Phosphate and nitrate sorption by amine-modified silica as the study of slow release fertilizer

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Abstract - The mud of Lapindo was reported consisted of significant amount of silica that have great capability to adsorb cations and anions due to their large surface areas. The properties of silica can be investigated in the purpose of binding cations and/or anions of micronutrients and releasing them when they are dissolved into water. The measurement of the release rate would give good description of the principle of slow release fertilizer. This research studied preparation of silica from Lapindo mud, that be treated become silica gel (SG) or whose surface were modified by use of (3-Aminopropyl)triethoxysilane (APTES) become amine-modified Silica (AMS). The sorbent was used for adsorption experiments of phosphate (H₂PO₄⁻) and nitrate (NO₃⁻) ions. Both ions bound the sorbent of AMS through hydrogen bonding. Phosphate formed monolayer surface complexes, but nitrate formed multilayers surface complexes with the sorbent. Desorption results showed that AMS bound phosphate ions stronger than SG. Rate constant of desorption (k) of phosphate by SG and AMS were 0.00507 and 0.000107 g.mmol⁻¹.min⁻¹ respectively. However, the nitrate desorption by SG were smaller than that by AMS as indicated by k of 0.000268 and 0.000836 g.mmol⁻¹.min⁻¹ respectively

1. Introduction
Since silica has a large surface, it can be utilized as a good sorbent. An adsorbate that already bonded by absorbent can be released again according to its each binding strength, and can be studied from desorption rate. This reaction rate is measured through desorption kinetics, as has been reported by pervious investigators [1-4]. Analysis to determine the sorption kinetic was performed by Lagergren rate equation. Adsorption experiments were conducted to study some factors affecting adsorption binding, to study adsorption process, to determine maximum loading of sorbent for the adsorbate, and to measure adsorption kinetics. Then, after the sorbate-which is nutrient anions- was successfully bonded, desorption experiment was conducted to examine its desorption rate.

This research used modified sorbent, e.i silica separated from Lapindo mud that was modified using reagents. The modification was conducted to study sorbent that able to bind nutrient anions optimally, and to release it again with small desorption rate. Small desorption rate is proportional to strong binding, meaning that the binding between adsorbent and adsorbate is strong. Among others, strong binding can occur on inner-sphere surface complexes with chemisorption between sorbent and sorbate. This research investigated the adsorption and the release of phosphate and nitrate anions kinetics by
modified silica sorbent, that can be used as basic knowledge on the preparation of slow release fertilizer.

Modification of silica can be performed by adding (Mercaptopropyl)trimetoxysilane [5-8], which is abbreviated as MPTS or (3-aminopropyl)trimetoxysilane [9], which is abbreviated as APTES. This research used APTES to modify the sorbent of silica from Lapindo mud.

2. Theoretical Background
Silica compound found in nature has crystal structure, while synthesized silica compound has amorphous structure can be separated to become silica gel. One of silica gel’s application is as adsorbent. Silica gel has great adsorptive properties due to the active sites on its surface, i.e. silanol (Si-OH) and siloxane functional groups (Si-O-Si). However, silica gel has a low affectivity and selectivity in interacting with ions. This is because silica gel has only silanol (Si-OH) and siloxane (Si-O-Si) functional groups as active sites. Oxygen atom which is active site has small size and low polarisability, thus interaction with heavy metal with big size and relatively high polarisability, is theoretically not strong enough [10]. In addition, O atom from active groups of silanol (=Si-OH) and siloxane (=Si-O-Si=) which is bound directly to Si atom in silica structure make its low capability as electron pair donor and caused a weak metal ion bonding on silica’s surface [11]. On the other hand, silanol (=Si-OH) and siloxane (=Si-O-Si=) groups are also beneficial in terms of probability of modification.

Modification towards silica surface can be performed after separation of silica from its sources. Silica separation from Lapindo mud need to be done before modifying it. Savita et al. [12] separated silica from rice husk.

Silica modification is performed by changing –Si-OH groups become –Si-OM, where M is simple or complex species, except hydrogen [13]. There are two kinds of compounds that can be used to modify silica, i.e. organofunctionalization (if the modifying substance is organic compounds) and inorganofunctionalization (if functional group which bound to surface is inorganic compound or metal oxide). Functional group modification method that can be used is impregnation and formation of covalent bond. Impregnation is related to physical interaction between modifier materials with solid surface by adsorption or electrostatic interaction. This modification process that has been done by Serano et al. [6] is not suitable for adsorption in water as medium.

