Field-based Detection and Monitoring of Uranium in Contaminated Groundwater using Two Immunosensors

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Abstract

Field-based monitoring of environmental contaminants has long been a need for environmental scientists. Described herein are two kinetic-exclusion based immunosensors, a field-portable sensor (FPS) and an Inline senor, that were deployed at the U.S. Department of Energy, Integrated Field Research Challenge Site in Rifle, CO. Both sensors utilized a monoclonal antibody that binds to a U(VI)-dicarboxyphenanthroline complex (DCP) in a kinetic exclusion immunoassay format; these sensors were able to monitor changes of uranium in groundwater samples from ~ 1μM to below the regulated drinking water limit of 126nM (30ppb). The FPS is a battery-operated sensor platform that could determine the uranium level in a single sample in 5-10 minutes if the instrument had been previously calibrated with standards. The average minimum detection level (MDL) in this assay was 0.33nM (79ppt); the MDL in the sample (based on a 1:200-1:400 dilution) was 66-132nM (15.7-31.4 ppb). The Inline sensor, while requiring a grounded power source, had the ability to autonomously analyze multiple samples in a single experiment; the average MDL in this assay was 0.12nM (29ppt); the MDL in the samples (based on 1:200 or 1:400 dilutions) was 24-48nM (5.7-11.4ppb). Both sensor platforms showed an acceptable level of agreement (r^2= 0.94 and 0.76, for the Inline and FPS, respectively) with conventional methods for uranium quantification.

Introduction

The ability to perform quantitative analyses of contaminants in groundwater samples while still in the field has been a long-term goal for environmental scientists. The need for field-portable assays that can detect heavy metals at concentrations close to their EPA action levels has been particularly hard to meet, since the instruments normally used for such analyses, ICP, ICP-MS, and/or AAS, cannot be easily miniaturized for field applications. Immunoassays have numerous advantages for rapidly determining levels of environmental contaminants. Immunoassay methods are rapid and simple to perform. Compact instruments can be designed to quantify antibody binding; such instruments are thus amenable for use in a field setting. Finally, immunosensors can be modularized such that many different contaminants can be measured using an identical sensor platform; if an antibody to a specific environmental contaminant can be generated, it can be used with this sensor technology. The two instruments described herein and shown in Figure 1 have the ability to meet many of the analytical needs of field investigators: they are relatively portable, easy to operate, acceptably precise, and amenable to the analysis of a wide range of contaminants. Both instruments are flow fluorimeters that use the previously described kinetic exclusion principle [1-3]. This method is a variation of a competitive immunoassay and the uranium assay developed for these instruments utilizes an antibody that binds to chelated U(VI) [4]. The antibody and U(VI)-chelate complex are allowed to incubate until the binding reaction reaches equilibrium (5 minutes or less), and the reaction mixture is then exposed briefly to chelated uranium immobilized on the surface of beads packed into an observation/flow cell. Unbound antibody, which can either be covalently modified with a fluorophore or labeled via a fluorescent anti-species antibody, binds to the chelated U(VI) complexes on the beads while antibody bound to environmental uranium is washed from the bead pack. The signal from the

fluorescent antibody remaining on the beads is transduced to an electrical signal by the immunosensor. These two sensors were used to assay aqueous uranium in groundwater samples collected during field experiments at a contaminated site near Rifle, CO.

The Rifle locale is a Uranium Mill Tailings Remediation Action (UMTRA) site that has been the location of ongoing *in situ* bioremediation experiments for the past three years as part of the U.S. Department of Energy's Integrated Field Research Challenge site (IFRC). Both the site and the field bioremediation research have been described extensively elsewhere [5-7] and a more complete description of the ongoing work at the Old Rifle UMTRA site may be found at http://www.pnl.gov/nabir-umtra/intro.stm. In the summer of 2008, much of the research activity was focused upon the collection of samples for proteomic analyses [5,8-11], and timely information about the extent of uranium immobilization or remobilization in groundwater samples was useful to the research team in the timing of sample collection.

This report details the development and validation of assays for hexavalent uranium utilizing both an Inline immunosensor previously described by our laboratory [12] and a newer Field Portable Sensor [13]. Both immunosensors provided data that was in good agreement with off-site analysis of duplicate samples by Kinetic Phosphorescence Analysis (KPA). A catalytic beacon sensor for U(VI) has also been reported that, under laboratory conditions, showed a sensitivity and selectivity approximately equivalent to the antibody-based sensors reported herein. The beacon sensor has not yet been validated in the field studies and preliminary tests on soil samples required a 20-hour sample extraction [14]. The data presented herein represent the first that has utilized an immunoassay to monitor heavy metal contamination during the progression of an active field experiment. These immunosensors were able to provide near real-

time data on the levels of hexavalent uranium from initial concentrations of ~1μM to those below the EPA permissible drinking water limit of 30ppb (126nM).

