A New Reusable Mercury-Sensitive Turn-On Nano-Chemosensor Based on Functionalized CoFe$_2$O$_4$@SiO$_2$ Magnetic Nanocomposite

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ABSTRACT

A novel "turn-on" nano chemosensor for Hg$^{2+}$ was developed based on CoFe$_2$O$_4$@SiO$_2$ nanocomposite. Cobalt nano ferrite particles were decorated with 1,8-naphthalimide dye conjugated with rhodamine dye. It was characterized using X-ray powder diffraction (XRD), transmission electron microscopy (TEM), FT-IR techniques. A fluorescence enhancement was observed upon binding Hg$^{2+}$ to two core chromogenic dyes. No significant change in the fluorescence intensity was observed in the presence of other metal ions. The results showed that the functionalized nanocomposite CoFe$_2$O$_4$@SiO$_2$/NR exhibited selective 'turn-on' fluorescent enhancements with Hg$^{2+}$. Also, the company of magnetic CoFe$_2$O$_4$@SiO$_2$ nanocomposite (with a wide range of applications such as biomedicine, magnetic fluids, magnetic energy storage) and catalysis facilitates the magnetic separation of the Hg (II) from the solution. Nano chemosensor exhibits high selectivity, high sensitivity and fast response to trace mercury ions. Designed nanosensor successfully applied for Hg$^{2+}$ determination at real samples with a linear range of 0.04-0.76 µM of Hg$^{2+}$ ions. It was successfully applied for the determination of mercury ion in real samples of tap water. It seems that the presence sensor is a suitable candidate for the detection of trace mercury ion in biomedical samples like a human serum. Also, it is fast and easy control for monitoring of water toxicity of pollutant industries. Prog. Color Colorants Coat. 15 (2022), 75-85 © Institute for Color Science and Technology.

1. Introduction

Mercury is one of the most toxic ions in the environment; its absorption in the human organs causes severe problems on the nervous system, kidneys, and endocrine. Because of high toxicity toward human health, the development of the quick, easy, and efficient detection method for mercury ion is strongly felt [1, 2]. Traditional quantitative methods, such as atomic absorption spectrometry (AAS) and inductively coupled plasma mass spectrometry (ICP-MS), have
been applied for mercury analysis [3-5]. The mentioned methods are efficient for Hg$^{2+}$ detection; however, they are time-consuming and require highly trained personnel and expensive instrumentation.

The design and development of chemosensors for detection of environmentally and biologically essential metal cations are currently of great importance since they allow the determination of metal cations by a simple absorbance and fluorescence enhancement (turn-on) or quenching (turn-off) response [6-10].

As well known, the rhodamine with spirolactam structure is non-fluorescent. Ring-opening of the spirolactam gives rise to strong fluorescence emission. This property provides an ideal model to construct OFF–ON fluorescent switch sensors. On the other hand, they have a longer emission wavelength (550 nm), which is often interested in serving as reporting probe for the analyte to avoid the influence of the background emission (below 500 nm). Thus the design of new rhodamine base structures is still considered [11-14].

1,8-Naphthalimides have attracted general interest in the design of fluorescent molecular devices, such as chemosensing, switching, and logic operations. 4-amino-1,8-naphthalimides have high emission intensity with significant stock shift with excellent stability among other fluorescence dyes and the possibility of targeted functionality. They are suitable for application as chemo-sensing probes [15-20].

The development of nanoscale materials for optical chemical sensing applications has emerged as one of the most critical areas of interest over the past decades. The nanomaterials’ chemical and physical properties are size- and shape-dependent, show unique surface chemistry, high surface area, and large pore volume per mass unit area. Their unique properties can help improve the sensitivity, response time, and detection limit of sensors. Suitable dispersion of functionalized nanoparticles gives rise to higher sensitivity and stronger signals in solid-state and solution.

Ferrite magnetic materials have been intensively studied because of the fundamental understanding as well as their applicability in a variety of areas such as high-density information storage, magnetic printing inks, ferrofluid technology, magnetic drug delivery, magnetic refrigeration, medical diagnostics, catalysts, magnetic resonance imaging enhancement, and gas sensors [21]. Cobalt ferrite has attracted considerable attention among spinel ferrites due to its unique physical properties (curie temperature and coercivity) and moderate saturation magnetization (large magnetostrictive coefficient). It has excellent chemical stability and mechanical hardness [22].

