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Ratiometric fluorescent probe for biothiol in aqueous medium with fluorescent organic nanoparticles

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ABSTRACT

A dipodal rhodamine-based mercury complex have been designed and synthesized, for the selective detection of 3-mercaptopropionic acid (**MPA**). To avoid the poor solubility of rhodamine-based ligand in pure water, the Hg^{2+} complex of fluorescent organic nanoparticles (FONs) of ligand have been developed using reprecipitation method and the formation of 1:1 complex has been confirmed with various spectroscopic techniques. The resultant chemosensor can detect **MPA** in a concentration range of 60 nM–1 μM (in buffered aqueous medium) with detection limit of 60 nM.

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1. Introduction

Thiol based molecules have significant roles in living organisms, and have proven to be an essential part in a number of biochemical processes [1]. It has been noticed that abnormal levels of mercaptan causes variety of diseases, such as liver damage, skin lesions, fat loss, hair depigmentation, lethargy, Alzheimer's and cardiovascular diseases [2–5]. Hence the designing and synthesis of sensitive probes for the detection of thiols is highly desirable. In recent years, considerable efforts have been devoted to develop different instrumental techniques for the detection of thiol level, which includes the fluorescent recognition of thiols based on reaction mechanisms between probes and thiols [6]. These reaction mechanisms include, Michael addition reactions [7,8], cyclization with aldehydes [9,10], cleavage of sulfonamide and sulfonate esters [11–13], cleavage of selenium–nitrogen bonds [14], cleavage of disulfide bonds [15], oxidation–reduction processes in metal complexes [16], and use of nanoparticles [17–19]. However, these methods have their own limitations such as insolubility in water and cannot be used in aqueous system. In literature there are only a few ratiometric probes that can work in aqueous system [20–22].

Recently the metal complex of rhodamine derivatives are being used for the anion recognition through "Cation Displacement Assay" [23,24], the next generation of "Indicator Displacement Assay" popularized with the fabulous work of Anslyn et al. [25–27]. The assay progression involves the formation of metal complex with receptor that further can be used as anion sensor. The metal ion binding, causes the change in fluorescent intensity of organic receptor and based on displacement approach the anionic guest displaces the metal ion from the coordination sphere of the receptor, this leads to restore the original fluorescence profile of receptor. This approach can be used to modify the simple organic receptors as efficient fluorescent probes; although mostly iodide, cyanide and phosphate are found to recognize through Cation Displacement Assay [28,29]. If the target is to recognize some biomolecules, through Cation Displacement Assay then the system is expected to suffer from the interference from these anions. The target of molecular recognition through "Cation Displacement Assay" can be achieved if the receptor offer stronger coordination sphere for metal ion through dipodal framework consisting of two units of rhodamine moiety. Thus two units may provide sufficiently large number of binding sites to complete the coordination sphere of metal ion to authenticate the strong complexation. Now, only strong ligands such as bifunctional thiols can eject out the metal ion, this may avoid the interference due to anions.

Using the same approach we have designed and synthesized dipodal rhodamine-based mercury complex, which can be used as chemosensor for the detection of 3-mercaptopropionic acid (**MPA**). Moreover to avoid the poor solubility of rhodamine-based probe in pure water, we developed fluorescent organic nanoparticles (FONs) of dipodal rhodamine-based receptor using reprecipitation method [30]. This involves an injection of receptor (1 ml of stock solution of organic receptor in pure DMF) to 99 ml of double distilled pure water. For metal complexation, the standard solution of FONs of organic receptor

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was further mixed with equal equivalent of mercuric nitrate salt in HEPES buffered aqueous solution.

2. Experimental section

2.1. General information

All solvents were dried by standard methods and chemicals were purchased from Sigma Aldrich and used without further purification. The dipodal Rhodamine based ligand **1** (Scheme 1) was prepared by our already reported method [31]. TLC was performed on glass sheets pre-coated with silica gel (Kieselgel 60 PF254, Merck). The ^{1}H and ^{13}C NMR spectra were performed in $CDCl_3$ and DMSO-d_6 with TMS as an internal reference, on a JNM-ECS400 (JEOL) instrument operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. The chemical shifts are reported as δ values (ppm) relative to TMS. The elemental analyses were performed on a Flash EA 1112 elemental analyzer. Mass spectra were recorded on Waters Micromass Q-Tof model of Mass Spectrometer. For recognition studies, the UV–vis absorption spectra were taken using dilute solutions in quartz cells (1 cm path length) on a Specord 250 Plus Analytikjena spectrometer. The fluorescence studies were performed on a Perkin Elmer L55 fluorescence spectrophotometer using 10 mm cell, with 400 scan speed. The GC–MS analysis was carried out using GCMS-QP2010 ultra spectrophotometer.

