



Title	Development of the simple analytical method for determination of arsenate(V) ion using fluorescence-labeled DNA and cerium oxide nanoparticles
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2

3 **Development of the simple analytical method for determination of Arsenate(V) ion**
4 **using fluorescence-labeled DNA and Cerium oxide nanoparticles**

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20

21 **Abstract**

22

23 Arsenic (As) contamination in groundwater presents a major health and
24 environmental concern. As is found in two oxidation states and most chemical tests for
25 inorganic arsenic are focused on As(III), and few have been developed for As(V). We
26 developed the simple analytical method for determining As(V) concentrations in
27 groundwater using CeO₂NPs and fluorescein (FAM)-labeled DNA. Prior to sample
28 measurements, we investigated the key operational parameters that affect the sensing
29 performance. The optimal CeO₂NPs final concentration, FAM-labeled DNA final
30 concentration, the sequence and length of FAM-labeled DNA, and incubation time were
31 15 µg/mL, 400 nM, 6-mer poly-cytosine sequence, and 6 min, respectively. After
32 optimizing the parameters, the total analysis time was about 20 min and the limit of
33 detection was 0.61 µM. This method has a high selectivity against the same
34 concentrations of Cu(II), Cd(II), Hg(II) and Pb(II). Pretreatment by cation extraction to
35 remove interfering ions was beneficial for determination of As(V) concentrations in
36 groundwater containing a variety of metal cations at high concentration. We could
37 determine As(V) concentration in groundwater. Modification of the reactions of the
38 method is necessary. This study provides the first step in the development of a simple

39 method for on-site As(V) analysis.

40

41 **Keywords:** Arsenate; Groundwater; Nanoparticles; Single-stranded DNA; Simple

42 analytical method

43

44 **Highlights**

45 ● Cerium oxide nanoparticles-based fluorescence method for As(V) determination was
46 developed.

47 ● Parameters that influence the method were optimized.

48 ● Most of groundwaters could determine As(V) concentrations roughly.

49

50 **Introduction**

51

52 Inorganic arsenic (As) is extremely toxic, leading to a serious threat including
53 cardiovascular, respiratory diseases, and cancers of skin, lung, liver and kidney (Chung,
54 Huang, et al., 2013; Singh, Singh, et al., 2015; Flora, 2015). Its contamination of drinking
55 water sources was estimated to affect human health over 144 million people around the
56 world (Clancy, Hayes, et al., 2013). Because of high toxicity of As, the World Health
57 Organization (WHO) and the US Environmental Protection Agency (USEPA) set the
58 primary maximum contaminant level (MCL) for total As in drinking water as low as 10
59 $\mu\text{g/L}$ (WHO, 2011; USEPA, 2018). Chowdhury *et al.* reported that more than 100 $\mu\text{g/L}$ -
60 As concentrations have been detected in about 47.9% of well water and the lower As
61 concentrations is less than 10 $\mu\text{g/L}$ and the upper As concentrations is higher than 1000
62 $\mu\text{g/L}$ in Bangladesh (Chowdhury, Biswas, et al., 2000). Amini *et al.* modeled probability
63 maps of global As contamination using a large database of measured As concentration in
64 groundwaters from around the world and the digital maps of physical characteristics such
65 as soil, geology, climate and elevation (Amini, Abbaspour, et al., 2008). They showed that
66 most of countries could contaminate As higher than 10 $\mu\text{g/L}$ (Amini, Abbaspour, et al.,
67 2008).

68 Some commercial instruments which are commonly used for As determination
69 include atomic absorption spectrometry (AAS), atomic fluorescence spectrometry and
70 inductively coupled plasma mass spectrometry (ICP-MS), hydride generation atomic
71 absorption spectrometry (HG-AAS), electrothermal atomic absorption spectrometry
72 (ETAAS), flow injection-hydride generation-inductively coupled plasma mass
73 spectrometry (FI-HG-ICPMS), anodic stripping voltammetry (ASV), cathodic stripping
74 voltammetry (CSV) using a hanging drop mercury electrode (Das and Sarkar, 2016).
75 Although these traditional techniques have excellent accuracy and sensitivity, they
76 require sophisticated, expensive and bulky equipment, specialized expertise for operation,
77 and high operating cost. Hence, they are not suitable to on-site analysis (Wu, Liu, et al.,
78 2012a; Kaur, Kumar, et al., 2015).

79 Inorganic As has two common oxidation states: arsenate (As(V)) and arsenite
80 (As(III)). Most of chemical and biological sensors have been developed to determine
81 As(III) (Baghbaderani and Noorbakhsh, 2019) or total As based on using DNA aptamer
82 (Matsunaga, Okuyama, et al., 2019; Zhan, Yu, et al., 2014; Wu, Liu, et al., 2012b) or the
83 redox properties and strong thiophilicity of As (Pena-Pereira, Villar-Blanco, et al., 2018;
84 Xu, Wang, et al., 2019). However, those assays could not determine As(V) alone although
85 some commercial kits are available for As detection. They rely on the reduction of As(III)

86 species in solution by zinc to form arsine gas (Lopez, Zhang, et al., 2017). While it is
87 more challenging to detect As(V) alone, there are a few studies to detect As(V) have also
88 been developed, which use polymer hydrogels, small molecules, gold nanoparticles, and
89 bimetallic NPs (Lopez, Zhang, et al., 2017).

90 Recently, there has been interests in using nanomaterials for analytical
91 applications. The nanomaterials in general have a high specific surface area and may offer
92 high sensitivity. One of the nanomaterials is metal oxide nanoparticles (MONPs). The
93 MONPs were carried out for their ability to adsorb DNA, quench fluorescence (Pautler,
94 Kelly, et al., 2013), and release DNA in the presence of target anions (Liu and Liu, 2015).
95 DNA-functionalized MONPs might be useful as a sensor platform for anion detection. In
96 previous studies, iron oxide nanoparticles ($\text{Fe}_3\text{O}_4\text{NPs}$) are used as the As(V) sensor
97 because As(V) binds to $\text{Fe}_3\text{O}_4\text{NPs}$ surface (Liu and Liu, 2014; Liu and Liu, 2015).
98 Another studies showed that cerium oxide nanoparticles (CeO_2NPs) were a general
99 oxidase that could oxidize many substrates (Pautler, Kelly, et al., 2013). CeO_2NP also
100 adsorbed As(V) on its surface and its DNA adsorption affinity was stronger than that of
101 $\text{Fe}_3\text{O}_4\text{NP}$ (Liu and Liu, 2015; Lopez, Zhang, et al., 2017; Bülbül, Hayat, et al., 2018).
102 However, their application for environmental monitoring has been extremely limited
103 because of the lack of selectivity (Lopez, Zhang, et al., 2017; Muppudathi, Perumal, et al.,

104 2019). Here, we developed a simple analytical method for determination of As(V)
105 concentrations in various kinds of groundwater by fluorescence spectroscopy.

106

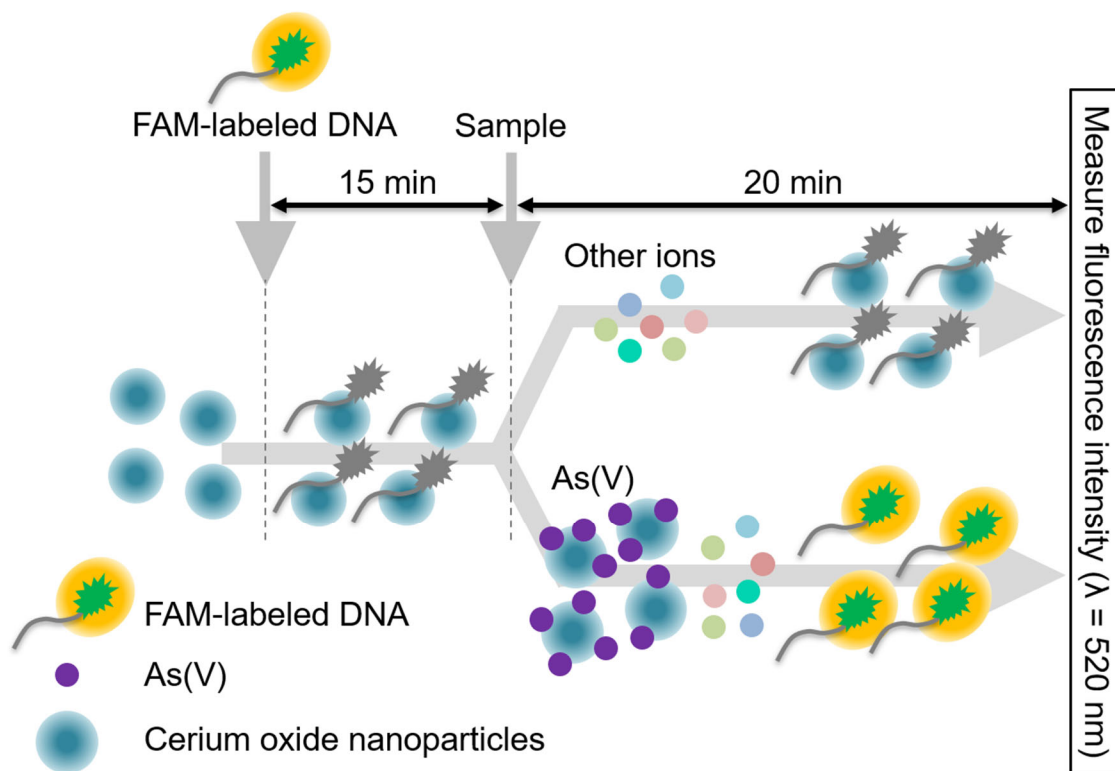
107 **Materials and methods**

108

109 *Principle*

110

111 Scheme 1 shows the sensing mechanism of the method. First, fluorescein (FAM)-
112 labeled DNA is incubated with CeO₂NPs. FAM-labeled DNA is adsorbed onto the
113 CeO₂NP surface and the fluorescence might be quenched. Second, samples are added into
114 the solution. In the absence of As(V), FAM-labeled DNA-CeO₂NPs complex remains in
115 the solution. In contrast, As(V) displaces the adsorbed FAM-labeled DNA from the
116 CeO₂NPs, resulting in recovery of fluorescence signal. Therefore, a quantitative analysis
117 of the As(V) concentration is possible by measuring the fluorescence intensity derived
118 from FAM (Ex: 495 nm, Em: 520 nm).



