Detection of Lead Contamination in Water using Fluorescence of Functionalized Gold Nanoparticles

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Introduction

With lead contamination in drinking water becoming an increasingly prevalent issue for residences and for human health, there is a growing concern that detection should be enabled for real-time analysis by the consumer. The lead water crisis of Flint, MI is one of the most recent incidents where lead contamination caused widespread health issues. In 2014, the city of Flint, MI changed its main water supply from Lake Huron to the Flint River. Due to changes in pH for the source, lead from the water lines began leaching into Flint's water system. Lead levels in some homes were as high as 397 ppb1, more than 25 times higher than the Environmental Protection Agency (EPA) limit of 15 ppb2. More timely analysis in the hands of residents could have averted some of the consequences of this excursion, including sick children throughout the community. The research presented here sought to establish a new method of lead detection, based on nanoparticle fluorescence. Currently, to have water reliably tested, a sample of the water would be sent to a lab to undergo atomic absorption spectroscopy or differential pulse anodic stripping voltammetry3. These methods are capital, labor, and expertise-intensive. Colorimetric methods exist, but the nuance of a color change can be misleading to the eye of the lay user. A large volume of work utilizing functionalized gold nanoparticles in colorimetric sensing does exist4 5 6. The novelty of the present approach was the use of excitation/emission matrix (EEM) fluorescent spectroscopy. Fluorescent spectroscopy features increased sensitivity and selectivity compared to colorimetry. The interactions between very small numbers of particles can be detected at length scales of nanometers or angstroms. In colorimetry, solutions which appear completely transparent before the introduction of a contaminant may change slightly afterward, but the human eye might have difficulty noting the difference. With fluorimetry, however, solutions which appear completely transparent to the human eye can still exhibit a detectable fluorometric response. Extension of this research would open the door to the creation of a device which is comprised of a membrane doped with fluorescent nanoparticles, the nanoparticles acting as the sensor. Testing to see if water is contaminated with lead would be as simple as turning on a UV flashlight (commonly sold even on eBay), or UV LED to see whether the membrane's fluorescent response has changed after exposure. One might conceive of a device in which the embedded membrane can be viewed through a window in a filter attached to the faucet, and half the membrane fluoresces in the presence of lead while the other half has control fluid and does not fluoresce. The human eye should be able to detect the comparative difference. Thus, this work will describe research in three parts: 1) identification through literature searches and experimentation of a nanoparticle/coating pair responsive to lead, 2) EEMs studies indicating a unique excitation-emission pair after proper corrections of the spectra, and 3) composition studies to verify sensitivity of the proposed method and quantification of the composition of lead ion in drinking water.

Background

Previous work using gold nanoparticles functionalized with 11-mercaptoundecanoic acid (MUA) has been performed suggesting its validity as a fluorimetric sensor for lead in water. First, Kim et al.4 showed an introduction to colorimetric detection by utilizing 2.4 nM suspension of 13.6 ± 4 nm gold nanoparticles capped with MUA, as well as an addition of a 1.0% poly(vinyl alcohol) stabilizer. Varying concentrations of lead were added to the suspension, and the resulting color change was analyzed using UV-visible spectroscopy. Kim et al.4 speculated that, in an aqueous solution, the –COOH groups found at the end of the MUA could create a chelate complex with the 2+ charge of a lead ion. This chelation between multiple nanoparticles would cause aggregates to form in the solution, which would therein change the color of the solution due to aggregation of the GNPs. A similar response was seen for Hg2+ and Cd2+ ions but not for Zn2+. One interesting aspect was the implementation of ethylenediaminetetraacetic acid (EDTA), where increasing amounts of EDTA were added to the lead-containing suspension of MUA-GNPs, and the lead chelation was reversible. They attained a limit of detection (LOD) as low as 400 µM. It should be noted that colorimetry has a relationship to fluorimetry in that the necessary (but not sufficient) condition for a molecule or nanoparticle to fluoresce is that it must first absorb light energy. Thus, the work by Kim et al.4 is a positive indication that this nanoparticle/coating pair is a) responsive to lead and b) has the potential to fluoresce as it satisfies the condition that it absorbs light energy.

