Sensing performance enhancement *via* chelating effect: A novel fluorescent film chemosensor for copper ions

Liping Ding, Xin'ai Cui, Yani Han, Fengting Lü, Yu Fang *

Key Laboratory for Macromolecular Science of Shaanxi Province, School of Chemistry and Materials Science, Shaanxi Normal University, Xi'an 710062, PR China

Abstract

A novel fluorescent Cu(II) sensing film was developed by covalently attaching dansyl (5-dimethylamino-1-naphthalenesulfonyl) moieties onto glass slide surface based on self-assembled monolayer technique. Fluorescence measurements showed the emission of the dansyl-functionalized film was selectively quenched by Cu(II) ions and slightly influenced by the presence of other similar divalent metal ions (Pb(II), Zn(II), Ni(II), Co(II), etc.). In addition, the sensitivity of the film towards Cu(II) was increased as expected since more binding sites, the diethylenetriamine moieties, were incorporated into the spacer. It was also found that the presence of organic anions like acetate, citrate, and tartrate, etc. could enhance the quenching efficiency of Cu(II) compared with inorganic anions such as chloride, sulfate, and nitrate. This observation was explained by considering the spacer screening effect as discovered previously. Moreover, the response of the film toward Cu(II) is fully reversible which makes it attractive for sensing application.
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1. Introduction

The past few decades have witnessed an intense investigation on the design and preparation of fluorescent chemosensors for the purpose of detecting transition metal ions in aqueous solutions [1–3], which is of significant importance for environmental sciences, medical diagnostics, and food technology. A basic ionic fluorescent sensor is usually composed of two intramolecularly linked functional units: an ionophore as a recognition part that is usually a metal-chelating structure designed to selectively bind the target ion and a fluorophore as a readout part that signals the binding event sensitively [4].

Among the relevant transition metal ions, the detection of copper ions has attracted particular attention as it is a significant metal pollutant and an essential trace element in biological systems. A great number of fluorescent chemosensors for copper ions which perform excellent sensing properties have been successfully designed in the past few years [4–7]. However, most of

these fluorescent chemosensors until now are designed to be used only in solution, which is hard to realize repetitive utilization. In this regard, solid surface-supported sensors offer several advantages over solution-used systems as they can be used repeatedly, easy to be made into devices and have no or less consumption of or contamination to the analyte solution. In recent years, there has been an expanding interest in the use of solid surface-supported fluorescent chemosensors for detecting copper ions. For instance, through surface modification, both Cu(II) binding ligands and fluorophores were immobilized on organic polymer nanoparticles [8] and on inorganic silica nanoparticles to realize sensing copper ions [9–11].

Compared to nanoparticles, planar substrates are much easier to handle in the process of utilization and more likely to be made into devices, which endow film-based fluorescent sensors to become increasingly prevalent. Leblanc et al. developed some thin-film fluorescent sensors for copper ions by employing poly(ethylene glycol) (PEG) films [12] and Langmuir–Blodgett (LB) films [13] as substrates, and then attaching fluorophore-connected ion-binding moieties onto the substrates. These film sensors exhibited excellent reversibility which is an important property in terms of practicability. However, PEG film and LB

* Corresponding author. Tel.: +86 29 85310081; fax: +86 29 85310079.
 E-mail address: yfang@snnu.edu.cn (Y. Fang).

film usually suffer from poor stability for being easily affected by temperature, pressure, pH, etc. due to their non-covalent attachment on solid surfaces. On the contrary, fluorescent films obtained through immobilizing fluorophores onto the functional end groups of self-assembled monolayers (SAMs) on solid substrates enjoy higher stability because of the chemical linkages throughout the whole system [14]. In this case, metal-binding moieties are usually covalently linked with fluorophores which are immobilized onto solid substrates through spacers that usually connect the fluorophore and the substrate, and thus become part of the spacer. The work of Reinhoudt and coworkers has shown how this strategy could be utilized for achieving fluorescent film sensors for metal ions [15–17]. By attaching various fluorophores like pyrene, dansyl (5-dimethylamino-1-naphthalenesulfonyl), coumarin or lissamine as well as binding groups (i.e., calix[4]arene, diamine moieties, aryl-urea, and alkyl-amide, etc.) onto SAM films on glass or quartz slide surfaces, they obtained some fluorescent film sensors selectively responding to sodium ions [15], plumbum ions [16], and other metal ions [17].

In comparison, the report on the film fluorescent sensors for copper ions through this approach is relatively rare. Our group has been especially interested in developing fluorophore-functionalized SAM films as well as investigating their photophysical properties and sensing abilities [18–22]. Very recently, we reported a selective pyrene-functionalized SAM film sensor for copper acetate based on the screening effect of the long, flexible spacer containing ethylenediamine moieties [23]. Unfortunately, the sensitivity of this system is low.

