Module 3
Measuring pH-dependent oxidation kinetics using a home-built spectrometer

Module Learning Outcomes

- Minimize experimental error using QA/QC techniques
- Automate repetitive tasks/calculations (II)
- Quantify the level of random error in any measurement or laboratory result (III)
- Create reliable standards for chemical analysis (II)
- Make quantitative measurements using spectroscopy
- Build and fully utilize calibration curves

Key Experimental Tasks:

- Construct working spectrometer with light source, sample holder, and cell phone camera as detector
- Select reflected light source color and RGB camera pixel type to limit wavelength range of detected light beam, given color of chemical to be measured
- Quantify and minimize stray light
- Calculate absorbance from raw measurements of beam intensities P_0 and P
- Manipulate reaction rate kinetics to be easily measurable within a 5-10 minute run
- Determine uncertainties in least squares linear fits

BACKGROUND

Literally billions of clinical chemistry tests are performed annually in the United States. The bulk of these analyses are performed by use of spectrophotometric methods. Of these, ordinary absorption spectroscopy in the visible region is the one most commonly used. The instrumentation for visible absorption spectroscopy is simple, stable, relatively inexpensive, and easy to automate, thus it can handle the large numbers and variety of tests to be done. The method has a large number of advantages and very wide applicability. Many substances absorb light, and if the analyte itself does not absorb, it can often be treated with reagents to generate a product with suitable absorbance characteristics.

The vast majority of chemical analyses are performed on systems that are at equilibrium, clinical or otherwise. The chemistry is "over." This is true whether the analysis method is spectrometric, electrochemical, or chromatographic in nature. When chemistry is performed to prepare samples for analysis, for example with the use of enzymes, the samples are normally allowed to react or to "incubate" for a sufficient period of time that the system is at or very near equilibrium. Once at equilibrium, precision can be improved by averaging multiple measurements if necessary.

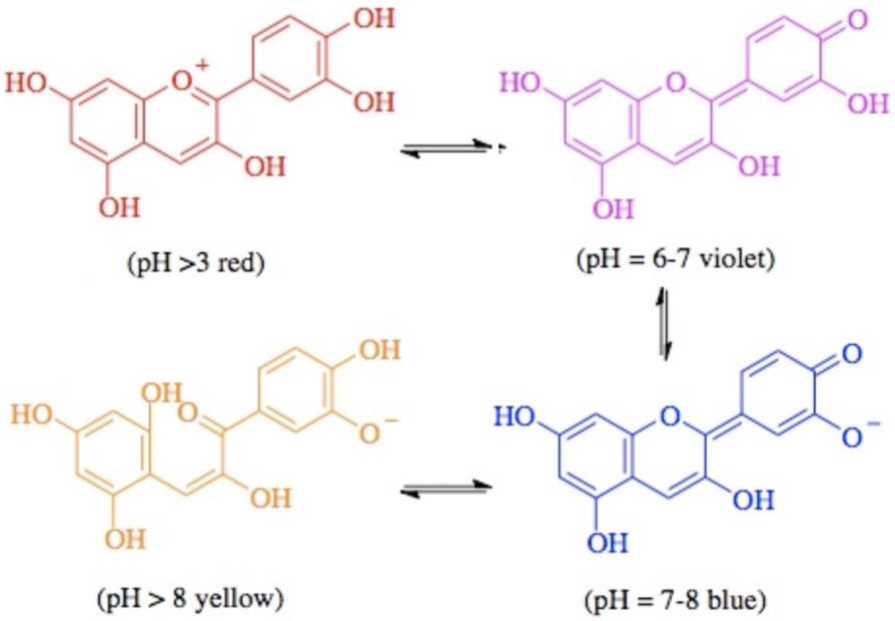
Kinetic methods of analysis are different. The goal here is to measure the rate of an ongoing chemical reaction to determine the quantity or concentration of an analyte of interest. The first, and probably most important, advantage of kinetic methods is flexibility and speed. You have the option of working with reactions that are relatively slow, and you don't have to wait for the reaction to go to completion to make your measurement. Kinetic methods are also particularly useful in samples in which some interfering compound is present, even if in large and variable concentrations from sample to sample. For example, take absorption spectroscopy as the method and a colored sample such as whole blood, soft drinks with dyes, or juice / food samples. If you can develop a kinetic method that reacts only with the analyte, or at least does not react with the interferent, modern instruments can often succeed in providing you with a decent analysis by measuring the rate of change in absorbance of a sample undergoing a reaction without having to do complicated and time-consuming chemistry to eliminate the interfering colored background or to resort to some separation method (the topic of Module 5).

