Nanoporous Sorbent Material as an Oral Phosphate Binder and for Aqueous Phosphate, Chromate, and Arsenate Removal

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Abstract

Phosphate removal is both biologically and environmentally important. Biologically, hyperphosphatemia is a critical condition in end-stage chronic kidney disease patients. Patients with hyperphosphatemia are treated long-term with oral phosphate binders to prevent phosphate absorption to the body by capturing phosphate in the gastrointestinal (GI) tract followed by fecal excretion. Environmentally, phosphate levels in natural water resources must be regulated according to limits set forth by the US Environmental Protection Agency. By utilizing nanotechnology and ligand design, we developed a new material to overcome limitations of traditional sorbent materials such as low phosphate binding capacity, slow binding kinetics, and negative interference by other anions. A phosphate binder based on iron-ethylenediamine on nanoporous silica (Fe-EDA-SAMMS) has been optimized for substrates and Fe(III) deposition methods. The Fe-EDA-SAMMS material had a 4-fold increase in phosphate binding capacity and a broader operating pH window compared to other reports. The material had a faster phosphate binding rate and was significantly less affected by other anions than Sevelamer HCl, the gold standard oral phosphate binder, and AG® 1-X8, a commercially available anion exchanger. It had less cytotoxicity to Caco-2 cells than lanthanum carbonate, another prescribed oral phosphate binder. The Fe-EDA-SAMMS also had high capacity for arsenate and chromate, two of the most toxic anions in natural water.

Keywords: Arsenate; Chromate; Phosphate; Sorbent; Hyperphosphatemia; Mesoporous silica; Oral phosphate binder

Introduction

Hyperphosphatemia is common in end-stage chronic kidney disease and dialysis patients totaling in 2 million worldwide [1]. The current prescribed phosphate binders in the market are calcium-based salts (acetate and carbonate), Sevelamer (hydrochloride and carbonate), lanthanum carbonate, aluminium salts and magnesium salts, which account for about $1 billion in global annual sales [2]. Yet they have several drawbacks including high risk of hypercalcemia and calcification [3,4], high costs, low-to-moderate efficacy, adverse gastrointestinal effects, and high pill burden [5]. A new oral phosphate binder with high efficacy, low adverse effects, low cost, and low pill burden is needed. Soluble iron based salts have recently been investigated as calcium-free oral phosphate binders. However, in clinical trials, they were linked to diarrhea, bloating, and constipation [6]. By employing a nanoporous sorbent, we can strongly attach iron on the benign silica, while maintaining high phosphate binding capacity via the high surface area of the silica. By ligand design of iron (Fe(III)) on ethylenediamine (EDA), we can achieve high phosphate binding efficacy that is less dependent on pHs and competing anions.

In addition to hyperphosphatemia in patients, excessive amounts of phosphate in water resources can lead to major problems of uncontrolled growth of aquatic plant and algae leading to the depletion of oxygen in aquatic ecosystems and subsequent decline in aquatic living organisms. Phosphate is widely used in industrial, agricultural and household products. The US Environmental Protection Agency (EPA) has limited the phosphate levels to be <20 mg/L in rivers, streams [7], lakes and reservoirs [8] and <2 mg/L in estuarine and coastal marine waters [9]. There have been extensive efforts in lowering phosphate levels in wastewater to meet these discharge limits. The methods exploited include phosphate adsorption, biological treatment, and chemical precipitation. Chemical precipitation is effective only at high phosphate levels, while biological treatment is very slow, expensive and requires multiple complicated operational processes [10]. Phosphate removal by the right adsorbent materials can have advantages over precipitation and biological processes because it can be effective at low phosphate concentration, it offers fast capture kinetics, it is potentially far less expensive, and it is easy to operate. In this regard, a wide array of materials have been investigated for phosphate removal sorbents such as iron oxide [11], binary metal oxides [12-15], alumina [16,17], chitosan [18,19], and silica-based materials [20-25] as summarized in Table 1. However, the phosphate removal properties of these materials, especially binding capacity, still need improvement. Herein, we report systematic optimization of advanced nanoporous sorbent materials, yielding much better phosphate capture efficiency than previously reported iterations and those of commercial sorbent materials in terms of phosphate binding capacity, capture rate, and less competition from competing anions.

