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Toxic Metals Chelation by 18-Crown-6 Ethers in Multiple Solutions and Quantification by Spectroscopic Techniques

Andrew L Cook[1], Fan Xue[1], Todd D Giorgio[1]
[1]Department of Biomedical Engineering
Vanderbilt University
2201 West End Avenue, Nashville, TN 37235, United States
Andrew.L.Cook.1@vanderbilt.edu; Fan.Xue@vanderbilt.edu; Todd.D.Giorgio@vanderbilt.edu

Abstract **-** Toxic metals exposure is a significant problem for military personnel, with increasingly prevalent embedded fragments due to improvised explosive devices. Current biomonitoring for military personnel with embedded fragments is centralized, limiting capacity and availability. Importantly, monitoring using this approach begins long after peak exposure, indicating a need for portable, multiplexed toxic metals detection that can be carried out closer to the time of exposure with increased frequency. Small molecule chelators such as crown ethers are known to selectively bind metal cations in solution. Crown ethers possess selective chelation of multiple metal ions and is dependent on molecular structure, solution properties, and other parameters. This selectivity extends to multiple ions and depends on not only molecular structure, but also the solution properties. The goal of this study is to assess the potential for metal sensing in solution as a function of crown ether structure and solution properties with future use for toxic metal sensing from embedded fragments as a potential translational objective.

Keywords**:** Crown Ether, Toxic Metal, UV-vis, Fluorescence, Embedded Fragment, Urine

1. Introduction

As of 2017, improvised explosive devices (IEDs) have accounted for almost 75% of all traumatic injuries to U.S. soldiers in recent conflicts in Iraq and Afghanistan [1]. This means that of the more than 50,000 military personnel wounded in action so far in those conflicts [2], almost 40,000 of them could have toxic embedded fragments [3]. In response to this growing need, the United States Department of Veterans Affairs (VA) established the Toxic Embedded Fragment Surveillance Center (TEFSC, Baltimore, MD) in 2008 with the overall mission to 1) identify veterans who may have embedded metal fragments, and 2) conduct long-term medical surveillance of this population [4]. The evaluation process for inclusion into the Embedded Fragments Registry (EFR) is predicated on the individual's knowledge or suspicion of retained fragments [5]. Thus, biomonitoring of toxic embedded fragments begins long after peak exposure and depends on incomplete knowledge concerning exposure and retention toxic fragments from IEDs, making inclusion into the EFR noncomprehensive. As a result, there are currently only around 16,000 veterans enrolled in the EFR [6]. Biomonitoring of these veterans is carried out via centralized urinalysis using inductively-coupled plasma mass spectrometry (ICP-MS)[1], [4], [7]. While ICP-MS is sensitive and precise, it is a large, research-grade instrument that requires significant power and highly trained technicians for operation[8], [9], making it unsuitable for use near locations where military blast injuries occur. Furthermore, there is a lack of information concerning the scope and extent of embedded fragments[10], [11] as well as their long-term health effects[3], [12]. To ensure more comprehensive and complete biomonitoring of embedded fragments, a portable, multiplexed toxic metals sensing strategy is required.

There are many techniques for toxic metals detection that can be implemented in a portable setting, including spectroscopic techniques such as fluorescence, colorimetry, and Raman spectroscopy. Many strategies utilizing these techniques make use of small-molecule chelators known to bind various metal ions in solutions. One common family of chelators are known as crown ethers, small molecules with a characteristic ring made up of carbons and oxygens which are best known for chelating alkali metal cations [13]. While these chelators are moderately selective, they still bind multiple different ions in solution. Small changes in crown ether structure can significantly affect which metals it will bind [14], [15]. Additionally, differences in solution affect crown ether morphology, changing chelation selectivity[16]. The purpose of this

study is to explore how changes in the structure and solution of 18-crown-6 (18C6) ethers can change its selectivity profile for metal ions.

