Project Proposal

Project Title: Using Acid Red 94 to Detect & Quantify Pb^{2+} Ion Concentration in Water
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Phrase 1 (Research question): How can changes in the fluorescence emission intensity of acid red 94 (AR94) be used to detect and quantify Pb^{2+} ion concentration in water?

Phrase 2 (Hypothesis): As Pb2+ concentration increases, the fluorescence emission intensity of an aqueous solution of AR94 decreases possibly because the fluorescent dye AR94 associates with Pb2+ ions to form a complex that is not fluorescent.

Background:

[Explain the context of the project and why is it needed (i.e. the problem statement, research question, or need statement).]

Research question: How can changes in the fluorescence emission intensity of acid red 94 (AR94) be used to detect and quantify Pb^{2+} ion concentration in water?

In recent years, ions of toxic heavy metals such as lead and mercury have gained much attention due to their negative effects on the environment and human health. Due to carcinogenic effects, these metals have been forbidden for use in electronics by the European Union's Restriction of Hazardous Substances (RoHS). Additionally, the United States Environmental Protection Agency (EPA) and World Health Organization (WHO, 2004) have established allowable limits on the basis of their toxicity in drinking water. (Rasheed et al., 2017). More specifically, the EPA's action level for Pb^{2+} ions in water is 15 micrograms/liter (Agency for Toxic Substances & Diseases Registry, 2017), so it is especially important that drinking water lead concentration does not exceed the EPA specification.

Lead and other heavy metals need to be detected before they can be removed from drinking water. Knowing how much lead is in water can help determine the optimum method of removal. Scientists often use fluorescent dyes to label chemicals of interest in biology, chemistry, and medicine (such as in cancer screening or apoptosis detection). They have done promising work on different chemical sensors, especially fluorescent ones, for heavy metals like lead. For example, researchers in China have done work on using dithizone functionalized CdSe/CdS quantum dots as a turn-on fluorescent probe for ultrasensitive detection of lead ions with success in environmental samples (Zhao, Rong, Ma, Tao, 2012). They note that conventional analytical lead detection methods, such as atomic absorption spectrometry, inductively coupled plasma mass spectrometry, and electrochemical methods, often require sophisticated equipment, trained personnel, high cost, and sample pretreatment. Consequently,

real-time and on-site monitoring of lead is difficult to accomplish. Therefore, easy, rapid, inexpensive and highly sensitive methods for measurement of lead are in demand.

Researchers from Egypt have used a dye called acid red 94 (AR94, also known as rose bengal) for simple, selective detection and efficient removal of toxic lead and silver metal ions (Kamel et al. 2019). Acid red 94 is a mildly toxic bright red stain often adsorbed to and absorbed by compromised epithelial cells, mucus and fibrous tissue (Efron, 2012). In certain eye drops, it is used to identify damage to the eye by staining damaged conjunctival and corneal cells. Additionally, the researchers also mention that AR94 dye is a good probe for monitoring electrostatic interactions and hydrogen bonding interactions and is widely used in photocatalytic reactions. To their knowledge, utilizing AR94 as a sensitive chemosensor is rarely reported in the literature. I want to further investigate the claim from the conclusion of their paper that AR94 can potentially become a sensitive and selective sensor for the qualitative and quantitative detection of lead and could be used as a visual indicator.

Fluorescent probes for heavy metals work because the heavy metals binding cause changes in the intensity of their fluorescence emissions.My research question is how can changes in the fluorescence emission intensity of acid red 94 (AR94) be used to detect and quantify Pb^{2+} ion concentration in water? I chose acid red 94 over other possible fluorescent probes from my research because it is inexpensive, water soluble, and easy to use.