Self-assembled monolayers on mesoporous supports (SAMMS®) are versatile hybrid materials generated by covalently grafting and cross-linking functional organosilanes to make a dense molecular coating inside nanoporous silica (SiO2). By varying the terminal

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organic functionality, SAMMS materials have been tailored to selectively capture oxometallate anions [20,26], toxic heavy metals [27-32], transition metals [33,34], lanthanides and actinides [35-40], and cesium and thallium [41-43]. For environmental phosphate removal, we have developed an iron functionalized nanoporous silica (Fe-EDA-SAMMS) [20], but its capacity needed improvement. Herein, we report the optimized synthesis of Fe-EDA-SAMMS to improve binding capacity (by 4-fold) and other characteristics, demonstrating Fe-EDA-SAMMS to be very highly attractive as an environmental phosphate removal sorbent and as an oral phosphate binder.

Materials and Methods

Chemicals and reagents

Potassium phosphate (KH$_2$PO$_4$ and K$_2$HPO$_4$), potassium chromate (K$_2$CrO$_4$), sodium arsenate (Na$_2$HAsO$_4$·7H$_2$O), sodium chloride (NaCl), sodium nitrate (NaNO$_3$), sodium bicarbonate (NaHCO$_3$), sodium sulfate (Na$_2$SO$_4$), sodium citrate (Na$_3$C$_6$H$_5$O$_7$·2H$_2$O), sodium acetate (NaC$_2$H$_3$O$_2$), potassium chloride (KCl), dimethoxyethane (glyme), [3-(2-aminoethylamino)propyl] trimethoxysilane, 3-isocyanatopropyl trimethoxysilane, then 3 g of the fumed silica was immediately added into the modified PEI mixture and stirred overnight under static nitrogen atmosphere. The material, PEI-fumed silica, was then washed with copious amount of methanol and air-dried. The Fe(III) incorporation was then conducted in the same manner as previously described.

Batch contact experiments

Sorbent materials developed in our lab as well as commercially available materials, Sevelamer HCl and AG® 1-X8 anion exchanger, were tested in batch contact experiments as described in our previous work [20]. Briefly, known weights of sorbent material were added into a phosphate solution prepared from KH$_2$PO$_4$ with a concentration range from 3 mg/L to 3000 mg/L of phosphate in a polypropylene vial to achieve a liquid-to-solid ratio (L/S) of 200, 1,000 or 2,000 mL/g. Batch contact was carried out on an orbital shaker (200 rpm) for 2 h and then filtered prior to analysis. Phosphate concentration of the filtrates was determined with inductively-coupled plasma mass spectrometer (ICP-MS) Agilent 7700c (Agilent, Santa Clara, CA). All experiments were performed with control (same solutions without sorbents added) in parallel and in triplicate. Sorbents' ability to capture phosphate was reported in terms of percentage of phosphate removal from the solution (Equation 1) or amount of phosphate adsorbed per gram of sorbent material (Equation 2):

$\text{Phosphate removal (\%)} = \frac{C_i - C_e}{C_i} \times 100$  \hspace{1cm} (1)

$\text{Phosphate adsorbed (mg phosphate/g sorbent)} = \frac{(C_i - C_e) \times L}{S}$ \hspace{1cm} (2)

where $C_i$ and $C_e$ are the initial and equilibrium concentrations of phosphate, respectively (in mg/L).

Typical testing conditions were 3 mg/L of phosphate prepared from KH$_2$PO$_4$ in DI water and pH of 5.3. In anion competition study, test matrices include chloride, nitrate, bicarbonate, acetate, sulfate and citrate; all from sodium salts. In the pH-dependent phosphate capture matrices includes chloride, nitrate, bicarbonate, acetate, sulfate and citrate; all from sodium salts. In the pH-dependent phosphate capture matrices include chloride, nitrate, bicarbonate, acetate, sulfate and citrate; all from sodium salts. In the pH-dependent phosphate capture matrices include chloride, nitrate, bicarbonate, acetate, sulfate and citrate; all from sodium salts.