Cobalt ferrite is of great interest for many disciplines, such as biomedicine, magnetic fluids, magnetic energy storage, and catalysis. It is vital to make a ceramic material of desirable microstructure in many electronic and magnetic applications, with a high sintered density, a small particle size, and a narrow particle size distribution [21-24]. Moreover, its structural, magnetic, and electrical properties can be tailored by selecting preparation, chemical composition, dopant ions, and thermal treatment [21].

The sol-gel process is a chemical route used to synthesize ceramic materials at relatively low temperatures based on wet chemistry. It is based on wet chemistry processing. Advantages of the sol-gel method may include better control of the structure, including porosity and particle size, the possibility of incorporating nanoparticles and organic materials into sol-gel-derived oxides. It allows the fabrication of any oxide composition and some non-oxides and the production of new hybrid organic-inorganic materials, which do not exist naturally, better homogeneity due to mixing at the molecular level. It has high purity and less energy consumption—also no need for special or expensive equipment [25]. The preparation of CoFe$_2$O$_4$/SiO$_2$ nanocomposites by sol-gel method using three short-chain diols (1,2-ethanediol, 1,3-propanediol, and 1,4-butanaediol) as chelators has been reported. The X-ray diffraction analysis showed that the chain length of the carboxylates embedded in the silica matrix influences the formation of crystallized cobalt ferrite as a single-phase at low temperatures.

Design and Synthesis of functional fluorescence dye as molecular and ion probe for chemosensing applications is undergoing by our research group. Design of the fluorescence chemosensors based on functionalized 1,8- naphthalimides is in progress in our group. Fluorescence probes were constructed to determine bio and environmentally essential ions such as fluoride and pH, molecular recognition sensors for DNA and drugs [26-29].

Herein we designed and synthesized the novel CoFe$_2$O$_4$@SiO$_2$ nanocomposite functionalized with 1,8-naphthalimide conducting rhodamine dye. Nano chemosensor exhibits a high selectivity toward mercury ions. After the addition of mercury, a turn-on
fluorescence enhancement takes place. Characterization and sensing behavior was studied briefly. Its magnetic properties enable it to be recovered and controlled easily.

2. Experimental

2.1. Materials and methods

All chemicals were of the reagent–grade from Merck (Germany). The nitrate salts of all cations used were used without any further purification. Tetraethyl orthosilicate (TEOS) was purchased from Merck. The 4-bromo-naphthalimide were synthesized using reported methods [28]. The \textsuperscript{1}H and \textsuperscript{13}C NMR spectra were recorded using a Bruker 500 spectrometer. The IR spectra were taken using a Perkin Elmer (KBr disks) within 4000–400 cm\textsuperscript{-1}.

X-ray diffraction (XRD) was performed on a Philips PNA-analytical diffractometer using Cu K\textalpha radiation. TEM images were recorded on a Philips CM 200 FEG transmission electron microscope. Selected area electron diffraction (SAED) patterns were obtained on the TEM to ascertain the crystallinity. Energy dispersion X-ray analysis (EDXA) was performed on the TEM. Magnetic measurements were carried out at room temperature by using a vibrating sample magnetometer (VSM).

The emission spectra were conducted on a Perkin-Elmer LS55 luminescence spectrometer. Fluorescence measurements were done in a quartz cuvette with a 1cm diameter. This solution was titrated with a standardized Hg\textsuperscript{2+} ion solution, and the system's fluorescence intensity was measured. The emission intensities were measured at an excitation wavelength of 480 and 580 nm.

2.2. Synthesis

2.2.1. Synthesis of 4-bromo-N-propyl (trithoxysilane) 1,8-naphthalimide (N)

Compound N was prepared according to the reported method in the literature [28]. The intermediate compound N was ready at 85% yield by the reaction of 0.275 mmol of compound 1 with 2.2 mmol of 3-aminopropyl-trithoxysilane, in ethanol. The solvent evaporated by rotary evaporation, and the crude product recrystallized with ethanol to give N (Scheme 1a).

