

Generate a Calibration Curve for Chlorophyll a

Purpose

To measure and graph the absorbance of light by chlorophyll at a specific wavelength (the absorbance maximum) versus chlorophyll concentration. Students will be given an unknown and determine its concentration from their calibration curve.

Overview

This exercise is designed to familiarize the student with the concept of spectroscopy or the study of the interaction of matter with electromagnetic radiation. Students will prepare solutions and use a colorimeter to measure absorbance by solutions of different concentrations.

Time

One class period.

Key Concepts

Matter absorbs electromagnetic radiation.

Absorbance is proportional to concentration and this relationship can be expressed mathematically (Beer's Law $A=abc$).

Scientists can use this relationship to measure concentrations of solutions if a calibration curve is generated.

Skills

Collecting data

Preparing serial dilutions

Making observations

Graphing data

Forming hypotheses

Materials

Mortar and pestle or glass blender (do not use plastic if you will be using nail polish remover as a solvent)

Spinach or dried algae preparations (can be obtained from health food store as dietary supplement. About five capsules will do)

Nail polish remover (**acetone or ethyl acetate**) or **ethanol or rubbing alcohol** (30 - 50 mL)

Balance

Erlenmeyer flask (or a jelly jar)

Funnel

Filter paper (or coffee filters)

Graduated cylinder (50 mL)

Vernier colorimeter interfaced to a CBL system.

Cuvetts

5 mL graduated pipettes and pipette bulbs

Pasteur pipettes
20 mL test tubes

Facilitator Preparation

This experiment works best if students work in groups. Some students will be responsible for operation of the colorimeter; others will collect and record data. Once the serial dilutions are prepared, ask the students which solution is most concentration. For most people this will be intuitively obvious based on the intensity of the color. Make the point that the more concentrated the solution, the more light is absorbed.

Students will generate a calibration curve for chlorophyll a. They will learn the principle of Beer's Law (yes, that's right, Beer's Law) $A=abc$

A = Absorbance

a = a constant

b = path length

c = concentration

You will prepare an unknown for the students to determine the concentration. If you have more than one group, you may wish to prepare an unknown for each group.

- Combine 3 mL of stock with 7 mL of solvent. The concentration of the unknown is 0.33 X stock.
- Combine 6 mL of stock with 3 mL of solvent. The concentration of the unknown is 0.67 X stock.

Background

When ocean color is observed from space, highly sensitive instruments such as the Sea-viewing Wide Field-of-View Sensor (SeaWiFS) monitor various wavelengths (depending on what one wants to look for). The absorbance of light at 650 nm is proportional to chlorophyll concentration. Thus, looking at the color of an area of the ocean allows us to estimate the amount phytoplankton in that area, and tells us about the health and chemistry of the ocean. Comparing images taken at different periods tells us about changes that occur over time.

The instruments don't really "see" color as we do. They detect intensities at various wavelengths. Images ("false color" images) are generated where different colors represent different ranges of concentrations. Usually with red representing the highest concentrations.

Procedure

Instructions are written for a Vernier colorimeter interfaced to a CBL system.

Weigh spinach (a handful) or dried algae.

(Record the weight here _____g).

1. Grind in a blender or mortar and pestle or blender with some of your solvent. Filter the suspension into the Erlenmeyer flask through filter paper or coffee filter. Pour the solution into the graduated cylinder and bring the volume to 50 mL. The concentration of your stock solution is (weight) g/50 mL. Record the concentration here _____.
2. Pipette 5 mL of your stock solution into a 20 mL test tube. Add 5 mL of solvent (label this tube ½ X). Mix well. The concentration of this solution is ½ of your stock. Record the concentration here _____.

3. Pipette 5 mL of your $\frac{1}{2}$ X solution into a 20 mL test tube. Add 5 mL of solvent (label this tube $\frac{1}{4}$ X). The concentration of this solution is $\frac{1}{4}$ of your stock. Mix well. Record the concentration here _____.
4. Set the wavelength to 635 nM.
5. With no sample in the colorimeter, (and the cover closed) set the %T at 0%.
6. Put a blank (solvent with no chlorophyll) in the Spec 20 and set the %T at 100%. The concentration of this solution is 0g/mL.
7. Place each cuvette with the chlorophyll solutions in the Spec 20. Read the (%T)
8. Convert %T to absorbance using the following equation; $A = -\log T$.
9. Plot absorbance (y axis) vs. Concentration (x axis) including the 0g/mL point. Draw the best straight line through these points.
10. Your instructor will provide you with a solution of unknown concentration. Measure the %T of this solution and convert that to A. From your calibration curve, determine the concentration of your unknown solution.

Student Assessment

Ask students to discuss how an instrument on a satellite might monitor for Harmful Algal Blooms. While the principles are the same, measuring ocean color from space is technically more complicated than measuring absorbance using a colorimeter. Ask students to discuss what some of these complications might be.