

Addition of Thiourea Host Monomer to Polymer Flocculants to Improve Selectivity of Phosphate Sorption

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Abstract

Inorganic phosphate is a common nutrient that is applied as a fertilizer to both agricultural fields as well as urban settings such as private yards, public parks and other urban landscaping. While phosphate typically binds tightly to soil, movement of phosphate off of application sites can occur through soil erosion. The soil and its bound phosphate can then end up in surface waters such as rivers and lakes. Phosphate found in surface water bodies exists both as bound to the suspended clay as well as that free in solution. Elevated phosphate concentration in surface waters can lead to algal blooms and eutrophication. While the phosphate bound to clay in suspension in surface water bodies can be removed by commercially available polymer flocculants, the phosphate that is free in solution is more challenging as it is usually found in low concentrations and other anionic salts are generally present in higher concentrations. To remove phosphate from contaminated water systems, where other anions exist at higher concentrations, it is favorable to have a method of removal that is selective for phosphate. As a proof of principle, thiourea derivatized polymer flocculants were examined for the selective removal of phosphate in the presence of competing anions. The polymer flocculants exhibited selectivity for phosphate through hydrogen bonding and were effective at removing up to 43% of phosphate from simulated wastewater. Computational studies and ¹H NMR were used to investigate the selectivity of the thiourea monomer for phosphate over competing anions such as chloride and sulfate.

Keywords

Molecular Recognition, Thiourea, Polymers, Flocculants, Phosphate

1. Introduction

Phosphate is an essential plant nutrient that is applied as a fertilizer to agricultural crops and to urban landscapes. While phosphate applied to a field benefits the crops being produced, it may also move off site into rivers and lakes [1] [2]. In rivers and lakes, phosphate is generally a limiting nutrient for the microorganisms present. The influx of phosphate can cause proliferation of cyanobacteria resulting in potential human health hazards as well as the aforementioned damage to the ecosystem via eutrophication [1].

Phosphate that is applied to fields is generally bound tightly to the soil and will have little movement through the soil profile [3]. Phosphate generally moves through the environment only when the soil it is bound to begins to move, usually through erosion processes. Once the soil moves into a surface water body it can become suspended and the phosphate will come to an equilibrium between the soil bound and aqueous portions based on environmental conditions such as pH and temperature of the water present [3]. Currently, polymer flocculants as well as other coagulants are effective at removing the suspended solids and the phosphate bound to them; however, they remove little if any of the phosphate that is free in aqueous solution [4] [5].

While phosphate is generally a limiting nutrient, it is not the only anion present in surface water systems. Other anions such as chloride (Cl^-) and hydrogen sulfate (HSO_4^-) are also present and are generally at higher concentrations than phosphate [6]. To remove phosphate from water systems, the method would be most efficient if it were selective for phosphate in the presence of other anions. Many methods have been investigated to remove excess phosphate from water such as chitosan-based flocculants, as well as, agricultural and industrial waste products such as mango seeds and even concrete [7] [8] [9]. Recently, molecularly imprinted polymers containing thiourea were shown to selectively remove phosphate from river water with up to 60% efficiency [10].

Based on previous studies, thiourea derivatized trapping groups are attractive, and have been shown to exhibit strong binding affinities to anions, specifically phosphate. The ability of thiourea to form strong hydrogen bonding interactions with oxyanions leads to high binding energies in solution. Based on these strong interactions, our laboratories have investigated the use of thiourea-derivatized polymer flocculants to remove both soluble and colloiddally sorbed phosphate from suspension [4]. This polymer flocculant was designed with a strategically placed 3rd site for hydrogen to favor and promote the binding of phosphate in the presence of other anions such as chloride and HSO_4^- . To expand on our previous studies, we devised a series of experiments as a proof of concept. Our ob-

jective was to test phosphate binding to thiourea-derivatized polymers immersed in simulated wastewater with high concentrations of competitive anions as well as, computational and ^1H NMR studies. The purpose of these experiments was to support selectivity of our newly developed thiourea trapping group.

