Procedure

Before SEFOS approved this project, preliminary testing was done in October 2019 using red food dye and table salt in place of acid red 94, AR94, and lead nitrate, $Pb(NO_3)_2$, to start developing and practicing procedures utilized later, during the collection of experimental data. The preliminary data collection was done using an Ocean Optics USB 4000 spectrometer (spectrofluorometer) connected to a laptop with a USB cable. One end of a blue fiber optic cable was connected to the spectrofluorometer while the other was connected to the cuvette holder.

A stock solution of red food dye was prepared in a similar way to which AR94 stock solutions were prepared later. The first step was half filling a 50 mL volumetric flask with deionized (dI) water. One drop of red food dye was added to the volumetric flask before it was capped and shaken to mix its contents and filled with dI water until the bottom of the meniscus rested on the fill line. The flask was then capped and shaken to mix its contents again.

Next, a 1 mL volumetric pipette was used to transfer enough stock solution from the flask to a glass cuvette so the cuvette was about ¾ full before closing the cuvette. The volumetric flask containing the red food dye stock solution was wrapped in aluminum foil because the dye is photosensitive (sensitive to light) and will degrade over time if exposed to light as is the case with AR94.

Then, the laptop connected to the spectrophotometer with a USB cable was turned on. The Spectra Suite program on the computer was started. The cuvette was placed in the

spectrophotometer so that the side marked with a Q was facing the blue fiber optic cable. An LED lamp used for excitation was turned on. The light entered the spectrometer through a hole that was 90 degrees from where the fiber optic cable was connected. The maximum fluorescence intensity of the sample was recorded from Spectra Suite and saved in a folder on the laptop. The volumetric flask and the cuvette were emptied into a waste beaker and rinsed with dI water.

The procedure was then repeated with 2 drops of red food dye, 3 drops, 4 drops, etc. The LED lamp was turned off after each fluorescence intensity measurement and then turn on right before taking the next one. The repetitions were stopped when additional drops decreased instead of increased the maximum fluorescence intensity measured by the spectrophotometer. The number of drops that resulted in the highest maximum fluorescence intensity measured by the spectrophotometer was recorded. The recorded number of drops of red food dye (3 drops) was used to make a new red food dye stock solution.

After that, a weigh boat was placed on a scale, and the scale was tared. About 0.02 g of table salt was weighed on the weigh boat. The salt was transferred from the weigh boat to another 50 mL volumetric flask. A dI water wash bottle was used to ensure all the salt from the weigh boat was transferred to the flask. The volumetric flask was filled with dI water so the bottom of the meniscus rests on the fill line before being capped and shaken to mix its contents.

Then, the volumetric pipette was used to transfer 3 mL of the red food dye solution into each of seven labelled 7 mL glass vials. The volumetric pipette was rinsed with dI water. It was then used to transfer incremental quantities of the saline solution to each vial such that

vial 1 received 0 mL, vial 2 received 0.5 mL, vial 3 received 1.0 mL, and so on until vial 7 received 3 mL. Each vial was filled with dI water, closed, and shaken to mix their contents.

A volumetric pipette was used to transfer enough solution from vial 1 to a cuvette so the cuvette was about ¾ full. The spectrophotometer was used to measure the fluorescence spectra in the same way as was done for the aforementioned procedure for determining the number of drops of red food dye that should be used to prepare the stock solution that results in the maximum fluorescent intensity reading by Spectra Suite. Then, the cuvette was taken out of the spectrofluorometer, emptied into a waste beaker, and rinsed with dI water. The process described in this paragraph was repeated for vials 2 – 7.

After SEFOS approved this project, experimental data collection for this project started in November 2019 with the supervision of Professor Buthelezi in her physical chemistry laboratory at Wheaton College. AR94 and $Pb(NO_3)_2$ were used instead of red food dye and table salt respectively. The same procedure used during preliminary data collection was used but with modifications and extensions. The experimental data collection took place over four separate days: November 16th and 23rd 2019, and January 3rd and 11th.

On November 16th , about 0.0098 g of AR94 was weighed on a scale and transferred to a 100 mL volumetric flask that was then filled with dI water until the bottom of the meniscus rested on the fill line. The flask was capped and shaken. That was then labeled as the AR94 stock solution and it had a concentration of 1.01×10^{-4} M. Next, about 0.0296 g $Pb(NO_3)_2$ was weighed on a scale and transferred to a 50 mL volumetric flask that was then filled with dI water until the bottom of the meniscus rested on the fill line. The flask was capped and shaken. That was then labeled as the $Pb(NO_3)_2$ stock solution and it had a concentration of 1.787×10^{-3} M.

Ten 7ml glass vials were labeled 1 – 10. Next, 0.7 mL of the AR94 stock solution was added in each vial. This will ensure [AR94] remains at a constant 1.01×10^{-5} M. Then, no $Pb(NO_3)_2$ stock solution was added to vial 1. In vial 2, 0.1 mL of the $Pb(NO_3)_2$ stock solution was added, then the volume added to vials 3 -10 were incremented by 0.1 mL such that 0.2 mL was added to vial 3, 0.3 mL was added to vial 4, and so on until 0.9 mL was added to vial 10. Then, fill each vial with dI water. All vials were capped and shaken to mix the contents.

