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Rhodamine-based chemosensing monolayers on glass as a facile fluorescent "turn-on" sensing film for selective detection of Pb^{2+}

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ABSTRACT

Rhodamine-based chemosensors **1** and **2** were synthesized and self-assembled onto glass surfaces for the selective fluorescent sensing of Pb^{2+}. The immobilized chemosensors showed fluorescent responses that were turned-on with Pb^{2+} in CH_3CN, selectively over various metal ions. The Pb^{2+}-selective fluorescent switch of the immobilized chemosensors was also reversible, allowing for repeated use for Pb^{2+} detection.

1. Introduction

A wide variety of diseases such as digestive, neurologic, and cardiac, along with mental retardation have been attributed to Pb^{2+} poisoning [1]. For Pb^{2+} detection, a range of instrumental techniques such as atomic absorption spectrometry (AAS) and inductive coupled plasma-mass spectrometry (ICP-MS) have been widely used [2]. Nevertheless, alternative easy-to-use detection methods have also been receiving much attention because the instrumental techniques require high costs and skillful operation. One particular promising approach for an alternative detection strategy would involve the use of a fluorescence-based chemosensor.

Fluorescent chemosensors provide several advantages over other analytical methods, such as high sensitivity, specificity, convenience, real time monitoring with fast response times, and low cost. In heavy metal ion detection, however, the use of fluorescence techniques are limited because heavy metal ions often act as fluorescence quenchers *via* spin–orbital coupling [3] and energy or electron transfer [4]. Therefore, it has been highly desirable to develop molecular switches whose fluorescence is turned-on (and not quenched) by the presence of a heavy ion such as Pb^{2+}, with high selectivity over other interfering heavy metal ions.

Use of rhodamine derivatives has recently gained much academic and industrial interest in constructing fluorescent chemosensors since the rhodamine framework offers selectivity based on its particular structural property [5]. Rhodamine derivatives bearing a spirolactam structure are non-fluorescent and colorless (turn-off), whereby ring-opening of the corresponding spirolactam gives rise to strong fluorescence emission and a pink color (turn-on). Thus far, considerable effort has been devoted to the development of rhodamine-based chemosensors to detect heavy metal ions [6], but we noticed that there are only a few reports on Pb^{2+}-selective chemosensors [7].

A great deal of interest currently exists regarding the surface assemblies of molecular switches whose fluorescent properties can be modulated in a controlled and reversible manner for the development of facile fluorescent chemosensors [8]. Assembly of the molecular switches on suitable surfaces has often employed formation of a self-assembled monolayer (SAM) with subsequent binding of the molecular switches to the monolayer. Formation of SAMs can be achieved on different materials such as gold, silicon, and glass. However, the use of SAM-based fluorescent switches on gold substrates is limited because of the strong quenching of the fluorescence of molecules by gold [9]. Glass (SiO_2) does not quench fluorescence and is even transparent to light. Thus, it would be an appropriate substrate for the development of fluorescent chemosensors [10]. Recently, an ultrathin platinum substrate has also been reported as a suitable substrate for fluorescent chemosensors since it does not quench fluorescence and allows formation of ordered and stable SAMs [11].

Here, we report the synthesis and surface assembly of rhodamine-based chemosensor **1** and **2** whose fluorescent properties are turned-on selectively with Pb^{2+} over various metal ions examined in CH_3CN. The Pb^{2+}-selective fluorescent switch of the

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immobilized chemosensors also showed excellent reversibility, enabling their repeated use for Pb^{2+} detection.

2. Experimental

2.1. Chemicals and materials

Rhodamine 6G, diethylenetriamine, tris(2-aminoethyl)amine, ethanolamine, Na_2SO_4, NaOH, and CH_3CN were purchased from Sigma–Aldrich, Inc. (USA). Deionized water (18 MΩ cm) was used to prepare all aqueous solutions (Ultra370, Younglin Co., Korea). Methanol (MeOH, HPLC grade) was purchased from J.T. Baker (USA).

