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Capped mesoporous silica nanoparticles for the selective and sensitive detection of cyanide

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Abstract: The development of easy and affordable methods for the detection of cyanide is of great significance due to the high toxicity of this anion and the potential risks associated with its pollution. Herein, optical detection of cyanide in water has been achieved by using a hybrid organic-inorganic nanomaterial. Mesoporous silica nanoparticles were loaded with $[Ru(bipy)_3]^{2+}$, functionalized with macrocyclic Nickel(II) complex subunits, and capped with a sterically hindering anion (hexametaphosphate). Cyanide selectively induces demetallation of Nickel(II) complexes and the removal of capping anions from the silica surface, allowing the release of the dye and the consequent increase of fluorescence intensity. The response of the capped nanoparticles in aqueous solution is highly selective and sensitive towards cyanide with a limit of detection of 2 µM.

Anions are vital partners of our biological system, but their abnormal concentrations always cause diseases for humans. One of the most toxic anions is cyanide. Concentrations of cyanide in the 0.5–3.5 mg/kg body weight interval are lethal to humans.[1] It is known that, at these concentrations, cyanide strongly binds to the cytochrome c active sites resulting in inhibition of cellular respiration[2] with subsequent central nervous system damage.[3] Taking into account these facts it is not strange that cyanide was used as chemical warfare agent.[4] Besides natural sources, cyanide is widely used in industry *e.g.* metal mining processes, organic chemical industries, iron and steel plants and wastewater treatment facilities.[5,6] All these industries can be sources of water and environmental contaminations with cyanide. At this respect, the maximum permissive concentration of cyanide in drinking water is set at 1.9 µM by the World Health Organization (WHO),[7] while in industrial effluents this level is also strictly regulated to be below 1 µM.[8]

In consideration of the potential risks associated with cyanide pollution, easy and affordable detection methods for this anion are of great concern. Different methods involving titrimetric, colorimetric, and electrochemical procedures are currently used for cyanide detection.[9] However, these methods are sometimes expensive, time consuming and require complex instrumentation. Recently, as a clear alternative, the use of optical chemical sensors and probes for the detection and quantification of cyanide anion has attracted great attention due to their easy synthesis and handling, the use of quite common instrumentation (UV-visible spectrophotometer or fluorimeter), and the high degree of sensitivity achieved.[10] In spite of these promising features, these chemosensors and probes have several drawbacks such as poor water solubility (that partially precludes their application for monitoring real samples), low selectivity and a non-immediate response (considering that most of the described probes takes advantage of the nucleophilic character of cyanide anion).[11] Very recently, as an alternative to molecular probes, the use of capped hybrid organic-inorganic nanomaterials in sensing protocols have been described.[12,13] These hybrid materials are mainly composed by three components, namely: (i) an inorganic porous support, (ii) a dye/fluorophore loaded inside the pores and (iii) molecular or supramolecular assemblies grafted onto the external surface of the solid. In these sensing materials the external grafted molecules control mass transport, from the inner of the pores to the bulk solution, after coordination/reaction with a certain analyte.[14] One of the most interesting features of these gated materials is the potential amplification effect taking into account that few analyte molecules could induce the release of a relatively high amount of entrapped dye/fluorophore.[15]

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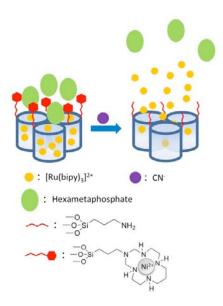   Molecular y Desarrollo Tecnológico (IDM)
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Scheme 1. Schematic representation of the sensing mechanism of solid **NP3** in the presence of cyanide.