Secondly, Huang et al.7 notes that gold nanoparticles coated with MUA can act as a fluorescent sensor for some heavy metal ion. GNPs of diameter 2.0 ± 0.1 nm capped with MUA were used to investigate the resulting fluorescent intensity response upon the addition of Hg2+. When Hg2+ was introduced to MUA-GNPs at a concentration of 10 nM, the fluorescent intensity was quenched. These GNPs also showed quenching of the fluorescent signal upon addition of lead. In order to drive the specificity towards Hg to prevent the occurrence of false positives from lead, pyrrole-2, 3-dicarboxylic acid (PDCA) was added to the solution. Using a 10 nM solution of MUA-GNPs in a buffer solution of 5 mM sodium tetraborate (pH = 9.2) with 1.0 mM PDCA, Huang et al.7 were able to achieve a remarkable tunable detector for Hg2+ ions in water at concentrations as low as 5.0 nM.

Materials and Methods

For the procedures described below, 5 nm unconjugated gold colloid was purchased from Ted Pella, Inc. (Redding, CA, USA). These nanoparticles were stabilized with citrate. HPLC grade water purchased from Fisher Scientific (Fair Lawn, NJ, USA) and was used for all dilutions and functionalizations. The as-purchased citrate coated gold nanoparticles were functionalized using 95% 11-mercaptoundecanoic acid purchased from Sigma Aldrich (Darmstadt, Germany) by a procedure described below. For the dialysis step described below, seamless cellulose dialysis tubing with a molecular weight cutoff of 12,000 D was used and purchased from Fisher Scientific (Fair Lawn, NJ, USA).

For the UV-Visible spectroscopy, a Varian Cary 3E UV-visible Spectrophotometer was used, made by Agilent (Santa Clara, CA, USA). A Varian Cary Eclipse Fluorescence Spectrofluorometer was used, also made by Agilent (Santa Cara, CA, USA).

In order to functionalize the as-purchased citrate coated gold nanoparticles with MUA, 1 mL of Cit-GNPs was combined with 1 mL of a 3.76 x 10-5 M solution of MUA, and 1 mL of an equimolar solution of NaOH. The resulting mixture was placed under sonication for 1 hr at 60 °C. After sonication, dialysis was used to purify the MUA-GNPs and remove any excess citrate and MUA.

Quartz cuvettes were used for all fluorimetric and UV-Visible testing. Prior to all testing, all cuvettes were cleaned using aqua regia. EEMs spectra were collected scanning from excitation wavelengths of 200-800 nm and emission wavelengths of 200-850 nm. UV-visible spectra were obtained scanning from 200-800 nm. For each data set, a sample of pure HPLC grade water (the solvent), MUA-GNPs, lead-contaminated water, and a sample of the MUA-GNPs with lead contamination were all separately tested.

Data Analysis

After the raw data from the EEMs were collected, corrections for primary and secondary inner filtering effects (IFEs), as well as for the Raman spectra of water were performed. A MatLab code was used on the data set in accordance with Tucker et al8. Eq. 3.6 gives the equation used to correct for primary inner filtering, and Eq. 3.7 gives the equation used to correct for secondary inner filtering.

$$f_{prim} = \frac{F_{corr}}{F_{obs}} = \frac{2.303A(y-x)}{10^{-Ax} - 10^{-Ay}} \qquad 3.6$$

$$f_{sec} = \frac{F_{corr}}{F_{obs}} = \frac{(v-u)/\left(\frac{1}{b}\right)\ln(T)}{T_v - T_u} \qquad 3.7$$

Here, A is the absorbance per centimeter at the excitation wavelength, T is the transmittance of the sample, b is the entire cell pathlength, and x, y, v, and u are the dimensions of the interrogation zone of the spectrofluorimeter (Figure 1).