2.2. Synthesis of compound **1**

Under nitrogen, a solution of rhodamine 6G (1.0 g, 2.0 mmol) and tris(2-aminoethyl)amine (3.0 g, 20 mmol) in methanol (10 mL) was refluxed until the solution changed from colorless to pink. After cooling to room temperature, the solvent was evaporated *in vacuo*. Then, CH_2Cl_2 (100 mL) and water (200 mL) were added and the organic layer separated. The CH_2Cl_2 layer was washed twice with water and dried over anhydrous Na_2SO_4. After filtration of Na_2SO_4, removal of the solvent *in vacuo* gave 0.8 g of **1** in 70% yield as a brownish oil. MP: 90–95 °C. IR (deposit from CH_2Cl_2 solution on a NaCl plate, cm^{-1}): 3352, 1683, 1615, 1515. ^1H NMR ($CDCl_3$, 300 MHz): δ 7.91 (m, 1H), 7.45 (m, 2H), 7.02 (m, 1H), 6.32 (s, 2H), 6.16 (s, 2H), 3.25 (br s, 2H), 3.25 (m, 6H), 2.52 (t, 4H, J = 2.70 Hz), 2.31 (t, 4H, J = 2.70 Hz), 2.21 (m, 2H), 1.85 (s, 6H), 1.33 (t, 6H, J = 3.69 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): 167.8, 153.4, 151.9, 147.6, 132.5, 131.6, 128.6, 128.2, 123.9, 122.7, 123.9, 122.7, 118.0, 106.0, 96.5, 65.1, 57.3, 51.5, 38.8, 38.4, 38.2, 16.8, 14.8 ppm. FAB MS m/z (M^+) calcd 542.3, found 543.6.

2.3. Synthesis of compound **2**

Under nitrogen, a solution of rhodamine 6G (1.0 g, 2.0 mmol) and diethylenetriamine (2.0 g, 20 mmol) in methanol (10 mL) was refluxed until the solution color changed from colorless to pink. After cooling to room temperature, the solvent was evaporated *in vacuo*. Then, CH_2Cl_2 (100 mL) and water (200 mL) were added and the organic layer separated. The CH_2Cl_2 layer was washed twice with water and then dried over anhydrous Na_2SO_4. After filtration of Na_2SO_4, removal of the solvent *in vacuo* gave 0.8 g of **2** in 76% yield as a brownish oil. MP: 88–92 °C. IR (deposit from CH_2Cl_2 solution on a NaCl plate, cm^{-1}): 3351, 1682, 1612, 1515. ^1H NMR ($CDCl_3$, 300 MHz): δ 7.87 (m, 1H), 7.42 (m, 2H), 7.04 (m, 1H), 6.34 (s, 2H), 6.18 (s, 2H), 3.51 (br s, 2H), 3.19 (m, 6H), 2.50 (t, 4H, J = 6.00 Hz), 2.35 (t, 4H, J = 6.00 Hz), 2.21 (m, 2H), 1.88 (s, 6H), 1.30 (t, 6H, J = 7.08 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): 168.7, 153.8, 151.8, 147.6, 132.5, 131.2, 128.9, 128.0, 124.0, 122.8, 118.4, 106.1, 96.5, 65.2, 51.9, 47.8, 41.9, 40.4, 38.5, 16.8, 14.8 ppm. FAB MS m/z (M^+) calcd 499.6, found 500.6.

2.4. Fabrication of chemosensor-immobilized glass slides and fluorescence imaging

The synthesized chemosensors were immobilized on *N*-hydroxyl succinimidyl (NHS)-modified glass slides (NSB NHS Slide, NSB POSTECH, Inc., Korea) *via* amine-NHS coupling. Briefly, the glass slide was incubated with a chemosensor solution (100 μM in DMSO) for more than 3 h at 25 °C. After incubation, the slide was rinsed with copious amounts of DMSO and CH_3CN, and blown dry with a N_2 stream. The slide was then placed in a blocking solution (50 mM ethanolamine in 0.1 M Tris buffer, pH 9.0) for 30 min at 50 °C to remove any unreacted NHS groups. After rinsing, the slide was blown dry with a N_2 stream. To investigate sensing properties, the chemosensor-immobilized slides were exposed to ClO_4^- salts of metal cations in CH_3CN for 30 min at 25 °C. The metal ion-exposed slide was then rinsed with copious CH_3CN and blown dry. Fluorescence micrographs were acquired with a fluorescence microscope (Nikon ECLIPSE Ti-U, Nikon Co., Japan) equipped with a mercury lamp (Nikon INTENSELIGHT C-HGFI, Nikon Co.) and a CCD camera (CoolSNAP EZ, Photometrics Ltd., USA). Micrographs were processed using NIS Elements imaging software (Nikon Co.) to show a forced red color.

