Title	Development of a simple analytical method to determine arsenite using a DNA aptamer and gold nanoparticles
Author(s)	Matsunaga, Koji; Okuyama, Yu; Hirano, Reiko; Okabe, Satoshi; Takahashi, Masahiro; Satoh, Hisashi
Citation	Chemosphere, 224, 538-543 https://doi.org/10.1016/j.chemosphere.2019.02.182
Issue Date	2019-06
Doc URL	http://hdl.handle.net/2115/81665
Rights	©2019. This manuscript version is made available under the CC-BY-NC-ND 4.0 license
Rights(URL)	https://creativecommons.org/licenses/by-nc-nd/4.0/
Type	article (author version)
File Information	Development of a Simple Analytical Method to Determine Arsenite Using a DNA 3 Aptamer and Gold Nanoparticles.pdf

[Instructions for use](#)

For submission to Chemosphere as a Research Paper

**Development of a Simple Analytical Method to Determine Arsenite Using a DNA
Aptamer and Gold Nanoparticles**

Koji Matsunaga^a, Yu Okuyama^b, Reiko Hirano^c, Satoshi Okabe^a, Masahiro Takahashi^a,
Hisashi Satoh^{a,*}

^a Division of Environmental Engineering, Faculty of Engineering, Hokkaido University,
North-13, West-8, Sapporo 060-8628, Japan

^b Douro Kensetsu K.K., New Hokkaido Building 12th Floor, 3-1 North-7, West-4,
Sapporo 060-7260, Japan

^c Cellspect Co., Ltd., 1-10-82 Kitaiioka, Morioka, Iwate 020-0857, Japan

E-mail addresses:

Koji Matsunaga: k-matsunaga_7dnd@eis.hokudai.ac.jp

Yu Okuyama: yu_okuyama@douken.co.jp

Reiko Hirano: hirano0317@gmail.com

Satoshi Okabe: sokabe@eng.hokudai.ac.jp

Masahiro Takahashi: m-takaha@eng.hokudai.ac.jp

Hisashi Satoh: qsatoh@eng.hokudai.ac.jp

** Corresponding author: Hisashi Satoh*

Tel. & Fax: +81-11-706-6277

E-mail: qsatoh@eng.hokudai.ac.jp

Abstract

A simple analytical method was developed to determine the arsenite (As(III)) concentration using a DNA aptamer and gold nanoparticles (AuNPs). Prior to sample measurements, the method sensing mechanism was confirmed by analyzing the particle size of the AuNPs at each step of the analysis procedure, and the key operational parameters that affect the method performance were optimized. The optimal final NaCl concentration, incubation time with NaCl and pH of a 3-(N-morpholino) propanesulfonic acid buffer were 60 mM, 10 min and 7.3, respectively. A calibration curve was created under optimized operational conditions. The calibration curve was linear from a 1.0- to 10- μ M As(III) concentration. The detection limit was 2.1 μ M (161 μ g/L). Using the calibration curve, we evaluated groundwater samples spiked with As(III). As(III) concentrations in groundwater pretreated with a 0.2- μ m-pore-size membrane filter and cation-exchange resin were determined by using the method, which suggests that the proposed method can be used to determine the As(III) concentration in groundwater.

Keywords: DNA aptamer; Arsenite; Simple analytical method; Groundwater

1. Introduction

Arsenic (As) contamination of drinking water poses a serious threat to public health (Flora, 2015). Symptoms of As poisoning include cardiovascular respiratory diseases, and cancers of the skin, lung, liver and kidney (Chung et al., 2013; Flora, 2015; Singh et al., 2015). Because of the high toxicity of As, the World Health Organization (WHO) and the US Environmental Protection Agency (USEPA) have set a primary maximum contaminant level for the total As in drinking water at as low as 10 µg/L (USEPA, 2018; WHO, 2011). One of the major sources of drinking water is groundwater. As contamination of groundwater is a global problem because As is a naturally occurring element from natural and anthropogenic sources (Mandal and Suzuki, 2002). For instance, the percentage of groundwater samples from tube wells with an As concentration above 100 µg/L exceeded 40% in Bangladesh (Chowdhury et al., 2000). Therefore, a measurement of the As concentration in groundwater in the sub-mg/L range is essential. As is determined mainly by atomic absorption spectrometry (AAS), atomic fluorescence spectrometry and inductively coupled plasma mass spectrometry (ICP-MS). These techniques have an excellent accuracy and sensitivity (Ma et al., 2014). However, they require sophisticated, expensive and bulky equipment, specialized expertise for operation,