2.2. Fabrication of FONs

Fluorescent organic nanoparticles (FONs) of **1** were prepared by re-precipitation method. Briefly, 1 ml of stock solution of **1** (0.1–1.3 mM, in pure DMF) was injected to 99 ml of double distilled pure water. Both the solutions were mixed under sonication to ensure the rapid mixing.

2.3. Preparation of fluorometric metal ion titration solutions

All the recognition studies were performed at 25 ± 1 °C and before recording any spectrum adequate time was given to ensure the uniformity of the solution. The cation recognition behavior of **1**(FONs).$\mathbf{Hg^{2+}}$ was evaluated from the changes in fluorescence spectra of fluorescent organic nanoparticles (FONs) upon addition of that thiol (10 μM) in aqueous medium. For titrations, volumetric flasks were taken each containing standard solution of **1**(FONs).$\mathbf{Hg^{2+}}$ along with varied amounts of a particular thiol (0–10 μM) in HEPES buffered aqueous solution.

2.4. Competition studies

To evaluate any possible interference due to different metal ions for the estimation of **MPA**, solutions were prepared containing **1**(FONs).$\mathbf{Hg^{2+}}$ and **MPA** (10 μM) along with both with and without other interfering metal ions (10 μM) in HEPES buffered aqueous solution (pH $=7.0 \pm 0.1$). The fluorescence intensity of each solution was recorded at 547 nm.

3. Result and discussion

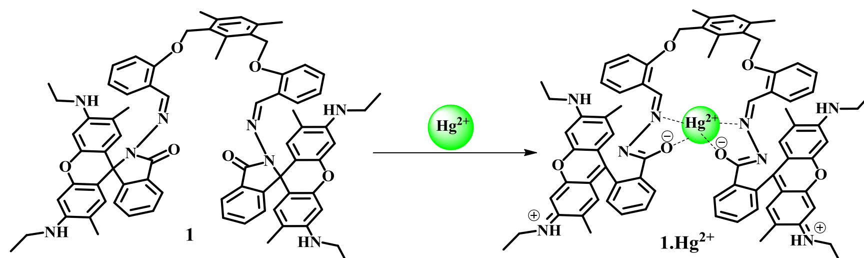
The reaction of already reported dipodal rhodamine-based **1** with mercuric nitrate generated dipodal rhodamine-based mercury complex $\mathbf{1.Hg^{2+}}$ (Scheme 1). The composition/purity of the complex $\mathbf{1.Hg^{2+}}$ was judged by its ^{1}H NMR, mass and CHN analysis. The mass spectrum showed $m/z=705$, which corresponds to [$\mathbf{1.Hg^{2+}}$], indicating 1:1 binding of receptor **1** with Hg^{2+} (Fig. S1). Similarly in ^{1}H NMR upon addition of Hg^{2+} ions the signal corresponding to H_m from the –NH protons (originally seen at δ 4.91 ppm) broadened and finally disappeared. The aromatic protons corresponding to the rhodamine moiety showed an upfield shift with $\Delta\delta$ 0.04–0.02 ppm, which can be attributed to the change in electron density due to the spirolactam ring opening. Moreover the imine proton H_l at 8.92 ppm, showed an upfield shift of 0.05 ppm, indicating the coordination of imine nitrogen to the metal ion. Moreover the appearance of symmetrical signals for $\mathbf{1.Hg^{2+}}$ complex in ^{1}H NMR spectrum confirmed the involvement of both the podes of receptor in complex formation (Fig. S2).