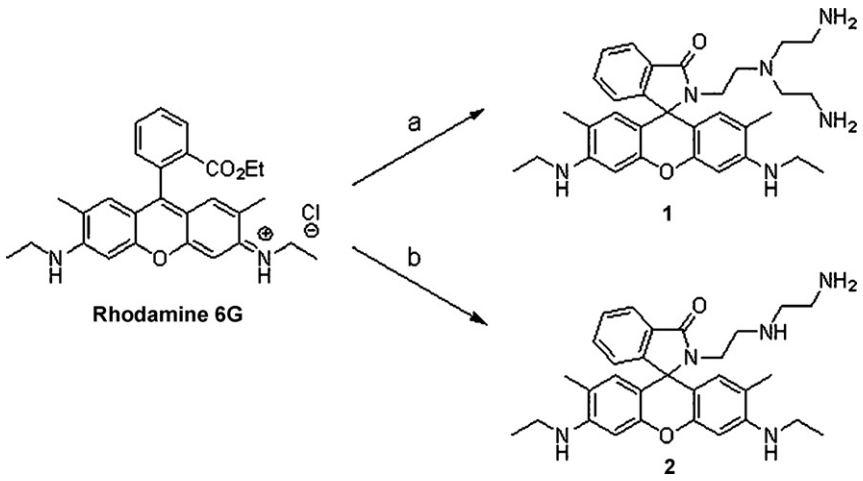
3. Results and discussion

3.1. Design and synthesis

Compound **1** was designed to function as a fluorescent colorimetric chemosensor for Pb^{2+} ions. The **1** is a *N*-tripodal structural compound consisting of a rhodamine moiety (spirolactam form) and two branched primary amine groups. Here, the rhodamine portion serves as a signaling unit while the alkyl amine spacer allows Pb^{2+} binding and immobilization onto the glass slide. In addition, we prepared the similar compound **2** (as a *N*-dipodal structure) for comparative investigation to explore which structure is preferable to the Pb^{2+} ion binding. As depicted in Scheme 1, reaction of rhodamine 6G with excess tris(2-aminoethyl)amine (tren) or diethylenetriamine in MeOH afforded **1** and **2** in 70 and 76% yields, respectively, after aqueous work-up. The final products (**1** and **2**) were well characterized by ^1H- and ^{13}C NMR, FT-IR, and FAB-MS. The spirolactam forms of **1** and **2** could be confirmed by the ^{13}C NMR spectra, where a peak at 65 ppm corresponding to the spiro-carbon atom was present.

3.2. Structure of immobilized chemosensor layers

It is important to investigate the structural properties of the immobilized chemosensors (**1** and **2**) on glass since their structural properties on surfaces would affect their binding selectivity to metal ions [12]. Accordingly, we carried out the key experiments illustrated in Fig. 1, parts a and b. Specifically, we immobilized chemosensor **1** on a NHS-modified glass slide *via* amine-NHS coupling reactions, and then exposed the **1**-immobilized slide to an amine-reactive organic fluorophore, 5-carboxyfluorescein *N*-succinimidyl ester (1.0 mM in DMSO). If the immobilized chemosensor **1** has available free amine groups, one would expect fluorescence from the fluorophore conjugated to the chemosensor as illustrated in Fig. 1a. Similarly, we exposed the **2**-immobilized slide to the amine-reactive fluorophore solution, expecting no significant fluorescence because the immobilized chemosensor **2** would not have any available free amines as shown in Fig. 1b. Indeed, only the chemosensor **1**-immobilized glass surfaces emitted significant fluorescence (2217 ± 298 counts) after the experiment described above was carried out. The chemosensor **2**-immobilized surface showed no significant increase in fluorescence (850 ± 70 counts) compared to that (842 ± 105 counts) of the chemosensor-free glass slide, which was the NHS-modified slide, but blocked with excess ethanol amines, even after being exposed to the amine-reactive fluorophore and subsequent washing. In Fig. 1c, the fluorescence signals obtained from the chemosensor-immobilized glass slides after these experiments were compared to that of the chemosensor-free slide treated identically. On the basis of these results, we concluded that the immobilized chemosensor **1** possesses free amine groups potentially available to interact with specific metal ions, while the immobilized chemosensor **2** has no available free amines.



