Indian Journal of Chemistry
Vol. 55A, June 2016, pp. 692-697

Naphthalimide based hybrid nanoparticles for selective recognition of Hg(II)

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Received 10 August 2015; revised and accepted 26 May 2016

1,8-Naphthalic anhydride and cystamine dihydrochloride have been used as precursors for the synthesis of a dipodal compound (**1**) which has been further processed into fluorescent organic nanoparticles (**N1**) by re-precipitation technique. This has resulted in the formation of spherical nanoparticles which have been characterized by TEM and DLS studies. These hybrid nanomaterials composed of organic and inorganic nanoparticles have been used as a sensing platform for determination of mercury(II). The sensing phenomenon shows a ratiometric response for the selective determination with Hg^{2+} ion among other heavy metal ions with enhancement in fluorescence emission at 340 nm and decrease in emission intensity at 469 nm. Other heavy metal show no interference towards recognition of Hg^{2+} ion.

Keywords: Nanomaterials, Hybrid nanomaterials, Selective recognition, Fluorescence, Mercury

The development of highly sensitive and selective fluorophores for molecular recognition is gaining wide attention[1-3]. Organic compounds show weak fluorescence emission when they exist in solid or aggregated state which cannot be employed for the sensing purposes. However, some organic dyes display excellent fluorescence profiles when they are in the aggregated form, known as aggregation induced enhancement emission[4-6]. Therefore, due to wide availability of organic fluorophores and their synthesis methods, fluorescent organic nanoparticles have motivated research interest. Such compounds exhibit good water solubility and photophysical properties[7,8]. Different methods are being used for the fabrication of organic nanoparticles like thermal evaporation, laser ablation, microemulsion in water, and re-precipitation method[9]. One of the easiest, simplest and least time consuming techniques that have been considered these days is re-precipitation method. The organic nanoparticles are developed by slowly injecting the solution of organic compound dissolved in organic solvent in aqueous solution with vigorous stirring at maintained temperature[10]. The criterion behind formation of aggregates is the insolubility of the organic compound in aqueous medium, leading to the development of spherical aggregates whose size can be optimized by controlling various parameters like concentration, temperature and the stirring rate[11-13]. Fluorescent organic nanoparticles are used as probes for the recognition of biologically and environmentally vital analytes due to their extraordinary photophysical characteristics.

Transition and heavy metal ions participate in biological processes in living systems and are broadly utilized for their domestic and industrial applications. While these metals are vital for functioning of biological systems, their existence in excess may lead to disorder in functioning of physiological processes[14]. Mercury is known to be the most toxic and pollutant metal among heavy and transition metal ions. It exists in metallic, inorganic, and organic forms. Its widespread availability in water, air and soil contaminates the environment. The accumulation of mercury in the body can damage central nervous system on conversion of inorganic Hg^{2+} into organic methyl mercury, which is neurotoxic. It bioaccumulates through the food chain[15-17]. The excessive exposure of Hg^{2+} affects the brain functions which results in the symptoms like irritability, anxiety, vision troubles, deafness and the loss of muscle coordination, sensation and memory. Apart from affecting the brain functioning, methyl mercury also harms the kidney, stomach, heart and intestines[18]. The World Health Organization (WHO) has set the limit for mercury in drinking water[19] to be 0.001 mg L^{-1}.

Different determination techniques have been employed to recognize Hg^{2+} ion. Some methods require very expensive instrumentation, reagents and trained technicians. Also, such techniques are less sensitive and selective with very tedious sample preparation is required. However, fluorescence spectroscopic methods overcome these drawbacks, and are highly sensitive and selective[20, 21].

Herein, we have synthesized a dipodal organic compound (**1**) using 1, 8-naphthalic anhydride and cystamine dihydrochloride, which has been processed into fluorescent organic nanoparticles (**N1**) by re-precipitation method. We have designed hybrid framework composed of organic-inorganic nanomaterials in which gold nanoparticles are reduced on the surface of organic nanoparticles (**N1**). This hybrid nanomaterial (**H1**) has been further evaluated for its photophysical behaviour towards heavy metal ions. **H1** results in the recognition of Hg^{2+} ion among various heavy metal ions without displaying any significant interference from other heavy metal ions.