In the present study, we were trying to get a more sensitive SAM film sensor for Cu(II). Prompted by the idea that polyamines have a generally high affinity for transition metal ions [24] and Cu(II) is a strong quencher to various fluorophores [25,26], diethylenetriamine (DETA) was employed as a Cu(II) binding structure and introduced onto an epoxy-terminated SAM film through surface reaction between its dansyl-functionalized derivative, *N*-dansyldiethylenetriamine (DDETA), and epoxy groups. The presence of more binding sites (amine groups) that could allow to achieve fluorescence quenching upon complexation with Cu(II) may resulted in a higher sensitivity.

2. Experimental

2.1. Chemicals

Dansyl chloride (99%), 3-glycidoxypropyltrimethoxysilane (GPTS, 97%), and diethylenetriamine were all purchased from Acros and used as received. Ethylenediamine was first washed with solid KOH and then distilled before use. Water used throughout was de-ionized and then double distilled. The solutions of metal ions were prepared from $Cu(NO_3)_2 \cdot 3H_2O$, $Pb(NO_3)_2$, $Zn(NO_3)_2 \cdot 6H_2O$, $Co(NO_3)_2 \cdot 6H_2O$, $Ni(NO_3)_2 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, $CuSO_4 \cdot 5H_2O$, $Cu(Ac)_2 \cdot H_2O$, $Pb(Ac)_2 \cdot 3H_2O$, $Zn(Ac)_2 \cdot 2H_2O$, $Ni(Ac)_2 \cdot 4H_2O$ and $Co(Ac)_2 \cdot 3H_2O$, respectively, and were dissolved in double distilled water. All other reagents were of at least analytical grade. Glass

slides (∼0.9 cm × 2.5 cm) used in the experiment were obtained by cutting microscope slides (25.4 mm × 76.2 mm, 1–1.2 mm thick) into the desirable pieces.

2.2. Measurements

All fluorescence measurements were conducted on a time-correlated single photon counting fluorescence spectrometer (Edinburgh Instruments FLS 920) which is equipped with a 450 W Xenon arc lamp for steady-state fluorescence measurement. A front face method was adopted throughout the fluorescence measurements. X-ray photoelectron spectroscopy (XPS) measurements were carried out on an ESCA Phi5400 (Perkin-Elmer) photoelectron spectrometer using a monochromatic Mg Kα X-ray source. The ^1H NMR spectra of the samples were obtained on a Bruker AV 300 NMR spectrometer. FTIR spectra were measured on the samples in KBr pellets by using a Bruker Equinox 55 Fourier transfer infrared spectrometer with a resolution of $1 cm^{-1}$ and scanned wavenumber ranging from 4000 to $400 cm^{-1}$. UV–vis absorption spectroscopy measurements were carried out on a Perkin-Elmer Lambda 950 UV–vis spectrometer to monitor the absorption of both dansyl-functionalized film and dansyl solution at 350 nm. The contact angles with water for the glass slide surfaces were measured by using a home-made JY-82 contact angle goniometer.

2.3. Preparation of dansyl derivative

The amino derivative of the sensing element, dansyl, was obtained by reacting DETA with dansyl chloride according to a method previously reported by others [27]. The product, DDETA, a kind of light-green crystals, was characterized, and the results are listed below: IR (KBr): $3339 cm^{-1}$, $2942 cm^{-1}$, $2840 cm^{-1}$, $1577 cm^{-1}$, $1317 cm^{-1}$, $1146 cm^{-1}$, $1089 cm^{-1}$; ^1H NMR (300 MHz, $CDCl_3$): 2.35 (t, 2H), 2.56–2.58 (m, 4H), 2.88 (m, 6H, $NH(CH_3)_2$), 2.92(t, 2H), 7.20 (d, 1H), 7.52 (dd, 1H), 7.57 (dd, 1H), 8.25 (d, 1H), 8.31 (d, 1H), 8.53 (d, 1H).

