Selective Cu$^{2+}$ adsorption and recovery from contaminated water using mesoporous hybrid silica bio-adsorbents

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A B S T R A C T

Metallothioneins (MTs) are low-molecular weight proteins (1–10 kDa), which are known to bind selectively metal ions such as Zn or Cd in metal–thiolate clusters. The present work describes the preparation of copper–metallothionein (Cu–MT) and its immobilization by covalent grafting on mesoporous silica for the selective uptake and recovery of Cu$^{2+}$ from water. The mesoporous silica used (SiDav) features 10 nm pore size suitable to accommodate Cu–MT (6 nm size) and 200 μm particle size adequate for flow processes. For the covalent coupling, SiDav was first functionalized with aminopropyl (SiDav–NH2) or glycidoxypropyl (SiDav–Gly) functions before to react with Cu–MT. After decomplexation of Cu, the resulting MT–SiDav–NH2 and MT–SiDav–Gly materials were used to adsorb Cu$^{2+}$ from aqueous solutions in the presence of various competing cations. The adsorption capacity of the hybrid biocomplexant silica materials was studied in batch and in column for flow process. Starting from a solution containing 2 mM of four cations, the maximum adsorption capacity under flow (1 mL/min, pH 6) was obtained for MT–SiDav–NH2 with a high selectivity for Cu$^{2+}$: Cu$^{2+}$ (0.210 mmol g$^{-1}$) > Cd$^{2+}$ (0.009 mmol g$^{-1}$) > Zn$^{2+}$ (0.005 mmol g$^{-1}$) > Pb$^{2+}$ (0.003 mmol g$^{-1}$). Furthermore, adsorbed Cu$^{2+}$ ions were quantitatively recovered by simply eluting the column with HCl. This column was also successfully used to preconcentrate Cu$^{2+}$ contained in different water samples as tap or mineral waters for an easier analysis of Cu traces.© 2011 Elsevier Inc. All rights reserved.

1. Introduction

The present work illustrates the feasibility of using selective biological chelators anchored at the surface of mesoporous silica supports as reusable materials for the selective removal and recovery of metal ions from contaminated waters. This material can also be used to preconcentrate traces of cations for an easier analysis of their content in different water samples, such as tap water or mineral waters. While synthetic ligands or chelators are widely studied, the use of bio-adsorbents prepared from biomass of bacteria, fungi and algae is less examined, but biocomplexants have several advantages over synthetic chemical ligands such as high selectivity to various ions depending on their tertiary structures and relatively mild conditions for adsorption and desorption [1]. Peptides and proteins could be efficient chelating ligands, due to their amino acidic residues that contain metal binding functional groups and because they can be produced at low cost. Previously, we had shown that a small protein, named pyoverdin ($M_w = 1.1$ kDa), a natural Fe$^{3+}$ chelator from a Pseudomonas fluorescens strain, covalently anchored through a glycidoxypropyl linker into mesoporous silica materials demonstrated very high selectivity and sorption capacity for Fe$^{3+}$ ions contained in an aqueous multimetallic solution. Amount as high as 1.33 mg of Fe$^{3+}$ per g of solid was adsorbed selectively from a solution of 40 mg/L of each metal (Fe$^{3+}$, Cu$^{2+}$, Ni$^{2+}$, Zn$^{2+}$, Co$^{2+}$, Pb$^{2+}$) and Fe$^{3+}$ was totally recovered after elution [2]. To complete this research in the selective uptake and recovery of metals contained in waters, our attention was focused on another family of biological chelators named metallothioneins. Metallothioneins are small metal-binding proteins found in all living organisms. They regulate the amounts of heavy metals in a cell. Metallothionein (MT) is a family of cysteine-rich, low-molecular weight proteins ($M_w$ ranging from 0.5 to 14 kDa). MTs have the capacity to bind both physiological (such as zinc, copper, selenium) and xenobiotic (such as cadmium, mercury, silver, arsenic) heavy metals through the thiol group of its cysteine residues, which represents nearly the 30% of its amino acidic residues [3]. Cysteine is a sulfur-containing amino acid, hence the name “-thionein”. When a metal enters a cell, it can be picked up by thionein, which thus becomes metallothionein. In humans, there are four main isoforms, which production is dependent on the type of metal exposure.

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(zinc, copper and selenium). Each MT molecule is able to capture several cations, such as 7 atoms/molecule for Zn, Cd, Pb metal ions and 18 atoms/molecule for Ag and Hg [4]. Their occurrence is reported not only for humans, but also throughout the animal kingdom, in plants and for several microorganisms, such as yeasts. MTs are produced by yeasts in response to metal threats provoked by elevated concentrations of some metals [5]. The yeast Saccharomyces cerevisiae has been commonly used to investigate copper incorporation in eukaryotic cells [6] and to study the properties of Cu–MT within the cellular environment. Cu–MT from yeast has been used in this work and is characterized by 53-residue polypeptide (5.6 kDa) containing 12 cysteines and 8 copper atoms/molecule [7]. Furthermore the unique feature of MT is the competition between protons and metal ions for protein binding. Protons can replace metals from MT and thus metal ions are released from the protein gradually with decreasing pH. Therefore, a simple addition of acid allows the metal recovery. As examples, Zn in MT is completely released at a pH lower than 4 and the same situation occurs for Cd at pH 2 [8]. Lower pH values for release are related to a higher binding affinity of the metal to MT. After removal of metals, in the presence of metal ions, the H+–MT can refold to the correct configuration when the pH is raised to neutral [9]. This characteristic makes MT a useful tool for the development of bio-adsorbents for the uptake and recovery of metals because the protein can be used repeatedly.

Since the MT capacity to bind metals such as zinc and cadmium has been demonstrated (see below Ref. [10]) with 7 metal ions/MT, we have attempted to achieve the selective and recyclable uptake of copper from water, by producing a Cu–MT from a culture medium with a high copper concentration and by using this natural ligand bounded to a mesoporous silica support. Among porous silica supports, MCM-41-like material demonstrated superior performance for iron uptake and release using pyoverdin as biochelator and compared to classical silica-gel due to a lower density of surface silanol groups [2]. In the present case however, silica-gel was preferred in order to fulfill two main conditions: a large pore diameter (10 nm) to accommodate MT (5.6 kDa, ~5 × 6 nm) (which is much larger than pyoverdin) and uniform particles of size large enough (200 μm) to perform studies in column under flow process without drop pressure. Indeed the existence of such uniform large particles of MCM-41 (without any aggregation) featuring large pores are not yet available, even by the pseudomorphic synthesis [11–13]. Cu–MT was expressed from yeast in a concentrated solution of copper to induce the formation of the isoform selective to copper, and the resulting Cu–MT was immobilized on silica support. Methods of physical or chemical immobilization of biomolecules on solid surfaces are well known [14]. For instance, MT has been immobilized on supports by several methods including unspecific adsorption/chemisorption, covalent binding or physisorption [15]. However, the supports were almost always polymeric resins or organic gels with limited capacity for reuse [16]. Inorganic carriers are more expensive than their organic commercial counterparts but have the advantages of being stable and reusable, which in some circumstances can decrease the effective cost of the process. The encapsulation of MT (from Sciszosaccharomyces pombe) into a sol–gel silica has been studied by Bahrami et al. [10] for Cd and Zn removal from water separately. Their experiments demonstrated that MT has a greater capacity for Cd and Zn than non-biological chelators such as polyethyleneimine or EDTA. Furthermore, the use of MT compared to non-specific biomass for metal adsorption offers the advantages of high metal binding capacities and selectivity.