119

120 Scheme 1: Schematic representation of the colorimetric detection of As(V) in aqueous
 121 solution based on an FAM-labeled ssDNA and cerium oxide nanoparticles.

122

123 *Chemicals and materials*

124

125 All of the FAM-labeled DNA were from Eurofins Genomics K.K. (Tokyo, Japan).

126 Table 1 shows the list of sequence which were used in this study. 4-(2-hydroxyethyl)-1-

127 piperazineethanesulfonic acid (HEPES) was from Nakalai tesque, INC. (Kyoto, Japan).

128 CeO₂NPs as a 10 wt.% in H₂O (catalogue number 643009-100ML) was from Sigma-

129 Aldrich Japan (Tokyo, Japan). As(V) as a 60% arsenic acid solution (H₃AsO₄, catalog

130 number 013-04675) was from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan).

131 All solutions were prepared with Milli-Q Water (Merck Millipore, Tokyo, Japan).

132

133 Table 1. The DNA sequences which we used in this study.

DNA Name	Sequences
FAM-C ₆	5'-[FAM]-CCCCCC-3'
FAM-C ₁₂	5'-[FAM]-CCCCCCCCCCCC-3'
FAM-C ₁₈	5'-[FAM]-CCCCCCCCCCCCCCCCCCCC-3'
FAM-C ₂₄	5'-[FAM]-CCCCCCCCCCCCCCCCCCCCCCCCCCCC-3'
FAM-C ₃₀	5'-[FAM]-CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3'

134

135 *Determination of As(V) using CeO₂NPs and FAM-labeled ssDNA*

136

137 The probe solution was prepared by adding CeO₂NPs dispersion and FAM-

138 labeled DNA solution to the 10-mM HEPES buffer solution (pH: 7.6). After 15 minutes,

139 a 20-μL probe solution was added into the microtubes. A 20-μL sample solution was

140 mixed with the probe solution in the microtube. After incubation of the mixture at room

141 temperature for 20 min, the fluorescence intensity at 518 nm in the test solution was

142 measured.

143 We examined the effects of the concentrations of CeO₂NPs and FAM-labeled
144 ssDNA, the length of FAM-labeled ssDNA and the incubation time with samples on the
145 method sensitivity. To optimize the concentration of CeO₂NPs and FAM-labeled ssDNA,
146 the final CeO₂NPs concentrations of the test solutions were changed from 0 to 60 µg/mL
147 and the final FAM-labeled ssDNA concentrations were changed from 0 to 500 nM after
148 the optimization of final CeO₂NPs concentration. We compared the fluorescence intensity
149 at 518 nm of the sample with 1-µM As(V) (POS) and one without As(V) (NEG). To
150 optimize the length of the DNA, FAM-labeled poly-cytosine DNAs (Table 1) were used
151 to study As(V)-induced DNA detachment reaction. All of the data were used to calculate
152 ΔF , which is the difference between the fluorescence intensity of the POS and the NEG
153 samples. To optimize the incubation time after adding the samples, three test solutions of
154 the POS and the NEG samples, respectively, were incubated for 30 min after adding the
155 sample, and the fluorescence intensities were measured every 6 min and ΔF were
156 calculated.

157 After optimization of the parameters described above, we created a calibration
158 curve of the method at a variety of As(V) concentrations. The fluorescence spectra of ten
159 blank samples (using Milli-Q water as the samples) and three samples of As(V) standard

160 solution at individual As(V) concentrations were measured. The fluorescence peaks at
161 518 nm were plotted against the corresponding As(V) concentrations. A linear regression
162 was also used to obtain a calibration curve at As(V) concentrations above 0.5 μM . Based
163 on the results, the limit of detection (LOD) value was estimated using an equation, $3\sigma/s$,
164 where σ is the standard deviation of ten blank samples and s is a slope of the regression
165 line.

166 Selectivity of the method was assessed by measuring the fluorescence intensities
167 of the test solutions with H_3AsO_4 , NaAsO_2 , H_3BO_3 , NaHCO_3 , Na_2CO_3 , NaNO_3 , NaF ,
168 K_2HPO_4 , Na_2SO_3 , Na_2SO_4 , Na_2SeO_4 , KBr and KI at 10 μM , which were against As(V)
169 for the environmentally important anions. For the cations, we used NaCl , $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$,
170 KCl , CaCl_2 , $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, CoCl_2 , $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$,
171 ZnCl_2 , HgCl_2 and $(\text{CHCOO})_2\text{Pb} \cdot 3\text{H}_2\text{O}$ as the selectivity test. ΔF were compared among
172 these samples by Steel test.

173 Groundwater samples (GW) were taken in February 2019. Since the GW did not
174 contain As, As(V) was spiked to the GW at different concentrations for determination
175 tests. The samples were filtered through a 0.2- μm pore-size membrane (Advantec Co.,
176 Ltd., Japan) and then passed through a cation-exchange column (MetaSEP IC-MC, GL
177 Sciences, Tokyo, Japan). Thereafter, As(V) concentrations were determined by using our

178 method and ICP-MS and HPLC-ICP-MS and both were compared.

179

180 *Instrumentation and software*

181

182 The fluorescence intensity was measured by using a fluorescence
183 spectrophotometer FP-6600 (JASCO Corporation, Tokyo, Japan). The metal-ion
184 concentrations in groundwater were measured by ICP-MS 8800 ICP-QQQ (Agilent,
185 United States) and HPLC-ICP-MS system, which were passed through a GelPack GL-
186 IC-A column (Hitachi Chemical) connected to a high performance liquid chromatograph
187 (Shimadzu, SLC-10Avp system), and introduced to an inductively coupled plasma mass
188 spectrometer (ICP-MS, Thermo, iCAP Qc) (Kamei-Ishikawa, Segawa, et al., 2017). R
189 version 3.5.2 was used for the statistical analysis in this study.

190

191 **Results and Discussion**

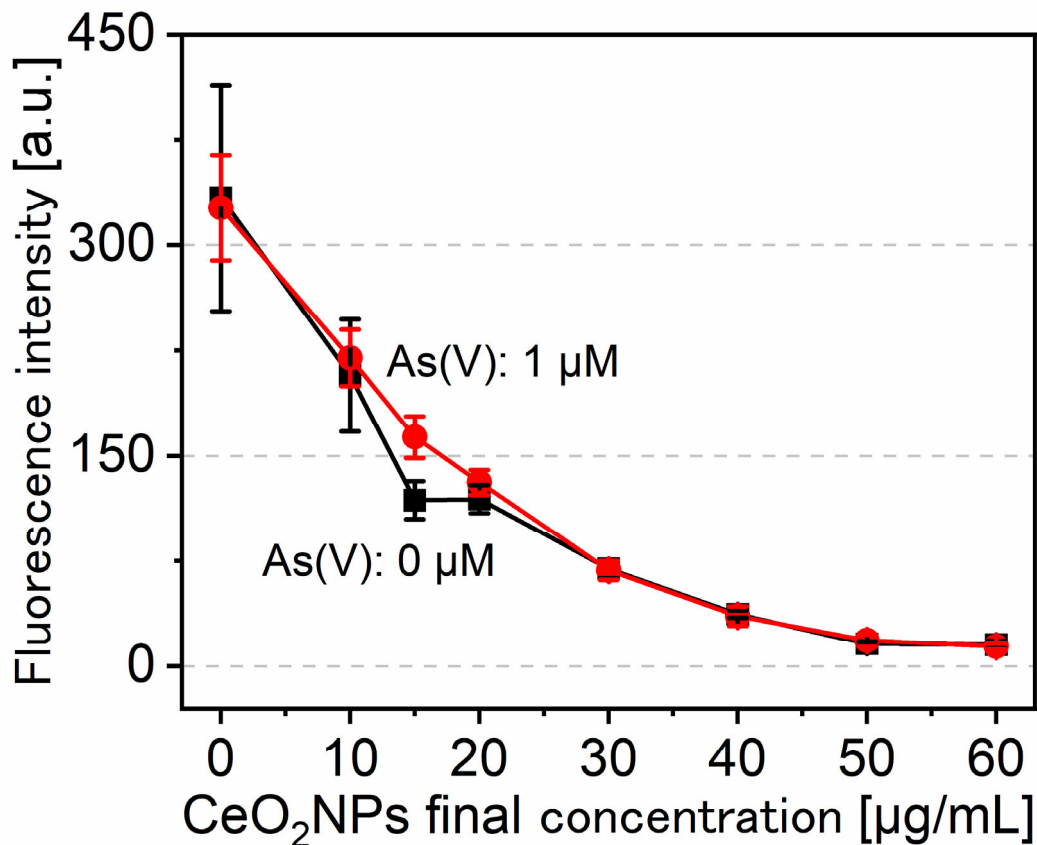
192

193 *Effect of CeO₂NPs and FAM-labeled DNA concentrations on fluorescence intensity*