2.4. Fabrication of dansyl-functionalized film

The dansyl-functionalized SAM film was prepared in the following steps: (i) The glass slide substrates were first rinsed with ethanol and distilled water and then activated by immersion in hot "piranha solution" (98% H_2SO_4/30% H_2O_2, 70:30 (v/v); 98 °C, 1 h). After that, they were rinsed thoroughly with double-distilled water and air dried (Caution: piranha solution is a very strong oxidant and reacts violently with many organic materials, and so must be handed with extreme care.). (ii) The epoxy-terminated SAM which can react with various amino-derivatized molecules [28] was obtained by immediately immersing the freshly activated glass slides into a toluene solution of GPTS (0.6% (v/v)) at 50 °C, containing a trace amount of water, for at least 12 h. Then the silanized slides were washed successively with fresh toluene and dichloromethane (CH_2Cl_2) for several times, to remove any physically adsorbed silanes. (iii) After the treatment, the epoxy-terminated slides were immersed into a CH_2Cl_2 solution of DDETA at room temperature for
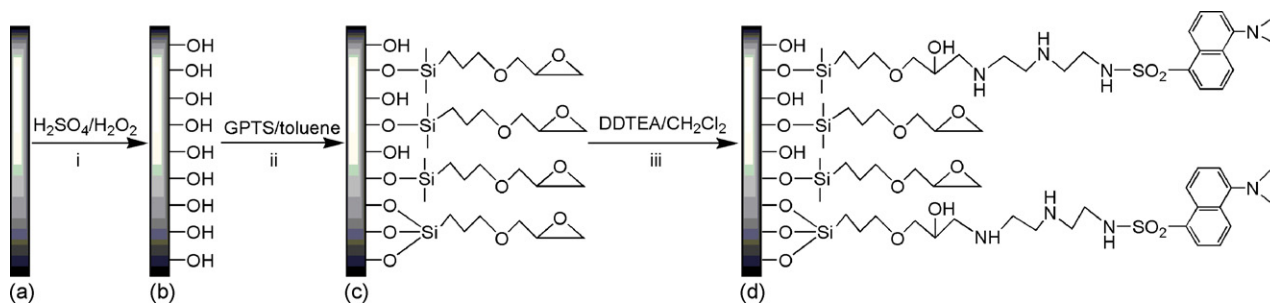
Fig. 1. Schematics for the synthesis of dansyl-functionlized film. (i) 98% H_2SO_4/30% H_2O_2 (v/v: 70/30), 98 °C, 1 h; (ii) GPTS, toluene, 0.6%, v/v, 50 °C, 12 h; and (iii) DDTEA, CH_2Cl_2, r.t., 48 h. (a) The original clean glass slide, (b) activated glass slide, (c) epoxy-terminated SAM-modified glass slide, and (d) dansyl-functionalized glass slide.

48 h. Therefore, through surface reaction between epoxy groups and aminos, the readout units, dansyl moieties, as well as the target ion-binding sites, diethylenetriamine functionalities, can be expected to be attached onto the epoxy-terminated SAM-modified slides. Finally, the dansyl-functionalized slides were washed with plenty of fresh CH_2Cl_2 and then rinsed with acetone and water successively. The whole coupling process is schematically shown in Fig. 1.

3. Results and discussion

3.1. Characterization of the glass slide surfaces

Contact angle measurement provides a convenient way to characterize surface polarity and then reflects surface functionality [28]. The advancing contact angles with water for the glass slides at different coupling stages are shown in Table 1. It can be seen that activation (step i) made the contact angle decrease from 10.8° to 2.5°, indicating the activated glass slide surface becomes more hydrophilic which should come from the existence of more surface hydroxyl groups. The contact angles of epoxy-terminated glass slide surface increased to 27.5° after treatment with the silane coupling agents (step ii), in accordance with the presence of more hydrophobic GPTS groups. Finally, the attachment of DDETA moieties onto the surface (step iii) further increased the contact angle to an even higher value, 31.5°. These results are consistent with the expectation from the chemical compositions of the glass slide surfaces as shown in Fig. 1.

The glass slide surfaces in different coupling steps were also examined by XPS technique to provide detailed information on the elemental composition of surface layers. Fig. 2 shows the representative XPS spectra of the three slide surfaces with differ-

ent structures. As for surface *b*, the spectrum was dominated by the peaks of O_{1s} (531.2 eV), Si_{2s} (153.4 eV) and Si_{2p} (103.3 eV) due to the silicon oxide substrate. The intensity of peak C_{1s} (284.6 eV) of surface c dramatically increased and was much larger than that shown in surface *b*, which apparently comes from the introduction of organosilanes onto the surface. In addition to the further increase in the intensity of peak of C_{1s}, the spectrum of surface d exhibits peaks of N_{1s} (400.8 eV) and S_{2p} (170.05 eV)

Table 1
Advancing water contact angles (θ) for (a) original clean glass slide, (b) activated glass slide, (c) epoxy-terminated SAM-modified glass slide, and (d) dansyl-functionalized glass slide

