Gradient Elution Moving Boundary Electrophoresis with Field Amplified Continuous Sample Injection

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The following pages contain data from repetitions of the experiments presented in the main text, as well as raw data corresponding to all figures included in the main text, and the supporting figures. Calculations and conductivity measurements are also included.
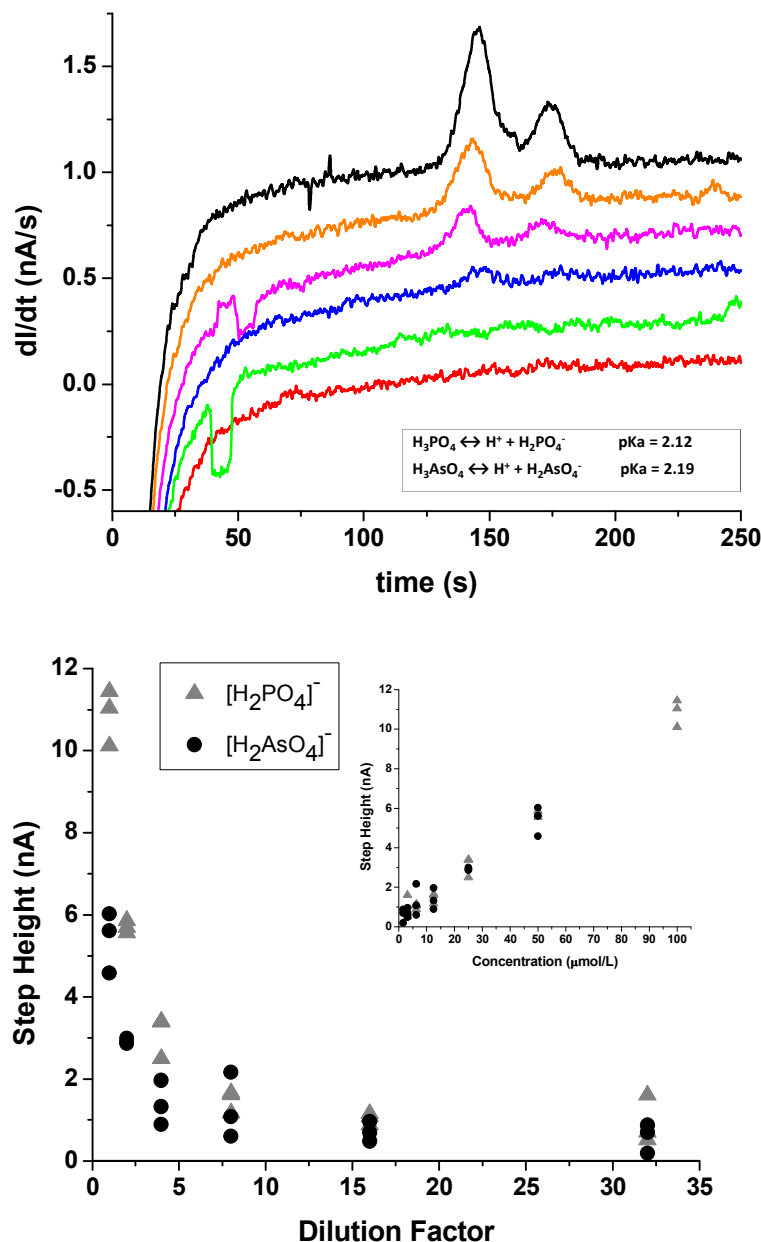
Figure S-1 (Figure 2 repeated):

The chemistry equilibria shown in the figure inset:

$H_3PO_4 \leftrightarrow H^+ + H_2PO_4^-$ pKa = 2.12

$H_3AsO_4 \leftrightarrow H^+ + H_2AsO_4^-$ pKa = 2.19

Figure S1. GEMBE data for samples with a serial 2-fold dilution of 100 μM NaH$_2$PO$_4$ (left peak), 50 μM Na$_2$HAsO$_4$ (right peak). Dilutions were performed with run buffer so that the sample conductivity was the same as the run buffer conductivity. (A) Time derivative of the current vs. time. The dilution factor increases from top to bottom starting with 1 x (100 μM NaH$_2$PO$_4$, 50 μM Na$_2$HAsO$_4$), 2 x, 4 x, 8 x, 16 x, and ending with 32 x (3.125 μM NaH$_2$PO$_4$, 1.5625 μM Na$_2$HAsO$_4$). (Inset – species of interest). (B) Step height (equivalent to peak area from A) vs. dilution factor. As the samples are progressively diluted and as the analyte concentration decreases, the signal decreases. The LOD for arsenate is approximately 12 μmol/L (signal to noise ratio equal to 3). (Inset – step height from (A) vs. concentration).
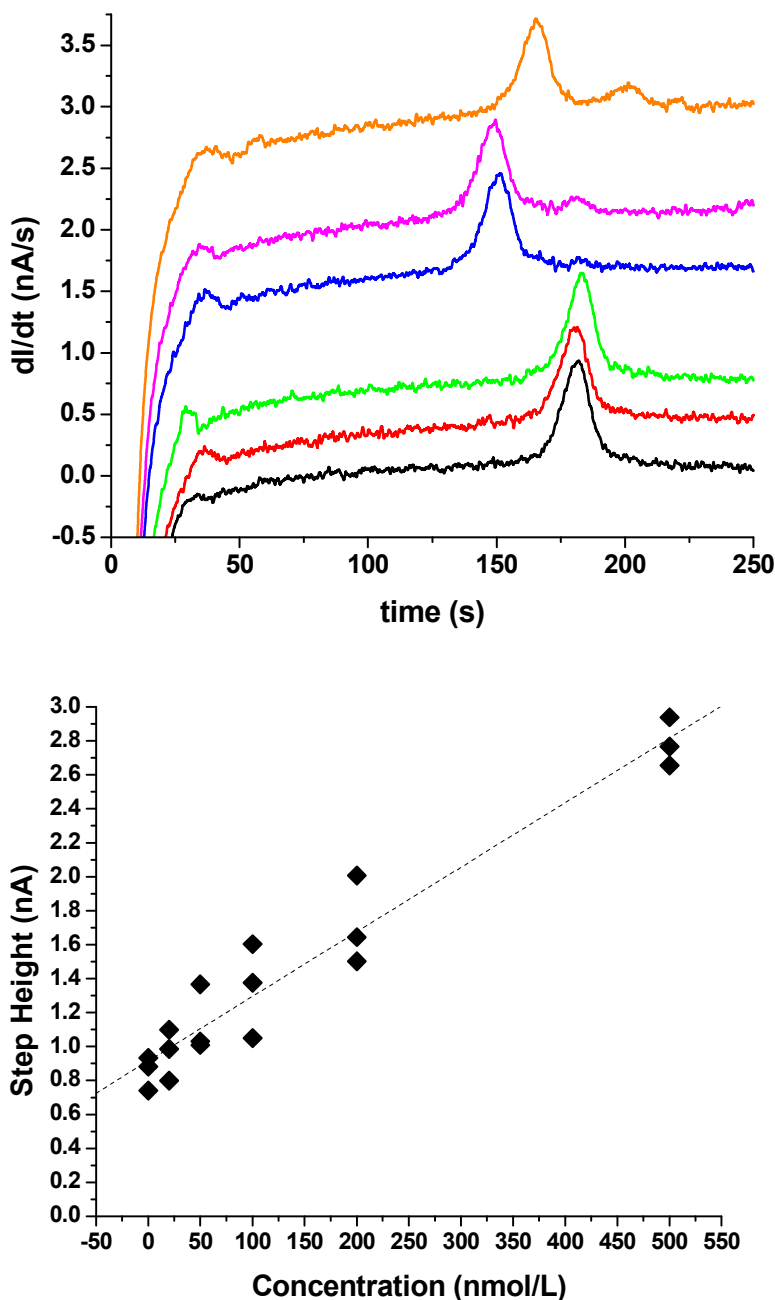
Figure S-2 (Figure 4 repeated):

Figure S-2. FACSI-GEMBE data with sample buffer diluted 10× (with DI water) relative to the run buffer. (A) Time derivative of the current vs. time. Phosphate concentration (left peak) is held constant at 2 µmol/L and arsenate concentration (right peak) is decreased from top to bottom: 500 nmol/L, 200 nmol/L, 100 nmol/L, 50 nmol/L, 20 nmol/L, 10 nmol/L, 0 nmol/L. (B) Step height (equal to peak area from A) vs. concentration. The LOD for arsenate is reduced approximately 67 fold from 10 µmol/L (Fig. 2) to 150 nmol/L. The dotted line represents a linear fit to all data points.

Raw Data (no smoothing): [Phosphate step on left, Arsenate step on right]

Figure S-3: Serial 2x Dilutions with Run Buffer – (Fig S-1)
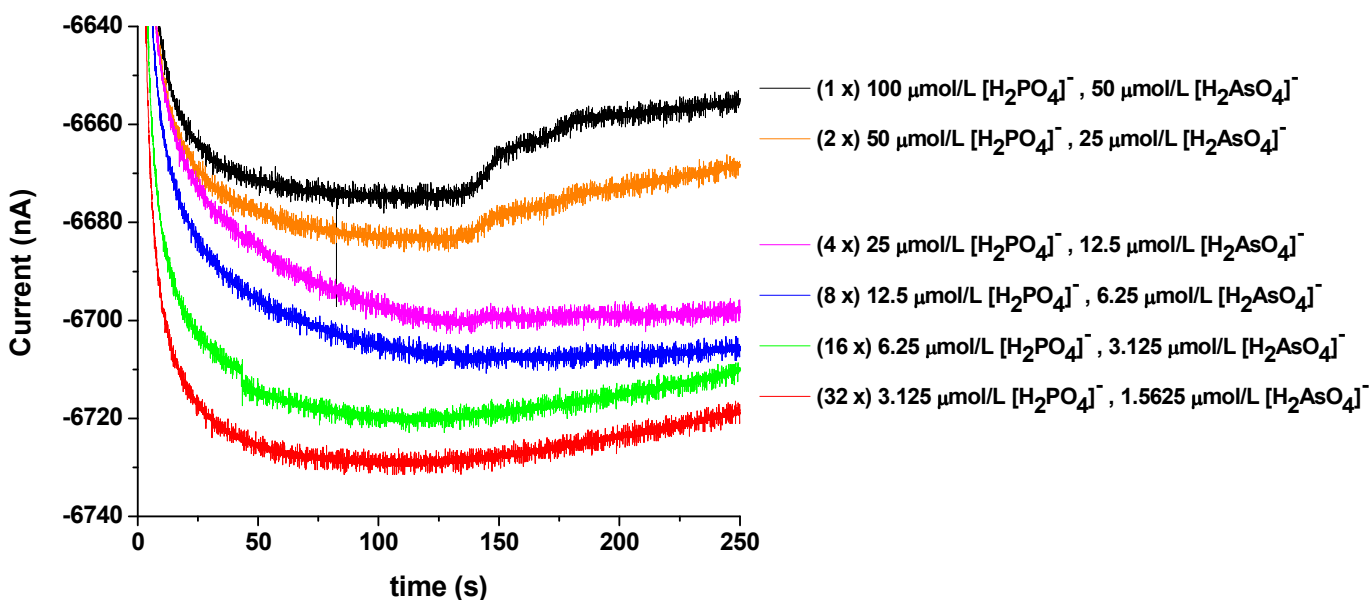