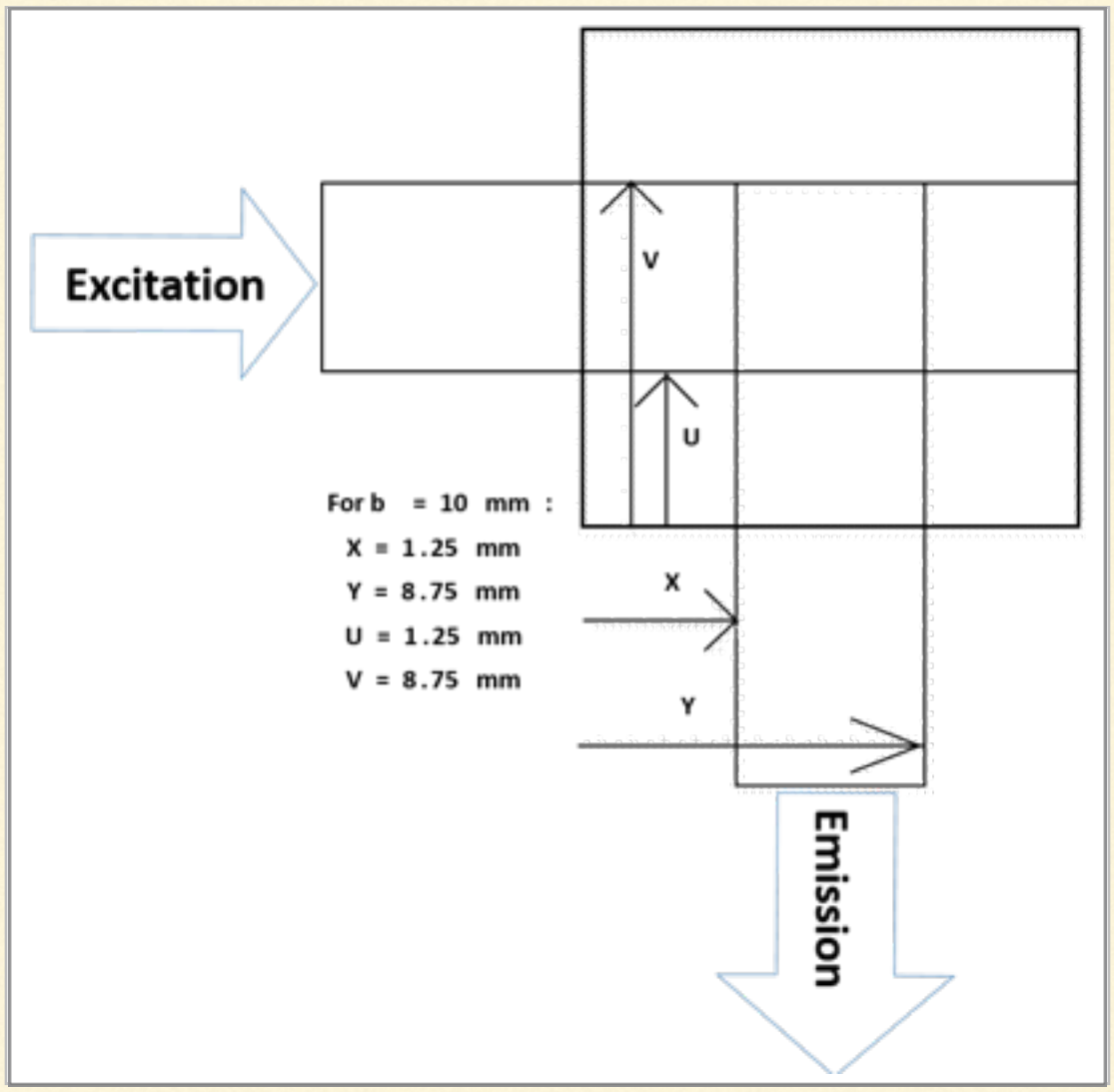

Figure 1. Dimensions of the spectrofluorimeter interrogation zone (not to scale).

The raw spectrum generated fluorescence is a three dimensional spectrum, excitation vs emission vs intensity. In order to determine whether quenching or enhancement occurs as a result of adding lead to the MUA-GNP solution, a 2 dimensional “predicted” curve was generated from the data. This predicted spectrum is taken of the intensity versus excitation wavelengths for a set emission wavelength, 342 nm. The predicted spectrum is the sum of the individual intensities of the lead contaminated water and the pure MUA-GNPs. If a comparison of the predicted spectrum and the intensity versus excitation wavelength for the MUA-GNP with Pb2+ samples are different, it can therefore be said that enhancement or quenching has occurred. Enhancement would be represented by the experimental intensity lying below the predicted, while quenching would be represented by the experiemental intensity appearing above the predicted.