The present work describes the immobilization of Cu–MT (from baker yeast) into a mesoporous silica previously functionalized with amino or glycidoxy groups to covalently bind MT to the material and its use for the selective uptake of Cu2+ from a multmetallic aqueous solution in batch and in flow process and the recovery of the Cu2+ ions.

2. Experimental

2.1. Reagents and materials

Analytical reagent-grade chemicals were used to prepare the solutions required for the biosynthesis and purification of MTs (from baker yeast). Freshly prepared Milli Q (Millipore) ultra pure water was used in all experiments. The support used was a commercial silica obtained as a gift from Grace-Davison named Davicat Si 1452 (named below SiDav) with 200 μm particle size, 390 m2/g specific surface area, 1.1 mL/g pore volume, 10 nm pore diameter. Before the covalent coupling of MT, this support was functionalized with 3-aminopropyl-triethoxysilane (APTES, Fluka) or 3-glycidoxypropyl-trimethoxisilane (GPTMS, Fluka) accordingly to Ref. [2]. Stock solutions (1000 ppm) of Cu(NO3)2, Cd(NO3)2, Zn(NO3)2 and Pb(NO3)2 were prepared in distilled water. The pH was adjusted between 2 and 8 using diluted solutions of HCl or NaOH.

2.2. Apparatus

Electronic absorption spectra were recorded on a UVikon XL UV–vis spectrometer from Bio-Tek Instruments and diffuse reflectance in the UV–vis range 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 nm thick cuvettes (100 QS, Helma, Mulheim, Germany). Atomic adsorption spectroscopy (FAAS) measurements were performed on a Spectra AA-220 Varian Spectrometer with an air–acetylene flame. Thermogravimetric analysis was carried out in a Netsch TG 209C thermostable. About 15 mg of solid sample was loaded, and the air-flow used was 50 cm3 min−1. The heating rate was 20 °C min−1 and the final temperature was 850 °C.

2.3. Procedures

2.3.1. Production, isolation and characterization of the metallothionein

Cu–MT was excreted from baker yeast cells grown in a medium containing 150 mg L−1 of Cu2+, according to the method of Weser and Hartmann [17]. Baker yeast was cultivated in a medium composed of 25 g yeast extract, 50 g gelatine hydrolysate, 175 g glucose, 1.5 g NaCl, 1.5 g KH2PO4 and 375 mg L−1 CuSO4 in 3 L of water. After 48 h, the cells were harvested by centrifugation at 3000 rpm for 15 min and then were washed three times, each in 10 vol. of deionised water. The total Cu2+ concentration in the yeast was determined by FAAS. Washed cells (20 mg dry weight of cells) were digested by adding 1 mL 6 N HNO3 in boiling water for 20 min. After this acid extraction, the samples were diluted to 5 mL with distilled water, centrifuged and the copper content was measured. In order to isolate Cu–MT, the copper loaded washed yeast cells were suspended in an equal volume of 20 mM Tris/HCl buffer, at pH 7 and ruptured in a Polytron homogenizer (0–4 °C). The homogenate, diluted two fold with 10 mM Tris/HCl buffer (pH 7), was centrifuged at 10,000 rpm for 1 h. The total supernatant solution was lyophilized. This powder was dissolved in a minimum volume (<2 mL) of N2-saturated 10 mM Tris/HCl, at pH 7. Then Cu–MT complexes were purified by size exclusion chromatography. The solution was applied to a Sephadex G-75 size exclusion chromatography column (80 × 2.5 cm) with a 10 mM Tris/HCl (pH 7) mobile phase in the presence of 0.1% 2-mercaptoethanol to avoid uncontrolled oxidation of thiolate sulfur. The fractions from the Mv 10 K region were chromatographed on Sephadex G-50 column (2.5 × 40 cm) equilibrated with
N2-saturated 10 mM Tris/HCl (pH 7). The detection and localization of Cu–MT during the preparation was controlled by copper analysis by FAAS in the respective chromatographic fractions and UV electronic absorption spectra. The presence of copper within these complexes was confirmed by FAAS. The H+–MT was prepared using the chelating agent diethyldithiocarbamate (DTC) [18]. The pH of the Cu–MT solution as isolated by G 50 gel filtration was adjusted to pH 5.0 by using 3 M sodium acetate (final concentration of acetate 0.1 M). One milligram of solid DTC/mL of solution was added and the sample was incubated at room temperature for 1 h. The colloidal Cu–DTC complex was removed by filtration through a 0.22 μm filter. The slightly yellow filtrate was desalted on a Sephadex G-25 column in 0.1% TFA and the fractions containing H+–MT were freeze-dried.

2.3.2. Immobilization of Cu–MT in mesoporous silica by adsorption

The SiDav support (1 g) was activated at 180 °C under vacuum for 1 h. A column was filled with the solid material and 14 mL of a solution containing 437 mg Cu–MT in 10 mM Tris/HCl buffer (pH 7) containing 0.1% of 2-mercaptoethanol was recirculated with a peristaltic pump. The copper concentration in this solution was determined by FAAS technique and the Cu–MT solution recirculation was continued until the inlet copper concentration is the same with the outlet concentration. The obtained adsorbent (Cu–MT–SiDav) was washed several times with water until no copper was detected in washing, and was then dried under vacuum at room temperature and stored at −5 °C.

2.3.3. Immobilization of Cu–MT into amino- or glycidoxy-functionalized mesoporous silica

Cu–MT was immobilized onto amino- or glycidoxy-functionalized silica by covalent binding. The silica support was firstly sialented with APTES or GPTMS according to the previously described procedure [2,19]. SiDav was freshly activated overnight at 180 °C under vacuum (1 g), and APTES or GPTMS (1 mL) were mixed in 50 mL of dry toluene. After stirring the solution (reflux, 2 h), the released ethanol was distilled off and the mixture was kept under reflux for 90 min. The functionalized silica (referred as SiDav–NH2 and SiDav–Gly, respectively) were filtered and washed with toluene, ethanol and then diethyl ether. They were then submitted to a continuous extraction run overnight in a Soxhlet apparatus using diethyl ether/dichloromethane (v/v, 1/1) at 100 °C and dried overnight at 110 °C.

The procedure of Cu–MT immobilization onto the amino-functionalized support involves the mixing of 1 g SiDav–NH2 with 4 mL of 0.1 M sodium phosphate buffer (pH 7). One millilitre of an aqueous 25 wt% glutaraldehyde solution was added leading to a total concentration of 0.54 M of glutaraldehyde (or 2.5 mmol glutaraldehyde/g SiDav–NH2) and the mixture was stirred for 30 min at room temperature. Then, the excess of glutaraldehyde was removed during three cycles of centrifugation/washing with 10 mL buffer solution each and the resulting solid was dispersed in 14 mL of 10 mM Tris/HCl buffer solution (pH 7) containing 460 mg lyophilized Cu–MT corresponding to 0.08 mmol Cu–MT per g of SiDav–NH2. The suspension was stirred at 25 °C for 24 h, centrifuged at 3000 rpm for 10 min to remove the buffer, and washed several times with water until no metallothionein was detected by UV in the washing. The final solid, referred as Cu–MT–SiDav–NH2, was dried under vacuum and stored at −5 °C.