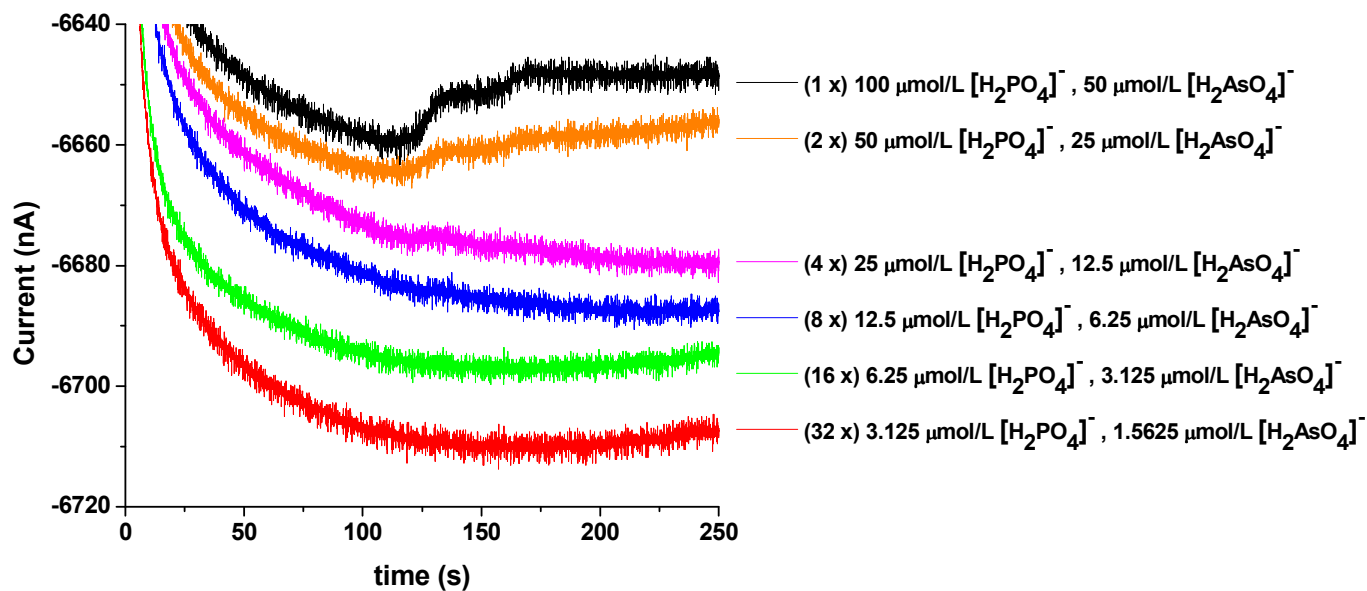**Figure S-4:** Serial 2x Dilutions with Run Buffer – (Fig 2 in main text)