Yield 85%, a pale yellow solid, m.p. 129.57 \textdegree C; \textsuperscript{1}H NMR (500 MHz DMSO), ppm: 8.44 (d, 1H), 8.39 (d, 1H), 8.20 (d, 1H), 8.08 (d,1H), 7.93 (t, 1H), 3.95 (t, 2H), 3.71 (m, 6H), 1.66 (m, 2H), 1.10 (t,9H), 0.61 (t, 2H); \textsuperscript{13}C NMR (125 MHz DMSO), ppm: 7.49, 18.15,20.90, 38.64, 38.92, 39.19, 39.47, 39.75, 40.03, 40.31, 42.29, 57.71, 121.84, 122.62, 128.09, 128.60, 128.95, 129.57, 130.76, 131.17, 131.38, 132.37, 162.66, 162.71; IR (KBr) cm\textsuperscript{-1}, 3066, 3092, 1989, 1702, 1571, 1590, 1082, 1166, 1346, 778.

2.2.2. Synthesis of rhodamine-ethylen diamine (R)

Ethylenediamine (3.6 mL, 56 mM) was added dropwise to a solution of Rhodamine 6G (4.4 g, 9.2 mM) in 120 mL of absolute ethyl alcohol at room temperature. The resulting solution was stirred at reflux for 5 h. After cooling down to room temperature the solid precipitate was filtered off, washed with water and dried to give 3.57 g (85%) R as pale pink crystals (Scheme 1b). \textsuperscript{1}H NMR (CDCl\textsubscript{3} 300 MHz) ppm: 7.88 (m, 1 H); 7.44 (m, 2 H); 7.01 (m, 1 H); 6.35 (s, 2 H); 6.19 (s, 2 H); 3.62 (s, 2 H); 3.20 (m, 6 H); 2.34 (t, 2 H); 1.91 (s, 6 H); 1.31 (m, 8 H). FTIR (KBr) cm\textsuperscript{-1}: 3218, 2936, 2842, 1682, 1618, 1522, 1480, Elementalanalysis: Calculated for C\textsubscript{28}H\textsubscript{32}N\textsubscript{4}O\textsubscript{2} (MW 456.58) C 73.66%, H 7.06%, N 12.27%; Found C 73.38%, H 6.98%, N 12.08%.

2.3.3. Synthesis of CoFe\textsubscript{2}O\textsubscript{4}@SiO\textsubscript{2} nanocomposite

Nanocomposites of cobalt-ferrite (CoFe\textsubscript{2}O\textsubscript{4}) were coated with silica matrix by the sol-gel process. Metallic nitrates were chosen as precursors for ferrite preparation. The sol was prepared by dissolving Fe(NO\textsubscript{3})\textsubscript{3}·9H\textsubscript{2}O and Co(NO\textsubscript{3})\textsubscript{2}·6H\textsubscript{2}O in the molar ratio of 2:1 at room temperature in an aqueous solution. The molar ratios of H\textsubscript{2}O and Fe: Co were precisely controlled at 1:5 and 2:1, respectively. The ratio of nanocomposites was 30 wt.% CoFe\textsubscript{2}O\textsubscript{4} in the silica matrix. Fe(NO\textsubscript{3})\textsubscript{3},9H\textsubscript{2}O and Co(NO\textsubscript{3})\textsubscript{2},6H\textsubscript{2}O were dissolved in deionized water, followed by adding the aqueous solution of THEOS. Then vigorous stirring for one h, the sol was allowed to gel at room temperature. The obtained alcogel was dried put into an oven for further drying at 110 \textdegree C for 24 h to get the xerogel. Finally, the xerogel was calcined at 500 \textdegree C for two h with a heating rate of 10 \textdegree C/min in the ambient atmosphere.
2.3.4. Synthesis of CoFe$_2$O$_4$@SiO$_2$/NR

Compound N (0.21 g) and CoFe$_2$O$_4$ (1.0 g) were dissolved in anhydrous toluene (50 mL), then stirred and refluxed for 24 h. Finally, the precipitate was filtered and washed three times with CH$_2$Cl$_2$. Then obtained precipitates were added to compound R (0.25 g) and dissolved in DMF with Et$_3$N (0.25 g). Then the solution was refluxed for ten h. Finally, the precipitate was filtered and washed three times with ethanol.