For the covalent coupling of Cu–MT onto GPTMS-functionalized silica, SiDav–Gly (1 g) was activated under vacuum at 130 °C for 1 h. The solid was dispersed in DMF (14 mL) before adding 460 mg of lyophilized Cu–MT (corresponding to 0.08 mmol Cu–MT per g of SiDav–Gly) dissolved in the minimum amount of water [2]. The mixture was heated at 60 °C under stirring, for 48 h. The resulting material was collected by filtration and washed several times with water. The final material, referred as Cu–MT–SiDav–Gly was then dried under vacuum and stored at −5 °C.

Aliquots of the rinsing waters were analyzed by FAAS to quantify the amount of complex that was not covalently coupled. In parallel to quantify the amount of Cu immobilised into the silica support, a decomplexation of the entrapped Cu was performed using the strong chelating agent DTC [20]. After the solubilization of Cu–DTC complex in CCl4, the amount of copper was quantified. The total amounts of immobilized Cu–MT in the different silica supports were evaluated from the TG/DTA curves and compared with the results of the FAAS analysis.

2.3.4. Decomplexation of copper from Cu–MT immobilized in the silica supports

In order to use the immobilized MT in adsorption studies, Cu2+ was decomplexed from the Cu–MT. For 1 g of Cu–MT–SiDav–NH2 (or other hybrid material containing Cu–MT), 50 mg DTC in 10 mL of a 0.1 M sodium acetate buffer (pH 5.0) were used. The solid was then washed several times with CCl4 and ethanol and dried at 80 °C.

2.3.5. Cations adsorption studies over MT–silica materials in batch mode

The sorption of Cu2+, Cd2+, Zn2+ and Pb2+ metal ions from aqueous solutions was investigated in batch mode at room temperature. All experiments were conducted in acid-washed PTFE bottles by stirring (300 rpm) 0.1 g of dried hybrid materials with 20 mL aqueous solutions (5 g L−1) with the desired initial concentration of metal ions. The contents of the bottles were equilibrated at room temperature for 2 h. After equilibration, samples for metal analysis were taken after filtration through a 0.45 μm nitrocellulose membrane. The samples where then diluted with distilled water to be within 0.05–20 mg/L. The concentrations of Cu2+, Cd2+, Zn2+ and Pb2+ were determined by flame atomic absorption spectrometry (FAAS).

2.3.5.1. Influence of pH. The influence of the pH on the adsorption capacity of Cu2+, Cd2+, Zn2+, Pb2+ ions onto the hybrid biochelating mesoporous silica MT–SiDav–NH2 has been performed at room temperature using separated solutions of 0.5 mmol L−1 of each cation. The pH in the range 2–8 was adjusted using diluted solutions of HCl or NaOH.

2.3.5.2. Influence of ionic strength. The influence of NaCl concentration at pH 6 on the adsorption capacity of Cu2+, Pb2+, Cd2+ and Zn2+ into MT–SiDav–NH2 has been performed using separated solutions of 1 mmol L−1 of each metal ions.

2.3.5.3. Adsorption isotherms. Adsorption isotherms of Cu2+ were established for SiDav, SiDav–NH2, MT–SiDav–NH2 materials at pH 6 by increasing Cu solution concentration from 0.1 to 5 mmol L−1. After adsorption, the concentration of remaining Cu was evaluated by FAAS and corresponds to the equilibrium concentration, Ce (mmol L−1). The Cu adsorption capacity of materials, qe (mmol g−1), was plotted as a function of the equilibrium concentrations of Cu2+ ions, Ce (mmol L−1).

2.3.5.4. Ions adsorption kinetics. Adsorption kinetics experiments were carried out to determine the adsorption rates and the reaction time to reach adsorption equilibrium. The effect of contact time on the uptake of metal ions by the hybrid materials was investigated at pH 6.0 by shaking 0.1 g of dried material in 20 mL of solutions with initial concentration in metal ions of 1 mM for each separated solution of cations. Samples were taken at different time intervals and the residual concentration of metal was determined by FAAS.
2.3.5.5. Competitive adsorption over MT–SiDav–NH$_2$. The competitive adsorption of ions in batch mode was performed with MT–SiDav–NH$_2$ at pH 6. Selectivity coefficients were determined by shaking 0.1 g of dried material in 20 mL of a multimetallic solution containing Cu$^{2+}$, Cd$^{2+}$, Zn$^{2+}$ and Pb$^{2+}$ ions with a concentration of 2 mM of each ion.

2.3.6. Cations adsorption studies over MT–silica materials in flow through column

Continuous flow experiments were performed using plastic columns with a length of 3 cm and a diameter of 1 cm. The hybrid materials (0.5 g) were placed between two small sand beds and were separated on top and bottom by two 45 $\mu$m nitrocellulose membranes.

2.3.6.1. Breakthrough curves. The Cu$^{2+}$ solution having an initial concentration of 0.8 mM (50 ppm) was allowed to flow downward through the column at the desired flow rate between 1 and 3 mL min$^{-1}$ by using a peristaltic pump. Samples were collected from the outlet of the column at different time intervals and analyzed for Cu$^{2+}$ concentration. The operation of the column was stopped when the initial Cu$^{2+}$ concentration matches the outlet Cu$^{2+}$ concentration. The outlet Cu$^{2+}$ concentrations were plotted versus volume to give the breakthrough curves. The outlet Cu$^{2+}$ concentration was also plotted against flow rate.

2.3.6.2. Competitive adsorption over MT–SiDav–NH$_2$. The competitive adsorption experiment of metal ions in continuous flow was carried out at pH 6 by passing through a column filled with MT–SiDav–NH$_2$, 100 mL of a multimetallic solution containing Cu$^{2+}$, Pb$^{2+}$, Cd$^{2+}$ and Zn$^{2+}$ ions with 2 mM concentration of each ion at a flow rate of 1 mL min$^{-1}$.

2.3.6.3. Column regeneration and copper recovery. Column regeneration and copper recovery was achieved by washing the column with 1 M HCl solution until no Cu$^{2+}$ was detected in the effluents. Thereafter the column was washed with water and the adsorbed copper was eluted into the minimum volume of 1 M HCl for which the metal ion recovery was the best. The preconcentration factor was calculated as the ratio between the volume of effluent used for the loading capacity and the volume of 1 M HCl solution necessary for Cu$^{2+}$ recovery.

2.3.6.5. Copper determination in natural and mineral water samples. Tap water (pH 6.4, conductivity 150 $\mu$S/cm) and commercial mineral water (pH 6.6, conductivity 460 $\mu$S/cm) were used without any pretreatment. Samples of 300 mL of tap or mineral water unspiked and spiked (with 10 $\mu$g L$^{-1}$ Cu$^{2+}$ solution) were passed through a column filled with MT–SiDav–NH$_2$ at a flow rate of 1 mL min$^{-1}$. Then the column was washed with 20 mL deionized water. The adsorbed copper was eluted with 6 mL of 1 M HCl and copper content determined by FAAS.

