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Using Quenching To Detect Corrosion on Sculptural Metalwork: A Real-World Application of Fluorescence Spectroscopy

Cory Hensen
Portland State University, chensen@pdx.edu

Tami Lasseter Clare
Portland State University, claret@pdx.edu

Jack Barbera
Portland State University, jbarbera@pdx.edu

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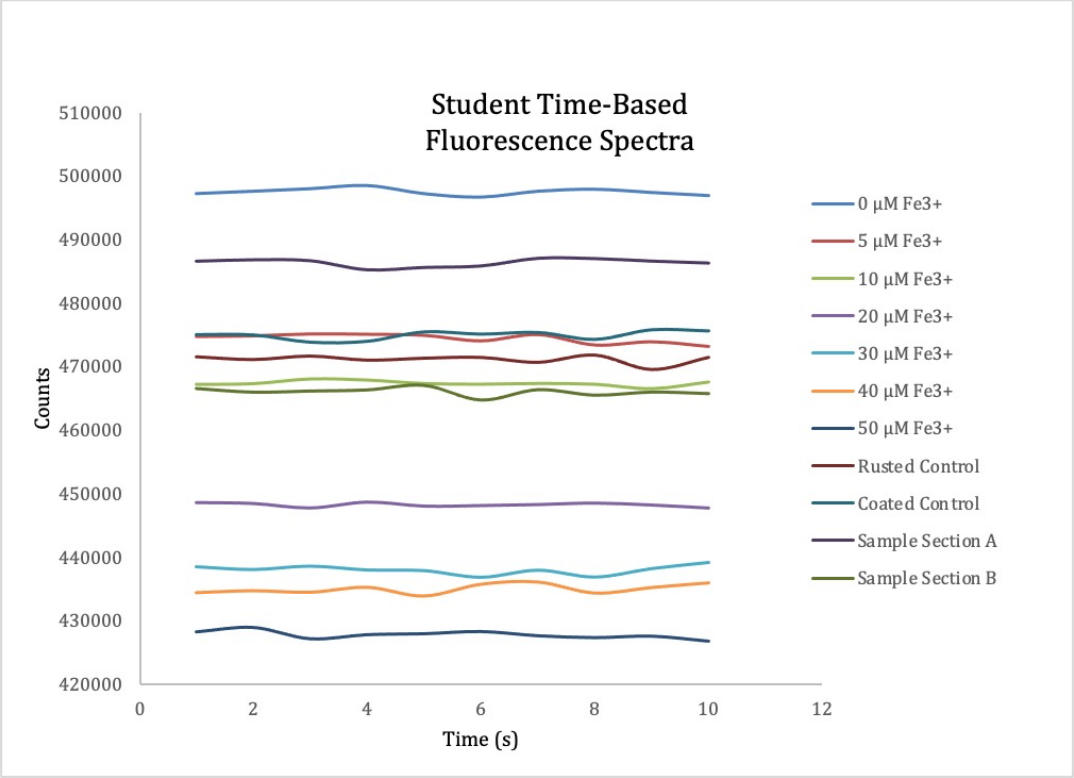
Cory Hensen, Tami Lasseter Clare, Jack Barbera*

Department of Chemistry, Portland State University, Portland, OR 97207, United States

ABSTRACT

Fluorescence spectroscopy experiments are a frequently taught as part of upper-division teaching laboratories. To expose undergraduate students to an applied fluorescence technique, a corrosion detection method, using quenching, was adapted from authentic research for an instrumental analysis laboratory. In the experiment, students acquire fluorescence spectra of sensing molecules in the presence of mock sculpture samples and discuss the condition of the sculptures based on the levels of soluble iron detected. This real-world based experiment allows students the chance to engage with ongoing research and further understand the challenges with early detection of corrosion. Most students successfully completed the experiment, wrote a journal-quality report, and met the learning outcomes.



KEYWORDS

Upper-Division Undergraduate, Laboratory Instruction, Problem Solving, Fluorescence Spectroscopy, Student-Centered Learning, Hands-On Learning/Manipulatives

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Corrosion is estimated to cause 276 billion dollars' worth of damage in the United States annually across industries ranging from aviation to civil engineering to art.[1] Currently, visual inspection is the most common method used to assess corrosion in the field[2], but it is not an ideal method, particularly in art conservation, as visible corrosion equates to loss of original material. In the laboratory, other methods are used, such as measurements of corrosion potential[3], which necessitates electrically induced corrosion of a metal to measure the voltage at which the metal oxidizes. If corrosion is detected, the coating must be removed in the damaged area, the corrosion mechanically removed, and a fresh coating reapplied. To reduce the frequency of this process, corrosion inhibitors are added to paint. Historically, chromates were used as corrosion inhibitors.[4] A desire to reduce heavy metal effluents drove the field to phosphates, which have their own negative impacts as runoff on water quality.[5] Effective replacements for phosphates are currently being investigated in the scientific literature and include long-chain fatty acids, among others.[6-7] Developing a non-destructive method to measure early corrosion remains an ongoing challenge in basic research.[8]

Research has shown numerous benefits for students who partake in authentic research experiences as an undergraduate student.[9-11] For example, in a study by Seymour et al., 91% of students self-reported receiving positive benefits ranging from gaining confidence in their scientific skills to further confirming or clarifying their career choice.[9] Popular methods for exposing a large number of students to aspects of research experiences are course-based undergraduate research experiences (CUREs)[12] and problem-based learning (PBL) experiments. However, these typically span several weeks to an entire course rather than one laboratory period. This real-world based experiment was developed to fit within a writing-intensive four-credit course designed to instruct students in writing journal-quality articles about their hands-on instrument experiences. To provide students the benefits of having exposure to current research in a single laboratory period, an experiment was designed around the research of Dr. Tami Lasseter Clare of Portland State University. One aspect of Dr. Clare's research works towards developing non-destructive methods for detecting early signs of corrosion on sculptures before the underlying metal is damaged.[13]

Semiconducting quantum dots have been employed as a colorful way to introduce fluorescence spectroscopy in the undergraduate instrumental analysis laboratory.[14] However, as toxicity concerns rise about the heavy-metal content of those quantum dots, environmentally-friendlier alternatives are sought.[15] In fact, a recently-published article incorporates carbon-based quantum dots instead of traditional heavy-metal quantum dots to teach fluorescence.[16] Other additional applications for this general methodology include glucose biosensors[17] and chemical sensors for monitoring water quality[18]. The range of applications for carbon quantum dots is owed, in large part, to the easy modification of the carbon dots' surface to display a range of chemical functional groups.[19] A proposed structure and image of the functionalized carbon dots used in this experiment are shown in Figure 1.

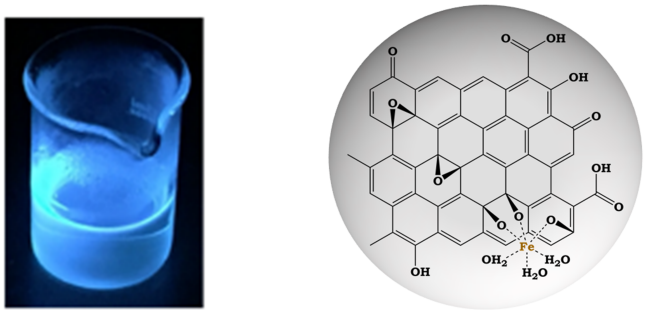

Figure 1. Student-synthesized quantum dot solution under illumination at 370 nm (left) and a proposed quantum dot structure (right).

Applying chemistry to art is a popular way of providing an engaging context for students to learn chemistry.[20-22] Currently, there are few undergraduate laboratory experiments that focus on corrosion[23-26] and none that use fluorescence to monitor corrosion or focus on non-destructive detection. This experiment presents a way to introduce fluorescence spectroscopy techniques while providing students

a chance to understand corrosion detection and work towards non-destructive methods. It is designed to be a real-world based experiment in which students are required to construct their own knowledge to tackle the real-world challenge of corrosion detection. Students are presented with two mock "sculpture sections" in the form of small painted metal coupons and asked to determine if the metal beneath the paint is corroding. To accomplish this, students must read primary literature and synthesize an approach from their own understanding to solve the problem.