2. Materials and Methods

2.1. General Methods

All reactions were performed in oven-dried glassware under an inert atmosphere of nitrogen. Solvents were dried by passing through a solvent dry system (MBRAUN MB-SPS). All chemicals were purchased from VWR and were used without further purification. ^1H NMR and ^{13}C NMR spectra were recorded on a 300 or 500 MHz (JEOL JNM-ECX-300/500) multinuclear spectrometer. ^1H NMR and ^{13}C NMR spectra were referenced to TMS, CD_3CN or DMSO. Fourier Transform Infrared spectroscopy (FT-IR) was performed on a Perkin Elmer Spectrum RX I using a thin film KBr pellet. Gas chromatography-TCD was performed on a Shimadzu GC-2010 with a Restek (RTX[®]-5MS, 15 m \times 0.25 mm ID \times 0.25 μm) column. Gas chromatography-mass spectrometry data was obtained from a Shimadzu GCMS-QP2010S equipped with a Restek (RTX[®]-5MS, 15 m \times 0.25 mm ID \times 0.25 μm) column. GC and GC-MS conditions were as follows: initial temp 60 $^\circ\text{C}$ hold 3 minutes, ramp 15 $^\circ\text{C}/\text{min}$, final temperature 300 $^\circ\text{C}$ hold 3 minutes. Reported melting points were conducted using a Mel-Temp apparatus and are uncorrected. Ion Chromatography (Conducted at Texas A & M Nutrient and Water Analysis Research Laboratory). Ion chromatography samples were run using a DIONEX ICS 2000 Ion Chromatography system equipped with AS20 and AG20 columns eluting with 35mM KOH at a flow of rate 1 mL/min with 25 μL injection volumes.

2.2. Preparation of *N*-(Aminophenylcarbamothioyl)acrylamide

Ammonium thiocyanate (0.500 g, 6.56 mmol) was dissolved in dry acetone (25 mL) and added to a flame dried round bottom flask under N_2 . The reaction was cooled to 0 $^\circ\text{C}$ and acryloyl chloride (0.550 mL, 7.22 mmol) was added slowly over 15 minutes. The reaction was allowed to stir at 0 $^\circ\text{C}$ for 30 minutes during which time a white suspension formed. After 30 minutes the suspension was vacuum filtered and the solution returned to a clean dry round bottom and cooled to 0 $^\circ\text{C}$. *o*-Phenylenediamine (0.708 g, 32.8 mmol) dissolved in dry acetone (3 mL) was added via syringe over 30 minutes and the reaction was allowed to stir for an additional 30 minutes at 0 $^\circ\text{C}$ under N_2 . The reaction was then added over 15 minutes to an ice/water bath (150 mL) and yellow crystals began to form. The resulting crystals were vacuum filtered to yield a yellow solid as the final product. Yellow solid (Yield 75%) mp = 134.2 $^\circ\text{C}$ - 136.0 $^\circ\text{C}$. ^1H NMR (300 MHz, DMSO): δ 5.02 (s, 2H), 5.96 (dd, $J_z = 9.6$ Hz, 1.8 Hz), 6.40 (dd, $J_z = 17.0$ Hz, 1.8 Hz), 6.51 - 6.65 (m, 2H), 6.72 (dd, $J_z = 8.0$ Hz, 1.4 Hz), 6.96 (m, 1H), 7.25 (dd, $J_z = 7.8$ Hz, 1.4 Hz), 11.53 (s, 1H), 11.87 (s, 1H). ^{13}C NMR (75 MHz, DMSO): δ

116.2, 116.4, 123.5, 127.7, 128.2, 130.6, 131.9, 143.8, 166.0, 180.6.

2.3. Preparation of Thiourea Based Polymer

To a stirring solution of 40 ml acetonitrile was added: 4.00 g (0.0206 mol) of (1), 2.98 g (0.0412 mol) acrylamide, and 8.458 g (0.0412 mol) acryloxyethyltrimethylammonium chloride. The solution was then sparged with nitrogen for 1 h, while the temperature was raised from room temperature to 30°C. To a stirring solution, 0.169 g (1.03×10^{-3} mol) azobisisobutyronitrile (AIBN) was added to initiate polymerization. The reaction temperature peaked at 38°C. The reaction was then heated to 50°C and allowed to react for 16 h. The reaction produced a slightly yellow solid that was ground in a mortar and pestle to a 1.2-mm diameter powder and analyzed by IR TGA/DSC. Other polymers with different densities and a different thiourea molecule were made using the same methods changing the mole percentage of monomers.

