The selective detection of uranium(VI) on a microchip using a derivatized 4-sulfonic calix[6]arene

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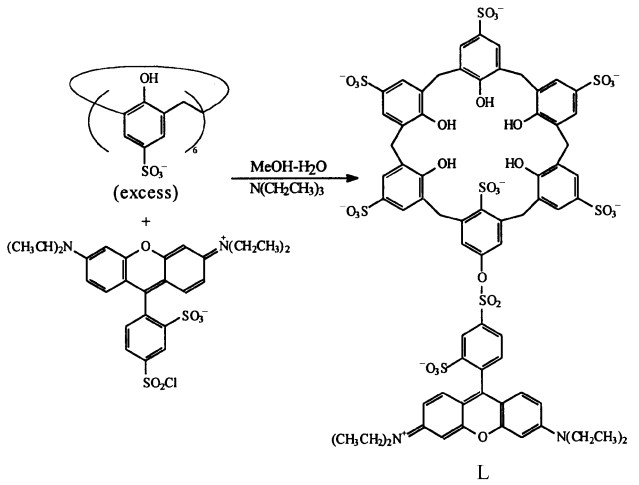
4-Sulfonic calix[6]arene is derivatized with lissamine rhodamine B to give a fluorescent ligand, L, which is applied to a capillary electrophoresis microchip for the selective detection of uranium(VI).

Environmental concerns associated with the remediation of radioactive waste sites have generated considerable interest in the development of a portable, rapid and selective monitor for radioactive contaminants, such as uranium. This need has been prompted by the costly and time-consuming delays which have resulted from submitting samples to external laboratories for analytical characterization before, during and after the application of remediation methodologies to contaminated groundwater or structural materials encountered at these sites. In this communication, we report the application of a derivative of the 'super-uranophile', 4-sulfonic calix[6]arene, which has been tagged with a long wavelength fluorophore, lissamine rhodamine B, for the selective detection of uranyl ion, UO_2^{2+}, on a glass microchip. The experimental design employed here combines the superior selectivity of 4-sulfonic calix[6]arene for the UO_2^{2+} ion,[1] the high sensitivity and diminished overlap of impurity fluorescence inherent with utilizing long wavelength fluorescence detection,[2] and the numerous advantages associated with performing glass microchip separations, including short analysis times, minimal sample consumption and waste generation, and portable instrumentation.[3]

The calixarene derivative, **L**, was synthesized through the coupling reaction of 4-sulfonic calix[6]arene with lissamine rhodamine B sulfonyl chloride in a mixed solvent of methanol and water in the presence of excess 4-sulfonic calix[6]arene (Scheme 1). The mono-derivatized product, **L**, was isolated by column chromatography (methanol/ethyl acetate) and verified by electrospray mass spectrometry.[4] Excitation of **L** at 532 nm gives an intense fluorescence peak at 593 nm in water, differing only slightly from the parent fluorophore, lissamine rhodamine B sulfonyl chloride, at 583 nm.



Scheme 1 Synthetic coupling of lissamine rhodamine B to 4-sulfonic calix[6]arene.

The microchips utilized for performing the separation were microfabricated in Borofloat glass to contain a simple cross pattern, lithographically etched into the glass substrate (20 μm deep × 50 μm wide) to define the sample loading and separation microchannels (see Fig. 1).[5] A glass cover plate pre-drilled to contain four access holes was thermally bonded to the microfabricated glass plate. Sample, buffer, and waste reservoirs were constructed by inserting pipette tips into these holes. The lengths of the loading and separation channels were 10 and 85 mm, respectively, with an effective separation length of 80 mm. Through computer control of the potentials applied to the different reservoirs, sample analyte was loaded into the intersection of the two channels and subsequently separated down the length of the separation channel. The separation was monitored by focussing light from a compact, 15 mW Nd/YAG laser (532 nm) onto the end of the separation channel, collecting the fluorescent light with a microscope objective (20×) positioned at 90°, and directing the light onto a miniature, red-shifted photomultiplier tube (Hamamatsu).