Experimental

All chemicals used were of analytical grade and were purchased from Sigma-Aldrich Co. NMR spectra were recorded on Avance-II (Bruker) instrument, operated at 400 MHz for ^1H NMR (chemical shifts are expressed in ppm). The fluorescence measurements were performed on a Shimadzu RF-5301 PC fluorescence spectrofluorometer. For determination of mass of the compound, LC-MS equipped with a Waters Micromass Q-Tof was used. TEM images were recorded on Hitachi (H-7500) instrument at 120 kV. This instrument has the resolution of 0.36 nm (point to point) with 40-120 kV operating voltage. A 400-mesh formvar carbon-coated copper grid was used for sample preparation. The particle size of nanoparticles was determined with dynamic light scattering (DLS) using external probe feature of Metrohm Microtrac Ultra Nanotrac particle size analyser.

Compound **1** was synthesised as follows: Cystamine dihydrochloride (51.69 mg, 0.2295 mmole) was dissolved in 20 mL DMF and to it was added triethylamine (160 µL, 1.147 mmole). The solution was refluxed and 1,8-naphthalic anhydride (100 mg, 0.505 mmole) was added to it. The reaction was stirred at 90 °C and refluxed overnight. The progress of the reaction was monitored over precoated TLC plate (chloroform:methanol:: 9.5:0.5). On completion of the reaction, the mixture was quenched in ice cold water and the off white solid of (1) which separated out was filtered (yield: 75%). Anal. for ($C_{28}H_{20}N_2O_4S_2$): Calc.: C, 66.54; H, 3.65; N, 5.85; O, 12.62; S, 12.88; Found: C, 65.61; H, 3.93; N, 5.46; O, 12.48; S, 12.51. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.60 (d, 4H, ArH), 8.20 (d, 4H, ArH), 7.75(t, 4H, ArH), 4.58 (t, 4H, $-CH_2$), 3.14 (t, 4H, $-CH_2$), ESI-MS m/z = 513.2 $[M+H]^+$, where M= $C_{28}H_{20}N_2O_4S_2$.

For the synthesis of the nanoparticles of compound (**1**) (**N1**), single step re-precipitation technique was used. To prepare the working solution, solubility of compound (**1**) was first checked in various solvents like acetonitrile, methanol, tetrahydrofuran, DMF and DMSO. It was found to be best soluble in DMSO. Hence, the ligand solution was prepared by dissolving the compound (**1**) (0.00512g, 1 m*M*) in 10 mL of DMSO. Further, 2 mL of the above working solution was injected in 18 mL of water using a micro-syringe with vigorous stirring. For the uniformity in size of the nanoparticles (**N1**), various parameters were maintained like temperature, rate of stirring and the speed at which the working solution was injected. The size of the organic nanoparticles (**N1**) was assessed by dynamic light scattering (DLS) and transmission electron microscopy (TEM) studies.

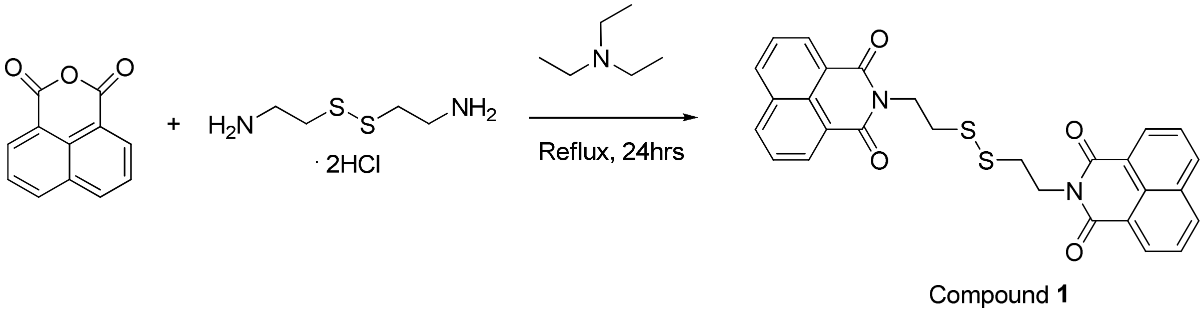
For the synthesis of the hybrid nanomaterial of compound (**1**).(**H1**), gold solution was prepared by dissolving $HAuCl_4$ (0.1 m*M*) in water. Ascorbic acid solution was prepared by dissolving ascorbic acid (0.1 m*M*) in water. To prepare the hybrid material, the solutions of gold, organic nanoparticles **N1** and ascorbic acid were mixed in the ratio of 9:0.1:9. A pink colored solution was formed which confirmed the formation of Au@**N1**, i.e., the hybrid material. The hybrid material **H1** was characterized using DLS and TEM studies.