have a high operating cost, and they are not suitable for on-site analysis (Kaur et al., 2015; Wu et al., 2012a). Moreover, they are not able to determine arsenite (As(III)) selectively, which is known to be more harmful than other As species (e.g., arsenate (As(V)) and organic As compounds) (Jena and Raj, 2008; Zhan et al., 2014). Therefore, there is a need for a simple, low-cost, on-site technology to analyze As(III) (Kaur et al., 2015).

Some biosensors to determine As have been reported owing to their advantages, such as a high sensitivity, specificity, simplicity, low manufacturing cost, rapid response time, ease of use and portability (Kaur et al., 2015). Recombinant whole cells, oligonucleotides and proteins are used as recognition elements in biosensors for As detection (Kaur et al., 2015). Among them, a DNA aptamer for As(III), which is termed Ars-3 (Kim et al., 2009), has attracted the attention of many scientists because of its high affinity and specificity to As(III). Kaur et al. suggested that adenine and guanine in Ars-3 contributed to specific binding of Ars-3 to As(III) via directed unsaturated hydrogen bonding with the nucleotide bases and their self-assembly-induced recognition behavior (Kaur et al., 2015). Thus far, colorimetric and electrochemical biosensors for As(III) that use Ars-3 have been developed (Cui et al., 2016; Wu et al., 2013, 2012a, 2012b). However, none of these studies measures groundwater samples. Previous studies have reported that false negative and/or false positive results occurred when using commercial kit assays of As because of

interference from other metal ions in environmental samples (Sankararamakrishnan et al., 2008; Van Geen et al., 2005). Therefore, a biosensor that is based on Ars-3 needs to be applied to groundwater and its applicability should be confirmed.

In this study, a simple analytical method to determine As(III) using Ars-3 and gold nanoparticles (AuNPs) has been developed to determine the As(III) concentration in groundwater. Scheme S1 shows the sensing mechanism of the method. In the absence of As(III), Ars-3 is adsorbed onto the AuNPs surface through a DNA base–gold interaction. The AuNPs that are covered with a negatively charged Ars-3 aptamer remain dispersed in the solution even at a high NaCl concentration. The dispersed AuNPs are red. In contrast, the Ars-3 forms an aptamer–As(III) complex in the presence of As(III). AuNPs that are added to the solution are no longer covered with Ars-3 and therefore are subjected to NaCl-induced aggregation. The aggregation of AuNPs leads to a color change of AuNPs from red to blue. Therefore, a quantitative analysis of the As(III) concentration is possible by measuring the ratio of absorbance that corresponds to the red (520 nm) and blue (600 nm) colors (*i.e.*, A_{600}/A_{520}). We investigated the key operational parameters (*i.e.*, final NaCl concentration, incubation time with NaCl and buffer-solution pH) to optimize the operational conditions. The As(III) concentrations in real groundwater samples were determined under optimized conditions.

2. Materials and Methods

2.1 Chemicals and Materials

The Ars-3 aptamer (Kim et al., 2009) with a sequence
5'-GGTAATACGACTCACTATAGGGAGATACCAGCTTATTCAATTTTACAGAAC
AACCAACGTCGCTCCGGGTACTTCTTCATCGAGATAGTAAGTGCAATCT-3'
was from Eurofins Genomics K.K. (Tokyo, Japan). 3-(N-morpholino) propanesulfonic
acid (MOPS) was from Dojindo Laboratories Co., Ltd (Kumamoto, Japan). The Ars-3
was dissolved in a 10-mM MOPS buffer solution of pH 7.3. AuNPs (10 nm) in phosphate
buffer solution (catalogue number 752584) were from Sigma-Aldrich Japan (Tokyo,
Japan). As(III) (NaAsO₂, catalogue number 35000, Fluka) and NaCl were from Fujifilm
Wako Pure Chemical Corporation (Osaka, Japan). All solutions were prepared with
Milli-Q Water (Merck Millipore, Tokyo, Japan). As(III) standard solutions were prepared
by diluting NaAsO₂ with Milli-Q water and used within 6 hours.