2.4. Phosphate Sequestration Experiment

A suspension of water and clay was made by adding 250 mg kaolinite to 19 ml of DI water and the clay was allowed to hydrate overnight. To this suspension 0.5 ml of a phosphate intermediate standard was added to the suspension to give a final concentration of 5 mg/L. To the phosphate suspension was added 0.5 ml of a polymer intermediate solution to give a final polymer concentration of 100 mg/L. The final mixture was vortexed for 5 seconds and then shaken for 12 hours. The mixture was allowed to settle and a 5 ml aliquot was taken and centrifuged on a Fischer Scientific Model 225 centrifuge at 305 g for 10 minutes. A 2 ml subsample was then taken and filtered through a 0.4-micron syringe filter prior to analysis by ion chromatography.

2.5. Computational Methods

All models for this work were computed using the Gaussian 09 suite of programs, including use of Gaussview 5 to generate three-dimensional figures [12]. Each molecule was modeled *separately* beginning with geometries optimized first using molecular mechanics and then using the PM3 semi-empirical method to generate starting structures for density functional theory. Each structure was then optimized using the M06-2X/6-311G (d) model chemistry. Geometries optimized with DFT were checked with frequency analysis at the same level of theory as the optimization.

3. Results and Discussion

3.1. Polymer Sorption Study

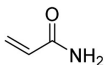
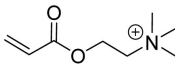
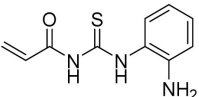
In this proof of concept study, the goal was to test the affinity of the polymer for phosphate in suspensions containing anions at concentrations typical of wastewater [7]. Suspensions of clay (kaolinite) were developed using 250 mg clay in 18.5 ml of water and allowed to hydrate overnight. To the clay suspension, 0.5

ml of an intermediate solution was added to give final anion concentrations of: 190 mg/L $(\text{NH}_4)_2\text{SO}_4$, 28 mg/L CaCl_2 , 180 mg/L MgSO_4 , and 5 mg/L NaH_2PO_4 . The suspension was vortexed, and 1 ml of an intermediate polymer solution was added to give a final polymer dose of 100 mg/L. The resulting suspension was vortexed and placed on a shaker for 12-h. The tubes were removed from the shaker and a 5 ml aliquot of the water above the floc layer was taken, centrifuged and filtered prior to analysis by ion chromatography. The polymers tested were: Magnifloc 494C⁺ a cationic polymer with 10% positive charge density (A), a control polymer C40-00 a cationic polymer with 40% positive charge density (B), and an experimental polymer C40-20 containing 40% positive charge density and 20% of the 3-point thiourea monomer (C), shown in **Table 1** [5].

The results from the control experiments were as follows: the clay adsorbed more than 30% of the phosphate from solution regardless of the presence of competitive anions (**Figure 1(a)**). This result was due to the interaction of phosphate with the exposed aluminum octahedral layer found on kaolinite [11]. The addition of Magnifloc 494C⁺, did not remove more phosphate from the suspension. In fact, addition of Magnifloc 494C⁺ appeared to decrease the amount of phosphate adsorbed to the clay, likely competing for adsorption sites on the exposed alumina layer (**Figure 1(b)**). The control polymer C40-00, containing the trimethyl ammonium monomer at 40%, removed an additional 20% of the phosphate from suspension without competing anions, likely from an interaction between the negatively charged phosphate and the positively charged quaternary ammonium ion present (**Figure 1(b)**). When competing anions were introduced into the suspension, C40-00 was not as effective at removing phosphate. The lack of selectivity of the quaternary ammonium ion resulted in an overall amount of phosphate removed similar to that of Magnifloc 494C. While the higher charge density on C40-00 results in more positively charged groups available to interact with phosphate in bulk solution, the available quaternary ammonium ion was not selective for phosphate.