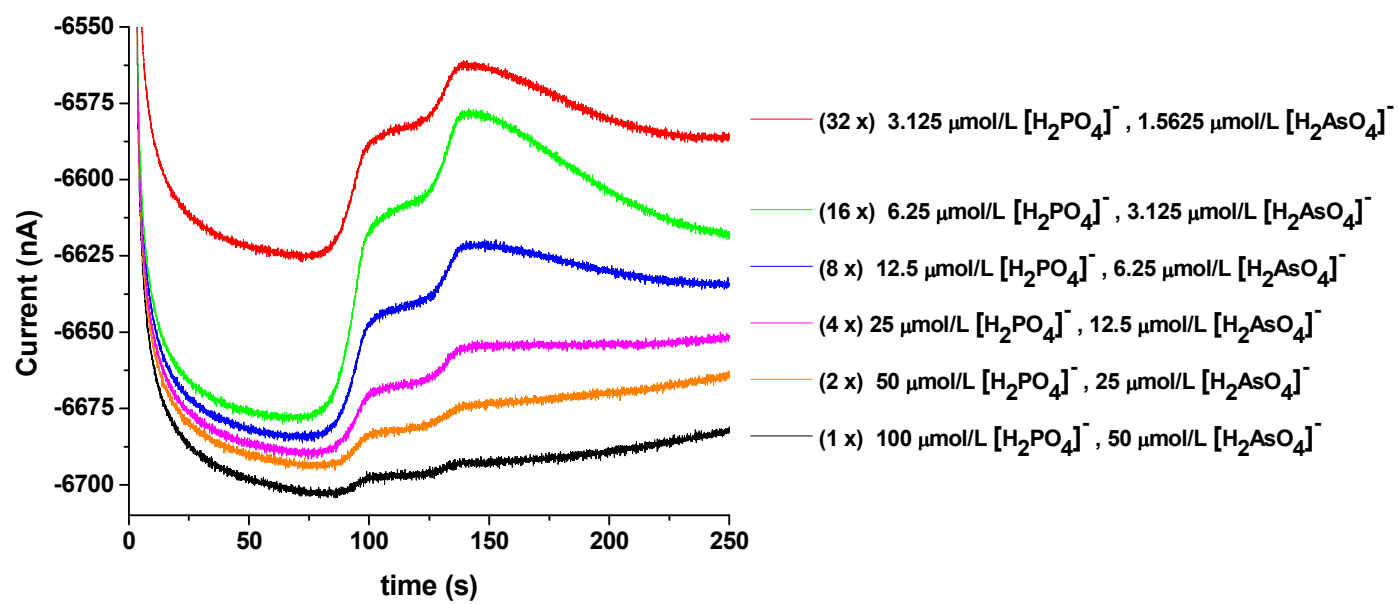**Figure S-5:** Serial 2x Dilutions with DI water – (Figure 3 in main text)

(32 x) 3.125 μmol/L $[H_2PO_4]^-$, 1.5625 μmol/L $[H_2AsO_4]^-$

(16 x) 6.25 μmol/L $[H_2PO_4]^-$, 3.125 μmol/L $[H_2AsO_4]^-$

(8 x) 12.5 μmol/L $[H_2PO_4]^-$, 6.25 μmol/L $[H_2AsO_4]^-$

(4 x) 25 μmol/L $[H_2PO_4]^-$, 12.5 μmol/L $[H_2AsO_4]^-$

(2 x) 50 μmol/L $[H_2PO_4]^-$, 25 μmol/L $[H_2AsO_4]^-$

(1 x) 100 μmol/L $[H_2PO_4]^-$, 50 μmol/L $[H_2AsO_4]^-$

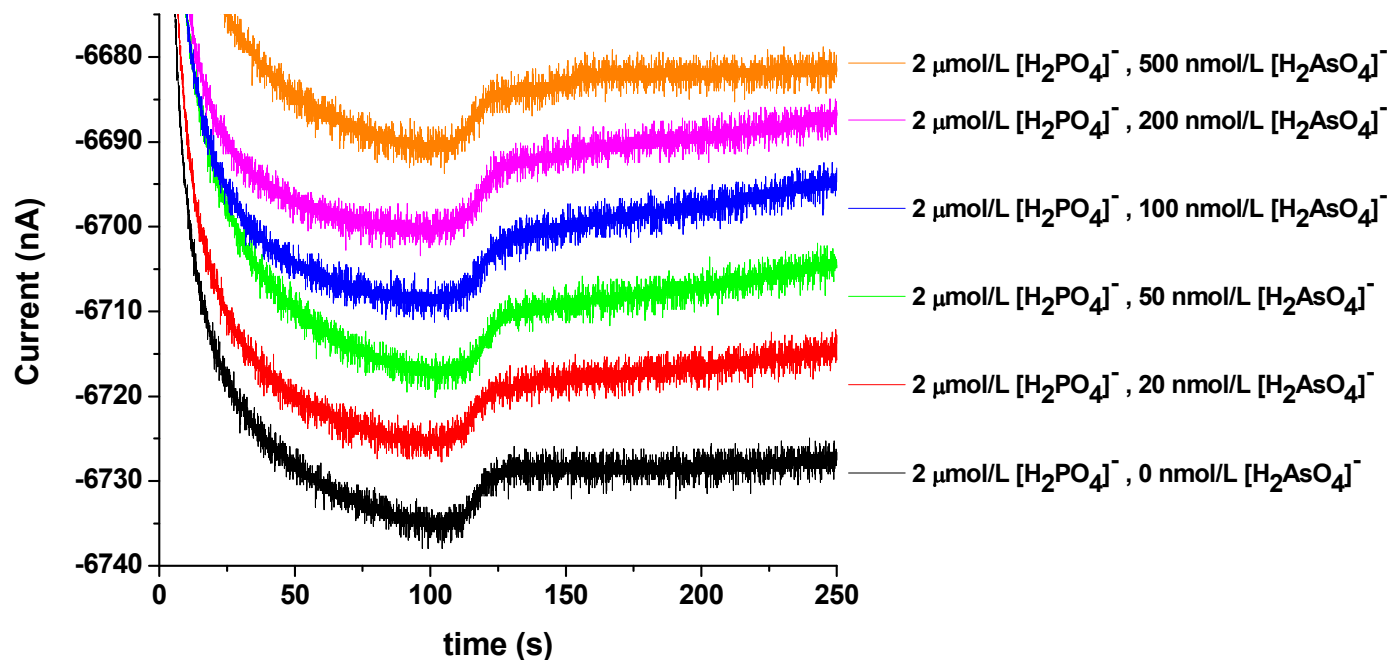**Figure S-6:** FACSI-GEMBE with sample buffer diluted 10x – (Figure 4 in main text)