In continuation to the marvelous work of Anslyn et al. for the concept of Indicator Displacement Assay [25–27], recently others and we have developed "Cation Displacement Assay" and "Counter ion Displacement Assay" for the determination of anions in semi-aqueous medium [32,33]. These assay are operating through displacement approach and the advantage of this assay lies to the fact that one can determine anionic species in semi-aqueous or aqueous medium. Hence to investigate the binding aptitude of **1**(FONs).$\mathbf{Hg^{2+}}$ as a selective sensor for thiols, the complex **1**(FONs).$\mathbf{Hg^{2+}}$ was tested for the recognition of different thiols such as 2-aminothiophenol, 4-aminothiophenol, 2-mercaptothiazoline, 2-mercaptopyridine, 4,6-diamino-2-mercaptopyrimidine, 2-mercaptopyrimidine, 2,5-dimercapto-1,3,4-thiadiazole, L-cysteine hydrochloride in HEPES buffered solvent system and no thiol was found to be perfect enough to bind with **1**(FONs).$\mathbf{Hg^{2+}}$.

Upon excitation of **1**(FONs).$\mathbf{Hg^{2+}}$ complex at 510 nm; an emission band was observed at 547 nm. However, addition of 3-mercaptopropionic acid (**MPA**) to the **1**(FONs).$\mathbf{Hg^{2+}}$ complex led to the quenching in band at 547 nm; which indicated the complexation between Hg^{2+} and **MPA** (Fig. 1A). The binding properties of **1**(FONs).$\mathbf{Hg^{2+}}$ with **MPA** were investigated by performing fluorescence titration experiments. Upon addition of increasing amounts of **MPA** from (0–10 μM) to the solution of compound **1**(FONs).$\mathbf{Hg^{2+}}$ resulted in the appearance of an emission band at 645 nm with the simultaneous quenching of the band at 547 nm (isosbestic point at 619 nm) as shown in Fig. 1B.

The chromogenic response of complex was also studied in the presence of different thiols. In the presence of **MPA**, **1**(FONs).$\mathbf{Hg^{2+}}$ showed significant change in absorption spectra with the formation an isosbestic point at 648 nm (Fig. 2).

Competition experiments were also conducted for **1**(FONs).$\mathbf{Hg^{2+}}$ complex in the presence of 10 μM of one particular thiol out of 2-aminothiophenol, 4-aminothiophenol, 2-mercaptothiazoline, 2-mercaptopyridine, 4,6-diamino-2-mercaptopyrimidine, 2-mercaptopyrimidine, 2,5-dimercapto-1,3,4-thiadiazole, L-cysteine hydrochloride along with same equiv of **MPA**. As shown in Fig. 3, the emission profile of the **1**(FONs).$\mathbf{Hg^{2+}}$ with **MPA** complex was unperturbed to



Scheme 1. Structures of **1** and $\mathbf{1.Hg^{2+}}$.