The experimental polymer C40-20, offering three-point binding with phosphate, removed 24% more phosphate than the control containing only clay without competing anions, and 13% in the presence of other anions (**Figure 1(b)**). The C40-20 polymer had two groups that could interact with phosphate:

Table 1. Mole percent of monomers for polymers tested.

| Monomer | Magnifloc 494C ⁺ (A) | C40-00 (B) | C40-20 (C) |
|---|------------------------------------|---------------|---------------|
|  | 90 | 60 | 40 |
|  | 10 | 40 | 40 |
|  | N/A | N/A | 20 |

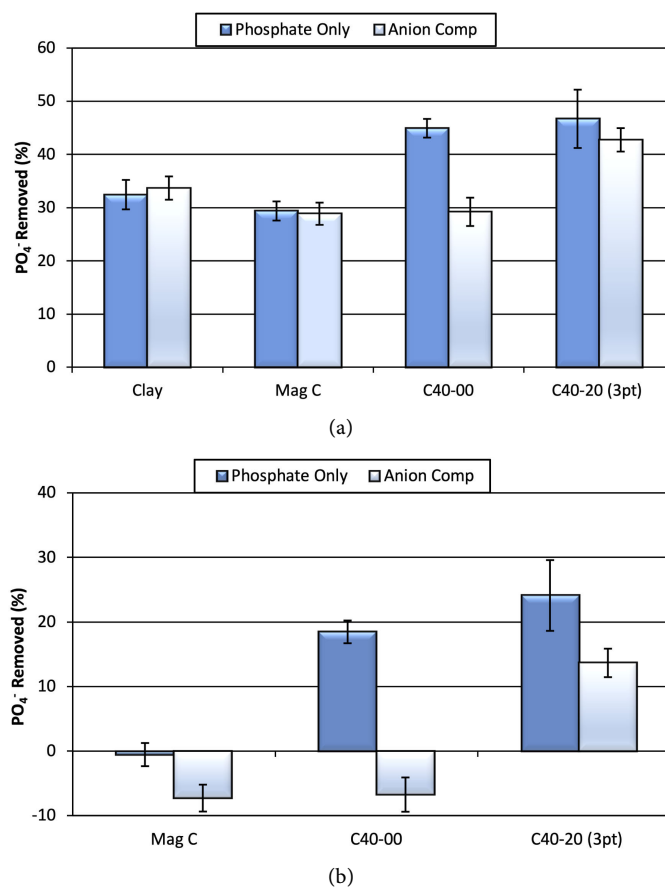


Figure 1. Phosphate removed by polymers from a clay suspension having an initial concentration of 5 mg/L H_2PO_4^- . (a) H_2PO_4^- removed in the presence of other anions at concentrations similar to wastewater and (b) the same results with the phosphate removed by the clay partitioned to examine the portion removed by the polymers.

the thiourea and the positively charged quaternary ammonium ion. The decrease in phosphate removal by the polymer when other anions were present was likely due to the interaction of anions with the quaternary ammonium group on the polymer. The control polymers showed the quaternary ammonium ion was unable to maintain phosphate sorption in the presence of competitive anions. These results indicated that the remaining 13% of phosphate removed in the presence of competing anions was due to the interaction of the thiourea with phosphate. This further indicated that the thiourea group may be selective for phosphate in the presence of other anions. To further investigate the selectivity of the thiourea for phosphate in the presence of competing anions, ^1H NMR and UV-V is experiments were conducted to examine the affinity of thiourea for phosphate in the presence of chloride and sulfate.

3.2. Computational and ^1H NMR Anion Binding Studies

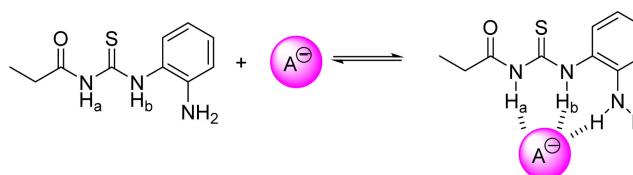
Density functional theory (DFT) computational studies were conducted in acetonitrile and water to determine the binding energies between the three-point hydrogen bonding thiourea trapping group and anions (Cl^- , HSO_4^- , and H_2PO_4^-)

[12] [13] [14] [15]. Each molecule was calculated for a base line energy and then the computations were conducted with binding of the anions in both acetonitrile and water (**Table 2**). The results exhibit the same trend observed with the flocculation studies. The strength of binding increases from chloride > hydrogen sulfate > dihydrogen phosphate in both acetonitrile and H₂O. Using acetonitrile as the solvent, the binding of phosphate is approximately 5 kcal/mol greater in comparison to sulfate and 9 kcal/mol for chloride respectively.