Scheme 1. Synthetic pathways of **1** and **2**. (a) Tris(2-aminoethyl)amine, MeOH, reflux; (b) diethylenetriamine, MeOH, reflux.

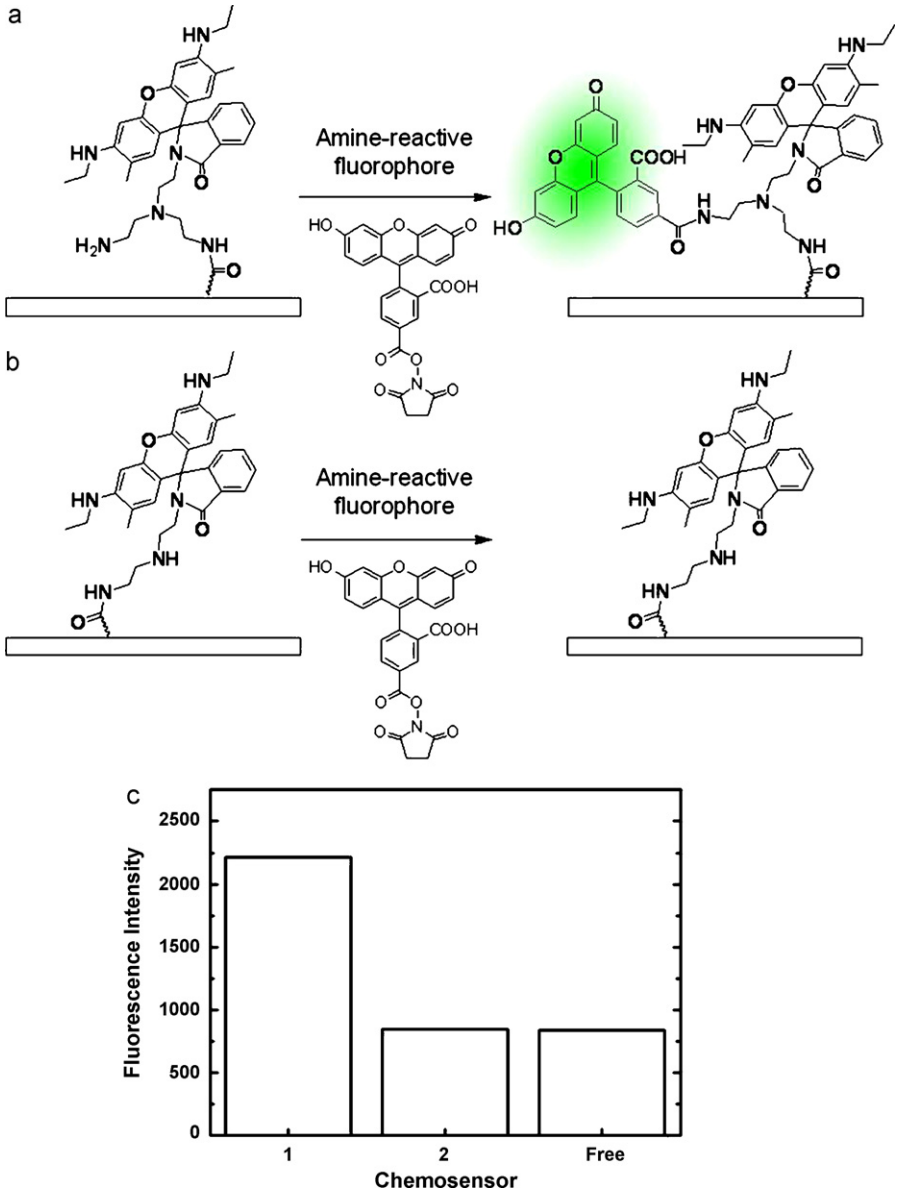
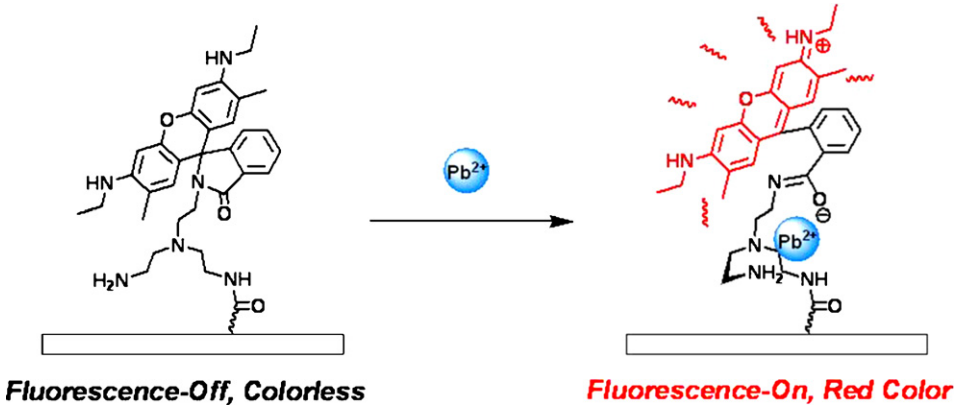