194

195 Because the CeO₂NPs and FAM-labeled ssDNA concentrations are the most
196 important of all parameters, the effect of final CeO₂NPs concentration and FAM-labeled
197 DNA concentration on the fluorescence intensity were investigated. Figure 1 shows the
198 effect of the final CeO₂NPs concentrations on fluorescence intensity of the POS and
199 NEG samples. The fluorescence intensity decreased as the concentration of CeO₂NPs
200 increased in both samples. Only at 15 µg/mL of CeO₂NP concentrations, the
201 fluorescence intensity of the POS sample became higher than the NEG sample and there
202 was statistically significant difference ($p = 0.05$). Because the difference in fluorescence
203 intensity of the POS and NEG samples is related to the sensitivity of the method, the
204 final CeO₂NPs concentration of a test solution was determined to be 15 µg/mL.

205



206

207 Figure 1: Effect of the final CeO₂NPs concentrations of the test solutions on
208 fluorescence intensity in the presence (POS) and absence of As(V) (NEG). FAM-C₆ as
209 FAM-labeled ssDNA was used. The final FAM-C₆ concentration was 375 nM. The
210 incubation time after sample addition was 20 min.

211

212 At 0-10 µg/mL, there were no difference between POS and NEG due to the low
213 amount of As(V) detached. At 20 µg/mL or higher, the amount of CeO₂NPs was higher

214 than that of FAM-C₆ in the test solution. We thought it is because the pentavalent As
215 adsorbed on the free CeO₂NPs and was not desorbed by adding FAM-C₆.

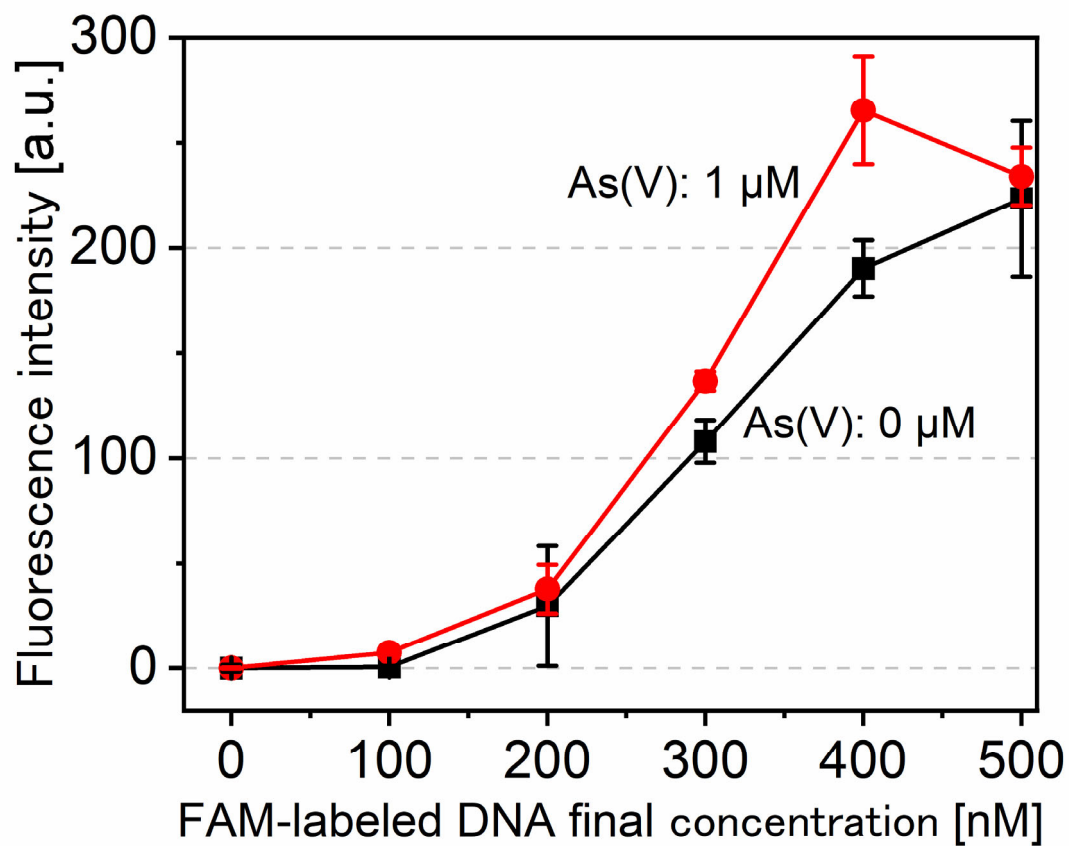
216 We performed the adsorption equilibrium experiments to calculate the
217 detachment constant (K_d) of the FAM-labeled ssDNA from CeO₂NPs. In the adsorption
218 equilibrium experiments, a 20- μ L aliquots of the probe solution were added into 1.5-mL
219 tubes. The probe solution was prepared by adding CeO₂NPs dispersion (0-60 μ g/mL) and
220 FAM-labeled ssDNA solution (400 nM) to the 10-mM HEPES buffer solution (pH: 7.6).
221 10-mM HEPES buffer solution (pH: 7.6) was prepared as a control sample. After
222 measuring the fluorescence intensity, we calculated the concentration of adsorbed ssDNA
223 using the calibration curve of the fluorescence intensity at 518 nm versus the
224 concentration of FAM-labeled DNA. The obtained data were fitted to the Langmuir
225 models using Equation (1) (Hafuka, Nagasato, et al., 2019);

$$226 \quad q_e = Q_m b C_e / (1 + b C_e) \quad (1)$$

227 where q_e is the concentration of ssDNA that adsorbed onto CeO₂NPs (nM), C_e
228 is the equilibrium concentration of non-adsorbed ssDNA (nM), Q_m is the maximum
229 adsorption capacity of ssDNA (nM) and b is the constant related to the energy of
230 adsorption. As a result, the binding curve was fitted to the plots of the NEG samples and
231 the K_d of DNA from CeO₂NPs was calculated to be 5.17 μ g/mL (Figure S1).

232 We also optimized the final FAM-labeled ssDNA concentration (Figure 2). The
233 difference of fluorescence intensity of the POS and NEG samples increased as the final
234 concentration of FAM-labeled DNA was increased. The ΔF were the highest (75.2) at
235 400 nM of FAM-labeled ssDNA. It decreased when FAM-labeled ssDNA concentration
236 was 500 nM, but we considered that it was a measurement error because there was no
237 statistically significant difference ($p = 0.05$) between the results at 400 nM and 500 nM.
238 Based on these results, the final concentration of FAM-labeled DNA was determined to
239 be 400 nM.

240



241

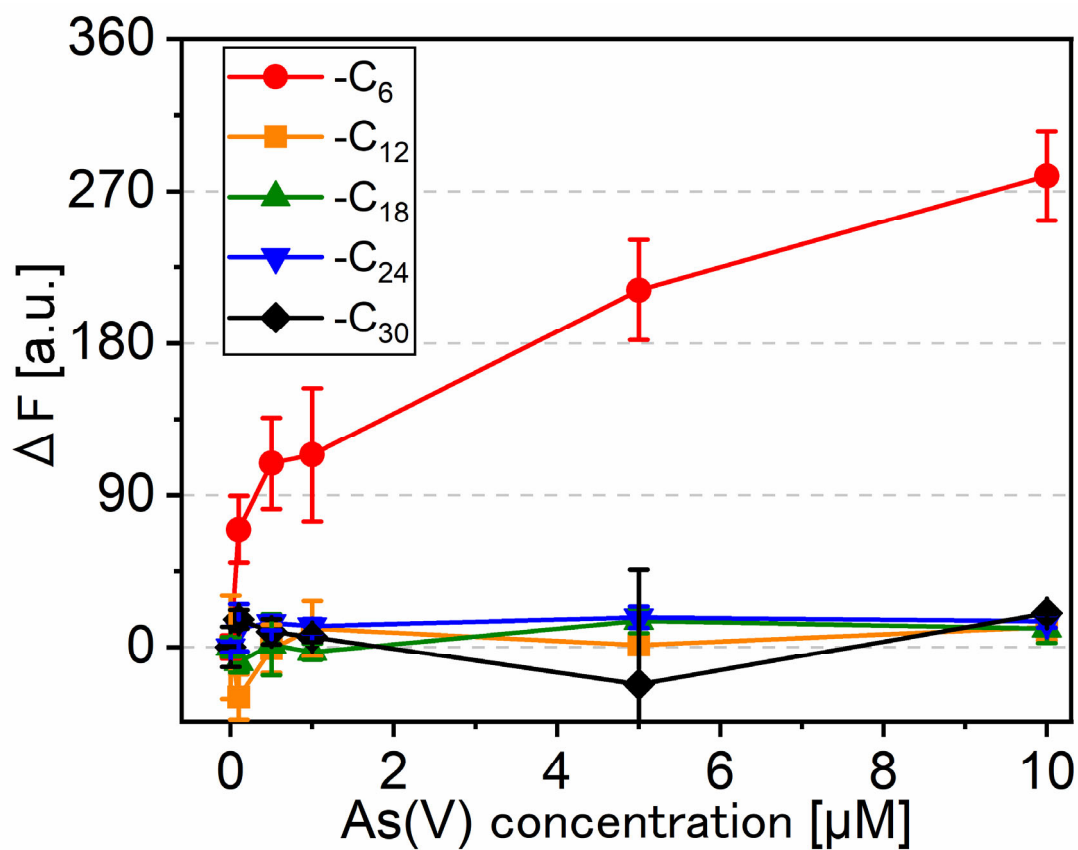
242 Figure 2: Effect of final FAM-labeled DNA concentration of test solutions on
243 fluorescence intensity in the POS and NEG. The final CeO₂NPs concentration was 15
244 μg/mL, FAM-C₆ as FAM-labeled DNA was used and the incubation time after sample
245 addition was 20 min.

