Analytical Methods

PAPER

Detection of PFOS and copper(II) ions based on complexation induced fluorescence quenching of porphyrin molecules†

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Yifeng Wang and Haiyan Zhu*

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Finding a highly sensitive and specific, but technically simple method for the detection of the anthropogenic pollutant, perfluorooctane sulfonate (PFOS), is a worthwhile yet challenging undertaking. In this article, it was found that both PFOS and copper(II) ions can form complexes with cationic porphyrin molecules, resulting in the fluorescence quenching of these molecules. However, when the quantitative analysis of PFOS was performed, the interference of copper(II) ions could be eliminated by additionally introducing 0.1 mM of EDTA into the sample, and similarly, the influence of PFOS on the quantitative analysis of the copper(II) ions could be eliminated using powder activated carbon to remove them from the samples. Therefore, a fluorescence quenching method for the detection of PFOS and copper(II) ions in the same sample was developed, using cationic porphyrin as the optical probe, which has the advantages of technical simplicity, high selectivity and sensitivity.

Introduction

Perfluorooctane sulfonate (PFOS), categorized as a persistent organic pollutant (POP) in the 4th meeting of the conference of the parties at the Stockholm Convention in May 2009, is an anthropogenic pollutant characterized by a fully fluorinated hydrophobic carbon chain attached to the hydrophilic head of the sulfonate group.[1] Due to its thermal stability, high resistance to degradation and environmental breakdown, and its ability to repel both water and oil, PFOS has been very useful in recent years for a wide variety of applications and products: as an additive in fire-fighting foam, insecticide formulations and food packaging, as a fat and water repellent for paper, textile and leather treatments, and as a polymerization aid for the production of fluorinated polymers such as polyvinylidene fluoride.[2–4] Consequently, PFOS is now a ubiquitous environmental contaminant as it tends to persist in the environment, showing bioaccumulation in wildlife and humans.[5] Thus, the development of methods for PFOS detection in different matrices is of critical importance, considering the potentially significant adverse impact it may have on both human and wildlife health.

Many analytical methods for PFOS detection have been described in the literature which are mainly based on capillary zone electrophoresis (CZE),[6] gas chromatography-mass spectrometry (GC-MS),[7,8] liquid chromatography-mass spectrometry (LC-MS),[9–12] and liquid chromatography-tandem mass spectrometry (LC-MS-MS).[13–18] Of these methods, the CZE method with indirect UV detection has low sensitivity. Although the GC-MS method is sensitive, it requires a derivatization step prior to analysis. On the other hand, LC-MS and LC-MS-MS methods are specific and sensitive, and their use is becoming increasingly widespread. However, most of these methods require time-consuming sample preparation procedures, such as liquid–liquid extraction or solid-phase extraction, to remove coexisting substances from the samples. Furthermore, an additional common defect of these methods is that the apparatus and operating costs are too expensive for routine analysis. Thus it remains a worthwhile yet challenging undertaking to find a sensitive and specific, but technically simple PFOS detection method.

As outstanding examples of aromatic molecules, porphyrin derivatives which have large extinction coefficients in the visible-light region, predictable rigid structures, and prospective photochemical electron-transfer abilities, have been extensively used in the sensing of various analytes of interest[19,20] and in areas of designing novel energy conversion architectures.[21–23] In this contribution, it was found that both PFOS and copper(II) ions can form complexes with cationic porphyrin molecules, resulting in the fluorescence quenching of these molecules. However, when PFOS was being detected, additionally introducing 0.1 mM of EDTA into the sample could eliminate the interference of the copper(II) ions, and similarly, when the copper(II) ions were being detected, the interference of PFOS could be eliminated using 0.1 g powder activated carbon (PAC)

Key Laboratory of Eco-enviroments in the Three Gorges Reservoir Region (Ministry of Education), Chongqing Key Laboratory of Plant Ecology and Resources Research in Three Gorges Reservoir Region, School of Life Science, Southwest University, Chongqing 400715, P.R. China. E-mail: zhylzd@swu.edu.cn

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to remove them from the samples. Therefore, a fluorescence quenching method for the detection of PFOS and copper(II) ions in the same sample was developed, using cationic porphyrin as the optical probe, which has the advantages of technical simplicity, high selectivity and sensitivity.

