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Title: Metal Ion Analysis Using Near-Infrared Dyes and the "Laboratory-on-a-Chip"

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Number of graduate students/post-doctorates: 1

RESEARCH OBJECTIVE: The primary objective of this project is to develop a sensitive and selective, portable sensor for radionuclides and heavy metals that utilizes a "laboratory-on-a-chip" platform for enabling low-cost, timely characterization of DOE remediation sites. The DOE has been tasked to deactivate and/or decommission nearly 10,000 buildings at 3314 EM release sites, and new analytical characterization tools are necessary in order to assist in the segregation of contaminated and non-contaminated scrap metal and concrete materials via the identification of the type and extent of radionuclide and heavy metal contamination apparent on these building materials. Current characterization techniques for radionuclides and/or heavy metals typically require lengthy analysis times at external laboratories, causing costly delays from a health and fiscal standpoint. This research objective will be accomplished by either synthesizing a new class of metal complexation ligands that are covalently linked to red fluorescing dyes, or by taking advantage of commercially available, red-shifted colorimetric metal complexation ligands, and utilizing these dyes for the selective quantitation of target metal pollutants through their selective separation down the microchannel of a "laboratory-on-a-chip." Inexpensive, compact light sources will be utilized as excitation sources, and detection will be achieved using compact, inexpensive, photodiode array detectors.

RESEARCH PROGRESS AND IMPLICATIONS: This progress report summarizes the present status as of July 11, 2000 for a 3 year project beginning October 1, 1998.

Synthesis and characterization: To date, four different metal complexation ligands, cyclen, calix[6]arene, iminodiacetic acid (IDA) and 18-crown-6 have been successfully linked covalently to the fluorophore, rhodamine. Each of these molecules have been purified by column chromatography, and fully characterized by electrospray mass spectrometry. Our initial synthetic efforts have utilized rhodamine as the linkage fluorophore of choice, primarily because, unlike near-infrared dyes, they are an inexpensive alternative for deriving the experimental protocols necessary to covalently link the metal complexation ligands and fluorophores together. Three different covalent linkage schemes have been investigated: succinimidyl ester, isothiocyanate, and sulfonyl chloride. Rhodamine sulfonyl chloride has demonstrated the most success with respect to its reactivity and final product stability. Difficulties have been encountered in obtaining the desired product for reactions involving EDTA, presumably due to the reactivity of the carboxylic acid functional groups. We are currently pursuing protective groups to overcome this problem.

Laboratory-on-a-Chip: Two separate microchip analysis platforms have been designed and fully interfaced to a computer for data collection, one for performing absorbance-based detection and the other for fluorescence. The absorbance-based detector utilizes either a red (560 nm) or green (540 nm) LED light source and a photodiode array detector, while the fluorescence based system utilizes a green Nd:YAG laser and a miniature photomultiplier tube. Three metal complexation ligands have now been examined on the microchip, PAR, Arsenazo III and rhodamine-calix[6]arene, each demonstrating excellent promise with respect to the selective, microchip detection of uranium and other transition metal ions.

With regards to fluorescence detection, rhodamine-calix[6]arene, a new, fluorophore tagged macrocycle synthesized in this program, has demonstrated excellent selectivity and sensitivity for the uranyl ion in the presence of a complex mixture of metal ions. Calixarenes form a barrel-shaped geometry which is well suited for uranyl ion metal complexation. The uranyl ion likely adopts a pseudoplanar penta- or hexa-coordinate structure that helps explain its enormous selectivity factors for uranium over other metal ions ($> 10^{12}$). It is for this reason that calix[6]arene has been coined the term, "super-uranophile." Our investigations on the microchip have established rapid separations (< 45 sec) of the free ligand from the uranyl-complexed ligand. Furthermore, the addition of various transition, alkali and rare earth metal ions does not result in the formation of any additional separation peaks, thereby, verifying the high selectivity of this ligand for uranium.

Simple absorbance based detection methods on a microchip have also been demonstrated using the commercially available, colorimetric metal complexation dyes, PAR and Arsenazo III. Arsenazo III is particularly well-suited for adaptation to the laboratory-on-a-chip in this project due to its capability for lanthanide/actinide metal complexation, and the overlap of these metal complex absorbance peaks with the output of a red LED excitation source. Arsenazo III has the additional advantage of not complexing the vast majority of transition and alkali/alkaline earth metal ions which predominate at any remediation site. With citric acid in the separation buffer, we have demonstrated the selective separation of a mixture of uranium from four other lanthanides, Eu, Dy, Ho and Yb in just over two minutes. If EDTA is additionally added to the separation buffer, Arsenazo III will only complex colorimetrically to uranium, making its identification on a microchip very simple, highly selective and sensitive (detection limit- 27 ppb). The colorimetric metal complexation ligand, PAR, has proven to be equally effective on the microchip for the separation and detection of transition metal ions. We have successfully separated seven transition metal ions (Co^{2+} , V^{+3+} , Ni^{2+} , Cu^{2+} , Fe^{2+} , Mn^{2+} , and Cd^{2+}) in the high ppb range in under 65 seconds with extremely high resolution.

PLANNED ACTIVITIES: Following our experience gained with the reactivity of rhodamine, we are planning to transition to the synthesis of near-infrared fluorophore linked ligands in the near future. The separation and quantitation of metal ions by ligands generated from this program continue to be examined on both a commercial electrophoresis unit and on the "laboratory-on-a-chip" platform. Possible avenues of research include longer channel lengths and polyacrylamide deposition within the microchannels in order to improve resolution. Efforts will begin to transition toward the packaging of the "laboratory-on-a-chip" into a portable sensing device ready for initial field testing. Remote sensing of radionuclides will be demonstrated via the application of a probe containing either micro-Nafion or micro-dialysis membrane tubing through which metal ions can diffuse and colorimetrically react with Arsenazo III reagent that is being transported inside the tubing to either the microchip or a fiber optic, liquid waveguide capillary cell.

INFORMATION ACCESS

- ✕ "The Selective Detection of Uranium (VI) on a Microchip Using a Derivatized 4-Sulfonic Calix[6]arene," Q. Lu, G.E. Collins and J.H. Callahan, *Chem. Comm.*, submitted March, 2000.
- ✕ "Radionuclide and Metal Ion Detection on a Capillary Electrophoresis Microchip Using LED Absorbance Detection," G.E. Collins and Q. Lu, *Sensors and Actuators, B*, submitted July 2000.
- ✕ "Separation of Uranium (VI) and Transition Metal Ions with 4-(2-thiazolylazo)-resorcinol by Capillary Electrophoresis," L. Evans III and G.E. Collins, *J. Chrom. A*, submitted June 2000.
- ✕ "Microchip Separations of Transition Metal Ions via LED Absorbance Detection of their PAR Complexes," Q. Lu and G.E. Collins, *Anal. Chem.*, in preparation.
- ✕ "Capillary Electrophoresis Microchip Separations of Uranyl and Lanthanide Metal ions using Arsenazo III," G.E. Collins and Q. Lu, *Anal. Chem.*, in preparation.