Polythiol-functionalized alumina membranes for mercury capture

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Abstract

Various materials (particles, resins, etc.) for Hg\textsuperscript{2+} sorption from aqueous streams have been reported in literature. Conventional sorbents are relatively inefficient because only a fraction of the immobilized ligands are accessible for metal complexation. Thus, our approach was to use open structures (0.2 \mum pore size alumina microfiltration membranes), immobilized with various ligands containing single or multiple thiol functional groups. Alumina has good chemical and thermal stability and abundant surface hydroxyl groups, necessary for chemical modification. Convective flow was used for all functionalization steps and Hg\textsuperscript{2+} sorption studies. Only 3-mercaptopropyl trimethoxy silane has been immobilized by direct silylation; the other ligands (cystine, cysteine, polycysteine, polyglutamic acid) required intermediate steps. Thus, via silylation with 3-glycidoxypropyl trimethoxy silane the membrane surface was functionalized with epoxy groups, which then reacted with the terminal amine group of each of the 4 ligands mentioned previously. In the case of polyglutamic acid, the carboxylic acid groups were activated with dihexylcarbodiimide and further reacted with cysteine, making it possible to synthesize a polythiol containing 240 repeat units. Hg\textsuperscript{2+} sorption studies on single thiol-functionalized membranes were used to analyze the interaction between Hg\textsuperscript{2+} and various functional groups. In addition, it was determined that Hg\textsuperscript{2+} bound to weak sites (disulfide, carboxylic acid) can be quantitatively removed by washing the membrane with water at pH = 3, making it possible to quantify the amount bound to the active sites (thiol). Polythiol-functionalized membranes showed high sorption capacities, high site accessibility, and fast sorption rates.

Keywords: Polypeptide; Water treatment; Immobilization; Microfiltration; Ligands; Sorbent; Silane

1. Introduction

Heavy metals in general and mercury in particular are important environmental pollutants, being a threat to human life and natural ecosystems. Various adsorptive matrices for metal capture have been proposed, including activated charcoal, zeolites and clays. The major disadvantages of these materials are low metal loading and small metal ion binding constants. The alternative is to use functionalized affinity membranes with strong binding affinities and high metal ion loading. With this approach one can use low pressure <2 \times 10^3 \text{ Pa} (<2 \text{ bar}) MF (microfiltration) membranes rather than high pressure RO (reverse osmosis) membranes for water permeation.

From the principles of hard–soft acid–base chemistry, it is very well known that mercury has a very strong affinity towards sulfur; consequently, various thiol-functionalized materials like resins \cite{1,2}, particles \cite{3,4}, or composite materials \cite{5,6} have been reported in the literature as mercury ion capture modalities.

One of the most important characteristics of any sorbent material is the total sorption capacity, which is directly proportional to the number of ligands in the matrix. The capacity can be increased by increasing the available surface area of the sorption material, which can be achieved by lowering the pore size of the material. Unfortunately, this increases the diffusion resistance to ion transport and some of the ligands...
may not be available due to pore narrowing and/or blocking during functionalization or operating steps.

Thus, an efficient sorbent should involve pore characteristics, such that high accessibility of the active sites can be achieved with a minimum pressure drop. Our recent research with various membrane supports (such as, cellulose, PE-Silica) functionalized with polypeptides (such as poly-(glutamic) acid, polyarginine, etc.) has demonstrated by Bhattacharyya et al. [7–12] high sorption capacities with various toxic metals.

The present work utilizes commercial alumina membranes with controlled pore structures as supports for covalent attachment of various mercury capturing ligands, containing single or multiple binding sites. The objectives of this study are to determine the accessibility of the active (thiol) sites for Hg$^{2+}$ capture, quantify the polythiol attachment efficiency, establish the permeate flux behavior and to compare the Hg$^{2+}$ binding characteristics with thiol and disulfide groups.

2. Materials and methods

2.1. Materials

Alumina membranes (Anopore) with pore diameters of 200 nm were used as the support material for thiol immobilization and were purchased from Whatman. All the chemicals used in membrane functionalization, 3-mercaptopropyl trimethoxysilane (MPS), 3-glycidoxypropyl trimethoxy silane (GOPS), cysteine, poly-$S$-benzyl-$L$-cysteine (PLC)–40 repeat units, poly-$L$-glutamic acid (PLGA)–241 repeat units, dihexylcarbodiimide (DCC), dithiobis(nitrobenzoic acid) [DTNB], and para-amino benzoic acid (PABA), were purchased from Aldrich, and mercuric nitrate monohydrate used in capture experiments was obtained from Fluka.