Experimental section

***Materials*.** 2,9-Dicarboxy-1,10-phenantroline (DCP) was purchased from Alfa Aesar (Heysham, UK). Uranyl diacetate was a product of Mallinckrodt Chemical Works (St. Louis, MO); standard solutions used as calibrators were referenced to NIST Standard Reference Material 3164 (Lot No. 891509). Goat anti-mouse IgG Fab conjugated to Cy5 and DyLight 649 were purchased from Jackson ImmunoResearch (West Grove, PA). HEPES-buffered saline (HBS, 137mM NaCl, 3mM KCl, 10mM HEPES, pH 7.4) was prepared using reagents from Sigma-Aldrich. (St. Louis, MO). All buffers were prepared using water purified by a Nanopure II water purification system (Barnstead International, Dubuque, IA). Bovine serum albumin (BSA, Fraction V, ultrapure) was purchased from Sigma-Aldrich (St. Louis, MO). UltraLink Biosupport beads (50-80 micron diameter) were purchased from Pierce (Rockford IL). Polystyrene beads (98 micron diameter) were obtained from Sapidyne Instruments (Boise ID). The isolation and characterization of the monoclonal antibody used in this study, 12F6, has been previously described [4]. The BSA-DCP conjugates were available from previous studies [4,12,15]. An artificial groundwater sample with an ionic composition similar to that at the Rifle site was prepared according to a formulation developed by Dr. Kate M. Campbell of the USGS, Menlo Park, CA. This formulation is provided in the Supplemental Materials as Table S1.

Instrumentation. The two prototype sensors employed in this study, as shown in Fig. 1, were developed in conjunction with Sapidyne Instruments and are based on the principle of kinetic exclusion [1-3]. The first instrument used in this study was a novel field-portable sensor (FPS) (Fig 1A). This instrument is lightweight, portable, and has a self-contained power supply. The

FPS employs a disposable flow/observation cell prepacked with a capture reagent especially designed for the assay of an individual environmental contaminant. Results are available within 3-5 minutes after sample injection if the sensor has been previously calibrated with standards. The complete analysis (calibration and sample analysis) requires ~1 hour. The current instrument has a relatively low sample throughput because the disposable flow/observation cell must be replaced after the analysis of 2-3 environmental samples. The second immunosensor deployed for this study, the Inline immunosensor (Fig 1B) is an autonomous instrument that can store and autonomously mix all reagents needed for the assay [12]. The instrument is controlled via a timing file created by the end-user; the timing file used for all experiments reported herein is included as Table S2 in Supplemental Materials. This is a higher throughput instrument that permits automated measurements of multiple samples in the course of one experiment.

***Collection and preparation of environmental samples*.** The test plot for the current study, shown in Figure S1, consisted of an area 12 x 18 meters that contained three tiers of four monitoring wells positioned down-gradient from an acetate injection gallery. The wells in the tier closest to the injection gallery were designated D-01 to D-04, those in the middle tier, D-05 to D-08, and those in most distal tier, D-09 to D-12. Three control wells located up-gradient of the injection gallery, designated U-01 through U-03, were also sampled during a period of acetate injection that spanned 71 days in the summer of 2008.

After the initiation of the bioremediation experiments, samples were collected from downfield and upfield monitoring wells at varying times and after purging ca. 12L of groundwater. Each groundwater sample was filtered using a 0.2μm IC-Millex filter (Millipore Inc., Billerica MA) and the pH of an aliquot (45 ml) of each filtered sample was adjusted to ~2 by the addition of 0.225mL of 8M nitric acid. A split of each sample was sent to the laboratory

(S.M. Stoller Corp., Grand Junction, CO) that performs U(VI) analysis for the Rifle IFRC via Kinetic Phosphorescence Analysis (KPA) [16]. Acidified samples were stable for at least 12 months at 4^{o}C. Prior to analysis by the immunoassay instruments, the environmental samples were diluted into HBS containing 400nM DCP. Neutralization of the acidified groundwater samples in the presence of a molar excess of DCP insured that all of the U(VI) in the groundwater was converted to the U(VI)-DCP complex recognized by the 12F6 antibody.

Analysis of environmental samples using the Field Portable Sensor. BSA-DCP was coated onto polystyrene beads and charged with a 1μM solution of uranyl acetate as described previously [12]. The presence of uranyl acetate resulted in the formation of a BSA-DCP-U(VI) complex on the bead surface. The U(VI)-DCP-coated beads were packed into disposable flow cells that had been fitted with circular 20 micron filters to contain the beads; both the disposable flow cells and filters were supplied by Sapidyne Instruments. Non-specific binding sites on flow cell were blocked by injecting 1mL of 3% BSA in HBS through the flow cells. The flow cell was subsequently rinsed with 5mL HBS before it was inserted into the sensor. Each disposable flow cell was calibrated before analysis of field samples. All calibrators and environmental samples were mixed by the operator. Calibration solutions contained 12F6 monoclonal antibody (1.5nM), Cy5 Fab (15nM), BSA (50μg/mL), DCP (200nM), U(VI) at 0, 1.0, 2.0 or 10nM (0-2.38ppb) and a 1:400 dilution (0.25%) of artificial groundwater (Table S1). In later experiments, 12F6 monoclonal antibody was reduced to 0.25nM and DyLight 649 Fab (5nM) replaced the Cy5 Fab; all other reagent concentrations were unchanged. For analysis of environmental samples, the U(VI) standard was replaced by a 1:200 or 1:400 dilution of acidified groundwater from the Rifle site. Each calibration curve included duplicates at each uranium concentration plus a determination of non-specific binding (12F6 monoclonal antibody omitted from the assay). After

the calibration curve was established, duplicate (Cy5 assays) or triplicate (Dylight 649 assays) determinations were performed for each environmental sample.

