# Arsenic Removal from Vietnamese Groundwater Using the Arsenic-Binding DNA Aptamer

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Single-stranded DNA aptamers were generated from a random library to remove arsenic from Vietnamese groundwater. On the basis of significant arsenic contamination levels, three areas in Ha Nam province (Vinh Tru, Bo De, and Hoa Hau) and five areas near the Mekong River Delta (MR1−5) were selected as study areas. The aptamers were in vitro selected using an arsenic aptamer affinity column created by immobilizing arsenic on Affi-gel 10 resin. Quantitative analyses of the aptamer candidates Ars-1 to Ars-8 by surface plasmon resonance (SPR) revealed the Ars-3 aptamer to have the highest affinity to arsenate [(As(V)] and arsenite [As(III)] with a dissociation constant ($K_d$) of 4.95 ± 0.31 and 7.05 ± 0.91 nM, respectively. The specific affinity interactions of the Ars-3 aptamer to arsenic were verified against other heavy metals. After obtaining successful removal results with a laboratory-prepared aqueous arsenic solution, Ars-3 was applied for removal of any arsenic present in the groundwater samples collected from the studied areas in Vietnam. Field results were also successful: various arsenic concentrations ranging from 28.1 to 739.2 μg/L were completely removed after 5 min of incubation with the arsenic-binding aptamer Ars-3.

## Introduction

Arsenic is a toxic carcinogen widely distributed in many parts of the world. Although it can exist in four oxidation states (−3, 0, +3, and +5), arsenate [As(V)] and arsenite [As(III)] are the most commonly existing forms of arsenic species in aqueous environments (*1*). Humans can be exposed to arsenic by direct and indirect ingestion, i.e., drinking arsenic-laden water and consuming crops grown from arsenic-accumulated soils (*2*), respectively. While arsenic can provoke acute and chronic health effects, the more serious effects from chronic ingestion or long-term exposure of arsenic can cause a variety of cancers (*3*), skin lesions (*4*), arsenicosis (*5*), and cardiovascular diseases (*6*). Arsenic that cannot be excreted from the human body accumulates in tissues with high keratin content such as skin, hair, and nails (*7, 8*). In Vietnam, for example, the Vietnam National Institute of Occupational and Environmental Health reported cases of arsenic poisoning in the local communities of Ha Nam province as a result of being exposed to arsenic-contaminated water (*9, 10*). High arsenic contamination levels were also reported in the Mekong River Delta of Vietnam (*11*). According to the study conducted by Nguyen in Ha Nam province, approximately 87% of the households in the affected areas used sand-filtered groundwater, which often failed to bring the water sources to safe levels by having residual arsenics that exceeded 10 μg/L of arsenic allowed in drinking water (*12*).

The current U.S. Environmental Protection Agency (EPA) standard of 10 μg/L is an adjustment from 50 μg/L, and such change created an urgent need for an improved arsenic removal system that can comply with the new standard. Coagulation, being the most widely used arsenic removal technology, shows over 99% removal efficiencies in laboratories under optimal conditions with residual arsenic concentrations of less than 1 μg/L (*13*). However, its removal efficiency will be affected by the pore size of the membrane filter disks used because coagulates smaller than the pores will remain unremoved (*14*). Likewise, another well-known removal technology such as ion exchange will have removal interference from its competing anion, sulfate (*15*). Various reports on the comparison of the existing arsenic removal technologies showed the importance of minimizing the interference factors for removal efficiency (*16*−*18*).

In an effort to provide an alternative removal method enhanced in removal efficiency with minimal interference, a new approach is made in this study with an aptamer serving as the removing agent. Aptamers are short single-stranded oligonucleotides with high specific affinity to a target molecule. On the basis of their three-dimensional structures, aptamers can be selected via the systematic evolution of ligands by exponential enrichment (SELEX) (*19, 20*) for different targets from a huge library of molecules containing randomly created sequences (*21*). After the DNA aptamer was synthesized with an arsenic-immobilized resin, a SPR assay was used to determine the specific affinity interactions of arsenic and aptamers. The removal tests using the synthesized arsenic aptamer were conducted with the laboratory-prepared aqueous solution and groundwater samples collected from various areas in Vietnam: Vinh Tru (VT), Bo De (BD), and Hoa Hau (HH) of Ha Nam province and five areas near the Mekong River Delta (MR1−5). With possible development as an alternative removal method, the studied aptamers are expected to bring beneficiary effects, including improved health conditions and a healthier living environment to many communities.