Although many PBL experiments occur over several laboratory periods to allow students ample time to construct their own knowledge, this real-world based experiment was designed to fit within a four-hour laboratory period due to curriculum constraints. Therefore, students were given more preparatory materials than a PBL experiment. However, the key elements of a PBL experiment[27] were included in this real-world based experiment.

EXPERIMENTAL OVERVIEW

Prior to attending the laboratory period, students watch the recorded pre-lab lecture video and complete the pre-lab exercise and quiz, which are included in the Supporting Information. Students begin the laboratory period by discussing their individually created procedures with their lab partners. After reaching consensus on a procedure, partners synthesize the carbon dots, prepare a calibration Stern-Volmer plot (Figure 2) and use the plot to test the mock sculpture samples for levels of corrosion (test samples are shown by the silver and red points on the plot). A Stern-Volmer plot is used for fluorescence quenching and is represented by the equation $F_0/F = 1 + K_{SV}[Q]$ where F_0/F is the ratio of unquenched signal to quenched signal, K_{SV} is the quenching constant and [Q] is the concentration of quencher. The intercept for a Stern-Volmer plot is set to 1 since the ratio between quenched and unquenched signal in the presence of no quencher is 1. Students working in groups of three to four should be able to complete their entire procedure within a four-hour laboratory period.

In the experiment, students are presented the problem from the standpoint that a museum needs a field-ready kit to assess the corrosion on their sculptures. Before the kit can be deployed to sculptures in the field, it is important to develop a method that is reliable. Therefore, to test the developed method, the museum has provided two small samples, known as coupons, from a mock sculpture. The museum is interested in if the developed method works to assess whether signs of corrosion are present. If the method works and produces consistent results, the museum can scale the research without the need to destructively remove metal from the sculpture for testing. The students write a journal-quality report to analyze how well their destructive method of early corrosion detection works and suggest a non-destructive alternative. This scenario, along with other useful information, is provided to students in the "Student Laboratory Guide", which is included in the Supporting Information.

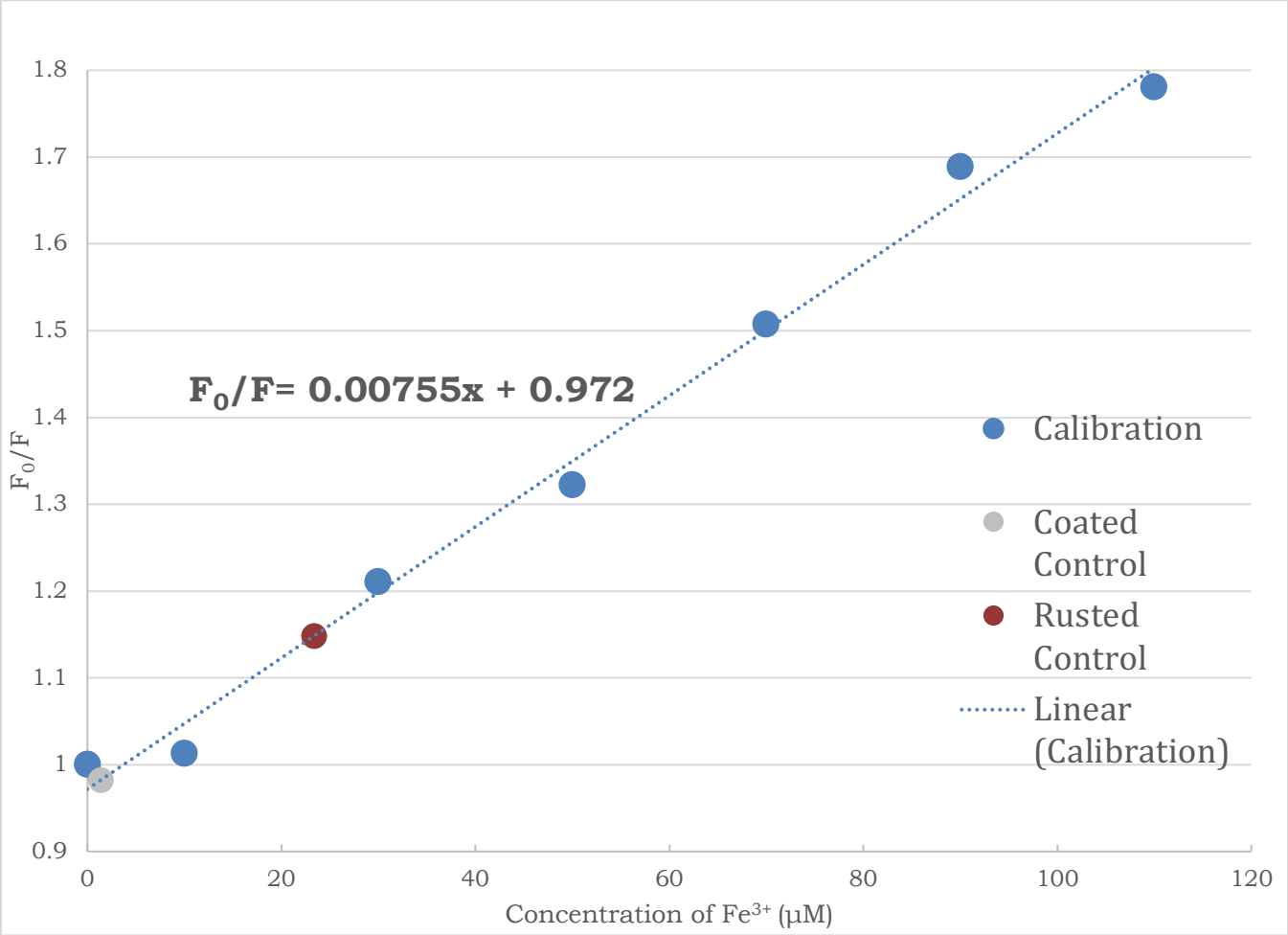

Figure 2. A sample student Stern-Volmer plot used to calculate the Fe^{3+} concentration in the samples.

LEARNING OUTCOMES

Through the use of quantum dots, students obtain knowledge about how fluorescence works along with how to obtain and analyze fluorescence spectra. After completing the experiment, students will be able to:

1. Read and apply primary literature to design a procedure
2. Describe fluorescence
3. Describe the process of quenching
4. Use a fluorometer and make a Stern-Volmer plot
5. Describe how a fluorometer works
6. Investigate the challenges presented in real-world problems
7. Understand the challenges presented in real-world problems
8. Gain insight to current research being performed
9. Write a scientific journal-quality article about their findings.

As seen in Table 1, these outcomes were successfully met by the students to varying degrees ranging from 69% of the students (learning outcomes 2 and 8) to 100% of the students (learning outcome 4).

EXPERIMENTAL COMPONENTS

Pre-lab Exercise

Before starting the experiment, students are given a sample Stern-Volmer data set and are required to process it in order to gain an understanding of the expected data output. Additionally, students complete a pre-lab quiz to determine how successful they were at reading the assigned primary literature and developing their own procedure. The pre-lab activity and quiz are provided in the Supporting Information.

Procedure

For this experiment, students are not given a procedure but rather are presented with a student guide that frames the problem they are attempting to address and provides primary literature resources on which to base the procedure. Once student groups have reached a consensus on a procedure, they are instructed on how to use the fluorometer and the associated software. There are several different types of scans that the students can chose to run from the software. The scan options are to either run full excitation/emission scans, if the optimal excitation and emission wavelengths are not known, or run time-based scans, if the excitation and emission wavelengths are known. When designing their procedure, the students are required to make several decisions including: how to synthesize the quantum dots, the selection of excitation and emission wavelengths, the slit widths to use, the standard concentrations for use in their Stern-Volmer plot, and the threshold concentration of iron. Common and uncommon questions students ask while making these decisions are found in Appendix C of the Supporting Information.

Each group is provided two small painted metal coupons from separate sections of a mock sculpture and two unpainted control samples for comparison (Figure 3). One control sample is visibly corroded, providing a control at one extreme of the possibilities while the other control is coated on both sides with a corrosion inhibitor for the other extreme (See Supporting Information for sample preparation).

