Immobilisation of a biological chelate in porous mesostructured silica for selective metal removal from wastewater and its recovery

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This study represents a preliminary stage in the development of a process aimed at the selective uptake and release of metal ions from wastewater. The process involves the immobilisation of highly selective natural chelates secreted by bacteria or other living species inside mesoporous micelle-templated silicas (MTS) that could be used as usual resins. To demonstrate the feasibility of the concept, a model system was used. It involves pyoverdin, a natural Fe(III) chelator from a Pseudomonas fluorescens strain, covalently anchored to a glycidoxypropyl linker into the large pores (13 nm) of a MTS material. The hybrid material obtained is very stable as no leaching of the pyoverdin complex was observed during repeated washings and repeated uses.

The native fluorescence of the pyoverdin allows a simpler follow-up and quantification of the iron uptake and release processes. The pyoverdin-anchored MTS is very selective towards Fe(III) if a multi-metallic solution is used. MTS materials allow a higher density of pyoverdin anchoring and consequently a higher metal uptake, compared to a high-grade silica gel.

Introduction

The discovery of highly structured mesoporous silica produced by self-assembly of micelles and silica, such as MCM-41 disclosed by Mobil researchers,1 has opened up new possibilities for their use as supports or adsorbents. Such nanostructured materials with large pores may encapsulate various biomolecules within the channels or may be used as supports for enzyme anchoring by covalent binding.2,3 Their large mesoporous volume and large surface area with a low silanol surface density favour a high loading in biomolecules while limiting their interaction with the surface silanols. Mass transfer considerations are also of paramount importance: these matrices are sufficiently porous to enable free diffusion into the pores and access to their surface. Furthermore, methods of physical or chemical immobilisation of biomolecules on solid surfaces are well known.4,5

Mineral oxides have been successfully used for the immobilisation of biomolecules. These materials often show good mechanical properties, thermal stability and resistance against microbial attack and solvents. Silica gels are of particular interest because they do not swell and have good mechanical strength and thermal stability.6 The modification of silica gels using inorganic or organic functional groups has been the subject of considerable interest. Surface modifications for enzyme immobilisation are usually achieved through silanisation by using an appropriate organosilane agent.7,8 One of the most widely used alkoxysilane derivatisation agents is 3-glycidoxypropyltrimethoxysilane. Its epoxide group is convenient for the covalent binding of enzymes. The O–C and N–C bonds formed following opening of the epoxide groups are extremely stable, so that the epoxide-containing material can be used for the immobilisation of enzymes and proteins.9,10

The present work focuses on the covalent grafting of bio-complexants into large-pore micelle-templated silicas (MTS)12,13 for their use in decontamination of heavy metals in wastewater as an original bioremediation process. The uptake of metal by such bio-complexants insures the further metal recovery by a decomplexation procedure. The grafting of bio-complexants is certainly the best choice for a recyclable metal “spoon”. Bio-complexants are highly selective natural chelates secreted by microorganisms such as bacteria. A wide variety of microorganisms possess the natural ability to sequester trace levels of heavy metal ions from dilute solutions for their needs or to neutralise toxic metals ions, which may penetrate the membranes.11 To reach that goal, they excrete natural ligands, which reversibly complex metal ions with a high selectivity. To show the feasibility of this original concept of bioremediation, we have chosen to immobilise into large-pore MTS a bio-complexant of iron, pyoverdin, which is a natural siderophore excreted by the bacteria Pseudomonas fluorescens.

Bacteria, like most living species require iron for their growth, but the insoluble nature of iron in neutral and alkaline environments limits its concentration to levels well below that required for growth. A response to this limitation is the extra-cellular release of a soluble, low molecular weight siderophore.14 For instance, Pseudomonas fluorescens are a major group of gram-negative bacteria.15 As a group, these bacteria produce water soluble yellow-green pigments, called pseudobactins or pyoverdins, known as siderophores.16,17 Pyoverdins are chromopeptides consisting of a 2,3-diamino-6,7-dihydroxyquinoline fluorescent chromophore linked to a short peptide.18 The length and composition of the oligopeptide varies among different pyoverdins. Binding of iron is mediated by the catecholate group of the chromophore and by two hydroxamate groups substituting the peptide backbone.19 The resulting neutral octahedral complex has a 1 : 1 stoichiometry and a stability constant of approximately 1024 mol−1 at neutral pH.20,21