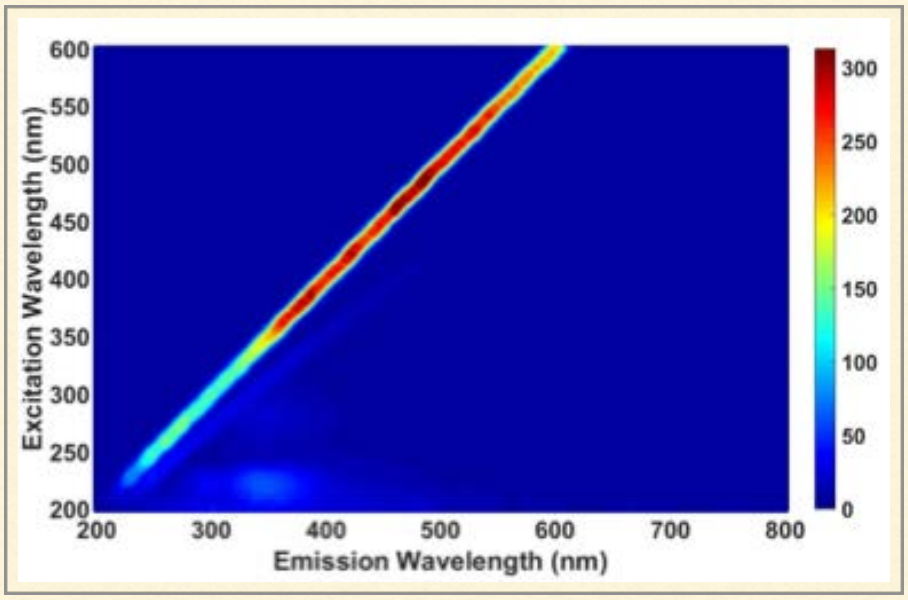

Figure 2. EEMs spectrum of HPLC grade water

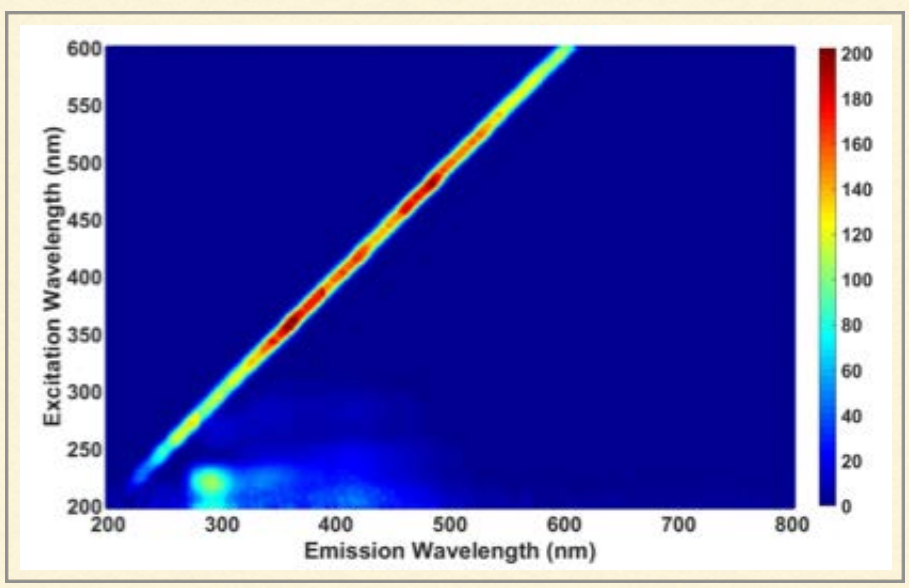

Figure 3. EEM spectrum of 100 µg/L Pb2+ in HPLC grade water.