3. Results and discussion

3.1. Properties of metallothionein and its copper chelate in solution

Copper–metallothionein (Cu–MT) was excreted from baker yeast and purified. Two significant copper containing fractions were identified by size exclusion chromatography. The more abundant fraction is a low-molecular (LMW) component with an elution time of 120–150 min. The second component is a high-molecular-weight (HMW) complex, which eluted from the column in the void volume (elution time ~60 min). The UV–vis absorption spectrum of the LMW component showed a resolved shoulder at 260 nm and a broad signal at approximately 340 nm while a single broad absorption shoulder at about 270 nm was obtained from the HMW copper-containing component. Agreement with previous studies only the LMW protein can be identified as Cu–MT [20].

The wet cells contained 1.1 mg copper/g cell (determined by FAAS analysis) that correspond to 12.55 mg MT/g cell if 8 Cu per MT has taken in the calculation. Indeed, the crystal structure of yeast Cu–MT has been resolved recently (in 2005) by Calderone et al. [21] and shows cluster of 8 copper atoms complexed through Cys–S–Cu bonds (Scheme 1). The Cu–MT structure shows the largest known oligonuclear Cu thiolate cluster in biology, consisting of
six trigonally and two diagonally coordinated Cu ions. Cu–MT shows a different structure of the metal cluster in comparison to other metallothionein, but also the main differences lie in the cysteine topology and in the conformation of some portions of the backbone.

The H+–MT was prepared using the chelating agent diethylidithiocarbamate (DTC) by the method proposed by Hunziker [18]. The amount of decomposed copper corresponded to that determined for the Cu–MT samples by FAAS. All the copper complexed by MT was depleted by DTC chelating agent. After extraction and purification 500 mg of Cu–MT were recuperated from 118 g of wet cells.

3.2. Metallothionein immobilized on mesoporous silica supports

Cu–MT was immobilized on mesoporous silica support (SiDav) by adsorption and different covalent graftings. SiDav features 10 nm pore diameter, large enough to accommodate Cu–MT (~5–6 nm), and particle size of 200 μm to allow the study of adsorption in flow process without pressure drop. For the covalent coupling of Cu–MT, SiDav was first functionalized with 3-aminopropyltriethoxysilane (SiDav–NH2) or with 3-glycidoxypropyltrimethoxysilane (SiDav–Gly) through a silanization reaction (Scheme 1) [2,19].

The TGA profile of SiDav, SiDav–NH2 and SiDav–Gly are presented in Fig. 1. The SiDav support exhibits a first mass loss (1.42%) in the temperature range 20–120 °C, corresponding to the physically adsorbed water. The second weight loss (2.77%) between 250 and 800 °C is attributed to water loss due to the condensation of the silanols. The amount of silanols is therefore estimated at 3.08 mmol SiOH/g silica and (if there are all in surface) to a density of 4.7 SiOH/nm², which is a classical value for silica gel materials. In the case of functionalized silica materials, the loss of physisorbed water (below 120 °C) was about 2%. Considering the TGA profiles, the degradation of the anchored organic moieties occurs between 150 and 800 °C. The corresponding weight losses were 8.2% and 11.7% for SiDav–NH2 and SiDav–Gly, respectively. This corresponds to an anchorage capacity of 91.3 mg of glycidoxy per g of silica for SiDav–Gly and through the glutaraldehyde intermediate coupling agent for SiDav–NH2 (Scheme 1) to obtain the hybrid biocomplexant silica materials, named Cu–MT–SiDav–Gly and Cu–MT–SiDav–NH2, respectively. Cu–MT was immobilized rather than H+–MT in order to preserve the conformation of the protein during the grafting, as it was previously done for the Fe–pyoverdin grafting in mesoporous silica materials [2], and to protect the functional groups involved in the metallic ions complexation, as the –SH groups of cysteine may oxidize into –S–S– bridges. The TGA profiles of all hybrid biocomplexant silica materials Cu–MT–SiDav–Gly and Cu–MT–SiDav–NH2, respectively, are presented in Fig. 2. The loss of the organic moieties occurred between 150 and 800 °C and corresponds to the loss of MT and the organic function used for the silica grafting.

The amount of immobilized Cu–MT was determined both by TGA and by copper mass balance by FAAS (Table 2). The results obtained by both methods stand in good agreement. Thus, for Cu–MT adsorbed on SiDav (Cu–MT–SiDav) only 1% of the Cu–MT engaged in the physical adsorption procedure was immobilized onto the support, which corresponds to 0.001 mmol Cu–MT g⁻¹ silica.

Table 1

<table>
<thead>
<tr>
<th>Material</th>
<th>Elemental analysis</th>
<th>Organic group (mg g⁻¹ silica)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>SiDav–NH2</td>
<td>6.30</td>
<td>2.46</td>
</tr>
<tr>
<td>SiDav–Gly</td>
<td>7.62</td>
<td>0.02</td>
</tr>
</tbody>
</table>

ᵃ Determined from N elemental analysis.
ᵇ Determined from C elemental analysis.
ᶜ Determined from TGA.

do to a density of 1.60 mmol NH2/g silica or 1.72 mmol NH2/g native silica (2.66 NH2/nm²), if additional silica due to the grafting is taken into account in the calculation with the hypothesis of a bidentate grafting (the value becomes 2.60 NH2/nm² for a tridentate grafting hypothesis). For SiDav–Gly, TGA result indicates a density of 1.18 mmol Gly/g silica or 1.27 mmol Gly/g native silica (1.96 Gly/nm²) with a bidentate grafting hypothesis (or 1.91 Gly/nm² with a tridentate grafting hypothesis). The grafting density values are in agreement with previous results found for the grafting of organic functions on silica gel and MCM-41 with values ranging from 1 to 2.5 molecules/nm² with an expected higher grafting density for aminopropyl groups in comparison to less polar functional chains [2,19].

The functionalized silica materials were used thereafter for the Cu–MT immobilization by direct covalent coupling for SiDav–Gly and through the glutaraldehyde intermediate coupling agent for SiDav–NH2 (Scheme 1) to obtain the hybrid biocomplexant silica materials, named Cu–MT–SiDav–Gly and Cu–MT–SiDav–NH2, respectively. Cu–MT was immobilized rather than H+–MT in order to preserve the conformation of the protein during the grafting, as it was previously done for the Fe–pyoverdin grafting in mesoporous silica materials [2], and to protect the functional groups involved in the metallic ions complexation, as the –SH groups of cysteine may oxidize into –S–S– bridges. The TGA profiles of all hybrid biocomplexant silica materials Cu–MT–SiDav–Gly and Cu–MT–SiDav–NH2, respectively, are presented in Fig. 2. The loss of the organic moieties occurred between 150 and 800 °C and corresponds to the loss of MT and the organic function used for the silica grafting.