Next, the contents of each vial were poured into individual disposable plastic cuvettes labelled with numbers to match the labelling of the cuvettes. The cuvettes were kept closed after they were filled. Then, to measure the fluorescence spectra of the solutions in the cuvettes, instead of using a handheld spectrofluorometer as was done during preliminary data collection, a Perkin Elmer LS-55 fluorescence spectrometer shown in the figure below was used for better accuracy.

The spectrometer was connected to a desktop computer with BL Studio version 1.04.02 software installed on it. That software was opened and used to record the spectra. The

spectrometer was set to use an excitation wavelength of 530 nm. Each cuvette was placed in the spectrometer. After recording each fluorescence spectrum from the cuvettes using BL Studio, they were exported to Microsoft Excel files saved in a folder on the desktop. Finally, the solutions were disposed of in a toxic metal waste bottle located in a satellite accumulation area (SAA) in the laboratory.

November 23rd was the day for testing for selectivity of using AR94 as a sensor for detecting Pb^{2+}. The first step completed to meet this goal was soaking filter paper in a concentrated AR94 aqueous solution. Then, the filter paper was covered in paper towels and left to dry in a hood. Next, the filter paper was cut into strips. Then 1×10^{-4} moles of nitrate salts of silver, aluminum, calcium, copper, iron, potassium, magnesium, sodium, and lead were weighed on a scale. The theoretical masses for each were calculated by multiplying 1×10^{-4} moles by the molar mass of each metal nitrate. The actual masses were recorded. Those masses of metal nitrates were used to make aqueous solutions in 10 mL volumetric flasks. Then, those solutions were diluted by a factor of 10 by adding 0.7 mL of each solution to 7 mL glass vials and then filling them with dI. Another 7 mL glass vial was filled with dI water so it could function as a control. An AR94 soaked filter paper strip was dipped in each of the vials. Which strips exhibited a color change from pink to purple or exhibited no color change other than becoming wet was noted.

Next, data was collected to determine the detection limit of the methods utilized so far for $[Pb^{2+}]$ detection and measurement using AR94. 0.7 mL of the AR94 stock solution was added to 10 vials and the fluorescence spectra of each was measured. The data collected this day was also exported to Excel and saved in the same folder as the data from November 16th.

On January 3rd , the procedure from November 16th was repeated to increase the homoscedasticity of the residuals of the data when linearized and fitted with a least squares-regression line and to decrease the detection limit so it is closer to the EPA's action level for lead. The first part of the procedure on this day was similar to how the preliminary data collection started with determining how many drops of red food dye should be used to make the stock solution. In this case, first new AR94 and $Pb(NO_3)_2$ stock solutions were prepared and had concentrations of 9.9×10^{-5} M and 1.83×10^{-3} respectively. Then, five pairs of solutions were prepared in 7 mL, each with a different [AR94]. In each pair, one solution contained no lead and the other contained 0.100 mL of the $Pb(NO_3)_2$ stock solution. The fluorescence intensity spectrum of each solution was recorded using BL Studio. The maximum fluorescence intensity was recorded for each solution and the difference was calculated for each pair. The pair that resulted in the greatest maximum fluorescence intensity difference was the one for which 0.98 mL of the AR94 stock solution was added to the 7 mL vial. Therefore, 0.98 mL of the AR94 stock solution was added to the ten vials instead of 0.7 mL. Furthermore, to minimize the effect of random variation in the data, three sets of ten such vials were prepared instead of one set. Additionally, the fluorescence spectra of each vial was measured three times and averaged to account for variation in the spectrometer measurements. Also, one glass cuvette was used and rinsed with dI water between fluorescence spectra measurements instead of different plastic cuvettes. The resulting data was exported to another Excel file and saved in the same folder as the data from November 23rd.

The final day of experimental data collection was January 11th. On this day, new stock solutions of AR94 and $Pb(NO_3)_2$ were prepared using 0.0100 g AR94 and 0.0034 g

$Pb(NO_3)_2$ respectively in 10 mL volumetric flasks to make 1×10^{-4} M solutions. Next, eight solutions were prepared in 10 mL such that the mole fraction of each stock solution ranged from 0.1 to 0.9. Each of those solutions and the AR94 and $Pb(NO_3)_2$ stock solutions were poured into a beaker and then aliquots were pipetted into a 7 mL glass vial. The content of each vial was then poured into one glass cuvette rinsed with dI water between fluorescence spectra measurements. The resulting data was exported to an Excel file and saved in the same folder as the data from January 3rd. Finally, two sets of ten solutions were prepared in 7 mL glass vials. One set had 1×10^{-4} M solutions of the same metal nitrates investigated on November 23rd different metals. The other set contained $Pb(NO_3)_2$ solutions with increasing lead concentrations, $[Pb^{2+}]$. Each set also contained a vial filled with only dI water as a control. An AR94 soaked filter paper strip was dipped in each vial. Strips that exhibited a color change from pink to purple or exhibited no color change other than becoming wet were noted.