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Pyoverdin has already been immobilised on controlled pore glass in order to obtain a biosensor for ferric ions28 and carry out a total inorganic iron determination.27 In our study on bioremediation, the choice of iron, which is not a toxic heavy metal, allows an easier control of the process since the free pyoverdin ligand emits a fluorescence that is totally quenched following its complexation with iron(III) ions. Recently, we reported the encapsulation of pyoverdin in a MTS by a direct synthesis procedure28 in which pyoverdin was first dissolved in the templating micelles. It was shown that the siderophore could reversibly bind iron and that the complexation and release of iron ions could be conveniently monitored. However the amount of encapsulated pyoverdin was too low to be practical in a large-scale operation. For this reason, the present work is devoted to the covalent immobilisation of pyoverdin on a glycidoxy-grafted large-pore MTS and, for the purpose of comparison, on a commercial high-grade glycidoxy-grafted silica gel. These solids were tested for uptake and release of iron and for the selective uptake of iron from a multi-metallic solution.

Experimental

Materials and methods

All chemicals were analytical reagent-grade products, including those used to prepare the solutions required for the biosynthesis and purification of pyoverdin from *Pseudomonas fluorescens*. Cetyltrimethylammonium bromide (CTAB, Aldrich), 1,3,5-trimethylbenzene (TMB, Aldrich), NaOH (Prolabo) and SiO2 (Aerosil 200, Degussa) were used for the synthesis of a large-pore MTS, MTS-1a. 3-Glycidoxypropyltrimethoxysilane (GPTMS, Sigma) was used for the functionalisation of the large-pore MTS to give the hybrid material MTS-2a. Commercial silica gel (500 m2 g−1 surface area and 6 nm diameter pores), functionalized with 3-(glycidoxy)-propyl, was a product from SiliCycleTM Inc. This grafted silica (40–63 μm particle size), hereafter named Si-2b, is usually a starting material to prepare chiral phases for chromatography. The amount of grafted GPTMS on the surface of Si-2b is 1.13 mmol g−1 SiO2. Sodium sulfite (Na2SO3) used for iron decomplexation and dimethylformamide (DMF) used for the anchoring of pyoverdin were from Prolabo.

Production, isolation and characterisation of pyoverdin

*Pseudomonas fluorescens* strains (IP-69-13T, Institut Pasteur, Paris) were a gift from Prof. J. Guiraud, Montpellier (France) and IP-69-13T, Institut Pasteur, Montpellier, (France) were a gift from Prof. J. Guiraud, Montpellier (France). Freshly prepared Milli Q (Millipore) ultra pure water was used in all experiments.

A 1000 mg L−1 Fe(III) stock solution was prepared by dissolving anhydrous FeCl3 (Prolabo) in 1% HCl. The multi-metallic stock solution was prepared by dissolving the required amount of K2Cr2O7, CuCl2, NiCl2, ZnCl2, 2H2O, Pb(NO3)2 and ZnCl2, 6H2O in 1% HCl. The Fe, Cr, Cu, Ni, Pb and Zn standard aqueous solutions were prepared following the recommended preparation methods for standard solutions for atomic absorption spectroscopy (AAS). The optimum pH for the formation of the Fe(III)–pyoverdin complex was obtained with a 0.01 M solution of phthalic acid/biphthalate (Prolabo) at pH 4.5.

Synthesis of the large-pore MTS-1a

The synthesis of large-pore MTS-1a (ca. 13 nm diameter), was carried out according to the procedure described by Desplan-tier-Giscard et al.16 The reactants were added under stirring at room temperature in the following order: H2O, NaOH, CTAB, TMB and SiO2 (Aerosil 200) (molar ratio: 21: 0.26: 0.1: 13: 1). Swollen micelles were formed using a TMB/CTAB molar ratio of 13: 1. After the addition of silica, the mixture was stirred for 30 min and then left to stand in an autoclave for 1 h at 115 °C. The gel was then filtered off, washed with distilled water until neutral pH and dried during 10 days at 115 °C.

Surfactants were removed by a thermal treatment at 550 °C, carried out under airflow during 8 hours.

Synthesis of the grafted MTS-2a

The grafted of large-pore MTS-1a was carried out according to the procedure of Brule et al.29 MTS-1a (3 g) was freshly activated overnight at 180 °C under vacuum, and GPTMS (3.74 mL) was added to dry toluene (50 mL). After stirring the solution at toluene reflux for 1 h 30 min, the methanol released was distilled off and the mixture kept under reflux for at least a further 1 h 30 min to reach completion. The grafted MTS-2a was filtered off and washed with toluene and then diethyl ether.