Modification by formation of covalent bond can be performed by silanization process or Sol-Gel process. This process is being done to silica gel, and usually in a non-water medium. For example, Tertykh and Yanishpolski [14] and Shylesh et al. [15] have done modification by using reagent containing mercapto group, by reacting (mercaptopropyl)trimetoxysilane with aerosol in toluene medium. Modification by Sol-Gel is being done to silica precursor. Modifier that will substitute silanol group is added into silica precursor during gel forming process. This process will results in compound containing siloxane, silanol, and modified silica functional group, which is notated as –S-OM.

Modifiers that can be used in silica modifying process, by impregnation, silanization or sol-gel method are (a) (mercaptopropyl)-trimetoxysilane (MPTS) to get mercapto functional group or –SH as substitution for –Si-OH [10] (b) (Aminopropyl)trimetoxesilane (APTES) to get amino group or –NH2 as replacement for –Si-OH. (c) (Chloropropyl)trimetoxysilane (CPTS) to get chloro functional groups or –Cl as replacement of –Si-OH.

In addition, modifier that does not contain silanol groups is 1-amino-2-hydroxy-4-naphtalena disulfonate acid, for instance. Modification process with this modifier needs other silane group as medium or as connecting bridge. To fulfill this need, (glicydoxypropil)-metoxysilane (GPTMS) can be used.

Amino-modified silica surface (AMS) in this research was used as adsorbent to bind phosphate and nitrate anions, and also as desorbent to release those bonded anions. Factors affecting the bond such as pH, contact time, and sorbate/sorbent concentration, were observed as control variables to determine optimum condition of binding.
3. Research Method
This research includes (1) the preparation of amine-modified silica (AMS) and (2) sorption of phosphate and nitrate anions by AMS to investigate nutrients anion desorption kinetics.

3.1. Preparation of amine-modified silica (AMS)
Experiment to produce amine-modified silica (AMS), was performed with following experiment procedures:
1. (3-Aminopropyl)triethoxysilane (APTES) (1g of dusk/1.5 mL of APTES) was added to sodium silicate from the previous separation, and 0.1M HCl was added with continuous stirring until gel formed, and neutral pH was formed, and was left for 18 hours [9]
2. 20 mL of water was added to the gel and was stirred for 15 minutes, and then was filtered with Buchner filter, and dried by oven at 80 °C temperature until the mass was constant, and amine-modified silica (AMS) was obtained.
3. Methylation process was repeated again, and the result was dried for 6 hours by oven at 80 °C temperature, and then being reacted with NaCl 10% solution with continuous stirring for 5 hours.
4. Precipitates were being separated, filtered by Buchner filter and being washed by water, ethanol and diethyl ether, respectively, and being dried in the air.
5. This dried result is sorbent quarternary amine-modified silica (AMS) as anion exchanger.
6. Spectroscopically confirmation was done by FTIR for group function, and by GSA to determine surface area, porous volume and porous distribution and by SEM EDS.

3.2. Sorption of phosphate and nitrate anions to investigate nutrients cation desorption kinetics.
Phosphate and nitrate anions sorption experiment was performed to investigate anion’s desorption kinetics. Adsorbed sorbate was being desorbed from sorbent with the following procedure.
1. 0.3 g of AMS was being diluted in 300 mL 0.001 M of KNO₃ on 30 °C temperature and its pH was maintained at 7.0. Suspension’s mixture was stirred continuously by magnetic stirrer until an optimum time of adsorption kinetics and 5 mL of sample was taken and measured for free anion concentration.
2. Suspension pH was being increased or decreased by dropping KOH or HCl to a contant pH at which minimum adsorption was occurred (from edge adsorption result) and maintained to be constant. After observed time range (5 minutes, 20 minutes, 30 minutes, 30 minutes, 1 hour, 3 hours, 5 hours, 10 hours, 20 hours, 30 hours, 36 hours, 48 hours and 72 hours), 5 mL of sample was taken, was centrifuged, and its filtrate then analyzed for free anion concentration. The difference of measured left free anion concentration with adsorbed anion concentration is amount of desorbed anion concentration by AMS in the observed time range.