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Fig. 1. Thermogravimetric analysis (TGA) of (1) mesoporous silica SiDav, and functionalized SiDav with (2) 3-aminopropyltriethoxysilane (SiDav–NH2) and (3) 3-glycidoxypropyltrimethoxysilane (SiDav–Gly). Curve (4) is SiDav–NH2 cross-linked with glutaraldehyde.

Fig. 2. Thermogravimetric analysis (TGA) of Cu–MT-immobilized silica supports: (1) Cu–MT immobilized by adsorption in SiDav, (2) Cu–MT covalently grafted on SiDav–Gly and (3) Cu–MT covalently grafted on SiDav–NH2.
Moreover, the solid prepared by simple adsorption Cu–MT–SiDav was deactivated after several metal ions adsorption/desorption cycles due to the progressive leaching of the biocomplexant. For Cu–MT grafted on SiDav–Gly, the difference of weight losses in the range 150–800 °C between the TGA analysis of Cu–MT–SiDav–Gly (307 mg/g silica) and SiDav–Gly (135 mg/g silica) allows to calculate an amount of 172 mg Cu–MT/g silica (calcined material), corresponding to 0.034 mmol Cu–MT/g silica or 0.037 mmol Cu–MT/g native silica (bidentate grafting hypothesis). This represents a density of 0.057 Cu–MT/nm² or 17.5 nm² of surface covered per Cu–MT molecule, equivalent to an average diameter of 4.7 nm per molecule, so to a densely packed Cu–MT monolayer at the surface of the silica support. Indeed the size of a Cu–MT molecule in a crystal is 5 × 6 nm [21].

For Cu–MT grafted on SiDav–NH₂, calculations are more complex as the amount and the way of the glutaraldehyde is anchored to the amino groups of the solid (1 or 2 bonds with two adjacent amino groups) is unknown. In the preparation, there is enough glutaraldehyde to cross-link all amino groups of the surface. However the time of reaction is very short (30 min at room temperature) and several washings have been performed before to introduce the solution of Cu–MT as well as after the cross-linkage with MT.

In a first hypothesis, we can assume that the amount of glutaraldehyde grafted corresponds to that necessary for the cross-linkage with Cu–MT. The weight loss due to this linker (66 g/mol) would be then negligible in comparison to the weight loss due to Cu–MT. The weight loss of organics of SiDav–NH₂ (91.3 mg/g silica) to that of Cu–MT–SiDav–NH₂ (321 mg/g silica). A value of 230 mg Cu–MT/g silica is obtained, which is in good agreement with the amount of Cu–MT calculated from the amount of copper in the material determined by FAAS (260 mg Cu–MT/g silica) (Table 2). The MT amount derived from TGA corresponds to 0.046 mmol Cu–MT/g silica or 0.049 mmol Cu–MT/g native silica (bidentate grafting hypothesis) and represents a density of 0.076 Cu–MT/nm² or 13 nm²/mm² of surface covered per Cu–MT molecule (equivalent to an average diameter of 4.1 nm per molecule), so to a higher amount of Cu–MT at the surface of SiDav–NH₂ in comparison to SiDav–Gly and a more densely packed Cu–MT monolayer. However, if the intermediate material SiDav–NH₂ cross-linked with glutaraldehyde is isolated and dried, TGA (Fig. 1) shows a weight loss of 118.6 mg/g silica. By taking the hypotheses that either (i) each glutaraldehyde is only cross-linked with one amino group or (ii) the majority of glutaraldehyde is cross-linked with two adjacent amino groups, the resulting amounts of Cu–MT immobilized in SiDav–NH₂ are 209 and 203 mg/g silica for each hypothesis, respectively. These values are far from the results obtained by FAAS copper analysis (260 mg Cu–MT/g silica). The result obtained using the TGA analysis of the intermediate SiDav–NH₂–glutaraldehyde material could arise from an excess of glutaraldehyde retained at the surface due to the drying step, whereas some glutaraldehyde could have been washed away during the entire preparation, which includes several washings, of Cu–MT–SiDav–NH₂. Therefore we have chosen for the discussion of the following sections to remain with the result of the first hypothesis (230 mg Cu–MT/g silica − 0.046 mmol Cu–MT/g silica).

Copper decomplexation was achieved by using diethyldithiocarbamate (DTC) as a chelating agent. All the copper complexed by MT was depleted by DTC making the resulting H⁺–MT available for a new complexation reaction.

3.3. Ions adsorption properties of the metallothionein immobilized into mesoporous silica supports

In order to establish that the chelation of metals occurs thanks to the presence of MT rather than on the silica matrix and/or on the amino groups, the removal of four metallic ions (Cu²⁺, Cd²⁺, Zn²⁺, Pb²⁺) from aqueous solutions was investigated over the parent mesoporous silica SiDav, the functionalized silica SiDav–NH₂, and the hybrid biocomplexant materials MT–SiDav–NH₂ and MT–SiDav–Gly (Table 3). MT covalently grafted on silica materials reveals much higher affinity for copper in comparison to SiDav and SiDav–NH₂ as demonstrated below (Section 3.3.3). The two hybrid biocomplexant materials MT–SiDav–NH₂ and MT–SiDav–Gly showed the same kind of behavior in the metal adsorption studies with a somewhat higher capacity for MT–SiDav–NH₂ due to a higher amount of MT on this material (Table 3). The amount of metal retained per unit mass of silica was evaluated using the following expression:

$$q_e = \frac{V (C_0 - C_e)}{W}$$  

where $q_e$ is the amount of metal ion adsorbed per g of silica (mmol g⁻¹); $V$ the volume of the aqueous phase (L); $W$ is the silica weight of the material (g); $C_0$ and $C_e$ are the concentrations of metal ions in the initial aqueous phase and equilibrium concentrations (mmol L⁻¹).

The adsorption of metal ions to the chelating agent MT immobilized onto the mesoporous silica is affected by several factors. These include the physicochemical parameters of solution such as pH, ionic strength, initial metal concentration, contact time, interaction with other ions, etc. The influence of these factors on the adsorption process is discussed below. For the sake of clarity, the only results described hereafter will be those obtained with MT–SiDav–NH₂.

3.3.1. Influence of pH

The influence of the pH on the adsorption capacity of separated ion (Cu²⁺, Cd²⁺, Zn²⁺, Pb²⁺) solutions onto the hybrid biocatalyst mesoporous silica is illustrated in Fig. 3. This study has been performed using separated solutions of 0.5 mM of each cation corresponding to a potential amount for adsorption of 0.10 mmol ion g⁻¹ silica therefore to 1/4 of the expected maximum capacity of MT–SiDav–NH₂ for copper ions (0.046 mmol of MT g⁻¹ silica with 8 Cu atoms per MT). The adsorption capacity of ions increased with the

<table>
<thead>
<tr>
<th>Parameters for the Langmuir and Freundlich isotherm models for the adsorption of Cu²⁺ in the hybrid biocatalyst MT–SiDav–NH₂ and MT–SiDav–Gly materials and in the parent mesoporous silica SiDav.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Materials</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>MT–SiDav–NH₂</td>
</tr>
<tr>
<td>MT–SiDav–Gly</td>
</tr>
<tr>
<td>SiDav</td>
</tr>
</tbody>
</table>
ph and presented a maximum around pH 6, this maximum of adsorption is maintained until pH 8. Metallothionein being an intracellular peptide, complexation of the metal ions occurs preferentially at pH values close to the physiological one. In the following, a pH of 6 was selected. The maximum of adsorption reached in this experiment was 0.066 mmol Cu g⁻¹ silica, corresponding to 66% of copper removal. For the other cations, the adsorption capacity was lower with 0.055, 0.023 and 0.006 mmol ion g⁻¹ silica for Pb²⁺, Cd²⁺, Zn²⁺, respectively.