The instrument was controlled wirelessly via a laptop computer, and a sensogram and a "delta" value were displayed for each U(VI) standard or environmental sample. The deltas for the standard curve were fit using SlideWrite® software (Advanced Graphics Software, Carlsbad, CA) and the following binding equation:

$$y = a0 - (a1x)/(a2+x) \qquad \text{(Eq. 1)}$$

where *a0* is the delta when no U(VI) is present in the sample (the y intercept), *a2* is the K_d value for the 12F6 antibody, and *a0* is x-dependent change in the delta as x approaches infinity. The delta of the environmental sample was then compared to this curve in order to determine U(VI) concentration. The average minimal level of detection for the FPS was estimated by determining the 95% confidence level for zero value from computer-fitted lines of all standard curves using a method described in more detail in Figure S2 and a previous publication [17].

Analysis of environmental water samples using the Inline sensor. Analysis for uranium with the Inline sensor was performed using antibodies and reagents identical to those employed with the FPS. In contrast to the FPS, however, the Inline instrument automatically packed a fresh column of beads in the flow/observation cell before the collection of each new data point. The instrument was programmed to generate a U(VI) standard curve in triplicate, and then analyze 7 individual environmental samples, also in triplicate. Once the reagents and environmental samples had been loaded into the instrument, all further operations were automatic. Total time required for the analysis of one sample was approximately 11 minutes; the entire program, which included generation of a 6 point standard curve and analysis of 7 environmental samples, all in triplicate, was 11-12 hours. Data analysis, which included a function that corrected for

instrumental drift during the duration of the experiment, was performed using the software developed by Sapidyne for the instrument. BSA-DCP was covalently conjugated to Ultralink Biosupport beads as described previously [12]. Beads for each day's experiment (50 mg) could be stored at 4^{o}C for up to 4 weeks in 1 mL of HBS containing 3% BSA and 0.1% NaN_3. These beads were diluted into 30 mL HBS containing 1μM uranyl acetate and loaded into the sensor. Excess uranyl acetate was washed away during automated bead handling steps performed by the sensor with a buffer comprised of 200nM DCP in HBS. The following reagents were also prepared and loaded into the sensor: 1) Purified monoclonal 12F6, 1.5nM (0.23μg/mL) in HBS containing 0.1mg/ml BSA; 2) Purified Cy5-labeled Fab, 15nM in HBS containing 0.1 mg/mL BSA; 3) U(VI) stock solution, 12nM uranyl acetate in HBS containing 400nM DCP and 1% artificial groundwater; 4) Diluent for U(VI) stock, 400nM DCP in HBS containing 1% artificial ground water; 5-11) Acidified environmental samples diluted 1:100 or 1:200 into 400nM DCP in HBS. The autonomous operation of the Inline instrument has been described elsewhere [12]. Briefly, the instrument packed U(VI)-DCP-coated beads into the capillary flow/observation cell, washed a mixing tube, added antibody solutions to either uranium standard solutions or to an environmental sample, and passed the resultant mixture over the beads in the observation/flow cell. Excess antibody was washed from the flow cell with buffer and the change in signal from the beginning and the end of each run, the "delta", was calculated for each reaction mixture. The final concentrations in the calibration assay mixtures applied to the flow cell were as follows: 12F6 mAb, 0.375nM; Cy5 Fab, 3.75nM; BSA, 50μg/mL, DCP, 200nM, U(VI) standard solutions, 0, 0.6, 1.2, 2.25 or 6nM (0-1.43ppb), and artificial groundwater, 0.25%, all in HBS. Assay mixtures with environmental samples contained 12F6 mAb, 0.375nM; Cy5 Fab, 3.75nM; BSA, 50μg/mL, DCP, 200nM, and a 1:400 dilution of the filtered, acidified groundwater sample,

all in HBS. Data analysis was performed by the Inline software, which calculated a binding curve using deltas from the U(VI) standards and automatically compared deltas from the environmental samples to this curve. The minimal level of detection (MDL) for each experiment was determined by calculating the mean and SD for the delta values obtained from samples without U(VI). The SD was multiplied by 2, and subtracted from the mean value. The U(VI) concentration that corresponded to this 0-2SD calculated delta was determined from the curve to be the MDL.

Results

Field portable sensor and uranium analysis. One of the main advantages of the FPS is its ability to operate independently from a grounded power supply. The instrument was transported in a backpack-like bag that also carried all of the supplies required for the assay, including buffers, mixing tubes, racks, syringes, pipettes, and reagents. The total weight of the instrument and all associated reagents and supplies was approximately 10kg. This resulted in a degree of portability not seen with many analytical instruments. Like the Inline sensor, the FPS measured the amount of fluorescence before and after injection by subtracting the pre-injection baseline from the post-injection baseline to determine a quantity known as the "delta". The delta value is inversely proportional to the amount of uranium present in the sample; a sample with no uranium provides the highest delta value. After determining the deltas for samples containing known quantities of uranium, a standard curve can be constructed and the amount of U(VI) in an environmental sample could be determined by comparison to this standard curve. A typical data trace and standard curve obtained with the FPS is seen in Figure 2A. Once the operator had loaded a syringe containing the assay mixture into the instrument, all further operations were automatic. The FPS was programmed to 1) flush any existing sample from the instrument; 2) fill