Bearing in mind our interest in the applications of gated materials in sensing protocols, we report herein the design of mesoporous silica nanoparticles functionalized with a Ni^{II} complex, capped with hexametaphosphate anion and the use of such nanodevice for the selective and sensitive detection of cyanide in water. Our proposed sensing protocol is shown in Scheme 1. MCM-41 mesoporous silica nanoparticles of ca. 100 nm diameter were selected as inorganic scaffold and the pores loaded with $[Ru(bipy)_3]^{2+}$ dye. Then, the external surface of the loaded scaffold was functionalized with a Ni^{II}-macrocyclic complex (solid **NP2**). Finally, the pores were capped upon addition of hexametaphosphate anion (solid **NP3**) through electrostatic/coordinative interactions with the grafted Ni^{II} complex. The signalling paradigm relies on a demetallation of the Ni^{II} complex induced by cyanide anion, which would uncap the pores with subsequent dye release. This paradigm has been recently used by us to prepare gated materials for the selective detection of hydrogen sulfide.[16] Besides, and as far as we know, **NP3** is the first capped material used for the selective and sensitive detection of cyanide anion.

In a first step, MCM-41 mesoporous nanoparticles were prepared according to a standard procedure. Then, the pores of the nanoparticles were loaded with $[Ru(bipy)_3]^{2+}$ by stirring a suspension of the calcined nanoparticles in an acetonitrile solution of the dye. Thereafter, the external surface of the loaded silica nanoparticles was functionalized by a modified two-step literature procedure:[17] (i) treatment with (3-aminopropyl)triethoxysilane yielding solid **NP1**; (ii) "template" reaction[18] of amino-functionalised **NP1** with $[Ni^{II}(2,3,2\text{-tet})]^{2+}$ (2,3,2-tet = N,N′-Bis(2-aminoethyl)propane-1,3- diamine) in the presence of formaldehyde and triethylamine, yielding nanoparticles functionalized with Ni^{II}-azacyclam[19] subunits **(NP2)**. The final solid **NP3** was obtained by stirring of **NP2** with hexametaphosphate in water (see Scheme 1 and Supporting Information for details).

Table 1. Content (α) of anchored complex and dye in hybrid materials.

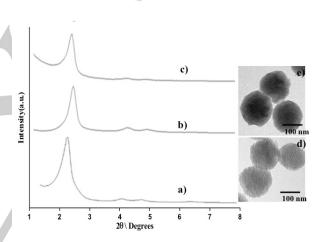| Solid	$\alpha_{\text{complex 3}}$ [mmol g^{-1} SiO$_2$]	α_{dye} [mmol g^{-1} SiO$_2$]	$\alpha_{\text{m-phosphate}}$ [mmol g^{-1} SiO$_2$]
NP2	0.107	0.37	-
NP3	0.106	0.34	0.05

The starting MCM-41 nano-sized scaffold and **NP3** solid were characterized using standard procedures (see Supporting Information). Powder X-ray diffraction (PXRD) and transmission electron microscopy (TEM) measurements carried out on the MCM-41 nanoparticles showed the presence of a mesoporous structure that persisted in solid **NP3** regardless loading with dye, functionalization with Ni^{II} complex, and capping with hexametaphosphate anion. TEM images of calcined MCM-41 and **NP3** nanoparticles are reported Figure 1 and show that **NP3** preserved the spherical shape of the calcined MCM-41 starting material, and a very similar average diameter of 99 ± 6 nm. On the other hand, thermogravimetric and elemental analysis were used to determine the contents of $Ru(bipy)_3^{2+}$, nickel(II) complex,

and hexametaphosphate in solids **NP2** and **NP3** (see Table 1). Main structural characteristics for MCM-41 and **NP3** such as surface area, particle diameter, pore volume and size were also determined (see Table 2 and Supporting Information).

Table 2. Main structural properties of nano-sized materials determined by TEM, PXRD and N_2 adsorption analysis..

Sample	Particle[a] diameter (nm)	Surface area, S_{BET} (m^2 g^{-1})	Pore Volume[b] (cm^3 g^{-1})	Pore size[b][c] (nm)
MCM-41	95.0 ± 5.0	970.5	0.89	2.49
NP3	99.0 ± 6.0	218.5	0.22	-

[a] Measured by TEM. [b] Pore volumes and pore sizes associated only with intra-particle mesopores. [c] Pore size estimated by using the BJH model applied to the adsorption branch of the isotherm.