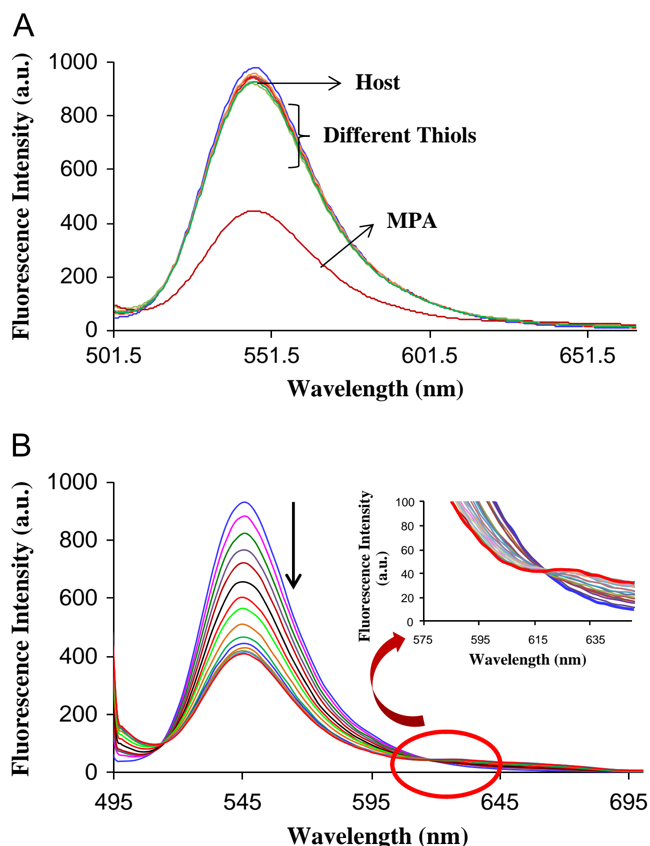


Fig. 1. (A) Changes in fluorescence intensity of **1**(FONs).$\mathbf{Hg^{2+}}$ upon addition of 10 μM of different thiols in DMF/H_2O (1:99, v/v) solvent system; (B) fluorescence emission spectra of **1**(FONs).$\mathbf{Hg^{2+}}$ upon addition of **MPA** (0–10 μM) in DMF/H_2O (1:99, v/v); inset represents the ratiometric change with isobestic point at 629 nm.

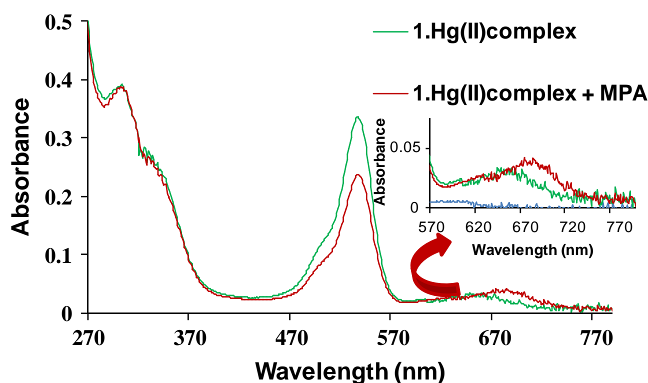


Fig. 2. Changes in absorption spectraof **1**(FONs).$\mathbf{Hg^{2+}}$ upon addition of 10 μM of **MPA** in DMF/H_2O (1:99, v/v) solvent system; inset represents the region of change upon binding with **MPA**.

the presence of different thiols. In cell or biological fluid, different types of anions and biomolecules are present and these analytes can interfere in recognition of **MPA**. To extend the scope of this sensor binding of **1**(FONs).$\mathbf{Hg^{2+}}$ was also executed with tetrabutylammonium salt of various anions (F^-, Cl^-, Br^-, I^-, CN^-, CH_3COO^-, $H_2PO_4^-$, NO_3^-, HSO_4^-, and $HClO_4^-$), sodium salts of SO_3^{2-}, SO_4^{2-}, $S_2O_3^{2-}$, $S_2O_8^{2-}$ and some biomolecules like 4,5-diamino-6-hydroxy-2-mercaptopyrimidine, thioacetic acid, succinic acid, malonic acid, terephthalic acid, isophthalic acid, oxalic acid, glutaric acid, adipic acid, suberic acid, pimelic acid, DL-homocysteine and L-glutathione reduced (GSH) as shown in Fig. S3A and B. Fig. S3 illustrated that **1**(FONs).$\mathbf{Hg^{2+}}$ did not show response towards any particular analytes. Further, detection of **MPA** through **1**(FONs).$\mathbf{Hg^{2+}}$ was also carried out in the presence of

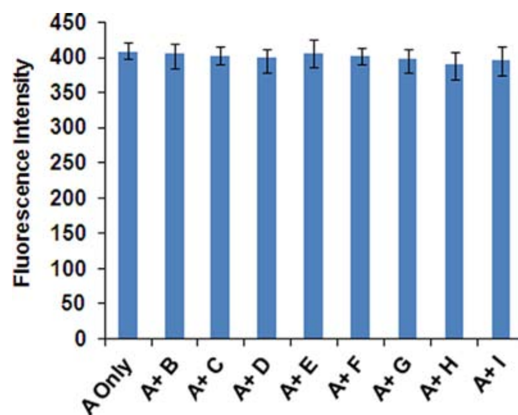


Fig. 3. Competitive binding assay of **1**(FONs).$\mathbf{Hg^{2+}}$ towards 3-mercaptopropionic acid (**MPA**) (10 μM) in the presence of other thiols (10 μM) with fluorescence spectroscopy, (A) **MPA** only, (B) 2-amino thiophenol, (C) 4-amino thiophenol, (D) 2-mercaptothiazoline, (E) 2-mercaptopyridine, (F) 4,6-diamino-2-mercaptopyrimidine, (G) 2-mercaptopyrimidine, (H) 2,5-dimercapto-1,3,4-thiadiazole and (I) L-cysteine hydrochloride.