the lines with fresh sample; 3) inject an aliquot of the fresh sample over the observation/flow cell; and 4) rinse the observation/flow cell with buffer provided from a reservoir. The instrument began recording the baseline fluorescence five seconds prior to injection of the sample and continued until after the bead rinse. A delta signal for an individual sample could be determined in 140 seconds. Concentrations of U(VI) equaling 0, 1, 2, and 10nM were used to generate a standard curve (+ in the *inset* of Fig 2A). Non-specific binding of the secondary antibody (shown as the closed squares in the inset) was determined by injecting a solution containing no uranium and only the Cy-5 labeled anti-species antibody. The equation used to fit the data for the standard curve (Eq. 1), describes a one-site homogeneous protein-ligand binding interaction [2] and the value of the equilibrium dissociation constant (K_d) of 12F6 with its ligand (U(VI)-DCP) is equal to the *a2* constant derived from fitting this equation to the data. The K_d determined for U(VI)-DCP (1.3nM from the curve fit, as compared to the published value of 0.9nM [1]) and the excellent fit of the data points to Eq. 1 ($r^2 = 0.99$) provided an internal control that the assay was functioning properly. The average minimum level of detection in the assay was estimated to be 0.33nM; the average MDL in the sample, based on a1:200 or 1:400 dilution, was 66-132nM (15.7-31.4ppb).

Uranium analysis using the Inline sensor. The curves generated automatically by the Inline software are shown in Figure 2B. The differences in the appearance of the standard curves shown in Figure 2A and 2B are a result of the software support available for each instrument. The FPS provided only the delta values for each experimental point, and these delta values were analyzed as described above. The Inline sensor, which is now available commercially from Sapidyne Instruments Inc, had an associated software package that automatically calculated the standard curve and the converted the delta values to relative proportions. The open diamonds in Fig. 2B

represent the U(VI) standards used to calibrate the instrument and the closed diamonds represent data from the environmental samples. Because of the log scale used by the software, the points determined for standards with no U(VI) are not shown on the curve, although they were used by the software for curve fitting. Early in the experiment the uranium concentrations were approximately the same in all samples, and the environmental data points on the instrument-generated curves appeared as a cluster (data not shown). As time passed and uranium immobilization was observed in some of the wells, soluble U(VI) levels decreased and the environmental data points began to spread out along the curve, as shown in Figure 2B. The mode of data presentation employed by the Inline software therefore made it simple to determine visually when the levels of soluble U(VI) had been affected by the bioremediation activities at the site. When the levels of uranium neared the limit of detection of the assay, samples could be re-run at a lower dilution (usually 1:200); doubling the amount of environmental sample in the assay mixture had no effect on the assay (data not shown). Minimum levels of detection (MDL) for individual assays ranged from 0.014 to 0.260nM (3-62ppt). The average assay MDL for experiments performed during the 2008 Rifle field activities was 0.12nM (29ppt). Since the samples were diluted 1:200 or 1:400 before analysis, the MDL for the environmental samples was 24-48nM (5.7-11.4 ppb). The Inline data analysis software also calculated the K_d of the antibody during each experiment. The K_d's determined during the experiments at Rifle ranged from 0.5 to 1.5nM, which compared well to the previously published value of 0.9nm and further validated that the antibody was performing in an acceptable manner.

Comparison of immunosensor data with KPA. Because of the extensive activity at the Rifle site during bioremediation experiments, the high throughput and automated operation of the Inline sensor made it the most convenient instrument for analysis of the large numbers of samples

collected at the site. Splits of all environmental samples were also sent to a contractor's analytical laboratory in Grand Junction, CO for analysis by KPA. The KPA method measures the phosphorescence emission from a uranyl chelate complex as a function of time [16,18]. Figure 3 shows a comparison of results obtained by Inline sensor and KPA in groundwater samples collected at varying times after the initiation of acetate injection from four separate wells at the Rifle site. Wells D-01 and D-04 were located in the first tier of wells downfield from site of acetate injection, and as expected were the first to show signs of uranium immobilization on day 4 after initiation of acetate injection. Later, as acetate began to migrate through the test site, uranium immobilization was also observed in a third tier well, D-11. The initial 14-day period of acetate injection was followed by an 8-day acetate-free groundwater flush, after which time the level of uranium began to rise in the first tier of monitoring wells approximately 22 days after the start of the injection. Uranium levels peaked at 35-40 days and then began to fall again once acetate injection resumed. This rise and fall in uranium levels was echoed in the next tier of downfield wells at slightly later dates reflecting the advective transport of acetate and uranium depleted groundwater. The level of uranium observed in U-02, a control well upfield from the acetate injection, remained constant throughout the course of the experiment at the Rifle site. The measurements obtained using the Inline sensor were consistently within two standard deviations of the measurements determined with KPA.

A global comparison of U(VI) values determined using the immunosensors with U(VI) values determined via KPA by an independent contractor is shown in Figure 4A and B. These graphs incorporate all of the uranium immunoanalyses performed on groundwater samples collected from the 18 wells present at the Rifle site. The immunoassay data is shown with its standard deviation, the KPA data provided by the contractor was a single value with no reported

error. In general, the two methods showed close agreement. Linear regression analysis of the data in 4A yielded a slope of 0.94, a y-intercept of 5.7, and a correlation coefficient of 0.94; analysis of the data in 4B yielded a slope of 0.99, a y-intercept of 11.3 and a correlation coefficient of 0.74.