Table 1: Performance comparison of previously reported phosphate removal sorbents in DI water.

| Sorbent                | pH  | Temperature (°C) | Adsorption Capacity (mg phosphate/g) | Reference |
|------------------------|-----|-----------------|-------------------------------------|-----------|
| Fe-EDA-SAMMS           | 5.0 | 25              | 156.3                                | This work |
| Fe-EDA-SAMMS           | 5.0 | 25              | 43.3                                 | [20]      |
| NH$_3$ on MCM-48       | N/A | 25              | 47.8                                 | [21]      |
| Fe-EDA on SBA-15       | N/A | 35              | 63.4                                 | [22]      |
| Fe-EDA on MCM-41       | 7.0 | 25              | 51.8                                 | [23]      |
| La-EDA on MCM-41       | 7.0 | 25              | 54.3                                 | [24]      |
| Magnetic Fe-Zr binary oxide | 4.0 | 25              | 41.8                                 | [15]      |
| Chitosan hydrogel      | 5.0 | 25              | 88.4                                 | [18]      |

For comparison, using the same conditions, Fe(III) was also incorporated into two other commercial ethylenediamine (EDA)-functionalized substrates: EDA-resin (StratoSpheres™ PL-EDA polystyrene resin) and EDA-porous silica gel (SiliaBond Diamine). Lastly, as an alternative to EDA, polyethyleneimine (PEI), having abundant amine groups, was grafted onto the surface of non-porous fumed silica (Cab-o-sil™ M5). Briefly, 5.2 g of 25-kDa branched PEI was pre-treated with 60 mL of DMSO in 60 mL of warm glyme (40–50°C) for 2 h. The warm mixture was treated with 120 µL of 3-isocyanatopropyl trimethoxysilane, then 3 g of the fumed silica was immediately added into the modified PEI mixture and stirred overnight under static nitrogen atmosphere. The material, PEI-fumed silica, was then washed with copious amount of methanol and air-dried. The Fe(III) incorporation was then conducted in the same manner as previously described.
(SIF) were also used as the test matrices. Both SGF and SIF were prepared following the recommendations of the U.S. Pharmacopeia for drug dissolution studies in stomach and intestine [44,45]. The SGF (pH 1.1) contained 0.03 M NaCl and 0.085 M HCl. The SIF contained 0.14 M NaCl, 0.005 M KCl and 0.008 M NaHCO3; its pH was adjusted to 4.0 and 6.5 with 0.1 M HCl. An additional SIF solution containing 0.08 M NaCl and 0.008 M NaHCO3, pH adjusted to 6.6 was also used [46].

### Sorption capacity

The sorption capacity of Fe-EDA-SAMMS was measured in batch contact at room temperature. Phosphate, arsenate, and chromate solutions with varying concentrations from 0 to 700 mg/L were in contact with the sorbent at an L/S ratio of 2000 mL/g for 2 h assuring equilibrium condition. Langmuir isotherm equation was used to fit sorption capacity data as shown in Equation 3.

\[
\frac{Q_e}{Q_{\infty}} = \frac{K_c Q_{\infty} C_e}{1 + K_c C_e}
\]

where \(C_e, Q_e, Q_{\infty}\), and \(K_c\) are equilibrium concentration (in mg/L), equilibrium uptake (in mg/g), maximum binding capacity (in mg/g) and Langmuir constant (in L/mg), respectively.

### Sorption kinetics

The kinetics of phosphate capture was measured in a modified batch contact experiment. Briefly, a 50 mL solution of 3 mg/L phosphate was mixed with 0.5 g sorbent at an L/S ratio of 2000 mL/g for 2 h assuring equilibrium condition. The kinetics of phosphate capture did not improve under SGF (pH 1.1) and SIF (pH 4.0) test matrices. To avoid fighting with the SGF (pH 1.1) and SIF, pH 4.0 and 6.5, consist of 0.14 M NaCl, 0.005 M KCl, 0.008 M NaHCO3, pH adjusted with 0.1 M HCl.

