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Selective removal and quantification of Cu(II) using fluorescent iminocoumarin-functionalized magnetic nanosilica†

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A newly prepared iminocoumarin-functionalized magnetic nanosilica (Ni@SiO$_2$-1) was found to form a selective stable complex with Cu^{2+} over other metal ions. Quantification of Cu^{2+} ions in aqueous solution using Ni@SiO$_2$-1 is demonstrated through a fluorescent demetallization ensemble process.

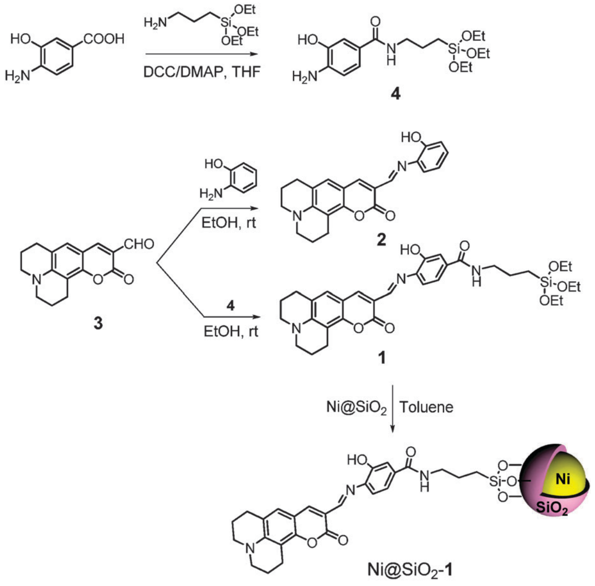
Copper is one of the abundant essential trace elements in the human body and can be commonly found as Cu^{2+} in natural water.[1] However, due to its potential to induce damages in proteins and DNA structures *via* ROS formation in biological systems,[2] excessive uptake of Cu^{2+} even for a short period of time may cause gastrointestinal disturbance and long-term exposure to it may result in liver and kidney damages.[3] Thus, efficient, economic and environmentally viable scientific techniques for selective monitoring and removal of Cu^{2+} from other metal mixtures or pollutants are highly desirable. Moreover, these methods are very useful in preventing copper poisoning in environmental and biological fields.[4]

Magnetic silica nanoparticles have been also of great interest in biomedical and environmental applications such as bioseparation, drug targeting, cell isolation *etc.*[5] Development of hybrid nanomaterials based upon a combination of their magnetic property with an additional function such as selective ligand binding ability, for example, that would endow diverse and noble functionalities to the nanomaterials is highly enviable. Indeed, studies on removal techniques of Cu^{2+} by using fluorescent nanoparticles have been introduced in biological and environmental systems.[6]

In this regard, herein we report the design and synthesis of core/shell type magnetic nanosilica (Ni@SiO$_2$-**1**) functionalized with an iminocoumarin moiety **2** which can form a stable complex with Cu^{2+}, but undergo fluorogenic hydrolysis in its absence in an aqueous environment. To prove the causative factors for this fluorescent response, Ni@SiO$_2$-**1** was tested with various metal ions in this study. Hence, we herein present the feasibility of Ni@SiO$_2$-**1** for selective capture of Cu^{2+} over other metal ions and quantification of the entrapped Cu^{2+} ions by virtue of fluorescence changes.

The synthetic pathways to Ni@SiO$_2$-**1** and **2** are depicted in Scheme 1. Compound **2** was prepared by adaptation of our previously reported synthetic procedures.[7] Nickel nanoparticles were prepared by a modified polyol process.[8] The particles were coated with silica shells through the Stöber method using tetraethyl orthosilicate (TEOS) as a silica source in water to yield Ni@SiO$_2$ core/shell particles[9] as detailed in ESI.† Finally, Ni@SiO$_2$-**1** was prepared by a coupling reaction of **1** with Ni@SiO$_2$ core/shell particles in toluene under reflux conditions. Morphological change of the Ni@SiO$_2$-**1** was observed by TEM. Detailed characterizations of the prepared compounds are provided in ESI† as well.