### 3.3.2. Influence of ionic strength

The ionic strength of the solution also affects the metal adsorption. Fig. 4 shows the influence of NaCl concentration on the adsorption capacity of Cu²⁺, Pb²⁺, Cd²⁺ and Zn²⁺ into MT–SiDav–NH₂. This study has been performed using separated solutions with a concentration corresponding to a potential amount for adsorption of 0.20 mmol ion g⁻¹ silica therefore half of the expected maximum capacity of MT–SiDav–NH₂ for copper ions (0.046 mmol of MT g⁻¹ silica with 8 Cu atoms per MT). For copper, the maximum of adsorption reached in this experiment was 0.186 mmol Cu g⁻¹ silica, corresponding to 93% of copper removal, for a NaCl concentration between 0 and 40 mmol L⁻¹. For higher NaCl concentration (>50 mmol L⁻¹), the amount of metal adsorbed on MT–SiDav–NH₂ materials drastically decreased (3–4-fold), indicating a conformational change of the tertiary structure of the protein and the metals binding sites and therefore a loss of the biocomplexant binding ability at high ionic strength.

![Fig. 4](image_url). Influence of NaCl concentration on metal ions removal at pH 6 using MT–SiDav–NH₂ as adsorbent (5 g L⁻¹) in batch experiments performed with separated aqueous metal solutions with initial concentrations of 0.5 mM for Cu²⁺, Cd²⁺, Zn²⁺, Pb²⁺ for each metal ions.

### 3.3.3. Adsorption isotherms

Adsorption isotherms of Cu²⁺ were established by plotting the adsorption capacities of MT–SiDav–NH₂, \( q_a (\text{mmol g}^{-1}) \) as a function of the equilibrium concentrations of Cu²⁺ ions, \( C_e (\text{mM}) \) (Fig. 5). The comparison of the adsorption capacity of MT–SiDav–NH₂ (0.046 mmol MT/g silica) with the parent mesoporous silica materials SiDav (3.08 mmol SiOH/g silica) and SiDav–NH₂ (1.60 mmol NH₂/g silica) has been performed. The maximum Cu²⁺ adsorption capacity of MT–SiDav–NH₂ amounted to 0.43 mmol g⁻¹ silica for an equilibrium concentration of 3.5 mmol L⁻¹ corresponding to a total occupancy of the adsorption sites by copper ions, whereas SiDav and SiDav–NH₂ adsorbed only 0.03 and 0.26 mmol g⁻¹ silica, respectively. The hybrid biocomplexant silica material MT–SiDav–NH₂ exhibited an enhanced adsorption performance for Cu²⁺ evidencing the higher affinity of Cu²⁺ for MT molecule in comparison to NH₂ and OH groups. As MT is able to complex 8 Cu ions per molecule, the loading value for MT–SiDav–NH₂ corresponds to a total occupancy of the adsorption sites by copper ions (0.37 mmol Cu/g silica) with some additional copper (0.08 mmol Cu/g silica) presumably linked on amino and/or silanol groups. It is to notice that a similar behavior is occurring for MT–SiDav–Gly (0.034 mmol MT/g silica) which complexes 0.35 mmol Cu/g silica (Table 3), corresponding to a total occupancy of the adsorption sites by copper ions (0.27 mmol g⁻¹ silica). There is therefore also some additional copper (0.08 mmol Cu/g silica) presumably linked to OH groups. Moreover, the isotherm of MT–SiDav–NH₂ shows a sharp initial slope, indicating that the material acts as highly efficient adsorbent even at low metal concentration.

The Langmuir and Freundlich isotherm models were applied to the experimental data in order to get a better insight into the sorption mechanism. The Langmuir adsorption isotherm describes a homogeneous surface, assuming that all the adsorption sites have the same activity and that the adsorption at a site does not affect adsorption at an adjacent site. Another assumption is that the adsorption occurs through the same mechanism and only a monolayer is formed at the maximum adsorption. The Langmuir equation is expressed in the following equation:

\[
\frac{C_e}{q_a} = \frac{C_r}{q_0} + \frac{1}{q_0b}
\]  

(2)

where \( q_a \) is the amount of solute adsorbed on the surface of the material (mmol g⁻¹), \( C_r \) is the equilibrium concentration of the solute (mmol L⁻¹), \( q_0 \) is the maximum surface density at monolayer coverage and \( b \) is the Langmuir adsorption constant (L mmol⁻¹) related to the adsorption energy. Plots of \( C_e/q_a \) versus \( C_e \) allow calculation of \( q_0 \) and \( b \). The Freundlich isotherm describes the equilibrium on heterogeneous surfaces and does not assume monolayer...
capacity. Such a model has been considered as it has been suggested that the hybrid adsorbent features a more heterogeneous microstructure than an unmodified one due to the coating by organosilica [22]. Moreover, the adsorption mechanisms for Cu²⁺ may involve not only the metal ion chelation by the thiol complexing functions of cysteine groups of the metallothionein, but as well a surface complexity by amino or Si-OH sites remaining uncovered. The sorption data representation according to the Freundlich equation takes the form:

\[ q_e = K_F C^{1/n} \]  

(3)

A linear form of the Freundlich equation is

\[ \ln q_e = \ln K_F + \frac{1}{n} \ln C_e \]  

(4)

where \( K_F \) is the Freundlich constant (mmol g⁻¹), which indicates the sorption capacity and represents the strength of the absorptive bond and \( n \) is the heterogeneity factor, which represents the bond distribution. According to Eq. (4) the plot of the \( \ln q_e \) versus \( \ln C_e \) gives a straight line and \( K_F \) and \( n \) values can be calculated from the intercept and slope of this straight line.

The Langmuir and Freundlich parameters for the sorption of Cu²⁺ onto hybrid biocomplexant silica materials MT–SiDav–NH₂ and MT–SiDav–Gly comparatively with parent mesoporous silica SiDav are listed in Table 3. It appears that the Langmuir isotherm model fits better the experimental data than the Freundlich model when the linearity coefficient \( R^2 \) values are compared, particularly for the MT–SiDav–NH₂ and MT–SiDav–Gly materials with \( R^2 > 0.997 \) for the Langmuir model. It can be then concluded that the metal adsorption on the hybrid biocomplexant silica material occurs most probably on a homogeneous surface and that Cu²⁺ adsorption on metallothionein immobilized onto mesoporous silica proceeds via a monolayer formation through a complexation mechanism.