2. Experimental

Two different solvents consisting of dimethyl sulfoxide (DMSO) and deionized (DI) water were used for all experiments in this study: 1:1 DMSO/water and 1:3 DMSO/water. 4'-aminobenzo-18-Crown-6 (AB18C6) and benzo-18-Crown-6 (B18C6) and the following 22 metals were examined as a part of this study: Al^{3+}, Ag^{+}, As^{3+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{3+}, Hg^{2+}, K^{+}, Li^{+}, Mg^{2+}, Mn^{2+}, Mo^{5+}, Na^{+}, Ni^{2+}, Pb^{2+}, UO_2^{2+}, W^{4+}, and Zn^{2+}. All metal salts used to obtain these ions were purchased from Sigma Aldrich. All 14 metals in the TEFSC biomonitoring panel [3], [4] are included, as well as metals commonly found in urine, such as Ca^{2+}, K^{+}, Mg^{2+} and Na^{+}. Also included are a number of common metals that could make their way into human urine, such as Al^{3+}, Mn^{2+}, and Zn^{2+}. 100 μM equimolar solutions of each metal individually with AB18C6 were formed in both 1:1 DMSO/water and 1:3 DMSO/water.

In this study, the chelation of metal ions by two crown ethers was studied in a single solution to explore how a small change in the crown ether's structure might change the crown ether's metal selectivity. Furthermore, chelation by chelation of metal ions by one of those crown ethers was examined in two solutions. Chelation of metals by AB18C6 and B18C6 was examined primarily using ultraviolet-visible extinction spectrophotometry. Preliminary examination of the extinction spectrum of using a quartz cuvette in a Varian Cary 50 Bio spectrophotometer. These measurements were acquired at a resolution of 5 nm over a range of 250 – 800 nm to confirm the absorption profile of AB18C6 reported by Sarfo et. al. [15], and to explore differences in chelation of metal ions between AB18C6 and B18C6. For these experiments, the extinctions from 100-μM concentrations of each crown ether in 1:3 DMSO:water were measured without metals. Subsequently, the extinctions of 100-μM equimolar concentrations of each crown ether and selected metal salts in 1:3 DMSO:water were acquired.

Full UV-vis spectrophotometry chelation sweeps of metal ions by AB18C6 were performed using Thermo Scientific™ Nunc™ UV-transparent plastic 96-well plates in a Tecan Infinite M1000 Pro plate reader. These measurements were acquired at a resolution of 1 nm over a range of 250 – 400 nm to determine which metals are chelated by AB18C6. For these measurements, 100-μM concentrations of AB18C6 in both solvents were used without metals, and 100-μM equimolar solutions of AB18C6 and each metal salt in each solvent were used for selectivity assessments. The same instrument was used to acquire fluorescence measurements of AB18C6 in the presence and absence of selected metals were acquired with an excitation wavelength of 295 nm over a range of 300 – 700 nm at a resolution of 2 nm. The same crown ether and metal solution strategy was used for fluorescent measurements, except only two metals known to be chelated from previous experiments were included. UV-vis spectrophotometry with the same spectral parameters described above was performed on 100-μM crown ether solutions with one of the metals known to be chelated in concentrations of 1 nM, 10 nM, 100 nM, 1μM, 10 μM, and 100 μM. Fluorescence spectroscopy was performed on these same solutions with an excitation wavelength of 295 nm over an emission range of 304 – 500 nm at a resolution of 1 nm.

3. Results & Discussion

The first experiment was a comparison via UV-vis spectrophotometry of which metals are chelated by AB18C6 and B18C6. It was reported by Sarfo et. al. [15] that AB18C6 crown ethers possess a strong extinction peak centered at 295 nm. When the crown ether chelates a metal, this peak is quenched and another emerges at ~280 nm. For this experiment, 100-μM solutions of AB18C6 and B18C6 by themselves and with equimolar concentrations of 13 selected metal salts prepared in 1:3 DMSO:water. As can be seen in Fig. 1, while AB18C6 has an extinction peak at 295 nm as Sarfo et. al. reported, B18C6 has a peak at ~275 nm. No chelation occurred for most of the metals investigated, as evidenced by a lack of quenching of the 295-nm peak for AB18C6 or the 275-nm peak for B18C6, as well as a lack of an additional peak. However, both Fe^{3+} and Hg^{2+} ions were chelated strongly by AB18C6, evidenced by the quenching of the 295-nm peak and appearance of a peak ~282 nm. Benzo-18-Crown-6 chelated none of the metals examined, indicating that even small differences in crown ether structure can lead to big differences in metal chelation.