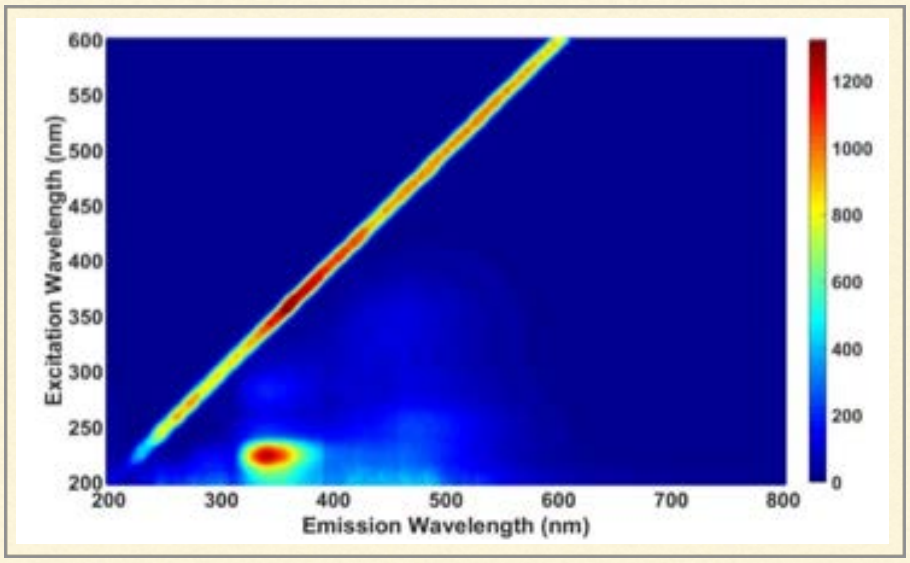

Figure 4. EEM spectrum of 5 nm MUA-GNPs at 11.375 µg/mL

Results and Discussion

Figure 2 shows an EEMs spectrum of HPLC grade water. The diagonal line shown in the spectrum below is representative of the Rayleigh scattering. For the purposes of this research, the Rayleigh scattering will effectively be ignored.

Figure 3 shows an EEMs spectrum of 100 100 µg/L Pb2+ contaminated water. This spectrum, along with that of pure MUA-GNPs, is used to generate theoretical predictions of mixtures where neither component interacted with the other.

Samples of 5 nm MUA-GNPs at 11.375, 9.6, and 5 µg/mL were prepared and tested. A representative EEMs spectrum of these different concentrations can be seen below in figure 4. For the pure MUA-GNP samples, the only difference between the concentrations lies in the intensity of the primary peak at EX220 EM 342.

Samples of the MUA-GNPs at the concentrations mentioned above were prepared with 100 µg/L Pb2+. The resulting 3D EEMs spectra was found to be difficult to determine whether quenching or enhancement had occurred. Therefore, the 2D curves are shown below for the different concentrations.