2.2. Support material characterization

The characteristics of the unmodified membrane are given in Table 1, based on the manufacturer’s data and our SEM imaging. SEM images of the cross-section of both membrane surfaces showed an ordered array of straight, monodisperse channels (Fig. 1). The membrane surface has a narrow pore-size distribution on each side, however the top side (Fig. 2A) has a porosity of 25–30% and smaller pores than the bottom layer (Fig. 2B) with a porosity of ~20%. This asymmetry was also mentioned in literature [13].

2.3. Membrane functionalization

In our studies, the membrane was functionalized with a single-thiol (MPS or cysteine), a disulfide (cystine) or a polythiol (PLC, Polythiol’s A and B) containing ligand (Fig. 3).

Among various procedures of chemical modification of ceramic (alumina) membranes reported in the literature [14,15], silylation was chosen because of its relative simplicity and flexibility to incorporate various functional groups. Aluminum oxide contains surface hydroxyl (–OH) groups, and is highly suitable in silane coupling. Firstly, the membrane is rinsed thoroughly with acetone prior functionalization. Then, various silanes dissolved in acetone (typically 0.5 mL silane in 100 mL acetone) were used in coupling. 3-Mercaptopropyl trimethoxysilane (MPS) requires one step (direct silylation), and Si–O bonds (1–3) are formed during this chemical reaction. The membrane was washed with ethanol to remove the unreacted silane.

In order to immobilize the ligands containing an amine group, intermediate steps are required. The hydroxyl groups on the alumina are reacted with the trimethoxy silyl groups on GOPS; this results in epoxy group formation on the membrane surface. The ring opening reaction of these epoxy

<table>
<thead>
<tr>
<th>Table 1 Unmodified alumina membrane characteristics</th>
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<tr>
<td>Pore diameter, (d) (nm)</td>
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<tr>
<td>Thickness, (L) (μm)</td>
</tr>
<tr>
<td>Top layer porosity (%)</td>
</tr>
<tr>
<td>Bottom layer porosity (%)</td>
</tr>
<tr>
<td>Pore density, (m) (pore/cm$^2$)</td>
</tr>
<tr>
<td>Mass (g) for 13.2 cm$^2$</td>
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</table>

Fig. 1. Cross-section for 200 nm diameter alumina membrane: (A) throughout the entire membrane thickness [15k x]; (B) inside the membrane [30k x]; (C) near the surface [30k x].
groups with the terminal amine functionality of the ligands mentioned above, making possible single point attachment to the membrane. The pH of these reactions (>10) ensures that the amino group is uncharged. In the case of PLC (supplied with a protective benzyl group) the reduction with NaBH₄ (1–2 g/L) is performed in order to reveal the terminal thiol moiety. Commercially available PLC’s have MW up to 10,000 (40 repeat units). An attractive alternative to make longer chain polythiols (241 repeat units) is to attach PLGA on the membrane, and subsequently reacting the carboxylic acid groups from each repeat unit with the amino group of cysteine (Fig. 4). In order for this reaction to occur, carboxylic acid groups are activated with DCC in CH₂Cl₂, one of the most common reagents used to make peptide bonds [16]. The excess DCC was washed from the membrane with CH₂Cl₂. Also, S-methyl-cysteine was used, in order to avoid a competitive nucleophilic attack (R–S⁻ is a better nucleophile than R–NH₂) at the activated carboxyl. The difference between polythiols A and B is that PLGA was immobilized in the presence of NaCl for the latter one, to increase the chain density on the membrane, as it will be discussed below.

Thiol-modified membranes were then used in metal ion capture studies. All functionalization steps as well as metal capture studies were performed under convective flow in a stirred, dead-end ultrafiltration cell Sepa ST (4.1 cm diameter and 300 mL capacity), obtained from Osmonics. Typical operating fluxes ranged from 7 × 10⁻⁴ to 220 × 10⁻⁴ cm³/cm²s, at pressures from 0.2 × 10⁵ Pa to 1.4 × 10⁵ Pa (0.2–1.4 bar), as a function of the type of ligand immobilized on the membrane.