2.2 Determination of As(III) using Ars-3

122

123 A probe solution was prepared by adding 5 μL of Ars-3 solution (200 nM) to 33 μL of the
124 10-mM MOPS buffer solution in a microtube. A 20- μL sample solution was mixed with
125 the probe solution. After incubation of the mixture for 15 min at room temperature, 40 μL
126 of the AuNPs solution was added to the mixture and incubated for 40 min at room
127 temperature. Finally, 2 μL of the NaCl stock solution (3 M) was added to the mixture and
128 incubated for 10 min. The absorption spectrum of 100 μL of a test solution was measured.
129 The final concentration of the Ars-3 in a test solution was 10 nM. Unless otherwise
130 mentioned, this measurement condition was used.

131 We examined the effects of NaCl concentration, incubation time with NaCl and MOPS
132 buffer-solution pH in a test solution on the method sensitivity. To optimize the NaCl
133 concentration, the final NaCl concentration of the test solutions was changed from 0 to
134 120 mM. To optimize the incubation time with NaCl, two test solutions at 0 μM and 100
135 μM of As(III) were incubated for 30 min after adding NaCl, and the absorbance spectra
136 were measured every 5 min. To optimize the MOPS buffer-solution pH, the absorbance
137 spectra of the test solutions with different As(III) concentrations at different pHs were
138 measured and As(III) calibration curves were created.

139 After optimization of the parameters described above, we created a calibration curve for

the method at a variety of As(III) concentrations. The absorbance spectra of ten blank samples (Milli-Q water) and three samples of As(III) standard solution at individual As(III) concentrations were measured. The ratio of the absorbance peaks at 520 nm and 600 nm (A_{600}/A_{520}) was plotted against As(III) concentration. A calibration curve was obtained by fitting a logistic equation to the plots. A linear regression was also used to obtain a calibration curve at As(III) concentrations below 10 μM . Based on the results, the limit of detection (LOD) value was estimated using an equation, $3\sigma/s$, where σ is the standard deviation of ten blank samples and s is a slope of the linear regression line.

To test the selectivity of the method against As(III) for the environmentally important ions, the absorbance spectra of the test solutions with H_3AsO_4 , HgCl_2 , $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, CaCl_2 , $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, ZnCl_2 , $(\text{CHCOO})_2\text{Pb} \cdot 3\text{H}_2\text{O}$, NaCl and AgNO_3 were determined at 6 μM .

Groundwater samples were taken at our campus in November 2016 (Groundwater-1) and December 2016 (Groundwater-2). Because no As was present in the samples, the samples were spiked with As(III) of different concentrations. The samples were filtered through a 0.2- μm pore-size membrane (Advantec Co., Ltd., Japan) and then passed through a cation-exchange column (MetaSEP IC-ME, GL Sciences, Tokyo, Japan) or ethylenediaminetetraacetic acid (EDTA) was added to the samples to stabilize the

cationic metal ions. After pretreatment, the As(III) concentrations were determined by using the method and compared with the known As(III) concentrations with which the samples had been spiked. Concentrations of metal ions in the groundwater samples before and after cation-exchange treatment are shown in Table S1. The recovery efficiency of As(III) in cation-exchange treatment was 94%.

2.3 Instrumentation

The absorption spectra were measured by using a spectrophotometer V-630 (JASCO Corporation, Tokyo, Japan). The AuNP particle size and its distribution in a test solution under a variety of measurement conditions was measured by dynamic light scattering (DLS) using a ZETASIZER NANO ZS90 (Malvern Panalytical Ltd, United Kingdom). The metal-ion concentrations were measured by ICP-MS 8800 ICP-QQQ (Agilent, United States).

