Development of a Dual Probe System for the Determination of Dissolved Oxygen in Biological Oxygen Demand Measurements

An Application for Randolph College Summer Research Program 2012
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Abstract:

This project builds on previous studies to produce a solid polymer-supported probe for luminescencebased oxygen sensing to be employed in Biological Oxygen Demand (BOD) measurements, an important analytical tool for assessing water quality. A previous Summer Research Project produced polymersupported probes, but found that there were unsuitable for oxygen measurements due to difficulties associated with the positioning of the polymers within the analytical samples. The irreproducibility of the positioning of the polymer made it impossible to achieve satisfactory calibration of the luminescence response because of the large fluctuations in instrumental parameters between measurements. In this project, we will attempt to correct that problem by integrating an internal standard in the polymer support, thus providing a constant signal against which the probe species can be compared. This dual-probe polymer will then be used as the basis of a new method for determining oxygen concentrations in BOD measurements.

Project Description:

Overview:

This project is a continuation of an SRP project undertaken in 2010 with student Poojan Pyakurel. In that project, Poojan synthesized solid silicon dioxide polymers doped with luminescent compounds suitable for use as oxygen probes. Our goals in that project were to complete the synthesis of the doped polymers, to measure their luminescence emission spectra, and to assess their utility as oxygen probes. The primary goals of that project were met, but our analysis showed that these polymers were not suitable as oxygen probes in their current formulation. Our primary goal this summer will be to modify the polymer systems to achieve a reliable oxygen response. If we can modify the probes to respond effectively, we will work to apply the new probes in Biological Oxygen Demand Measurements.

Luminescence:

Luminescent compounds are those which are capable of absorbing light and then re-emitting light of a slightly different color (see figure, on next page). Luminescent compounds are fun to work with and make for interesting presentation topics, but are also extraordinarily useful analytical tools. The usefulness of these compounds arises from the fact that there is very small delay (typically on the order of thousandths of a second) between the absorption of light and its re-emission, and during that brief time interval, the luminescent molecule is constantly interacting with its surroundings. These interactions can affect the molecule in ways that alter it ability to re-emit light, and can therefore change the color of the emitted light, its intensity, or both. For the last few decades, chemists have been investigating ways to use this change in emitted light as a tool in investigate the environment of the luminescent species. Our proposed project will be geared toward improving some of those techniques.

Examples of luminescent objects: (Fig. 1) a glow-in-the-dark dragon toy, (Fig. 2) commercially available luminescent paint, and (Fig. 3) a luminescent polymer made by Poojan Pyakurel in the 2010 Randolph College Summer Research Program. Each of these samples is being irradiated with ultraviolet light, and is re-emitting light in the visible region of the spectrum.

Figure 1 glow-in-the-dark dragon toy,

Figure 2commercially available luminescent paint

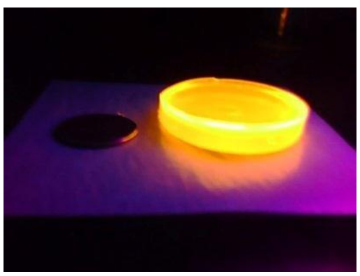

Figure 3 luminescent polymer made by Poojan Pyakurel

Luminescent Compounds as Analytical Tools

One of the most common species to be studied by luminescence is oxygen. Oxygen is a good quencher of many simple luminescent compounds, and interacts with them in such a way as to reduce their ability to re-emit light. Oxygen can therefore be detected and quantified by observation of luminescent signals from these species. The more oxygen resent in the environment of the probe species, the lower its emission intensity will be.

The detection of oxygen by a luminescent probe solution is clearly demonstrated in the two photographs

Figure 4 Photograph showing a luminescent probe species in solution with normal lighting

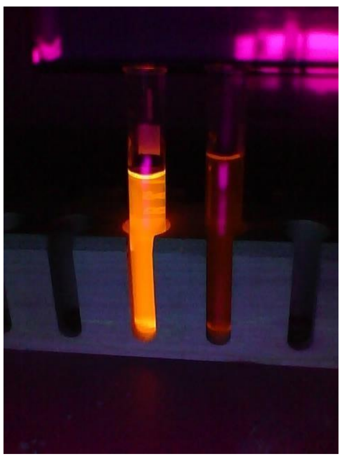

Figure 5 Photograph showing a luminescent probe species in solution with ultraviolet lighting