3.3.4. Ions adsorption kinetics over MT–SiDav–NH₂

This study has been performed using separated solutions of each cation with a concentration corresponding to a potential amount for adsorption of 0.20 mmol g⁻¹ silica, therefore the half of the expected maximum capacity of MT–SiDav–NH₂ for copper ions (0.05 mmol of MT g⁻¹ silica with 8 Cu atoms per MT). Fig. 6 shows the specific adsorption capacity of MT–SiDav–NH₂ as a function of time. The hybrid biocomplexant silica material reached the saturation capacity after one hour. The highest affinity was for Cu²⁺ and followed the sequence: Cu²⁺ > Pb²⁺ > Cd²⁺ > Zn²⁺, with cations uptake values of 0.18, 0.16, 0.06, 0.02 mmol g⁻¹ silica, respectively.

Adsortion rates have been analyzed by using two common semi-empirical kinetic models, which are based on adsorption equilibrium capacity: the pseudo-first-order and pseudo-second-order models, proposed by Lagergreen [23] and Ho and McKay [24], respectively. The pseudo-first-order equation relates the adsorption rate to the amount of metal adsorbed at time \( t \) as

\[ \frac{dq_t}{dt} = k_1(q_e - q_t) \]  

(5)

where \( q_e \) and \( q_t \) are, respectively, the adsorbed amounts of metal at equilibrium and time \( t \), expressed as mmol g⁻¹; \( k_1 \) is the pseudo-first-order kinetic constant, expressed as min⁻¹. By integration and rearrangement the linear form is obtained:

\[ \ln(q_e - q_t) = \ln q_e - k_1 t \]  

(6)

The pseudo-second-order equation may be written in the form:

\[ \frac{dq_t}{dt} = k_2(q_e - q_t)^2 \]  

(7)

where \( k_2 \) is the pseudo-second-order kinetic constant, expressed as g mmol⁻¹ min⁻¹. By integration of the differential equation, the linear form is obtained:

\[ \frac{t}{q_t} = \frac{1}{k_2 q_e^2} \cdot \frac{t}{q_e} \]  

(8)

\[ h = k_2 q_e^2 \]  

(9)

where \( h \) is the initial adsorption rate and is expressed as mmol g⁻¹ min⁻¹.

The linear forms (Eqs. (6) and (9)) are commonly used to check the validity of these models and to obtain the model parameters when the corresponding linear plot is adequate. From the applied models, the pseudo-second-order model showed the best correlation with the experimental data (Table 4).

The parameter that influence the efficiency of the biocomplexant material at the short contact times associated with dynamic adsorption systems is the initial sorption rate, \( h \). Furthermore, this parameter becomes particularly important for selective sorption since the retention of the metal ion can be modulated by changing the contact time with the solid. The \( h \) values for the four ions gave the following sequence: \( h_{\text{Cu}^{2+}} > h_{\text{Pb}^{2+}} > h_{\text{Cd}^{2+}} > h_{\text{Zn}^{2+}} \).

3.3.5. Competitive adsorption over MT–SiDav–NH₂ in batch mode

The competitive adsorption experiments by the MT–SiDav–NH₂ hybrid adsorbent were first carried out in a batch reactor from a multimetallic solution containing all the four investigated metal ions (Cu²⁺, Pb²⁺, Cd²⁺, Zn²⁺). This study has been performed using cation concentrations corresponding to a potential amount for adsorption of 0.40 mmol g⁻¹ silica, therefore the total capacity for copper ions in MT–SiDav–NH₂ (0.05 mmol of MT g⁻¹ silica with 8 Cu atoms per MT). The selectivity coefficients, \( k_i \), of the Cu²⁺ in the presence of the other metal (Me²⁺) ions were calculated according to the following equation:

\[ k_i = \frac{q_i \text{Cu}^{2+}}{q_i \text{Me}^{2+}} \]  

(10)

The results obtained in batch are presented in the first two columns of Table 5.

Table 4

<table>
<thead>
<tr>
<th>Metal ion solutions</th>
<th>( h ) (mmol g⁻¹ min⁻¹)</th>
<th>( k_i ) (g mmol⁻¹ min⁻¹)</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu²⁺</td>
<td>0.0160</td>
<td>0.245</td>
<td>0.9936</td>
</tr>
<tr>
<td>Pb²⁺</td>
<td>0.0076</td>
<td>0.137</td>
<td>0.9608</td>
</tr>
<tr>
<td>Cd²⁺</td>
<td>0.0027</td>
<td>0.256</td>
<td>0.9345</td>
</tr>
<tr>
<td>Zn²⁺</td>
<td>0.0021</td>
<td>0.356</td>
<td>0.9994</td>
</tr>
</tbody>
</table>
The Cu²⁺ adsorption capacity (0.34 mmol g⁻¹) of MT–SiDav–NH₂ from a multimetallic solution is very high and reaches 85% of the total adsorption capacity of the bio-adsorbent with a very low adsorption for the other metal ions (>0.01 mmol g⁻¹). The selectivity coefficients of Cu²⁺ in the presence of other divalent metal ions, \( k_{\text{i}} \), are in the following order: \( k_{\text{i}} \text{Pb}^{2+} > k_{\text{i}} \text{Zn}^{2+} > k_{\text{i}} \text{Cd}^{2+} \) showing the very high selectivity of MT–SiDav–NH₂ toward the Cu²⁺ ions. The adsorption behavior of Pb ions is noteworthy. In the case of the adsorption from a monometallic solution, the amount of Pb²⁺ adsorbed (0.14 mmol g⁻¹) was close to the one of Cu²⁺ ions (0.18 mmol g⁻¹) (see Fig. 6). By contrast, Pb²⁺ ions are barely adsorbed (0.001 mmol g⁻¹) in MT–SiDav–NH₂ from a competitive multimetallic solution. This behavior is most probably due to the greater ionic radius of Pb²⁺ with respect to the other metallic ions in the solution, which may limit its diffusion towards the active adsorption site.

### 3.3.6. Adsorption over MT–SiDav–NH₂ in flow processes

Continuous metal ions removal was investigated with MT–SiDav–NH₂ introduced in a packed-bed column passing aqueous metallic solutions at controlled flow rates.

#### 3.3.6.1. Breakthrough curves over MT–SiDav–NH₂ in flow processes

Continuous Cu²⁺ removal was investigated with MT–SiDav–NH₂ in the packed-bed column from an aqueous solution containing 0.8 mM metal at different flow rates from 1 to 3 mL min⁻¹ (Fig. 7). As comparison, in batch mode (with an infinite contact time), the effective copper adsorption capacity was 0.18 mmol g⁻¹ silica (8.51 mg Cu/g material) for this metal concentration, which will correspond to the adsorption of the copper contained in 84 mL of solution for 0.5 g of bio-adsorbent as used for the experiment reported in Fig. 7. Effluent Cu²⁺ concentration levels were plotted as a function of passed volume in the form of breakthrough curves (Fig. 7) for the different flow rates. Breakthrough describes the increase in effluent concentration of a metal ion due to the reduced capacity of the adsorbent for the metal ion. The equilibrium loading will determine the maximum sharpness of breakthrough that is, how efficiently the ion will be separated from other components. The sharpness of breakthrough curves is indicative as well as the thickness of the adsorption zone indicating that the bed is loaded more completely. In the case of the adsorption of Cu²⁺ on MT–SiDav–NH₂ in the packed-bed column, the breakthrough curve is very sharp, which is the evidence of a complete loaded bed and a high efficiency of this adsorbent for copper removal. The breakthrough points stand at 20, 40 and 55 mL of solution for flow rates of 3.2 and 1 mL min⁻¹, respectively, corresponding to adsorption capacities of 0.04, 0.09 and 0.12 mmol g⁻¹ silica, therefore to 23%, 48% and 66% of the adsorption capacity obtained in batch mode. Lower flow rate increased the adsorption capacity of the column. After the first breakthrough, the column adsorbed additional ions on a large volume zone before to reach the maximum of adsorption obtained when the concentration of the solution is equal to the initial concentration (\( C/C_0 = 1 \)). For all flow rates the maximum dynamic loading capacity of 0.18 mmol g⁻¹ silica (8.5 mg Cu g⁻¹ bio-adsorbent) was attained after passing 84 mL of solution (Fig. 7), showing a very high level of copper adsorption corresponding to 100% of the batch mode (for the same initial concentration) and to 37% of the maximum achievable loading capacity of the bio-adsorbent.