For the recognition studies, the photophysical studies were carried out at 25±1 °C. Before recording the spectra, the solutions were shaken and kept for about 30 minutes. The binding efficiency of **H1** (0.1 m*M*) in aqueous medium was determined by addition of 20 µ*M* of metal nitrate salts to 5 mL solutions taken in volumetric flasks. For the titrations, the metal nitrate salt of Hg^{2+} was added to volumetric flasks containing (**H1**) in aqueous medium. The interference due to different heavy metal ions was checked by the addition of interfering cations (20 µ*M*) to the (**H1**) solution containing Hg^{2+} ion. The effect of ionic strength was explored by recording the spectra at different concentration of tetrabutylammonium perchlorate (0-100 equivalent).

Results and discussion

The formation of compound (**1**) (Scheme 1) was confirmed by various spectroscopic techniques such as ^1H NMR and mass spectroscopy (Supplementary data, Figs S1 and S2).

(**N1**) was prepared by the reprecipitation method, in which a dilute solution of compound (**1**) dissolved in good solvent was vigorously injected into a poor solvent with sonication. The difference in solubility of the compound (**1**) between the good and poor solvent led to the nucleation and thus formation of nanoparticles. The solubility of compound (**1**) was checked in different solvents and it was found to have good solubility in



Scheme 1

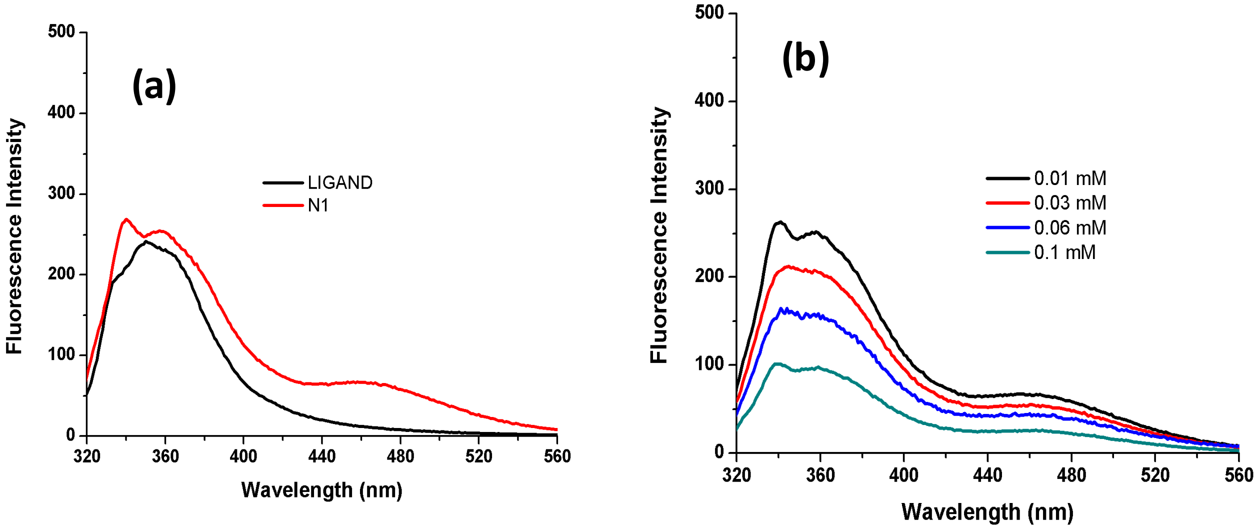


Fig. 1 – (a) Fluorescence spectra of compound (**1**) in DMSO, and nanoparticles (**N1**) in pure water, and, (b) Variation in fluorescence emission intensity at varying concentrations of (**N1**) [0.01, 0.03, 0.06 and 0.1 m*M*].

DMSO. Compound (**1**) dissolved in DMSO was then injected in water with sonication which led to the formation of organic nanoparticles (**N1**).

To assess the effect of water content on compound (**1**), fluorescence spectrophotometer was employed. Compound (**1**) displays emission peak at 350 nm in DMSO. Upon changing the solvent system from DMSO to water, formation of the nanoparticles (**N1**) has taken place, which shows two emission peaks at 340 and 357 nm and a weak shoulder at 469 nm (Fig. 1a).