3. Result and Discussion

3.1. Design and synthesis of CoFe$_2$O$_4$@SiO$_2$/NR nanocomposite

The XRD patterns of the nanocomposite are shown in Figure 1. The diffraction peaks approved the pure single-phase cobalt ferrite formation with the face-centered cubic spinel phase and Fd3 m (2 2 7) space group. No diffraction peaks were observed in the patterns. The vast peak observed at 2θ of around 23° in XRD patterns for all samples was attributed to the characteristic diffraction peak of the amorphous SiO$_2$ matrix.
The crystallite size for the sample was 9 nm estimated from XRD peak broadening using Scherrer's formula (Eq. 1),

\[
D = \frac{0.9 \lambda}{\beta \cos \theta}
\]  

(1)

Where \(D\) is the crystallite size, \(\lambda\) the wavelength of X-ray radiation (CuK\(\alpha\)), \(\theta\) the Bragg angle and \(\beta\) is the full width at half maximum (FWHM) of the most intense diffraction peak (311). The Scherrer equation is limited to nanoscale crystallites, or more strictly, the coherently scattering domain size, which can be smaller than the crystallite size [26]. XRD is applicable in the determination of phase identification, but porosity determines by the porosimeter device. But according to one classification, it is possible to say if the starting peak is higher than 8 or 9°, then this implies a small pore, but if it is in between 6 to 7 medium pore, 5 to 6 could be large pore, and 5 to 5.5 huge pore [27]. Therefore since starting peak is higher than 9°, then the nanocomposite implies small pores.

Fourier transforms infrared (FTIR) spectroscopy is an essential tool to explore local chemical bonds' nature in a material. As XRD does not provide evidence of amorphous SiO\(_2\) surface coating, we used FTIR spectroscopy to unfold the features of SiO\(_2\), ferrite, and also nanoparticle/SiO\(_2\) interactions. FT-IR spectra of CoFe\(_2\)O\(_4\)@SiO\(_2\) and CoFe\(_2\)O\(_4\)@SiO\(_2\)/NR were shown in Figure 2. The absorption bands at 467 and 474 cm\(^{-1}\) are due to the vibration of the chemical O-Co-O bond at octahedron position and O-Co-O chemical bond at tetrahedron position of CoFe\(_2\)O\(_4\) nanoparticle, respectively. The presence of these absorption bands indicates the formation of the CoFe\(_2\)O\(_4\) spinel ferrite structure.

It can be seen that the FT-IR spectrum of the calcined CoFe\(_2\)O\(_4\)@SiO\(_2\) sample shows the disappearance of the absorption at 1384 cm\(^{-1}\) could be related to the complete decomposition of the nitrate species after calcination. The peak at 605 cm\(^{-1}\) assigned to the Si-O-Fe vibration and \(\equiv\)Si-O-Si\(\equiv\) absorption band of the SiO\(_2\) tetrahedron of the silica network observed at 1079 cm\(^{-1}\). There are no signs of interaction band such as Fe–O–Si, which is usually found around 950 cm\(^{-1}\).

FTIR also characterized magnetic nanocrystallites of CoFe\(_2\)O\(_4\)@SiO\(_2\)/NR to confirm the successful conjugation of NR onto the surface of the CoFe\(_2\)O\(_4\)@SiO\(_2\) nanocomposite. The FTIR spectra of CoFe\(_2\)O\(_4\)@SiO\(_2\)/NR compared with the spectrum of CoFe\(_2\)O\(_4\) before grafting show that new bands refer to N's bonding on the CoFe\(_2\)O\(_4\) surface. The bands observed at 2921 and 2851 cm\(^{-1}\) are attributed to the C-H stretching frequency. The bands at 3300-3500/ cm and 1000-1300/cm of both samples are ascribed to the O-H

![Figure 2: FT-IR spectrum of CoFe\(_2\)O\(_4\)@SiO\(_2\) and CoFe\(_2\)O\(_4\)@SiO\(_2\)/NR samples.](image-url)
and Si-O stretching vibration on silane. CoFe$_2$O$_4$@SiO$_2$/NR exhibited a lactone (C=O) vibration at 1424/1385 cm$^{-1}$, and it is attributed to the naphthalimide and rhodamine ring. Observed results showed both CoFe$_2$O$_4$ and CoFe$_2$O$_4$@SiO$_2$/NR. CoFe$_2$O$_4$@SiO$_2$/NR was synthesized according to the procedure shown in Scheme 1.

