem. C*, 2008, 112, 1772-1781.

(38) L. Sun, Y. Du, L. Fan, X. Chen and J. Yang, *Polymer* , 2006, 47, 1796-1804.

(39) L. Zhang, Y. Feng, H. Tian, M. Zhao, M. Khan and J. Guo, *J. Polym. Sci. Pol. Chem.*, 2013, 51, 3213-3226.

(40) X.-T. Dong, W.-T. Shi, H.-C. Dang, W.-Y. Bao, X.-L. Wang and Y.-Z. Wang, *Ind. Eng. Chem. Res.*, 2012, 51, 14037-14046.

(41) B. Arredondo, B. Romero, A. Gutiérrez-Llorente, A. I. Martínez, A. L. Álvarez, X. Quintana and J. M. Otón, *Solid State Electron*, 2011, 61, 46-52.

(42) J.-S. Yang and T. M. Swager, *J. Am. Chem. Soc.*, 1998, 120, 11864-11873.

Table of Contents

Self-assembled Conjugated Polymer/Carboxymethyl Chitosan Graft Poly (p-dioxanone) Nanomicelles and Their Functionalized Indicator Paper for Fast and Visual Detection of Banned Food Dye

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The fluorescent nanoparticles composed by self-assembled polysaccharide derivative and conjugated polymer show greatly improved sensitivity for the selective detection of a banned food dye in aqueous media.

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