| Iteration | Fe Incorporation conditions | | | % Phosphate Removal |
|-----------|-----------------------------|----------------|-------------------|
| I [20]    | 0.023 Water                 | 25°C           | 1                 | 0.83              | 34 ± 5 | 63 ± 2 | 14 ± 4 |
| II        | 0.02 Water                  | 25°C           | 1                 | 0.017 ± 0.000     | 5 ± 2 | 58 ± 2 | 74 ± 1 |
| III       | 0.02 Acetonitrile           | 25°C           | 1                 | 0.122 ± 0.003     | 51 ± 4 | 83 ± 1 | 61 ± 2 |
| IV        | 0.02 Acetonitrile           | 65°C           | 1                 | 0.140 ± 0.013     | 75 ± 2 | 96 ± 1 | 98 ± 1 |
| V         | 0.02 Acetonitrile           | 65°C           | 20                | 0.224 ± 0.015     | 93 ± 0 | 96 ± 1 | 93 ± 1 |
| VI        | N/A Acetonitrile            | 65°C           | 20                | N/A               | 0 ± 0 | 19 ± 2 | 8 ± 3 |

Table 2: Phosphate removal efficiency of Fe-EDA-SAMMS

#### Results and Discussion

##### Optimization of Fe(III) incorporation

Varied conditions of Fe(III) incorporation onto EDA-SAMMS and the phosphate binding ability of the resulting materials are summarized in Table 2. In an attempt to increase the Fe loading on EDA-SAMMS, we increased the amount of initial Fe(III) in the solution by 20-fold compared to our earlier work (Iteration I) [20]. However, this did not result in increased Fe loading (0.83 mmol/g vs. 0.017 mmol/g). This is likely due to the fact that the pH of the loading solution dropped at the higher Fe(III) concentration (i.e., from pH 1.96 to 1.46), and the EDA-SAMMS are more likely to be protonated under the more acidic conditions. Accordingly, the phosphate capture did not improve under the SGF (pH 1.1) and SIF (pH 4.0) test matrices. To avoid fighting the pH issues associated with aqueous solutions of Fe(III) ion, we employed acetonitrile as our solvent for the Fe incorporation. As an anhydrous, polar aprotic solvent, acetonitrile does not have any readily dissociable protons to increase the acidity of the reaction mixture upon dissolution of Fe(III) salts, resulting in no competing protonation of the EDA ligand. This solvent change led to a 7-fold increase in Fe loading after 1 h of contact time (Iteration III), which is associated with improved phosphate removal efficacy. Next we show that increasing the temperature of Fe(III) in acetonitrile from room temperature to 65°C (Iteration IV) and contact time from 1 h to 20 h (Iteration V), we could substantially increase Fe loading to 0.140, and 0.224 mmol/g, respectively. The high reaction temperature and increased reaction time helped overcome kinetic barriers associated with charge accumulation (i.e., cation-cation repulsion) within the monolayer, thereby facilitating integration of Fe into the EDA monolayer inside the pores of SAMMS. The most optimal Fe-EDA-SAMMS (Iteration V) achieved 93 – 96% of phosphate removal in all three matrices. However, increasing the
temperature of reaction to boiling point (82°C) of acetonitrile did not further increase phosphate removal efficacy of the material (data not shown). It is worth noting that, although Fe loading of original material (Iteration I) was extremely high (0.83 mmol Fe/g) compared to others, it did not yield the best phosphate capture perhaps due to the iron oxide formation which did not attribute to the phosphate adsorption of the material. A control experiment (Iteration VI) was carried out similarly to Iteration V but without Fe(III) in the solution. The Iteration VI material likely contained protonated EDA as a functional group around fumed silica in thick layers, preventing the penetration of both EDA and Fe(III) into the pores, and hence was loaded on fumed silica (Cab-o-sil® M5). The fumed silica is a non-porous material, thus the PEI was loaded on the external surface. We hypothesize that the lower surface area of fumed silica (200 m²/g) could not supply enough amine groups for Fe(III) to bind to, leading to lower phosphate binding capacity. This was not the case with EDA-SAMMS since the amount of EDA ligands used was limited by design to only a monolayer deposition.