3. Results and Discussion

3.1 Confirmation of Sensing Mechanism by Analyzing Size Distribution and Absorbance

Spectra of AuNPs

The first step to confirming the As(III) detection mechanism described above was to determine the AuNP size after incubation with and without Ars-3 and NaCl and to compare the resulting AuNP size distribution. Figure 1 shows the number-weighted particle-size distribution of AuNPs in 10 mM MOPS buffer determined by DLS. The scattered-light-intensity-weighted-particle-size distribution of AuNPs in each sample determined by DLS is shown in Fig. S1 of the Supporting Information. The hydrodynamic diameter (D_h) of the bare 10-nm AuNPs was 10.1 nm. The mixture of AuNPs and Ars-3 exhibited a monomodal peak at 10.1 nm. The D_h was shifted to 18.2 nm by NaCl addition to the mixture. The D_h of the mixture of AuNPs and Ars-3 with As(III) was 8.7 nm. This result indicates that As(III) did not affect the particle-size distribution of a mixture of AuNPs and Ars-3. NaCl addition to the mixture caused the D_h shift to 164 nm. The scattered-light-intensity-weighted-particle-size distribution of the AuNPs in the solutions shows a similar trend to that of the number-weighted particle-size distribution (Fig. S1). The addition of Ars-3 and As(III) did not have a significant effect on the particle-size distribution of the AuNPs, NaCl-aggregated AuNPs and the presence of As(III) stimulated AuNP aggregation by NaCl.

It is well known that the ultraviolet-visible (UV-Vis) spectrum of a AuNP solution shows a strong absorption band in the visible region (500 nm–600 nm) because localized surface-plasmon resonances and aggregation states of the AuNPs can be detected by observing a spectrum, because AuNP aggregation results in a red-shift in the spectrum. Figure 2 shows the absorbance spectra of a colloidal solution of AuNPs dispersed in 10 mM MOPS buffer (pH 7.3). The typical absorbance spectrum of a mixture of AuNPs and Ars-3 without As(III) and NaCl showed a maximum peak at 520 nm, which was identical to that of the bare AuNPs, and which indicates that Ars-3 addition did not affect the AuNP size. NaCl addition to the mixture resulted in a slight decrease in absorbance at 520 nm, but no absorbance peak shift was observed. As(III) was mixed with Ars-3 before AuNP addition. As(III) addition to the AuNPs and Ars-3 mixture did not affect the absorption spectrum. In contrast, NaCl addition to the mixture made a significant change to the spectrum; the absorption peak intensity around 520 nm decreased and the peak intensity around 600 nm increased, which shows that the presence of As(III) stimulated AuNP aggregation by NaCl. These results agree well with those obtained by DLS measurements. Based on the results described above, we concluded that the sensing mechanism described above (Scheme S1) was correct and that the ratio of absorbance of a mixture at 520 nm and 600 nm (A_{600}/A_{520}) could be used as a sensor output signal.

212

213 *3.2 Effect of NaCl Concentration*

214

215 Because the NaCl concentration is higher than the Ars-3 and AuNP concentrations in the
216 above tests, and they modulate the aggregation degree of the AuNPs, the effect of final
217 NaCl concentration on the absorbance spectra was investigated. Figure 3 shows the effect
218 of final NaCl concentration on the A600/A520 of the test solutions with and without
219 As(III). The incubation time after NaCl addition was 20 min. In the absence of NaCl, the
220 A600/A520 of the test solutions with and without As(III) was approximately 0.4. With
221 NaCl addition to the test solutions at a final NaCl concentration of 30 mM and higher, the
222 A600/A520 of the test solutions with 100 μ M As(III) increased significantly to
223 approximately 1.3. In contrast, the A600/A520 of the test solutions without As(III)
224 increased slightly when the final NaCl concentration increased to 120 mM. This result
225 confirms the sensing mechanism of the analytical method in Scheme S1. The difference
226 between the A600/A520 with and without As(III) was ~0.80, 0.85 and 0.77 at 30 mM, 60
227 mM and 90 mM NaCl, respectively. The difference in A600/A520 decreased to 0.64 at 90
228 mM NaCl because the higher NaCl concentration led to an increase in the A600/A520 of
229 the test solutions by AuNP aggregation even without As(III). Because the difference in