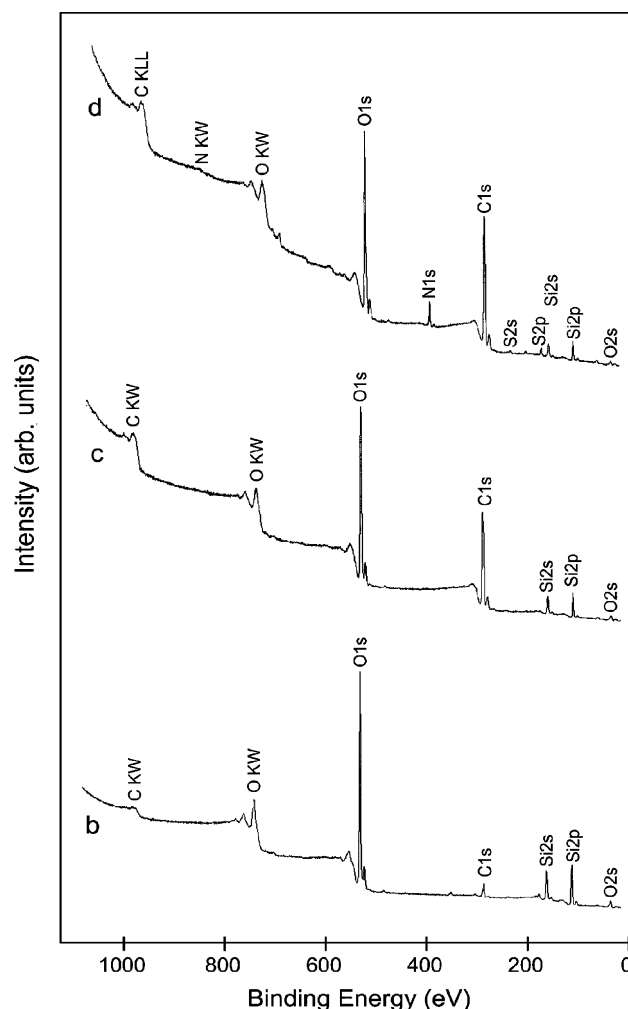| Slides	θ (°)
(a)	10.8 ± 2.0
(b)	2.5 ± 0.5
(c)	27.5 ± 1.0
(d)	31.5 ± 2.0

Fig. 2. XPS spectra of (b) the activated glass slide, (c) the epoxy-terminated SAM-modified glass slide, and (d) the dansyl-functionalized glass slide.

after modification with DDETA. Moreover, the relative surface percentages of the two elements is 4.84% and 1.26%, which is just in agreement with the ratio of 4:1 for N and S elements in DDETA species. Consequently, these results further confirmed that the fluorophores and the ion-binding sites are successfully attached on the glass slide surface.

The surface coverage of dansyl was evaluated by measuring the UV–vis spectroscopy of the dansyl-functionalized film. According to the Beer–Lambert law ($\rho = A\varepsilon^{-1}$), the absorption at 350 nm of the film in chloroform was used to estimate the surface density of dansyl moieties (ρ), using the absorption coefficient of dansyl chloride (0.1 mmol l^{-1}) in chloroform at 350 nm ($\varepsilon = 3809\,M^{-1}\,cm^{-1}$) which was determined through UV-vis measurements. The resulting density is 3.4 dansyl groups per 100 Å^2. The theoretical value (or the maximum value) of the density was 5.8 molecules per 100 Å^2 [23], and thereby about 58% of the epoxy groups had reacted with DDETA.

3.2. Steady-state fluorescence of the dansyl-functionalized film

The steady-state fluorescence excitation and emission spectra of the dansyl-functionalized film in water were recorded by varying the analysis and the excitation wavelengths and are depicted in Fig. 3, which exhibit better spectral properties than those dansyl-functionalized PEG films or LB films investigated in other reports [12,13]. Similar to our previous studies [22], the profiles of the excitation spectra and those of the emission spectra of the film are independent of the analysis wavelengths or the excitation wavelengths adopted, indicating, according to Karsh' rule, that dansyl moieties are immobilized on the substrate surface in a uniform state [29]. In addition, a similarly large Stokes shift, 175 nm, is also observed in this case, as the excitation maximum and the emission maximum are at 343 nm and 518 nm, respectively, which means that the dansyl moieties in this film is less likely to self-quench [13]. Throughout the experiments, a longer wavelength, 350 nm, was chosen at the excitation wavelength as to reduce the interference of absorbance of glass
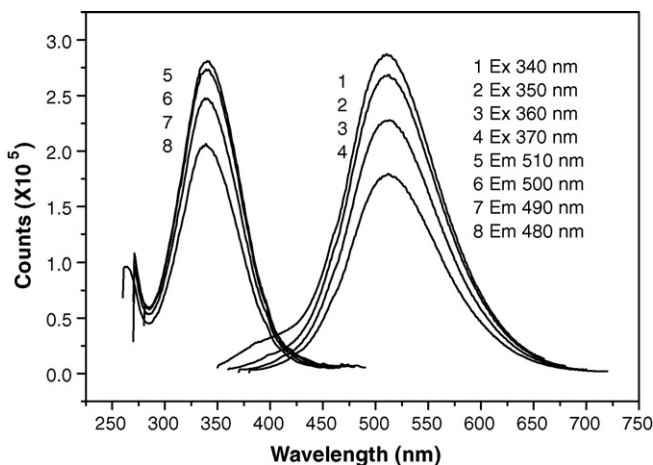
substrate and the emission intensity of the film at 518 nm was investigated.