Fig. 1. (a) Illustration of an experiment in which a **1**-immobilized surface was exposed to the amine-reactive fluorophore 5-carboxyfluorescein *N*-succinimidyl ester. (b) Illustration of an experiment in which a **2**-immobilized surface was treated identically to that shown in part a. (c) Comparison of fluorescence intensities obtained after the two experiments (parts a and b) with that from chemosensor-free surface that was treated identically. The exposure time for fluorescence measurements was 300 ms.



Scheme 2. Expected binding mechanism of Pb^{2+} to chemosensor **1**-assembled monolayer on glass.

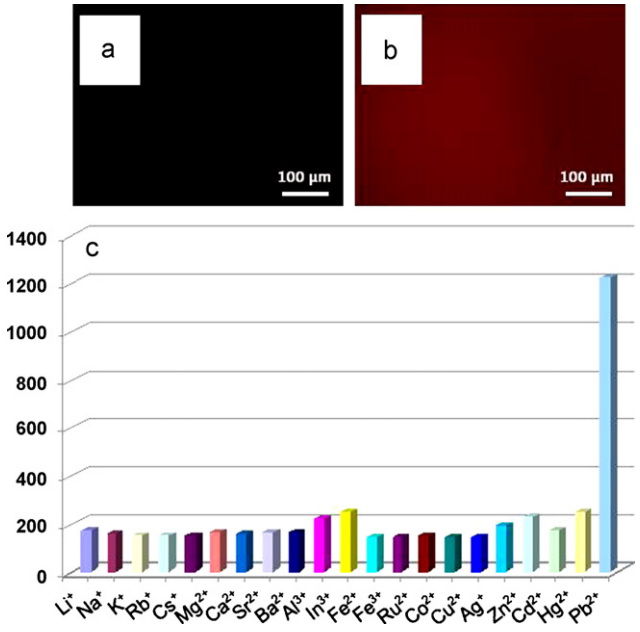


Fig. 2. Fluorescence micrographs of a **1**-immobilized surface (a) before and (b) after exposure to the Pb^{2+} ion (10 mM in CH_3CN) for 30 min and subsequent washing. (c) Comparison of fluorescence intensities obtained from the **1**-immoblized surfaces after exposure to various metal cations (10 mM each in CH_3CN) and subsequent washing. The exposure time for fluorescence measurements was 100 ms.