2.4. Availability of epoxy groups

With the exception of MPS, which is attached by direct silylation, the amount of thiol/polythiol ligands immobilized on the membrane is limited to the number of attached epoxy
groups that are available to react with their amine groups. The number of epoxy groups present on the membrane surface is quantified from the reaction with the amine group of PABA [9]. The concentration of PABA in the feed and permeate is measured by UV–vis at 274 nm with an error <0.5%, and the amount of PABA reacted can be estimated from material balance. This allows the estimation of the number of epoxy groups available for coupling.

2.5. Estimation of the thiol content

Thiols react with DTNB (Ellman’s reagent) by the following reaction:
\[
\text{R-SH} + \text{R-S-S-R'} \rightarrow \text{R-S-S-R'}^+ + \text{R-SH}
\]

Thiolate ion (2-nitro-5-thiobenzoate) produced during this reaction, gives a yellow color at pH 8 that can be readily analyzed by UV–vis at 412 nm [17]. The reaction with simple thiols (i.e., cysteine) gives a fully developed color within 2–3 min. One thiolate ion is formed per thiol group, and the number of thiol groups immobilized on the membrane surface can be determined from material balance.

2.6. Metal capture studies

Aqueous solutions of Hg(NO₃)₂, H₂O or Ca(NO₃)₂·2H₂O, were used as a feed, with volume ranging from 100 to 300 mL and concentration from 90 to 180 mg/L. Hg²⁺ sorption studies were performed at pH = 5–5.5 to avoid precipitation [11], which can occur at higher pH’s for a feed concentration of 180 mg/L. The concentration of feed and permeate was measured by using a Varian SpectAA200 FS atomic absorption spectrometer. Three replicates were taken for each measurement, the errors were <2%.

3. Results and discussion

Alumina membranes were functionalized with various ligands containing single or multiple active sites. Schematic diagrams of arrangement of chains on the membrane surface and inside the pore are presented in Fig. 5. The chains bearing sorption sites are extended from the pore wall towards the pore center. The chains do not cover the entire pore volume. Two distinct regions are thus formed: the chain region, containing the ligands, and the channel region without chains. The size of the channel region will have a dramatic effect on sorption efficiency, since in this region metal ions can freely pass through the membrane without encountering any sorption sites, causing a so-called “leakage”. The size of the channel region can be adjusted by varying the ratio of ligand chain length/pore radius. The gaps between chains, directly related to the chain density, might affect the sorption efficiency significantly, for loose membrane gaps can be another source of ion “leakage”.

3.1. Ligand attachment efficiency

Metal sorption capacity is limited by the number of available active sites, and in order to ensure a large number of sites, a high degree of attachment (high chain density) must be obtained. In our case, the limiting factor is the amount of available epoxy groups, after GOPS immobilization. As mentioned previously, the amount of epoxy groups available for amine-coupling is quantified by reaction with PABA. The reaction between epoxy groups on GOPS and the amine functionality of PABA is 1:1 (m/m), and is analogous with the reaction with all of the ligands used in metal sorption studies. The amount of epoxy groups was estimated to be 0.12 mmol epoxy/g (1 g of membrane corresponds to 165 cm² external membrane area) membrane.

Since the PABA molecule is much smaller than the polythiols used, the amount of PLC or PLGA that can be immobilized on the membrane cannot exceed the amount of PABA, by reacting the terminal amine group with the epoxy functionality. The polythiol chain density is dependent on the chain length, due to steric and electrostatic effects. Steric effects cause the terminal amine group to be less accessible for the reaction with the epoxy on the membrane surface, so longer chains will have a lower density. Electrostatic effects are due to the fact that functionalization reactions must be carried out at high pH (above 10, where the terminal amine group is deprotonated). At this pH the repeat units are charged, and the electrostatic repulsion between chains will lower their density. Also, for longer chains the repulsion will be stronger (more repeat units, all negatively charged). However, the charge and steric effects on the degree of attachment can be reduced if the functionalization is conducted either in a mixture of water–ethanol [11], or using high ionic strength (salts). In both cases, inter-chain repulsion is reduced because the charge is shielded. Table 2 shows the amount of ligand immobilized per gram dry membrane, pore volume (Vₚ), total area (Aₜ), and membrane volume (Vₚₐ). The latter three have been calculated as: 
\[
V_p = \sigma A_t \pi r_p^2, A_t = 2\pi L r_p, V_p = \frac{\pi}{6} L (r_d^3 - r_p^3),
\]