In our earlier study, we found that a complex of **2**–Cu^{2+}, where the *o*-OH unit of **2** as an additional binding site is essential to make the stable metal complex, is resistant to hydrolysis in aqueous solution. The 1 : 1 complex of **2**–Cu^{2+} was evidenced by the ESI-MS spectrum (Fig. S1, ESI†) and X-ray crystal structure (Fig. S2, ESI†), respectively. However, upon Cu^{2+} elimination, non-fluorescent **2** was rapidly hydrolyzed to produce a strongly fluorescent **3** revealing an absorption band



Scheme 1 Synthetic routes to **1**, **2** and Ni@SiO$_2$-**1**.

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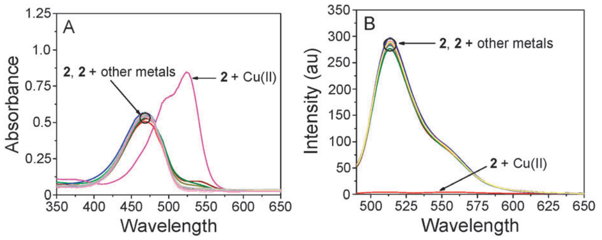

Fig. 1 (A) UV-Vis and (B) fluorescence spectra of **2** (10 μM) with addition of Li^+, Na^+, K^+, Rb^+, Cs^+, Ag^+, Cd^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+}, Zn^{2+}, Ca^{2+}, Pb^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Hg^{2+}, Fe^{2+}, Al^{3+}, Fe^{3+}, and In^{3+} (5 equiv., respectively) in aqueous solution (10 mM PBS buffer, pH 7.4, containing 1.0% CH_3CN) after 10 min.

at 465 nm and an emission band at 515 nm (Φ_f = 0.67) in aqueous solution (10 mM PBS buffer, pH 7.4, containing 1.0% CH_3CN) (Fig. S3 and Table S2, ESI†). From this result, it can be envisaged that **2** would play an important role in the selective detection and removal of Cu^{2+} unless it is hydrolyzed by Cu^{2+} complexation in aqueous solution. Thereby, the hybrid nanomagnet **1** would form a stable complex with Cu^{2+} followed by separation of Cu^{2+} by its magnetic property. Then, the exact amount of entrapped Cu^{2+} ions by Ni@SiO_2-**1** can be easily estimated by fluorescence changes after Cu^{2+} ion stripping.

At first, to investigate the metal ion selectivity of **2**, absorption and emission spectra of **2** in aqueous solution (10 mM PBS buffer, pH 7.4, containing 1.0% CH_3CN) were examined by the addition of various metal ions. As indicated in Fig. 1A, hydrolysis of **2** is exclusively inhibited in the presence of Cu^{2+} whereas the hydrolysis was observed with other metal ions (Li^+, Na^+, K^+, Rb^+, Cs^+, Ag^+, Cd^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+}, Zn^{2+}, Ca^{2+}, Pb^{2+}, Ni^{2+}, Co^{2+}, Hg^{2+}, Fe^{2+}, Al^{3+}, Fe^{3+}, and In^{3+}). Absorption and emission bands of each product formed from the addition of metal ions to a solution of **2** are the same as those of **3** (Fig. S4, ESI†). In addition, the ^{1}H NMR spectra of the hydrolysis products are identical to that of **3** (Fig. S5, ESI†). The fluorescence quantum yields (Φ_f = 0.65) of a mixture of **2** with other metal ions are also similar to that of **3** (Φ_f = 0.69) (Table S2, ESI†). On the other hand, upon the addition of Cu^{2+}, the fluorescence intensity of **2** declined (Fig. 1B). These results confirm again that **2** selectively encapsulates the Cu^{2+} ion which is not hydrolyzed by aqueous media. Studies of fluorescence-enhancement factor (FEF) of **2** (10 μM) with and without Cu^{2+} (5.0 equiv.) in the presence of other metals (Li^+, Na^+, K^+, Rb^+, Cs^+, Ag^+, Cd^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+}, Zn^{2+}, Ca^{2+}, Pb^{2+}, Co^{2+}, Cu^{2+}, Hg^{2+}, Fe^{2+}, Al^{3+}, Fe^{3+}, and In^{3+}) in aqueous solution (10 mM PBS buffer, pH 7.4, containing 1.0% CH_3CN) are also implemented and their results are shown in Fig. S7 (ESI†). Both free **2** and metal complexes of **2** except Cu^{2+} ion were found to be hydrolyzed to give strongly fluorescent **3**. To the best of our knowledge, this ensemble system based on the blocking hydrolysis caused by Cu^{2+} ion complexation has been rarely exploited so far.