A600/A520 with and without As(III) indicated the sensitivity of the method, the final NaCl concentration of a test solution was determined to be 60 mM. This concentration fell into a NaCl range of 50 mM to 70 mM to aggregate AuNPs with an average particle diameter of 15 nm (Wu et al., 2012a), but was lower than 120 mM of the optimal final NaCl concentration to aggregate AuNPs with a diameter below 15 nm (Zhan et al., 2014). The optimal final NaCl concentration may depend on AuNP size, buffer-solution type and/or incubation time.

3.3 Effect of Incubation Time with NaCl

Figure S2 shows the change in A600/A520 of the test solutions with time with and without As(III) after NaCl addition. The A600/A520 of the test solutions with As(III) increased significantly immediately after NaCl addition and stabilized at 10 min. In contrast, the A600/A520 of the test solutions without As(III) did not increase with NaCl addition. Based on these results, the incubation time after NaCl addition was determined to be 10 min.

3.4 Effect of Buffer-Solution pH

248

249 Figure 4 shows the effect of pH of the MOPS buffer solution on the calibration-curve
250 slope, *i.e.*, the method sensitivity. The slope of the calibration curve at pH 6.5 was $2.5 \times$
251 $10^{-2} \mu\text{M}^{-1}$. As the pH increased from 6.5 to 7.3, the slope increased and was highest ($8.2 \times$
252 $10^{-2} \mu\text{M}^{-1}$) at pH 7.3. A further increase in pH led to decrease in the slope of the
253 calibration curves. Therefore, the MOPS buffer pH for the highest method sensitivity was
254 determined to be 7.3.

255

256 3.5 Calibration Curve

257

258 Figure 5 shows a typical calibration curve for As(III) for the method. The A600/A520 of
259 the test solution without As(III) was 0.40. The A600/A520 remained unchanged below 1
260 μM As(III) and increased linearly from 0.41 to 1.0 for a 1.0- to 10- μM As(III)
261 concentration. When the As(III) concentration exceeded 10 μM , the A600/A520
262 increased further and remained almost unchanged at 100 μM As(III) (Fig. S3). The LOD
263 was calculated to be 2.1 μM (161 $\mu\text{g/L}$).

264

265 3.6 Method Selectivity

266

267 The A600/A520 of the test solutions supplied with As(III) or the environmentally
268 important metal ions is shown in Figure 6. The A600/A520 of the solution with As(III)
269 was significantly higher than that of the blank sample. The A600/A520 of the solutions
270 with 13 other different metal ions were almost identical to that of the blank sample. These
271 results show a high selectivity of the method toward As(III), and even against As(V).

272

273 *3.7 Analysis of Environmental Water Samples*

274

275 When a groundwater sample with no As(III) was subjected to the method, the AuNPs
276 aggregated significantly (Fig. S5). The aggregation may be attributed to aggregation of
277 the AuNPs by metal cations in the sample (Table S1). Therefore, the groundwater sample
278 was pretreated with a 0.2- μ m-pore-size membrane filter and cation-exchange resin.
279 Membrane filtration did not avoid the interfering effect of groundwater (Fig. S4). In
280 contrast, the absorption spectrum of the sample that was subjected to filtration followed
281 by cation exchange overlapped with that of a probe solution (Fig. S4), which indicates
282 that the filtration followed by cation exchange removed the interfering metal cations, and
283 uncharged or negatively charged As(III) remained in a sample as described below. Table

S1 shows the metal-ion concentrations in the groundwater samples before and after cation-exchange treatment. The raw groundwater samples contained divalent cations (*i.e.*, Mg and Ca), which may have resulted in AuNP aggregation. The results show a reduction in metal-cation concentrations in groundwater samples by cation exchange and an increase in Na-ion concentration. However, As(III) concentration was decreased approximately 10% after cation-exchange treatment when the groundwater including 10 μ M of As(III) treated (data not shown).