4. Result and Discussion
4.1. Preparation of silica from Lapindo mud and its modification
Lapindo mud was being calcinated, before silica was separated. AMS was obtained by modification of (3-aminopropyl)triethoxysilane. Obtained silica without modification was crystal with white colored. Table 1 below depicts the rendement of silica synthesis results with and without amine modification.
### Table 1. Synthesis results of silica from lapindo mud

| Sorbent | Obtained Mass (from 20 g of calcinated mud) |
|---------|---------------------------------------------|
| SG      | 1.536 gram                                  |
| AMS     | 2.73 gram                                   |

Spectrogram results from FTIR for characterization of synthesis result is shown in Table 2.

### Table 2. Spectrogram of SG and AMS

| Functional group          | Wave number (cm\(^{-1}\)) |
|---------------------------|-----------------------------|
| SG                        | AMS                         |
| Stretch vibration of –OH from Si-OH | 3448.72 | 3425.58 |
|                           | 1095.57 | 1072.42 |
| Asymmetric stretch vibration of Si-O from Si-O-Si | 794.67 | 786.96 |
| Symmetric stretch vibration of Si-O from Si-O-Si | 1635.64 | 1627.92 |
| Bending vibration of –OH from water molecule | - | 3286.70 |
| Stretch Vibration of –NH\(_2\) | - | 1527.62 |
| Symmetric bending vibration of –NH from –NH\(_2\) | - | 2939.52 |
| Stretch vibration of –CH\(_2\) | - | 1473.62 |

SEM results of both sorbents are given below (Figures 1 and 2).

![SEM AMS with 10000 Times Zoom](image)
Figure 2. SEM SG with 10000 Times Zoom

Specific surface area and volume of silica sorbent of SG and AMS porous was determined by using Brunauer–Emmett–Teller (BET) and nitrogen adsorption-desorption BJH adsorption on 77.3K temperature by using Quantachrome Nova2000 instruments. The results are presented in Table 3 below.

Table 3. Surface area and porous volume analysis results

| Properties                        | SG       | AMS     |
|-----------------------------------|----------|---------|
| Surface area (m$^2$/g)            | 143.251  | 32.121  |
| Total porous volume (mL/g)        | 0.533    | 0.215   |
| Porous average radius (1 \times 10^{-10} m) | 74.821   | 15.129  |

4.2. Nitrate anion sorption by SG and AMS

Nitrate ion adsorption experiment in several contact times was used to determine adsorption kinetics model, either on SG or AMS. Plotting the adsorption kinetics data was referring to Pseudo-Second-Order equation:

\[
\frac{t}{q_t} = \frac{1}{kq_e^2} + \frac{1}{q_e^2} t.
\]

By plotting the time t (minutes) versus \(\frac{t}{q_t}\), a linear plot was obtained [16], as given on Figure 3.

Figure 3. Pseudo-second order of nitrate ion bonding
Obtained straight line equation was then interpreted by Lagergren equation to obtain adsorption kinetics parameter as shown in Table 4, which describe bonding reaction order by SG and AMS.

**Table 4.** Pseudo-second order reaction kinetics parameter of nitrate ion adsorption by SG and AMS

| Adsorption Kinetics Parameter | Pseudo Second Order |
|------------------------------|---------------------|
| Experiment’s $q_e$ (mmol g$^{-1}$ of adsorbent) | 36.230 | 43.067 |
| determined result’s $q_e$ (mmol g$^{-1}$ of adsorbent) | 35.714 | 41.667 |
| k (g mmol$^{-1}$ minutes$^{-1}$) | 0.000268 | 0.000836 |
| Coefficient of determination ($R^2$) | 0.991 | 0.993 |

Based on adsorption kinetics determinant coefficient which is referred to *Lagergren Pseudo-Second-Order*, i.e. 0.991 for SG and 0.993 for amine-modified silica. These can be seen from measured $q_e$ which are comparatively nearer with experiment’s $q_e$ [17]. Adsorption process following pseudo second order kinetics model means that binding rate of SG and AMS towards NO$_3^-$ ion per time unit is proportional to square of still-empty adsorbent capacity ($q_e-q_t$). Thus, in the beginning of adsorption process, solution’s concentration was decreased drastically and adsorption rate continually decreased until equilibrium condition was achieved. Based on their finding, Kurniawati et al. [18] stated that pseudo second order kinetics model depended on adsorption ability to every solid phase, and assumes that adsorption capacity was proportional for active sites in absorbent. In adsorption process, difference of concentration between adsorbate in solution phase with adsorbate concentration in adsorbate’s surface will continually occurred until adsorbent’s surface was filled by adsorbate and the adsorption reached equilibrium condition when there was no more adsorbate available for adsorption. Table 5 shows that the constant values of pseudo second order k for SG and AMS are very small, which mean that adsorption process in this research occurred slowly.