TEM image of the nanocomposites calcined at 500 °C was shown in Figure 3. TEM results indicate that almost spherical CoFe$_2$O$_4$ nanoparticles were embedded in the silica network. According to Scherrer's formula, the average particle sizes (obtained from XRD analysis) of the calcinated nanocomposites calcined at 500 °C were about 11 nm.

The magnetic properties measured by vibrating sample magnetometer at room temperature for CoFe$_2$O$_4$@SiO$_2$ sample showed in Figure 4. It is found that the M-H curves of the sample take on incomplete reversibility, and corresponding Mr values are about 0.01 emu/g, which means that sample is superparamagnetic. For samples, most nanoparticles' size is smaller than the critical size to remain ferromagnetic; hence they will show superparamagnetic behavior.

3.2. Spectroscopic properties and optical responses

To evaluate whether CoFe$_2$O$_4$@SiO$_2$/NR could be used as a selective fluorescent chemosensor for Hg$^{2+}$ ion, we recorded the emission fluorescence spectra during the addition of the cations (1×10$^{-4}$ M) to an of the probe (1×10$^{-5}$ M) at 25.0±0.1 °C. The resulting fluorescence intensity in the presence of cations is shown in Figure 5. As it is evident from Figure 5, the intensity of the emission band at 580 nm enhanced with the addition of Hg$^{2+}$ ions. Other metal ions revealed no such addition in the emission band under the same conditions.

The mode of coordination of CoFe$_2$O$_4$@SiO$_2$/NR with Hg$^{2+}$ has been investigated by spectrophotometric titration at 298 K (0.2 g/L). Figure 5a shows typical fluorimetric titration curves of Co-ferrites@SiO$_2$/NR with different concentrations of Hg$^{2+}$ added. It is evident from Figure 5 that the intensity of CoFe$_2$O$_4$@SiO$_2$/NR at 480 and 580 nm gradually decreases and increases as the concentration of Hg$^{2+}$ increases stepwise. Also, there were well-defined isosbestic points at 518 nm, respectively.

Upon excitation at 400 nm, the free N displayed a single emission band centered at 480 nm, attributed to the emission of naphthalimide. There was no FRET in the free N, as the rhodamine acceptor was in the ring-closed form. In addition to Hg$^{2+}$, the fluorescence intensity at 480 nm significantly decreased with a simultaneous appearance of a new red-shifted emission band at around 580 nm (Figure 5 a and b), ascribed to the emission of the rhodamine acceptor. This energy transfer was also accompanied by a resonance color change from green to red under 360 nm illumination of light (Figure 5b photograph). Also, the intensity of fluorescence emission at 480 and 580 nm decreased and increased. Linearly range of probe response was obtained between 0.04-0.76 µM of Hg$^{2+}$ ions (Figure 5d). Thus, the addition of Hg$^{2+}$ elicited a sizeable redshift (100 nm) in emission, which almost wholly resolved the two emission peaks. This fluorescence enhancement at 580 nm is typically attributed to the opening of the spirolactam ring of rhodamine-6G (Scheme 2).
Scheme 2: Purposed mechanism of interaction of chromogenic NR with Hg$^{2+}$.

Figure 5: a) The effect of gradual addition of Hg$^{2+}$ ions on Co-ferrites @SiO$_2$/NR (b) Addition of Hg$^{2+}$ ions on CoFe$_2$O$_4$@SiO$_2$/NR, c) The linear changes in the fluorescence intensity vs. [Hg$^{2+}$] at 485, 585 nm and d) The linear changes in the fluorescence intensity vs. [Hg$^{2+}$] at I$_{480}$/I$_{580}$.

The selectivity behavior is one of the essential characteristics of a chemosensor, the relative sensor response for the primary ion over other ions present in the solution. The selectivity of the proposed nanochemosensor was studied against other metal ions. The influence of interfering ions such as Cu$^{2+}$, Zn$^{2+}$, Cd$^{2+}$, Hg$^{2+}$, Al$^{3+}$, Co$^{2+}$, Pb$^{2+}$, Cr$^{2+}$ on the fluorescence behavior of CoFe$_2$O$_4$@SiO$_2$/NR (20 mg/L) as shown in Figure 6.