246

247 *Effect of the length of FAM-labeled DNA on fluorescence intensity*

248

249 It was expected that the ssDNA length would affect the As(V)-induced ssDNA
250 detachment reaction (Liu and Liu, 2014). Therefore, we investigated the effect of
251 ssDNA length on ΔF . We used FAM-C_n (n changes from 6 to 30) (Figure 3). The
252 previous studies have shown that adsorption took place via the phosphate backbone
253 (Lopez, Zhang, et al., 2017; Liu and Liu, 2014). Moreover, C₆ had the highest
254 adsorption and detachment affinity among four types of bases (Lopez, Zhang, et al.,
255 2017; Liu and Liu, 2014). We found that the fluorescence intensity increased as the
256 As(V) concentration increased (Figure 3) due to the FAM-C₆ adsorbed onto CeO₂NPs
257 was detached (Scheme 1). The ΔF of the POS samples with C₁₂ to C₃₀ did not increase
258 as the As(V) concentration increased due to C₁₂ and longer ones adsorbed too tightly
259 onto CeO₂NPs surfaces to detach. The FAM-C₆ could produce a higher sensitivity
260 because it was easier to the adsorbed than the FAM-C₁₂ and the longer ones. Previous
261 studies showed that FAM-A₁₅ was used for MnO₂NPs (Wang, Huang, et al., 2018) and
262 FAM-C₁₅ were used for Fe₃O₄NPs (Liu and Liu, 2014) to achieve fluorescence
263 quenching and recovering. We concluded that CeO₂NPs have the unique property to
264 achieve higher sensitivity using shorter FAM-labeled DNA length.



266

267 Figure 3: Effect of DNA length on calibration curves. The final CeO₂NPs concentration
 268 was 15 μg/mL, the final concentration of each FAM-labeled DNA was 400 nM and the
 269 incubation time after sample addition was 20 min.

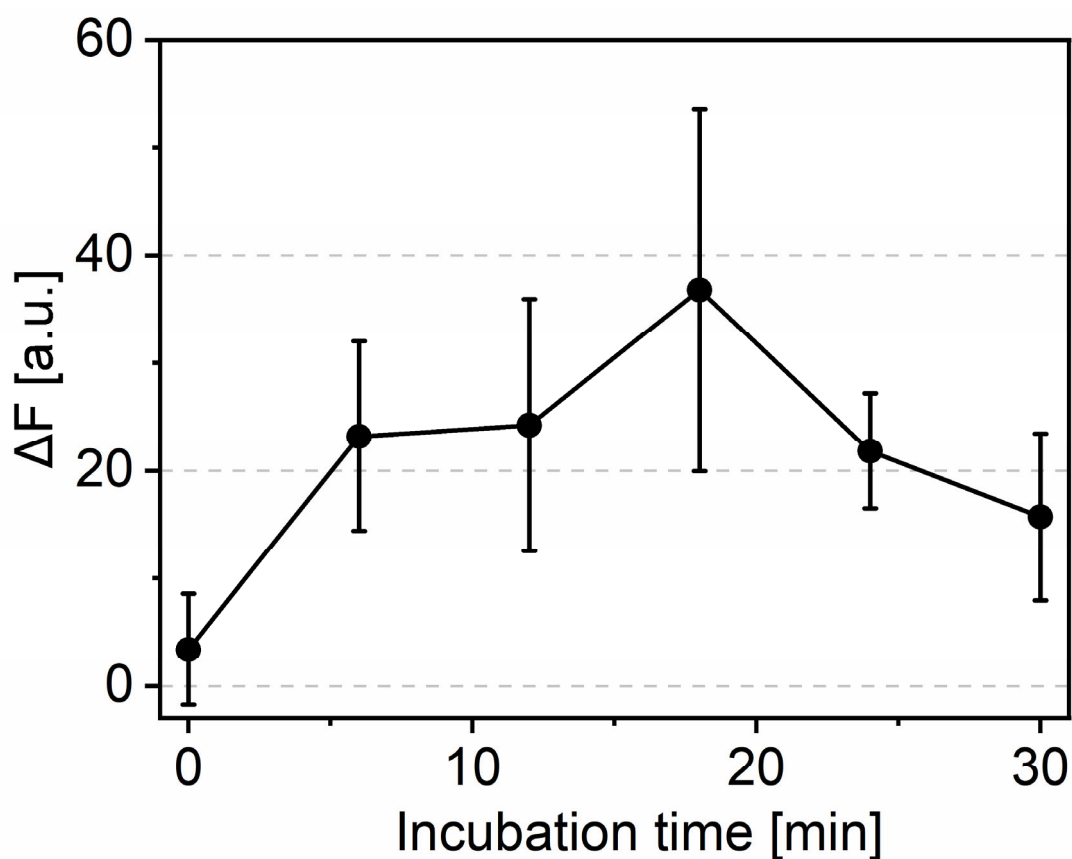
270

271 *Effect of the incubation time after adding samples on fluorescence intensity*

272

273 Figure 4 shows the time course change in ΔF. The ΔF increased immediately
 274 within the initial 6 min, indicating that the detachment of FAM-labeled ssDNA from

275 CeO₂NPs occurred within the initial 6 min. The ΔF maximum value at 18 min. In
276 contrast, the ΔF did not increase until 18 min and increased thereafter. Based on these
277 results, the optimal incubation time after sample addition was determined to be 6 min.
278 The optimal incubation time was shorter than that in the previous studies using
279 Fe₃O₄NPs and MnO₂NPs (over 30 min) (Liu and Liu, 2014; Wang, Huang, et al., 2018).
280 Such fast signaling kinetics is advantageous for analysis of As(V). This is likely because
281 the density of FAM-C₆ on the CeO₂NPs surface was higher than Fe₃O₄NPs and
282 MnO₂NPs, which would accelerate the displacement reaction.



283

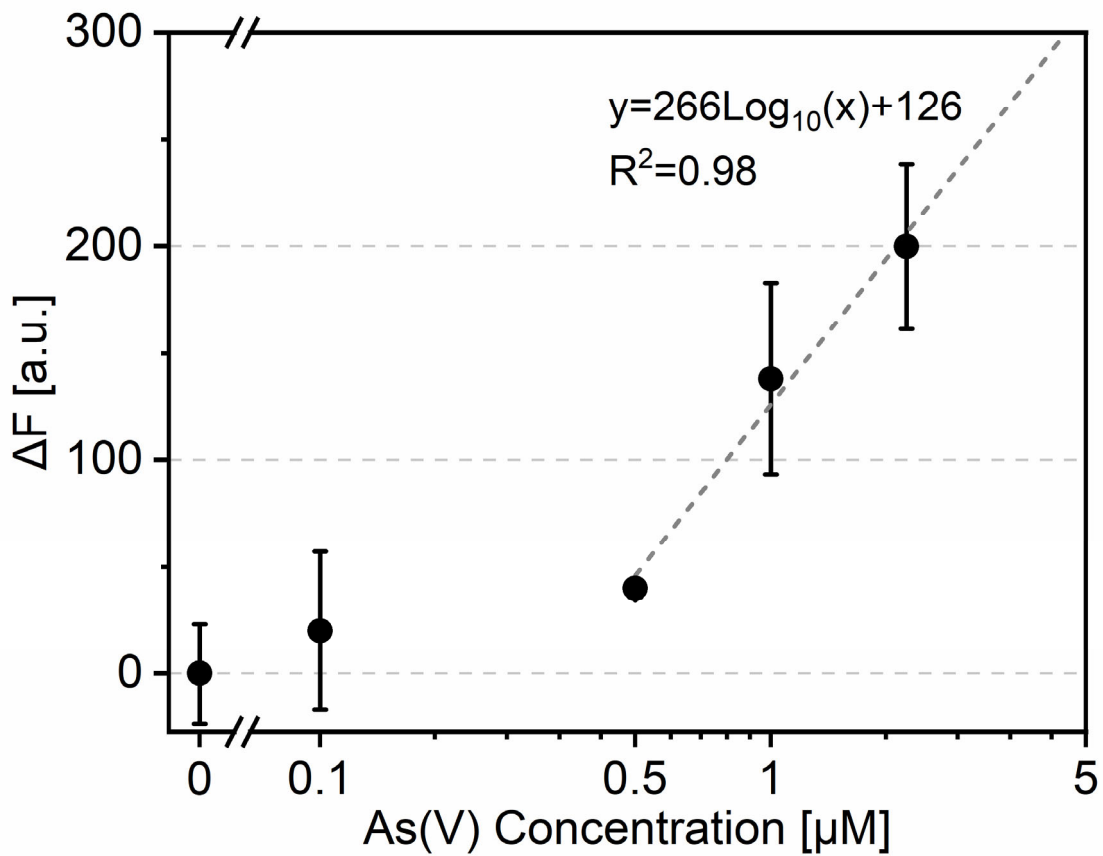
284 Figure 4: Variation of ΔF of test solutions with time after sample addition in the POS
285 and NEG. The final CeO₂NPs concentration was 15 $\mu\text{g/mL}$, FAM-C₆ as FAM-labeled
286 DNA was used and the final FAM-C₆ concentration was 400 nM.