Figure 3. Two metal coupons from a mock sculpture (left) and two unpainted control samples (right).

After the students have been briefly trained on the fluorometer and made decisions about how to carry out their procedure, students synthesize the quantum dots. While each group's procedure may vary, on average the synthesis ranges from 20 to 40 minutes and is carried out in a fume-hood. (See Supporting Information for synthesis information)

To quantify the amount of soluble iron on the four samples (two controls and two mock sculptures), the students make a Stern-Volmer plot by adding known concentrations of Fe^{3+} to the synthesized quantum dots.

To determine the reproducibility of their findings, students are given their peers' data after completion of the week's experiments and were required to discuss the reproducibility in their lab reports. This emphasizes the importance of high reproducibility in research so that the museum is provided with reliable information regarding the condition of the samples.

After completing their procedures, students are asked to write a journal-quality laboratory report while considering several discussion questions such as thinking critically about how their quantum dots and metal samples can be scaled non-destructively to real sculptures that do not fit inside a cuvette. The rubric to score the reports and more details about the report can be found in the Supporting Information.

Equipment

A Photon Technology International Fluorometer with a LPS-220B lamp was used in this experiment. Other models of fluorometers would be acceptable as long as the lamp source is capable of emitting photons at 350-400 nm and the detector can sense photons at 450-500 nm. The metal coupons were prepared from a McMaster-Carr Low-Carbon Steel sheet and then painted to mimic a painted steel structure. Information regarding sample preparation can be found in the Supporting Information.

Post-Lab Exercise

After completing the experiment, the students are asked to write a journal-quality article no longer than six pages. Questions for the students to consider when writing the discussion are included near the end of the Student Laboratory Guide.

Hazards

This experiment is relatively low risk but does use both acids and bases to prepare the quantum dots. Additionally, spray paint is used to prepare the metal samples. Therefore, appropriate PPE should be worn including laboratory coat, gloves, and safety goggles. The sample preparation step using spray paint should be completed in a well-ventilated area as a precaution.

RESULTS AND DISCUSSION

Students across four, four-hour, instrumental analysis laboratory sections completed this experiment in groups of three to four students. The students enrolled in instrumental analysis have successfully completed organic chemistry and the majority of them have completed a quantitative analysis lecture and laboratory as well. Of these 38 students, 32 consented to have their laboratory reports collected as part of this Portland State University Institutional Review Board approved project. After completing the experiment, most students were able to quantify the soluble iron in the two control and two mock sculpture samples. More inconsistent, however, was that students presented a variety of opinions regarding what iron threshold was sufficient to warrant repair or restoration of the sculpture. Ideally, students would understand that the repair threshold should be the limit of quantification. That is, that if *any* iron is detected through the paint, the metal underneath is already being damaged. However, a large minority of students were okay with tolerating minimal amounts of damage. These varied opinions highlight that in real-world applications it is a challenge to identify and justify an appropriate threshold iron concentration, which is an aspect of the ongoing research in the Clare group.

The variety of opinions was further evident when students wrote their reports. When asked to interpret their numbers in terms of a repair threshold (learning outcome 7), 84% of students were able to explain their rationale while unsuccessful students struggled to justify a threshold value. As the mock samples were designed to fit inside of a cuvette, but in the actual application of this research the sculptures need to remain intact, students were asked to think critically about how to expand their procedure into a field-ready test kit. Successful students (91%) were able to read the assigned primary literature and suggest a possible synthesis of their quantum dots that would allow iron ions to be detected on the mock sculpture sections (learning outcome 1), while unsuccessful students (9%) arrived at the laboratory completely underprepared for the experiment. Although a large majority can read and apply the primary literature, only 69% of the students successfully read current primary literature and then applied their new knowledge to a different situation (learning outcome 8).

This student-centered experiment provides a novel approach for introducing fluorescence spectroscopy. The students are challenged to construct their own knowledge about fluorescence spectroscopy and quenching while applying it to the problem of corrosion detection. While some students struggled with not being able to simply report a value, as they would at the end of a confirmation-type experiment, most students welcomed a real-world example of how fluorescence spectroscopy is important and relevant. It is often too easy for students to treat the undergraduate laboratory space as artificial, therefore this experiment challenges students to consider the real-world applications of chemistry for early detection of corrosion on sculptures.

Although there was a relatively low percentage of students that were able to apply their knowledge to a different situation in this experiment, the percentage should increase with an increase in the number of student-centered experiments in the curriculum. As students shift their approach away from looking to report a value and towards creating their own knowledge on the topic throughout a course, the number of students successfully reaching all learning outcomes for a given experiment should

increase. This can be further increased with instructors providing plenty of feedback as students work on developing new approaches to chemistry labs.

STUDENT LEARNING OUTCOMES

As seen in Table 1, students met the learning outcomes for this experiment to varying levels of success, with learning outcomes 3 and 8 being the lowest with 69% and outcome 4 with 100%. Success was defined, in this context, as a student receiving a rank of Fair or above on the scoring rubric. This rubric and more detailed information are provided in the Supporting Information. This experiment was designed to give students a chance to *evaluate* threshold values (learning outcome 7) and *create* new ways of approaching early corrosion detection (learning outcome 8). These are the two highest levels in Bloom's Taxonomy of Educational Objectives.[28] While 84% of the students reached the *evaluate* level and 69% of the students reached the *create* level, some students failed to meet the lower *describe* level outcomes (learning outcomes 2 and 3).

Table 1. Comparative Results for Student Performance on the Learning Outcomes

Learning Outcomes	Successful Students (N = 32)	Successful Students, %
1. Apply primary literature	29	91
2. Describe fluourescence	26	81
3. Describe quenching	22	69
4. Make a Stern–Volmer plot	32	100
5. Describe a fluourometer	26	81
6. Investigate a real-world problem	28	88
7. Understand obstacles in real-world problems	27	84
8. Gain insight into current research	22	69
9. Write a journal-quality report	29	91

This experiment allowed students to construct their own knowledge without directing them explicitly towards describing the techniques used to reach their decision about the corrosion level on the mock sculpture sections. The students that failed to meet the lower level outcomes (2 and 3) tended to not meet them successfully (or meet them with a rank of fair) because they focused their writing on how this experiment fit into the real world and less on the specifics of the experiment. Thus, some students did not focus on describing the techniques used but rather focused on the application of that technique in their reports. Additionally, students are given a six-page limit for their reports, forcing them to decide what is important to include. Keeping in mind that this was the first-time students in this course completed a student-centered experiment like this, it is expected that issues in meeting the lower level outcomes will be reduced as students gain more experience with the requirements for successfully completing these types of experiments and reports.

This experiment took place in a writing-intensive course that included a supplemental writing workshop. Throughout the workshop, students are trained to write journal-quality reports (learning outcome 9). Therefore, the expectations of what a report looks like change over the term. During the first-week the expectation is that students write a report that has some logical organization to it. However, by the end of the term, students are expected to be writing concise, well-structured reports that logically flow. As this experiment was conducted near the end of the term (during weeks 5-8 in a 10-week term), it is not surprising that a majority of students (91%) were successful in meeting this learning goal.

SUMMARY

This experiment was designed to give undergraduate instrumental analysis students an opportunity to engage with current research being performed to detect early corrosion on metal sculptures. Most students successfully obtained fluorescence spectra, created a Stern-Volmer plot, and made decisions about the threshold value for corrosion on the mock sculpture samples.

ASSOCIATED CONTENT

Supporting Information

The following files are available in the supporting information:

- Appendix A: Learning outcomes rubric
- Appendix B: Pre-lab lecture
- Appendix C: Instructor's laboratory guide
- Appendix D: Sample preparation
- Appendix E: Report rubric
- Appendix F: Student Laboratory Guide
- Appendix G: Pre-lab activity
- Appendix H: Sample student plots

AUTHOR INFORMATION

Corresponding Author

*Jack Barbera. E-mail: jbarbera@pdx.edu

Notes

The authors declare no competing financial interest.