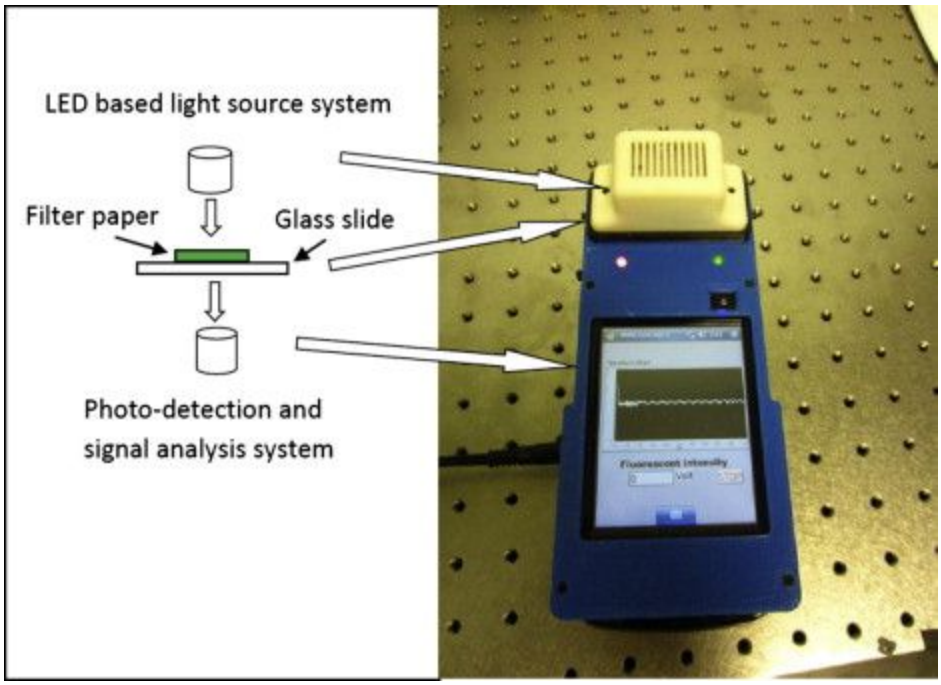

Figure 1. A portable device for measuring chloride ion concentration with a miniature fluorescent detection system.

Researchers in China have worked on a miniature fluorescent sensor (shown above in figure 1) for chloride ion concentration in sweat determination based on a modified Stern–Volmer Equation (Wang et al., 2013) and I plan on using similar methods to analyze the data I will gather for my project.

Project Definition:

[Provide a succinct summary of the main aim of the project, objectives, and intended outcomes. A statement of purpose] The overall aim of this project….

The overall aim of this project is to use the fluorescence emission intensity of aqueous solutions containing a given concentration of the dye acid red 94 (AR94) to detect and quantify the Pb^{2+} ion concentration in that solution.

Expected outcomes:

- The Pb^{2+} ion concentration and the resulting AR94 fluorescence emission intensity will follow a Stern–Volmer relationship that can be modeled using an equation.

Overall implications:

- The equation derived from this experiment can be used to calculate the Pb^{2+} ion concentration in an AR94 aqueous solution given the AR94 fluorescence emissions intensity.
- Hopefully, one can determine if water contains lead by adding AR94 and observing its fluorescence with the naked eye and, if it does, can determine what the lead concentration is by using the Stern–Volmer relationship equation.

Potential Roadblocks:

- The relationship between lead ion concentration and the fluorescence of the dye acid red 94 may not be immediately clear from experimental data.
- There may be unexpected chemical reactions that affect the fluorescence spectra.

Experimental Design/Research Plan Goals:

Major Parts of the Project (rough outline) will continue to evolve over time and should be updated frequently. Make sure the goals are SMART oriented.

- Prepare the following materials
  - UV-Vis absorption spectrometer
  - Ocean Optics USB 4000 spectrometer (spectrofluorometer)
  - USB cable
  - Fiber optic cable
  - Cuvette holder
  - 50 mL volumetric flask
  - Deionized (dI) water
  - Wash bottle
  - Red food dye
  - 1mL volumetric pipette

- A laptop with Spectra Suite installed on it
- LED lamp
- Aluminum foil
- Table salt - NaCl
- Weigh boat
- Mass scale
- Seven 7 mL vials labeled with numbers 1 - 7
- Regular cuvette
- Disposable cuvettes or small vials (small enough to fit in the cuvette holder)
- Acid Red 94 (AR94 also known as Rose Bengal)
- Lead Nitrate - $Pb(NO_3)_2$, KNO_3, $Mg(NO_3)_2$, $Ca(NO_3)_2$, and $Cu(NO_3)_2$

- Follow the following preliminary procedure:

1. Gather the materials listed above.
2. Connect the Ocean Optics USB 4000 spectrometer (spectrofluorometer) to the laptop with the included USB cable.
3. Connect one end of the blue fiber optic cable to the spectrofluorometer by one end to the cuvette holder. Turn the cable at each end so the connection is tight.
4. Half fill a 50 mL volumetric flask with deionized (dI) water.
5. Add 1 drop of red food dye to the dI water in the volumetric flask.
6. Cap the flask and mix its contents.
7. Add dI water to the flask until the bottom of the meniscus rests on the fill line.
8. Repeat step 6.
9. Use the 1mL volumetric pipette to transfer enough solution from the flask to a cuvette so the cuvette is about ¾ full and then close the cuvette.
10. Wrap the volumetric flask in aluminum foil because the dye is photosensitive (sensitive to light) and will degrade over time if exposed to light.
11. Turn on the laptop and open Spectra Suite.
12. Place the cuvette in the spectrophotometer so that the side marked with a Q is facing the blue fiber optic cable.
13. Turn on the LED lamp, lay it horizontally, and support it so the light is horizontal as shown in the picture below. Make sure the light enters the spectrometer through a hole that is 90 degrees from where the fiber optic cable is connected.

Figure 2. Ocean Optics USB 4000 spectrometer and LED lamp setup.

14. Record the maximum fluorescence intensity.
15. Empty the volumetric flask and the cuvette into a waste beaker and rinse both the flask and the cuvette with dI water.
16. Repeat steps 4 - 15 with 2 drops of red food dye, 3 drops, 4 drops, etc. Stop when additional drops decrease instead of increase the maximum fluorescence intensity measured by the spectrophotometer.
17. Record the number of drops that resulted in the highest maximum fluorescence intensity measured by the spectrophotometer.
18. Repeat steps 4 - 8 using the recorded number of drops of red food dye from the previous step.
19. Place a weigh boat on a scale and tare the scale.
20. Measure about 0.02g of table salt on the weigh boat.
21. Transfer the salt from the weigh boat to another 50 mL volumetric flask. Use a dI water wash bottle to ensure all of the salt from the weigh boat is transferred to the flask.
22. Fill the flask with dI water so the bottom of the meniscus rests on the fill line.
23. Cap the flask and mix its contents.
24. Use the volumetric pipette to transfer 3 mL of the red food dye solution into each of the seven labelled 7 mL vials.

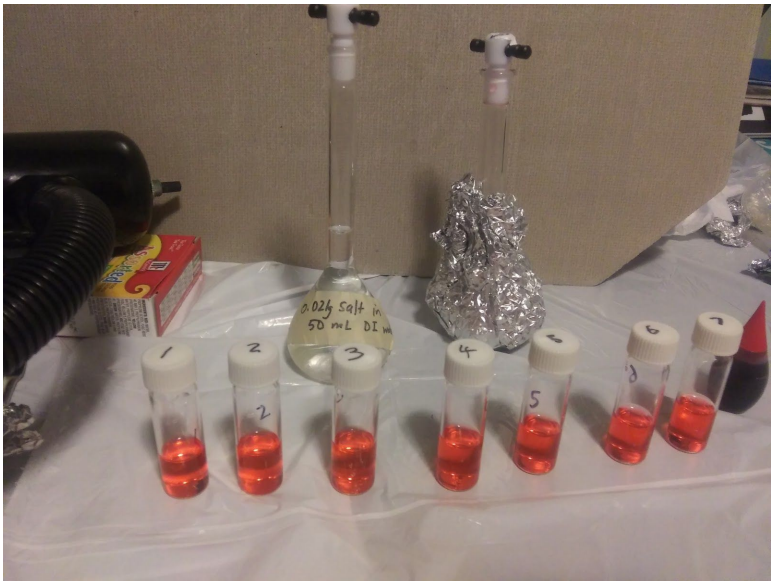

Figure 3. A 50mL volumetric flask with a salt solution, a 50mL volumetric flask with a red food dye solution and covered in aluminum foil, seven labelled 7mL vials each containing 3mL of red food dye solution.

25. Rinse the volumetric pipette with dI water.
26. Use the volumetric pipette to transfer incremental quantities of the saline solution to each vial such that vial 1 receives 0 mL, vial 2 receives 0.5 mL, vial 3 receives 1.0 mL, and so on until vial 7 receives 3 mL.
27. Fill each vial with dI water, close them, and shake them to mix their contents.

Figure 4. Seven labelled 7mL containing food dye and salt solutions and dI water as described in steps 24 and 26 in this procedure.