Each of the test tubes in the two photographs contains a solution of tris (bipyridine) ruthenium (II) chloride, a luminescent transition metal complex. The only difference between the two solutions is the concentration of oxygen in each. In the test tube on the left, all oxygen has been removed from the solution, while that on the right has a relatively high oxygen concentration (approx. 100 micrograms per milliliter). The photo on the left was taken under normal light conditions, while that on the right was taken in a darkened room under ultraviolet (UV) light. The UV light excites the ruthenium compound and triggers a re-emission of orange light. The presence of oxygen in the solution on the right, however, impedes this re-emission process, resulting in a clearly noticeable decrease in the emission intensity.

The difference in emission intensity as a function of oxygen concentration obeys a well-defined mathematical relationship (as seen in Figure 3) so that quantitative oxygen measurements can be made with high precision by measuring the emission intensity.

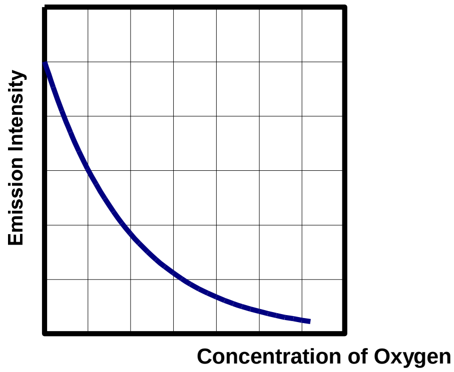

Figure 6 Relationship between Emission Intensity and Oxygen Concentration for a Solution of Ruthenium tris-bipyridine in water

In a 2003 Summer Research project, second-year student Chi Pham employed emission intensity measurements to measure the change in oxygen concentrations during the oxidation of glucose. Those measurements allowed for accurate real-time monitoring of oxygen concentrations during the reaction and led to very good measurements of the important kinetic parameters of the enzyme-catalyzed reaction. This work was subsequently published in the Journal of Chemical Education.[1]

Luminescent Probes in Polymer Supports:

A consistent challenge for luminescent sensing has been to transfer the technology outside the test tube. In the last ten to twenty years, applications for this technique have been developed for sensing oxygen in air, on surfaces, and even in intravenous samples. These methods have all required the luminescent material to be encased in an organic polymer and affixed to a suitable substrate for measurements. In a 2010 Summer Research project, Poojan Pyakurel synthesized a relatively new type of luminescent polymer composed of silicon dioxide and an ionic liquid, which was then doped with a luminescent ruthenium compound. We were pleased to find that these polymers showed the expected luminescence response to variations in oxygen concentrations.

The luminescent polymer system, however, does have an important disadvantage compared to the older solution phase probes. The amount of UV light reaching the polymer and the amount of emitted visible light reaching the instrument detector are both very sensitive to minor variations in the position of polymer support within the instrument. As a result, calibrations made with standard solutions cannot be applied to the measurement of new samples, since the repositioning of the polymer in the sample solutions changes the parameters upon which the calibration curve is based. This is the crucial problem that must be overcome if these polymers are to become usable oxygen probes.

The Proposed Dual-Probe System:

Our solution to the problem of instrumental instability is to incorporate a second luminescent species within the polymer. This second luminescent compound is called the "reference" and is one that is insensitive to changes in oxygen concentrations (many such compounds are known and are readily available). When this new dual-probe polymer is irradiated with UV light, both species (the probe species and the reference) will

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[1] "An Improved Method for Studying the Enzyme-Catalyzed Oxidation of Glucose Using Luminescent Probes", W. D. Bare, C. V. H. Pham, M. Cuber, and J. N Demas, *J. Chem, Ed.* **2007**, 84, 1511

absorb and re-emit light. Both species will also be affected by changes in the relative position of the polymer within the instrument. The reference will be affected by both instrumental parameters and oxygen concentration. Our strategy will be to measure both emission spectra simultaneously, and determine the oxygen concentrations based on the RATIO of the probe signal to that of the reference. I feel confident that we will be able to synthesize and characterize a dual-probe luminescent polymer in the eight-week summer research program. After we have this new polymer (or polymers), we will begin working to apply this to the measurement of dissolved oxygen in Biological Oxygen Demand (BOD) measurements.