Fig. 5. Schematic diagrams of arrangement of chains on the membrane surface and inside pore: (a) cross-section; (b) top view.
Table 2
Thiol attachment on alumina membrane

<table>
<thead>
<tr>
<th>Ligand</th>
<th>mmol SH/Vm</th>
<th>mmol SH/Vp</th>
<th>mmol SH/At</th>
<th>mmol SH/m</th>
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<tbody>
<tr>
<td>MPS</td>
<td>0.69</td>
<td>2.21</td>
<td>0.11</td>
<td>0.69</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.5</td>
<td>1.6</td>
<td>0.08</td>
<td>0.5</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.5</td>
<td>1.6</td>
<td>0.08</td>
<td>0.5</td>
</tr>
<tr>
<td>PLC</td>
<td>1.23</td>
<td>3.94</td>
<td>0.20</td>
<td>1.23</td>
</tr>
<tr>
<td>Polythiol A</td>
<td>1.01</td>
<td>3.21</td>
<td>0.16</td>
<td>1.01</td>
</tr>
<tr>
<td>Polythiol B</td>
<td>1.77</td>
<td>5.62</td>
<td>0.28</td>
<td>1.75</td>
</tr>
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</table>

Vm is the membrane volume (external surface area \times thickness) (7.9 \times 10^{-2} cm^3); Vp the total pore volume (2.5 \times 10^{-2} cm^3); At the calculated total membrane area (0.50 m^2); m the membrane mass (0.08 g for a 13.2 cm^2 area membrane and 60\mu m thickness).a As mmole S-S-group.

V_m = A_s L\sigma, where \sigma is pore density, and A_s, L and r_p are the membrane external surface area, thickness and pore radius, respectively.

Even if the amount of polythiols immobilized is smaller than for simple ligands, the number of active sites is higher because each chain contains 40 or 241 thiol groups, respectively. The number of thiol groups was determined from the reaction with DTNB. Highest chain density is obtained for MPS, since it requires a single step of functionalization. The other ligands’ density depends on the availability of epoxy groups. Because GOPS and MPS are similar molecules, the same degree of attachment is expected; however, PABA analysis shows that only 20% of this amount represents available epoxy groups. Epoxy is a very reactive functional group; it may undergo decomposition (i.e. hydrolysis), making it unavailable for amine coupling. Also, the packing density of PABA may be less than for MPS, due to the presence of bulky benzene rings. The degree of attachment for polythiols is expressed relative to the available epoxy (or PABA) groups which for PLC this corresponds to 24%, and for longer polythiols A and B, is 3.7% and 6.3%, respectively.

3.2. Metal sorption studies

As it was mentioned earlier, single thiol group ligands, attached to the membrane surface, cannot be efficiently utilized in these very open structures due to large leakage in the channel region. Their purpose is to study the effect of operating conditions on Hg^{2+} capture and the interactions between metal and various functional groups. Besides MPS which has just the active site (thiol), all other ligands have multiple functionalities, like disulfide, amine, or carboxylic acid sites, which will be referred to as weak binding or non-specific binding sites.

3.2.1. The effect of permeate water flow rate on mercury sorption

Due to the rapid reaction of Hg^{2+} with sulfur sites, it is convenient to perform the sorption under convective conditions, avoiding the mass transfer to become the limiting factor. The Hg^{2+} concentration in the feed decreases almost instantaneously since some active sites (thiol groups) are immobilized on the membrane external surface. A sample profile for cumulative Hg^{2+} sorption versus time is presented in Fig. 6. Linear profile of the sorption curve suggests that sorption takes place at a constant rate. The sorption efficiency is influenced by the solution residence time in the pores, \tau, calculated as \tau = P\, A_s\, L / \nu, where P, A_s, L are membrane porosity, external surface and thickness, and \nu the flux through the membrane. The effect of residence time on sorption efficiency is more significant as the channel region is larger and the degree of attachment is lower. The overall sorption capacity will not be affected, the efficiency will be lower because more feed solution is needed (longer time) to reach the active site saturation, since most of the ions are lost through the channel region (Fig. 7). The residence times of 0.08 and 0.22 s correspond to initial flux values of 220 \times 10^{-4} cm^3/cm^2 s at 0.3 \times 10^5 Pa (0.3 bar), and 90 \times 10^{-4} cm^3/cm^2 s at 0.14 \times 10^5 Pa (0.14 bar), respectively.

