

# Hexametaphosphate-capped silica mesoporous nanoparticles containing Cu<sup>II</sup> complexes for the selective and sensitive optical detection of hydrogen sulfide in water

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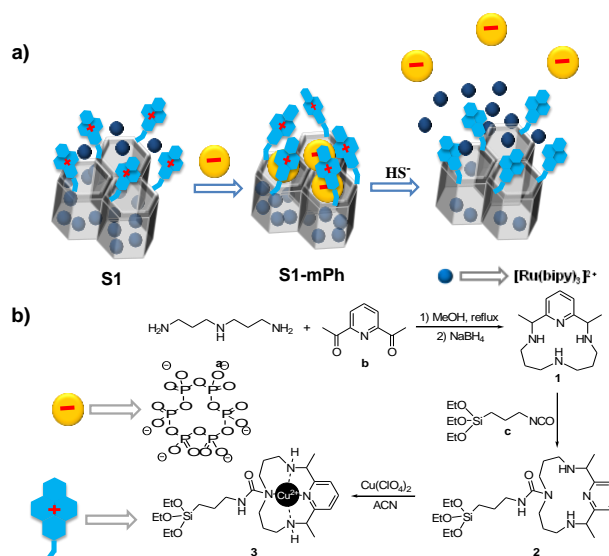
**Abstract:** Cu<sup>II</sup>-macrocyclic functionalized hexametaphosphate-capped silica mesoporous nanoparticles have been prepared and used for the selective and sensitive detection of hydrogen sulfide in aqueous environments.

Hydrogen sulfide, a colourless and flammable gas, is well known for its toxicity, for its presence in several environmental sites and for its use in certain industrial protocols. Moreover, the role of hydrogen sulfide as endogenous gasotransmitter and its participation in relevant biological processes (neurotransmission, vasorelaxation, cardioprotection, anti-inflammation) has been discovered in recent years.<sup>[1]</sup> Besides, certain diseases (such as chronic kidney disease, liver cirrhosis, Alzheimer<sup>[2]</sup> and Down's syndrome<sup>[3]</sup>) have been associated to abnormal hydrogen sulfide levels.<sup>[4]</sup> Taking into account the above mentioned roles, the development of selective and sensitive probes for the optical detection of hydrogen sulfide has recently received great attention from the international researcher's community.

In this context, some probes constructed under the chemodosimeter paradigm for the chromo-fluorogenic detection of hydrogen sulfide have been described in the last years. These probes used reduction (of azide,<sup>[5,6]</sup> hydroxylamine<sup>[7]</sup> or nitro moieties<sup>[8]</sup> to amine) and hydrolysis (of disulfide bonds,<sup>[9]</sup> dinitrophenyl ethers<sup>[10]</sup> or cyanovinyl moieties<sup>[11]</sup>) reactions as main mechanisms for the optical recognition of hydrogen sulfide. Unfortunately, some of these probes presented certain drawbacks such as poor selectivity and cross-reactivity with other thiol-containing biomolecules (such as Cys, Hcy and GSH) and anions (SO<sub>3</sub><sup>2-</sup> and S<sub>2</sub>O<sub>3</sub><sup>2-</sup>).<sup>[12]</sup> An interesting alternative, recently developed, consisted on the use of hydrogen sulfide-induced demetallation reactions of non-fluorescent complexes.<sup>[13]</sup>

From another point of view, there is an increasing interest in the design and synthesis of gated nanoscopic hybrid materials

with the ability to release an entrapped guest upon application of certain external stimuli.<sup>[14]</sup> These nanodevices are usually formed by a silica mesoporous support (in which the guest is stored) and molecular or supramolecular entities, attached on the external surface that act as "gates", allowing the controlled release of the entrapped cargo at will. These functional materials have been used for drug delivery applications but we have recently suggested the use of gated materials in optical recognition protocols.<sup>[15]</sup> Here the underlying idea is that coordination or reaction of a target analyte with the "gate" could modulate dye delivery from the pores to the solution resulting in an optical response. The potential existence of amplification features is one of the advantages of these solids. In particular, it has been reported that the presence of few analyte molecules may induce the release of a relatively high amount of entrapped dye molecules. In spite of these interesting features, the use of gated solids for sensing purposes is still a barely studied area of research especially for the detection of anions.



**Scheme 1.** (a) Schematic representation of the sensing paradigm of HS<sup>-</sup> anion using solid **S1-mPh**. (b) Synthesis of macrocyclic **2** and complex **3**.