It was then submitted to a continuous extraction run overnight in a Soxhlet apparatus using diethyl ether/dichloromethane (v/v, 1/1) and dried overnight at 160 °C.30

Anchoring of the iron–pyoverdin complex onto MTS-2a and Si-2b

The hybrid MTS-2a (1.5 g) and the commercial amorphous silica gel functionalised with GPTMS Si-2b (1.5 g) were freshly activated overnight at 180 °C under vacuum, and GPTMS (3.74 mL) was added to dry toluene (50 mL). After stirring the solution at toluene reflux for 1 h 30 min, the methanol released was distilled off and the mixture kept under reflux for at least a further 1 h 30 min to reach completion. The grafted MTS-2a was filtered off and washed with toluene and then diethyl ether.

It was then submitted to a continuous extraction run overnight in a Soxhlet apparatus using diethyl ether/dichloromethane (v/v, 1/1) and dried overnight at 160 °C.

Two different protocols were used to quantify the amount of pyoverdin anchored onto solids MTS-3a and Si-3b. In the first one, pyoverdin recovered in the rinsing waters was quantified by UV-visible absorption and the amount grafted was inferred by subtracting this value from the initial amount added. In the second one, the solids were first dissolved in a 10% w/w HF solution and a 20% w/w NaHSO3 solution was then added to reduce Fe(III) into Fe(II) ions. The latter, that have no affinity for pyoverdin, are released from the chelate. The resulting solutions were analysed by AAS.
Quantification of Fe(III) uptake and release

AAS calibration graphs for the determination of Fe(III) and of the other metallic ions were obtained by plotting the increase in absorbance versus the metal ion concentration. In order to determine the maximum iron adsorption capacity of the solids MTS-3a and Si-3b, Fe(III) was first released from the solids using a 20% w/w NaHSO₃ solution. The iron-free solids (0.5 g) were then mixed with 5 mL of a 40 mg L⁻¹ iron solution, left to equilibrate and centrifuged for 15 min at 3000 rpm. Fe(III) was quantified in the supernatant by AAS. The procedure was repeated until the concentration of Fe(III) in the supernatant became constant, indicating the saturation of the solid. The solids were washed with de-ionised water before the beginning of each cycle. The amount of Fe(III) complexed by the anchored pyoverdin was quantified by difference.

In order to test the selectivity of the metal complexation, the same procedure was carried out for a multi-metallic solution containing 40 mg L⁻¹ of each metal (Fe³⁺, Cr³⁺, Cu²⁺, Ni²⁺, Zn²⁺, Co²⁺, Pb²⁺).

The same procedures were applied for the large-pore MTS-1a (without bio-complexant) in order to determine the amount of ions trapped by the bare mineral support.

Characterisation

Electronic absorption spectra were recorded on an Uvikon XL UV-Visible spectrometer from Bio-Tek Instruments. Diffuse reflectance spectra in the UV-visible range (DRUV) were obtained on a Lambda 14 spectrometer (Perkin-Elmer Inc., Shelton, USA) with an integrating sphere (Labsphere, North Sutton, USA) for solid samples. The latter were held in 0.05 mm thick quartz cuvettes (100 Q, Hellma, Mulheim, Germany) and barium sulfate was used as reference. Atomic adsorption spectroscopy (AAS) measurements were performed on a Spectra AA-220 Varian spectrometer. Nitrogen adsorption–desorption isotherms at 77 K were recorded on a Micromeritics ASAP 2000 for samples previously outgassed at 373 K under vacuum, overnight. Average pore diameters were evaluated from the nitrogen desorption branch according to the method of Broekhoff and De Boer, which has been shown to provide reliable results for MCM-41 materials. FTIR spectra of self-supported wafers previously heated at 150 °C under vacuum were performed on a Bruker Vector 22 spectrometer. FTIR spectra have been normalized with respect to the overtones of the silica bands at 1861 cm⁻¹ and 1960 cm⁻¹. Thermogravimetric studies (TGA) were carried out under airflow, on a Netzsch IRIS TG 209 C.