Leaking of the fluorophore moieties from the film was investigated by monitoring the fluorescence emission of the remained solvent after immersion of the film in the solvent for 24 h. Satisfactorily, no noticeable dansyl emission from the solvent was detected, indicating that the leaking problem is negligible in the present study and the sensing molecules had been chemically attached to the substrate surface.

3.3. Sensing properties of the dansyl-functionalized film to copper ions

The influence of the addition of an aqueous $Cu(NO_3)_2$ solution on the fluorescence emission of the dansyl-functionalized film is depicted in Fig. 4 (inset). As increasing the concentration of $Cu(NO_3)_2$, the fluorescence intensity of the film decreases dramatically. The responses of the film against the nitrate salts of other divalent transition metal ions such as Pb(II), Zn(II), Ni(II), and Co(II) were also investigated. Plots of I_0/I against the concentration of the nitrates are shown in Fig. 4, where I_0 and I represent the fluorescence intensity of the film analyzed at 518 nm in the absence and presence of metal ions, respectively. It is evident from the figure that compared with other metal ions the fluorescence quenching efficiency was much higher for Cu(II) ion. This is not a very surprising result because Cu(II) is a well known highly efficient fluorescence quencher due to its paramagnetic property *via* electron or energy transfer [23,30,31]. Furthermore, the stronger affinity of the film to Cu(II) than to other ions due to the chelating effect of the polyamine in the spacer could also contribute to this selectivity [6,10].

It is interesting to note that the emission of the present film is more sensitive to the presence of Cu(II) than that of the film reported earlier where ethylenediamine was employed as the subunit in the spacer [23]. The better performance of the present
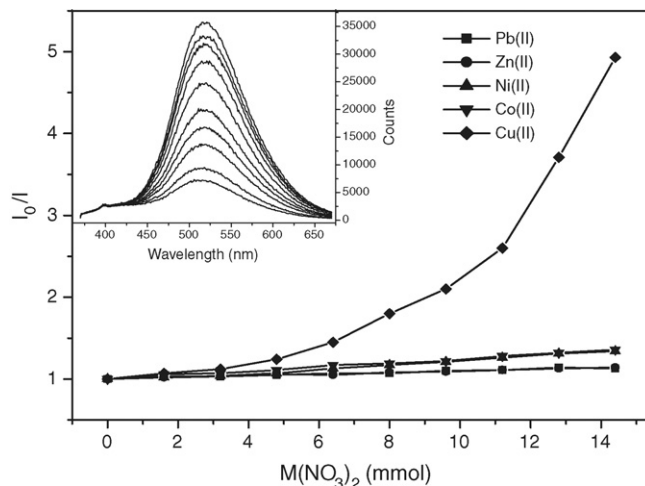

Fig. 3. Steady-state excitation and emission spectra of the dansyl-functionalized film in water.

Fig. 4. Plots of the ratio, I_0/I, measured at 518 nm, for the dansyl-functionalized film against the concentration of metal nitrates ($\lambda_{ex} = 350$ nm). Inset: fluorescent spectra of the dansyl-functionalized film upon the addition of $Cu(NO_3)_2$ in water ($\lambda_{ex} = 350$ nm).

film can be attributed to the stronger binding ability of diethylenetriamine. It was found that 82.5% of the initial fluorescence intensity was quenched for the present film and only 16.7% was quenched for the previous film when both films were tested in an aqueous 14.4 mM $Cu(NO_3)_2$ solution.

In order to investigate the dependence of metal-ion sensitivity on the structure of the chelating group in the spacer of the fluorescent film, a comparison experiment was conducted. The response to $Cu(NO_3)_2$ of a controlled film which was also a dansyl-functionalized film but with ethyldiamine as binding sites was examined (the preparation of this film was reported elsewhere [22]). As expected, the sensitivity of the controlled film is far lower than the present film as the addition of 8 mM $Cu(NO_3)_2$ produced a decrease of only 13% of the initial intensity of the fluorescence emission from the former but 45% of the later, proving again more nitrogen binding sites present in the spacer enhanced the sensitivity of the present film toward Cu(II) [32].

It is worthwhile to note that a blue shift of the maximum wavelength of the film occurred when the concentration of the $Cu(NO_3)_2$ increased. That is the maximum wavelength shifted from 518 nm to a shorter wavelength, 513 nm. However, the increase in the amount of the other four nitrates ($Zn(NO_3)_2$, $Pb(NO_3)_2$, $Ni(NO_3)_2$, and $Co(NO_3)_2$) caused no variation to the position of the maximum wavelength of the film, which remained at 518 nm over the whole range of concentration tested (cf. Table 2). This result was quite contrary to the report where the presence of Zn(II) rather than Cu(II) caused a blue shift to the fluorescence of dansylated polyamine detected in solution phase [33]. The reason for this is still unclear and needs further investigation. But one possibility could be that a complex was formed between copper ions and the diethylenetriamine ligands in the spacer which provided chances for energy transfer from dansyl to metal ions.

