In-line Uranium Immunosensor

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The goal of this project is to develop an in-line uranium immunosensor that can be used to determine the efficacy of specific biostimulation procedures by measuring the amount of hexavalent uranium [U(VI)] present at varying times during the treatment process. An alpha prototype of this sensor has been developed. The instrument consists of a capillary flow/observation cell fitted with a microporous screen. An antibody "capture ligand" is immobilized onto uniform particles, and the particles are subsequently deposited above the screen in a packed bed. A mixture that contains a fluorescently labeled antibody and soluble ligand is rapidly passed through the bead pack. Only those antibody molecules without soluble ligand in their binding sites are available to bind to the "capture ligand," and the fluorescent signal generated is inversely proportional to the amount of soluble ligand in the original mixture of antibody and ligand. A fresh bed of beads is used for each determination. For the measurement of U(VI), the ligand is hexavalent uranium complexed with 2,9-dicarboxyl-1,10-phenanthroline [U(VI)–DCP], and the antibody is one of three monoclonal antibodies (8A11, 12F6, or 10A3) that recognizes this metal-chelate complex.

Stability tests of the reagents used in the assay (capture ligands, chelators, diluent buffers) has shown that these assay components are stable for at least six weeks at 4°C. Frozen antibody stocks are stable for more than two years at –80°C, and diluted stocks for approximately two weeks at 4° C. An alpha prototype of this sensor has the ability to autonomously run a standard curve for hexavalent uranium from stock reagents, and to prepare environmental samples for analysis. When the 8A11 monoclonal antibody is utilized, the assay measures hexavalent uranium at concentrations from 0.5 to 4.8 nM (0.12–4.8 ppb). The coefficients of variation (CV) in the assay range from 3.5 to 5.9%, with an average of 4.6%. The immunoassay results are comparable to those obtained using the Kinetic Phosphorescence Assay (KPA).

The spike-and-recover experiment using natural groundwater from the ORNL FRC background site, and an artificial groundwater designed to stimulate other FRC samples, have indicated that high concentrations of calcium stimulate the binding of the 8A11 antibody to the "capture ligand." Such stimulation may lead to false negatives in the analysis of environmental samples. The binding of the 12F6 monoclonal antibody to the capture ligand is neither stimulated nor inhibited by calcium concentrations up to 31 mM, and future assay development will most likely utilize this antibody.