Biological Oxygen Demand is a measure of oxygen used by microorganisms like bacteria in rivers, streams, and wastewater. Sources such as organic matter, nutrients, sunlight, urban runoff, and fertilizers serve as nutrients for aerobic bacteria and increase their consumption of dissolved oxygen. As microbes demand a greater and greater supply of oxygen, dissolved oxygen levels drop, making the water inhospitable for higher organisms. Most fish, for example, die when the dissolved oxygen level falls below 5 parts per million. Thus, BOD measurements are strongly correlated with general “water quality” and are important indicators for ecological heath of environmental samples, as well as a measure of efficiency of wastewater treatment.

Currently, EPA approved protocols for BOD measurements require expensive oxygen probes. It is our hope to demonstrate that these measurements can be made easily and less expensively with our dualprobe luminescent polymers to be synthesize this summer. I expect that we will be able to make significant progress on this goal, although we will not likely progress enough to develop a complete luminescence-based protocol for these measurements.

Dissemination Goals:

My past students have enjoyed presenting at the MARCUS conference, and I anticipate that Mimansha will do that as well. The last SRP project that I completed was also presented at the National ACS conference, which the student found to be very rewarding. If Mimansha is willing to travel, I expect that we will be able to do this again.

The main goal of this project is to determine if the dual-probe system works to correct the problems found in previous polymers, and the BOD samples will serve as an environment in which to test them. It is unclear whether or not we will progress to devising a completed procedure for luminescence-based BOD measurements. However, if the polymers ultimately work very well and we are fortunate enough to be able to work out a new BOD protocol, I would like to adapt this project as a student lab in my Environmental Chemistry course.

External Funding:

I do not have any external funding for this project, although I may be willing to have the project considered for VFIC funding.

Budget:

Instrumental Needs Dissolved Oxygen probe and meter $985
BOD bottles $115
Chemicals Bathophenanthroline (1 g) $100
1,10-Phenanthroline (25 g) $86

Total $1286

I believe that we have many of the required chemicals for the synthesis portion of the project on hand already. The dissolved oxygen meter and probe are those used in the current EPA-approved BOD protocols. We will need these in order to compare our results to determine if our measurements are of comparable quality.

Aaron Whalen and I have looked for oxygen probes and are hopeful that we can find a cheaper probe that will be suitable for this project, but I am not convinced that we will be able to do so. Most of the probes that we found were actually nearly $1500. I believe that the one we finally found for $985 will suffice. The project probably cannot be completed without the items listed here. If the number of proposals is small enough to allow some excess funds above the $1000 cap, then it would be very helpful to have this funded at the requested amount. If this is not possible, then I believe we can cover the $286 gap from the chemistry budget, because we may be able to find uses for the non-consumables in the chemistry teaching labs.

Past Outcomes:

I believe that I have submitted two previous SRP proposals dealing with luminescence. The first of these, in 2002, was completed with Chi Pham. That project employed solution phase luminescent measurements to track the progress of an enzymatically-catalyzed reaction. That project was completed in the eight-week program (or was it ten back then?). I presented the results of the project at the National American Chemical Society (ACS) conference in Boston shortly after the completion of the project. Chi presented the work at the MARCUS conference and also at a regional ACS conference at UVa. The project was written up and published in a peer-reviewed journal in 2006. Chi was later accepted in the PhD program at the University of Southern California.

The second luminescence project that I have completed in the SRP program was done two years ago with Poojan Pyakurel. This work reached a suitable conclusion (but revealed some problems that we are attempting to address in this year"s project). This project has not been submitted for publication because it did not reach a publishable conclusion. We did however make some important progress, which was presented by Poojan both at MARCUS and at a National ACS conference in California. Poojan is currently working as a lab technician in a research lab at the University of Virginia, and has applied to and been accepted in the PhD programs at both UVa and Virginia Tech.

In the summer of 2011, I worked on a joint project with Karin Warren and student Adam Eller. This project was mainly run by Karin Warren, so I am not sure what her ultimate dissemination goals are or what her plans are for continuing the project. I do know that Adam presented his work at a conference out of state with Ludo Lemaitre and Karin.