The addition of EDTA was examined as a pretreatment to mask metal cations. EDTA (10 nM) addition to the raw groundwater sample prevented AuNP aggregation in As(III)-free groundwater and in groundwater with As(III) (data not shown). Therefore, EDTA was unsuitable as a masking agent in this method, possibly because of the protection of the AuNP surface with EDTA.

Figure 7 shows the relationship between the concentrations of groundwater samples spiked with As(III) and those determined by the method. Because As(III) was not present in all samples, sample were spiked with As(III) with a variety of concentrations.

Groundwater samples with an As(III) concentration above 1.0 μ M yielded As(III) concentrations as determined by the method that were almost identical to those spiked solutions, although the As(III) concentrations were overestimated by the method below

1.0- μ M As(III). Thus, it could be concluded that the method could be applied to a simple determination of As(III) concentrations in groundwater samples greater than 1.0 μ M. Zhan *et al.* determined the As(III) concentrations in water samples using Ars-3 and AuNPs (Zhan et al., 2014). The detection limit of their method was lower (0.017 μ M) than that of our method. However, they could not overcome a problem with interfering ions at high concentrations. Wu *et al.* developed a colorimetric biosensor using Ars-3, AuNPs, a cationic polymer (Wu et al., 2012b) and a cationic surfactant (Wu et al., 2012a) to aggregate AuNPs instead of NaCl. Song *et al.* developed a novel biosensor based on Au@Ag core-shell nanoparticles (Song et al., 2016). The detection limit of their method was lower than that of our method. However, they did not determine As(III) concentrations in environmental samples. These results from previous studies on As(III) determination using Ars-3 indicate that the use of Ars-3 and AuNPs allows us to determine As(III) concentrations in water samples, however, it cannot be applied to water samples that contain metal cations at relatively high concentrations, such as in groundwater. We determined As(III) concentrations in groundwater samples using Ars-3 and AuNPs combined with pretreatment by cation extraction.

4. Conclusions

320

321 We developed a simple analytical method to determine As(III) concentrations in
322 groundwater using a DNA aptamer and AuNPs. The parameters that affect the sensitivity
323 of the method, such as the final concentration of and incubation time with NaCl and the
324 buffer solution pH, were optimized. The total analysis time was 65 min and the LOD was
325 161 µg/L. Pretreatment by cation extraction enabled a determination of As(III)
326 concentrations in groundwater that contained a variety of metal cations at high
327 concentration. As(III) concentrations in groundwater with a variety of metal cations at
328 high concentration could be determined for the first time using Ars-3.

329

330 **Acknowledgements**

331

332 This research was supported financially by JSPS KAKENHI [grant numbers
333 JP17H03328 and 26289178], and by JFE 21st Century Foundation. This work was
334 supported by Platform for Drug Discovery, Informatics, and Structural Life Science from
335 the Ministry of Education, Culture, Sports, Science and Technology, Japan. We would
336 like to thank OPEN FACILITY (Hokkaido University Sousei Hall) for allowing us to use
337 the 8800 ICP-QQQ to determine the cadmium concentrations.

338

339 **Declarations of interest**

340

341 None.

342

343 **References**

344

345 Chowdhury, U.K., Biswas, B.K., Chowdhury, T.R., Samanta, G., Mandal, B.K., Basu,
346 G.C., Chanda, C.R., Lodh, D., Saha, K.C., Mukherjee, S.K., Roy, S., Kabir, S.,
347 Quamruzzaman, Q., Chakraborti, D., 2000. Groundwater arsenic contamination in
348 Bangladesh and West Bengal, India. *Environ. Health Perspect.* 108, 393–397.
349 <https://doi.org/10.2307/3454378>

350 Chung, C.J., Huang, Y.L., Huang, Y.K., Wu, M.M., Chen, S.Y., Hsueh, Y.M., Chen,
351 C.J., 2013. Urinary arsenic profiles and the risks of cancer mortality: A
352 population-based 20-year follow-up study in arseniasis-endemic areas in Taiwan.
353 *Environ. Res.* 122, 25–30. <https://doi.org/10.1016/j.envres.2012.11.007>

354 Cui, L., Wu, J., Ju, H., 2016. Label-free signal-on aptasensor for sensitive
355 electrochemical detection of arsenite. *Biosens. Bioelectron.* 79, 861–865.