Figure S-7: FACSI-GEMBE with sample buffer diluted 10x – (Figure S-2)
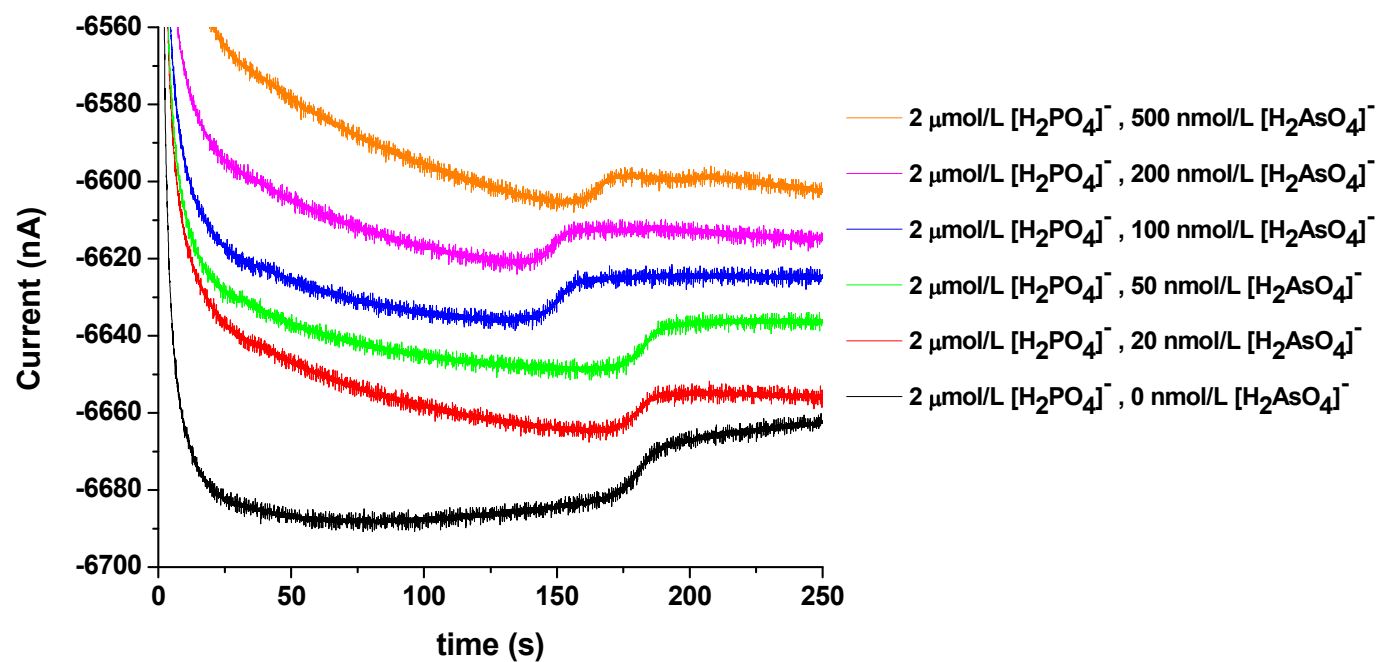
Figure S-8: FACSI-GEMBE of tap water samples – (Figure 5 in main text). The large peak around 150 seconds represents an unknown species in the tap water samples. Phosphate elutes around 250 seconds, followed by arsenate.
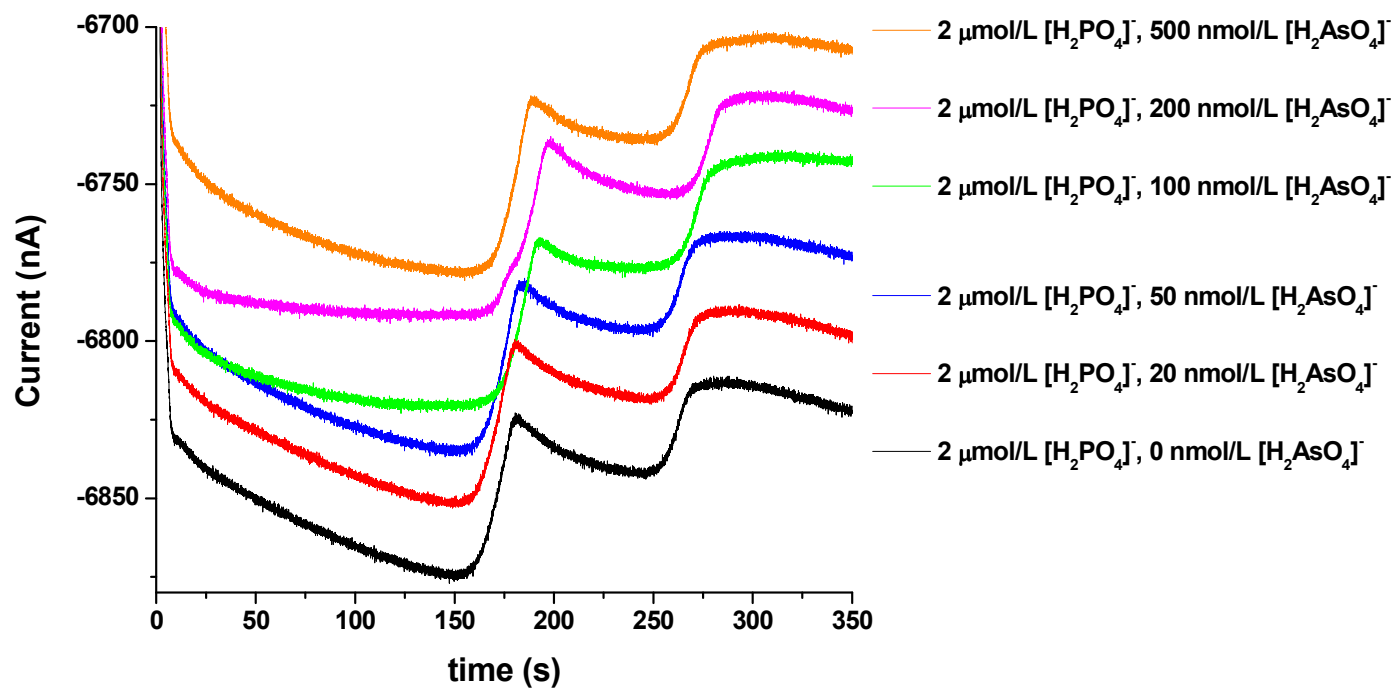
Figure S-9: Tap water – including unknown peak at multiple pHs: FACSI-GEMBE of tap water (sample = 9 parts tap water, 1 part run buffer). Run buffer: 100mM beta-alanine, 