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Department of Chemistry, Portland State University, Portland, OR 97207, United States

Supporting Information

Introduction:

This experiment is designed to give undergraduate analytical chemistry students exposure to current research being done that uses fluorescence spectroscopy of quantum dots. It is important for the students to be able to read primary literature and then develop their own procedure for making and using a fluorescence dye. The instructor should support the development of the procedure and challenge the students to think about the application of their procedure to detect early signs of corrosion. Since there is no set procedure, the instructor should guide the students and be a resource to help them tackle the real-world problem. This guide will provide you with the resources to help give your students exposure to early detection of corrosion on metal samples such as art sculptures. However, there may need to be adjustments made based on the number of student-centered experiments in the curriculum. Students being exposed to student-centered experiments for the first time may need more support than students use to a student-centered format.

Important Resources:

Appendix A: Learning outcomes rubric

Appendix B: Pre-lab lecture

Appendix C: Instructor's laboratory guide

Appendix D: Sample preparation

Appendix E: Report rubric with comments on expectations

Appendix F: Student Laboratory Guide

Appendix G: Pre-lab activity

Appendix H: Sample student plots

Appendix A: Learning outcomes rubric

Student lab reports were assessed with the following rubric to determine how successful they were at meeting the experiment's learning outcomes. The number in each box represents the number of students out of the 32 total students that were scored in that column for each learning outcome.

After completing this experiment, students will be able to:	**Not present**	**Poor**	**Fair**	**Good**	**Excellent**
Apply primary literature	0	3	3	17	9
Describe Fluorescence	0	6	12	6	8
Describe quenching	0	10	12	8	2
Make a Stern-Volmer plot	0	0	1	0	31
Describe a fluorometer	0	6	5	14	7
Investigate a real-world problem	0	4	26	2	0
Understand obstacles in real-world problems	0	5	18	9	0
Gain insight into current research	0	10	8	14	0
Write a Journal-quality report	0	3	14	14	1

The ranks were broken down by using the following general definitions:

- **Excellent** - The student goes above and beyond expectations
  - Example: A student who goes beyond the references given to them to design the procedure
  - Example: A student who spends significant time on their plots to succinctly show their findings
- **Good** - The student is meeting expectations but is not going above and beyond what is being asked of them
  - Example: A student who reads the additional reference on hydrogels and mentions how they could be used to make the procedure non-destructive
- **Fair** - The student is understanding the general learning outcome but is missing a key element
  - Example: A student who is asked to consider a painted sample and what the repair threshold should be but instead struggles to understand paint interference and the repair threshold
- **Poor** - The student is not showing an understanding of the learning outcome but gave an attempt
  - Example: A student who struggles to describe the quenching process or does so too briefly in their report
- **Not present** - The student did not make any progress towards the learning outcome
  - Example: A student who did not turn in a report or missed a significant part of the laboratory

Appendix B: Pre-lab lecture

The pre-lab lecture has been recorded on YouTube for use and the link is https://youtu.be/wgytRjUPAPY

The transcript is provided below in case modifications are desired.

1. Hello! My name is _____ and I am the [Insert role] for Instrumental Analysis here in the Chemistry Department at [Insert University]. Today I am going to be talking to you about a laboratory method to detect corrosion using fluorimetry by detecting the quenching of luminescent carbon quantum dots when they interact with soluble iron ions. This is an experiment that Cory Hensen helped develop along with Dr. Lasseter Clare and the rest of her research group. This lab has been designed to incorporate current research being done by a faculty member into the teaching laboratory. This allows you to get a glimpse into what research looks like along with all the challenges and successes that come along with doing research.
2. So first I am going to begin with some background on what the Lasseter Clare Lab is interested in. Dr. Lasseter Clare is interested in detection of corrosion before any signs of corrosion can be seen visually. Corrosion is a huge issue globally. Here in the US, the 2013 corrosion (direct and indirect) costs were 3.1% of the Gross domestic product, which was about 500 billion dollars. Those 500 BILLION dollars were used mainly to treat already corroded metal and for maintenance, not towards prevention of corrosion. Currently, most detection methods for corrosion rely on visual markers such as an inspector noticing some rust. However, once the corrosion is visible, part of the metal has already been lost, weakening the metal structure. Thus, there is a real need for a method capable of detecting corrosion early, before any signs of it can be seen. And hopefully, by detecting it early, researchers can develop materials that resist corrosion better, either through more impervious protective coatings, the use of corrosion inhibitors, by new metal alloys, or mixtures of different metals and other elements, that make the final material more corrosion resistant.
3. For outdoor sculptures, there are three different types of metal that are of particular interest, since they are the most commonly used materials. These three metals, steel, bronze, and aluminum in addition to being used in sculptures are also used in bridges, buildings, architecture, and aircraft to name a few other important applications for this research. Each of these metals produce different corrosions products; the presence of different types of products poses one of the challenges in developing an early corrosion detection method. Ideally, there would be a **single** tool that could detect the corrosion products from all three metals. This tool could then be used by inspectors in a variety of situations to detect early corrosion. One strategy that Dr. Lasseter Clare and her students are developing is a method to evaluate the protective **quality** of coatings on outdoor metalwork, including paints and clear coats. When protective coatings start to fail, electrolytes can then penetrate to the substrate and start the corrosion process. Dr. Lasseter Clare is using electrochemical impedance spectroscopy as a way to assess the permeability of coatings based on impedance measurements. In **this** experiment we will not be using electrochemical impedance spectroscopy but instead we are interested in developing a complementary method, in which we will detect the presence of early

markers or signs of corrosion, to determine if corrosion is actually occurring, and to assess how much is occurring. It is her vision to use both techniques simultaneously, first to assess the protective quality of coatings and then if the protective quality is questionable, to detect and quantify the amount(s) of transition metal ions present. Using two types of instrumentation, such as electrochemical and spectroscopic would allow one to assess both the protective quality and corrosion markers on the same sample and possibly simultaneously. Ideally there would be a tool that could be set on a sculpture, and produce a signal that scales based on the quantity of corrosion products present.

4. One of the first tasks, for me, in thinking about developing this new tool was to turn to the primary literature and see what had already been done, that might be helpful with our goals. We then found a promising paper by Chen et. al. for a synthetic method to produce glowing or luminescent nanoparticles, using citric acid. These nanoparticles are a specific type of particle known as graphene quantum dots or GQD as it is labeled on the screen. Essentially, citric acid is heated and water is lost in order to form the quantum dots pictured on the screen. Upon further heating, the quantum dots will go through complete carbonization and form the graphene oxide sheet. For our purposes, we do not want the graphene oxide sheet, but rather the quantum dots. These dots are of particular interest because the research group on the next slide uses these dots to detect iron ions.
5. This research group used the previous quantum dots to detect iron ions, but they incorporated nitrogen into the synthesis method for the dots, which increased their sensitivity to the analyte. The detection of iron was of interest because iron ions are one of the early corrosion products for steel. Their synthetic method can be seen in the figure presented from their paper. They start with citric acid and get the same quantum dots that the previous research group got. They then nitrogen dope the dots using hydrazine. However, hydrazine can be very explosive, so for this experiment we are not interested in nitrogen doping the dots. Nitrogen doping the dots changes the homo-lumo gap, which helps make the dots more sensitive to iron, but it is not necessary for detection of iron. Therefore, graphene quantum dots from citric acid will be used to detect early corrosion products from steel.
6. Once promising primary literature is discovered, those methods can provide useful starting points for projects. Reading the primary literature is also important to understand the current research in the context of previous work, related applications, along with the theory and background. Here is a bit of background for this project: the quantum dots both groups used are described as blue luminescent graphene quantum dots and it is their luminescent properties that allowed for iron to be detected. These quantum dots fit into a broader category of luminescent molecules, which includes fluorophores such as Texas Red. Texas Red is used for staining cells as is shown in the figure on the right. Fluorophores are chemical compounds that can be excited by absorbing light and can then return to ground state by emitting light (usually at a different wavelength than was used in excitation). The end result of what quantum dots do is the same as a fluorophore: they absorb and emit light at a different wavelength. But, exactly which wavelengths a quantum dot absorbs and emits is usually dependent

on the size of the dots, as is the case for cadmium selenide dots. With larger cadmium selenide dots, there are more bonds, thus more orbitals, which narrows the homo-lumo band gap, and reduces the energy of light emission, causing larger dots to luminesce red and smaller ones to luminesce blue. For the carbon quantum dots produced from citric acid, they are not tunable based on their size, which suggests that the mechanism by which they emit light is not based on the homo-lumo band gap, but rather on something else, possibly it is the presence of chemically unique bonds, called defect states. While the exact cause and mechanism of carbon quantum dot luminescence remains an interesting and ongoing topic in the literature, the end result is that these carbon quantum dots produce a blue luminescence, giving them the name, "blue luminescent graphene quantum dots".