287

288 *Calibration Curve*

289

290 Figure 5 shows a calibration curve of As(V) for the method. The ΔF of the test
291 solutions remained unchanged below 0.5 μM As(V) and logarithmically increased from
292 40 to 200 with increase in As(V) concentrations from 0.5 to 2 μM . The detection limit
293 (LOD) was calculated to be 0.61 μM . When the As(V) concentration exceeded 2 μM ,
294 the ΔF increased further and remained almost unchanged at 50 μM As(III) (data not
295 shown).



296

297 Figure 5: Method calibration curve. The dotted line is a regression line.

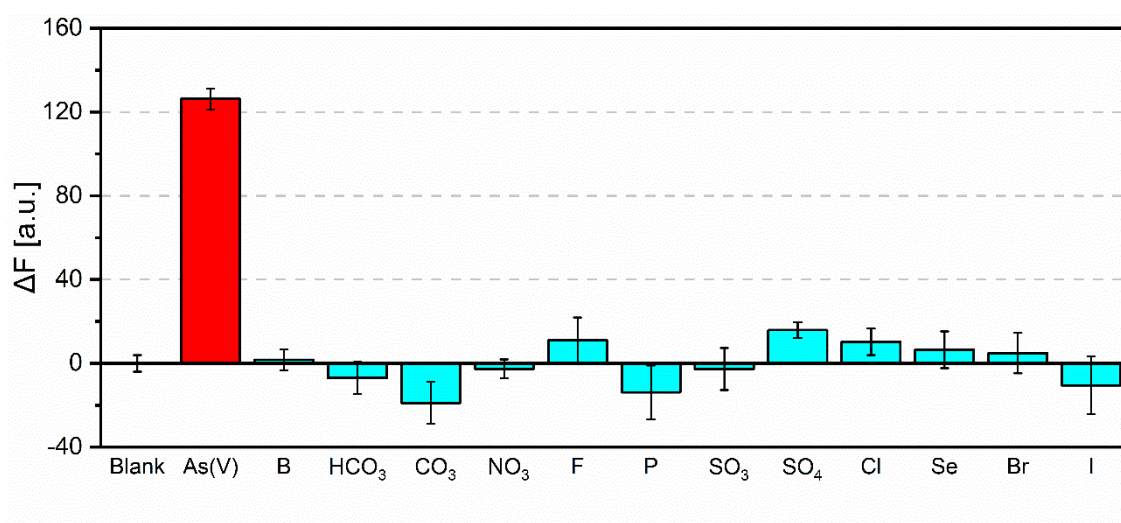
298

299 *Method Selectivity*

300

301 Figure 6 shows the ΔF of the samples with different anions. The ΔF of the
 302 solution with As(V) was significantly higher than that of the blank sample. The ΔF of
 303 the samples with sulfate were slightly higher and the samples with carbonate and
 304 phosphate were slightly lower than that of the blank sample. We conducted a statistical
 305 analysis among the anions. There were no statistically significant differences ($p > 0.05$)

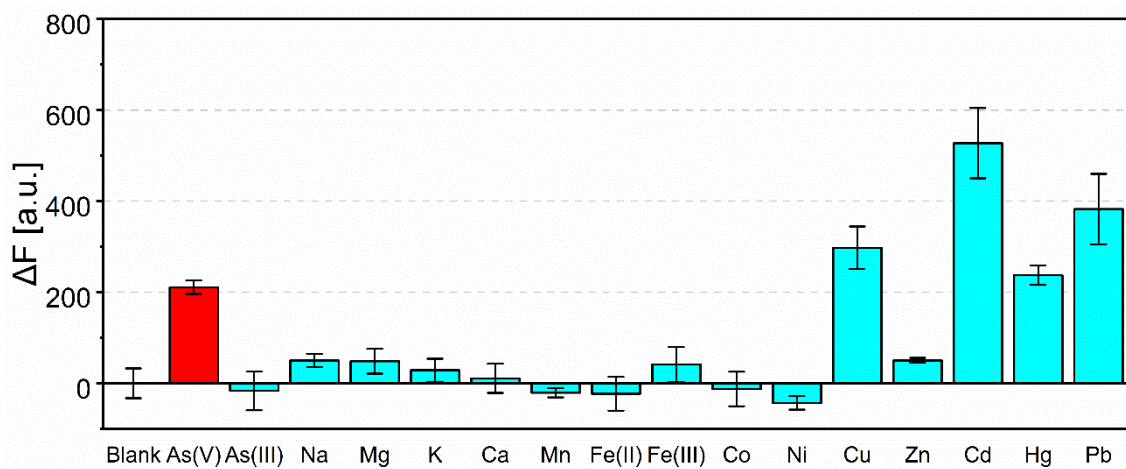
306 among blank and the other anions (except As(V)). However, when the inhibitory test
307 was conducted using 100 μM of the anions, borate and phosphate interfered the method
308 (Figure S2). When we made the calibration curve of the method using borate and
309 phosphate instead of As(V), the fluorescence intensity increased with increase in these
310 anions (Figure S3). Therefore, we found that borate and phosphate were the main
311 interfering substances in this method.



312
313 Figure 6: Method selectivity for anions. The concentration of anion was 10 μM .

314
315 Figure 7 shows the ΔF of the samples with different cations. The ΔF of the
316 solution with As(V) was significantly higher than that of the blank sample. There were
317 no statistically significant differences ($p = 0.05$) in the ΔF of the samples with As(III),
318 K(I), Ca(II), Mn(II), Fe(II), Fe(III), Co(II) and Ni(II) solutions against the blank sample.
319 In contrast, ΔF values were slightly higher in the solutions of Na(I), Mg(II), Fe(III) and

320 Zn(II) and significantly higher in the solutions with Cu(II), Cd(II), Hg(II) and Pb(II).
321 However, we could remove these ions by pre-treatment applied in this study (see Figure
322 S4). These results showed a high selectivity of the method toward As(V) after the pre-
323 treatment.



324
325 Figure 7: Method selectivity for cations including As(V) and As(III). The concentration
326 of each ions was 10 μ M.