## Materials and Methods

**Selection of Aptamers by Systematic Evolution of Ligands by Exponential Enrichment (SELEX).** The aptamers were generated using in vitro selection and amplification technology known as SELEX (*19, 20*) from a random DNA library designed and prepared by Bioneer, Korea (chemical synthesis,

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purified by PAGE): 5′-GGT AAT ACG ACT CAC TAT AGG GAG ATA CCA GCT TAT TCA ATT-$N_{40}$-AGA TAG TAA GTG CAA TCT-3′. The arsenic affinity colum used for the selection of the arseinc-binding ssDNA was created by immobilizing 4-aminophenylarsine oxide (PAO) on an Affi-gel 10 resin as described by Hoffman and Lane (*22*). During a total of 10 rounds of the SELEX process, a negative selection step, which involved incubation of the ssDNA aptamer eluted from round 7 and activated Affi-gel 10 resin, was carried out between rounds 7 and 8 to eliminate or minimize nonarsenic binding of the ssDNA aptamer to the Affi-gel 10 resin. The arsenic aptamer affinity interaction was visualized and examined using the fluorescent Cy5-labeled aptamer with the arsenic-immobilized affinity column and arsenic-absent activated Affi-gel 10 resin column. The optimal SELEX round was determined by real-time PCR that quantified the arsenic-binding ssDNA aptamer eluted from SELEX rounds 0, 8, 9, and 10 using a MiniOpticon Real-Time PCR fluorescence signal detection system (Bio-Rad, U.S.A.). The aptamer pool from the optimal SELEX round 9, which eluted the largest amount of arsenic-binding ssDNA, was subsequently amplified by PCR using the unmodified primers and cloned into the vector pGEM-T Easy vector system (Promega, Germany). Sequence analysis and alignments were performed using the Vector NTI (Invitrogen, U.K.). Secondary structure analyses of several aptamers were performed using Zuker's free energy minimization algorithm (*23*) utilizing the Internet tool Mfold (http://mfold.bioinfo.rpi.edu/cgi-bin/dna-form1.cgi). Detailed experimental procedures and materials are described in the Supporting Information.

**Binding Measurements Using Surface Plasmon Resonance (SPR).** *Linking the Arsenic.* The binding affinities of the eight selected aptamers (Ars-1 to Ars-8 from SELEX round 9) for their target arsenic were analyzed by surface plasmon resonance (SPR) at 25 °C using a Biacore 2000 instrument (Biacore AB, Sweden). Detailed experimental procedures and materials are described in the Supporting Information. The kinetic parameters were obtained using the BIA evaluation program.

*Linking the Arsenic-Binding Aptamer.* To immobilize the biotinylated arsenic-binding aptamer, the chip was pre-equilibrated with HEPES then activated with 50 mM NaOH and 1 M NaCl at 5 $\mu$L/min for 10 min. The activation of the chip was followed by the injection of 1 mg of the aptamer with the highest affinity to arsenic (Ars-3) onto the sensor chip. After immobilizing the aptamer, various heavy metal solutions were injected individually onto the aptamer-bound SA sensor chip to determine the $K_d$ of the aptamer with each of the following heavy metal solutions: 1 M $FeCl_2$, 1 M $MnCl_2$, 1 M $NiCl_2$, 1 M $CdCl_2$, 1 M $CoSO_4$, 1 M $ZnCl_2$, 1 M $CaCl_2$, 1 M $MgCl_2$, 1 M $CuSo_4$, 1 M $NaAsO_2$, and 1 M $Na_2HAsO_4$. After each measurement, the sensor chip was regenerated with 0.5% SDS. The BIA evaluation program, version 3.1, was used to analyze the binding kinetics of the arsenic-binding aptamer and each of various heavy metals.

*Arsenic Removal from Aqueous Solutions Using the Arsenic-Binding Aptamer Ars-3.* In order to remove any arsenic available in the aqueous solution, Ars-3 was immobilized on streptavidin agarose resin, using the following procedure: (1) A total of 400 $\mu$L of streptavidin agarose resin (Thermo scientific, U.S.A.) was added to 1.5 mL of propylene microtube (Axygen Scientific, CA, U.S.A.). (2) After centrifuging the microtube for 1 min, the supernatant was removed, and the resin was washed twice with 1 mL of an arsenic aptamer selection buffer. (3) Another 600 $\mu$L of the arsenic aptamer selection buffer containing 2 mg of the biotinylated aptamer Ars-3 was added to the resin for adsorption, while a negative control was prepared using the same reagents but the Ars-3 aptamer. The incubation of the mixtures at 4 °C for 2 h on a thermomixer (Eppendorf, Germany) at 1300 rpm was followed by 1 min of centrifugation, removal of the supernatant, and two successive washes with 1 mL of the arsenic aptamer selection buffer. After As(V) and As(III) solutions were prepared in 0.5, 1, 2, 4, 6, 8, 10, 20, and 30 mg/L, the experimental errors in preparing various arsenic concentrations were determined. The mixtures were then loaded into separate tubes containing 1 mL each of laboratory prepared arsenic solutions [As(V) and As(III)] in 0.5, 1, 2, 4, 6, 8, 10, 20, and 30 mg/L. Five more tubes were prepared identically for 1, 6, 10, 20, and 30 mg/L to measure the arsenic level after a given incubation time. Initial arsenic concentrations were measured at $t = 0$. Then after 0.5, 1, 2, 5, and 30 min of incubation at 25 °C on a thermomixer (Eppendorf, Germany) at 1300 rpm, tubes loaded with 1, 5, 10, 20, and 30 mg/L were centrifuged for 1 min to determine the arsenic content from the supernatant buffer using inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7500ce, Agilent, U.S.A.). After 60 min of incubation, the final arsenic levels from the supernatants of all concentrations were measured. All arsenic measurements were done in triplicate and compared to 1 mL of various arsenic concentrations prepared for each reaction mixture.