Figure 1. Left: powder X-ray patterns of the solids (a) MCM-41 as synthesized (b) calcined MCM-41 and (c) solid **NP3**. Right: TEM images of (d) calcined MCM-41 sample and (e) solid **NP3**.

After characterization of hybrid materials, the sensing features of solid **NP3** in the presence of selected anions were tested. For this purpose, in a typical assay, 1 mg of **NP3** was suspended in 2 mL of buffered solution (pH 7.5, HEPES 10 mM) in absence (control) and in the presence of cyanide anion. In both cases suspensions were stirred at room temperature and, at scheduled times (up to 6 hours), aliquots were taken, centrifuged, and dye delivered to the bulk solution was detected by monitoring the emission band of $[Ru(bipy)_3]^{2+}$ at 610 nm (upon excitation at 451 nm). In the absence of cyanide anion, a negligible dye release was observed due to a thight pore closure as a consequence of the strong interaction between hexametaphosphate anion and the grafted Ni^{II}-macrocyclic complex. Conversely, in the presence of cyanide anion, a remarkable $[Ru(bipy)_3]^{2+}$ delivery was observed even after just 45 min. The release of $[Ru(bipy)_3]^{2+}$ dye from solid **NP3** in the presence of cyanide anion is directly related with the demetallation of Ni^{II}-azacyclam complexes, the consequent

decomposition of the macrocyclic moieties,[19] and the removal of bulky anions. This demetallation process is highly favoured taking into account the strong stability constant for the formation of $[Ni(CN)_4]^{2-}$ complex (log β_4 = 30.5 at 25°C)[20] in water when compared to that of the macrocyclic complex (log K_f = 22.2 at 25 °C for $[Ni^{II}(cyclam)]^{2+}$ taken as reference).[20] In order to confirm the demetallation process, energy dispersive X-ray spectroscopy (EDX, 20 kV) was used to determine Ni and P content in the nanoparticles before and after cyanide-induced pore opening and dye release. In the initial **NP3** nanoparticles EDX measurements showed values of weight percentage of 0.59 % and 4.88 % for Ni and P respectively. The presence of Ni and P in the surface of **NP3** nanoparticles indicated the proper grafting of the Ni^{II}-azacyclam complexes and the further capping of the pores with hexametaphosphate anion. As clear contrast, a marked decrease in Ni (weight percentage of 0.02 %) and P (weight percentages of 0 %) content in the final solid (after pore opening in the presence of cyanide anion) was measured. These reductions in Ni and P contents are clearly ascribed to a cyanide-induced demetallation process with the subsequent detachment of hexametaphosphate anion, pore opening and dye release. Besides, the ruthenium content (as weight percentage) also decreased from 0.54 (in **NP3** nanoparticles) to 0.14 (nanoparticles after reaction with cyanide anion and proper washing) as a result of pore uncapping and $[Ru(bipy)_3]^{2+}$ complex delivery.
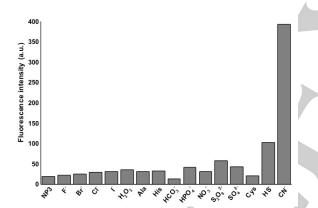

Figure 2. Fluorescence intensity at 610 nm of $[Ru(bipy)_3]^{2+}$ dye released from **NP3** (HEPES 10 mM at pH 7.5) after 6 h from addition of selected anions, amino acids and oxidants (1 mM).

Table 3. Determination of cyanide spiked in tap water samples using **NP3**.

Sample	CN$^-$ spiked (μM)	CN$^-$ determined (μM)	Recovery (%)
1	4.0	3.41 ± 0.44	85
2	6.0	5.34 ± 1.87	89
3	12.0	10.93 ± 2.93	91

Besides, the crucial role played by Ni^{II} cation in the capping protocol was demonstrated using solid **NP1** (loaded with $[Ru(bipy)_3]^{2+}$ dye and functionalized in its outer surface with aminopropyl moieties). With solid **NP1** none of the anions tested (including hexametaphosphate) were able to block the pores and to inhibit $[Ru(bipy)_3]^{2+}$ release.