Discussion

A number of technical problems had to be solved before this immunoassay performed properly in the field. Antibody 12F6 was irreversibly inactivated by covalent modification of its amino groups (data not shown), but could be rendered fluorescent via the addition of a 10-fold molar excess of a fluorescently labeled anti-species antibody. We used commercially available Cy5 or DyLight 649-labeled Fab fragments rather than an intact Cy5-IgG to avoid the cross-linking and subsequent precipitation that can occur in reaction mixtures containing bivalent secondary antibodies. The immunosensors have very sensitive detection systems, and more than 200 experimental samples (including all calibrators) could be assayed with a single milligram of 12F6 antibody.

The 12F6 antibody recognizes U(VI) in a complex with DCP with subnanomolar sensitivity [4]. Thus, all of the uranium in the environmental samples from the Rifle site had to be dissociated from natural complexants present in the groundwater [19,20] and subsequently transformed to DCP complexes. A variety of pretreatment strategies were tested (data not shown), and the most successful involved a simple addition of acid to bring the pH of the environmental sample to ~2, and then a neutralization via dilution of the environmental sample into buffer containing a molar excess of DCP. The high affinity of 12F6 for the U(VI)-DCP complex meant that the environmental sample could be significantly diluted (1:200 to 1:400)

before analysis. This large dilution mitigated matrix effects, as demonstrated by the good agreement of our immunoassay results with those determined by KPA.

The two immunoassay instruments tested at the Rifle site provided complementary functions. The Inline sensor, which required a grounded power source, provided automated analysis of a large number of samples; however, once the pre-programmed cycle had begun, it usually required 10-12 hours to finish an experiment. We therefore used the FPS primarily to "break queue" when other scientists at the site required a rapid analysis of a small number of samples. One operator could easily handle both instruments, since the Inline sensor required only 30-60 minutes of setup before autonomous operation for 10-12 hours. The FPS was battery powered and hand operated, making it useful for rapid analysis of a small number of samples. The design of FPS requires that multiple assay mixtures to be applied to the same column of beads during the analysis. When too many samples were injected onto the same bead column, however, sensor response began to decrease. In order to deal with the limited capacity of the FPS bead packed into each disposable flow/observation cell, only single injections of each U(VI) concentration were used to generate the standard curve; environmental samples were analyzed in triplicate. Under field operations at the Rifle site, only 2 environmental samples could be analyzed with one prepacked flow/observation cell. Since each change of beadpack required a recalibration with U(VI) standards, sample throughput with the FPS was low. Increasing the capacity of the disposable flow/observation cell used in this instrument is an area of active investigation in our laboratory.

The immunosensors described in this study had the ability to determine the levels of a contaminant, in this case U(VI) in environmental samples. While the Inline sensor showed a higher degree of precision than the FPS, both immunosensors produced measurements that were

consistent with KPA. The total reagents costs per sample for the Inline instruments were less than $1.00, which is comparable to that of KPA analysis and consistent with what has been reported for other Inline sensor assays [21]. The cost for FPS analysis was even lower because this instrument used a lower concentration of primary and secondary antibody in the assay mixtures and fewer calibrators. Unlike the KPA, however, whose capabilities are limited to the detection of uranium and other lanthanides, these immunosensors can be adapted for use in the detection of a wide variety of low molecular weight contaminants. A significant strength of the Inline and FPS instruments are their ability to be rapidly reconfigured for the analysis of other analytes. Assays for other heavy metals, PCB's, 2,4-dichlorophenoxyacetic acid, environmental estrogens, organophosphate pesticides, imidazolinone herbicides and TNT have been published using the KinExA™ technology (for a review, see [22]). These new field deployable sensors will provide researchers and resource managers with an invaluable tool for generating near real-time data and modifying field experiments already in progress.

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Supporting information is available including a detailed description of the Rifle site, more information about how MDL's were determined for the FPS, the composition of Rifle artificial groundwater and the timing file used for the Inline sensor. This information is available free of charge via the Internet at http://pubs.acs.org.

Brief

Antibody-based sensors provided near real-time data on the groundwater levels of uranium from ~1μM to less than 126nM (EPA action level) during a field bioremediation experiment.

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Figure Legends

Figure 1. Two kinetic exclusion-based instruments useful for field-based studies. A. ***The beta prototype field-portable sensor.*** This battery-operated sensor permits sample analysis in remote areas without a power source. The sensor interfaces with a laptop computer or other control device through a wireless connection and weighs less than 7 kilograms. After the generation of a standard curve, sample analysis can be completed in minutes. ***B. The Inline sensor***. This instrument has the ability to autonomously run a standard curve from stock reagents and to prepare and analyze environmental samples [12].