*Arsenic Removal from Groundwater Samples of Vietnam Using the Arsenic-Binding Aptamer Ars-3.* After conducting a successive arsenic removal test with laboratory prepared arsenic solutions, the arsenic-binding aptamer Ars-3 was applied to remove arsenic present in Vietnamese groundwater. From Ha Nam province, the following three areas were selected to collect groundwater samples: Vinh Tru (VT, 20°33′38″ N, 106°01′48″ E), Bo De (BD, 20°29′32″ N, 106°06′44″ E), and Hoa Hau (HH, 20°28′51″ N, 106°10′38″ E). An additional five sampling points named MR1 (10°42′48″ N, 105°20′27″ E), MR2 (10°42′50″ N, 105°20′25″ E), MR3 (10°42′51″ N, 105°20′24″ E), MR4 (10°43′13″ N, 105°19′39″ E), and MR5 (10°43′02″ N, 105°19′53″ E) were selected from the Mekong River Delta. The groundwater samples from eight areas in Vietnam were collected and analyzed according to the method reported by Nguyen et al. (*12*). The removal method employed for these groundwater samples were identical to the method used to remove arsenic from the laboratory-prepared aqueous solutions of various arsenic concentrations, except that 1 mL of arsenic solutions at various concentrations were replaced by that of eight groundwater samples with only 5 min of incubation time. The concentrations of arsenic and other constituents present in eight groundwater samples were measured before and after 5 min incubation.

## Results and Discussion

**Selection and Identification of Arsenic-Binding Aptamers.** DNA aptamers needed for the affinity interaction study with arsenic were selected using the SELEX approach as described in Materials and Methods. The target arsenic (in the form of PAO) was immobilized on Affi-gel 10 resins, which allows easy and effective handling of the arsenic aptamer selection process that involves association and dissociation of the aptamer with the target (Figure 1). In each selection round, the immobilized ssDNA aptamer was washed with a selection buffer to remove any unbound species and was subsequently eluted with the selection buffer. The interaction between the aptamer pool and the arsenic immobilized on the affinity column was examined using a fluorescently (Cy5) labeled aptamer pool from round 8, which had already undergone a negative selection step. The purpose of having a negative selection that involves the incubation of the ssDNA aptamers with targetless Affi-gel 10 resins is to minimize the selection or the accumulation of ssDNA aptamers with nonspecific bindings before moving on to the next round of selection. Cy5, a reactive water-soluble fluorescent dye, was used to visualize the aptamers that had a specific binding interaction