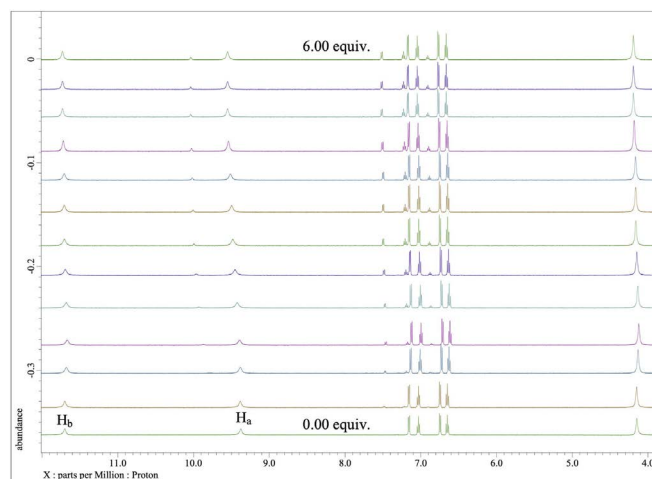
When the solvent is changed to water, the binding energy difference between phosphate and sulfate are again approximately the same as acetonitrile, however, chloride decreased by approximately 4 kcal/mol. The lower binding affinity for chloride can be attributed to the stronger solvation effects of Cl⁻ in H₂O compared to acetonitrile.

To further test anion binding affinity, ¹H NMR titration experiments were conducted with Cl⁻ (tetrabutylammonium chloride/TBACl), HSO₄⁻ (tetrabutylammonium hydrogen sulfate/TBAS), and tetrabutylammonium dihydrogen phosphate/TBAP) [16]-[27]. To examine these interactions by ¹H NMR, the thiourea samples were prepared at a 4 millimolar concentration and the anions were titrated up to 6.00 equivalents (24.0 mM) in CD₃CN and DMSO_{d6}. A stronger hydrogen bonding interaction was observed between the monomer and phosphate, in comparison to Cl⁻ and HSO₄⁻ (**Figure 2**). Increasing the concentration (0.0 - 6.0 equiv.) of Cl⁻, leads to a downfield shift in both H_a and the aromatic region. H_a shifts from 9.40 ppm to 9.90 ppm upon the addition of 3.00 - 6.00 equivalents displaying the interaction of the chlorine with one of the thiourea protons. For the titration using TBAS little change is observed using Hydrogen Sulfate. Upon titration with dihydrogen phosphate a significant shift in both the thiourea H_a and H_b protons is observed. An upfield shift of H_b from

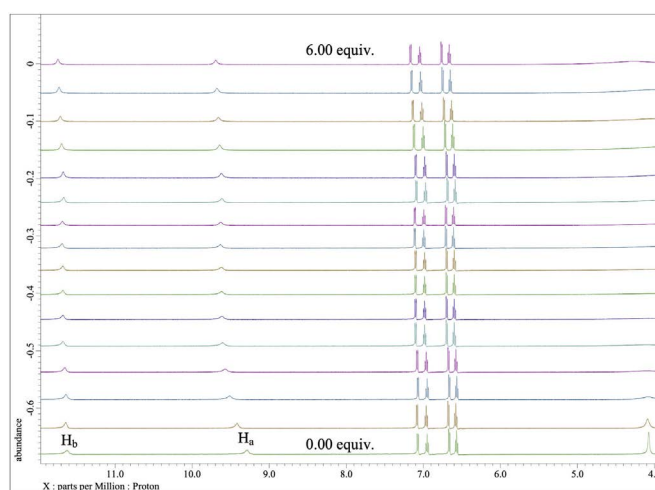
Table 2. Calculated binding energies for anions with thiourea host in acetonitrile and water. Relative energies obtained from DFT calculations were performed on each of the molecules and then with hydrogen binding. These energies were then subtracted to obtain the calculated values (M06-2X/6-311G (d)).