Experimental

Apparatus

The fluorescence and absorption spectra were recorded with a Hitachi F-7000 fluorescence spectrophotometer (Tokyo, Japan) and a Shimadzu UV-2450 spectrophotometer (Tokyo, Japan), respectively. Atomic absorption measurements were performed on a TAS-990 atomic absorption spectrometer (AAS, Purkinje General Instrument Co., Ltd., Beijing). A Fangzhong pHS-3C digital pH meter (Chengdu, China) was used to measure the pH value of the solution and a vortex mixer QL-901 (Haimen, China) was used to blend the solution.

Reagents

α,β,γ,δ-Tetrakis [4-(trimethylammoniumyl) phenyl] porphyrin (TAPP), 5,10,15,20-tetrakis (1-methyl-4-pyridinio) porphyrin (TMPyP), and 5,10,15,20-tetrakis (4-sulfopheny) porphyrin tetrasodium hydrate ($TPPS_4$) were purchased from Sigma-Aldrich. Perfluorooctane sulfonate (PFOS) was obtained from Shanghai Tixian Co., Ltd (Shanghai, China). $Cu(AC)_2$ was purchased from Shanghai Shengong Genetech Co., Ltd (Shanghai, China). Powder activated carbon (PAC) was obtained from Sinopharm Chemical Regent Co., Ltd (Shanghai, China), and represents particles below 1.0 mm. Britton–Robinson buffer solution (BR, pH 5.5) was used to control the acidity of the system. All other routine reagents were of analytical grade and were used without further purification, and ultrapure water (18.2 ΩM) was used throughout.

Procedures

100 μL of 24 μM TAPP and 50 μL of BR buffer (pH 5.5) were first pipetted into a 1.5 ml vial. Subsequently, an appropriate volume of PFOS or copper(II) ion working solution or sample solution was added, diluted to 500 μL with Milli-Q purified water and vortex-mixed thoroughly. The mixture was placed for 5 minutes and then transferred for fluorescence and absorbance measurements.

Pretreatment of samples

The concentration of PFOS and copper(II) ions in some real samples was determined to further validate our present method. Water samples including river water, lake water and industrial waste water, were sampled from Jialingjiang River in Chongqing of China, Chongde Lake in the campus of Southwest University, and Maanxi Stream in the vicinity of several factories, respectively. They were collected in 250 ml pre-cleaned amber glass bottles. In order to evaluate the accuracy of the method, another quantity of the copper(II) ion standards was added to the water samples for recovery tests. Prior to the detection of copper(II) ions, the interference of PFOS was eliminated through the procedures reported by Yu and coworkers.[24] Briefly, 100 ml of the sample solution in the flask was mixed at 150 rpm in an orbital shaker with 0.1 g powder activated carbon for 2 h, and then successively filtrated with ordinary filter paper and a 0.22 μM microporous membrane. Subsequently, the clear water samples were used for the detection of copper(II) ions according to the general procedure without additional special treatment.

The fish samples used were *Navodon septentrionalis* purchased from the local market and prepared using the procedures reported by Viviana[25] with a slight modification. In brief, aliquots of homogenated fish muscles (3 g) were weighed in 10 ml glass tubes, and another quantity of PFOS standards was added to the fish samples. Similarly, to eliminate the interference of copper(II) ions, an additional 0.1 mM of EDTA was introduced into the sample solutions. The tubes were vortexed for 1 minute and ultrasonicated for 40 minutes to improve the diffusion of the standards and analytes. The samples were then centrifuged at 6000 rpm for 10 minutes, the supernatants were filtered with a 0.22 μM microporous membrane, and the clear solutions were analyzed according to the procedures described above.

Results and discussion

Spectral characteristics

As shown in Fig. 1, the cationic porphyrin molecule TAPP exhibits strong fluorescence emissions at 645.0 nm when excited at 412.0 nm. However, obvious fluorescence quenching of TAPP is observed in the presence of PFOS (Fig. 1a) or copper(II) ions (Fig. 1b), and the extent of this fluorescence quenching increases gradually as the concentration of PFOS and copper(II) ions varies in the range of 0.05–20 μM and 0.08–30 μM, respectively. It is worth mentioning that when using porphyrins as the spectrophotometric reagents for the determination of metal ions, a certain disadvantage is that the incorporation reaction of the metal ion in the porphyrin ring is very slow and the rate of metalloporphyrin formation is several orders of magnitude lower than for common ligands.[26] However, the occurrence of fluorescence quenching of TAPP in the presence of copper(II) ions is prompt, indicating that the copper(II) ions are being chelated very quickly by the TAPP moieties. Similar phenomena were observed in the interaction between another cationic porphyrin molecule, TMPyP, and PFOS (Fig. S1†), and the same obvious fluorescence quenching was observed in the presence of PFOS. However, the fluorescence of the anionic porphyrin molecule, $TPPS_4$, was almost not quenched with the addition of the negatively charged PFOS (Fig. S2†), indicating that electrostatic interactions play an essential role in the complex formation between porphyrin molecules and PFOS.