anions like F^-, Cl^-, Br^-, I^-, CN^-, CH_3COO^-, $H_2PO_4^-$, NO_3^-, HSO_4^-, $HClO_4^-$, SO_3^{2-}, SO_4^{2-}, $S_2O_3^{2-}$, $S_2O_8^{2-}$ and some biomolecules like 4,5-diamino-6-hydroxy-2-mercaptopyrimidine, thioacetic acid, succinic acid, malonic acid, terephthalic acid, isophthalic acid, oxalic acid, glutaric acid, adipic acid, suberic acid, pimelic acid, DL-homocysteine and L-glutathione reduced (GSH). The competitive titrations were accomplished in the presence of **MPA** along with equal equiv of other anions and biomolecules as shown in Fig. S4. The results indicated that a negligible interference was observed from these anions and biomolecules. Thus, **1**(FONs).$\mathbf{Hg^{2+}}$ can recognize the **MPA** in complex matrix which represent the high selectivity of **1**(FONs).$\mathbf{Hg^{2+}}$ for **MPA**.

$$\ln[(F-F_0)/(F_\infty-F)] = n\ln[Ag^+] + n\ln(K_{asscn}) \quad (1)$$

Lehrer and Chipman purposed an equation for calculation of binding constant and stoichiometry for (**MPA***n*.**1**(FONs).$\mathbf{Hg^{2+}}$) complex (Eq. (1)) [34,35]. Here n belongs to number of **MPA** attached with each molecule of **1**(FONs).$\mathbf{Hg^{2+}}$, K_{asscn} refer to binding constant, F_0 corresponds to fluorescence intensity of receptor **1**(FONs).$\mathbf{Hg^{2+}}$ only, F corresponds to fluorescence intensity of **1**(FONs).$\mathbf{Hg^{2+}}$ at particular concentration of **MPA** and F_∞ refer to fluorescence intensity of **1**(FONs).$\mathbf{Hg^{2+}}$ at maximum concentration. The slope of graph between $\ln[(F–F_0)/(F_\infty–F)]$ and ln[**MPA**] gave stoichiometry and it was about 1.2, which corresponds to 1:1 ratio between host and guest (Fig. S5). The intercept of the plot gave the binding constant of **1**(FONs).$\mathbf{Hg^{2+}}$ **MPA** and it was about $1.77\ (\pm 0.5) \times 10^5\ M^{-2}$. The detection limit of **1**(FONs).$\mathbf{Hg^{2+}}$ complex as a fluorescent sensor for the analysis of **MPA** was determined and found to be 60 nM. Among various thiols, the **MPA** has significant biological importance. A lower dose (97.5 mg/kg i.p.) of 3-**MPA** increases heart rate and arterial pressure in mammals. 3-Mercaptopropionic acid (3-**MPA**) is an inhibitor of the synthesis of gamma-aminobutyric acid (GABA). The decreased GABA levels in the hypothalamus and in the medulla pons are responsible for the increase and decrease in heart rate, respectively, after systemic administration of 3-mercaptopropionic acid [36,37]. Hence, the sensing and recognition of **MPA** forms an important part of the chemical and biological research. The Hg^{2+}complex of **1** showed a fluorescence band at 547 nm due to spirolactam ring opening of rhodamine moiety. The quenching of fluorescence intensity upon addition of **MPA** to the solution of **1** (FONs).$\mathbf{Hg^{2+}}$ can be attributed to the stronger binding affinity of thiols towards Hg^{2+}. As evident from the mass spectrum (Fig. S1), although counter anion ($–NO_3$) is not participating into the coordination sphere of metal complex; however the addition of