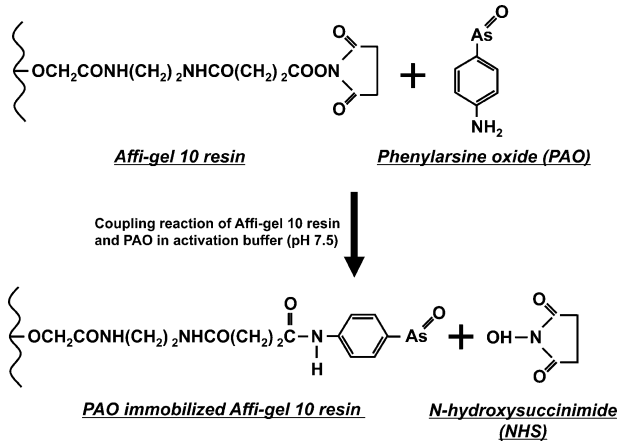


**FIGURE 1. Coupling reaction of PAO on Affi-gel 10 resin.**

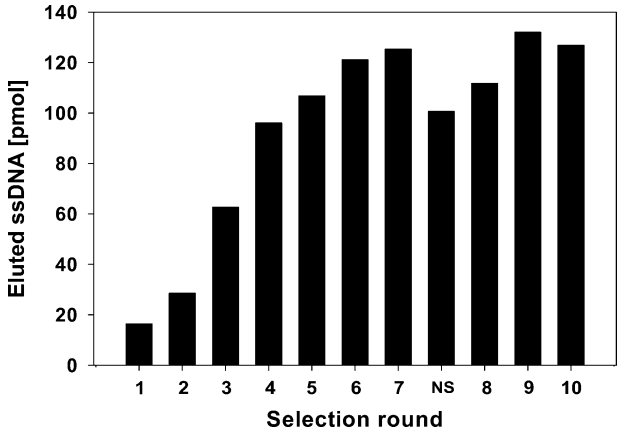


**FIGURE 2. Concentrations of the eluted arsenic-binding ssDNA from SELEX selection rounds. NS: negative selection.**

with its target immobilized on the column. Figure S1 of the Supporting Information shows the Cy5-labeled round 8 aptamer pool after its incubation with the arsenic affinity column (Figure S1a of the Supporting Information) and arsenic-absent Affi-gel column (Figure S1b of the Supporting Information). Rounded fluorescent spots clearly observed for Figure S1a of the Supporting Information is an indication that the Cy5-labeled aptamers were properly bound to the arsenic affinity column and collected after the elution step. However, mere traces of fluorescence observed for Figure S1b of the Supporting Information is a result of properly selected aptamers having no target to bind to on arsenic-absent Affi-gel 10 resins. The most of Cy5-labeled specific aptamers were washed away from the targetless column before they were eluted; only mere traces of Cy5-labeled aptamers were seen for Figure S1b of the Supporting Information. This verification of the interaction of the selected aptamer to the arsenic affinity column allowed further selection of the best binding aptamers.

After round 10 of the SELEX process, the arsenic-binding ssDNA aptamer pools eluted from rounds 8, 9, and 10 were quantified by real-time PCR in $1{:}10^3$, $1{:}10^4$, and $1{:}10^5$ serial dilutions. Standard curves of the DNA pools from the selected rounds in 10-fold dilutions drawn with the nontemplate control showed that only the template diluted in $1{:}10^3$ had the ability for amplification. The concentration of eluted arsenic-binding ssDNA aptamer, which reflects the direct affinity between the ssDNA aptamer and its target arsenic, was used as a guideline to determine the optimal round for cloning and sequencing. As shown in Figure 2, the concentrations increased progressively after each SELEX round until a negative selection was introduced after round 7 to eliminate

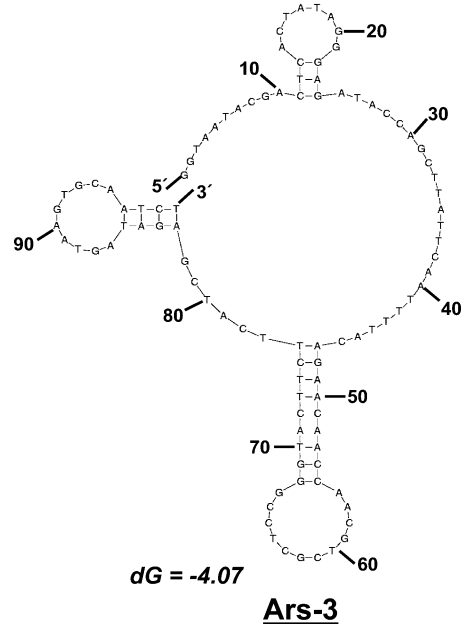


**FIGURE 3. Secondary structures of the selected DNA aptamers predicted using Zuker's algorithm, Mfold.**

the nonspecific bindings of aptamers. Increasing ssDNA concentrations is an indication of successive SELEX rounds because each SELEX round amplifies the aptamers eluted from a previous round, hence enriching the affinity progressively. After the ssDNA aptamers having nonarsenic specific bindings were removed by negative selection, the concentrations returned to their increasing pattern in rounds 8 and 9 (111.8 pmol and 132.0 pmol, respectively), but a slight decrease in concentration to 126.9 pmol in round 10 suggested no further enrichment of the aptamer affinity after round 9. The SELEX rounds were also evaluated by the enrichment efficiencies of real-time PCR products. The real-time PCR efficiencies of rounds 8, 9, and 10 were 84.16%, 85.23%, and 80.16%, respectively (data not shown). Just as the eluted ssDNA concentration increased until round 9, the efficiencies also increased progressively until round 9 but decreased approximately 5% thereafter. Both the eluted ssDNA concentrations and the real-time PCR efficiencies suggested round 9 as the optimal state that can be used for cloning and sequencing. Because round 9 was selected as the optimal round, no further SELEX round was carried out. Forty clones obtained from the cloning procedure were sequenced for a more detailed study of the DNA aptamer, of which eight nonrepeating sequences (named Ars-1 to Ars-8) were finalized. Table S1 of the Supporting Information shows the sequences of the selected arsenic-binding aptamers. Within this collection of sequences, the Ars-3 sequence was found among 12 of the 40 clones, and this similarity outnumbered that of the others. Ars-4 and Ars-8 had the next highest number; these sequences were found in seven clones. The rest of sequences were identically found among five or less clones, the sequence of Ars-2 being unique. Arsenic binding assays were carried out for all aptamers selected.