Figure 2. Analysis of environmental samples with the immunosensors. ***A. Sensogram and standard curve from FPS.*** The data trace shown was typical for an experiment performed on this instrument. Pre-injection baseline (0-5 sec) was subtracted from the post-injection baseline (55-60 sec) to determine a delta signal. *Inset,* ***Standard curve generated using the FPS.*** Samples containing known concentrations of U(VI) (✚) were loaded into the sensor; the resulting data points were fit as described in the *Experimental section.* After generation of a standard curve, environmental samples (●) were loaded into the sensor. The delta signals obtained were compared to the standard curve to determine the concentration of U(VI) in the environmental samples. The closed squares (■) on the y-axis represent non-specific binding, the delta obtained in the absence of primary antibody. ***B. Standard curve generated by Inline sensor.*** This panel represents an actual standard curve generated by the instrument software. U(VI) concentrations in the environmental samples were automatically reported. The open diamonds represent the U(VI) standards used to calibrate the instrument and the closed diamonds represent data from the environmental samples.

Figure 3. Comparison of Immunosensor and KPA results. Results are shown for 3 wells downgradient of the injection wells (***D-01, D-04 and D-11***) and one up-gradient well that was used as a control in these bioremediation experiments (***U-02***). The numbers on the x-axis represent the days after the initiation of acetate injection at the site. Values for U(VI) determined by immunosensor and KPA are shown by solid and hatched bars, respectively. Each immunosensor determination was performed in triplicate and error bars represent the standard deviation of three measurements of the same sample.

Figure 4. Immunosensor data correlates with KPA data. AU(VI) values in groundwater samples, as determined using the Inline sensor (Panel A) or the FPS (Panel B) were plotted versus KPA data for 112 groundwater samples analyzed from the summer 2008 experiment. Linear regression analysis of the data in 4A yielded a slope of 0.94, a y-intercept of 5.7, and a correlation coefficient of 0.94; analysis of the data in 4B yielded a slope of 0.99, a y-intercept of 11.3 and a correlation coefficient of 0.74. The immunosensor points are plotted as the mean $\pm$ SD of three replicates; KPA data was provided without error analysis.