![Fig. 6. Cumulative Hg^{2+} capture for MPS-functionalized alumina membranes.](image-url)
3.2.2. Metal interaction with various binding sites

The metal ion (Hg$^{2+}$, Ca$^{2+}$) sorption on cysteine and cystine-immobilized membranes provided information about metal interaction with thiol, carboxylic acid, amine and disulfide groups (Table 3). The amount of Ca$^{2+}$ sorbed on cysteine is two times higher than for cystine. This is expected because cysteine has two carboxylic acid groups per molecule, compared to one for cystine, and the only site to bind Ca$^{2+}$ is the carboxylic acid group. In the case of Hg$^{2+}$, the binding sites are the thiol, disulfide and carboxylic acid groups. Even if there are more potential sites on cystine (1 extra carboxyl and 1 amine group), the overall sorption capacity is lower than for cysteine. The amount of Hg$^{2+}$ sorbed is much greater than Ca$^{2+}$, meaning the disulfide linkage plays a role in Hg$^{2+}$ complexation, stabilizing the structure, however Hg$^{2+}$ binds covalently only to thiol groups. It has been shown that in metallothioneins, which contain numerous cysteine residues, COOH groups are involved in Cd$^{2+}$ complexation, and carboxylic acid and thiol sites are more stable than binding by the thiol group alone [18]. After washing with deionized water at pH 3, 100% Hg$^{2+}$ is removed from cystine, and 55% from cysteine. These mild stripping conditions are enough to desorb Hg$^{2+}$ from weak binding sites; in order to remove quantitatively Hg$^{2+}$ sorbed on thiol, concentrated acid and high ionic strength solutions are frequently required [10,19].

3.2.3. Polythiol metal sorption studies

As mentioned above, only long-chain polyligands are suitable to operate in these very open structures, and the discussion will be focused on sorption capacity and efficiency, active site accessibility and metal selectivity.

Sorption experiments for polythiol–PLGA A (referred also as Polythiol A) and PLC were conducted with Hg$^{2+}$ solutions of 180 mg/L feed concentration and volumes between 600 and 900 mL to reach the equilibrium capacity. In the case of polythiol–PLGA B (Polythiol B), metal sorption was conducted with a feed solutions containing single salt (Hg$^{2+}$ and Ca$^{2+}$), as well as Hg$^{2+}$/Ca$^{2+}$ mixture in a 1:3 molar ratio.

Sorption curves for the three polythiols and a single-thiol containing ligand are presented in Fig. 8 as mmol Hg$^{2+}$ captured per unit of active (thiol) group versus mmol of Hg$^{2+}$ solution permeated; the steeper the slope, the higher is the sorption efficiency. Solid lines shown are best-fit lines for each ligand. From the slope of the best-fit lines for MPS and PLC-immobilized alumina membranes, both containing SH groups, it can be observed that sorption capacity per unit of thiol group is very similar, approaching unity at saturation. PLC contains also one COOH group per chain (0.0025 mmol total); this is too small to make a significant difference. Polythiols A and B contain a carboxylic acid functionality per repeat unit in addition to thiol groups; as a consequence the total amount of Hg$^{2+}$ captured per mmol thiol is >1.

In terms of total sorption capacity, it can be observed that all of the immobilized polythiols show not only higher metal loading (more active sites) compared to single thiol ligands, but also more efficient sorption, due to a better pore coverage. Also, the amount of Hg$^{2+}$ sorbed initially on polythiol-immobilized membranes equals or is greater than the total sorption capacity for single ligand immobilized ones.