To examine the influence of increasing concentration of compound (**1**) on the photophysical properties of (**N1**), four different concentrations of (**N1**) (0.01, 0.03, 0.06 and 0.1 m*M*) were prepared and their fluorescence spectra were recorded. On increasing the concentration of compound (**1**), a regular increase in emission intensity is seen with almost no shift in fluorescence intensity (Fig. 1b).

Dynamic light scattering (DLS) and transmission electron microscopy (TEM) were used to evaluate the formation of (**N1**). TEM image shows the formation of spherical sized particles 18 nm in size (Fig. 2a). The average particle size with DLS was found to be 25 nm (Fig. 2b).

Hybrid assembly of Au nanoparticles on the surface of organic nanoparticles **N1** were prepared using (**N1**), $HAuCl_4$ and ascorbic acid. Solutions of (**N1**), $HAuCl_4$ and ascorbic acid were mixed with continuous sonication and the appearance of pink confirmed the formation of the hybrid assembly (**H1**). TEM image recorded to estimate the size of (**H1**) showed the particle size to be 28 nm (Fig. 3a). Also, DLS analysis showed the average particle size to be 35 nm (Fig. 3b).

In this study, we aimed to investigate the sensing ability of (**H1**) towards heavy metal ions in aqueous medium. In order to carry out the photophysical

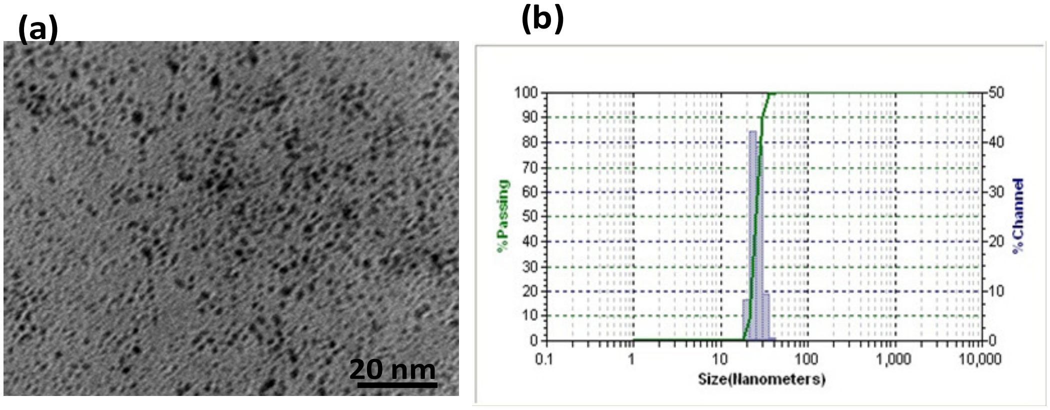


Fig. 2 – (a) TEM image of nanoparticles (**N1**), and, (b) distribution of particle size of (**N1**) by dynamic light scattering, showing average particle size of 25 nm.

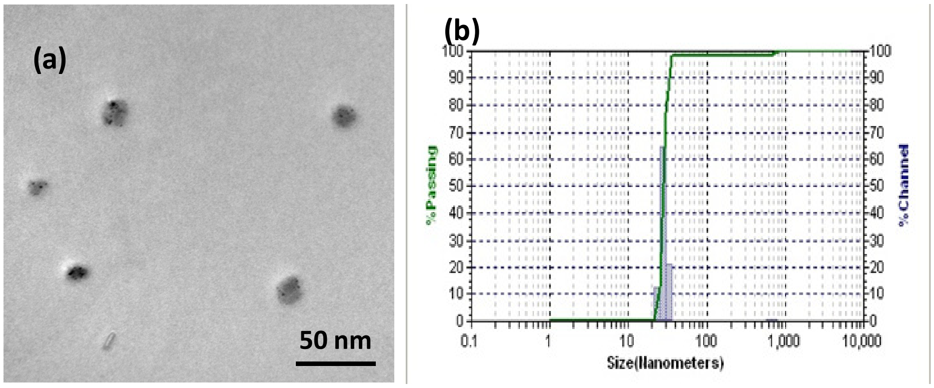


Fig. 3 – TEM image of (**H1**) with particle size 28 nm, and, (b) distribution of particle size of (**H1**) by dynamic light scattering, showing average particle size of 35 nm.