Results and discussion

Properties of pyoverdin and its Fe(III) chelate in solution

Purified pyoverdin extracted from a Pseudomonas fluorescens strain that was selected and cultivated in an iron-poor medium, shows an electronic absorption spectrum typical of pyoverdins (Fig. 1a). Its absorption maximum is observed at λmax = 380 nm (ε = 24 530 M⁻¹ cm⁻¹) at pH 5.0. The chromopeptide (without Fe(III)) emits a strong fluorescence with a maximum at λem = 452 nm for an excitation at 400 nm (Fig. 1b). The spectrum is independent of the exciting wavelength. The native fluorescent pigment used (Scheme 1) forms a 1 : 1 complex with Fe(III). After control, the reddish complex (Fe(III)-pyoverdin), free in solution or simply adsorbed on mesoporous silica, was shown to be non-fluorescent. The absorption spectrum of the iron complex of pyoverdin has a maximum at λmax = 400 nm (ε = 24 800 M⁻¹ cm⁻¹) and two shoulders at 480 nm (ε = 6620 M⁻¹ cm⁻¹) and 550 nm, (ε = 2950 M⁻¹ cm⁻¹) (Fig. 2).
in the mixture. In the present work, it was decided to anchor the Fe(III)-pyoverdin complex rather than the free pigment (i.e. without iron), in order to prevent the functional groups that are involved in the binding of iron from reacting with the epoxy groups or with the silanols. Interestingly, the formation of the iron-pyoverdin complex leaves only one free amino group on the peptide backbone. This amino group reacts covalently with the epoxy group of the spacer during the anchoring process. It is expected that even after the release of iron, the covalently anchored pyoverdin will be kept at a distance from the pore walls and will not interact strongly with silanols. Such interactions certainly occurred in the previously made MTS hybrid material obtained by the direct synthesis of MTS templated by micelles containing solubilized pyoverdin, and this could explain its reduced loading capacity. 28

The amount of iron-pyoverdin complex anchored on MTS-3a and on Si-3b was determined by TGA and AAS analysis (Table 2). For MTS-3a, there are ca. 40 mg pyoverdin per g SiO2 (0.04 mmol per g SiO2) (Table 2), which corresponds to an anchoring of 80% of the pyoverdin initially introduced in the reaction medium and to the linkage of 2.5% of the glycidoxypropyl groups. The amount of iron-pyoverdin complex anchored to silica-gel Si-3b, 6 mg pyoverdin per g SiO2 (0.006 mmol per g SiO2) (Table 2), is much lower than on MTS-3a, and corresponds to an anchoring of only 12% of the pyoverdin initially present in the reaction mixture. Only 0.5% of the glycidoxy groups were involved in the anchoring of pyoverdin onto the silica-gel Si-2b. The lower amount of pyoverdin covalently bound to Si-3b compared to MTS-3a can be rationalized on the basis of the two following explanations. The first one is that the epoxy groups of the spacer are more accessible in the larger pores (12.9 nm) of MTS than in those of the silica-gel (6 nm pore size), which has some restricted pore entrance. The second one is that the higher amount of remaining silanols present on silica-gel leads to an activation of the epoxide ring opening which competes with the anchoring reaction between epoxide and pyoverdin, and at the same time to stronger pyoverdin-silanol and water-silanol interactions. Indeed, at low coverage, the MTS silica surface presents less water adsorption sites characterised by a large differential adsorption enthalpy than silica gel does. 36 This is in agreement with the quasi absence of defects in MTS materials revealed by the very low Q2/Q4 ratio determined by 29Si MAS NMR. 35

The iron-pyoverdin complex anchored MTS-3a and silica-gel Si-3b were characterised at each step of their synthesis by their porosity (Table 1). The nitrogen adsorption-desorption isotherms of the native large-pore MTS-1a features a sharp step characteristic of the filling of mesopores at ca. 12.9 nm (Figs. 3 and 4), with nevertheless the presence of some smaller pores revealed by a hysteresis loop in the range 0.42 < p/p0 < 0.72. This secondary porosity is due to a small fraction of the material formed when the CTAB/TMB emulsion has demixed during the large-pore MTS synthesis. The glycidoxypropyl-grafted MTS-2a shows a 2.1 nm decrease in pore size (pore diameter ca. 10.8 nm) that nicely fits with the size of the glycidoxypropyl linkers grafted into the pores. Unlike the linker grafting, the formation of the pyoverdin-anchored MTS-3a involves only a small decrease in pore size (pore diameter ca. 10.4 nm). The conservation of the shape of the nitrogen isotherms for the different MTS clearly indicates that the mesostructure of MTS is preserved during the different surface modifications. The smaller pores present on the initial

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<tr>
<td></td>
<td>mmol g⁻¹ SiO₂</td>
<td>molecule nm⁻²</td>
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⁵ Determined by ATG. ⁶ Determined by FAAS. ³ Taking into account the molecular weight of pyoverdin (Mw = 1103). ³⁵,³⁸