The second experiment performed was an examination of how different solutions would affect the chelation of metal ions by AB18C6. 100-µM solutions of AB18C6 were prepared in 1:1 DMSO:water and 1:3 DMSO:water, without and with equimolar concentrations of 22 metals. UV-vis spectra were acquired of each solution and plotted in Fig. 2. While the chelation profile for AB18C6 was very similar in both solutions, there were some significant differences. AB18C6 chelated

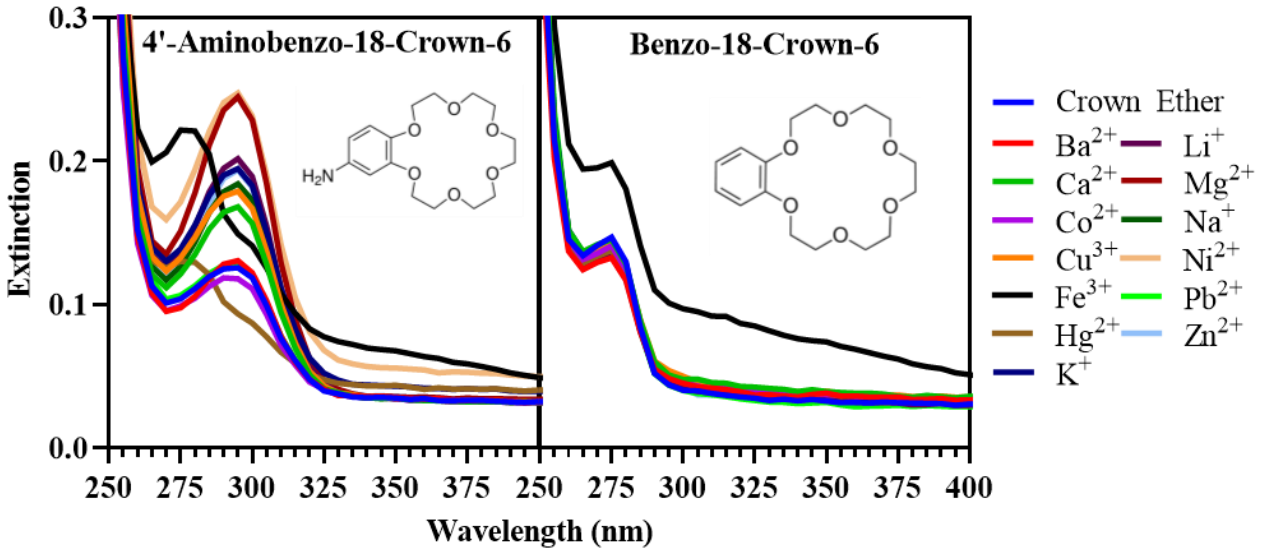

Fig. 1: UV-vis extinction spectra of 100-µM AB18C6 and B18C6 in 1:3 DMSO:water without and with equimolar concentrations of selected metals.

