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

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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. This will be accomplished by synthesizing a new class of metal complexation ligands that are covalently linked to red fluorescing dyes, 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 work summarizes the present status as of March 1, 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 a red LED light source and a photodiode array detector, while the fluorescence based system utilizes a green Nd:YAG laser and a miniature photomultiplier tube. Two metal complexation ligands, in particular, have demonstrated excellent promise with respect to the selective, microchip detection of uranium, and these will be discussed here.

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

(< 50 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 are also being pursued based upon the commercially available metal complexation dye, Arsenazo III. This dye 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 three other lanthanides, Sm, Gd, and Ce in under 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 and highly selective.

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.