3.3. Sensing properties

The fluorescence intensity changes of immobilized chemosensors **1** and **2**, upon exposure to ClO_4^- salts of a wide range of metal cations in CH_3CN, which include Li^+, Na^+, K^+, Rb^+, Cs^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Al^{3+}, In^{3+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Cu^{2+}, Ag^+, Zn^{2+}, Cd^{2+}, Hg^{2+}, and Pb^{2+}, were measured. Fig. 2a and b are fluorescence micrographs obtained from the chemosensor **1**-immobilzed glass surface before and after exposure to the Pb^{2+} ion and subsequent washing, respectively. The significant increase in fluorescence intensity (OFF to ON transition) in the micrographs indicates that the Pb^{2+} complexes with immobilized **1** and induces the spirolactam moiety (OFF) of **1** to be opened (ON). Interestingly, the significant changes in fluorescence intensity were not observed with other metal ions. As shown in Fig. 1c, we observed the selective fluorescent change (1223 ± 166 counts) only for Pb^{2+} over other cations. The detection limit of Pb^{2+} (S/N = 3) was approximately 10^{-4} M. The chemosensor **2**-immobilized glass slides were also treated identically to that shown in Fig. 2, and showed a selective fluorescent change for Pb^{2+} over other cations. However, the fluorescence intensity was significantly lower (543 ± 136 counts) than that obtained from the chemosensor **1**-immobilized slides (Supporting Information Fig. S5). This might be attributed to the lack of a free amine moiety of the immobilized chemosensor **2**. A plausible Pb^{2+}-selective sensing mechanism is therefore depicted in Scheme 2.

The immobilized chemosensors showed reversible transitions between the spirolactam (OFF) and open-ring amide (ON) structures, providing reversible fluorescent switches of the immobilized chemosensors. Fig. 3a shows a fluorescence micrograph obtained from the chemosensor **1**-immobilized glass surface after exposure to Pb^{2+} (1.0 mM in CH_3CN) and subsequent washing. The Pb^{2+}-induced ring opening of the spirolactam of immobilized **1** resulted in the fluorescent surface (ON). The chemosensor **1**-immobilized surface could be then fully regenerated (OFF) by rising with 0.1 M NaOH, which caused the open-ring amide to return to the spirolactam structure (Fig. 3b). The refreshed surface was exposed to Pb^{2+} again and showed regeneration of fluorescence (ON), indicating a Pb^{2+}-induced ring reopening of the spirolactam (Fig. 3c). However, we also observed progressive degradation of the chemosensor **1**-immobilized surface upon repeated exposure to NaOH solution, which could be attributed to hydrolysis of the Si–O bonds on glass upon exposure to basic aqueous solution [12]. These results clearly demonstrate that the Pb^{2+}-selective fluorescent switch of

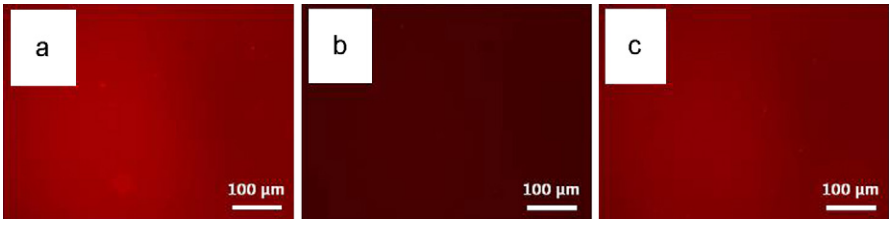


Fig. 3. Fluorescence micrographs of a **1**-immobilized surface after successive exposure to: (a) 1.0 mM Pb^{2+} in CH_3CN for 1 h and washing; (b) 0.1 M NaOH for 1 min and washing; (c) 1.0 mM Pb^{2+} in CH_3CN for 1 h and washing. The exposure time for fluorescence measurements was 900 ms.

the immobilized chemosensor **1** was reversible, allowing for its use for Pb^{2+} detection multiple times as a molecular switch.

4. Conclusions

In this report, we described the synthesis and surface assembly of rhodamine-based chemosensors **1** and **2** that showed molecular switching behaviors for the fluorescent sensing of Pb^{2+}. The immobilized chemosensors presented fluorescent responses that were turned-on selectively with Pb^{2+} over various metal ions in CH_3CN. The fluorescent switch was also fully reversible, allowing for repeated use of the immobilized chemosensors for Pb^{2+} detection. At present, we are improving water solubility of the chemosensors for possible application to selective fluorescent sensing of metal ions in aqueous samples.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2010.11.016.

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