The most accurate reaction rate measurements are usually done during only the first 3 to 10% of the reaction, before possible back-reactions start to become significant or before conditions change too much. In this situation, the *initial* reaction rate can be measured with minimal error. However, if using the integrated rate law

or graphical method to determine reaction orders, the reaction must be allowed to go through 2-3 half-lives in order to be able to see curvature in some of the plots. This means that the reaction must continue until the limiting reactant has dropped to 25 to 12.5% of its initial concentration. Under these conditions, perfect linearity in one of the analysis plots is not usually observed, because of back-reactions or changing conditions.

THE OXIDATION REACTION

The anthocyanins shown at right are the compounds that give red cabbage juice its colors at each pH. (Typo: the red form should say "pH < 3.") In this experiment, our ultimate goal is to measure the rate constants that govern the rates at which these compounds are oxidized by hydrogen peroxide (HOOH) in solution. These rates will not necessarily be the same for the four compounds! In order to determine rate constants, we also need to determine if the reactions are 1st or 2nd order with regard to the anthocyanins. We will use the integrated rate law method to find the reaction orders.



The oxidant molecule in this experiment, HOOH, reacts to form water as it oxidizes other molecules. In UV sunlight, HOOH can break apart into two hydroxyl radicals (•OH) that are even faster at oxidizing other molecules, so we'll do this experiment indoors to avoid these effects of UV. Since redox reactions often involve producing or using up H^+ or OH^- ions, we will have to control the pH during the reactions to make sure that we don't start a reaction with one anthocyanin and shift to another one part way through – this would cause a noticeable change in rate, or a bend in a kinetics graph that should have been linear. Finally, since the rates of chemical reactions typically double for a 5℃ increase in temperature, rate constants are always reported with temperatures. Be sure to record a measured or estimated temperature of your reaction mixtures.

Reagents and supplies needed

You will need the following solutions and supplies for this lab. The first 6 items are leftovers from Module 2 – you only need to remake these if you run out! The items listed in red will need to be gathered for this lab.

- *Toilet cleaner solution (for low pH measurements)*
- *pH 4.3 buffer*
- *pH 5.4 buffer*
- *pH 9.8 buffer*
- *Oven cleaner solution (for high pH measurements)*
- *Indicator solution (red cabbage or blueberry juice)*
- *3% hydrogen peroxide solution (available at drug stores or supermarkets)*
- *1 clear plastic cup or glass, the smaller the better*
- *1 small cardboard box, plus tape/glue and scissors/knife for rigging up a sample holder / stray light blocker*
- *Smart phone with Color Name AR app loaded (free at App Store, or try Color Picker or Color Identification at GooglePlay – any app that can give the RGB pixel readouts from a selected area of the camera image), and a way to keep phone stationary during experiment*

Prelab Assignment:

Build your spectrometer: See video instructions on Blackboard. Once you have put your spectrometer together, set it up for measuring the **red** (acidic) form of the anthocynanin compound in the indicator solution. Be sure to record the

following info in the ELN: To detect the red form of the indicator, what color light will you be using? What type of pixel (R, G, or B) will you be reading? Upload a photo of your setup.

Procedure:

Optimize your spectrometer: Place tap water in the sample holder so that it is about an inch deep. Using the live view camera feature on the ColorName app, align your camera so the pixel (the tiny white box where the app will read the light) sees through the lower part of the cup in the sample holder, straight to the colored object. Adjust the lighting, the object, and/or the camera position to get the highest beam strength reading along the correct beam path, while minimizing stray light. Record any important adjustments in the ELN.

If you can remove some liquid from the cup and still have the camera pixel beam path easily pass through the liquid, do so. (The less volume you need, the less likely you will run out of any of your solutions during this module.) Measure and record this minimum volume – this will be the total reaction volume you will use in each experiment.

Guided by our discussion in class, devise the best method you can to *quantify the amount of stray light* ***S*** being detected by the camera pixels in your optimized setup. Describe your method in the ELN. To make this measurement useful for later parts of this experiment involving different colors of light, record the amount of stray light detected by each of the 3 colors of camera pixels (R, G, and B). (To take readings with ColorName, click on the box containing the color name to read out the pixel color (R, G, and B are all listed, so always record whichever you need). Press the back symbol to go back to the color name box / live view.)