3.4. Influences of acetate ions to the sensing properties of the dansyl-functioanlized film to metal ions

The response of the fluorescent film toward other copper salts like $CuCl_2$ and $CuSO_4$ is similar to that observed for $Cu(NO_3)_2$.

Table 2
Emission maximum of the dansyl-functionalized film in the presence of various divalent metal ions

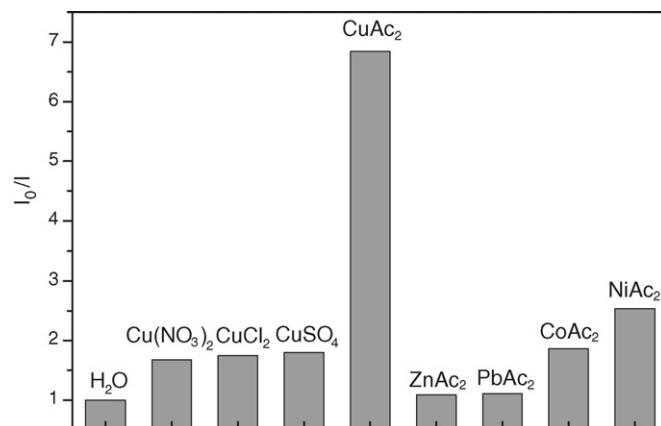| Metal salts	λ_{max} (nm)
$Pb(NO_3)_2$	518
$Zn(NO_3)_2$	518
$Co(NO_3)_2$	518
$Ni(NO_3)_2$	518
$Cu(NO_3)_2$	513
$CuCl_2$	512
$Pb(Ac)_2$	518
$Zn(Ac)_2$	511
$Co(Ac)_2$	513
$Ni(Ac)_2$	512
$Cu(Ac)_2$	511
$CuSO_4$	513

Fig. 5. Quenching effect to the dansyl-functionalized film induced by metal acetates and different copper salts at concentration of 8 mM (except for copper acetate at 7.2 mM) ($\lambda_{ex} = 350$ nm, $\lambda_{em} = 518$ nm).

In each case, a dramatic fluorescence decrease was observed, and an equivalent amount of quenching was obtained for all these three copper salts at the same concentration as shown in Fig. 5. However, as discovered in our previous work [23], the presence of $Cu(Ac)_2$ resulted in a much larger quenching effect (Fig. 5). The quenching effect of $Cu(Ac)_2$ is nearly four times as great as those of inorganic copper salts. The possible reason for this was discussed in the later part of this section. Interestingly, compared to the results of our previous study [23], the present film also showed a higher sensitivity toward $Cu(Ac)_2$, since the fluorescence intensities of the present one and the previous one were quenched to 14.6% and 52.9% of their respective initial values in the presence of 7.2 mM of $Cu(Ac)_2$. A similar result was found in the case of the controlled dansyl-functionalized film which showed a smaller response to copper acetate (7.2 mM of $Cu(Ac)_2$ induced a quenching to 51.8% of its initial fluorescence intensity) compared to the present film. Therefore, the importance of introduction of target-binding sites into the film sensor for improving sensitivity is quite obvious.

Surprisingly, an increase in the quenching effects is also observed for Co(II) and Ni(II) ions when their acetate salts were added in the solution tested (Fig. 5). A similar phenomenon was previously reported by Prodi et al. [33] who studied a series of dansylated amino acids and polyamines as copper sensors used in solution phase [26,34,35]. It was proposed that energy-transfer from dansyl to Co(II) and Ni(II) should be attributed to the quenching. It is beyond the scope of the present work to discuss in detail the mechanism of the quenching of cobalt and nickel ions to the present film. Compared with the other four divalent metal acetates, the film still has the largest response to copper ions, although its selectivity towards Cu(II) decreased in this case.

A similar blue shift of the fluorescence spectra not only occurred for the film in the presence of copper salts ($CuCl_2$, $CuSO_4$, $Cu(Ac)_2$) but also in the presence of $Co(Ac)_2$, $Ni(Ac)_2$ and $Zn(Ac)_2$ (Table 2). This surprising result indicated that a complexation with the functional subunits in the spacer also happened in the case of $Co(Ac)_2$, $Ni(Ac)_2$ and $Zn(Ac)_2$. Compared to the results observed for $Co(NO_3)_2$ and $Ni(NO_3)_2$, acetate

ions might make contribution to the quenching effect of Co(II) and Ni(II) to the fluorescence of the film as observed for copper acetate. However, even a complexation may form between Zn(II) and the spacer, the presence of $Zn(Ac)_2$ showed slight quenching effect to the film, which may be ascribed to its d^{10} structure.