To increase phosphate binding capacity, branched PEI (25-kDa) was investigated as an alternate to EDA since it contains a high content of amines. 25-kDa PEI was too big to be loaded effectively inside the pores, and hence was loaded on fumed silica (Cab-o-sil® M5). The fumed silica is a non-porous material, thus the PEI was loaded on the external surface. We hypothesize that the lower surface area of fumed silica in comparison to SAMMS could be compensated by the considerably higher amount of amine groups of PEI than that of EDA. Despite the substantial surface area of fumed silica (200 m²/g) and the high loading of PEI (6.97 mmol N/g), PEI-fumed silica had low Fe loading (0.047 mmol Fe/g) and the resulting material (Fe-PEI-fumed silica) yielded low phosphate binding capacity compared to Fe-EDA-SAMMS (45 vs. 157 mg/g). This is mostly due to PEI wrapping the pores, and hence was loaded on fumed silica (Cab-o-sil® M5). The fumed silica is a non-porous material, thus the PEI was loaded on the external surface. We hypothesize that the lower surface area of fumed silica in comparison to SAMMS could be compensated by the considerably higher amount of amine groups of PEI than that of EDA. Despite the substantial surface area of fumed silica (200 m²/g) and the high loading of PEI (6.97 mmol N/g), PEI-fumed silica had low Fe loading (0.047 mmol Fe/g) and the resulting material (Fe-PEI-fumed silica) yielded low phosphate binding capacity compared to Fe-EDA-SAMMS (45 vs. 157 mg/g). This is mostly due to PEI wrapping around fumed silica in thick layers, preventing the penetration of both Fe(III) and phosphate to the available amine groups in the inner layers. Only the outer layer amine groups remained active, resulting in low Fe loading and low phosphate binding capacity. Based on the finding in Table 3, SAMMS, produced from MCM-41, proved to be the best substrate for Fe-amine loading, yielding the highest phosphate binding capacity. It was therefore used in all subsequent experiments.

### Adsorption isotherm

The phosphate binding capacity of Fe-EDA-SAMMS was established with adsorption isotherm experiments conducted in DI water (pH 5.3) with an L/S ratio of 2000 mL/g and room temperature. The phosphate uptake of Fe-EDA-SAMMS increased sharply with increasing initial concentrations of phosphate from 0 to 90 mg/L (or 22 mg/L of equilibrium concentration) and began to saturate thereafter.

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**Table 3: Phosphate removal capacity of Fe-amine on various substrates**

| Material         | Capacity (mg/g) | N content (mmol/g) | Fe content (mmol/g) | Specific surface area (m²/g) | Pore size (Å) | Particle size (µm) |
|------------------|-----------------|--------------------|---------------------|-----------------------------|---------------|-------------------|
| Fe-EDA-SAMMS     | 157 ± 6         | 2.64 ± 0.06        | 0.224 ± 0.015       | 880                         | 48            | 20-75             |
| Fe-EDA-resin     | 126 ± 3         | 6.35               | 0.053 ± 0.005       | N/A                         | N/A           | 194               |
| Fe-EDA-porous silica gel | 66 ± 5 | 1.98 ± 0.02   | 0.113 ± 0.014       | 500(1)                      | 60(1)         | 40-63(1)          |
| Fe-PEI-fume silica | 45 ± 5 | 6.97 ± 0.23 | 0.047 ± 0.001       | 200(1)                      | N/A           | 0.2-0.3(1)        |

**Figure 1:** Adsorption isotherm of phosphate on Fe-EDA-SAMMS in DI water (pH 5.3), L/S of 2000 mL/g, symbols represent data and dash-line represents Langmuir isotherm fitting.