#### 3.3.6.2. Competitive adsorption over MT–SiDav–NH₂ in flow processes

The competitive adsorption experiment of metal ions in continuous flow was carried out by passing through the column filled with MT–SiDav–NH₂ a multimetallic solution containing Cu²⁺, Pb²⁺, Cd²⁺ and Zn²⁺ ions with 2 mM concentration of each ion at a flow rate of 1 mL min⁻¹. As comparison, in batch mode (with an infinite contact time), the effective copper adsorption capacity was 0.34 mmol g⁻¹ silica for this metal concentration (Table 5). In flow process for this flow rate, a capacity for copper adsorption of 0.21 mmol g⁻¹ silica was reached corresponding to 62% of the adsorption capacity of the batch mode and to 52% of total adsorption capacity of the bio-adsorbents. A very high selectivity for copper in flow is proved by the low adsorption capacity of the other metals, below 0.009 mmol g⁻¹. The most significant difference between batch and continuous flow experiments was observed for the Pb²⁺ ions (selectivity coefficient of 38 in flow compared to 340 in batch mode). Most probably, the equilibrium displacement for the more voluminous lead ions by the other metal ions with a greater affinity for the adsorbent could not be attained due to the shorter contact time than that achieved in batch mode. For the other ions, the values of selectivity coefficients were in the same range than those obtained in batch mode (Table 5).

#### 3.3.6.3. Column regeneration and copper recovery

When the effluent concentration passes the breakpoint values, the feed of the column is discontinued and the column should be regenerated. The column containing 0.5 g of bio-adsorbent was regenerated by circulating

### Table 5

<table>
<thead>
<tr>
<th>Metal ions</th>
<th>Batch mode</th>
<th>Continuous flow mode</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{q}_{\text{D}} )ₐₙₐₙ \left( \text{mmol silica}^{-1} \right)</td>
<td>( \text{q}_{\text{D}} )ₚₑₙ \left( \text{mmol silica}^{-1} \right)</td>
</tr>
<tr>
<td>Cu²⁺</td>
<td>0.340</td>
<td>0.210</td>
</tr>
<tr>
<td>Cd²⁺</td>
<td>0.011</td>
<td>0.009</td>
</tr>
<tr>
<td>Zn²⁺</td>
<td>0.008</td>
<td>0.003</td>
</tr>
<tr>
<td>Pb²⁺</td>
<td>0.001</td>
<td>0.005</td>
</tr>
</tbody>
</table>

### Table 6

<table>
<thead>
<tr>
<th>Sample</th>
<th>Spiked (( \mu \text{g L}^{-1} ))</th>
<th>Found (( \mu \text{g L}^{-1} ))</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized water</td>
<td>0</td>
<td>n.d.</td>
<td>-</td>
</tr>
<tr>
<td>Tap water</td>
<td>10</td>
<td>10.11 ± 0.84</td>
<td>101</td>
</tr>
<tr>
<td>Mineral water</td>
<td>0</td>
<td>6.54 ± 0.60</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>16.45 ± 0.24</td>
<td>99</td>
</tr>
</tbody>
</table>

* Average of three determination ± standard deviation, n.d. = not detectable.
6 mL of 1 M HCl solution and 98% of the adsorbed copper was recovered.

3.3.6.4. Preconcentration effect of MT–SiDav–NH₂ bio-adsorbent and quantification of Cu²⁺ traces. The very high selectivity of Cu²⁺–MT and the easy recovery of the adsorbed copper by using small amount of acidic solution prompted us to explore the possibility of using the bio-adsorbent for traces analysis. Estimation of the preconcentration factor of the column was made by using a diluted solution of copper with a concentration of 0.08 mM (5 ppm) Cu²⁺ which was passed through the column containing 0.5 g MT–SiDav–NH₂ adsorbent. The maximum dynamic loading capacity was found equal to 0.13 mmol g⁻¹ material (8.26 mg Cu g⁻¹ material, equivalent to that found in the breakthrough experiments) and was attained after passing 850 mL solution with a quantitative copper recovery (98.66%). Based on these data a preconcentration factor of 142 was obtained. Such a material is therefore suitable for metal ion detection and quantification from diluted solutions.

To demonstrate the promise of the MT–SiDav–NH₂ material for traces analysis, samples of 300 mL of dionized water, tap water and mineral water were used for Cu²⁺ preconcentration. After elution with 6 mL of 1 M HCl the recovered copper was determined by FAAS. The data obtained for the native and spiked (with added copper) water samples are presented in Table 6. The results of triplicate analysis of each water sample showed that the copper recovery by MT–SiDav–NH₂ bio-adsorbent was quantitative (case of spiked samples) and that the material can indeed be useful for traces analysis (case of plain tap and mineral waters).

4. Conclusion

The anchoring of copper thioneine grown from baker yeast to the surface of a mesoporous silica support leads to a highly selective bio-adsorbent for the removal of Cu²⁺ from a multitemetallic water solution and its recovery as a pure component. The covalent immobilization of the biochelator to the silica surface can be readily achieved by using efficient spacers as glycidoxypropyl or amino-propyl and allows multiple recycling and operation under continuous flow without loss of performance. Related recent works describing the selective removal of Cd²⁺ and Zn²⁺ by bio-adsorbents consisting of immobilized metallo-thionein from S. pombe [10] and of Fe³⁺ by bio-adsorbents consisting of immobilized pyoverdin from P. fluorescens [2] on mesoporous silica matrices from multitemetallic contaminated waters show that this type of hybrid biomaterials could be implemented in biotechnologies processes, not only for the decontamination of polluted waters but for the recovery of pure metals as well. Processes in flow can be easily developed for all of these mentioned bio-adsorbents. Furthermore, thanks to the high preconcentration factor achieved, analytical devices for the measurement of the copper content of spiked tap water and mineral waters have been already successfully evaluated. Other metallothioneins, selective for other metals (Se, Ag, …) could be prepared as well and complete the spectrum of bio-adsorbents prone to be immobilize. The high selectivity of the biocomplexants can authorize the design of processes combining several connected columns specific for a single metal for its uptake and recovery.

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References