7. Now that we know it is possible to use blue luminescent graphene quantum dots to detect an early corrosion product from steel, it is important to understand how to measure these products using an instrument, after all this is instrumental analysis. The instrument we will use to quantify fluorophores is called a fluorometer. A fluorometer is similar to a standard UV-vis in terms that it is measuring the detection of photons through a sample. However, there are some major differences between the two instruments. In a fluorometer, the detector is 90 degrees from the source instead of 180 degrees. The 90 degree angle is important because you really want to avoid the source directly shining into the detector. The light from the source is many orders of magnitude brighter than the luminescence from the dots is, and so any stray light from the source would make it impossible to see any change in the emission intensity of the dots. Here you can see the block diagram where the source is coming in and then passed through a monochromator which controls the excitation wavelength. The sample is then excited and emits light which is measured 90 degrees from the source after passing through another monochromator. This monochromator controls the emission wavelength that is measured. These two wavelengths are very important for florescence. The excitation wavelength controls the energy levels of the incoming photons while the emission wavelength controls where the detector measures the signal. Therefore, it is possible to run different combinations of scans using a fluorometer. For example, you can keep the emission wavelength constant while running a full spectrum of the excitation wavelength in order to determine the maximum wavelength. Here is an example of what the spectrum would look like and it can be seen that the maximum wavelength is right under 400 so that would be what the source should be set at. However, you can also hold the excitation wavelength constant while scanning through the entire range of emission in order to collect a full spectrum of how the fluorophore emits light. Here is an example of what the spectrum would look like and it can be seen that the maximum wavelength is right under 500 so that would be what the detector is set to look for. When the two spectra are overlaid on the same graph you get a figure that looks like this. The two maxima are separated by a fixed distance of about 100nm in this example. This distance is called the Stokes' shift, the larger the Stokes' shift, the bigger the separation between the excitation and emission peaks. Now that we have seen images of fluorescence and how a fluorometer works, it is also important to understand how fluorescence takes place on the atomic scale. It can be seen in this Jablonski diagram

that the electron gets excited to a higher energy state by the source of the instrument and then transitions back down to an energy state but not the ground state. The electron absorbs some of the energy, which is why there is a Stokes' shift. When the electron falls back down from the excited state, it fluoresces and allows us to use the fluorometer to quantify its fluorescence.

8. Now that you know what fluorescence is and how it is detected, the question is what happens to the fluorescence of the graphene quantum dots when iron is present? Here is an image of 7 vials that have increasing concentration of iron from left to right. Hopefully you can tell that as iron concentration increases, the blue luminescence decreases. We can say that iron quenches (or stops) the fluorescence emission of these quantum dots. This amount of luminescent emission can be measured using fluorometer as seen in the image on the right. Why do the dots quench? Instead of releasing absorbed energy in the form of emitted photos, energy absorbed from the source must have been lost through non-radiative pathways, such as vibrational modes, instead of by the fluorescence pathway. However, the exact pathways for this system are still debated in the literature. Quenching can be thought of like turning off the fluorescence when iron is present with the more iron present the more the fluorescence is turned off. There is a linear relationship that exists based on how much quenching is taking place in relation to the original signal. This relationship can be plotted using the Stern-Volmer equation, which is shown on screen. You take the ratio of the original signal over the quenched signal in order to establish a linear curve. A sample Stern-Volmer plot for this experiment is shown on the screen. This equation then allows us to solve for unknown concentrations of iron much like another calibration curve was used to measure unknown concentrations in UV-vis experiments. It also shows us the quenching constant, k-sub-q, or the slope of the equation. This relationship with known standards can then be used to detect low concentrations of soluble iron that exists as an early corrosion product from steel samples.
9. How does this help with the global problem of corrosion? Because Dr. Lasseter Clare's lab is actively developing a methodology to detect the early signs of corrosion, through this lab you are helping to evaluate a methodology and the reproducibility of these experimental results. It is our hope that within a few years, methods similar to those that you are using today will be used to detect corrosion, on outdoor metalworks, like sculptures and bridges. The samples that you will test in the lab have a range of conditions that we find on sculptures, including the case where no corrosion is visible by eye, yet is detectable by quantum dots. Myself and Dr. Lasseter Clare hope that in the near future, using data similar to that which you will produce, maintenance staff will be able determine if a sculpture needs to be entirely recoated, repainted in a specific damaged location or whether nothing at all needs to be done. To prepare yourself to do the lab, you need to read the primary literature articles cited, write an experimental procedure to make quantum dots and describe how you will use the dots to detect iron that might be present as an early corrosion product on test samples.

Appendix C: Instructor's laboratory guide

Although this research is based on actual work done by Dr. Tami Lasseter Clare at Portland State University (https://www.pdx.edu/clarelab/), it is a simplification of the research. The goal of this experiment is to expose students to some aspects of the research rather than completely mimic the current research. Out of necessity, the metal samples were not taken from an actual sculpture as this would be a destructive method of analysis.

The procedural considerations section of the Student Laboratory Guide has been designed in such a way that mimics the results of beginning research into this project by explaining common pitfalls and how to learn from them. In this experiment, students are not provided with a procedure, so that they will start from scratch, by building off primary literature. In this way, students have the opportunity to learn how an actual research project may start.

A general overview procedure for this experiment is as follows:

1. Start synthesizing GQDs
2. Start setting up and making standard solutions of iron
3. Warm-up instruments
4. End the synthesis, dilute the GQDs and adjust the pH to neutral
5. Add GQDs to standard solutions and sample cuvettes
6. Set sample cuvettes aside for designated soaking time
7. Run standards while the samples are soaking
8. Run the samples
    a. The samples do not need to be removed but should be shaken to ensure homogeneity
9. Plot the Stern-Volmer plot and solve for unknown sample concentrations

The instructor should help the students set the appropriate slit-widths for the instrument. It is suggested that the slits be wide enough to give a good signal-to-noise ratio. At wide slit widths, the students have better success at separating out their unquenched dye from their lowest concentration. As such, the initial concentration for the Stern-Volmer plot may need to be adjusted based on the instrument if the signal is not distinguishable from the unquenched dye.

The synthesis of the dye and the collection of the spectra can be completed in under an hour. However, the majority of the time is spent soaking the metal samples in quantum dot solution to reach an equilibrium between the iron ions and the dots. It is suggested that the students allow their metal to soak in the cuvette with quantum dots for at least 2 hours. It may be advantageous to let the students start the soaking a week prior to the experiment and collect the spectra the following week.

It is suggested that students start the synthesis at the beginning of the laboratory period. There is an opportunity to walk the students through the fluorometer while the quantum dots synthesize. The average synthesis will take between 20-40 minutes. While only one group was ever running the experiment at a given time in this implementation, an entire class could do this experiment given enough fume-hood space. If synthesizing the dots is not an important step for the instructor, the students can also be provided with a pre-made quantum dot solution.

It is also important that the instructor looks over the pre-lab quizzes to ensure that the students successfully designed their procedure and know how to synthesize the dots before coming to class and reaching a consensus with their group.

While it is not possible to predict everything the students may suggest, most students do not deviate too far from the procedural considerations and may ask questions such as what pH should the GQDs solution be and why.

There are also uncommon questions students may consider based on their background and previous exposure such as:

- Does the dissolved iron in rain deposit on outdoor sculptures when it rains?
    - If this is considered, it is suggested that students find articles on what amount of iron is dissolved in rain and further, what amount of the iron is deposited on surfaces when it rains.
- Are there other ions present on the sculpture that could interfere with the quenching of the GQDs?
    - If this is considered, it is suggested that the students find more articles about citric acid GQDs, as some of these articles include competitive binding assays that show how other ions interact with the GQDs.