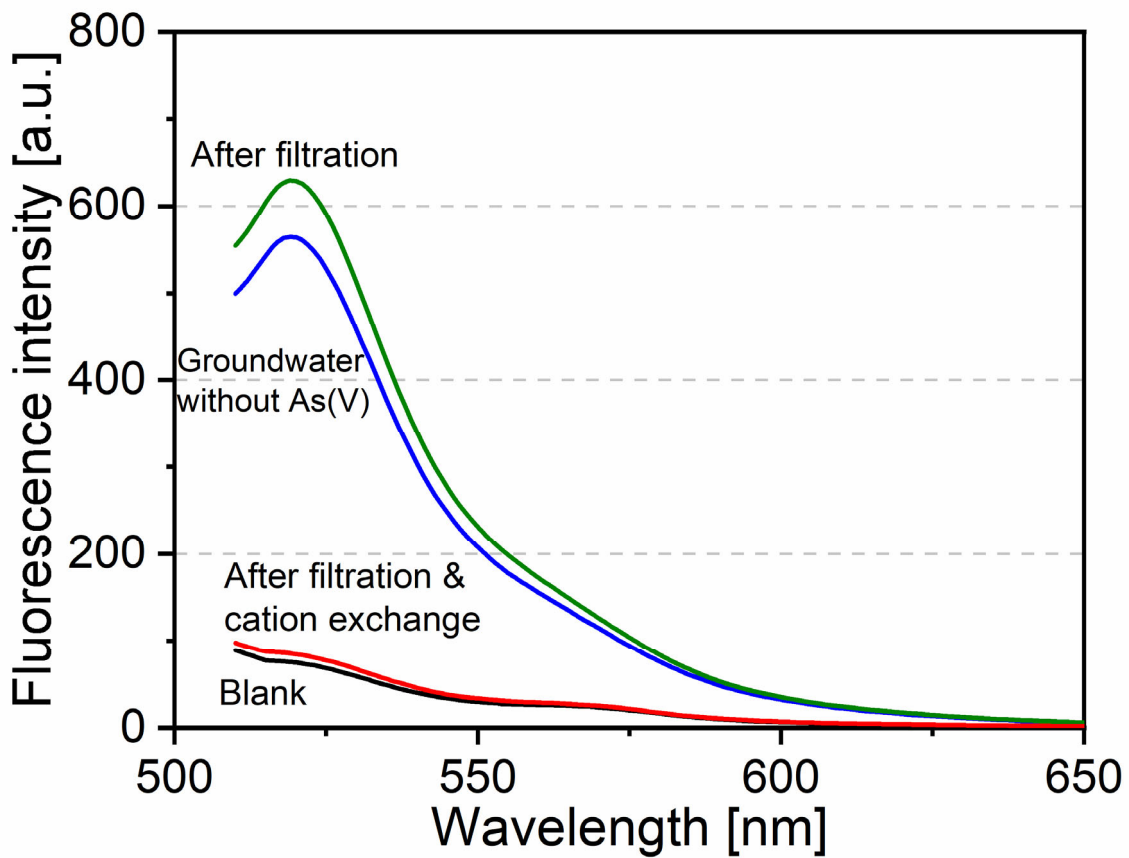
327

328 *Analysis of Groundwater Samples*

329

330 Contrary to expectations, when GW without As(V) was subjected to the assay,
331 the peak of fluorescence intensity at 518 nm increased significantly (Figure 8). The
332 increase in the fluorescence band might be attributed to detachment of the FAM-C₆ by
333 the ions in the sample (Table S1). Therefore, the GW was pretreated with a 0.2- μ m-

334 pore-size membrane filter and cation-exchange resin. Membrane filtration did not
335 remove the interfering ions in the GW (Figure 8). In contrast, the peak of fluorescence
336 intensity of the sample subjected to filtration followed by cation exchange almost same
337 as that of a blank sample (Figure 8), indicating that the filtration followed by cation
338 exchange could significantly reduce interfering effects of matrix of GW. Table S1 shows
339 the ion concentrations in the GW before and after cation-exchange treatment. The GW
340 contained divalent cations (*i.e.*, Mg(II) and Ca(II)), which might explain increasing the
341 peak of fluorescence band (Figure S5). The results show that metal-cation
342 concentrations in GW were efficiently removed by cation exchange and alternatively
343 Na(I) was released.



344

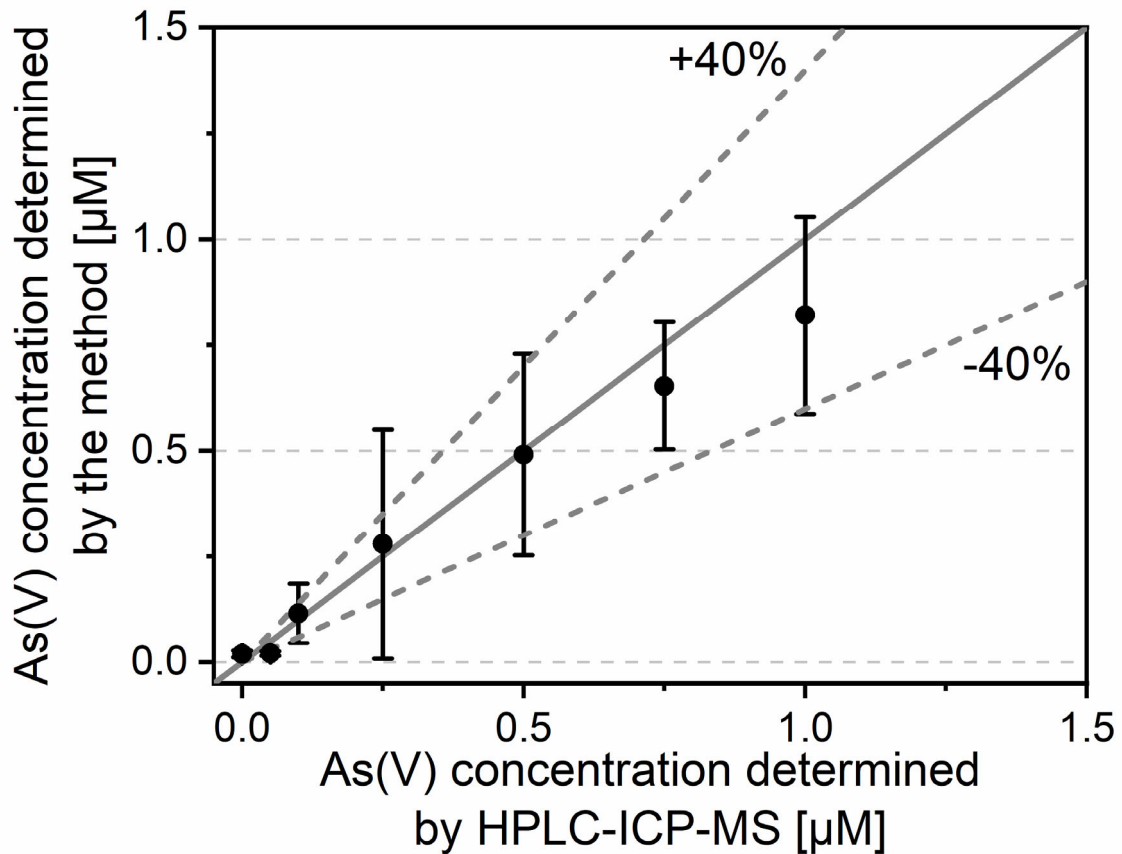
345 Figure 8: Fluorescence intensity of FAM-labeled DNA in groundwater, groundwater
 346 filtered with 0.2-µm-pore-size membrane filter, and groundwater filtered with 0.2-µm-
 347 pore-size membrane filter and passed through cation-exchange resin.

348

349 Figure 9 shows the relationship between the concentrations of As(V) in GW
 350 determined by the method and HPLC-ICP-MS. Some plots show that As(V)
 351 concentrations determined by the method were almost identical to those by HPLC-ICP-
 352 MS. Our methods could determine As(V) concentration in groundwater between 0.1 µM
 353 and 1.0 µM. On the other hand, our methods could not determine As(V) concentration

354 in groundwater above 2.5 μM because the standard deviations were larger and greatly
355 overestimated (data not shown).

356 However, As(V) concentrations of most of the samples have the large
357 measurement error because Na(I) was sole ions present in the pre-treatment samples
358 (Table S1) and standard error of the results was large. Therefore, we need to modify the
359 method to reduce the measurement error.



360
361 Figure 9: Relationship between concentrations of As(V) determined by HPLC-ICP-MS
362 and those determined by using the method.

363

364 Lakatos *et al.* developed the simple analytical method for As(V) using S-layer
365 functionalized gold nanoparticles (Lakatos, Matys, et al., 2015). Das *et al.* developed
366 the As(V) sensor based on antimonyl–arseno–molybdate complex in the presence of
367 ammonium molybdate, potassium antimonyl tartrate and ascorbic acid (Das and Sarkar,
368 2016). However, As(III) is also adsorbed onto gold nanoparticles (Zong and Liu, 2019)
369 and the selectivity between As(III) and As(V) is not shown in the study (Lakatos,
370 Matys, et al., 2015). The As(V) sensor which Das et al. developed can measure As(V)
371 concentration by color shading, which is not suitable for low concentration As(V)
372 analysis because the LOD is higher than that of our method (Das and Sarkar, 2016).

373 The merit of our method is that it is selective for As(III). It is because the
374 simple analytical methods for As have been developed only for As(III) and total As, but
375 not for As(V). On the other hand, the disadvantage of our method is that it is subject to
376 interference by other anions such as borate and phosphate at high concentrations. Since
377 there is no simple technique to separate As(V) from borate and phosphate, our method
378 cannot be applied to samples with high concentrations of borate and phosphate.

379 Determining various chemical species such as As(III) and As(V) is a quite
380 challenging task. The instrumental methods, such as LC-ICP-MS, are not common to
381 determine the concentration of various As species. In this regard, developing the

382 methods for analysis of various As species is of importance. Our method relies on the
383 strong interaction between As(V) and the surface of metal oxide nanoparticles and
384 provides an inexpensive, simple, and easy-to-use platform.

385

386

387 **Conclusion**

388

389 In this study, we developed a simple analytical method to determine As(V)
390 concentrations using CeO₂NPs and a FAM-labeled ssDNA and firstly attempted to
391 measure the concentration of As(V) in groundwater by the method. The parameters that
392 affect the method performance, such as the final concentration of CeO₂NPs (15 µg/mL)
393 and FAM-labeled DNA (400 nM), the sequence and length of FAM-labeled DNA (FAM-
394 C₆) and incubation time (6 min-) with samples were optimized. After optimizing the
395 parameters, the total analysis time was about 20 min and the LOD was 0.61 µM. This
396 method has a significant selectivity against the same concentrations of Cu(II), Cd(II),
397 Hg(II) and Pb(II) and a slight selectivity against the same concentrations of sulfate and
398 carbonate. For cations, pre-treatment by cation extraction to remove interfering ions was
399 beneficial for determination of As(V) concentrations in groundwater containing a variety

400 of metal cations at high concentration. We could underestimate the As(V) concentrations
401 in As(V)-spiked GW by the method. In the future, we should reduce the standard
402 deviation and stabilize the adsorption and detachment of FAM-labeled DNA and As(V)
403 onto CeO₂NPs. This method has the potential in the development of an As(V) sensor for
404 application to on-site analysis.