Figure 1

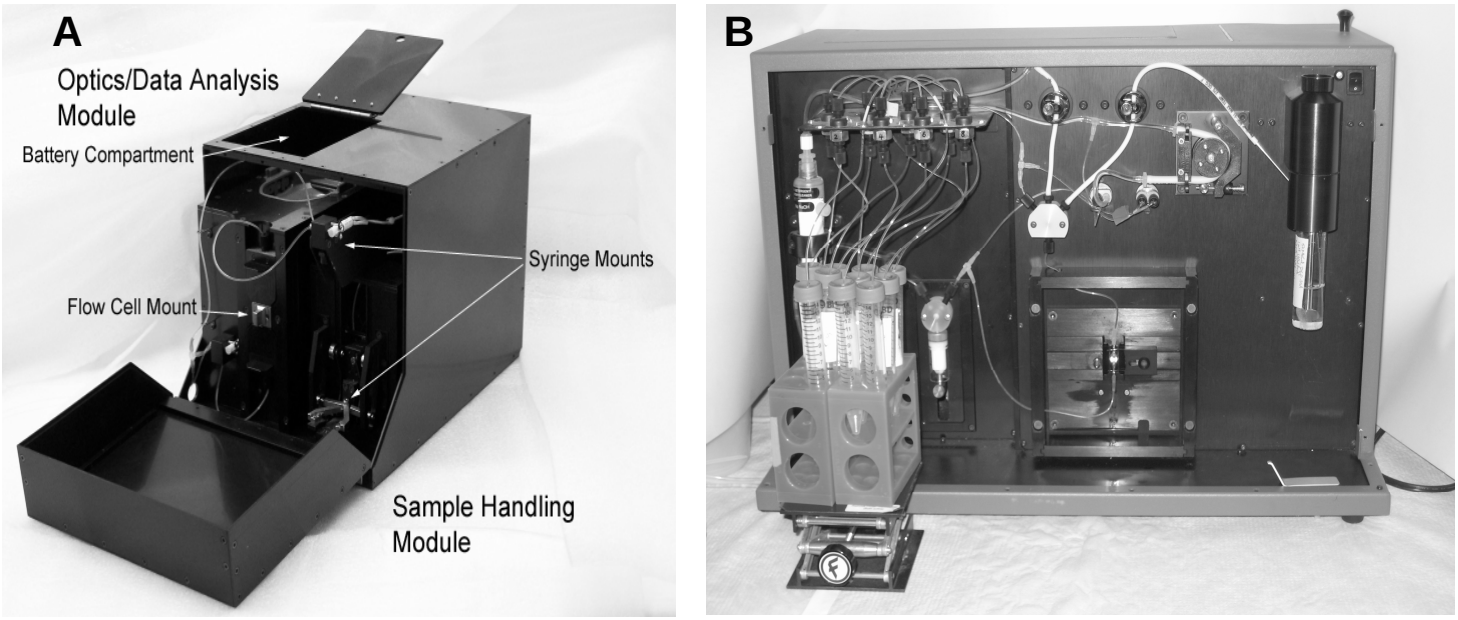


Figure 2

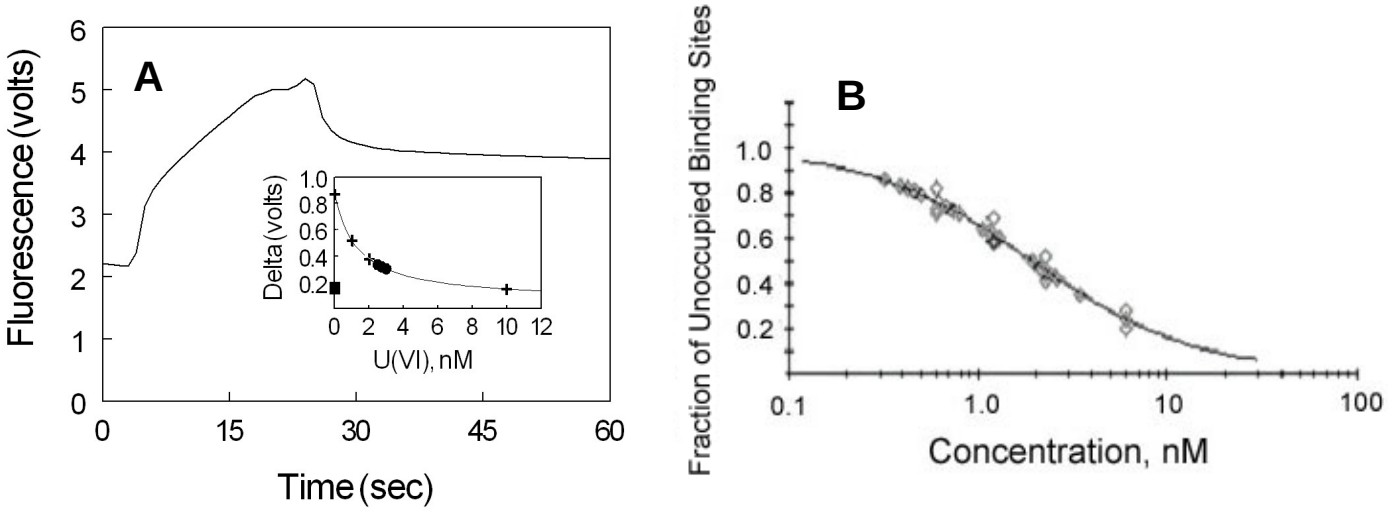


Figure 3.