Once you have everything optimized, draw a quick diagram of your setup, labeling the light source (window? desk light?), the wavelength selector (object and its color), the sample holder, and the detector. Upload the diagram to LabArchives.

Designing a reaction mixture: It will take some trial and error to get a strongly absorbing solution that reacts with HOOH at a convenient rate. To stay organized, make a table with that contains the following information for every reaction that you run:

- Target volume of reaction mixture: determined in previous part.
- Volume and concentration of toilet cleaner (or other pH-controlling solution) needed: The target pH = 2 for measuring reaction rates with the red form of the indicator. For toilet cleaner, calculate how much solution you must add to achieve the desired pH, using the concentration determined in Module 2 by titration. (Hint: this is a dilution problem. What is the H_+ concentration in a pH 2 solution? How much toilet cleaner will you need to add to achieve this target pH, given your target volume for the reaction mixture?)
- Volume of HOOH solution: You'll need to play with this to get a reaction rate that is convenient to measure (neither too fast nor too slow). Anywhere from 10 to 75% of the volume may need to be HOOH solution, depending on the pH. Assign your group members to test out different percentages, then compare results to find the optimum.
- Volume of indicator solution: 15 to 30% of the total solution volume should be red cabbage indicator solution. But don't add this to the mixture yet – this would start the reaction with HOOH! First, you need to measure P_0.
- Volume of water: if the volumes of the other 3 ingredients don't add up to the target volume, you can add water to make up the difference, but remember to leave room for the indicator – it *must* be added last, when you're ready to start the reaction!

Setting up and running reactions: Set up a table in the ELN to record raw data every 15 s over several minutes. (It is good to also record the time of day that the reaction is started!) Note that you can open Excel from within the ELN, even if you don't have Excel yourself (Insert → Office Document → Spreadsheet). Mix the toilet cleaner, hydrogen peroxide, and any water needed and place in the sample holder. Check that the ColorName live view pixel sights the colored object through the sample liquid, and fix the position of the phone so that it won't move. Take a reading with ColorName – but make sure to record the right color pixel value! Try this a couple of times to make sure you are getting consistent values – the average of these readings is $\boldsymbol{P_0}$', the power of the light beam without the indicator present. (I'm using the apostrophe to designate a reading that hasn't been corrected for stray light.) Measure $\boldsymbol{P_0'}$ at the start of every reaction.

Once you have $\boldsymbol{P_0}$' recorded, you may start the reaction by adding the planned volume of indicator solution and immediately stirring (this is time = 0). As quickly as possible, click on the color name box to take the first reading of $\boldsymbol{P'}$. Continue to take and record readings of $\boldsymbol{P'}$ every 15 seconds for a few minutes. If the reaction mixture is proportioned well, the $\boldsymbol{P'}$ values will continue to increase the entire time as the indicator color fades. Later in the reaction you can take readings less

often, if you so choose. When the reaction ends, measure the pH. If the ***P'*** values stop rising, the reaction may be over, and if they stay low and never seem to rise, the reaction is probably too slow, and needs more HOOH. (Note: your initial reading of ***P'*** should be a much lower number than P_0'. In fact, to get an optimal starting absorbance of 0.6 or larger, ***P'*** should be at least 4 times smaller than P_0'. If it is not, you may have too much HOOH, making the reaction too fast, or too little indicator in your reaction mixture.)

Compare results with members of your group, troubleshoot your results, and decide what other reaction mixtures you should each try to optimize the rate measurements at pH 2. Repeat this process until everyone in the group has at least 2 good runs of their own.