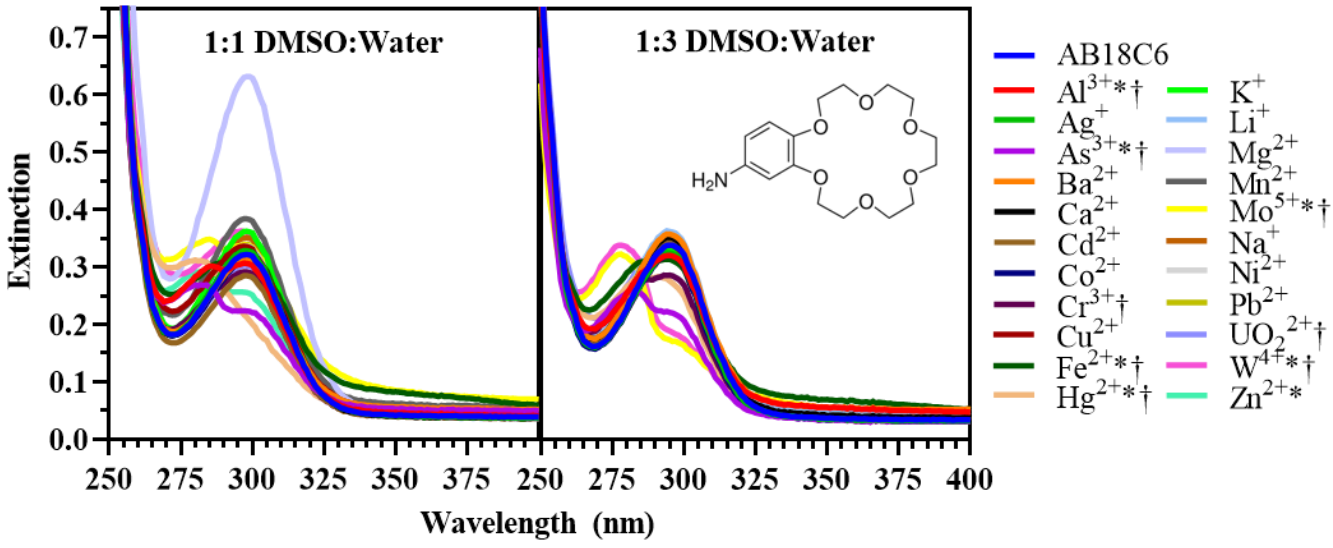

Fig. 2: UV-vis extinction spectra of 100-µM AB18C6 alone and with equimolar concentrations of 22 metal salts in two different solutions. Metals chelated by AB18C6 in 1:1 DMSO:water are denoted with "*", while metals chelated by AB18C6 in 1:3 DMSO:water are denoted with "†".

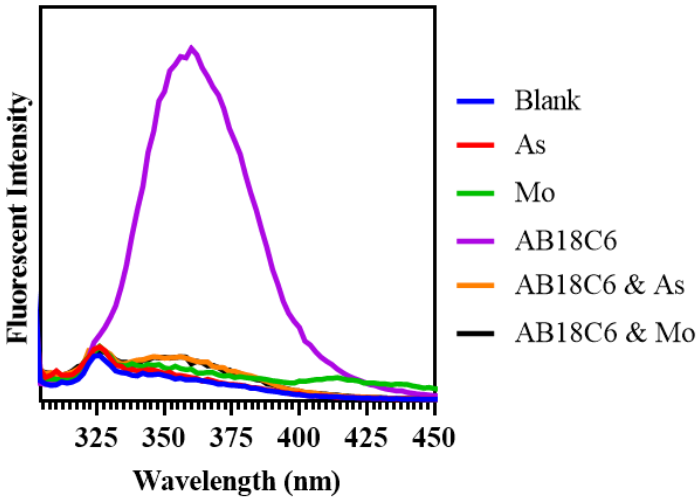

Fig. 3: Fluorescence spectra of 100-µM AB18C6 without and with equimolar concentrations of As and Mo in 1:3 DMSO:water.

Al, As, Fe, Hg, Mo, and W in both solutions, while it chelated U in only 1:1 DMSO:water and Zn in only 1:3 DMSO:water. Even among metals chelated in both solutions, there were differences in the strength of chelation. Notably, Al is more strongly chelated in 1:1 DMSO:water while Hg is more strongly chelated in 1:3 DMSO:water. Interestingly, while Sarfo et. al. reported that AB18C6 chelates Pb^{2+} [15], these experiments demonstrate a lack of Pb^{2+} chelation in either solution. Because of the dependence chelation has on solution, it is likely that this discrepancy with reported results result from an unreported difference in solution.

While UV-vis is excellent for qualitatively determining whether a crown ether is chelating a metal ion, it is difficult to extract any meaningful quantitative information on the amount of chelation taking place in a given solution. However, Sarfo et al. reported that AB18C6 possesses a fluorescence peak at ~370 nm that gets quenched in the presence of chelated metals [15]. To confirm this, fluorescence measurements of 100-µM solutions of AB18C6 without and with equimolar concentrations of As^{3+} and Mo^{5+} (known to be strongly chelated from Fig. 2) in 1:3 DMSO:water were acquired. As can be seen in Fig. 3, AB18C6 with no metal ions present fluoresces as Sarfo et. al. reported. This fluorescence was strongly quenched when As and Mo were present. However, even at equimolar concentrations of metal ions, this fluorescence was not quenched completely, indicating that fluorescence can be used for quantification of toxic metals chelation by AB18C6.