Given our interest in the development of capped mesoporous materials and their application in recognition and sensing protocols<sup>[16]</sup> we report herein the design of silica mesoporous nanoparticles functionalized with a Cu<sup>II</sup> complex, capped with the anion hexametaphosphate and the use of such ensemble for the selective and sensitive detection of hydrogen sulfide. Our proposed sensing protocol is depicted in Scheme 1. MCM-41 mesoporous silica nanoparticles (MSN) of ca. 100 nm diameter were selected as inorganic scaffold. The

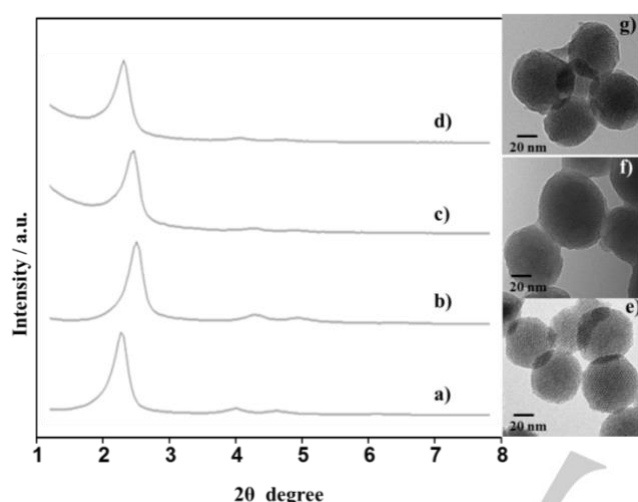
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pores of the MSN were loaded with a suitable dye (in this case  $\text{Ru}(\text{bipy})_3^{2+}$ ) and the outer surface was additionally functionalized with a copper(II) macrocyclic complex (solid **S1**). The final capped sensing solid was prepared via strong electrostatic interactions of the highly charged anion hexametaphosphate with the positively charged anchored  $\text{Cu}^{\text{II}}$  complexes. The signaling paradigm relies on a demetallation of the copper(II) complex from the grafted macrocycle in the presence of hydrogen sulfide, that would uncap the pores and induce release of the entrapped dye. Demetallation reactions have been commonly used in the development of hydrogen sulfide molecular fluorogenic probes<sup>[17]</sup> but, as far as we know, is the first time that a hybrid capped materials use this sensing mechanism.



**Figure 1.** Left: powder X-ray patterns of the solids (a) MCM-41 as synthesized (b) calcinated MCM-41, (c) solid **S1** and (d) solid **S1-mPh**. Right: TEM images of (e) calcinated MCM-41 sample, (f) solid **S1** and (g) **S1-mPh** showing the typical hexagonal porosity of the MCM-41 mesoporous matrix.

MCM-41 mesoporous nanoparticles were synthesized according to well-known procedures and then, the pores of the calcined mesoporous scaffold were loaded with the  $\text{Ru}(\text{bipy})_3^{2+}$  dye by simply stirring a suspension of the nanoparticles in an acetonitrile solution of the dye. In a second step, the external surface of the nanoparticles was functionalized with the  $\text{Cu}(\text{II})$ -macrocyclic complex **3** yielding solid **S1**. Complex **3** was synthesized using a three step procedure (see Supporting Information for details). In a first step bis(3-aminopropyl)amine (**a**) was reacted with 2,6-diacetylpyridine (**b**) in methanol yielding compound **1** (see Scheme 1). In a second step **1** was reacted with (3-isocyanatopropyl)triethoxysilane (**c**) in order to prepare compound **2** that, finally, yielded complex **3** upon reaction with copper(II) perchlorate. The final sensing solid **S1-mPh** was prepared by simply mixing **S1** in a solution containing an excess of the highly charged anion hexametaphosphate.

**Table 1.** Content ( $\alpha$ ) of anchored molecules and dye in  $\text{mmol g}^{-1}$  of  $\text{SiO}_2$ .

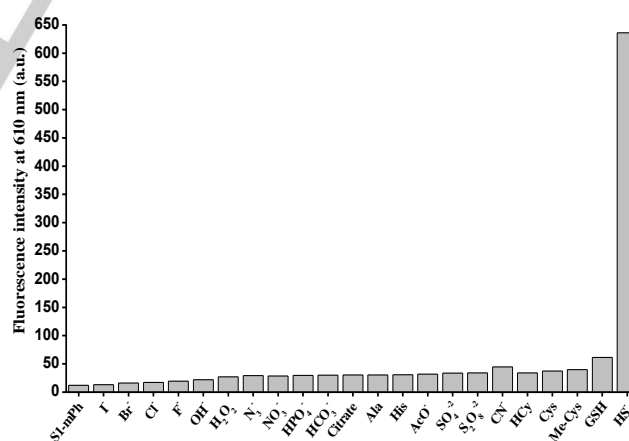
Solid	$\alpha_{\text{complex 3}}$ [ $\text{mmol g}^{-1} \text{SiO}_2$ ]	$\alpha_{\text{dye}}$ [ $\text{mmol g}^{-1} \text{SiO}_2$ ]	$\alpha_{\text{m-phosphate}}$ [ $\text{mmol g}^{-1} \text{SiO}_2$ ]
<b>S1</b>	0.107	0.39	-
<b>S1-mPh</b>	0.105	0.38	0.040