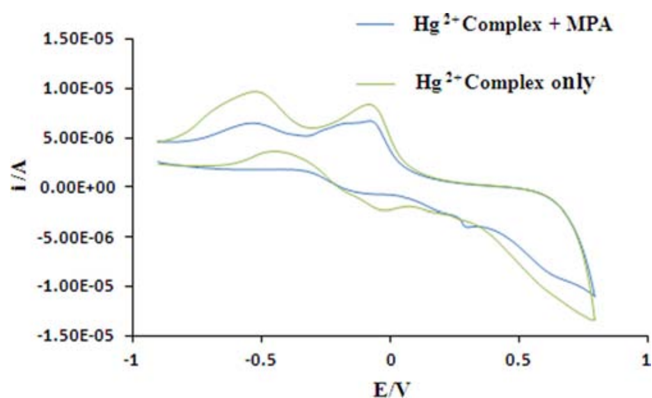


Fig. 4. CV profiles of receptor Hg^{2+} complex of **1**(FONs) and Hg^{2+} complex of **1** (FONs)+**MPA**.

Table 1
A comparison of results obtained from **1**(FONs).**Hg^{2+}** with GC–MS.

Sample code	**1**(FONs).**Hg^{2+}** (average)[a] (nM)	GC–MS (average)[a] (nM)
HS1	80.1 ± 0.4	80.0 ± 0.3
HS2	409.8 ± 0.2	410.0 ± 0.1
HS3	96.01 ± 0.4	96.8 ± 0.2

[a] Average of three different reading.

MPA leads to modulate the photophysical properties of metal complex. The observed phenomena could be the consequence of some displacement assay. The possibility of cation displacement can be ruled out because after titrations (UV–vis and fluorescence) system could not regain the original profile of the pure host. The other alternate seems to be the direct coordination of **MPA** with Hg^{2+} to some binding sites of receptor.

To comment upon the mechanism of **MPA** binding, we have performed the cyclic voltametery and as per expectation different profiles of **1**.Hg^{2+} and **1**.Hg^{2+}+**MPA**, were observed (Fig. 4). Hence it can be inferred that binding with Hg^{2+} changes the electrochemical structure of the sensor. In addition, the **1**.Hg^{2+}complex also interact with **MPA**. To gain more insight about the responsible binding sites of ternary complex **1**.Hg^{2+}+**MPA**, the ^{1}H NMR titration was performed (Fig. S6). Addition of 1 equivalent of **MPA** to the solution of **1**.Hg^{2+} in DMSO-d_6 led to change the ^{1}H NMR of **1**.Hg^{2+} to an appreciable extent. The signal at δ 8.68 assigned for imine linkages, split into two signals. The similar type of splitting was also observed for the aromatic signals. This implies that the two podes of receptor are not in uniform environment, which is quite possible when the coordination of **MPA** occurs with Hg^{2+}, with the simultaneous displacement of one pod of receptor from the coordination sphere of metal (Fig. S7). Thus the suggested mechanism is not purely Cation Displacement Assay rather the occurrence of more than one phenomenon.

A 3-**MPA** is widely used as stabilizer in the production of oil, PVC, grease and sealants. Therefore, it is essential to detect the 3-**MPA** in environmental samples. For real sample analysis, samples were collected on three different days of week from drain of industrial area, Sonipat, India. These samples were filtered through Whatman filter paper (4 μm) followed by centrifugation for 10 min at 500 rpm and whole process was repeated thrice. This process removed most of insoluble organic part. These samples were divided into two sets; one set was evaluated on fluorescence spectroscopy using **1**(FONs).**Hg^{2+}** probe and other set through GC–MS. For GC–MS analysis, a known compound (2-hydroxybenzaldehyde) having known concentration (400 nM) was added into each samples. The concentration of **MPA** was calculated relative to the standard sample (using area under the peak) as shown in Table S1. Table 1 illustrated that concentration calculated from fluorescence spectroscopy using **1**(FONs).**Hg^{2+}** probe is more or less similar to GC–MS data.

4. Conclusions

In conclusion, a dipodal rhodamine based Hg^{2+} complex has been prepared and characterized.The formation of 1:1 complex has been confirmed with various spectroscopic techniques as well as through visual color changes. The Hg^{2+} complex was found to detect 3-mercaptopropionic acid (**MPA**) in a concentration range of 60 nM–1 μM with detection limit of 60 nM. Thus the present probe can be useful for various applications in the field of toxicology and environmental sciences.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.05.036.

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