An initial survey of the changes in optical absorbance and fluorescence resulting from AB18C6 chelation of a range of molybdenum concentrations between 1 nM and 100 µM, as shown in Fig. 4. Mo was selected for this assessment based on the quenching of extinction at 295 nm and the presence of another extinction peak at ~280nm (Fig. 2). Mo concentrations of 1 nM, 10 nM, 100 nM, 1 µM, 10 µM and 100 µM were prepared with 100-µM solutions of AB18C6 in 1:3 DMSO:water. UV-vis spectrophotometry was performed at a resolution of 1 nm over a range of 250 – 400 nm (Fig. 4), and spectra of each concentration of Mo were subtracted from their corresponding spectra of mixed AB18C6 and Mo. Fluorescence used an excitation wavelength of 295 nm over a range of 304 – 450 nm at a resolution of 1 nm. A clear peak shift from ~295 to ~275 nm at 100 µM Mo concentration was evident in addition to a small peak shift at 10 µM. Large fluorescence quenching occurred at 100 µM at ~360 nm with additional, slight quenching at 10 µM Mo concentration. Other concentrations of Mo did not produce evident changes in either UV-vis or fluorescence (Fig. 4).

To expand the characterization of Mo chelation by AB18C6, the same experiment was performed using Mo concentrations (in µM) of 6.25, 12.5, 25, 50, 100, and 200. UV-vis spectrophotometry and fluorescence spectroscopy used

the same resolution and range, as shown in Fig. 5. The extinction peak at ~295 nm steadily decreased with increasing Mo concentration between 6.25 µM and 50 µM. This decrease reached a minimum at 50 µM and did not continue to decrease for 100 or 200 µM. The extinction peak at ~277 nm increased in conjunction with the decrease of the 295-nm peak, steadily intensifying between 6.25 µM and 50 µM and remaining steady for 100 and 200 µM. Gradual quenching of AB18C6's

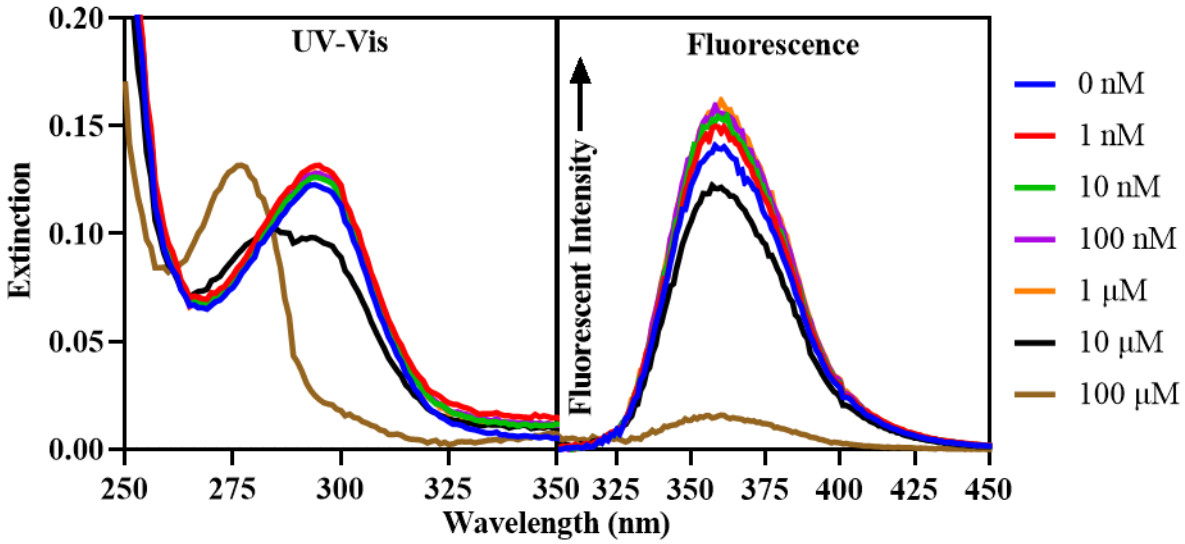

Fig. 4: UV-Vis and fluorescence spectra of 100-µM AB18C6 with concentrations of Mo between 1 nM and 100 µM, with a control solution of 100-µM AB18C6 unmixed with Mo.