**Table 2.** Main structural properties calculated from TEM, PXRD and  $\text{N}_2$  adsorption analysis.

Sample	Diameter particle <sup>a</sup> (nm)	$S_{\text{BET}}$ ( $\text{m}^2 \text{g}^{-1}$ )	Pore Volume <sup>b</sup> ( $\text{cm}^3 \text{g}^{-1}$ )	Pore size <sup>a</sup> (nm)
<b>MCM-41</b>	$94.0 \pm 5.0$	980.9	0.79	2.58
<b>S1</b>	$95.0 \pm 8.0$	320.1	0.28	-
<b>S1-mPh</b>	$98.0 \pm 6.0$	268.5	0.18	-

<sup>a</sup> Measured by TEM. <sup>b</sup> BJH model.

The starting MCM-41 scaffold and solids **S1** and **S1-mPh** were characterized following standard procedures (see Supporting Information for details). Powder X-ray diffraction (PXRD) and transmission electron microscopy (TEM) carried out on the MCM-41 starting nanoparticles showed clearly the presence of a mesoporous structure that persisted in the final solid **S1-mPh** regardless of the loading process with the dye, further functionalization with the  $\text{Cu}(\text{II})$ -macrocyclic complex **3** and capping with hexametaphosphate (see Figure 1). Besides, from SEM, thermogravimetric and elemental analyses, contents of  $\text{Ru}(\text{bipy})_3^{2+}$ , **3** and hexametaphosphate were determined and are shown in Table 1. Moreover Table 2 list main structural properties such as particle diameter, BET specific surface area, pore volumes and pore sizes obtained for MCM-41 starting nanoparticles, **S1** and **S1-mPh**.

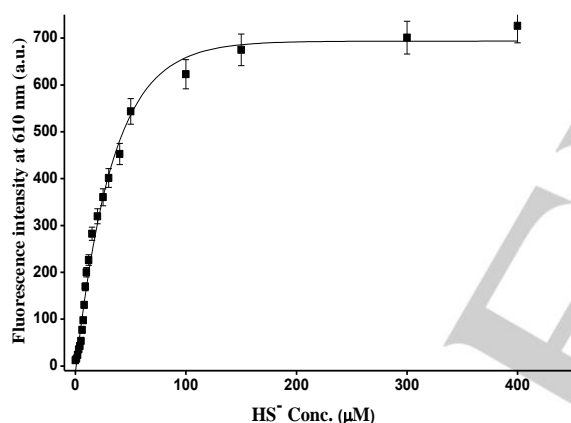


**Figure 2.** Fluorescence intensity at 610 nm of  $\text{Ru}(\text{bipy})_3^{2+}$  dye released from **S1-mPh** (HEPES 10 mM at pH 7.5) after 6 h of adding 1 mM of selected anions, amino acids and oxidants.

In a typical sensing experiment, 1 mg of **S1-mPh** was suspended in 2 mL of buffered solution (pH 7.5, HEPES 10 mM) in absence and in the presence of  $\text{HS}^-$  anion. In both cases, suspensions were stirred at room temperature and at certain

times, fractions were taken and filtrated to remove the solid. Dye delivery to the bulk solution was easily detected by monitoring the emission band of Ru(bipy)<sub>3</sub><sup>2+</sup> at 610 nm upon excitation in the spin allowed d-π metal-to-ligand charge transfer (MLCT) transition at 451 nm.<sup>[18]</sup> The obtained results are depicted in Figure 2. As it can be seen, in the absence of HS<sup>-</sup> a negligible dye release was observed due to tight pore closure due to a strong electrostatic interactions between the hexametaphosphate anion and the grafted Cu<sup>II</sup> macrocyclic complex. However, when HS<sup>-</sup> anion was present a remarkable dye delivery was observed. The release of the entrapped dye from the pore voids was attributed to a HS<sup>-</sup>-induced demetallation reaction of the grafted Cu<sup>II</sup> macrocyclic complex.