**Structural Analysis of Selected Aptamers.** The secondary structures of the eight sequences were predicted at 25 °C, 150 mM NaCl, and 0.5 mM $MgCl_2$ using Zuker's algorithm, Mfold. The similarity shared by the architectures of the predicted secondary structures was that they all had hairpin loops, which can be classified into tight and branched types (Figure 3 and Figure S2 of the Supporting Information). Three regions of base-pairing or hairpin structures, named 1, 2, and 3 starting from the 5′ end, were observed for Ars-3. While hairpin 2 was a unique formation found in Ars-3, hairpin

loops 1 and 3, which formed from the primer sequences, were also observed in Ars-4 and 6. Ars-2, 5, and 8 shared loop 1 with Ar-3, whereas Ars-1 that has a stem-loop sequence of GGAGATACC (hairpin 4) shared loop 3 only with Ars-3. Ars-7 shared no hairpin loop with Ars-3, but Ars-7 shared hairpin 4 (part of branch loop) with Ars-1. Ars-4 and 6 had a similar secondary architectural structure by having hairpin loops formed in a similar direction.

**Identification of the Arsenic Aptamer with the Highest Affinity Using SPR.** A quantitative assessment of the arsenic-binding properties of Ars-1 to Ars-8 obtained from the round 9 aptamer pool was done by a modified SPR assay that did not utilize the protein linkers. A study on direct SPR coupling by Win et al. reported that the presence of a protein linker such as BSA or biotin/streptavidin might introduce artifacts or nonspecificity in the small molecule−aptamer interaction, thereby preventing an accurate assessment of the intact affinity of the aptamer to its target molecule (*24*). While previously reported direct coupling strategies employed target molecules containing an amine group (*25, 26*), a commonly used functional group in Biacore sensor chip immobilization strategies, the arsenic that lacks an amine group was coupled to the CM5 sensor chip via 1,8-diaminooctane, which was first attached to the chip surface to create arsenic linkage based on an electrostatic interaction between the negatively charged arsenic complexes and positively charged amine groups as shown in Figure S3 of the Supporting Information.

The eight sequenced aptamer clones were tested for their ability to bind arsenic immobilized on a CM 5 sensor chip. All of the aptamers could bind immobilized arsenic, but their affinities determined in terms of the dissociation constants, $K_d$, varied. The nanomolar scaled $K_d$ values of the eight arsenic-binding aptamers ranged from $4.95 \pm 0.31$ to $96.6 \pm 2.16$ nM for arsenate [As(V)], and from $7.05 \pm 0.91$ to $51.2 \pm 3.21$ nM for arsenite [As(III)] (Table S1 of the Supporting Information). While the Ars-1, 3, 4, 6, and 8 aptamers exhibited higher $K_d$ values to As(III) than to As(V), the results were opposite for Ars-2, 5, and 7. Because the $K_d$ values and affinities are inversely related, the Ars-1, 3, 4, 6, and 8 aptamers with lower $K_d$ values to As(V) suggested a better aptamer binding affinity to As(V) than to As(III). The approximate As(V) to As(III) $K_d$ ratio was as much as 1:2.75 for Ars-8 with a higher affinity to As(V). However, Ars-5, an aptamer with a higher affinity to As(III), showed an As(V) to As(III) ratio of 3.63:1. Most closely related $K_d$ values were found with Ars-3 having an As(V) to As(III) ratio of 1:1.42. Not only are two $K_d$ values of Ars-3 to As(V) and As(III) ($4.95 \pm 0.31$ and $7.05 \pm 0.91$ nM, respectively) closely related, they are also the lowest values found for each chemical. From a total of eight selected aptamers, Ars-3 showing the highest affinity to As(V) and As(III) was selected for further studies. Appropriate $K_d$ values and their relative ratios are shown in Table S1 of the Supporting Information.

**Determination of the Specific Affinity of the Ars-3 Aptamer Using SPR.** The high affinity interaction of Ars-3 to arsenic was verified by examining the $K_d$ values. However, its specificity must be confirmed against other chemicals individually so that the selected aptamer Ars-3 can detect and capture arsenic only. Nine different metals present in abundance in the environment were chosen as nontarget chemicals. The affinity interactions of Ars-3 with nine different metals, As(V), and As(III) were hence monitored (Figure 4) in terms of $K_d$. Because low $K_d$ values suggest a high affinity to its target, As(V) with the lowest $K_d$ value, had the highest affinity to Ars-3 ($6.15 \times 10^{-16} \pm 5.45 \times 10^{-16}$ M). The $K_d$ value of Ars-3 to As(III) was slightly higher ($1.32 \times 10^{-15} \pm 8.20 \times 10^{-16}$ M) by comparison with the $K_d$ value of Ars-3 to As(V). The $K_d$ values of the Ars-3 aptamer against arsenics were $\sim 10^{-3}$ times lower than that against $Co^{2+}$, $Mg^{2+}$, $Mn^{2+}$, and $Ni^{2+}$. The $K_d$ value in the presence of As(V) was