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**References:**

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**Table 2:** Specific surface area, pore size and particle size are of substrates.

| Material         | Specific surface area (m²/g) | Pore size (Å) | Particle size (µm) |
|------------------|-----------------------------|---------------|-------------------|
| Fe-EDA-SAMMS     | 157 ± 6                     | 48            | 20-75             |
| Fe-EDA-resin     | 126 ± 3                     | N/A           | 194               |
| Fe-EDA-porous silica gel | 66 ± 5 | 60(1)         | 40-63(1)          |
| Fe-PEI-fume silica | 45 ± 5 | N/A           | 0.2-0.3(1)        |
The adsorption data fitted the Langmuir adsorption model quite nicely, as shown in Figure 1 (R²=0.999). This confirmed that phosphate adsorption on the Fe-EDA-SAMMS followed a monolayer adsorption model similar to the original Fe-EDA-SAMMS previously reported [20]. However, the new Fe-EDA-SAMMS offered much higher phosphate binding capacity than the commercial AG® 1-X8 resin, having simple ammonium as the binding site, which has little selectivity. Coexisting anions such as chloride (Cl⁻), nitrate (NO₃⁻), bicarbonate (HCO₃⁻) and sulfate (SO₄²⁻), whereas the most common anion species in gastrointestinal tract are chloride (0.10 – 0.13 M) and bicarbonate (0.006 – 0.040 M) [47]. These anions can compete with phosphate for binding is a function of anion basicity [48]. However, as illustrated in Table 4, Fe-EDA-SAMMS was much less affected by the presence of coexisting anions than the commercial AG® 1-X8 resin, having simple ammonium as the binding site, which has little selectivity. Evidently, the addition of Fe(III) to the EDA group increases selectivity of the binding site for phosphate compared to simple ammonium which acts as an anion exchanger.

Adsorption kinetics

A fast adsorption rate is highly desirable for field-deployment of sorbent materials, especially for flow system operations. Likewise, for oral phosphate binders, fast phosphate capture (onto the sorbent) reduces the degree of phosphate available to be absorbed into the body. The adsorption kinetics of Fe-EDA-SAMMS was measured in 3 mg phosphate/L in DI water (pH 5.3) with an L/S of 1000 mL/g as shown in Figure 2A. We also benchmarked against two commercially available materials: Sevelamer HCl (the gold standard oral phosphate binder) and AG® 1-X8 resin (a commercial anion exchange resin). Both materials had slower phosphate binding kinetics than Fe-EDA-SAMMS. Specifically, while Fe-EDA-SAMMS removed over 98.7% of phosphate in 1 min, Sevelamer HCl and AG® 1-X8 only removed 79.2% and 31.7%, respectively. To remove more than 99% of phosphate, Fe-EDA-SAMMS took 5 min, while Sevelamer HCl took 10 min and AG® 1-X8 resin took 30 min. Similar to our previous reports on other types of SAMMS materials [20,34,36-39,42], fast kinetics is a signature of SAMMS performance, which is owed to the rigid and open pore structure, allowing easy access of metal ions to the binding sites inside the pores. These unique characteristics of SAMMS provides advantages over other recently developed phosphate removal materials, which require much longer time to reach equilibrium (e.g., more than 24 h for Fe-Zr binary oxide [15] or 7 h for chitosan hydrogel [18]). The binding kinetics on SAMMS was slightly affected by the coexisting anions (Figure 2B), to be described in subsequent section.

Effect of coexisting anions

Wastewaters and other natural waters typically contain several coexisting anions such as chloride (Cl⁻), nitrate (NO₃⁻), bicarbonate (HCO₃⁻) and sulfate (SO₄²⁻), whereas the most common anion species in gastrointestinal tract are chloride (0.10 – 0.13 M) and bicarbonate (0.006 – 0.040 M) [47]. These anions can compete with phosphate for Fe-EDA binding sites. The effect of coexisting anions was measured in 3 mg/L phosphate solution (0.00003 M) and 0.01 M coexisting anions, including chloride, nitrate, bicarbonate, sulfate and citrate, with an L/S ratio of 1000 mL/g. All of the initial and final pH of the solution fell within 3.4 – 8.7. As shown in Table 4, there were no significant decreases in phosphate removal with chloride and nitrate even when the anions were 300 folds by mole in excess of phosphate. Only bicarbonate, sulfate, and citrate ions could hamper phosphate removal. This finding is in agreement with our earlier report on Cu-EDA-SAMMS that anion binding is a function of anion basicity [48]. However, as illustrated in Table 4, Fe-EDA-SAMMS was much less affected by the presence of coexisting anions than the commercial AG® 1-X8 resin, having simple quaternary ammonium as the binding site, which has little selectivity. Evidently, the addition of Fe(III) to the EDA group increases selectivity of the binding site for phosphate compared to simple ammonium which acts as an anion exchanger.