Furthermore, the formation of complexes between the cationic porphyrin molecule TAPP and the negatively charged PFOS was supported by UV-visible spectra. As shown in Fig. 2a, the TAPP spectrum features an intense Soret band at 411.0 nm together with weak Q bands, and with the addition of PFOS, the intensity of both the original Soret band and Q band decrease

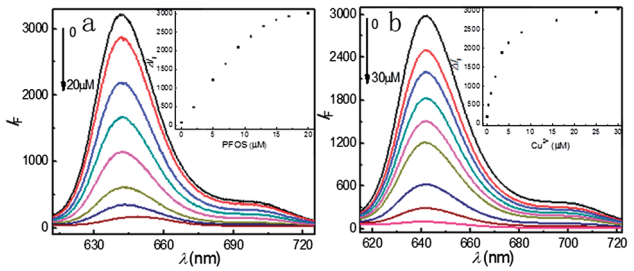

Fig. 1 Fluorescence spectra of TAPP in the absence and presence of different concentrations of PFOS (a) and copper(II) ions (b). The insets show that the fluorescence quenching (ΔI_F) at 645.0 nm varies with the increasing concentration of PFOS (a) and copper(II) ions (b). Concentration: TAPP, 4.8 μM; pH 5.5. λ_{ex}, 412.0 nm.

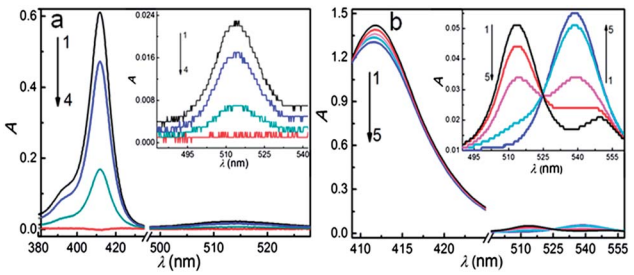

Fig. 2 Absorbance spectra of TAPP in the absence and presence of different concentrations of PFOS (a) and copper(II) ions (b). The insets show the detailed changes in the absorbance spectra in the long wavelength regions. Concentration: TAPP, 4.8 μM in (a) and 9.6 μM in (b); PFOS from curve 1 to 4, 0.0, 2.0, 12.0, 20.0 μM; copper(II) ions from curve 1 to 5, 0.0, 0.8, 4.0, 10.0, 15.0 μM; pH 5.5.

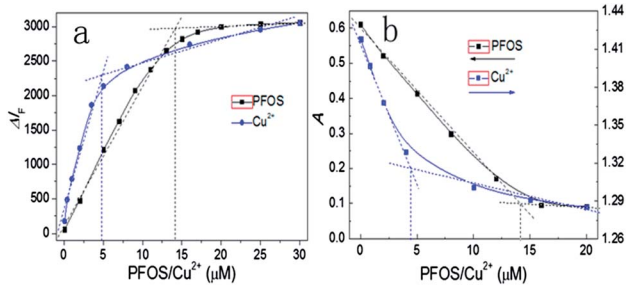

Fig. 3 The molar ratio of binding between PFOS or copper(II) ions and the TAPP molecule. The fluorescence data were obtained at 645.0 nm and the absorption data at 411.0 nm. Concentration, TAPP, 4.8 μM; pH 5.5.

gradually. Similarly, when copper(II) ions were introduced into the system, the intensity of the original Soret band at 411.0 nm and Q band at 516.0 nm gradually decrease, and the Q band at 540.0 nm increases. This is simultaneously accompanied by the appearance of an obvious isosbestic point at 525.0 nm, which firmly confirms the incorporation of copper(II) ions into the porphyrin ring and the formation of the metal chelate.