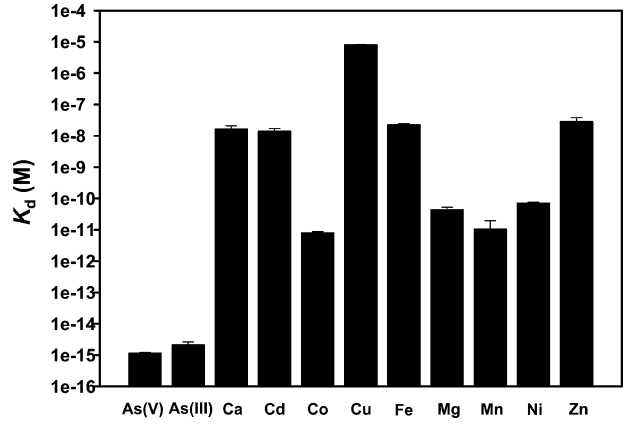


**FIGURE 4. $K_d$ values of Ars-3 to arsenate, arsenite, and nine different metals determined individually.**

$\sim 10^{-8}$ times lower than that in the presence of $Ca^{2+}$, $Cd^{2+}$, $Fe^{2+}$, and $Zn^{2+}$. The largest difference in $K_d$ value was observed with As(V) and $Cu^{2+}$, with an approximate difference of $4.08 \times 10^{-6}$ M. The distinct $K_d$ values of Ars-3 to arsenics compared with those of Ars-3 to various metal ions confirmed that the Ars-3 aptamer has specific affinity to arsenic as its only target.

**Arsenic Removal from Aqueous Solutions Using the Arsenic-Binding Aptamer Ars-3.** The specific binding of the Ars-3 aptamer to arsenic was confirmed using a variety of techniques. In order to apply such specific affinity in removal of arsenic available in the aqueous conditions, the removal efficiencies of Ars-3 at different arsenic concentrations were examined under two different conditions: streptavidin agarose resin prepared with 2 mg of biotinylated Ars-3 aptamer and the same resin without the addition of the arsenic-binding aptamer. Table 1 summarizes the arsenic concentrations determined from the supernatants of reaction mixtures after a 60 min incubation. The arsenic concentrations obtained from the aptamer-absent streptavidin agarose resin were similar to the loaded concentrations of arsenics as the arsenics that have no arsenic-binding aptamer to bind to were all detected by ICP-MS. However, no significant arsenic concentration was detected from the supernatants of the Ars-3-bound mixtures when 1 mL of As(V) and As(III) solutions at concentrations ranging from 0.5 to 20 mg/L were incubated, indicating successive bindings of As(V) and As(III) to Ars-3 aptamer. Traces of both arsenic species were detected when the As(V) and As(III) concentrations were increased to 30 mg/L ($0.74 \pm 0.06$ and $1.9 \pm 0.17$ mg/L, respectively). More As(III) than As(V) was detected due to the higher As(V) aptamer affinity.

The successive results shown with an arsenic aptamer-saturated resin allow further application of Ars-3 aptamer in removal of arsenic from the aqueous environment. The arsenic interactions with both aptamer-saturated and aptamer-absent resins are shown in Figure S4 of the Supporting Information. The removal of arsenic was also studied under various incubation times with arsenic concentrations in 1, 6, 10, 20, and 30 mg/L (see Table S2 of the Supporting Information for results). The time required for Ars-3 to capture or remove As(V) and As(III) from the aqueous solution was as short as 0.5 min for 1 and 6 mg/L of arsenic. From solutions containing 10 mg/L of arsenic, 99.8% of As(V) and 97.2% of As(III) were removed after 0.5 min of incubation, leaving $0.02 \pm 0.01$ and $0.28 \pm 0.03$ mg/L of As(V) and As(III), respectively, in the solution. As the loading concentration of arsenic doubled to 20 mg/L, the removal efficiencies also dropped to 60.6% and 56.7% for As(V) and As(III), respectively, during the same 0.5 min. For the maximum arsenic concentrations of 30 mg/L, more than half of the arsenic or $17.23 \pm 0.15$ mg/L of As(V) and $16.47 \pm 0.01$ mg/L of As(III) still