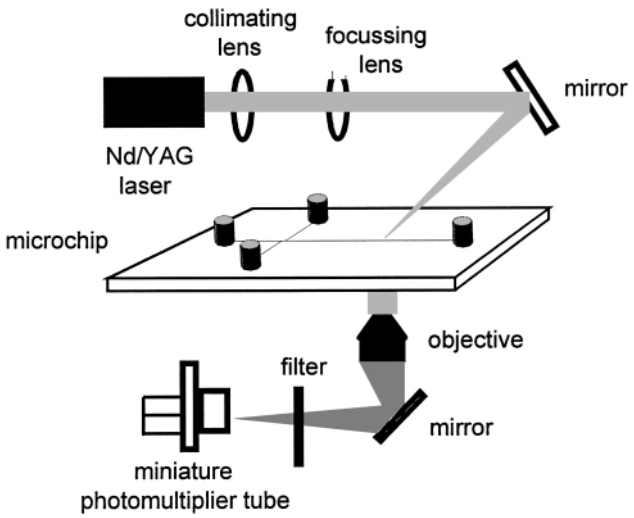


Fig. 1 Instrumental layout for performing microchip separations.

Microchip separations were performed using a buffer solution containing 10 mM sodium borate, 50 mM boric acid, and 2 mM $MgCl_2$ (pH = 8.3). Under this pH condition, in addition to the complete deprotonation of the six sulfonic groups of ligand **L**, previous studies establish that two of the five phenol groups will also likely be deprotonated to give $\mathbf{L}^{8-}$.[6] In experiments run without the addition of $MgCl_2$ in the buffer solution, **L** was detected under positive polarity (+1000 V cm^{-1}) with long migration times (over 100 s) and poor peak shapes. Furthermore, following the addition of UO_2^{2+} to the analyte reservoir, the resolution obtained on the microchip was insufficient to separate the uranyl bound ligand from the free ligand, **L**. The addition of 2 mM $MgCl_2$, however, allowed **L**

and its uranyl ion complex to be completely resolved under negative polarity (−1000 V cm^{-1}) with significantly reduced migration times and improved peak shape (Fig. 2a, b). A similar enhancement in the resolution was reported for the capillary electrophoretic separation of *p*-sulfonated calixarenes following the addition of Mg^{2+} to the background supporting electrolyte.[7] The uranyl complex formed with **L** is believed to further deprotonate the remaining three phenols, giving a planar complex, $[\mathbf{L}\cdot UO_2]^{9-}$, which is more negatively charged than the parent molecule, $\mathbf{L}^{8-}$. This observation helps explain the shorter retention time obtained for the uranyl complex, $[\mathbf{L}\cdot UO_2]^{9-}$, due to its enhanced electrophoretic mobility under the applied electric field. Microchip evaluations of the electroosmotic flow using the neutral marker, rhodamine B, demonstrate that the electroosmotic flow was significantly reduced by the addition of $MgCl_2$, but not reversed. The detection limit for UO_2^{2+} under these conditions was 4.2 μM.

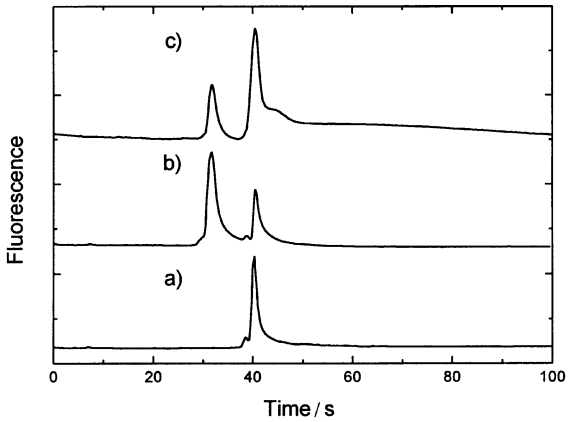


Fig. 2 Microchip separations obtained using 10^{-4} M **L** in a buffer solution containing 10 mM sodium borate, 50 mM boric acid and 2 mM $MgCl_2$ (pH = 8.3), (a) 0 mg L^{-1} UO_2^{2+}; (b) 10 mg L^{-1} UO_2^{2+}; (c) 10 mg L^{-1} UO_2^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+}, Th^{4+} and Nd^{3+}.