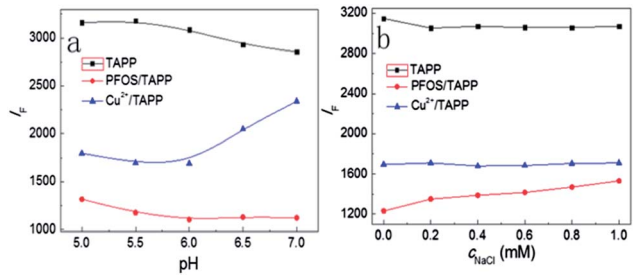

Fig. 4 The effect of pH (a) and ionic strength (b) on the fluorescence intensity of TAPP in the absence and presence of PFOS and copper(II) ions. All data were obtained at 645.0 nm. Concentration: TAPP, 4.8 μM; PFOS, 8.0 μM; copper(II) ion, 4.0 μM.

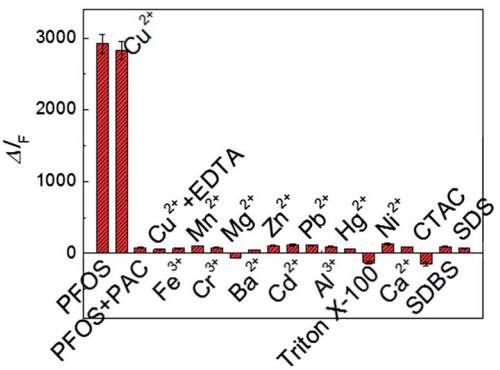

Fig. 5 Fluorescence response of the TAPP molecule at 645.0 nm towards PFOS, various metal ions and common surfactants. Concentration, TAPP, 4.8 μM; PFOS, 16.0 μM; copper(II) ion, 8.0 μM; SDBS and SDS, 100 μM; other species, 80 μM. pH 5.5.

The molar ratio of binding between PFOS or copper(II) ions and the TAPP molecule

Fig. 3a and b show the binding molar ratio of PFOS and copper(II) ions with the cationic porphyrin molecule TAPP. When the concentration of PFOS is three times that of the TAPP molecule, both the intensity of the fluorescence quenching and the absorbance do not show significant changes, as shown by the black curve in Fig. 3a and b, illustrating that the complexes between the cationic porphyrin molecule TAPP and the negatively charged PFOS are formed at a molar ratio of 1 : 3. Similarly, the metal chelate between the TAPP molecule and the copper(II) ions is formed at a molar ratio of 1 : 1 as shown by the blue curve in Fig. 3a and b, which is in agreement with the fact that almost all metals form 1 : 1 complexes with porphyrin molecules, except for Na, K and Li.[26]

Optimal conditions

The impact of the pH of the medium on the fluorescence of TAPP in the presence of PFOS or copper(II) ions was investigated. As Fig. 4a shows, the fluorescence intensities of the TAPP molecules alone exhibit a decreasing tendency in the pH range of 5.0–7.0, and in the presence of PFOS they display obvious fluorescence quenching and remain relatively stable. However,

Table 1 Results for the determination of PFOS in fish and stream water samples ($n = 3$)[ab]

Sample	Added (μM)	Found (μM)	Recovery (%)	Mean ± SD
Fish	0.25	0.26, 0.24, 0.23	92.0–104.0	0.24 ± 0.02
	2.50	2.36, 2.67, 2.74	94.4–109.6	2.59 ± 0.20
Stream water	0.13	0.14, 0.13, 0.12	92.3–107.7	0.13 ± 0.01
	0.75	0.68, 0.82, 0.83	90.7–110.7	0.78 ± 0.08
	4.50	4.23, 4.62, 4.78	94.0–106.2	4.54 ± 0.28

[a] Mean of three determinations. [b] SD, standard deviation. Concentration: TAPP, 4.8 μM; pH 5.5.

the fluorescence intensities of TAPP solutions containing copper(II) ions present different traits. That is, when the pH-value is less than 6, obvious fluorescence quenching was observed as a result of the incorporation of copper(II) ions into the porphyrin ring, and the extent of fluorescence quenching lessens distinctly with a further increase in pH, as a result of copper(II) ion hydrolysis. A pH 5.5 buffer medium was therefore selected for the determination of both PFOS and copper(II) ions.