The influence of acetate ions was caused as explained in our previous study by the spacer screening effect [23]. It was supposed that a long, flexible and hydrophobic spacer that is linked with a polycyclic aromatic hydrocarbon tends to bend over to form an intermediate phase between the bulk phase and the solid substrate when it connects with polar bulk solution, where the dansyl moieties may be buried to enjoy a more hydrophobic environment [22,36]. Moreover, the emission maximum of the surface-bound dansyl moieties at 518 nm showed a dramatic blue shift as compared to its parent dansylated amine, DDETA which had an emission maximum at 560 nm, suggesting again that the surface-bound fluorophore experienced a less polar environment. Therefore, the screening effect of the long, flexible hydrophobic spacer also existed in the present study as discovered previously [23]. Consequently, the quenching effect of organic salts like $Cu(Ac)_2$, $Co(Ac)_2$, and $Ni(Ac)_2$ becomes larger than their corresponding inorganic salts. But it was worth to mention that the higher sensitivity of the present film toward $Cu(Ac)_2$ was a combined result of chelating effect and spacer screening effect.

In order to confirm the influence of organic anions on the quenching effect of copper ion to the sensing film, a more detailed experiment was conducted. Firstly, 0.2 M aqueous $Cu(NO_3)_2$ was proportionally added into the cell containing 2.5 ml of water and the film until the final concentration is 6.4 mM. As shown in Fig. 6, the intensity ratio, I_0/I, gradually increased with the addition of $Cu(NO_3)_2$ in the testing aqueous solution. Then, the concentration of $Cu(NO_3)_2$ was kept unchanged, the same volume of pure water was added into the system and showed slight influence on the quenching process,

however, the increase in the concentration of NaAc induced dramatic enhancement on the quenching effect. The responses of the film to some other organic copper salts like copper tartrate and copper citrate were also investigated. It was found that the presence of these organic anions, tartrate and citrate, could also induce a larger quenching effect to the fluorescence emission of the film. This illustrates that organic anions do enhance the quenching efficiency of copper ions to the film due to the spacer screening effect.

3.5. Selectivity, reversibility of the dansyl-functionalized film towards copper ions

The responses of the film to the analyte solution containing mixtures of competing species were investigated. As shown in Fig. 7, the presence of mixture of 16 mM of each of $Ni(NO_3)_2$, $Co(NO_3)_2$, $Pb(NO_3)_2$, and $Zn(NO_3)_2$, caused only 11.5% decrease of the initial intensity of the film in 10 min. However, the presence of one half of $Cu(NO_3)_2$ (8 mM) quenched nearly 50% of the fluorescence intensity in 2 min. The addition of 16 mM of NaAc produced a further remarkable decrease in the fluorescence intensity. This result indicates that the film may act as a sensor for Cu(II) even in the presence of elevated levels of other competing divalent metal ions provided their counters are inorganic anions. The presence of organic anions, however, would decrease the selectivity of the film towards Cu(II), indicating that not only the functionality (e.g. ion binding sites) but also the conformation of the spacer has a great effect upon the sensing performance of a fluorophore-functionalized film chemosensor.

Furthermore, the quenching on the dansyl-functionalized film could be recovered by simple rinsing the film with an aqueous solution of EDTA. The reversible sensing ability of this functional film was investigated by alternatively exposing the film to an aqueous solution of copper acetate and pure water, and the corresponding fluorescence intensity at 518 nm was measured
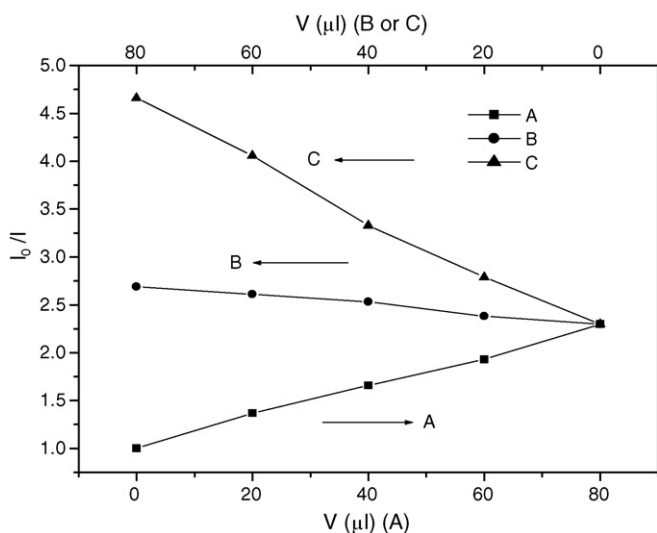