80mM HCl, pH = 3.0. The GEMBE data is cut off at 200 seconds to allow visualization of the arsenate shoulder eluting with the unknown peak.
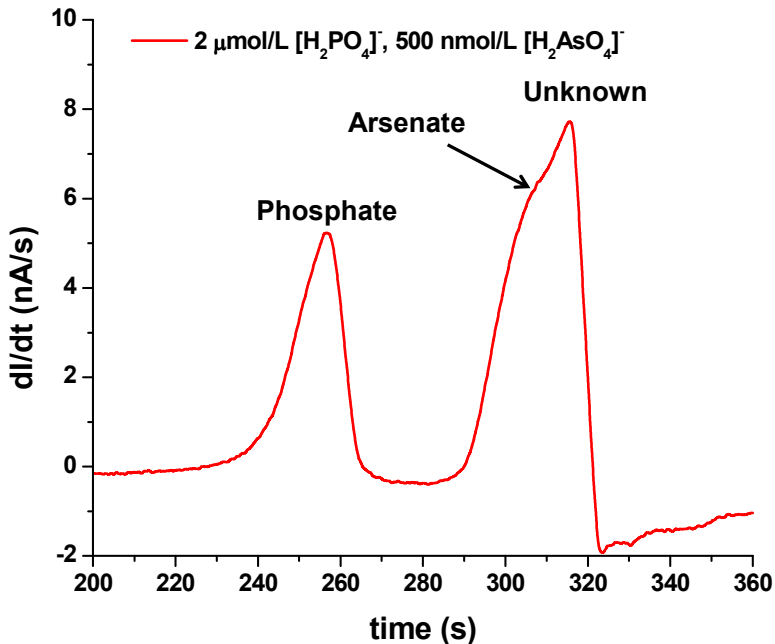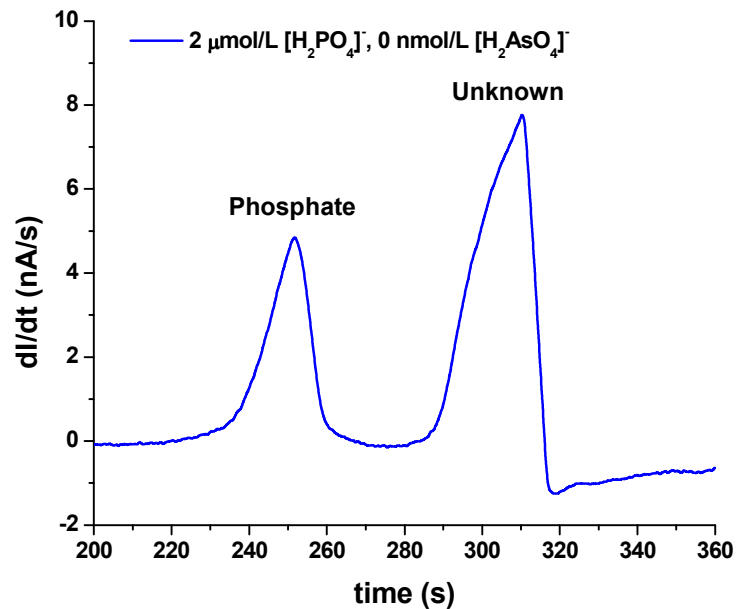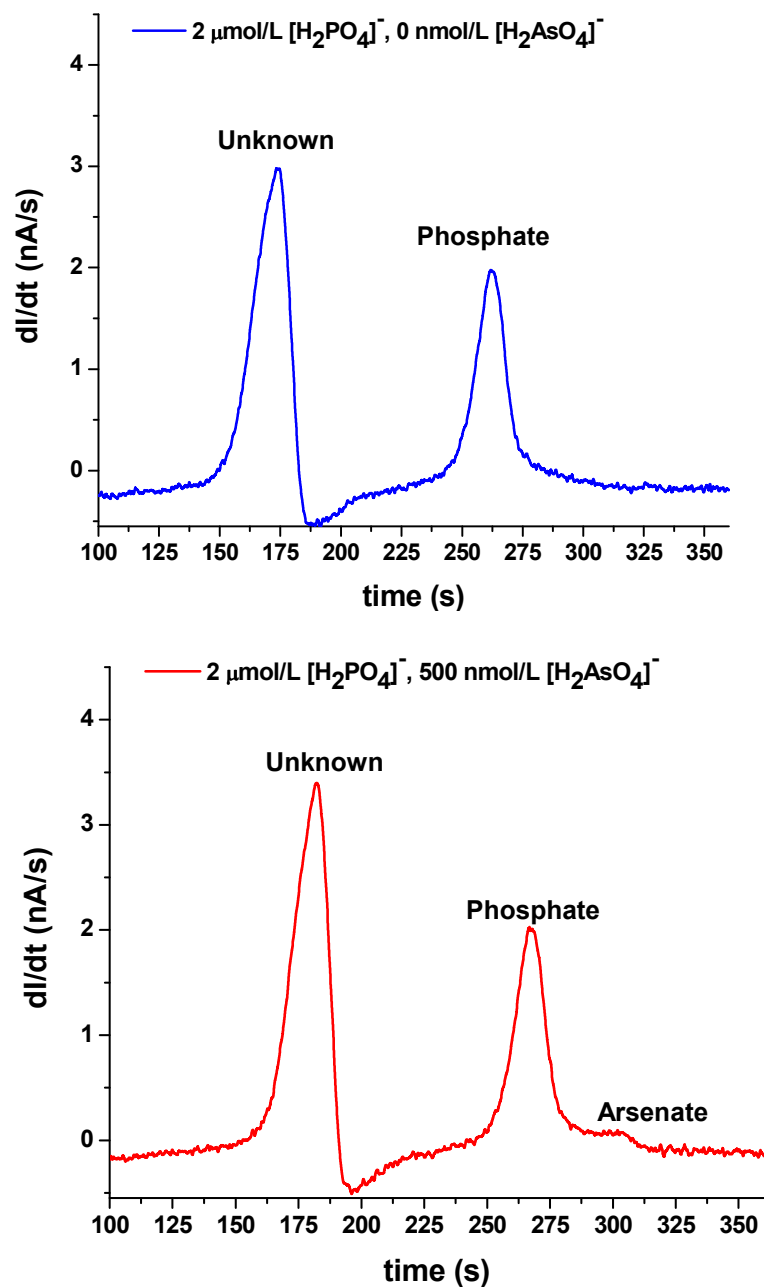
Figure S-10: FACSI-GEMBE of tap water (sample = 9 parts tap water, 1 part run buffer). . Run buffer: 100mM beta-alanine, 70mM HCl, pH = 3.2. The GEMBE data is cut off at 100 seconds to allow visualization of the arsenate peak eluting after the unknown and phosphate peaks.