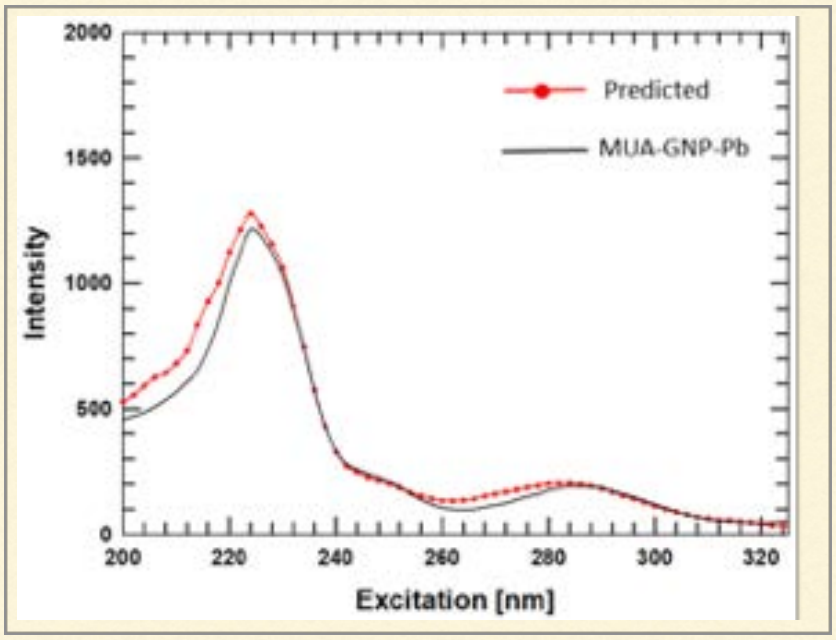

Figure 5. 2D spectrum of MUA-GNPs at 11.375 µg/mL with Pb2+ at 100 µg/L

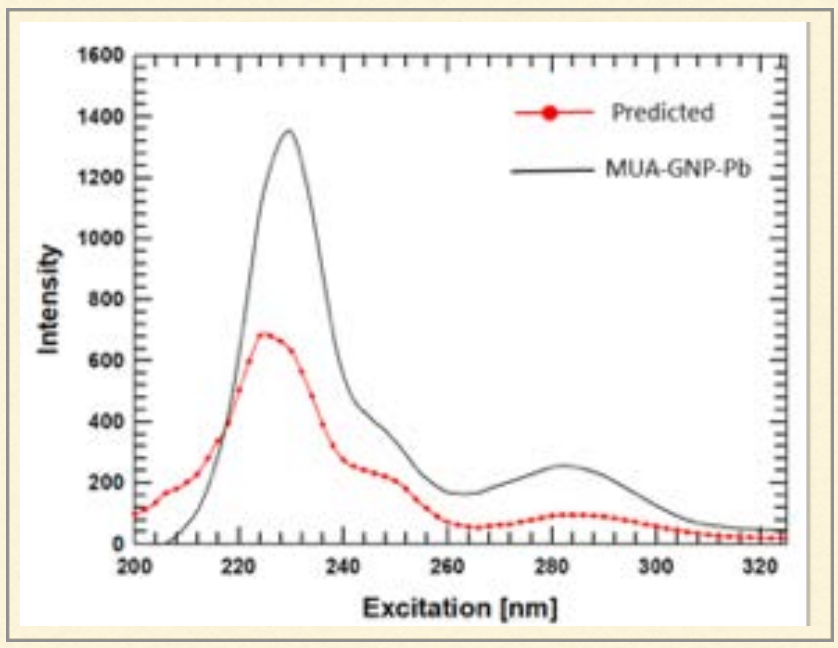

Figure 6. 2D spectrum of MUA-GNPs at 11.375 µg/mL with Pb2+ at 100 µg/L

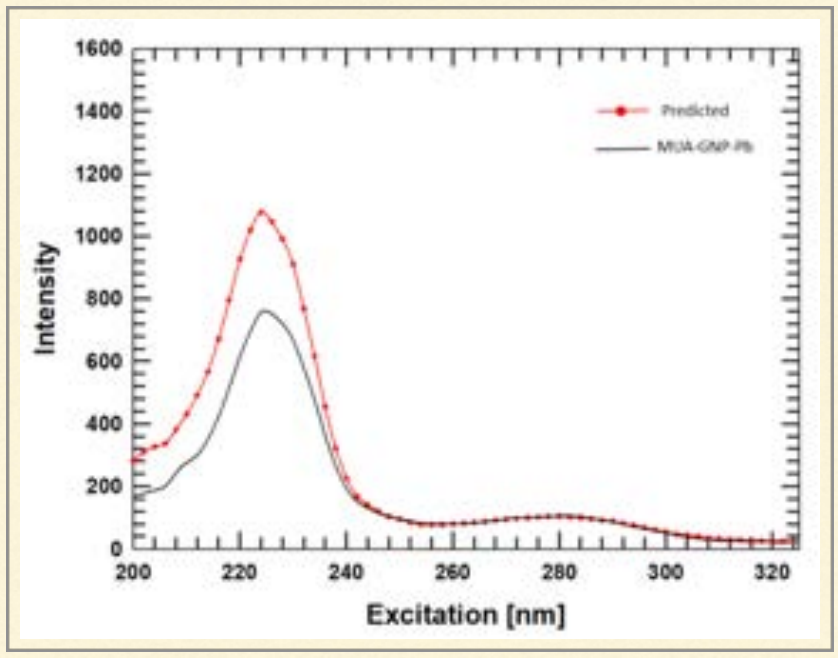

Figure 7. 2D spectrum of MUA-GNPs at 11.375 µg/mL with Pb2+ at 100 µg/L

From the above figures, it can be seen that all three possible responses are exhibited (no change, enhancing, quenching, respectively).

Conclusion

The goal of this research is to determine whether the fluorescence of gold nanoparticles functionalized with 11-mercaptoundecanoic acid can be used as a sensor for lead in water. The preliminary results of this study shown above are promising. The addition of lead to varying concentrations of gold nanoparticles does have an effect on their fluorescent intensity. The issue which now arises is in consistency of data. Future work will be to conduct these experiments again, at the same concentrations, to determine whether these results are reproducible. Once reproducability has been established, the research will focus on sensitivity and selectivity studies. This research is clearly still in the early phases, but the preliminary work is hopeful. We have successfully shown that the presence of a lead ion in a solution of gold nanoparticles functionalized with 11-mercaptoundecanoic acid can affect the fluorescent intensity of the nanoparticles. This result suggests that these MUA-GNPs can in fact be used as a form of detecting lead in water.

References

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