To prepare the hybrid nanomagnet (Ni@SiO_2-**1**), the non-fluorescent **1** was immobilized on the Ni@SiO_2 core/shell particles under reflux in toluene for 24 h. In this process, the triethoxylsilyl group of **1** undergoes hydrolysis to covalently attach to the surface of the Ni@SiO_2 core/shell particles. Cooling the reaction mixture to room temperature and filtering the red precipitates provided Ni@SiO_2-**1** in quantitative yield.

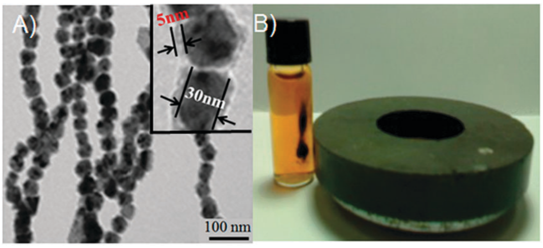

Fig. 2 (A) TEM image of Ni@SiO_2-**1**. (B) Photograph of a magnet attracting Ni@SiO_2-**1** in MOPS buffer solution (pH 7.0).

A transmission electron microscopic (TEM) image of Ni@SiO_2-**1** revealed that it appears to have a spherical structure with narrow size distribution (*ca.* 35 nm) with a 30 nm Ni core, thereby it maintained a nanocrystalline appearance (Fig. 2). IR and TOF-SIMS results are in good accordance with a bond formation between Ni@SiO_2 and **2**. The IR spectrum showed new strong bands at 3632, 3282, 2918, 2848, 2369, 2330, 1698, 1564, 1515 and 1094 cm^{-1} which originated from **2** (Fig. S10, ESI†). The TOF-SIMS spectrum of **1** residing on the Ni@SiO_2 core/shell nanoparticles displayed the signals at m/z = 484, 543 attributable to **1** and its fragment, implicating that **1** is anchored onto the surface of the Ni@SiO_2 core/shell nanoparticles (Fig. S11, ESI†). The amount of **2** on the Ni@SiO_2 surface was estimated to be 1.66 ± 0.05 nmoles per mg (n = 4) of the nanomagnet, based on the recovered fluorescence intensity from the complete hydrolysis of Ni@SiO_2-**1** compared to that of **2** (Fig. S12, ESI†).

Absorption and emission spectroscopic measurements of Ni@SiO_2-**1** in the presence of biologically and environmentally relevant metal ions were performed in 0.2 M MOPS, buffer pH 7. The absorption spectrum of free Ni@SiO_2-**1** in the absence of metal ions showed a single absorption band at 465 nm and a corresponding emission maximum at 515 nm (Fig. S13, ESI†). In the aspect of fluorescence changes, the Ni@SiO_2-**1** emits a strong fluorescence in the presence of metal ions except the Cu^{2+} ion due to its rapid hydrolysis to generate **3** in aqueous media as seen in Fig. 3 and 4. However, the Ni@SiO_2-**1**–Cu^{2+} complex was not indeed hydrolyzed, but maintained its fluorescence intensity. In this regard, it should be also noteworthy that