The phosphate adsorption capacities reported were measured in DI water.

| Matrix                  | Fe-EDA-SAMMS | AG® 1-X8 |
|-------------------------|--------------|----------|
| Initial pH              | Equilibrium pH | Phosphate removal (%) | Equilibrium pH | Phosphate removal (%) |
| 0.00003 M phosphate     | 5.6          | 3.6      | 99.5 ± 0.0 | 9.6 | 99.8 ± 0.2 |
| + 0.01 M sodium chloride| 5.5          | 3.8      | 97.7 ± 0.3 | 11.2 | 79.9 ± 9.6 |
| + 0.01 M sodium nitrate | 5.7          | 3.9      | 93.8 ± 0.5 | 11.3 | 67.5 ± 10.6 |
| + 0.01 M sodium bicarbonate | 8.7   | 7.7      | 28.7 ± 5.7 | 9.4 | 29.1 ± 1.0 |
| + 0.01 M sodium sulfate | 5.7          | 5.8      | 24.2 ± 0.8 | 11.0 | 0.0 ± 0.6 |
| + 0.01 M sodium citrate | 7.2          | 7.2      | 25.3 ± 3.2 | 10.7 | 1.3 ± 0.3 |

Table 4: Effect of coexisting anions on phosphate removal by Fe-EDA-SAMMS and AG® 1-X8 anion exchanger resin.

(A) Initial phosphate concentration of 3 mg phosphate/L (0.00003 M from KH₂PO₄), L/S ratio of 1000 mL/g.

(B) pH 6.3, L/S ratio of 1000 mL/g.

Figure 2: Phosphate adsorption kinetics on Fe-EDA-SAMMS, Sevelamer HCl and AG® 1-X8 in DI water (A) pH 5.3 and in SIF (B) pH 6.3, L/S ratio of 1000 mL/g.
SIF containing 0.145 M Cl\(^-\) and 0.008 M HCO\(_3\)\(^-\) phosphate concentration (3 mg/L) but with higher anion content (i.e., much more affected by increasing concentrations of the interfering ions). The predominant species is HPO\(_4\)\(^{2-}\) when considering initial pH, our Fe-EDA-SAMMS had a larger operating pH window (pH 2.0 – 11.0) than Fe-diamino-functionalized SBA-15 with an operating initial pH of 3.0 – 6.0 [22] and ammonium-functionalized MCM-48 with an operating initial pH of 4.0 – 6.0 [21].

**Flow removal of phosphate and regeneration of Fe-EDA-SAMMS**

In a field deployed water treatment system, the ability to regenerate and reuse sorbent materials is highly desirable for cost-effectiveness. As mentioned earlier, the pK\(_a\) of phosphoric acid is 2.14 [49], so at pHs below 2.0, phosphate exists primarily as the neutral phosphoric acid, meaning there is no Coulombic driver for binding to a metal cation. Thus we chose to evaluate the efficacy of a mild acid wash for stripping the bound phosphate from Fe-EDA-SAMMS. Fe-EDA-SAMMS was operated in a packed bed column allowing phosphate capture in a flow system. A 3 mg/L phosphate solution was flowed through the 0.01 g of Fe-EDA-SAMMS at a flow rate of 2 ml/min to allow phosphate capture by the sorbent bed. Regeneration was performed with 0.2 M HCl at the same flow rate to leach captured phosphate, followed by pH equilibration of the bed with 0.01 M sodium acetate. Results are presented in Figure 4. The Fe-EDA-SAMMS captured 2.96 mg phosphate/g which accounted for more than 95% of the initial phosphate solution. The subsequent cycles on regenerated material captured at an average of 2.85 mg phosphate/g (93% of initial solution). The amount of bound phosphate leached out by 0.2 M HCl was an average of 2.53 mg phosphate/g (88.5%). This confirmed that the condition used was sufficient to elute most of the captured phosphate under flow conditions. The amount of Fe leached out from acid treatment in each cycle was at an average of 2.8% of the total amount of Fe on original Fe-EDA-SAMMS. These experiments also showed that Fe-EDA-SAMMS is stable and can withstand exposure to 0.2 M HCl used to strip off bound phosphate. The performance (Figure 4) in terms of % removal efficacy was maintained after 10 cycles of study, indicating that most of the binding sites were preserved after the acid elution.