405

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407

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412 ICP-MS.

413

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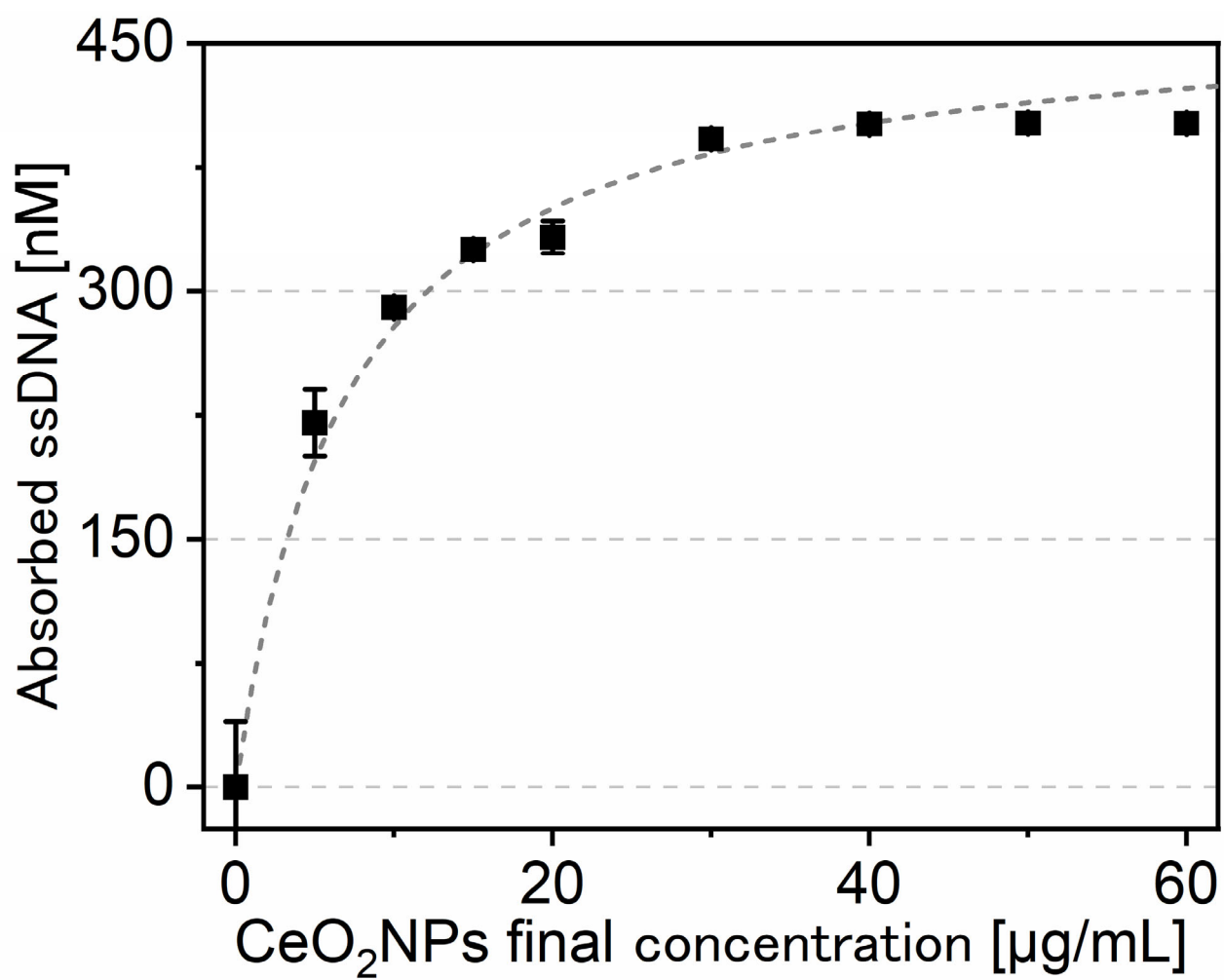
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517
518

519 Table S1: Concentration of ions (μM) in groundwater samples. “Bef” and “Aft” indicate
520 the samples before and after cation-exchange treatment, respectively. The ions
521 concentrations were measured by ICP-MS.

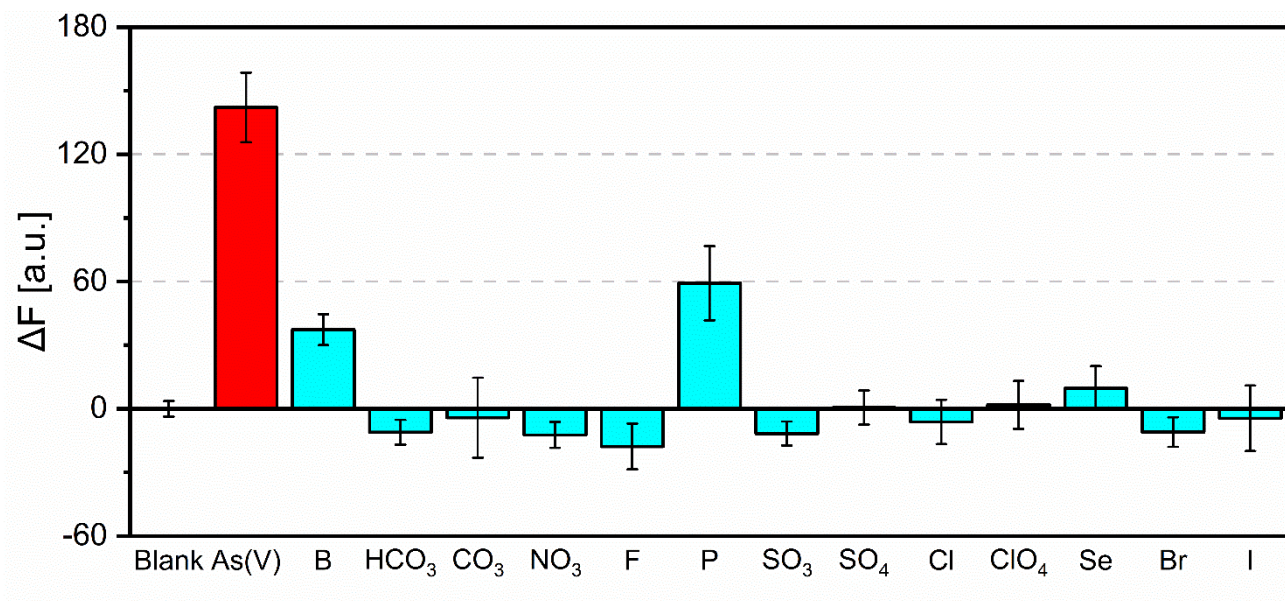
Ions	GW	
	Bef	Aft
B	-	-
Na	940	1,904
Mg	590	0.0667
Al	1.69	0.40
P	<0.00	<0.00
K	128	0.62
Ca	1,085	<0.00
Mn	0.242	<0.00
Fe	3.68	<0.00
Cu	0.09	<0.00
Zn	0.35	<0.00
As	<0.00	0.04