pH is one of important factors in adsorption process. Adsorption in this research was performed to investigate effects of pH towards NO$_3^-$ ion’s adsorption by SG and AMS (Figure 4). Changes of active sites in silica’s surface is affected by PZC (*point zero of charge*) value. Adsorbent’s PZC can be used to explain NO$_3^-$ ion’s adsorption process in SG and AMS, where silica’s PZC is known to be 2.5 [19] and AMS’s PZC is 10 [20]. If pH value > PZC, then the surface is negatively charged and if pH value < PZC, then the surface is positively charged [21]. This negative charge increases with increasing solution’s pH until pH 10. Above it, the increase of solution’s pH gives relatively small effect to negative charge of surface. In this research, maximum pH was found to be pH 10, either in SG and AMS.
Figure 4. The adsorption of 0.001 M NO$_3^-$ ion’s by SG and AMS in various pHs.

SG’s charge in adsorbent’s surface is found to be negative because PZC’s value was relatively smaller than pH. Meanwhile, charge of AMS’s surface is found to be positive because PZC was bigger than pH. In this research, as pH was being increased into basic pH values after the equilibrium contact time, the suspension’s pH tends to decrease. On the other hand, when the pH was being decreased in acid’s pH after the equilibrium contact time, the pH tends to increase. This means that in high pH protonation is occurred and in low pH deprotonation is occured. Surface’s charge difference is due to the difference of PZC in SG and AMS. This difference of PZC does not make any difference in NO$_3^-$ ion’s binding concentration in initial concentration of 0.001 M as shown in Figures 17 and 18.

Isotherm adsorption also shows that nitrate binding towards AMS tends to form multilayer, however it does not show specific binding pattern towards SG based on Langmuir and Freundlich isotherm analysis.

In SG, adsorbent’s surface tends to be negatively charged due to deprotonation of silanol group (Si-OH) and becomes Si-O$^-$ [22]. Most likely, NO$_3^-$ anions that are bonded together with adsorbent form an aggregat first [23] by hydrogen binding between water molecule and three oxygen atoms in NO$_3^-$ ion, and atom H in water molecule was bound by Si-O$^-$ which exist at adsorbent’s surface. Water molecule has an important role in binding NO$_3^-$ anions by adsorbent so it can be proposed that the binding occurred gradually at adsorbent’s surface which is heterogeneous in suraface energy. However on the adsorbent of AMS, adsorbent’s surface tends to be positively charged due to -NH$_2$ group is protonized and become –NH$_3^+$ [20, 23], and because of large value of PZC [23], NO$_3^-$ ion on amine-modified silica can be bound directly to the adsorbent’s surfaces, i.e. –NH$_3^+$ aligns with adsorption kinetics result where amine-modified silica adsorbs NO$_3^-$ faster. Reactions that occur are described in the Figures 5 and 6.
Either SG and AMS bound NO$_3^-$ ions without releasing or taking up proton. It was shown by adsorption experiments in various NO$_3^-$ ions concentrations on pH 7 that were not experiencing significant increase or decrease of pH values.

Desorption results showed that nitrate anions that has been bounded by AMS are less desorbed than that by SG during the duration time of desorption experiments of 9 days (Figure 7). This means that AMS the modified surface of silica have slower desorption rate of nitrate that has been bounded significantly than SG.
4.3. Sorption of phosphate anion by SG and AMS

Figure 8 shows kinetics modelling to the experiment data of the adsorption of phosphate by SG and AMS, and it shows that reaction of phosphate and SG or AMS has pseudo second order rate.

