

Project ID: **64982**

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

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RESEARCH OBJECTIVE

This project addresses the need for developing a highly sensitive and selective, portable radionuclide analyzer which would permit a low-cost and timely characterization of DOE remediation sites. Through the application of near-infrared fluorophore tagged macrocycles, in combination with the capillary electrophoretic separation of radionuclide and heavy metal complexes on a microchip, we propose an innovative, low cost characterization approach to gaining timely characterization data in the field. The research goals for this program are summarized as follows:

- a) To synthesize a new class of near-infrared tagged macrocycles that will take advantage of the inherent metal complexation properties of the macrocycle, while fluorescing in a region of the spectrum with very little background fluorescence.
- b) To characterize the fluorescence and complexation behavior of this new class of complexation agents.
- c) To implement these new materials into the design of a portable monitor for radionuclide and heavy metal analytes that utilizes the "laboratory-on-a-chip" technology for performing capillary electrophoresis on a microchip.

RESEARCH PROGRESS AND IMPLICATIONS:

This work summarizes the present status as of June 11, 1999 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 different mask patterns have been lithographically etched and packaged into glass slide microchips in order to investigate the separation of fluorescent metal complexes down microchannels. The two different etched patterns permit the investigation of two alternate injection schemes within a simple cross pattern: pinched mode and “Z”-type injection. We have experimented with various potential applications in order to provide injection volumes of variable size onto the separation column. The microchip analysis platform has been arranged on a small, laser breadboard with the focussing optics, glass microchip, collection optics and photomultiplier tube optimized using various translation tools. LABView has been used as the interfacing software of choice, and several programs have been written to enable full computer control of the separation and injection potentials, in addition to the rapid collection of signal intensity variations from the photomultiplier tube as the fluorescent metal complexes are separated and migrate passed the detection zone.

As an initial demonstration of the effective operation and separation efficiency of the microchip, two rhodamine B based ligands were rapidly separated within a 20 second separation time. Investigating the conditions necessary to optimize this separation helped demonstrate the factors influencing electroosmotic and electrophoretic flow, and how changes in these parameters influence the separation of fluorophores. The first fluorophore tagged ligand synthesized in this program was an 18-crown-6 linked rhodamine fluorophore, which was theoretically proposed to complex alkaline earth metal cations, such as Sr^{2+} . Difficulties were encountered separating the uncomplexed ligand from the fluorescent metal complex on the microchip, however. The addition of metal resulted in only slight perturbations of the chromatographic retention times for the uncomplexed fluorophore. This suggested that the stability constant for metal complexation in this ligand was too small to enable the complete resolution of the complexed and uncomplexed ligands. We are currently investigating whether operation of the microchip in an organic solvent such as methanol will sufficiently enhance the metal complexation stability constants for the 18-crown-6 linked rhodamine fluorophore in order to enable a separation.

Investigations on the microchip with ligands bearing much stronger stability constants for metal complex formation, such as cyclen-rhodamine and Calcium Orange, are just beginning, and have enabled the separation of metal complexes, e.g. Hg and Pb, in a reasonably rapid fashion. These preliminary studies demonstrate that the types of metal complexation ligands being proposed here should very efficiently form metal complexes with radionuclide and heavy metals, in addition to permitting their separation and quantitation.

PLANNED ACTIVITIES:

Efforts are currently focussed on quantitatively determining the various stability constants for metal complexation by the newly synthesized fluorophore-tagged ligands, in order to ascertain the impact of the fluorophore on metal complexation. While each ligand synthesized to date has had the fluorophore covalently linked directly to the metal complexation ligand, in the future we plan to synthetically introduce a short linkage or alkyl unit between the fluorophore and the ligand. This will help separate these two entities, improving our metal complexation and fluorescence properties. 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.