28. Use the volumetric pipette to transfer enough solution from vial 1 to a cuvette so the cuvette is about ¾ full.
29. Repeat steps 12 and 13.
30. Make a folder for experimental data on the laptop.
31. Open an excel file in that folder.
32. Save the spectra as a txt file in the folder from step 30.
33. Export the txt files from the raw spectra data folder to Microsoft Excel, and copy the raw spectral data from each new excel file into the experimental data excel file from step 31 such that there is one column for wavelengths and one column for the fluorescence intensity for each solution. The columns should all start in the same row. Above that row, add another row. Label the leftmost column wavelength (nm). Label the subsequent columns solution 1, solution 2, solution 3, and so on until solution 7.
34. Graph the processed spectral data as a scatter plot with smooth lines.
35. Locate the wavelength that corresponds with the maximum fluorescence intensity for most of the solutions.
36. Make a new table in the same excel file with four columns. The rows of leftmost column should be labeled from top to bottom as solution 1, solution 2, solution 3, and so on until solution 7. The next column to the right should have the corresponding concentrations in molarity (M). The next column to the right should have the fluorescence intensity at the wavelength chosen in the previous step. The rightmost column should contain the ratio of the fluorescence intensity for each solution to the fluorescence intensity of the solution with a salt concentration of 0 M.
37. Repeat steps 4 - 36 with the dye acid red 94 and lead nitrate instead of with red food dye and table salt and instead of first making solutions in vials and transferring them to a cuvette one by one, make solutions in enough either vials small enough to fit in the spectrophotometer or disposable cuvettes. Then put those in the spectrophotometer to collect spectra. Keep the vials or disposable cuvettes closed after solutions are made. Dispose of the solutions in a toxic metal waste bottle located in a satellite accumulation area (SAA) in the chemistry lab at Wheaton College at the end of the experiment.

38. Test for selectivity by repeating the experiment with AR94 and other metal ions that could be found in drinking water, such as the metal ions K+, Mg2+, Ca2+, and Cu2+.
39. Determine the detection limit for the concentration of lead ions using AR94 in water.

- Analyze the data in using equations (such as the Stern-Volmer equation), tables, and graphs in Microsoft Excel.
- Write a final report.
- Practice presenting results on a poster board.

Risks/Hazards:

- I will work under the supervision of Professor Buthelezi at Wheaton college in her lab for the creating the lead nitrate and AR94 dye solutions..
- I will make those solutions in vials or disposable cuvettes and keep them closed for the rest of the experiment.
- I will dispose of the solutions in a toxic metal waste bottle located in a satellite accumulation area (SAA) in the chemistry lab at Wheaton College at the end of the experiment.

Timeline: (with action steps identified- sub-deadlines will continue to evolve):

Rough timeline of major phases. As these phases get established, specific tasks under these phases will be defined further.

- Brainstorming
- Reading research articles and other informative text related to my project,updating project notes, updating and editing project proposal
- Completing and editing paperwork
- Collect preliminary data
- Collect experimental data
- Data analysis and conclusion
- Prepare and practice presentation.

Background Knowledge Goals:

Date	Topic	Completed Date
9/24/19	AR94 and Heavy Metal Complexes	10/19/19
9/24/19	Fluorescence	10/19/19
9/24/19	Stern-Volmer Equation	10/19/19

Brainstorming Reflections:

What do you not want to use?	Why?
Prototype	I would rather utilize currently available materials and devices for my project than design my own. My inexperience in synthesizing materials and the time constraints for this project would make developing a prototype (i.e. synthesizing my own dye to use for this project) infeasible.
Reverse Osmosis	This requires complex and costly equipment I do not currently have access to. Acid Red 94 dye is comparatively cheap and simple to use.
Biomimicry of bioluminescence	This is also no longer related to my project topic since I am no longer investigating light production for this project.

What do you want to use?	Why?	What assumptions are being made?	How can you challenge those assumptions?
Low cost	I must use materials within my family's budget.	Others can afford the materials and devices I will use in this experiment.	People may not know some of the materials or devices exist or where to find them.
Look at ways it has been done before	I could possibly build off of or improve what has already been done.	Current methods of detecting and quantifying lead concentration in	Possibly, current methods of detecting and quantifying lead concentration in

		water can be improved upon.	water may already be as good as they can be and doing better requires a new method.
Think about the target user/audience.	I want to find a way of detecting and quantifying lead concentration in water that as many people as possible with the right materials and devices can do easily.	Other people can afford and have access to the materials and devices I will use in this experiment.	People may not know some of the materials or devices exist or where to find them.

References:

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