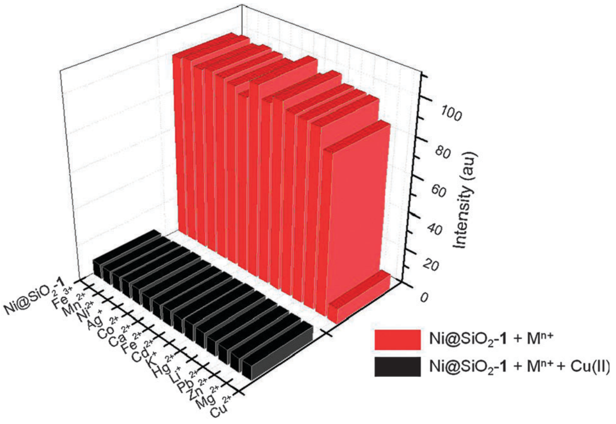

Fig. 3 Fluorescence intensities of Ni@SiO_2-**1** (0.1 mM) (red bar) and Ni@SiO_2-**1** with Cu^{2+} (10 equiv.) (black bar) upon addition of Li^+, K^+, Ag^+, Cd^{2+}, Mg^{2+}, Zn^{2+}, Ca^{2+}, Pb^{2+}, Ni^{2+}, Co^{2+}, Hg^{2+}, Mn^{2+}, Fe^{2+}, and Fe^{3+} (10 equiv., respectively) in MOPS buffer solution (pH 7.0) after 10 min.

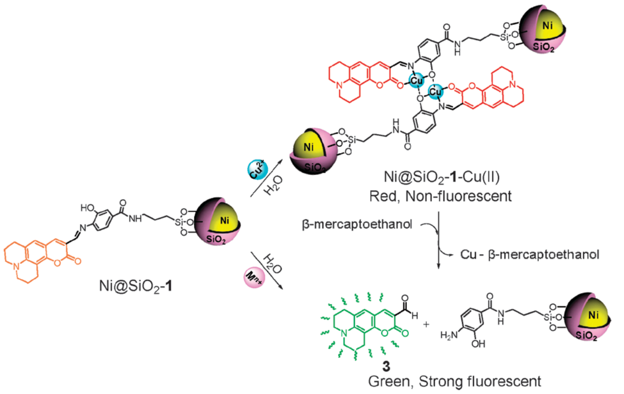

Fig. 4 Schematic representation of formation of a stable complex of Ni@SiO$_2$-**1** with Cu^{2+} ion followed by hydrolysis by a thiol to generate fluorescence-on.

the Ni@SiO$_2$-**1** would be a great promise as a useful selective chemosensor for detection and removal of Cu^{2+}.

We also examined the pH dependency of Ni@SiO$_2$-**1** on the Cu^{2+} uptake. As seen in Fig. S14 (ESI†), the fluorescence band was constantly observed in the pH range of 4–11, suggesting that complexation of Ni@SiO$_2$-**1** with Cu^{2+} readily undergoes in the pH range of 4–11, which is applicable for biological and environmental fields. From the fluorescence changes of the Ni@SiO$_2$-**1** upon binding with the Cu^{2+} ion, the detection limit was calculated to be 0.15 ppb of [Cu^{2+}] (Fig. S15, ESI†).

To get insight into the linear relationship of fluorescence intensity *vs.* Cu^{2+} concentration, emission intensities of the Ni@SiO$_2$-**1** were measured with various concentrations of Cu^{2+} ions (Fig. 5A). The fluorescence intensity of the Ni@SiO$_2$-**1** decreased at 515 nm as the [Cu^{2+}] reached 1.0 equiv. (inset of Fig. 5A). In order to quantify the entrapped Cu^{2+} ions in the Ni@SiO$_2$-**1**, the Cu^{2+} ion embedded Ni@SiO$_2$-**1** was separated and treated with 1.0 mM of β-mercaptoethanol solution. We then in turn observed a marked enhancement in fluorescence from the solution, which is obviously attributed to the hydrolysis of Ni@SiO$_2$-**1** to give compound **3**. Within this implementation, we noticed that variation of Cu^{2+} concentration provides linearity as depicted in Fig. 5B, which should be one of the useful and important standards to determine metal ions in aqueous media. These results led us to conclude that Ni@SiO$_2$-**1** can form a stable complex with Cu^{2+} ions in the aqueous solution, and the amount of Cu^{2+} ions entrapped can be quantified based on the measurement of the fluorescence after treatment with β-mercaptoethanol (see also Fig. 4).