The effective detection of UO_2^{2+} on the microchip requires a pre-complexation step between the ligand, **L**, and uranyl ion. In general, the binding rates of UO_2^{2+} by calixarenes are relatively slow, due to the geometric rearrangement requirements imposed on the calixarene for conforming to the pseudoplanar geometry which permits binding of the uranyl ion in a hexacoordinate structure. The half-life of the binding reaction between the super-uranophile, 4-sulfonic calix[6]arene, and UO_2^{2+} is about an hour.[1b] The high thermodynamic stability constant and slow dissociation kinetics of the uranyl ion complex, $[\mathbf{L}\cdot UO_2]^{9-}$, however, explain why the complex does not dissociate and become undetectable as it travels down the separation column, a problem which plagues the majority of capillary electrophoresis separations of strongly complexed metal ions when the complexing ligand is absent from the separation buffer.[8]

Studies were performed on the detection of UO_2^{2+} in the presence of six competing metal ions, Cu^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, Th^{4+}, and Nd^{3+}, each at a concentration of 10 mg L^{-1}. Introduction of these six impurity metal ions to **L** in the absence of UO_2^{2+} resulted in no change to the electropherogram recorded for **L**. The subsequent addition of UO_2^{2+}, however, gave the electropherogram shown in Fig. 2c, indicating the capability for **L** to selectively complex UO_2^{2+} despite the presence of a matrix of six impurity metal ions. The underivatized form of the ligand utilized here, 4-sulfonic calix[6]arene, has been termed a super-uranophile, due to the extremely large selectivity factors, 10^{12-17}, determined for the binding of uranyl ion in the presence of competing Ni^{2+}, Zn^{2+}, and Cu^{2+} ions.[1] The selective binding of UO_2^{2+} ion by **L** in the presence of other metal ions results from the fact that **L** provides a pseudoplanar, hexacoordinate geometry which suits UO_2^{2+} uniquely. Any weakly bound metal complexes and/or complexes bearing fast dissociation kinetics will be unresolved due to their rapid dissociation from the fluorescent ligand as the complexes travel down the separation column.

In conclusion, by synthetically coupling the super-uranophile, 4-sulfonic calix[6]arene, to the long wavelength, fluorescent dye, lissamine rhodamine B, we have developed a new ligand, **L**, which is ideally suited for selectively detecting uranium on a glass microchip platform. **L** has a coordination site which is highly selective for binding UO_2^{2+} ion in the presence of numerous impurity metal ions, and a fluorescent tag whose red shifted emission wavelength enables a compact, portable laser to be utilized, minimizing any background fluorescence arising from impurities apparent in real samples. The results of this study have exciting implications with respect to methods for preparing molecular and metal recognition molecules for application to the burgeoning field of microchip capillary electrophoresis.

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4 Compound **L** was verified by NMR and electrospary mass spectrometry. The negative ion mode of the mass spectrometer yielded three major clusters of peaks, corresponding to doubly, triply and quadruply charged species. The most abundant cluster of peaks corresponded to the following species: $[M - 2H]^{2-}$, *m/z* 828.1; $[M - 3H + Na]^{2-}$, *m/z* 839.1; $[M - 4H + 2Na]^{2-}$, *m/z* 850.1; $[M - 5H + 3Na]^{2-}$, *m/z* 861.1; $[M - 6H + 4Na]^{2-}$, *m/z* 872.1. A second cluster of peaks, corresponding to triply charged ions, was observed at the following *m/z* ratios: $[M - 3H]^{3-}$, *m/z* 551.7; $[M - 4H + Na]^{3-}$, *m/z* 559.0; $[M - 5H + 2Na]^{3-}$, *m/z* 566.4; $[M - 6H + 3Na]^{3-}$, *m/z* 573.7. The cluster of quadruply charged ions was observed at the following *m/z* ratios: $[M - 4H]^{4-}$, *m/z* 413.5; $[M - 5H + Na]^{4-}$, *m/z* 419.0; $[M - 6H + 2Na]^{4-}$, *m/z* 424.5. Mass assignments were confirmed to 0.1 u using a high resolution scanning mode.

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