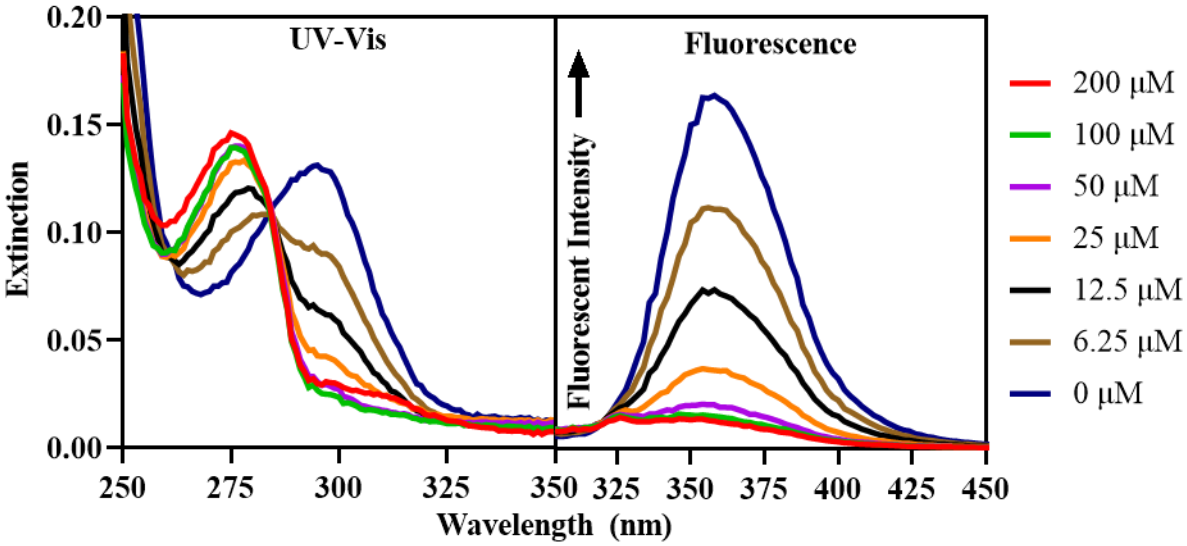

Fig. 5: UV-Vis and fluorescence spectra of 100-µM AB18C6 with concentrations of Mo between 6.25 and 200 µM, with a control solution of 100-µM AB18C6 unmixed with Mo.

fluorescence peak at ~360 nm was observed under increasing Mo concentrations, as shown in Fig. 5. Full quenching of this fluorescence peak occurred at the 100 μM concentration of Mo, indicating greater range of chelation quantification for fluorescence than for UV-vis. The absorption decrease at ~295 nm or increase at ~277 nm did not directly correlate with the increase in Mo concentration, suggesting that absorbance may be a complicated indicator of concentration for this metal. However, these experiments indicate that a quantitative relationship between AB18C6's extinction profile and Mo concentration exists. Furthermore, fluorescence quenching is directly correlated with increasing Mo concentration, that demonstrates the quantitative potential for fluorescent detection of Mo through crown ether chelation.

4. Conclusions

This study demonstrated that minor structural changes in 18C6 ethers modulate metal ion chelation with crown ethers. Furthermore, solution characteristics influence crown ether morphology and the strength of metal ion chelations. Crown ether structure and the solvent environment determine metal chelation characteristics. Optimal quantitation of metal ion concentrations will require additional studies of crown ether chelation under various conditions. The quantitative spectroscopic response of single metal ion species with crown ethers, as demonstrated here, can also be expanded to include multi-composition solutions characterized by suitable mathematical analysis. This study demonstrated the potential for UV-vis and fluorescence spectroscopy to quantify chelation of toxic metal ions by crown ethers. These results inform the design of future portable detection and quantification techniques for toxic metal ions in solution.

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