The diameter of the channel region, $d_c$, which plays a critical role in metal sorption, can be estimated applying Hagen–Poisseuille’s law of capillary flow, assuming straight
cylindrical pores and using the manufacturer’s data for thickness, \( L \) and pore density, \( \sigma \).

\[
d_{c} = 2\left(\frac{8J_{v} \mu L}{\pi \Delta P \Delta A_{s}}\sigma\right)^{1/4}
\]

where \( J_{v} \) is water flow rate, \( \mu \) the water viscosity, \( A_{s} \) the membrane surface area and \( \Delta P \) the transmembrane pressure. Thus, the estimated diameters of the channel region (no ligands present) for polythiols A and B were 125 and 112 nm, respectively, and 156 nm for PLC. It should be noted that this estimation was made using initial flow rate. However, it can be observed that the flux during Hg\(^{2+}\) sorption is decreasing for all sorbents. The system used was free of any foul-
ing agents, since the feed contained only Hg(NO\(_{3}\))\(_{2}\) and/or Ca(NO\(_{3}\))\(_{2}\) dissolved in ultrafiltered and deionized water. The flux drop was attributed to the Hg\(^{2+}\) binding to the active sites (thiols). This would result in a decrease of the void fraction in the chain region, increasing the resistance to water permeation.

As expected, the lowest flux decrease is observed for MPS-functionalized membranes, due to very low chain length/pore size ratio. For PLC-immobilized membrane flux decreases 4 times from \(31 \times 10^{-4} \) to \(7 \times 10^{-4} \) cm\(^3\)/cm\(^2\)/s, whereas for polythiols A and B the flux reduction is 2-fold (Fig. 9). PLC is more hydrophobic and even though it has shorter chains (40 repeat units) than polythiols A and B (240 repeat units), the flux decrease is more pronounced. Because for PLC the chain density is higher than that for both polythiols, the void fraction between PLC chains is expected to be reduced more, due to Hg\(^{2+}\) sorption. Also, polythiols were synthesized on a PLGA backbone, which is known to form helix in presence of divalent cations [11]. Thus, in addition to increased resistance in the chain region of the pore during Hg\(^{2+}\) sorption, helix conformation may increase the channel region, and the overall effect will be a lower reduction in flux compared to
PLC. Our assumption that the flux drop is caused primarily by Hg\textsuperscript{2+} sorption was verified monitoring the flux change in the absence of Hg\textsuperscript{2+} ions. For Polythiol B, when the feed solution contained only Ca\textsuperscript{2+} ions, the reduction in flux is less than 20% from the original flux (Fig. 9, open symbols). In contrast to Hg\textsuperscript{2+} ions Ca\textsuperscript{2+} binds only to COOH groups (ion exchange) and thus one would not expect significant water flux drop.

The cumulative Hg\textsuperscript{2+} sorption for polythiol-functionalized membranes was calculated in the following manner. The amount of ions escaping through the channel was subtracted from the total amount in feed and a constant sorption rate per unit of active site was assumed. Thus, the parameters used in this calculation were initial water permeability, equilibrium sorption capacity and the initial rate of sorption. Using these data, the sorption curves were calculated. As can be observed in Fig. 10, calculated curves fit well Polythiol B experimental data, thus confirming our assumption that sorption takes place at constant rate.

Comparing the experimental sorption curves for PLC and Polythiol A, which contain 0.098 and 0.08 mmol SH, respectively, initially their Hg\textsuperscript{2+} uptake trend is similar. However, as sorption proceeds, the overall capacity for Polythiol A is higher. Polythiol A has an additional COOH group per repeat unit, whereas PLC has just 1 per chain (terminal). Lower sorption efficiencies for both Polythiol A and PLC compared to Polythiol B are discussed below.

In the case of PLC, because metal sorption experiments are conducted at a pH of 5.5, the repeat units are protonated, (→SH has a pK\textsubscript{a} of 8), and intramolecular forces may lead to chain folding, thus hindering some binding sites over a period of time. As sorption process proceeds, Hg\textsuperscript{2+} binds to SH groups forming (→SHg\textsuperscript{2+}). The repeat units become positively charged, and the electrostatic forces lead to chain unfolding, gradually revealing the hindered sites. This may be the reason that after an initial high sorption rate, when all the sites are available (at the membrane surface and at the pore mouth), inside the pore the sorption occurs at an approximately constant rate.