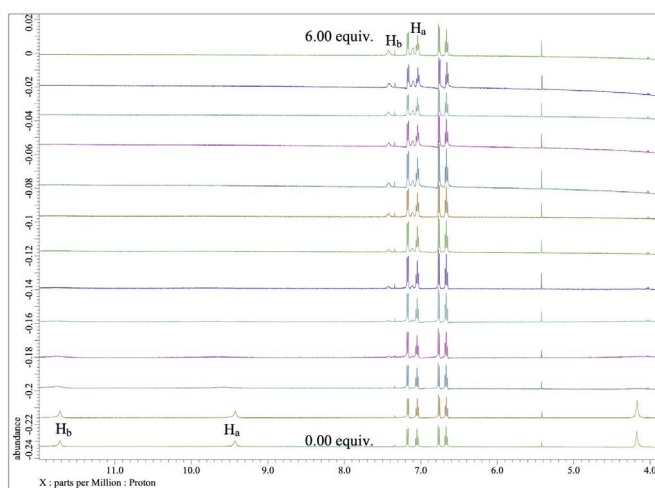
| Anion | Solvent | Binding Energy (Kcal/mol) |
|---|------------------|---------------------------|
| Chloride (Cl ⁻) | Acetonitrile | -6.76 |
| Hydrogen Sulfate (HSO ₄ ⁻) | Acetonitrile | -10.15 |
| Dihydrogen Phosphate | Acetonitrile | -15.30 |
| Chloride (Cl ⁻) | H ₂ O | -2.65 |
| Hydrogen Sulfate (HSO ₄ ⁻) | H ₂ O | -10.42 |
| Dihydrogen Phosphate (H ₂ PO ₄ ⁻) | H ₂ O | -15.02 |



(a)



(b)



(c)

Figure 2. ^1H NMR of: (a) Three-point thiourea and tetrabutyl ammonium chloride (Cl^-) (6.00 equiv.), Titration with Cl^- ; (b) Tetrabutylammoniumhydrogensulfate (HSO_4^-) (6.00 equiv.), Titration with HSO_4^- ; and (c) Tetrabutyl ammonium dihydrogen phosphate H_2PO_4^- (6.00 equiv.) in CD_3CN , Titration with H_2PO_4^- .

11.80 ppm to 7.40 ppm and H_a from 9.40 ppm to 7.50 ppm was observed. The large shift of the thiourea protons indicates stronger hydrogen bonding interaction in comparison to Cl^- and HSO_4^- supporting both the flocculation and computational studies.

The acid/base reaction of inorganic phosphate with thiourea in DMSO has been prevalent in the chemical literature [28]. To ensure that a hydrogen bonding interaction is occurring and not acid/base chemistry, tetrabutylammonium hydroxide was added to a thiourea host in $DMSO_{d_6}$. A significant change was observed in the removal of the thiourea proton the aromatic region of the 1H NMR. Due to the absence of these changes in the anion titrations, it is evident that the interaction of inorganic phosphate occurs via hydrogen and not Bronsted-Lowry acid/base chemistry. Both the computational studies and the titration support the polymer flocculent results in that dihydrogen phosphate has a higher affinity for the thiourea trapping group in comparison to the hydrogen sulfate and chloride.

4. Conclusion

The study described herein show that the addition of thiourea to a polymer flocculent results in a material that can be used to remove both the sorbed and soluble phosphate from wastewater. In contrast to our thiourea derivatized polymer flocculants, the control polymers tested had no selectivity for phosphate in the presence of competing anions. From the computational and 1H NMR titration studies, it appeared that the thiourea monomer had a higher affinity for $H_2PO_4^-$ than Cl^- or HSO_4^- , supporting our observation of selectivity in the polymer flocculent studies. It is believed that the higher affinity of the monomer for phosphate is due to the ability of a third hydrogen bonding interaction that is not existent with sulfate or chloride. This interaction would make this an enthalpically driven process where the ability to form the third hydrogen bond provided the anion selectivity. Currently, an investigation into the interaction of phosphate with the monomer using calorimetry is underway to determine the thermodynamics of this interaction.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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