After running the samples, students may get a negative concentration for their samples. This means that the signal from their sample is slightly higher than the unquenched solution. It is an opportunity to talk to the students about noise from the instrument and other possibilities of a higher signal. One possibility is that there is something in the clear protective coating that also slightly fluoresces.

Appendix D: Sample preparation

All samples were punched out of the same sheet of low carbon steel and prepared as noted below.

Sample A:

1) Sanded and allowed to soak in saturated salt water for 1 week
2) Dried and then spray painted with Montana gold spray paint (color=brick)

Optional: If paint is too thick you can poke holes in paint layer with a needle to ease the solution reaching the metal

Sample B:

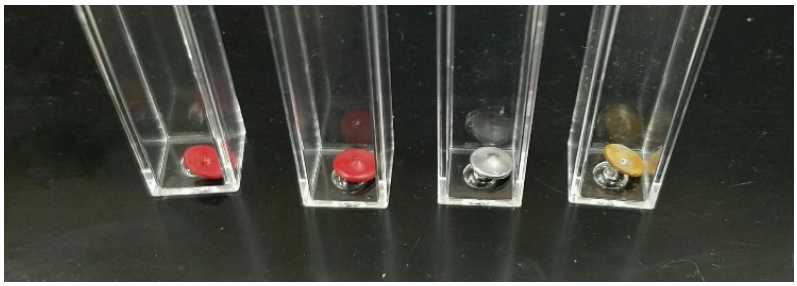
1) Coated with a spray clear enamel (Brand=Rust Oleum)
2) Dried and spray painted with Montana gold spray paint (color=brick)

Coated:

1) Coated with a spray clear enamel (Brand= Rust Oleum)

Rusted:

1) Sanded and allowed to soak in saturated salt water for 1 week
2) Dried



Note: The thicker the layer of paint on the samples, the harder it is to detect iron ions underneath the paint. It is advised to have a very thin uniform layer of paint on the samples.

Appendix E: Report rubric

Student lab reports were scored for a grade based on this point distribution.

Section Points		
1		**Title**
	1	Original title that describes content concisely, adequately, appropriately
4		**Abstract**
	2	Brief Statement of problem & methods used
	2	Brief Summary of Results (numbers!) and Conclusions
10		**Introduction**
	2	Experimental Purpose, Objectives, Hypothesis
	2	Importance of Ion Detection
	2	Introduce fluorometer and technique. (w/ block diagram)
	2	Basics of Fluorescence
	2	Citations and references adhere to proper format
2		**Experimental**
	1	Gives (just) enough details to allow for replication of procedure
	1	Instrumental & Reagent Specs
14		**Results/Discussion**
	2	Quenching plots
	2	Addresses follow-up question 1
	2	Addresses follow-up question 2
	2	Addresses follow-up question 3
	2	Error Analysis
	4	Feedback about mock sculpture
7		**Presentation**
	2	Proper tense throughout
	2	Tables and Figures use right format
	1	Report is written in scientific style: clear and to the point
	2	Grammar and spelling are correct
2	2	**Conclusion**
40		**Total**

The students are scored using this rubric and are given a score for each row on the rubric. Students can earn an “Excellent”, a “Good”, a “Fair”, a “Poor”, and a “Not Present”. The excellent mark is worth 100% of the points for that row, the good mark is worth 85% of the points for that row, the fair mark is worth 70% of that row, the poor mark is worth 55% of that row and the not present mark is worth 30% of that row. The not present row is used for attendance points, which is why it is 30% and not 0%. The students earn 30% of their grade by being present for the experiment. The rows are then summed up for a total grade out of 40 points.

Early Detection of Corrosion via Fluorescence Quenching

Instructions:

This experiment is not like any other experiment this term. For this experiment, you are **not** provided with a procedure. Instead, you are asked to read the primary literature and the other documents provided in order to come up with your own procedure. It is important to draw on your analytical skills learned up to this point when designing the procedure. In addition to giving you the opportunity to experience research as it truly is (without explicit instructions as to how to do it), this lab focuses on a real research question by a faculty member at PSU. In this way, you are exposed to authentic research – to solve a current problem using a synthetic procedure and analytical methodology that you design. Please feel free to ask any questions you may have as you work on coming up with your procedure – asking questions and making revisions can be an important part of the learning process!

Table of Contents

Learning outcomes

After completing this experiment, students will be able to:

- Read, understand, and apply primary literature to design a procedure that begins to solve a real-world problem.
- Describe fluorescence and the process of quenching.
- Use a fluorometer and make a Stern-Volmer plot from data.
- Describe how a fluorometer works.
- Begin to investigate a real-world problem that mimics current research.
- Understand the challenges and obstacles present in investigating real-world problems
- Gain insight to current research.
- Write a scientific journal-quality article about their findings.

Statement of the Problem

The Olympic Sculpture Park's conservation department and Dr. Tami Lasseter Clare of Portland State University are collaborating to figure out if corrosion can be detected before it becomes visible to sculpture park visitors. Dr. Clare's research group is in the process of developing a luminescent method of detecting early stages of corrosion and you can help her to figure out this problem. You need to determine if corrosion products are present on metal samples and, if so, how much. To complete your task, you will design a procedure from primary literature and use the procedure to analyze metal samples from two sections of a steel sample, composed of paint and metal similar to a real sculpture, to determine if corrosion products are detected. After the analytical methodology is refined through experimentation and replication, similar experiments can be carried out on actual sculptures and with the data that you provide, collections care personnel (such as conservators in an art museum) will use it to figure out what, if anything must be done: nothing at all, locally treat an area for corrosion, or repaint the entire sculpture.

Background information

Sculpture Information

Dr. Tami Clare and her research group are working on developing a method for early detection of corrosion on metal sculptures. The idea of using luminescent particles to detect soluble transition metal ions is of interest to many researchers: to answer biological questions and for environmental monitoring in addition to corrosion detection. The methodology that you will be using is from Professor Yuwu Chi and his team,[1] who published a synthesis for producing blue luminescent graphene quantum dots (GQDs) and graphene oxide sheets. For the purposes of this part of Dr. Clare's research, graphene oxide is not of interest. These blue luminescent graphene quantum dots were later used by a different research group[2] to show that their luminescence can be quenched, when Fe^{3+} is present. Thus, these two methods seemed like a promising starting point in that they might be used to detect early corrosion products from sculptures. However, detection of iron by the previously published methods takes place in the solution phase, where soluble iron is mixed with soluble luminescent dots. Figuring out how to detect corrosion when one's sample is non-aqueous presents a challenge for how to adapt this research to analyze corrosion on metal sculptures.

Quantum Dots

Before you begin tackling this challenge, it is important for you to know a little information behind the two methods this research is built upon. The first piece of information that will be helpful is explaining what a "blue luminescent graphene quantum dot" is. Graphene is a planar material that is one-atom thick composed of six-membered carbon rings in a honeycomb lattice. A quantum dot is a nanoscale particle that has different optical and electronic properties than their macroscopic counterparts. For this system, that means that the graphene quantum dot (GQD) has very different properties than the citric acid that it started from. An example of a Transmission Electron Microscopy (TEM) image of a graphene quantum dot can be found in Reference 2 (page 221 Figure 1). This image shows individual quantum dots on a 5 nm scale.

Although there is some debate in the literature, one hypothesized synthetic pathway is found in Reference 1. Generally, what happens is that when heated, citric acid goes through pyrolysis and then

incomplete carbonization to form the GQDs. After seeing the proposed structure in Reference 1, it can be seen why they are called graphene quantum dots. When you look at the center of the proposed quantum dot, it looks exactly like graphene. Now that we have a better understanding of quantum dots and graphene, it is important to understand the rest of the phrase "blue luminescent graphene quantum dot".