Qualifications of Student Researcher:

Mimansha is a sophomore Environmental Science major with a GPA [redacted] Her transcript shows consistently high academic performance, with no grades (unless I missed one somewhere) below a B in her first year and a half at the college.

Mimansha entered Randolph with A-Level credit for both CHEM 105 and CHEM 106, and as a result did not take the General Chemistry sequence here. However, she did take my Environmental Chemistry course this past fall and was the top student in the course. I occasionally notice some apparent gaps in her content knowledge as a result of the year-long gap between her A-level course and the 200-level chemistry course that she took here, but these have obviously not been a problem for her. She probably has the best chemistry knowledge of any non-chemistry major that I have worked with. Moreover, her lab skills are good, and

that is probably more important for this project. I have absolutely NO reservations about her ability to perform well on this project.
Mimansha‟s interests lie in the area of Environmental Science, and she is currently doing an internship with the City of Lynchburg as a water quality analyst at the wastewater treatment center. This experience makes her ideally suited for this project because BOD measurements are one of her responsibilities in this new position. I have had BOD measurements in the back of my mind for years as a possible application of the polymer-supported probes, but I had not pursued it until now, partly because I did not have a great deal of experience with BODs myself. When Mimansha told me that she was doing water quality measurements for the City, including BODs, I couldn‟t believe it. It seems to be a perfect match between project and student. The expertise that she will be developing this spring working in this position will allow her to bring something to this project that no other student could.

Mimansha‟s responsibilities will be to synthesize luminescent compounds and polymers according to some well established synthetic methods (as well as a few newer methods developed by Poojan Pyakurel in 2010) and to perform spectral analyses on these products using the Fouromax-3 spectrofluorimeter. She will also be in charge of all BOD preparations and will work together with me on adapting the luminescent probes to the BOD protocol.

Student Statement:

My name is Mimansha Joshi, and I am a junior at Randolph College with an Environmental Science major. Working with professors has always been something I wanted to do in order to get greater experiences and know a lot. To grab one of the best opportunities provided by the college- Summer Research - wasn‟t a difficult decision, and I have been eager to participate in the program. Now that I have been given the chance, I am looking forward to doing my best in the research.

Last semester in all my environmental science classes, I found my niche. Studying various methods and experiments in classes that I took with Dr. William Bare on Environmental Chemistry, Dr. Tatiana Gilstrap on Environmental Methods and Dr. Karin Warren on Quantitative Environmental Problems captured my interest more. I was also involved in writing for a national newspaper in my country related to alternate energy resources, rain water harvesting and waste water treatment. This widened my perspective on both research and the environment. Similarly, the classes I have taken so far with I look forward to applying everything I studied thus far to diminish environmental problems.

In my two years at Randolph College, I have enjoyed a lot of opportunities to learn about sciences as I developed an interest in environment. I got a great deal of knowledge from professors which engaged my mind in learning more. I have been motivated by studying environmental chemistry and it has generated a greater appreciation for the environment. My interest has led me to become more curious about materials related to chemistry's role in measurement of different aspects of environmental issues.
Although I have carried out research in various fields, I wish to do something more tangible in the field of environmental science and chemistry. I have been doing a research on Ground Penetrating Radar with Dr. Tatiana Gilstrap, chair of the Department of Physics at Randolph College. We have been working to analyze a fault near a river at Bedford County's Claytor Nature Study Center. As an intern in Lynchburg Department of Utilities, I have been working as a Lab Assistant to carry out wastewater laboratory tests like Biological Oxygen Demand, Dissolved Oxygen in water samples, run colorimetric machines to get phosphorus and Nitrogen concentration in effluents from various sectors from the City of Lynchburg. I am also involved in collecting samples from industries and streams. Having discussed about research possibilities with Dr. Bare, we have decided to find a different method to measure Biological Oxygen Demand in water samples.

All my lab and research experiences have been fascinating, but I believe that a few experiences cannot satisfy me. I thus chose to work on a summer research project so that I will get good laboratory experiences. Because I have chosen to work on my favorite arena of science, I am positive that my knowledge will be useful for the project. I look forward to giving the best effort for the opportunity that I have been given.