**Cytotoxicity of Fe-EDA-SAMMS to intestinal tissue culture cells**

We sought to establish a safety profile for Fe-EDA-SAMMS in vitro. The Caco-2 cell line resembles the epithelial cells lining the small intestine both morphologically and functionally and has been previously used to establish drug safety profile of several oral drugs [32,51]. We found that Fe-EDA-SAMMS was well-tolerated by Caco-2 cells.
cells and did not cause significant toxicity up to 2500 µg/mL both for 24 h and 48 h of contact time while Sevelamer HCl was tolerated up to 5000 µg/mL (Figure 5). Fe-EDA-SAMMS was better tolerated than another prescribed oral phosphate binder, lanthanum carbonate, which started to show some toxicity at 1000 µg/mL. Lanthanum carbonate is an inorganic salt which is dissociated in GI tract and is readily taken up by cells, making it more toxic to cells. On the other hand, the micron-scale particle sizes of Fe-EDA-SAMMS and Sevelamer HCl (both having similar zeta potential of 30–40 mV in water) made them not easily taken up by the cells, which limited cytotoxicity. This agrees with our previous work [31] that SAMMS (with thiol groups) having particle size of 1–2 µm could be taken up by Caco-2 cells after 3 h of contact time whereas particles of larger size (>5 µm) were not. This is why we chose SAMMS of 20–75 µm to avoid uptake by the GI tract. Increases in dose and contact time might enhance the uptake, resulting in the increased cytotoxicity of SAMMS at high dose (e.g., at 5000 µg/mL at 24–48 h contact time, Figure 5). Preliminary clearance study of one SAMMS material (with thiol groups) of the same particle sizes (20–75 µm) after oral administration to rats (given as 0.1% by weight of food for 24 h, 4 animals) indicates that most of SAMMS administered was recovered (i.e., 91% within 2 days and 99% within 4 days, by silicon (Si) analysis of daily collected feces and urine using an ICP-MS). Out of the total Si excreted after 4 days, 0.98 fraction was found in feces, and 0.2 fraction was found in urine. Hence, the majority of SAMMS material does not absorb to the body and is cleared through fecal excretion.

Arsenate and chromate adsorption on Fe-EDA-SAMMS

Although not a main focus of this paper, we have found Fe-EDA-SAMMS to be very efficacious at capturing arsenate and chromate, the two most hazardous oxometallate anions found in environmental water bodies [52]. We have previously shown that Cu-EDA-SAMMS was able to adsorb both arsenate and chromate in aqueous solution at high capacity [26]. With the improved iron incorporation method reported in this work, we chose to evaluate whether arsenate and chromate adsorptions could be improved as well. We found that the new Fe-EDA-SAMMS has a capacity of 286 mg/g for arsenate and 139 mg/g for chromate in DI water (pH 7.5) (Figures 6A-B), representing a significant improvement over the Cu-EDA-SAMMS's capacity for arsenate (vs. 140 mg/g), but same for chromate (vs. 130 mg/g).

Conclusions

The improved Fe-EDA-SAMMS has great potential for both environmental phosphate removal and as an oral phosphate binder. Using warm acetonitrile as a solvent during the Fe(III) incorporation step eliminated the issue of competing EDA ligand protonation and resulted in higher quality sorbent materials. The new material had (i) 4-fold higher phosphate binding capacity, (ii) high selectivity to phosphate in simulated gastrointestinal fluids, (iii) rapid phosphate binding kinetics, (iv) wide range of working solution pH suitable for phosphate removal in stomach and intestinal tract as well as in wastewater, (v) smaller interference from competing anions, (vi) low cytotoxicity to in vitro intestinal epithelial cell model, and (vii) an enhanced ability to bind arsenate and chromate.

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