Polythiol B was synthesized in the presence of 0.5 M NaCl, and the amount of SH attached was 0.14 mmol, 75% more than Polythiol A. This increased →SH content and thus higher chain density undoubtedly is responsible for the higher sorption capacity and better sorption efficiency than those for Polythiol A. Compared to PLC, the higher sorption capacity and efficiency are due to longer chains and higher thiol and carboxylic groups content. It is interesting to notice from the sorption curve for Polythiol B that initially, when all groups are available, the amount of Hg\textsuperscript{2+} collected in the permeate was smaller than the amount sorbed. The sorption rate decreases gradually, as more sites become occupied, and at this point the curve plateaus.

For Polythiol B the repeat units are charged at all times under our operating conditions (pH of 5.5), whereas the pK\textsubscript{a} value of the COOH group of cysteine, on each repeat unit is ∼2. This means the chains are elongated, making the active sites available at all times and the rate of sorption decreases as more and more active sites are occupied. The high accessibility of sorption sites, combined with fast sorption rates, implies that for a very dilute feed concentration, which is the case for most application purposes, there would be a very sharp breakthrough.

Another important item to mention is the selectivity between Hg\textsuperscript{2+} and Ca\textsuperscript{2+}, which is required to ensure that sorption capacity is not exhausted by common divalent cations (i.e. Ca\textsuperscript{2+}, Mg\textsuperscript{2+}). As can be observed in Fig. 11, Ca\textsuperscript{2+} is captured only initially, when all the binding sites are available. Thiol is a soft base, so it does not have affinity for hard acids (Ca\textsuperscript{2+}); therefore, the only accessible sites for Ca\textsuperscript{2+} are carboxylic acid groups. Despite being in great excess, the rate of sorption is smaller than that of Hg\textsuperscript{2+} at all times. When free sites become limited, Hg\textsuperscript{2+} sorption becomes dominant and...
Ca\(^{2+}\) is displaced from the sorbent. Carboxylic acid groups are involved in mercury complexation, and in combination with thiol gives a more stable binding than for thiol alone, previously observed for Cd\(^{2+}\)-binding peptides containing cysteine [18]. Since both Hg\(^{2+}\) and Cd\(^{2+}\) are soft acids with high affinity for thiols, this is a similar situation. The selectivity Hg\(^{2+}\)/Ca\(^{2+}\) is 4:1.

Desorption experiments were conducted at pH 3 to identify the amount of metal sorbed on weak binding sites (i.e., COOH groups). Low pH is not sufficient to desorb Hg\(^{2+}\) bound to thiol sites, due to the large binding constant for mercury–thiol complexes. More aggressive conditions are required for quantitative desorption [19]. Even at pH 3, the carboxylic acid group of cysteine is deprotonated (pK\(_a\) \(\sim\) 2), so the metal bound is not recovered quantitatively, due to weak electrostatic interactions (Table 4). PLC shows only 9% Hg\(^{2+}\) desorption (only a small fraction of Hg\(^{2+}\) is bound to terminal COOH groups on each chain). Polythiol’s A and B show 52% and 28% Hg\(^{2+}\) recovery, respectively. The latter also has –COOH sites complexed with Ca\(^{2+}\), 95% of which is recovered under these mild conditions. After desorption, the selectivity Hg\(^{2+}\)/Ca\(^{2+}\) sorbed increases to 56:1.

### 4. Conclusions

Alumina membranes are attractive support materials because of their good chemical and thermal resistance and abundant surface hydroxyl groups, necessary for ligand attachment. Our results show that sorbents with extremely high capacities can be created by covalent attachment of ligands containing multiple binding sites (polythiols). Sorption efficiency can be significantly improved by optimizing the ratio of ligand chain length/pore radius, and operating conditions (permeate flux). The presence of hydrophilic groups (i.e. COOH on Polythiols A and B) not only creates additional number of binding sites, but also decreases the resistance to ion transport, resulting in lower operating pressures. In addition, fast sorption kinetics and high accessibility of binding sites are other supportive attributes of this approach for designing high-performance sorption materials. Cystine (disulfide) in contrast to cysteine (thiol) provided additional benefit in terms of complete sorbent regenerability (after mercury capture) under mildly acidic (pH \(\sim\) 3) condition.

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