**TABLE 1. Arsenic Concentrations after Removing the Arsenic in Aqueous Solutions Using Ars-3**

| | As(V) (mg/L) | | | As(III) (mg/L) | | |
|---|---|---|---|---|---|---|
| arsenic[a] | prepared arsenic[b] | aptamer-absent streptavidin agarose resin | aptamer-saturated streptavidin agarose resin | prepared arsenic | aptamer-absent streptavidin agarose resin | aptamer-saturated streptavidin agarose resin |
| 0.5 | 0.48 ± 0.01 | 0.48 ± 0.02 | N.D. | 0.49 ± 0.01 | 0.49 ± 0.01 | N.D. |
| 1.0 | 0.98 ± 0.02 | 0.98 ± 0.03 | N.D. | 0.96 ± 0.02 | 0.98 ± 0.01 | N.D. |
| 2.0 | 2.04 ± 0.07 | 2.01 ± 0.10 | N.D. | 1.97 ± 0.11 | 2.06 ± 0.07 | N.D. |
| 4.0 | 3.86 ± 0.07 | 3.88 ± 0.06 | N.D. | 3.83 ± 0.10 | 3.71 ± 0.11 | N.D. |
| 6.0 | 5.90 ± 0.02 | 5.75 ± 0.06 | N.D. | 5.89 ± 0.14 | 5.81 ± 0.12 | N.D. |
| 8.0 | 7.78 ± 0.17 | 7.82 ± 0.12 | N.D. | 7.79 ± 0.16 | 7.85 ± 0.18 | N.D. |
| 10.0 | 9.74 ± 0.30 | 9.74 ± 0.29 | N.D. | 9.98 ± 0.27 | 9.83 ± 0.31 | N.D. |
| 20.0 | 19.82 ± 0.78 | 19.72 ± 0.57 | N.D. | 20.12 ± 0.17 | 19.99 ± 0.54 | N.D. |
| 30.0 | 30.11 ± 0.77 | 28.79 ± 0.75 | 0.74 ± 0.06 | 28.29 ± 0.38 | 29.21 ± 0.65 | 1.9 ± 0.17 |

[a] Theoretical arsenic concentration for each reaction mixture. [b] Experimental arsenic concentration prepared for each reaction mixture.

**TABLE 2. Removal of Arsenic Present in Groundwater Samples of Vietnam using Ars-3**

| | Ha Nam province | | | | | | Mekong River Delta | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Vinh Tru | | Bo De | | Hoa Hau | | MR1 | | MR2 | | MR3 | | MR4 | | MR5 | |
| parameters | raw | treated | raw | treated | raw | treated | raw | treated | raw | treated | raw | treated | raw | treated | raw | treated |
| As-total ($\mu$g/L) | 365.8 | N.D. | 287.6 | N.D. | 376.7 | N.D. | 552.1 | N.D. | 739.2 | N.D. | 476.0 | N.D. | 28.1 | N.D. | 61.1 | N.D. |
| As−III ($\mu$g/L) | 331.7 | N.D. | 261.7 | N.D. | 369.4 | N.D. | 454.5 | N.D. | 561.1 | N.D. | 398.4 | N.D. | 25.2 | N.D. | 57.0 | N.D. |
| Ba (mg/L) | 0.6 | 0.6 | 1.9 | 1.9 | 1.1 | 1.2 | 9.2 | 9.1 | 8.9 | 8.9 | 15.1 | 15.2 | 17.4 | 17.3 | 7.3 | 7.3 |
| Fe (mg/L) | 19.4 | 19.3 | 22.4 | 22.3 | 16.0 | 15.9 | 1.7 | 1.6 | 1.9 | 1.8 | 8.0 | 7.9 | 7.8 | 7.8 | 80.5 | 80.4 |
| Mn (mg/L) | 0.2 | 0.2 | 0.2 | 0.2 | 0.4 | 0.4 | 7.0 | 7.1 | 10.7 | 10.6 | 3.9 | 3.8 | 9.3 | 9.2 | 3.5 | 3.4 |
| Ca (mg/L) | 79.9 | 80.1 | 169.9 | 169.5 | 109.9 | 109.8 | 622.2 | 622.0 | 648.2 | 648.3 | 1144.0 | 1144.2 | 602.0 | 601.8 | 622.3 | 622.1 |
| Na (mg/L) | 14.7 | 14.8 | 38.9 | 38.8 | 77.9 | 78.2 | 267.1 | 267.0 | 264.1 | 264.2 | 410.1 | 410.0 | 172.3 | 172.1 | 362.1 | 362.0 |
| Mg (mg/L) | 46.4 | 46.5 | 73.0 | 72.9 | 62.5 | 62.6 | 178.1 | 177.8 | 191.0 | 191.8 | 358.2 | 358.3 | 56.1 | 56.0 | 159.0 | 159.1 |
| K (mg/L) | 2.5 | 2.4 | 1.9 | 2.1 | 2.7 | 2.4 | 55.3 | 55.2 | 62.2 | 62.3 | 102.2 | 102.1 | 18.0 | 18.1 | 35.2 | 35.1 |
| $NH_4$ (mg/L) | 84.1 | 84.5 | 60.1 | 60.4 | 43.6 | 43.4 | 0.1 | 0.1 | 4.0 | 3.9 | 3.5 | 3.5 | 0.1 | 0.1 | 0.8 | 0.8 |
| $PO_4$ (mg/L) | 7.1 | 7.2 | 2.1 | 2.2 | 4.0 | 3.9 | 1.1 | 1.2 | 1.1 | 1.1 | 0.7 | 0.8 | 1 | 1.1 | 0.6 | 0.6 |
| $SiO_2$ (mg/L) | 33.5 | 33.6 | 55.2 | 55.5 | 27.5 | 27.3 | 169.2 | 169.6 | 165.0 | 165.1 | 255.1 | 254.8 | 147.3 | 147.1 | 257.2 | 257.1 |
| pH | 6.86 | 6.85 | 6.98 | 6.98 | 7.26 | 7.26 | 7.30 | 7.31 | 7.25 | 7.27 | 7.23 | 7.23 | 7.26 | 7.24 | 7.16 | 7.19 |