Calculations

To analyze the data for a reaction, you will need to do the following calculations in a spreadsheet:

- Calculate the initial HOOH concentration (in M) in the reaction mixture, given that a 3% HOOH solution contains 3g HOOH / 100 mL solution.
- Since you measured the stray red light at the beginning, you can now correct your P_0' and P' values. All of the readings you just collected should contain the same amount of stray light, so $P + S = P'$ and $P_0 + S = P_0'$. Use these equations to solve for P and P_0, the corrected values.
- Use P_0 and each P value to calculate the absorbance at each measurement time during the reaction.
- Use the Beer-Lambert law to determine the concentration of red anthocyanin at each measurement time. (Note: you will have to guestimate the path length b in cm in your setup. $\varepsilon_{anthocyanin} = 28000\ M^{-1}\ cm^{-1}$.[1])
- Perform a graphical analysis to determine whether the data is 0, 1st or 2nd order with respect to anthocyanin concentration. Here's how: graph time on the x-axis, and ln(concentration) on the y-axis. If the data falls in a straight line, it's 1st order. Then, graph time on the x-axis, and 1/(concentration) on the y-axis. If this data falls in a straight line, it's 2nd order. Graph concentration vs time – this graph will be linear for zero order reactions. Which graph has a better R^2 value relative to a linear fit? (Note: if using Excel within LabArchives, you'll need to copy your data into an external Excel or GoogleSheets spreadsheet in order to do linear fitting and get R^2 values.) Once you have determined the reaction order, check if your results agree with other group members.
- Take the most linear of the three graphs and get the slope of the linear fit. This slope is equal to the "pseudo" 0, 1st or 2nd order rate constant k', and has units of conc/time, 1/time, or 1/(conc x time), respectively. Record this value of k' along with the measured pH of the reaction mixture in a table with extra rows for future reaction data.
- If we assume that the reaction is 1st order with respect to HOOH, the pseudo rate constant k' is related to the real rate constant k by the following equation: $k' = k[HOOH]$. Use this equation to solve for the real rate constant, with units.

Measurements at other pH's

Once calculations are complete, you may begin work on measuring reaction rates at pH 4.3 using the buffer made at this pH instead of toilet cleaner. At each new pH, test a small sample of indicator to see its color (or check your observations from Module 2), and determine what color of object you should be using as your reflective light source, and what color of pixel you should use for taking P_0' and P' readings. Record these critical decisions in the ELN. Then, start at "Designing a reaction mixture" to repeat the process. If the buffer is less concentrated than your toilet cleaner solution, you may want to use proportionally more volume of buffer solution to make sure that it can control the pH throughout the reaction. (No dilution calculation is necessary with buffers, because buffers stay at nearly the same pH when diluted.) The HOOH volume will probably not require re-optimization, since pH 2 and pH 4.3 are not too far apart.

During **week 2** of this module, we will conduct rate measurements at pH 5.4, 9.8, and 12. For some of these pH's the HOOH volume *will* require re-optimization!

Instructions for R&A section

- Upload your summary graph of rate constants as a function of pH, along with a figure caption that includes the temperature at which your measurements were conducted.

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[1] J. Lee et al., *Journal of AOAC International* **88** (5) 1269-1278 (2005)

- Use your Python code to determine uncertainties in the slope of each graph, and report these uncertainties in a table.
- Propagate these uncertainties to the rate constants k determined at each pH.
- Make a summary table listing pH, rate constants (k), and uncertainties in k values. Include a title, units, and a few sentences of explanation (the "guided tour" of interesting things to notice).

Questions

1. What are typical concentrations of HOOH and anthocyanin in your reaction samples, in M? Did you successfully limit control of reaction rates during your measurements to anthocyanins, by making the concentrations of other reactant comparatively large, and therefore nearly constant?
2. Graph the k values calculated for each reaction you conducted as a function of pH. What trends do you observe from this graph? Try to explain these trends using your growing knowledge of chemistry.
3. The molar extinction value (epsilon) we used for anthocyanin was for the red form (measured at a green wavelength of light). Molar extinction values are only valid at the wavelength at which they are measured. Does the fact that we used the same molar extinction value at other pH's, when other forms / colors of anthocyanin are present, which you measured with other colors of light, cause an error in our calculated rate constants? (Test out your answer by changing the molar extinction value used in a spreadsheet calculation for one of the experiments. When you do this, which results change, and which do not?)
4. Based on your error propagation, what is the dominant source of error contributing to uncertainty in your measured rate constants? For example, is it pH, volume measurements, fitting a straight line to your data, or the original concentration of HOOH in the bottle you picked up at the store? Based on your answer, how could you improve your results if you had a lot more time to work on this in lab?
5. Once you know the rate law and the rate constant, it is possible to use this reaction to quantify HOOH in other mixtures. Describe how you could now measure the HOOH concentration in a sample of toothpaste.