![Fig. 2](solution-uv-visible-electronic-absorption-spectra-of-the-fe(III)-pyoverdin-complex-and-of-free-pyoverdin.png)
large-pore MTS are left unaffected by the different grafting processes. The surface area and mesoporous volume of MTS-2a and MTS-3a decrease as the amount of grafted organic increases (Table 1). Surface and volume have been standardized versus their pure silica content to check that the grafting was not occurring only on the material surface, a conclusion that could be borne out by the sole consideration of the total weight. The decrease of the mesoporous volume standardized versus pure silica weight and the decrease of the pore diameter, are consistent with the location of the grafted species (glycidoxypropyl linkers and iron–pyoverdin complexes) inside the mesopores and not only on the outer surface. This result is in agreement with the initial large pore diameter of MTS-1a, which allows embedding large molecules. Moreover, the \( C_{\text{BET}} \) parameter value decreases at each step of the surface modification procedure (Table 1). This parameter, derived from the adsorption branch of the nitrogen isotherm, has been shown to be a global index of the hydrophobic/hydrophilic character of the surface. \(^{39,40}\) Here, its decrease from 90 to 58 reveals the gradual surface modification toward a more hydrophobic/organophilic character.

The DRUV spectra of MTS-3a (Fig. 5) and Si-3b display the same characteristics as the absorption spectrum of the iron–pyoverdin complex in solution (i.e. a maximum at \( \lambda_{\text{max}} = 400 \) nm and two shoulders at 480 nm and 550 nm, respectively) (Fig. 2). So, it appears that the iron–pyoverdin complex is not affected in its structure and co-ordination properties following its covalent anchoring.

The grafting of GPTMS onto the large-pore MTS-1a surface and the iron–pyoverdin anchoring onto MTS-2a was also investigated by FTIR (Fig. 6). The FTIR spectrum of grafted MTS-2a displays as expected absorption bands at 3747 cm\(^{-1}\) due to some remaining free silanol groups, absorption bands from aliphatic CH\(_2\) groups at 2880 cm\(^{-1}\) (symmetric stretching) and 2955 cm\(^{-1}\) (asymmetric stretching) and bands from the CH\(_2\) group of the epoxy ring at 3000 cm\(^{-1}\) and 3050 cm\(^{-1}\). For the pyoverdin-anchored MTS-3a, the bands relative to aliphatic CH\(_2\) groups (2880 cm\(^{-1}\) and 2955 cm\(^{-1}\)) remain, but the bands relative to the CH\(_2\) group of the epoxy ring (3000 cm\(^{-1}\) and 3050 cm\(^{-1}\)) have disappeared. This shows that all the epoxy rings have been opened during the reaction. As only 2.5% of glycidoxy groups were involved in the anchoring of pyoverdin, the remaining epoxy rings were opened into diols due to the presence of the small amount of water needed to carry the pyoverdin anchorage reaction. Water was introduced into the reaction medium to counteract the low solubility of pyoverdin in organic solvents. A blank reaction was performed with glycidoxy-grafted silica \( 2b \) under the same conditions as the pyoverdin anchorage but without pyoverdin, using a DMF/water mixture (20/0.3; v/v). The FTIR spectrum (not shown) reveals that more than 60% of epoxy rings were opened to form diols during that reaction. This means that most of the linkers that did not react with pyoverdin are left with two hydroxyl groups on their free end (1.58 mmol of diol per g SiO\(_2\) for MTS-3a and 1.12 mmol of diol per g SiO\(_2\) for Si-3b). These diols did not take part in the pyoverdin anchoring, as the acidity of the alcohol function is too low.

Apart from the epoxy ring opening, another proof of the pyoverdin covalent anchoring on MTS-3a material is indirectly given by both the appearance of a C=O absorption band at 1720 cm\(^{-1}\) characteristic here of the presence of pyoverdin, and by the stability of pyoverdin after repeated washings and uptake-and-release cycling tests.
Uptake and release of Fe(III) ions