remained unremoved in the solution after 0.5 min of incubation. After being incubated for 1 min, the complete removal of 10 mg/L arsenic from their supernatants was observed. Interestingly, while no arsenic was detected from the mixture containing 20 mg/L of As(V) after 1 min of incubation, 0.09 ± 0.11 mg/L of As(III) was detected from the supernatants of the same mixture. In fact, Ars-3 was able to remove more As(V) than As(III) under a given time for all concentrations. Therefore, when tubes loaded with 30 mg/L of each arsenic were incubated for 1 min, 6.13 ± 0.75 mg/L of As(V) and 6.42 ± 0.07 mg/L of As(III) were detected. These remaining concentrations of As(V) and As(III) were reduced to 0.85 ± 0.27 and 1.98 ± 0.15 mg/L, respectively, after 2 min incubation. Though arsenic levels were continuously monitored after 5 and 30 min of incubations, only small changes that fall within the corresponding standard deviations were made until the final incubation time of 60 min was reached.

**Arsenic Removal from Vietnamese Groundwater Using the Arsenic-Binding Aptamer Ars-3.** The arsenic removal ability of the arsenic-binding aptamer Ars-3 was evaluated with Vietnamese groundwater samples collected from private tubewells of various areas in Vietnam. These locations include three villages in Ha Nam province located near the Chau Giang River (the unconfined sub-brand of the Red River) in northern Vietnam and five more locations near the Mekong River Delta in southern Vietnam. Previous reports of significant arsenic contamination and the adverse effects of arsenic exposure on human health in those areas (*9, 10*) contributed to the selection. The arsenic contamination found in the groundwater of VT, BD, and HH (all in Ha Nam province) were 365.8, 376.7, and 287.6 $\mu$g/L, respectively. As compared to Ha Nam province, even higher arsenic concentrations were found in the areas close to the Mekong River: MR1, MR2, and MR3. The corresponding arsenic levels in those Mekong River areas were as high as 739.2 $\mu$g/L in MR2, followed by 552.1 and 476.0 $\mu$g/L in MR1 and MR3, respectively. Interestingly, the arsenic contamination found in the remaining two target areas of Mekong River, MR4 and MR5, that are located further away from the Mekong River were at a significantly lower range (28.1 and 61.1 $\mu$g/L, respectively), yet still higher than the EPA's standard for arsenic level in drinking water. The removal condition for arsenic present in Vietnamese groundwater samples was limited to 5 min, yet the results were outstanding. After a 5 min incubation, the complete removal of arsenic was achieved by 2 mg of Ars-3 aptamer with arsenic concentrations ranging from 28.1 $\mu$g/L in MR4 to 739.2 $\mu$g/L in MR2. Other than arsenic, the groundwater from these studied areas was also contaminated by iron, manganese, and ammonium. All groundwater samples were neutral in pH, ranging between 6.86 and 7.30. After the incubation with the arsenic-binding aptamer, other parameters remained unchanged as the Ars-3 aptamer is designed to target arsenic specifically. This is a great advantage over other removal techniques, of which their removal efficiencies are reduced by many interfering factors such as other ions. For instance, phosphate interference of the passive removal method reported by Robert et al. (*27*) can be avoided with the DNA aptamer. The pH of raw groundwater also stayed neutral after the removal of arsenic. The chemical composition, arsenic concentrations, and pH

of all groundwater samples before and after the removal test are summarized in Table 2. As aptamers can be engineered chemically without altering its existing properties even for multiple targets, the studied DNA aptamer can be extended as an emerging biosensor and removal agents. For cost efficiency, the current DNA aptamer can be prepared alternatively with silicates and silicon that are readily available. Continuous developments in techniques that can minimize the accumulation of hazardous substances are essential.

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## Supporting Information Available

Four figures, two tables, and detailed procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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