According to Figure 8, the model of data of phosphate adsorption by SG, linear regression with equation of \( Y = 0.053X + 0.554 \) and determinant coefficient \( (R^2) \) of 0.999 were obtained. \( q_e \) value can be determined by substituting slope in the equation, i.e. 18.8679. Meanwhile, reaction rate constant \( (k) \) was determined through its intercept, i.e. 0.00507. In AMS, the line equation is \( Y = 0.0089X + 0.596 \) with determinant coefficient of 0.996. Adsorption capacity value in equilibrium condition \( (q_e) \) is 125 and reaction rate coefficient \( (k) \) 0.000107.
By using *Lagernon Pseudo-first Order Kinetic* and *Lagernon Pseudo-second Order Kinetic* equations on phosphate binding by SG and AMS, several parameter values were obtained, such as $q_e$, $k$, and $R^2$, as can be seen in Table 5.

| Binding Kinetics Parameter | Pseudo Second Order  |
|----------------------------|---------------------|
|                           | SG  | AMS |
| Determined $q_e$ (mol g$^{-1}$ of adsorbent) | 18.9822 | 127.3632 |
| Experiment’s $q_e$ (mol g$^{-1}$ of adsorbent) | 18.86792 | 125 |
| $k$ (g mol$^{-1}$ minutes$^{-1}$) | 0.00507 | 0.000107 |
| Determinant Coefficient ($R^2$) | 0.999 | 0.996 |

Based on the table above, it can be seen that phosphate binding kinetics of SG and AMS were aligned with *Lagernon Pseudo-second Order Kinetic* equation, which was indicated by high value of determinant coefficient ($R^2$) of *Lagernon Pseudo-second Order Kinetic*, and the value of calculated $q_e$ that close to the experiment’s $q_e$.

In *Lagernon Pseudo-second Order Kinetic*, phosphate binding rate either by SG or AMS per unit of time is proportional to square of empty adsorbent capacity ($q_e$), thus in the beginning of adsorption process, solution’s concentration was decrease dramatically. Adsorption rate was then decreasing until equilibrium condition was achieved [1].

Phosphate binding by silica and AMS was performed in range pH of 3-10 to investigate pH’s effects. Phosphate binding by either SG or AMS was optimum in the pH of 5, and tends to decrease at higher pH values. Phosphate binding percentage by SG with 0.001 M phosphate is higher, compared to that of 0.01 M phosphate as reported by previous investigation [Jaslin xx]. This describes binding ability shortage of surface’s binding ability towards phosphate, thus phosphate’s concentration addition after binding rate maximum will not increase amount of bounded phosphate, and thus bounded phosphate’s concentration will get smaller. This is aligned with obtained isotherm data where adsorption was following Langmuir isotherm pattern, monolayer formation and optimally formed when all of the surface was covered by phosphate.

pH’s effect towards adsorption is determined by the dependence of sorbent’s and sorbate’s surface characteristics due to the changes of observed pH. The high and low pH value that determines protonation-deprotonation of silica group either by SG or AMS was determined by point zero charge (PZC), i.e., pH value (point) where surface’s charge value is neutral or zero.

For SG, surface tends to be negatively charged in experiment’s pH range. But for AMS, amine functional group is in neutral form at pH 6 thus AMS’s surface is negatively charged (Buhani, 2010:180). This causing amine functional group can be functioned as electron pair donor and results in interaction between phosphate anion and active sites at AMS on covalent coordination. In addition, in AMS’s adsorbent –OH (cylanol) and –Si-O-Si (cyloesan) groups was still found and can be functioned as donor in that condition. Interaction between anion phosphate with AMS’s adsorbent is a strong interaction.
As shown by Figure 9 that the optimal phosphate binding by silica and AMS was occurred at pH 5. After that, phosphate binding percentage was decrease as the pH increase.

![Figure 9](image)

**Figure 9.** The effect of pH on the adsorption of 0.01 M phosphate by SG and AMS.

Based on phosphate species distribution, phosphate ions was dominated by anion species in pH range of 3-10. In low pH, \( \text{H}_2\text{PO}_4^- \) was species which its distribution is the most dominant. While in pH of 7.5-9 species \( \text{HPO}_4^{2-} \) was species which its distribution is the most dominant. Above pH of 8, species \( \text{PO}_4^{3-} \) was significant. Meanwhile, SG’s surface tends to be negatively charged and AMS to be positively charge in low pH (pH<PZC) and negatively charge at high pH (pH>PZC). Those species existence are significantly affects the phosphate binding by SG or AMS.