Luminescence and Quenching

When a substance has luminescent properties, it means that the substance emits light through fluorescence or phosphorescence. Since these quantum dots are "blue luminescent", they emit blue light as shown in Figure 1. However, what this picture also demonstrates is that the luminescence can be quenched, or reduced. This can be seen by noting that there are increasing concentration of corrosion products from left to right. As seen, the blue luminescence decreases with the presence of soluble iron, which is a marker for the presence of corrosion products. Not all quantum dots are quenched by the same substances however. Looking back on the structure of these dots in Reference 1, it can be seen that they have carboxylic acid groups on the edges of the dots. This means that at higher pH, the surface of the dots will have some negatively charged carboxylic (COO^-) groups present. Therefore, when cations are present, it is possible for them to interact with these groups and quench the luminescence. While this is a simple way to think about the interaction, the true mechanism is much more complex and is debated in the literature. However, these debates do not impact the use of carbon quantum dots in solving the problem presented here.

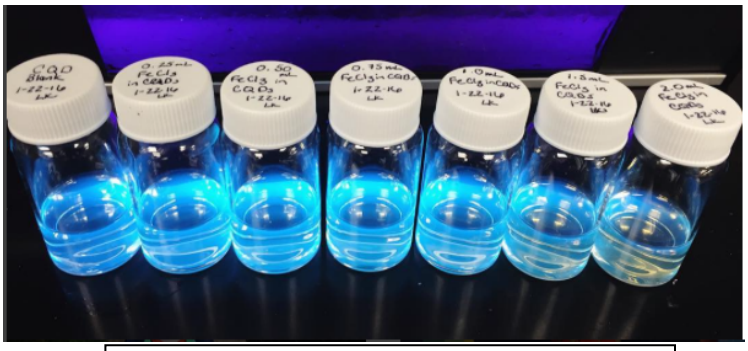


Figure 1: Samples of quantum dots with a range of concentrations of quencher

Detecting Iron Ions

In early detection of corrosion for sculptures, the iron III cation (Fe^{3+}) and the copper II cation (Cu^{2+}) are of particular interest. These cations are of particular interest because most outdoor sculptures, as well as other outdoor metal objects such as bridges, are made out of iron alloys (such as steel) or copper alloys (such as bronze or brass). When these metals corrode, they release cations from their surface. Because these metals are made of alloys of copper or iron, some of the cations being released from the surface when corrosion happens, are Cu^{2+} and Fe^{3+}. An illustration of corrosion on steel is shown in Figure 2.

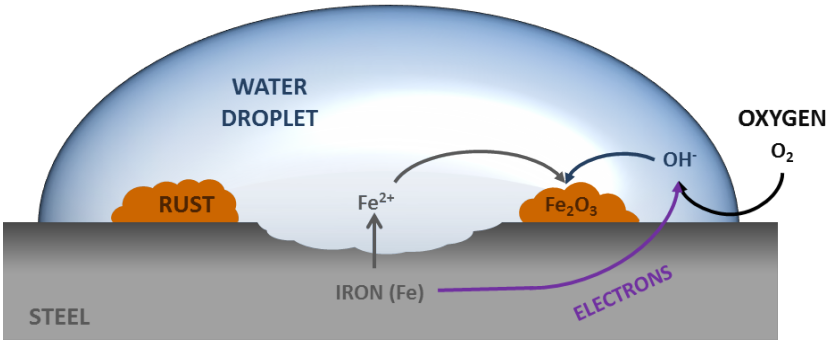


Figure 2: Corrosion process on steel (image provided by Dr. Alice England, Clare lab.)

The process of rust forming is illustrated in Figure 2 and described by Equations 1-4.

$$4\ Fe^{2+} + O_2 \rightarrow 4\ Fe^{3+} + 2\ O^{2-} \text{ (Eq. 1)}$$

$$4\ Fe^{3+} + 12\ H_2O \rightleftharpoons 4\ Fe(OH)_3 + 12\ H^+ \text{ (Eq. 2)}$$

$$4\ Fe(OH)_3 \rightleftharpoons 4\ FeO(OH) + 4\ H_2O \text{ (Eq. 3)}$$

$$4\ FeO(OH) \rightleftharpoons 2\ Fe_2O_3 + 2\ H_2O \text{ (Eq. 4)}$$

As can be seen in Figure 2 and Equations 1-4, Fe^{3+} is not the major contributor in early corrosion. The Fe^{2+} ion (the major contributor to early corrosion) rapidly forms Fe_2O_3 after being released from the metal. Rust is composed of solid, insoluble iron oxide (Fe_2O_3), which the GQDs do not detect as it is the interaction between ion and dot that is needed for luminescence quenching. Despite that fact that Fe^{3+} is present only as a minor component in early iron corrosion, there is still some Fe^{3+} present in small quantities during early corrosion, and even in low quantities, the presence of Fe^{3+} is detectable due to the sensitivity of GQDs. As the corrosion process continues, the initial Fe^{2+} will be oxidized to Fe^{3+} allowing the GQDs to be further quenched beyond the quenching that was due to the initial low concentration of Fe^{3+}.

Materials

- Two samples from a steel sheet made to mimic a sculpture
- Rusted and fresh steel samples
- Fluorometer
- Cuvettes

Note: The other materials will be based on your prelab quiz where you will let us know what the stock room needs to prepare for your procedure

References:

1) Dong, Y., Shao, J., Chen, C., Li, H., Wang, R., Chi, Y., ... & Chen, G. (2012). Blue luminescent graphene quantum dots and graphene oxide prepared by tuning the carbonization degree of citric acid. *Carbon*, *50*(12), 4738-4743.
2) Ju, J., & Chen, W. (2014). Synthesis of highly fluorescent nitrogen-doped graphene quantum dots for sensitive, label-free detection of Fe (III) in aqueous media. *Biosensors and Bioelectronics*, *58*, 219-225.

Additional Resources:

1) Lim, S. Y., Shen, W., & Gao, Z. (2015). Carbon quantum dots and their applications. *Chemical Society Reviews*, *44*(1), 362-381.
2) Wang, H., Maiyalagan, T., & Wang, X. (2012). Review on recent progress in nitrogen-doped graphene: synthesis, characterization, and its potential applications. *ACS Catalysis*, *2*(5), 781-794.
3) https://chem.libretexts.org/Textbook_Maps/Analytical_Chemistry_Textbook_Maps/Map%3A_Analytical_Chemistry_2.0_(Harvey)/10_Spectroscopic_Methods/10.6%3A_Photoluminescence_Spectroscopy
4) https://corrosion.ksc.nasa.gov/corr_fundamentals.htm

Procedural Considerations

Here is some information to keep in mind as you design and run your procedure:

- Heating should be done gently. These Variacs should never exceed an output of 80 volts to prevent overheating during the synthesis of the GQDs.
    - Hint: The color of the solution should be a very dark orange (almost brownish-orange). If your synthesis has been going for less than 30 minutes, it is probably orange, but needs more than 30 minutes to reach the proper darker orange color.
    - If you are unsure of the proper color, please ask a TA.
    - It should look something like this: 
- The pH of the solution will vary from synthesis to synthesis, depending on the rate of stirring and oxygen incorporation. However, all syntheses should be neutralized. There is NaOH and HCl present to neutralize the solution to pH 7 based on your measured pH.
- The GQDs become highly viscous (meaning like a syrup) when cooled. It is advised to keep the round bottom flask warm when transferring. The GQDs can be re-solublized by placing small amounts of hot water in the flask if the solution cools down too much.
- The unknown samples require a minimum of 30 minutes of soaking in solution (which can be done in a cuvette) in order for the ions to leach through the paint and equilibrate with the GQDs.
    - It is advised to start the samples soaking first and then move on to preparing the standard solutions.
    - All solutions will need to be properly mixed at the end of the soaking time to ensure homogenous solutions.
- The lamp on the instrument takes a minimum of 30 minutes to warm up. Plan ahead so you can warm it up for at least 30 minutes before running your first sample.
- The ability of the GQDs to quench metal ions is dependent on the concentration of GQDs. To maximize the quenching, it is recommended to prepare a GQD solution that is 5% (v/v).
- As a reminder, to reduce the error in concentrations you should not be preparing standard solutions within the cuvettes. Appropriate glassware is provided to make the standard solutions prior to transferring to the cuvettes. If more glassware is needed, just ask.
    - Exception: The metal samples can be placed directly into the cuvettes.
    - Exception: There are no 5 mL beakers available. A 25 mL round bottom can be provided instead.
- The total volume for all samples should remain consistent.
    - You are provided with 3.5 mL cuvettes; these should never be completely filled.
- The emission and excitation wavelengths will vary from synthesis to synthesis. It is recommended that you start with the wavelengths given to you in the original synthesis literature (see reference 1) and then adjust the wavelengths based on the first emission and excitation scan.
    - You never want the source to shine directly into the detector (it is very bright and could damage the detector by overloading it), therefore, ensure your wavelength collection is always 50 nm away from the excitation or emission wavelength

(depending on type of scan). For example, if you are doing an emission scan at an excitation of 360 nm, start the scan at least at 410 nm.