Calculations

LOD calculation

Figure 2(B): LOD was determined using arsenate data in the concentration range from zero to 50 µmol/L. The slope of the fit line was 0.100 nAL/µmol. The standard deviation (SD) of the fit residuals was 0.300 nA. The LOD was taken to be 3×SD/slope.

Figure 4(B): LOD was determined using arsenate data in the concentration range from zero to 500 nmol/L. The slope of the fit line was 2.760 nAL/µmol. The standard deviation (SD) of the fit residuals was 0.132 nA. The LOD was taken to be 3×SD/slope.

Figure 5: LOD was determined using arsenate data in the concentration range from zero to 500 nmol/L. The slope of the fit line was 2.136 nAL/µmol. The standard deviation (SD) of the fit residuals was 0.144 nA. The LOD was taken to be 3×SD/slope.

Comparison of Figure 2 and Figure 3 data: Conductivity Ratio and corresponding estimated increase in signal.

For FACSI-GEMBE with sample buffer diluted (with DI water) 16 x relative to run buffer:

Measured conductivity:

      1x run buffer = 6.54 mS/cm (no dilution with DI water)

      16x run buffer = 0.796 mS/cm (16 x dilution with DI water)

Calculated conductivity Ratio: (6.54 mS/cm) / (0.796 mS/cm) = 8.21

Step height (phosphate signal) at final concentration of 6.25 µmol/L:

      With FACSI (sample buffer diluted 16x with DI water) = 75.5795

      Without FACSI* (sample buffer equal to run buffer) = 0.1097 * 6.25 = 0.685625

*signal extrapolated to low concentration using the slope of the calibration curve (see Figure 2 inset)

      Signal enhancement = 75.5795/0.685625 = 110.23

LOD improvement (Run buffer/Tap water comparison)

Measured conductivity:

      Run buffer: 6.54 mS/cm

      Run buffer diluted 10 x with tap water: 1.1023 mS/cm*

*determined from fit of conductivity data for run buffer diluted with tap water (See supplemental Figure S11)

Calculated conductivity ratio: (6.54 mS/cm) / (1.1023 mS/cm) = 5.93

LOD for arsenate without FACSI (samples prepared in run buffer) = 11.577 µmol/L

(Figure 2 and S1: 8.958 and 14.196 µmol/L)

LOD for arsenate with FACSI (samples = 9 parts tap water, 1 part run buffer) = 202.732 nmol/L = 0.202732 µmol/L

(Figure 5: 202.732 nmol/L)

LOD improvement: (11.577 µmol/L) / (0.202732 µmol/L) = 57.1x improvement

LOD improvement (Run buffer/DI water comparison)

Measured conductivity:

Run buffer: 6.54 mS/cm

Run buffer diluted 10 x with tap water: 1.0036 mS/cm*

*determined from fit of conductivity data for run buffer diluted with DI water (See supplemental Figure S11)

Calculated conductivity ratio: (6.54 mS/cm) / (1.0036 mS/cm) = 6.52

LOD for arsenate without FACSI (samples prepared in run buffer) = 11.577 µmol/L

(Figure 2 and S1: 8.958 and 14.196 µmol/L)

LOD for arsenate with FACSI (samples = 9 parts tap water, 1 part run buffer) = 139.992 nmol/L = 0.139992 µmol/L

(Figure 4 and S2: 143.32 and 136.664 nmol/L)

LOD improvement: (11.577 µmol/L) / (0.139992 µmol/L) = 82.7x improvement

Figure S-11: Conductivity of tap water/DI water diluted buffer (pH = 3.0)
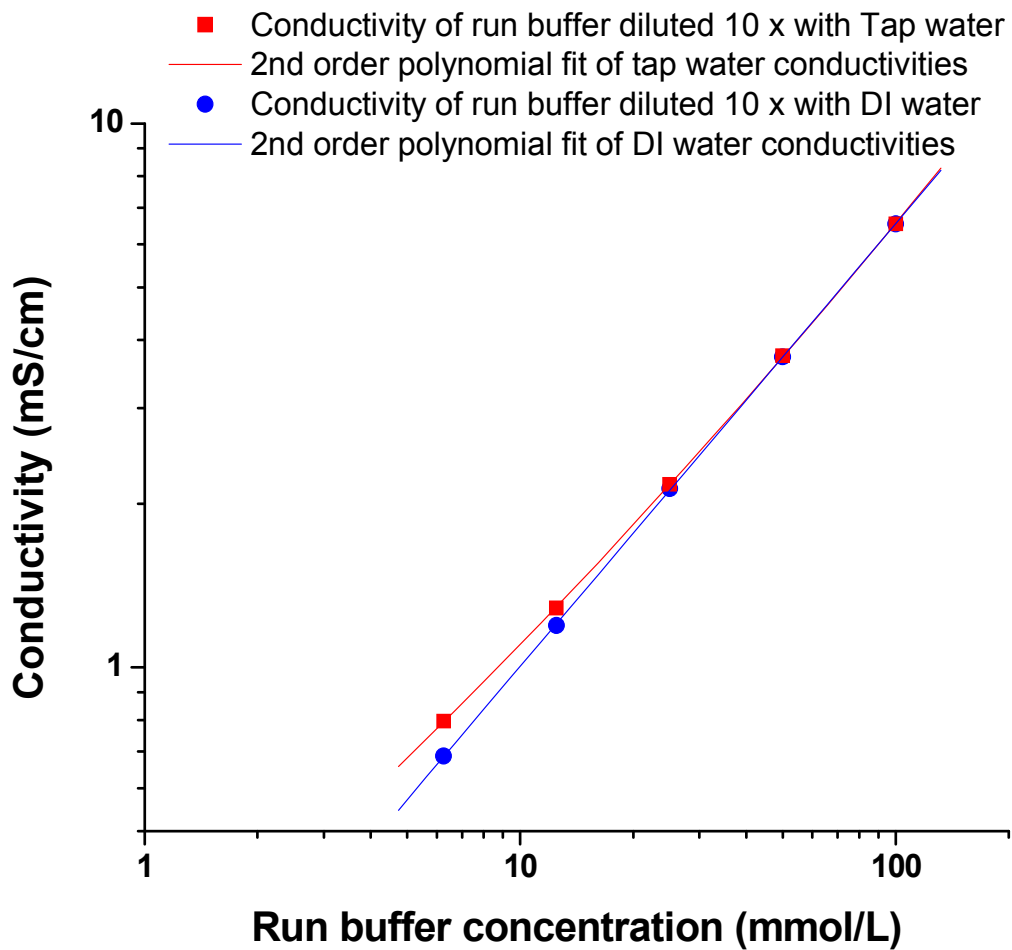
DI water fit:

Log(conductivity) = $(8.87846 * 10^{-4} * (Log(concentration))^2) + (0.81177 * Log(concentration)) - (0.81108)$

Tap water fit:

Log(conductivity) = $(0.06171 * (Log(concentration))^2) + (0.58929 * Log(concentration)) - (0.60862)$