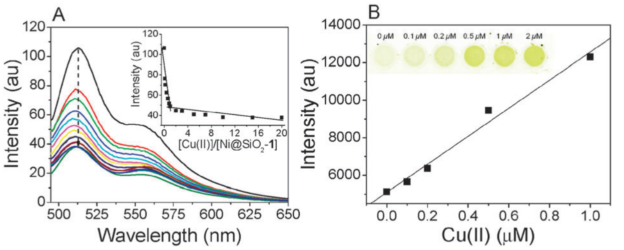

Fig. 5 (A) Fluorescence titrations of Ni@SiO$_2$-**1** (0.1 mM) upon addition of increasing Cu^{2+} concentrations (0, 0.1, 0.2, 0.4, 0.6, 0.8, 1, 2, 3, 5, 7, 10, 15, and 20 equiv.) in MOPS buffer solution (pH 7.0) after 10 min. The inset: plot for fluorescence intensity of Ni@SiO$_2$-**1** as a function of Cu^{2+} concentration. (B) Fluorescence intensities of Ni@SiO$_2$-**1**–Cu^{2+}. The fluorescence intensities and the image of 96 well plate were obtained by fluorometry and a scanner.

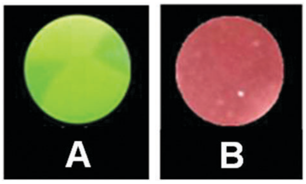

Fig. 6 Photogram of Ni@SiO$_2$-**1** (10 mg) (A) before and (B) after immersion into Cu^{2+} (10 μM) by irradiation with a UV lamp.

In order to extend the above performance to a portable chemosensor kit, a disk-type pellet has been prepared with Ni@SiO$_2$-**1** (Fig. 6). Upon dipping in Cu^{2+} (10 μM) solution the pale yellow pellet of Ni@SiO$_2$-**1** changed into red with fluorescence quenched due to its Cu^{2+} ion complexation (Fig. 6B). On the other hand, in the parallel experiments with Co^{2+}, Mn^{2+}, Cd^{2+}, Ag^{+} and Pb^{2+} solutions (10 μM each), pale yellow fluorescence was observed caused by its hydrolysis. The result implies that the disk-type pellet prepared from Ni@SiO$_2$-**1** is applicable as a portable chemosensor for the detection of Cu^{2+} in the environmental field as well.

In conclusion, we synthesized a core/shell type magnetic nanosilica (Ni@SiO$_2$-**1**) functionalized with iminocoumarin moiety **2** which can form a stable complex with Cu^{2+} in an aqueous environment. The stable complex is found to be not hydrolysed even in an aqueous solution. We then found that the Ni@SiO$_2$-**1** is capable of selectively entrapping Cu^{2+} ions over other metals and the captured Cu^{2+} ions can be quantified by means of fluorescence spectroscopy. These are promising findings for the development of a new category of biocompatible systems built by immobilizing appropriate fluorogenic receptors on the surfaces of novel magnetic nanomaterials for detection, recovery and separation of other heavy metal ions from environmental pollutants.