356 <https://doi.org/10.1016/j.bios.2016.01.010>

357 Flora, S.J.S., 2015. Handbook of Arsenic Toxicology, 1st ed. © Academic Press 2015.

358 Jena, B.K., Raj, C.R., 2008. Gold nanoelectrode ensembles for the simultaneous

359 electrochemical detection of ultratrace arsenic, mercury, and copper. *Anal. Chem.*

360 80, 4836–4844. <https://doi.org/10.1021/ac071064w>

361 Kaur, H., Kumar, R., Babu, J.N., Mittal, S., 2015. Advances in arsenic biosensor

362 development - A comprehensive review. *Biosens. Bioelectron.* 63, 533–545.

363 <https://doi.org/10.1016/j.bios.2014.08.003>

364 Kim, M., Um, H.J., Bang, S., Lee, S.H., Oh, S.J., Han, J.H., Kim, K.W., Min, J., Kim,

365 Y.H., 2009. Arsenic removal from Vietnamese groundwater using the

366 arsenic-binding DNA aptamer. *Environ. Sci. Technol.* 43, 9335–9340.

367 <https://doi.org/10.1021/es902407g>

368 Ma, J., Sengupta, M.K., Yuan, D., Dasgupta, P.K., 2014. Speciation and detection of

369 arsenic in aqueous samples: A review of recent progress in non-atomic

370 spectrometric methods. *Anal. Chim. Acta* 831, 1–23.

371 <https://doi.org/10.1016/j.aca.2014.04.029>

372 Mandal, B.K., Suzuki, K.T., 2002. Arsenic round the world: A review. *Talanta* 58, 201–

373 235. [https://doi.org/10.1016/S0039-9140\(02\)00268-0](https://doi.org/10.1016/S0039-9140(02)00268-0)

374 Sankararamakrishnan, N., Chauhan, D., Nickson, R.T., Tripathi, R.M., Iyengar, L.,

375 2008. Evaluation of two commercial field test kits used for screening of

376 groundwater for arsenic in Northern India. *Sci. Total Environ.* 401, 162–167.

377 <https://doi.org/10.1016/j.scitotenv.2008.03.042>

378 Singh, R., Singh, S., Parihar, P., Singh, V.P., Prasad, S.M., 2015. Arsenic contamination,

379 consequences and remediation techniques: A review. *Ecotoxicol. Environ. Saf.* 112,

380 247–270. <https://doi.org/10.1016/j.ecoenv.2014.10.009>

381 Song, L., Mao, K., Zhou, X., Hu, J., 2016. A novel biosensor based on Au@Ag

382 core-shell nanoparticles for SERS detection of arsenic (III). *Talanta* 146, 285–290.

383 <https://doi.org/10.1016/j.talanta.2015.08.052>

384 USEPA, 2018. National Primary Drinking Water Regulations [WWW Document]. URL

385 [https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-](https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations)

386 [water-regulations](https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations)

387 Van Geen, A., Cheng, Z., Seddique, A.A., Hoque, M.A., Gelman, A., Graziano, J.H.,

388 Ahsan, H., Parvez, F., Ahmed, K.M., 2005. Reliability of a commercial kit to test

389 groundwater for arsenic in Bangladesh. *Environ. Sci. Technol.* 39, 299–303.

390 <https://doi.org/10.1021/es0491073>

391 WHO, 2011. Guidelines for Drinking-Water Quality - Fourth Edition.

392 Wu, Y., Liu, L., Zhan, S., Wang, F., Zhou, P., 2012a. Ultrasensitive aptamer biosensor
393 for arsenic(iii) detection in aqueous solution based on surfactant-induced
394 aggregation of gold nanoparticles. *Analyst* 137, 4171–4178.
395 <https://doi.org/10.1039/c2an35711a>

396 Wu, Y., Wang, F., Zhan, S., Liu, L., Luo, Y., Zhou, P., 2013. Regulation of hemin
397 peroxidase catalytic activity by arsenic-binding aptamers for the colorimetric
398 detection of arsenic(III). *RSC Adv.* 3, 25614–25619.
399 <https://doi.org/10.1039/c3ra44346a>