In order to determine the maximum iron adsorption capacity for the pyoverdin-anchored MTS-3a and silica-gel Si-3b, Fe(III) ions were first released as described in the Experimental section. The amount of iron that was released per gram of solid was 1.30 mg Fe per g solid (0.033 mmol Fe per g SiO₂) for MTS-3a and 0.14 mg Fe per g solid (0.004 mmol per g SiO₂) for Si-3b. This agrees with the amount of pyoverdin anchored on the solids, 0.038 and 0.006 mmol pyoverdin per g SiO₂, respectively and with the 1 : 1 stoichiometry of the complex of the natural fluorescent pigment with Fe(III),34 All iron ions trapped in the solids are removed by treatment with NaHSO₃ solution. After decomplexation, the materials containing uncomplexed pyoverdin were further used for iron uptake from a 40 mg L⁻¹ Fe(III) solution. Even though the optimum pH for the formation of the Fe(III)-pyoverdin chelate is pH = 4.5,8,28 FeCl₃ in an unbuffered water solution was used to contact the solids for the successive complexation steps. With 1 g of MTS-3a, 1.30 mg Fe(III) (0.033 mmol Fe per g SiO₂) were adsorbed from the solution, versus 0.14 mg Fe(III) for 1 g of Si-3b (0.004 mmol per g SiO₂). One concludes that almost all the immobilised pyoverdin is available for Fe(III) complexation. When blanks were run with the reference material MTS-1a, 0.10 mg Fe(III) only per g of solid (0.002 mmol per g SiO₂) were adsorbed. The amount of iron adsorbed by Si-3b is larger than that of the blank. So the depletion of iron from the solution results from the Fe(III) complexation by the pyoverdin molecules immobilised on MTS-3a.

In the conditions where the amount of Fe(III) in solution is insufficient to saturate the bound pyoverdin, the amount of metal adsorbed is affected only by the pyoverdin loading of the solid and not by the initial metal concentration in the solution. After iron adsorption, the solids could be regenerated again as above and be used for another iron adsorption step. Each solid was submitted to three adsorption–desorption cycles. For both solids tested, iron recovery after the decomplexation step was 100% and the amount of adsorbed iron per g of solid remained the same for all successive cycles. This means that pyoverdin is stabilised by covalent anchoring as it does not interact irreversibly with the pore surface.

Selectivity for Fe(III) ions tested against a multi-metallic solution

With single heavy metal recovery as a goal, it was necessary to test the selectivity of the complexation of the biological chelator. After decomplexation, MTS-3a and Si-3b containing the free pyoverdin were exposed to a multi-metallic solution containing 40 mg L⁻¹ of each of the following ions: Fe, Cr, Cu, Ni, Zn, Co and Pb. The iron loading of the solids was determined by AAS (Table 3). A high selectivity for Fe(III) was found with an uptake of 1.33 mg g⁻¹ of solid for MTS-3a. Leaving out Cr(III) ions that are adsorbed to some extent (0.56 mg g⁻¹ solid), none of the other ions tested interfere as they have no affinity or an extremely reduced affinity for the pyoverdin ligand, compared to iron. It must be noted that in solution, Cr(III) ions are not complexed by the free pyoverdin ligand. The fixation of the low amount of Cr(III) ions could be explained by the presence of traces of proteins acting as impurities in our pyoverdin preparation. These proteins could even originate from other Pseudomonas fluorescens strains able to chelate Cr(III) ions.41,42 In the case of silica-gel Si-3b, 0.15 mg Fe per g solid were recovered together with a very small amount (0.03 mg) of Cr(III).

Conclusions

The concept of using selective chelators of biological origin, anchored on a mesostructured porous silica (MTS type), as recyclable materials for the selective removal of metal ions from wastewater and their recovery has been demonstrated. By using Fe(III) ions and a ligand of bacterial origin, namely pyoverdin, iron has been selectively removed from a multi-metallic solution and its recovery has been performed. The use of an efficient spacer of the glycidoxypropyl type, reduces unwanted interactions of the chelator with the silica surface. Furthermore, the covalent anchoring of the bio-chelator is strong enough to allow multiple recycling without loss of capacity. The advantages of such ligand-anchored MTS silica are multiple. Ligands such as pyoverdin are easily prepared using standard methods from biotechnology and their covalent immobilisation is easy. Silica supports are generally resistant to bacterial attack. The extensive surface area and the low silanol density of large pore MTS allow for a high loading in the anchored ligand while minimizing the competing interactions with silanols. In this respect, the present study shows clearly that mesostructured silicas of the MTS type perform better than high-grade commercial amorphous mesoporous silicas. Furthermore, diffusion in large mesoporous channels provides easy access to the complexation sites. The successful trapping properties of covalently anchored pyoverdin on mesostructured silica of the MTS type for selective iron removal is a
milestone for the development of such strategy as it opens new possibilities in the field of toxic heavy metal removal from wastewater using anchored biological chelates.

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