Field-portable and Automated Immunosensors for Hexavalent Uranium, Other Heavy Metals and Chelators

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Introduction

A better understanding of remediation processes depend upon accurate detection and measurement of contaminants prior to, during, and following the remediation process. Detection and measurement costs are presently high due to the complex methodologies required for analyses. The immunosensor-based assays being developed and refined in this project will yield reliable data in real time (i.e., less than 1 hour), be field ready (i.e., simple, durable, and accurate), and have relatively low acquisition costs (~$5000 and $15,000 for the handheld and in-line sensors, respectively), and disposable costs of less than $100/assay.

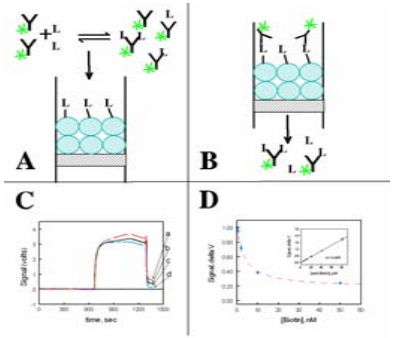


***Figure 1. The immunosensor method*. Panel A**, fluorescently labeled antibody (**Y**) is incubated with its specific ligand (**L**) **Panel B**, The incubation mixture is passed through a microcolumn of beads that contains an immobilized version of the ligand **(-L**). Panel C, fluorescent signal (measured in volts) is monitored; the amount of antibody bound to the column (see a-d) is inversely proportional to the concentration of ligand in the original sample. **Panel D**, The amount of antibody bound to the beads can be expressed as the difference (delta) in the average signal in the background portion of the trace and the signal after the automated wash step. When this delta signal is replotted versus ligand concentration, a standard curve is generated. *Inset,* Delta signal is a linear function of the concentration of antibody that flows through the column.

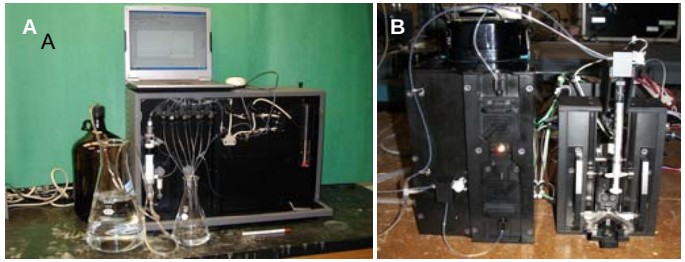


Figure 2. Completed In-line and field-portable sensor under development with Sapidyne Instruments Inc. **Panel A**. An automated sensor that has the ability to autonomously run a standard curve from stock reagents and to prepare and analyze environmental samples (Yu et al. (2005) *Int. J. Env. Anal. Chem.* **85**:817) . **Panel B**. A field-portable device that will analyze environmental samples on-site. The operator mixes antibody with the environmental sample, and loads the mixture into a syringe. The sample is injected over the flow cell by the instrument and the concentration of contaminant is determined by comparison to calibrators.

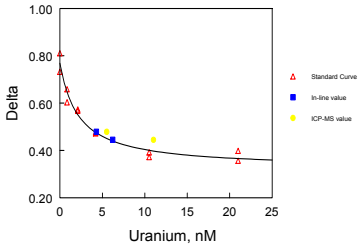
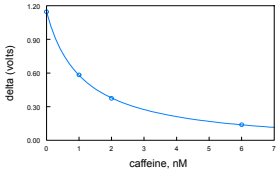
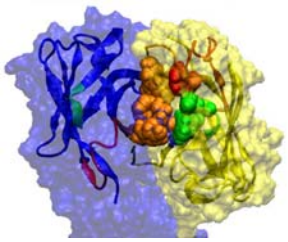


Figure 3. Results from field test at ORNL. The in-line device shown in Panel A above was transported to ORNL and set up in the laboratories there. After signal tests to insure that the sensor was not damaged, the instrument was loaded with reagents. The sensor autonomously mixed samples with known quantities of uranium (VI) (0-21 nM), and determined the delta (Δ) then mixed two samples from the Criddle site with reagents (buffer, chelator, 12F6 antibody and Cy5-labeled anti-mouse Fab) for comparison to the instrument-generated standard curve.

Figure 5. Results from preliminary testing of field-portable sensor. These tests were performed using commercially available antibodies and capture reagents (anti-caffeine and caffeine-BSA). The use of these nontoxic reagents allows researchers at Tulane and Sapidyne Instruments to compare sensor performance at both research sites.



New technologies in antibody engineering that will enhance this sensor project



Light Chain	Heavy Chain	Activity (%)
none	none	100
L- Y54V		>5
L- K55A		155
L F101A		126
L- F103A		>5
	H- F107A	69
L- Y37A		33
L- H39A		>5
	H- Y102A	33
	H- S104A	84

Figure 6. Molecular model of the ligand-binding site of 12F6, an antibody that binds chelated uranium (VI). 12F6 was cloned, sequenced and expressed as a recombinant Fab fragment. Molecular dynamics were used to predict which amino acid side-chains in the light and heavy chain of the antibody molecule were important for uranium recognition. **Table**, Site-directed mutagenesis was used to confirm predictions made from the molecular simulations. In two cases, changes in light chain amino acid sidechains (mutation of lysine 55 to alanine and of phenylalanine 101 to alanine) actually improved the binding activity of the antibody.

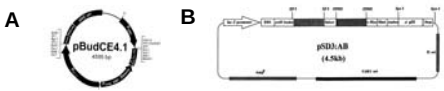


Figure 7. Vectors used in the laboratory to construct recombinant forms of uranium-binding antibodies. Panel A. Commercial dicistronic vector used to express Fab fragments. **Panel B**. Vector used to construct phage-displayed recombinant antibody libraries.

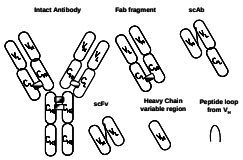


Figure 8. Antibodies and antibody fragments isolated in our laboratories that have the ability to bind chelated uranium (VI).

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