A fluorescence enhancement was observed for CoFe$_2$O$_4$@SiO$_2$/NR upon binding Hg$^{2+}$ by comparing only CoFe$_2$O$_4$@SiO$_2$/NR in the solution. No noticeable change in the fluorescence intensity was observed even when double equivalents of other metal ions were used.

The competition measurements were carried out by the subsequent addition of 5×10$^{-4}$ M metal ions to the solution of CoFe$_2$O$_4$@SiO$_2$/NR in an aqueous solution. The fluorescence spectra were recorded at 580 nm with the addition of these metal ions and the subsequent addition of (5×10$^{-4}$ M) Hg$^{2+}$ ions to the above solutions. Figure 6 illustrates that mono, bivalent metal ions have not significantly influenced the enhancement in the fluorescence intensity resulting from the addition of Hg$^{2+}$ ion. Consequently, the new hybrid material CoFe$_2$O$_4$@SiO$_2$/NR can be used as a chemosensor to detect different concentrations of Hg$^{2+}$ ions with high selectivity.
The high degree of mercury selectivity exhibited by the proposed sensor makes it potentially useful for monitoring low-level mercury ion concentrations in different water samples. These samples were filtered two times through qualitative filter paper before use. The 10.0 mL of each water sample (tap and wastewater samples) was taken and diluted within a 25.0 mL volumetric flask. Different amounts of mercury ions were added to water samples. The proposed method was used for the determination of mercury content of the samples by using the calibration method. The results are summarized in Table 1.

Some recent studies performed on fluorescence probes for the detection of mercury have been shown in Table 2. As demonstrated in Table 2, the performance of the fluorescence probe designed in the present work is comparable with the literature. Different strategy conducted for sensing mercury. Fluorescence enhancement and quenching are reported in the most sensing mechanism. An enhancement system upon addition of analyte is a more sensitive method than quenching. Various interferes capable of quenching the chromophores, so enhancing systems are interested. As mentioned before, the opening of the lactam ring in rhodamine leads to remarkable fluorescence enhancement. Some rhodamine derivatives presented in Table 2 show that two core probes with large stock shift emissions show high sensitivity with low detection limits [13, 27-36]. It was found that the accuracy of mercury detection in different solution samples is almost quantitative. Also, complete magnetic separation of CoFe$_2$O$_4$@SiO$_2$/NR was achieved in 50 s by placing a magnet near the vessels containing the nanoparticles' water dispersion (Figure 7). CoFe$_2$O$_4$@SiO$_2$/NR nanoparticles' magnetic separation capability in this detection method can also offer a simple route to separate Hg$^{2+}$-CoFe$_2$O$_4$@SiO$_2$/NR system from various environment real sample analyses.

Table 1: Real sample analysis results.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Hg$^{2+}$ added (µM)</th>
<th>Hg$^{2+}$ found (µM)</th>
<th>Recovery (%)</th>
<th>R.S.D. $^a$(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water</td>
<td>0.2</td>
<td>0.25</td>
<td>108</td>
<td>1.8</td>
</tr>
<tr>
<td>Synthesized water</td>
<td>0.2</td>
<td>0.19</td>
<td>95</td>
<td>1.6</td>
</tr>
</tbody>
</table>

$^a$ n = 4.
4. Conclusion

A magnetic nanocomposite of CoFe$_2$O$_4$@SiO$_2$, functionalized by two-core chromogenic dyes of NR, was developed. To the best of our knowledge, this is the first study that uses CoFe$_2$O$_4$@SiO$_2$ magnetic nanocomposite as a fluorescent chemosensor for Hg$^{2+}$ ions. Two core fluorescence sensing caused a specific wavelength response. Nano chemosensor exhibits high selectivity and sensitivity with a fast response to trace mercury ions. Its answer is based on a turn-on fluorescence enhancement. Designed nanosensor successfully applied for Hg$^{2+}$ determination at real samples with a linear range of 0.04-0.76 µM of Hg$^{2+}$ ions. It was successfully used for the determination of mercury ion in actual samples of tap water. A remarkable change in the fluorescence emission wavelength of CoFe$_2$O$_4$@SiO$_2$/NR upon the addition of Hg$^{2+}$ makes it a right candidate for trace mercury detection. Its magnetic properties enable it to be recovered and controlled easily.

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