Ionic strength was investigated as it can supply reference information on the binding mechanism of the two interacting components. As shown in Fig. 4b, the fluorescence intensity of the TAPP solution containing copper(II) ions is immune to variations of ionic strength, indirectly highlighting the coordination interaction between the TAPP molecule and the copper(II) ions. The complex formation between the TAPP and PFOS molecules is triggered by electrostatic attractions as described above, however, the extent of the fluorescence quenching of the TAPP solution containing PFOS exhibits a slow decrease with increasing ionic strength, indicating that hydrophobic interactions play an important auxiliary role in the binding mechanism of the two components.

Validation of analytical method

Under the optimal conditions, the quenched fluorescence intensity (ΔI_F) for PFOS at 645.0 nm can be fitted to the equation of $\Delta I = 115.9 + 205.6c$ (PFOS, 10^{-6} M) over the range of 0.05 to 16.0 μM with a detection limit (3σ) of 8.0 nM ($r = 0.9917$), and similarly for the copper(II) ions $\Delta I = 324.9 + 395.7c$ (Cu^{2+}, 10^{-6} M) over the range of 0.08 to 5.0 μM with a detection limit (3σ) of 10.0 nM ($r = 0.9916$).

The specificity of our analytical approach towards both PFOS and copper(II) ions against other metal ions and common surfactants, including negatively charged surfactants such as sodium dodecyl sulphonate (SDS) and sodium dodecyl benzene sulphonate (SDBS), was also studied. As shown in Fig. 5, although the concentration of other species used was 5–6 times that of PFOS, the fluorescence quenching of TAPP towards PFOS or copper(II) ions was 21–60 times more than those towards them. It is worth mentioning that both PFOS and copper(II) ions can result in the fluorescence quenching of TAPP molecules due to their complex formation and therefore they cause mutual interference in the process of their quantitative analysis. However, it was found that when PFOS was being determined, additionally introducing 0.1 mM of EDTA into the sample could eliminate the interference of copper(II) ions, and similarly, when copper(II) ions were being determined, the interference of PFOS could be eliminated using 0.1 g PAC to remove them from the samples. Therefore, we can detect the concentration of PFOS and copper(II) ions in the same sample based on the complexation induced fluorescence quenching of the TAPP molecules.

This point was further proven by the detection of PFOS and copper(II) ions in real samples. The proposed method for the detection of PFOS works quite well as shown in Table 1, with recoveries in the range of 90.7–110.7. Similarly, the results for the detection of copper(II) ions in real water samples are shown in Table 2, with recoveries in the range of 91.6–114.8, which are consistent with the results determined by atomic absorption spectrometry (AAS). These results indicate the high selectivity of this method against competing species, and therefore, the present method may be used for the detection of PFOS and copper(II) ions as a way to monitor the quality of water and the safety of aquatic products.

Conclusions

In summary, the complex of the positively charged porphyrin molecule TAPP and the negatively charged PFOS was formed at a molar ratio of 1 : 3. Electrostatic interactions play an essential

Table 2 Results for the determination of copper(II) ions in several water samples using the proposed method and AAS ($n = 3$)

	Copper(II) ion (μM)				
Sample	Proposed mean[a] + SD[b]	AAS mean[a] + SD[b]	Added	Found mean[a] + SD[b]	Recovery (%)
Waste water	2.23 ± 0.11	2.21 ± 0.08	4.50	6.70 ± 0.35	91.6–107.1
River water	0.28 ± 0.01	0.27 ± 0.01	0.75	1.06 ± 0.05	97.3–110.7
Lake water	1.46 ± 0.07	1.44 ± 0.04	2.50	4.12 ± 0.21	98.0–114.8

[a] Mean of three determinations. [b] SD, standard deviation. Concentration: TAPP, 4.8 μM; pH 5.5.

role in the binding mechanism of these two components and hydrophobic interactions play an important auxiliary role. However, the complex formed by the porphyrin molecule TAPP and the copper(II) ions was formed at a molar ratio of 1 : 1, and the binding mechanism involved requires the incorporation of copper(II) ions into the porphyrin ring and the formation of a metal chelate. On the basis of this complexation induced fluorescence quenching of the cationic porphyrin TAPP molecules, a fluorescence quenching method for the detection of PFOS and copper(II) ions in the same sample was developed. This method has the advantages of technical simplicity, high selectivity and sensitivity.

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