studies of (**H1**), various heavy metal ion solutions (Zn^{2+}, Ag^{+}, Cd^{2+}, Pb^{2+}, Al^{3+}, Hg^{2+}) were taken. Their solutions (20 µ*M*) were added to volumetric flasks containing 5 mL of 0.1 m*M* of (**H1**). It was observed that upon addition of Hg^{2+} ions there was an increase in fluorescence emission profile at 340 nm and a slight decrease in fluorescence intensity at 469 nm, showing ratiometric response (Fig. 4). However, no such response for other heavy metal ions was observed when binding studies were performed.

To further explore the binding affinity of Hg^{2+} ion towards (**H1**), titrations were carried with incremental addition of Hg^{2+} ion to (**H1**). With the successive addition of Hg^{2+} ion (0–10 µ*M*) to (**H1**), there was a regular enhancement in the fluorescence intensity at 340 nm and a slight decrease in the emission profile at 469 nm (Fig. 5). Titration studies showed linearity in the range of (0–10 µ*M*) of Hg^{2+} ion. From titration studies, the limit of detection for Hg^{2+} ion was calculated to be 1.9 n*M*.

For a practical and efficient system in a complicated environment and presence of competing species, development of a highly selective and sensitive sensing system is necessary. To check the recognizing ability of (**H1**) for Hg^{2+} in the presence of other competing metal ions, competitive binding studies were performed. There was negligible effect on recognition of Hg^{2+} ion by other interfering species which confirms that (**H1**) works efficiently for determining Hg^{2+} ion. The response of (**H1**) towards Hg^{2+} as a function of time was monitored. Varying concentrations of Hg^{2+} (3 µ*M*, 6 µ*M* and 9 µ*M*) were added to a fixed concentration of **H1** (0.1 m*M*) and the emission spectra were recorded at regular intervals of time. The results show that Hg^{2+} ion binds and interacts with (**H1**) within the first 20 s and then no change in fluorescence emission intensity was noticed up to 140 s (Fig. 6). Further, the emission response towards varying *p*Hs was examined. The results revealed that (**H1**) showed

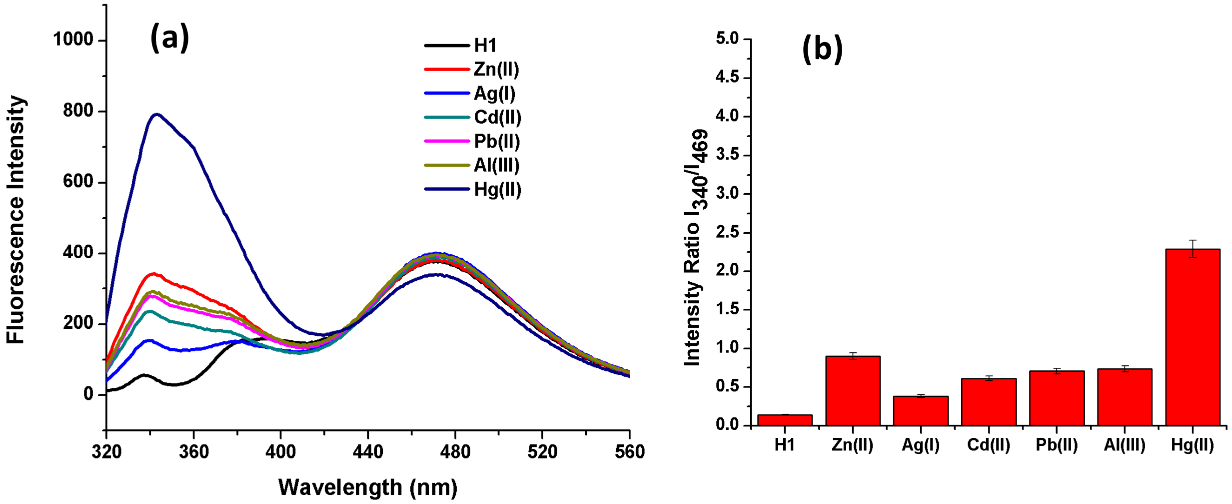


Fig. 4 – (a) Variation in the fluorescence intensity of (**H1**) upon addition of 20 μ*M* of the metal ion (Zn^{2+}, Ag^{+}, Cd^{2+}, Pb^{2+}, Al^{3+}, Hg^{2+}), and, (b) fluorescence ratiometric response of (**H1**) upon addition of 20 μ*M* of the metal ion (Zn^{2+}, Ag^{+}, Cd^{2+}, Pb^{2+}, Al^{3+}, Hg^{2+}).