Fig. 6. Enhancement effect of sodium acetate upon the sensing properties of the film for $Cu(NO_3)_2$ (A: 0.2 M $Cu(NO_3)_2$, B: water, and C: 0.4 M NaAc).
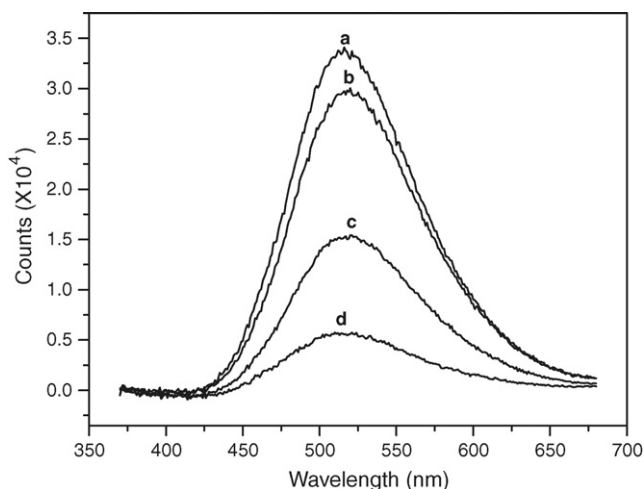

Fig. 7. Effect of addition of Cu(II) in the presence of other divalent transition metal ions ($\lambda_{ex} = 350$ nm). Curve a stands for the initial fluorescence spectrum, curve b for the effect of addition of 16 mM each of $Ni(NO_3)_2$, $Co(NO_3)_2$, $Pb(NO_3)_2$, and $Zn(NO_3)_2$, curve c for the effect of 8 mM of $Cu(NO_3)_2$, and curve d for the effect of 16 mM of NaAc.
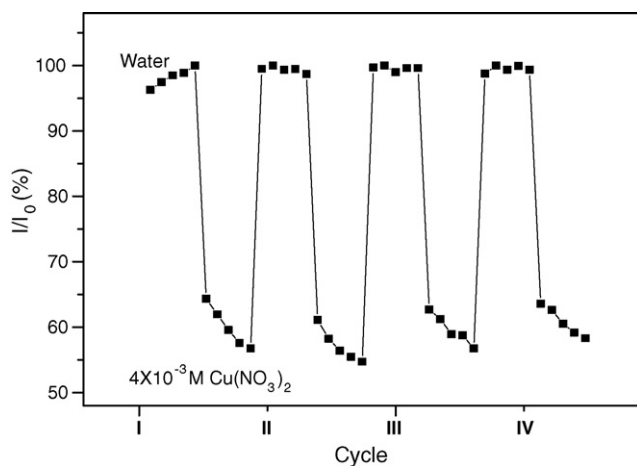
Fig. 8. Reversible sensing process of the dansyl-functionalized film toward Cu(II).

every 3 min in each case for five times. After each measurement of the salt solution, the film was washed with an EDTA solution and pure water for several times. The intensity ratio, I/I_0, was calculated and reported in Fig. 8. It was found that the response of the film to copper ions could be fast and fully restored. Compared with homogenous solution fluorescent sensors, the reversibility of this film opens up the possibility for making reversible optical film sensors based on this approach.

As the binding ligand, diethylenetriamine, was introduced in the spacer and directly connected with the readout unit, dansyl chromophore, the sensitivity of the film towards Cu(II) was enhanced than the results of our previous work. The limiting detection can be as low as 7.6×10^{-5} M, a concentration suitable for the detection of copper ions in wastewater [37] (e.g. chemical wastewater, electroplate wastewater, and metalworking wastewater, etc.) and contaminated soil [38]. Although this result is not as low as that observed for most of the homogeneous fluorescent sensors [6], it is still worthwhile to try new design strategies due to the promising future and advantages of using fluorescent film sensors. The results from the present work further indicate that the nature of the spacer as well as the subunits have a great influence on the sensing properties of a fluorescent SAM film, which promises a possibility to develop novel fluorescent film sensors for copper ions with much higher sensitivity by changing the functionality of the spacer and may realize application in environmental monitoring and waste management.

4. Conclusion

It has been shown that SAM film can be effectively employed as template for the preparation of fluorescent film sensor for copper ions. The chemical attachment of the fluorophore, dansyl and the ligand, diethylenetriamine to the solid glass surface modified with an epoxy-terminated SAM not only ensures the stability of the sensor, but also increases the sensitivity of film towards Cu(II) ions. Fluorescence measurements in the present study showed that the quenching efficiency of a fluorescent film

toward a metal ion strongly depends on the functionalities of the subunits in the spacer as well as the properties of the spacer such as flexibility, polarity and conformation, etc. As a result, the sensing ability of a film sensor toward some certain metal ion can be easily obtained by simply adjusting the above properties of the spacer. Moreover, this modular approach allows for the easy variation of the components: thus giving access to sensors for other metal ions.

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