400 Wu, Y., Zhan, S., Wang, F., He, L., Zhi, W., Zhou, P., 2012b. Cationic polymers and
401 aptamers mediated aggregation of gold nanoparticles for the colorimetric detection
402 of arsenic(iii) in aqueous solution. *Chem. Commun.* 48, 4459–4461.
403 <https://doi.org/10.1039/c2cc30384a>

404 Zhan, S., Yu, C.M., Lv, C.J., 2014. Colorimetric Detection of Trace Arsenic (III) in
405 Aqueous Solution Using Arsenic Aptamer and Gold Nanoparticles. *Aust. J. Chem.*
406 67, 813–818. <https://doi.org/10.1071/CH13512>

Fig. 1 Number-weighted particle-size distributions of AuNPs in 10 mM MOPS buffer determined by DLS. (i) AuNP solution, (ii) AuNPs incubated with 10 nM of Ars-3, (iii) AuNPs incubated with 10 nM of Ars-3 and 60 mM of NaCl, (iv) AuNPs incubated with 10 nM of Ars-3 and 100 μ M of As(III), and (v) AuNPs incubated with 10 nM of Ars-3, 100 μ M of As(III) and 60 mM of NaCl.

415

416 Fig. 2 Absorption spectra of AuNPs in solutions incubated with and without 10 nM of

417 Ars-3, 100 μ M of As(III) and 60 mM of NaCl. Figure legends ((i)–(v)) are identical to

418 those in Fig. 1.

419

Fig. 3 Effect of final NaCl concentration of test solutions on A600/A520 in the presence and absence of As(III).

Fig. 4 Effect of pH of MOPS buffer on calibration curves. Before conducting tests, the initial MOPS buffer-solution pH was adjusted to the value indicated on the right of each line.

429

430 Fig. 5 Method calibration curve. The dotted line is a regression line.

431

432

433 Fig. 6 Method selectivity. The concentration of each metal ion was 10 µM.

434

Fig. 7 Relationship between concentrations of As(III)-spiked groundwater samples and those determined by using the method.

442

443 Scheme S1 Schematic representation of the colorimetric detection of As(III) in aqueous solution based on an aptamer for As(III) (Ars-3)

444 and gold nanoparticles.

445

446

447

448 Table S1: Concentration of metal ions (μM) in groundwater samples. “Raw” and “Eluent” indicate the samples before and after cation-exchange treatment,
449 respectively. The metal-ion concentrations were measured by ICP-MS.

450

Metal ions	Groundwater-1		Groundwater-2	
	Raw	Eluent	Raw	Eluent
Na	1,562	4,741	1,087	1,579
Mg	572	0.66	601	0
Al	134	1.78	5.23	0
K	188	4.94	143	0
Ca	272	0.27	103	1.38
Fe	5.35	1.02	7.93	0
Ni	1.35	0.002	0	0
Cu	0	0.06	0.16	0.008

451

452
 453 Fig. S1 Scattered-light-intensity-weighted-particle-size distribution of AuNPs in 10 mM MOPS buffer determined by DLS. The legends ((i)–(v)) are
 454 identical to those in Fig. 1.

Fig. S2 Variation of A_{600}/A_{520} of test solutions with time after NaCl addition in the presence and absence of $As(III)$.

Fig. S3 Typical calibration curve of As(III) for the method in a wider range of As(III) concentrations. The dashed-line calibration curve was sigmoidal and the data plots were fitted with a logistic regression model with a determination coefficient of 0.99. The logistic regression equation is:

$$y = \frac{-0.74}{1 + \left(\frac{x}{15.4}\right)^{1.44}} + 1.43$$

$$R^2=0.99$$

Fig. S4: Absorbance spectra of AuNPs in (i) Milli-Q water, (ii) groundwater, (iii) groundwater filtered with 0.2- μ m-pore-size membrane filter, and (iv) groundwater filtered with 0.2- μ m-pore-size membrane filter and passed through cation-exchange resin.