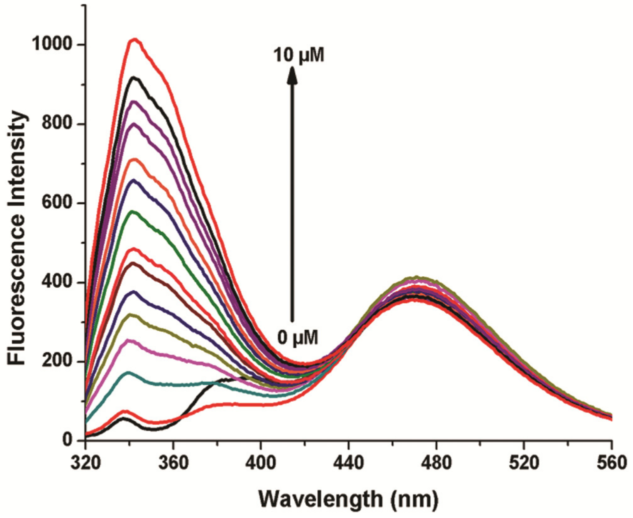


Fig. 5 – Changes in fluorescence emission intensity of (**H1**) upon successive addition of Hg^{2+} ion (0-10 μ*M*).

negligible effect when the solution *p*H was varied from 4-10 (Supplementary data, Fig. S3). Thus, the precise and accurate results can be obtained for sensing of Hg^{2+} ion using (**H1**) in the *p*H range of 4–10. Similarly, the consequence of variation in salt concentration was checked by fluorescence spectroscopy by addition of increasing concentration of the electrolyte, viz., tetrabutylammonium perchlorate (TBAP). It was observed that addition of even a very high concentration of TBAP (100 equiv.) did not influence the performance of (**H1**) in determination of Hg^{2+} ion in aqueous medium (Supplementary data, Fig. S4).

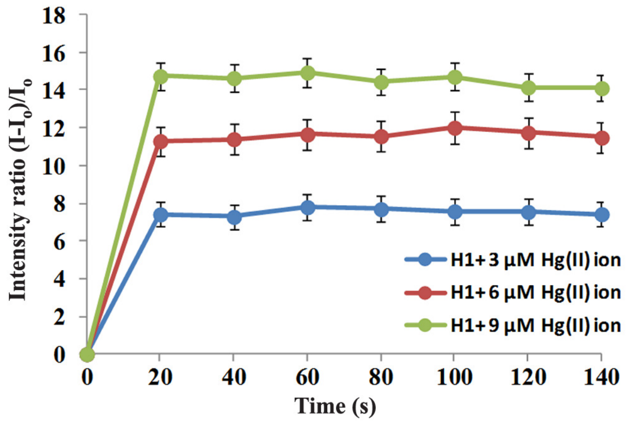


Fig. 6 – Plot of relative intensity ratio of (**H1**) of various concentrations of Hg^{2+} ion as a function of time.

In this study, we have prepared hybrid nanomaterial (**H1**) and evaluated its recognition behaviour towards heavy metal ions (Zn^{2+}, Ag^{+}, Cd^{2+}, Pb^{2+}, Al^{3+} and Hg^{2+}). The hybrid nanomaterial (**H1**) showed selective ratiometric response towards Hg^{2+} ion. The titrations have displayed linear binding towards Hg^{2+} ion and limit of detection obtained is 1.9 n*M*. Interference studies showed that other heavy metal ions showed no interference in the estimation of the (**H1**).Hg^{2+} complex, indicating that (**H1**) is highly selective towards Hg^{2+} ion.

Supplementary data

Supplementary data associated with this article, i.e., Figs S1-S4, are available in the electronic form at http://www.niscair.res.in/jinfo/ijca/IJCA_55A(06)692-697_SupplData.pdf.

Acknowledgement

NS is thankful to Department of Science & Technology, New Delhi, India, for research funding through research project (File N: EMR/2014/000613). AS acknowledges DST-PURSE for fellowship.

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