523

524 Figure S1: Adsorbed ssDNA as a function of the final CeO₂NP concentrations. The

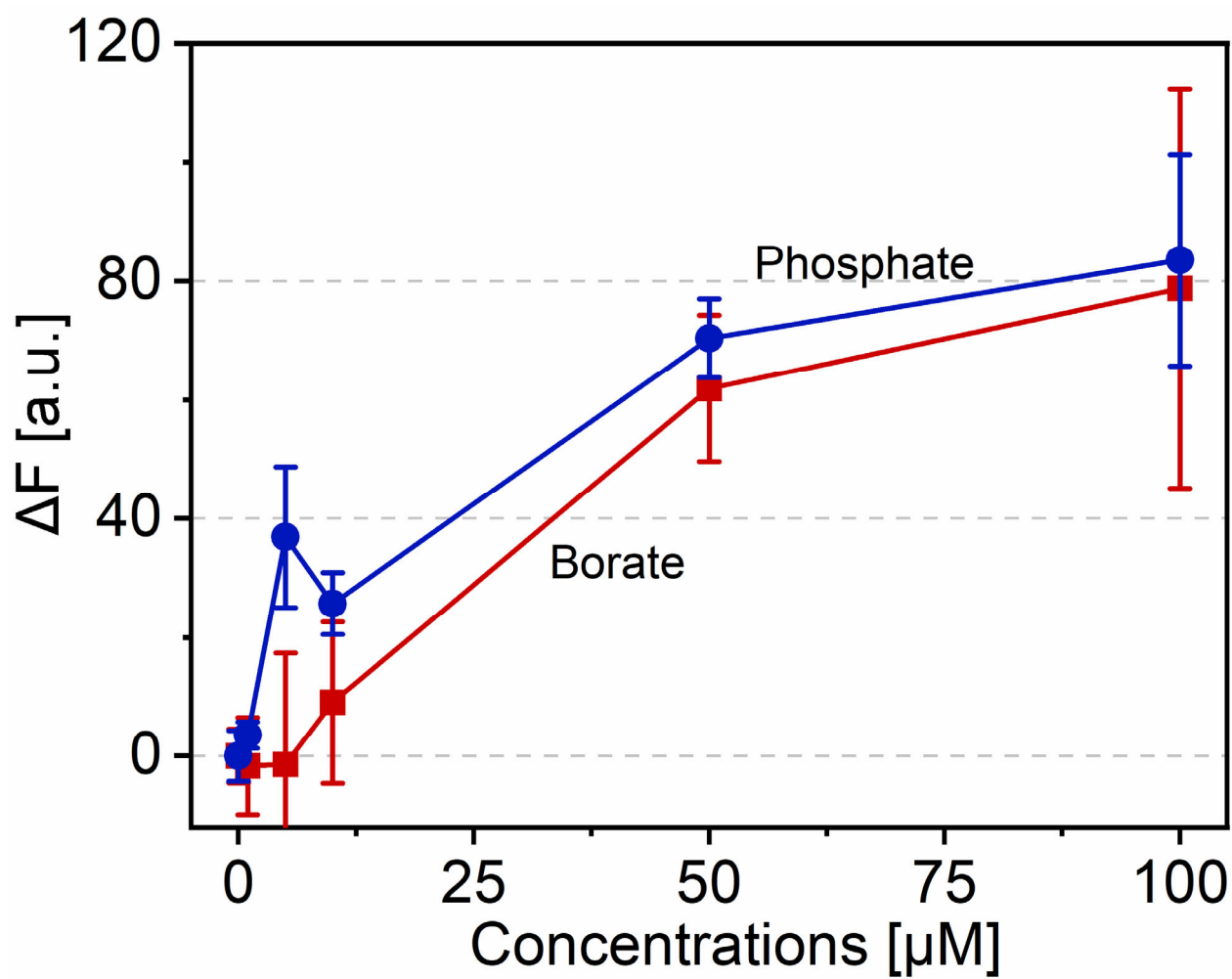
525 dotted line is the Langmuir isotherm.



526

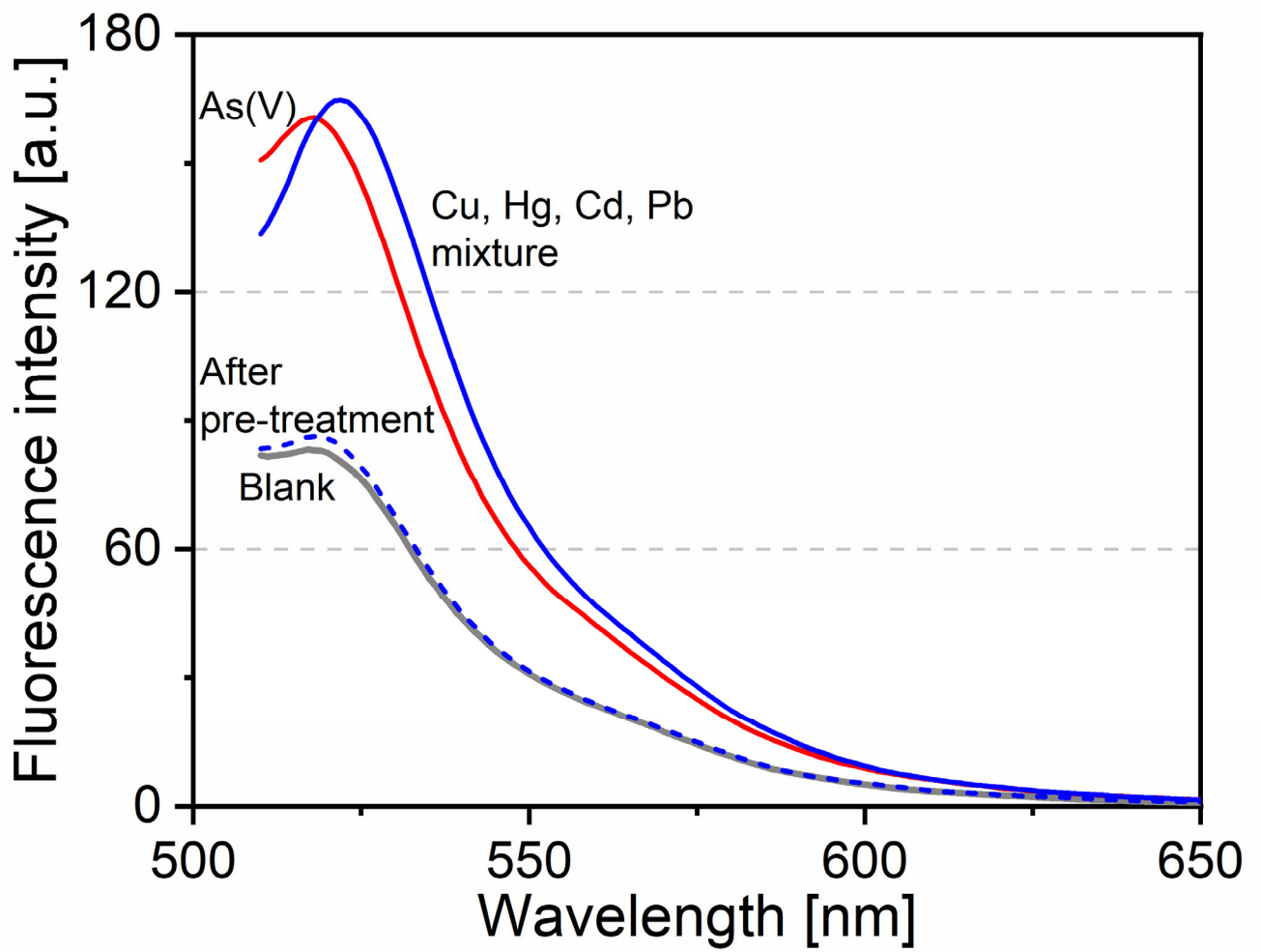
527 Figure S2: Method selectivity for anions. The concentration of As(V) was 10 μ M and

528 the others were 100 μ M.



529

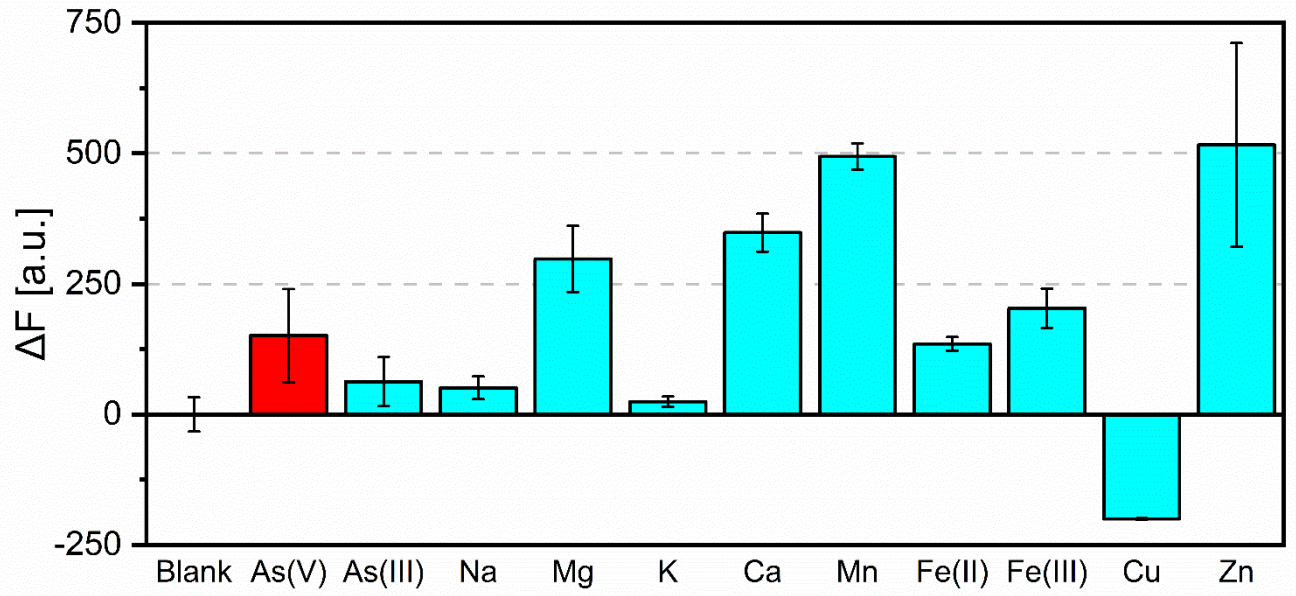
530 Figure S3: Effect of borate and phosphate on this method.



531

532 Figure S4: The effect of pre-treatment (cation-exchange) via inhibitor.

533



534

535 Figure S5: Effect of the metal ions that contain in groundwater at high concentration. The

536 concentration of As(V) was 10 μ M and the others were 100 μ M.

537