Moreover, the crucial role played by copper(II) cation in the capping protocol was demonstrated by the preparation of solid **S2** (see Supporting Information for details). This material contains the Ru(bipy)<sub>3</sub><sup>2+</sup> complex in the inner of the pores and the macrocycle **2** (that lacks Cu<sup>2+</sup>) in the outer surface. None of the anions tested in water (including hexametaphosphate) were able to block the pores and inhibit dye release to a significant extent (data not shown).



**Figure 3.** Calibration curve of Ru(bipy)<sub>3</sub><sup>2+</sup> dye released from **S1-mPh** (HEPES 10 mM, pH 7.5) upon addition of increasing quantities of HS<sup>-</sup>.

Moreover, in order to assess the selectivity of the method, the response of solid **S1-mPh** in the presence of selected anions (HS<sup>-</sup>, F<sup>-</sup>, Cl<sup>-</sup>, Br<sup>-</sup>, I<sup>-</sup>, N<sub>3</sub><sup>-</sup>, CN<sup>-</sup>, OH<sup>-</sup>, HPO<sub>4</sub><sup>2-</sup>, NO<sub>3</sub><sup>-</sup>, HCO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, AcO<sup>-</sup> and citrate), amino acids (Ala, His), thiol-containing biomolecules (Cys, Hcy, GSH, Me-Cys) and oxidants (H<sub>2</sub>O<sub>2</sub> and S<sub>2</sub>O<sub>8</sub><sup>2-</sup>) was studied. Figure 2 shows the emission of the ruthenium dye in the solution at 610 nm upon addition of these selected chemicals (1 mM) to buffered suspensions of **S1-mPh**. As seen, these species were unable to induce cargo delivery, pointing out that **S1-mPh** displays a remarkable highly selective response to HS<sup>-</sup>.

In a second step the fluorogenic response of solid **S1-mPh** was tested upon addition of increasing quantities of HS<sup>-</sup> anion by using a similar protocol to that described above. As seen in Figure 3, a clear correlation between the concentration of HS<sup>-</sup> and the dye delivered was observed in agreement with an uncapping protocol involving a demetallation reaction. From the titration

profile shown in Figure 3 a remarkable limit of detection for HS<sup>-</sup> as low as 1.85 μM was determined. As stated above one appealing characteristic of analyte-induced uncapping protocols in gated mesoporous supports is the possibility to observe signal amplification. In particular in our case it was confirmed that the presence of one HS<sup>-</sup> molecule (at a concentration of ca. 1.0 × 10<sup>-5</sup> moldm<sup>-3</sup>) results in the release of ca. 220 molecules of the Ru(bipy)<sub>3</sub><sup>2+</sup> dye.

Encouraged by these results we attempted to detect HS<sup>-</sup> in a more complex and realistic environment. For this purpose, tap water was spiked with known amounts of HS<sup>-</sup> and the concentration was determined using **S1-mPh** following the method of standard addition. Results are shown in Table 3. As it can be seen, **S1-mPh** was satisfactorily applied to the detection of HS<sup>-</sup> with rather high recovery ratios ranging from 83 to 92 %.

**Table 3.** Determination of HS<sup>-</sup> spiked in tap water samples using **S1-mPh**.

Sample	HS <sup>-</sup> spiked (μM)	HS <sup>-</sup> determined (μM)	Recovery (%)
1	0.5	0.83 ± 0.34	83
2	4.0	3.58 ± 1.03	89
3	8.0	7.40 ± 2.86	92

In summary, we reported herein the synthesis, characterization and sensing behaviour of a new hybrid material functionalized with a Cu<sup>II</sup> macrocyclic derivative and capped, through electrostatic interactions, with the bulky anion hexametaphosphate. Of all the chemicals tested, only HS<sup>-</sup> was able to induce pore opening and dye release. The fluorogenic response obtained is ascribed to a demetallation reaction, selectively induced by HS<sup>-</sup> anion. The response of the capped hybrid material is highly selective and sensitive to HS<sup>-</sup> anion with a limit of detection of 1.85 μM. Moreover, the possible use of different metal complexes combined with different capping anions and choice of different dyes or other sensing molecules as indicators makes this new protocol highly appealing for the preparation of new sensing systems for sulfide detection in different environments.

## Acknowledgements

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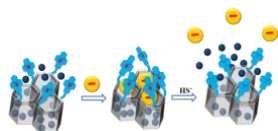
**Keywords:** gated materials • sensing • hydrogen sulfide • displacement reaction • signal (???) amplification

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## Entry for the Table of Contents

## COMMUNICATION



Selective and sensitive fluorogenic hydrogen sulfide detection has been achieved by using hexametaphosphate-capped mesoporous silica nanoparticles

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