On the other hand, the selectivity of **NP3** nanoparticles toward cyanide was assessed by testing the controlled release performance in the presence of other anions (HS$^-$, F$^-$, Cl$^-$, Br$^-$, I$^-$, HPO$_4^-$, NO$_3^-$, HCO$_3^-$, SO$_4^{2-}$), selected amino acids (Ala, His, Cys), and oxidants (H$_2$O$_2$ and S$_2$O$_8^{2-}$). Figure 2 shows the emission of $[Ru(bipy)_3]^{2+}$ dye at 610 nm in the solution after addition of selected species (1 mM) to buffered suspension of **NP3**. As it can be seen, none of the tested chemicals were able to induce cargo delivery, pointing toward a highly selective response of **NP3** solid toward cyanide anion. We recently reported hexametaphosphate-capped nanoparticles functionalized with a Cu^{II} macrocyclic complex, which were selectively uncapped only upon hydrogen sulfide addition.[16,21] Previous and current results indicate that the selectivity of the hexametaphosphate-capped nanoparticles can be properly addressed by changing the metal-macrocyclic subunits grafted on their surface. In particular, the presence of Cu^{II} complexes make nanoparticles to respond selectively to hydrogen sulphide, whereas on changing to Ni^{II} the uncapping process is only observed with cyanide.

The controlled release performance of **NP3** was tested upon addition of increasing quantities of cyanide and using the same protocol described above. Results depicted in Figure 3 show a clear correlation between cyanide concentration and the amount of released $[Ru(bipy)_3]^{2+}$. This correlation agrees with the above-mentioned uncapping mechanism involving a demetallation reaction and the formation of $[Ni(CN)_4]^{2-}$ complex. From the titration profile shown in Figure 3 a limit of detection for cyanide of 2 μM was measured. As cited in the introduction one powerful characteristic of analyte-induced uncapping protocols in gated solids is the possible presence of signal amplification features. In particular, for solid **NP3**, it was confirmed that the presence of one cyanide ion results in the release of ca. 180 molecules of $[Ru(bipy)_3]^{2+}$ dye.
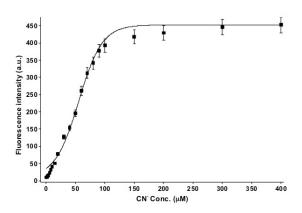

Figure 3. Calibration curve of $[Ru(bipy)_3]^{2+}$ dye released from **NP3** (HEPES 10 mM, pH 7.5) upon addition of increasing quantities of cyanide.

Finally, in order to test the possible applicability of **NP3** nanoparticles for cyanide sensing in real environments, tap water was spiked with known amounts of this anion and the concentration determined using the standard addition method. The obtained results, shown in Table 3, indicate that **NP3** was able to detect cyanide with recovery ratios ranging from 85 to 91%.

In conclusion, we report in this communication a new hybrid organic-inorganic material made by silica nanoparticles loaded with [Ru(bipy)$_3$]$^{2+}$ dye, functionalized with a Ni^{II}-macrocyclic complex and capped with bulky hexametaphosphate anion. Among all the tested species, only cyanide is able to induce pore opening and dye release. The observed delivery can be clearly ascribed to a cyanide-induced demetallation reaction with a consequent detachment of hexametaphosphate anion from the nanoparticles surface. The response of the capped nanoparticles is highly selective and sensitive to cyanide anion with a limit of detection of 2 μM. Moreover, the capped nanoparticles have been used to detect cyanide in tap water with high recovery rates.

Acknowledgements

Conflict of interest

The authors declare no conflict of interest.

Keywords: optical detection • hybrid materials • mesoporous silica nanoparticles • cyanides • macrocyclic ligands •
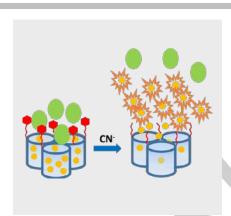
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COMMUNICATION

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