- It is good analytical technique to ensure that your Stern-Volmer plot covers an evenly spaced range.
- A typical limit of quantification is around 10 µM of Fe^{3+} in the cuvette for this instrument.
- To obtain good resolution between the peak intensity of different samples, a TA can adjust the slit widths on the instrument.
    - If the slits are too narrow the intensities of samples run the risk of overlapping due to noise in the instrument.
    - If the slits are too wide the detector will be over saturated and damage the detector.
    - **Because the shutters that define the slit width are delicate, to protect the equipment for future use, a TA needs to adjust the slits. Please do not touch the slit widths yourself.**

Follow-up Questions

1) Experiments are rarely done without replications. Therefore, on Thursday of the week you do the experiment, a Teaching Assistant will send you the data from the other groups that ran this experiment. How do your real-world samples compare with replications of the experiment? Does this change your conclusion about the test sculpture?

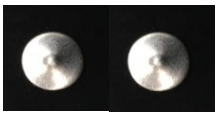
2) What potential problems are there in trying to detect rust under paint?

    a. How did your controls compare against the real-world samples?

    b. Which combination of images below do you think best matches the two samples that you tested?

1

2



3

    c. Compare the concentration of iron the GQDs detected with the images above. Does the concentration match the level of rust in the image? If not, how does this change your thoughts about the ability to detect corrosion?

3) In conservation of artwork, destructive analytical methods (which are methods that destroy the sample in the process of doing the analysis) are not ideal. After searching current literature for examples, how would you recommend this method be adapted for non-invasive corrosion detection?

Appendix G: Pre-lab activity

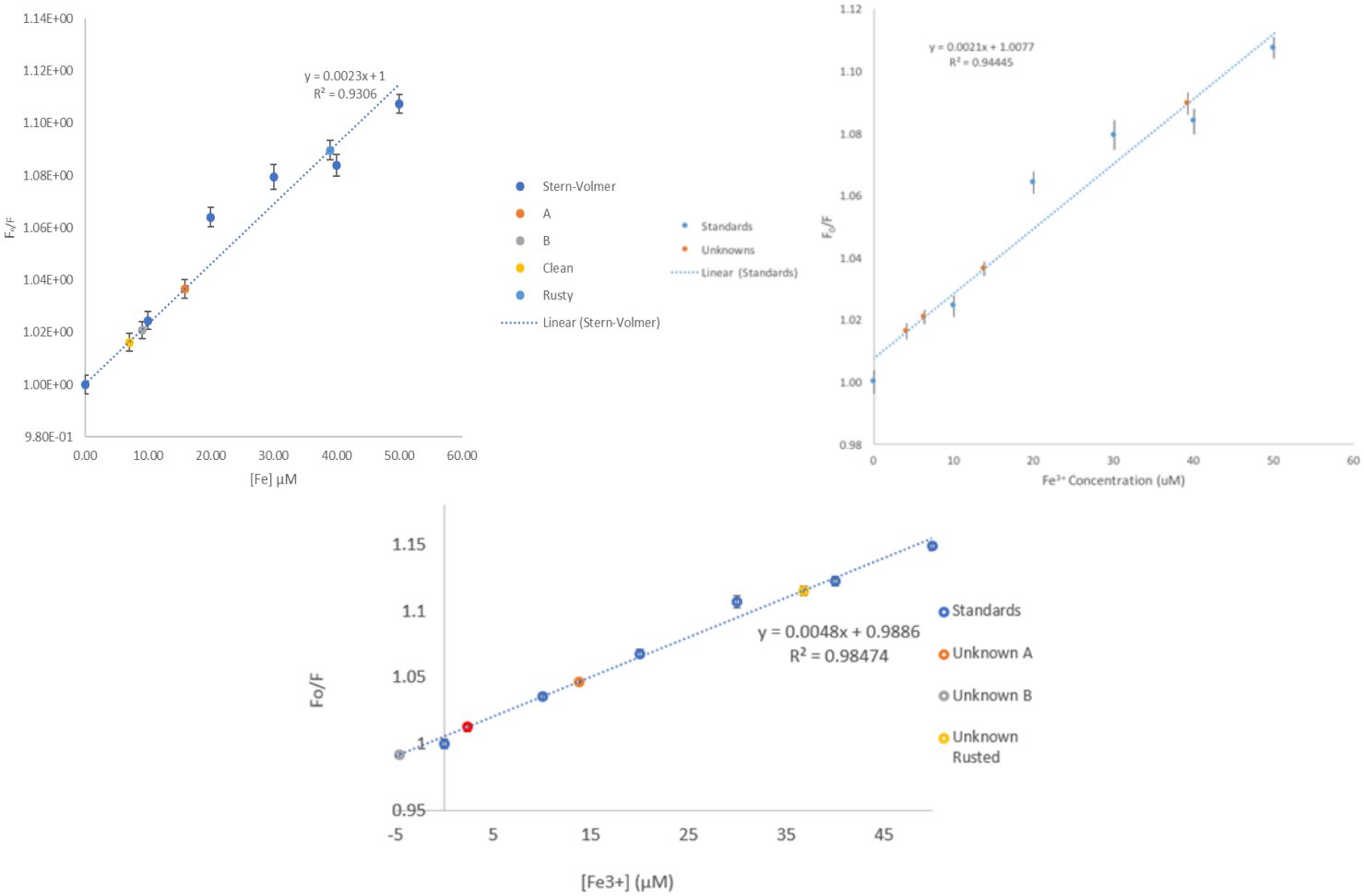
This data is given to the students to plot the data and determine the concentration of sample on the unknown sample before coming to class.

		Scan 1	Scan 2	Scan 3	Scan 4	Scan 5	Scan 6	Scan 7	Scan 8	Scan 9
	Background	1027.28	1100.32	1142.35	1081.31	1071.30	1084.31	1131.34	1156.35	1060.30
Final Fe^{3+} Concentration (uM)	0.00	267486.10	266704.84	268749.78	267637.60	267191.30	268166.38	267050.13	267453.10	267817.97
	10.00	259353.58	259434.92	260013.67	258861.38	259135.27	259706.78	259963.20	258190.05	259580.13
	20.00	251791.30	251478.53	250643.17	250695.64	251866.40	251689.45	251166.81	251204.88	251216.19
	30.00	234902.22	234948.42	234467.84	235792.58	235280.13	234647.55	234519.19	235756.64	234486.33
	40.00	225909.10	224970.48	225574.67	226096.83	225477.22	224744.83	224782.78	225329.50	225688.53
	50.00	219655.63	219661.78	219993.94	218082.19	218099.63	217382.19	217644.55	216313.31	216621.77
	unknown corrosion sample	246608.78	245888.50	247773.83	246748.45	246336.99	247235.96	246206.83	246578.35	246914.74

The quiz questions are below.

1) What is the excitation and emission wavelengths used in the references for GQDs? (in nm)
2) Please list the materials you will need for your procedure
3) Summarize the procedure you designed
4) What concentration of GQD (in % by volume) should you use for the soaking solution?

Appendix H: Sample student plots



Three representative student data plots demonstrating linearity with an average R^2 of 0.96 $\pm$ 0.03. While this is not as high as other experiments, students still successfully used the line of best fit and fluorescence signal to determine concentration. The techniques used in this experiment are techniques requiring high levels of precision and students may need more than one laboratory period to master these techniques.