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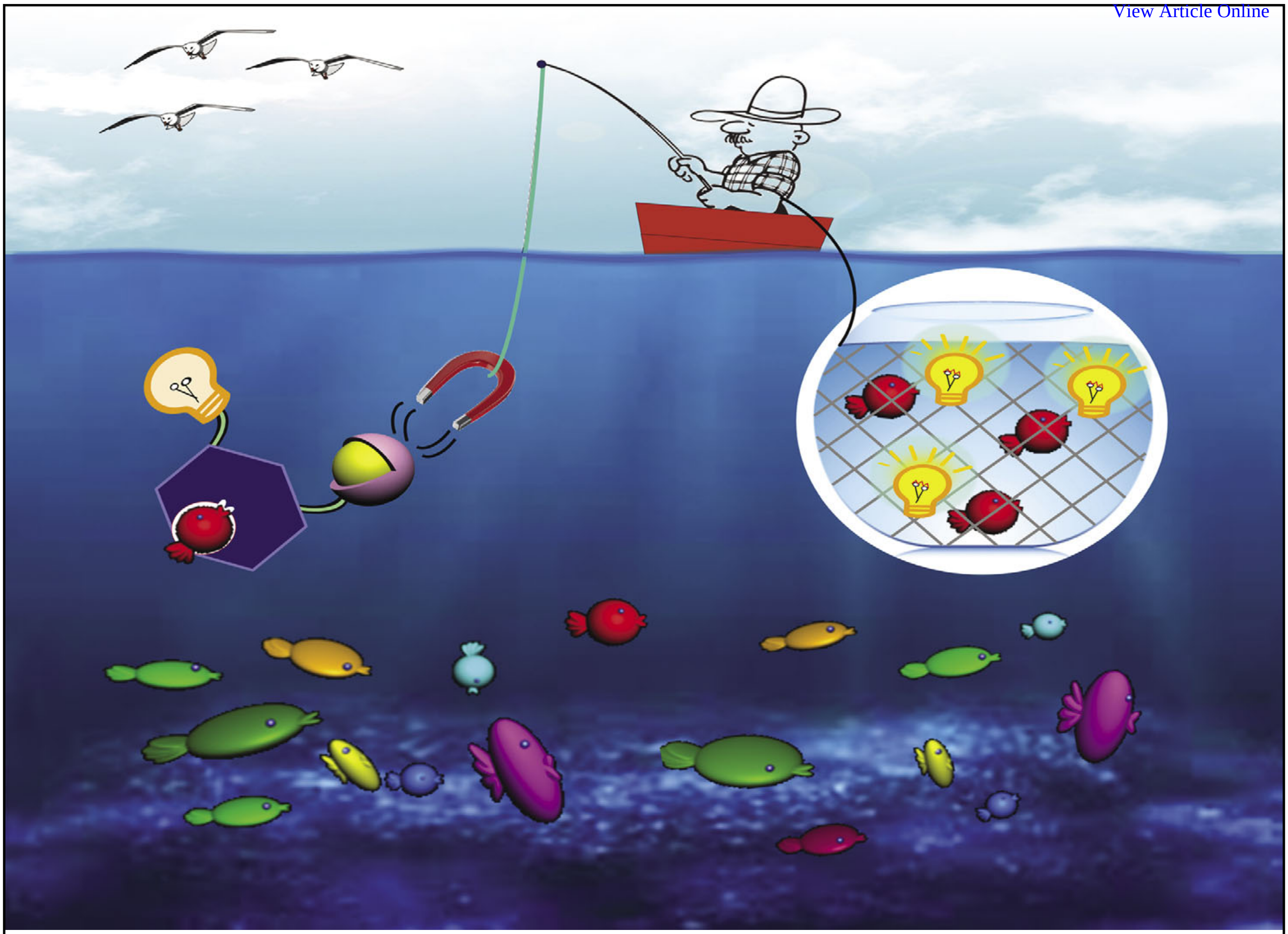

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Selective removal and quantification of Cu(II) using fluorescent iminocoumarin-functionalized magnetic nanosilica

A newly prepared iminocoumarin-functionalized magnetic nanosilica (**Ni@SiO$_2$-1**) forms a selective stable complex with Cu^{2+} over other metal ions. Quantification of removed Cu^{2+} ions from aqueous solution using **Ni@SiO$_2$-1** is demonstrated through a fluorescent demetallization ensemble process.

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