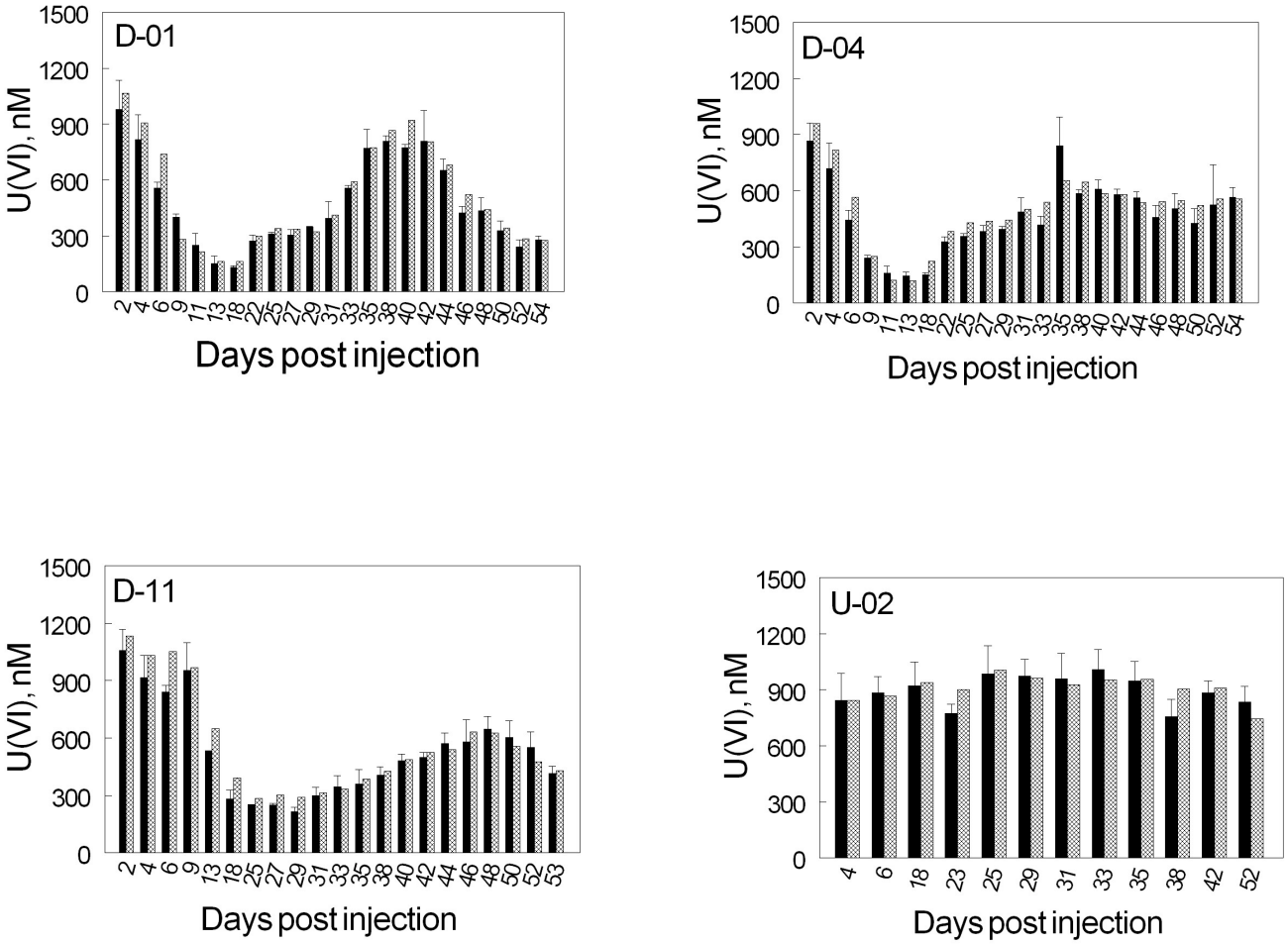
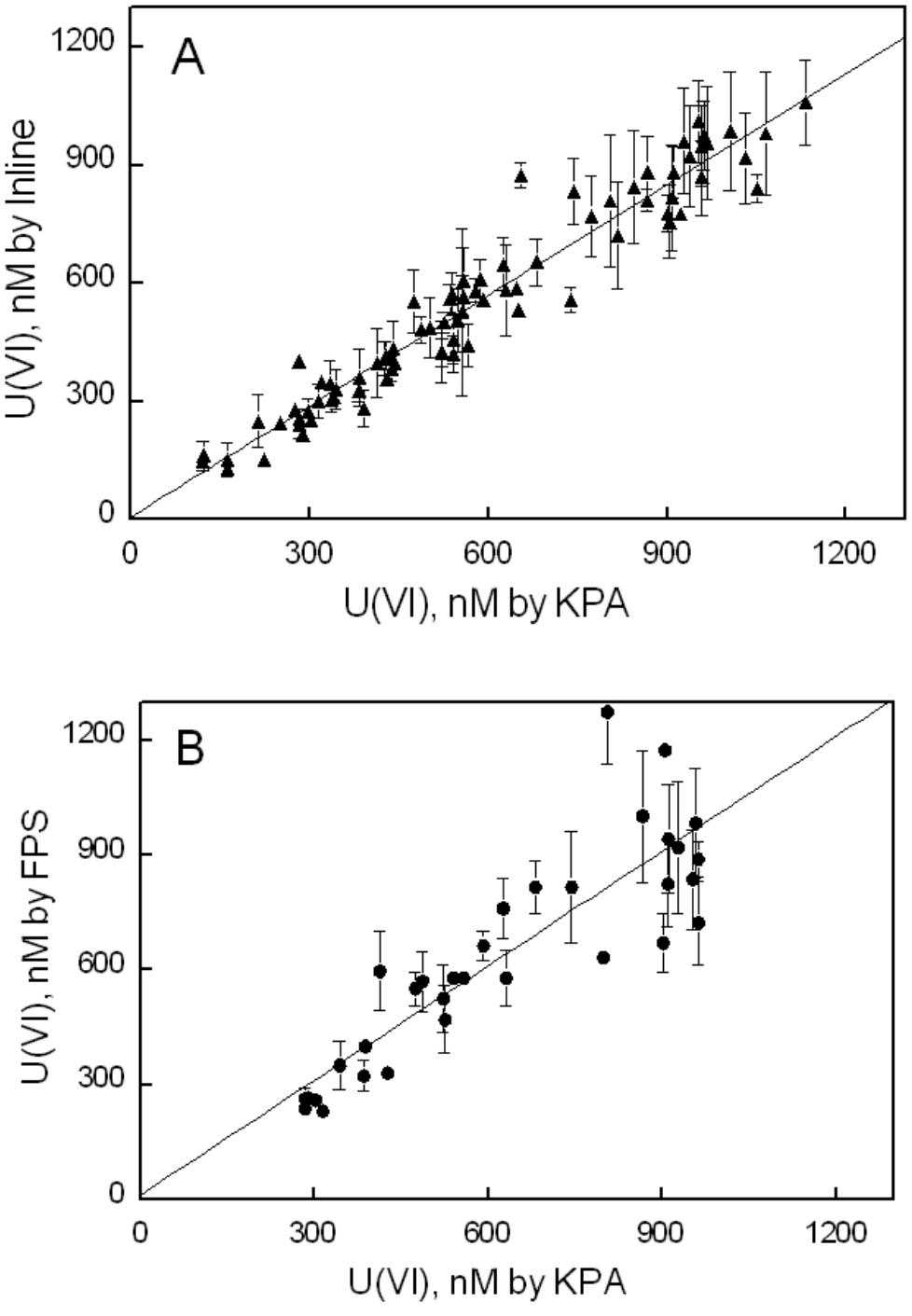

D-01
U(VI), nM
1500
1200
900
600
300
0
2 4 6 9 11 13 18 22 25 27 29 31 33 35 38 40 42 44 46 48 50 52 54
Days post injection
D-04
U(VI), nM
1500
1200
900
600
300
0
2 4 6 9 11 13 18 22 25 27 29 31 33 35 38 40 42 44 46 48 50 52 54
Days post injection
D-11
U(VI), nM
1500
1200
900
600
300
0
2 4 6 9 13 18 25 27 29 31 33 35 38 40 42 44 46 48 50 52 53
Days post injection
U-02
U(VI), nM
1500
1200
900
600
300
0
4 6 18 23 25 29 31 33 35 38 42 52
Days post injection

Figure 4


A
U(VI), nM by Inline
U(VI), nM by KPA
0
300
600
900
1200
B
U(VI), nM by FPS
U(VI), nM by KPA
0
300
600
900
1200