Isotherm experiment of phosphate binding by silica and AMS was performed by adding \( \text{KH}_2\text{PO}_4 \) 0.01 M solution with certain volume. This experiment was aimed to measure phosphate concentration that was bounded by silica and AMS in various phosphate’s concentration. Phosphate binding isotherm was performed at pH of 7. To maintain the experiment pH, \( \text{HNO}_3 \) and \( \text{NaOH} \) were added to phosphate solution. In binding isotherm experiment, the amount of added \( \text{NaOH} \) and \( \text{HNO}_3 \) was very small and can be ignored thus not involved significant amount of proton. The species distribution of phosphate in the pH 7, the amount of proton in \( \text{H}_2\text{PO}_4^- \) is still significant thus the reaction did not release/bind proton.

Langmuir adsorption model was shown by plot of Ce to Ce/N, where Ce is free phosphate concentration at equilibrium, and Ce/N is the amount of bounded phosphate molecules per weight unit of adsorbent. Isotherm Langmuir model for SG and AMS was depicted on Figure 10.
Figure 10. Langmuir model for phosphate binding by SG (♦) and AMS (■).

Based on Langmuir isotherm graph, linear equation $Y=122006x+11.558$ was obtained with the determinant coefficient values of 0.998 for SG, and equation of $Y=119762x+5.9188$ with determinant coefficient of 0.995. Langmuir isotherm parameters in phosphate binding by SG and AMS; $N_{\text{max}}$, $K_L$, and $R^2$ were calculated with the results are listed in Table 6.

Table 6. Langmuir parameters in phosphate adsorption by SG and AMS

| Isotherm Model | SG       | AMS       |
|---------------|----------|-----------|
| **Langmuir**  |          |           |
| $N_{\text{max}}$ (M g$^{-1}$) | $8.20 \times 10^{-3}$ | $8.35 \times 10^{-3}$ |
| $K_L$ (L g$^{-1}$)       | 1056.28  | 2023.66   |
| $R^2$                   | 0.998    | 0.995     |
| **Freundlich**          |          |           |
| $K_F$ (L g$^{-1}$)       | $1.40 \times 10^{-5}$ | $1.36 \times 10^{-5}$ |
| $1/n$                  | 0.09     | 0.08      |
| $R^2$                  | 0.869    | 0.692     |

Silica modification was done by reacting it with APTES ligands. The purpose was to increase adsorption capacity. This adsorption capacity increase could occur because of the increase of active sites for the adsorption, beside cyanol (-Si-OH) and cylocsan (Si-O-Si) groups in the SG. In AMS, the new functional groups that can improve adsorption capacity, i.e. –NH$_2$. –NH$_2$ is more basic than cyanol and cylocsan groups. Figures 11 and 12 show binding that occur between phosphate and SG and between phosphate and AMS.

Figure 11. Phosphate binding reaction by SiO- sites in SG
Phosphate desorption experiment by SG and AMS in this research was performed to investigate desorption rate of phosphate. Desorption results shows that the phosphate desorption percentage by AMS is smaller than that by SG. This indicates the binding strength between adsorbate and adsorbent. In AMS, covalent coordination binding was occurred between atom N⁺ (active group in AMS) and atom O⁻ (active group in phosphate). In SG, hydrogen binding was occured between atom O⁻ (active group in SG) and atom H⁺ (active group in phosphate). Hydrogen’s binding strength is weaker than covalent coordination binding thus phosphate was bounded to AMS stronger than that by SG, so phosphate binding by SG is easier to break than that by AMS.

5. Conclusion
Amine modified silica (AMS) that separated from Lapindo mud has been investigated, and AMS has smaller specific surface area and porous volume than SG. Phosphate is bounded by forming monolayer form, thus maximum adsorption power shows plentiful amount of functional groups in silica’s surface. Adding phosphate concentration in silica’s surface, either in SG and AMS caused the increasing free phosphate ions that are not bounded by sorbent, thus binding percentage grows smaller. Bounded nitrate ion by sorbent after hydrated and formed agregate, is then bind sorbent’s surface. This means that nitrate was bounded by sorbent by forming multilayer at surface. Thus, with higher nitrate ion concentration, the binding percentage is also higher. Based on desorption kinetics data, AMS sorbent is proven to increase the binding strength towards anion phosphate, but not towards anion nitrate.

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