MASSACHUSETTS INSTITUTE OF TECHNOLOGY

Department of Chemistry

5.310 Laboratory Chemistry

EXPERIMENT #2

An Analysis of Fish, Sediment and Water Samples for the Presence of Trace Essential & Toxic Elemental Substances[1,2]

Courtesy of MIT Museum. Used with permission.

[1] This experiment was designed by John J. Dolhun and includes contributions from course textbooks, current literature, and others affiliated with 5.310 Updated by John Dolhun June, 2019.

[2] Photo from: https://webmuseum.mit.edu/detail.php?module=people&type=popular&kv=12360

I. Dedication

This experiment is dedicated to Ellen Swallow Richards the first woman to graduate from MIT in 1873 with a Bachelor of Science degree, in addition, a top engineer, environmentalist, and Chemistry Instructor at MIT during the later 19th century. Her most important contributions included working on behalf of women establishing the first all woman's laboratory at MIT and starting a career path for women in science education with the Boston Public School Department. As an Instructor at MIT in 1884 she taught a course on Sanitary Chemistry at MIT a position she held up until her death in 1911.[3] She became interested in the analysis of water samples collecting upwards of 20,000 samples from various waterways across Massachusetts, which led to the establishment of the first program on sanitary engineering at MIT. Way ahead of her time and with the interest of protecting people from toxic chemicals in their homes and environment she concentrated on consumer products late in her life studying such things as the arsenic content in wallpaper and textiles and anything related to nutrition. Eventually she established and directed the first Home Economics Convention in Lake Placid (1899-1908). In December 1908, The American Home Economics Association was formed and Richards was elected the first President.[4]

I. PURPOSE OF THE EXPERIMENT

This is an integrated experiment, which combines techniques from Organic, Biological, Physical, Food and Analytical Chemistry. Its purpose is to introduce students to: https://www.britannica.com/biography/Ellen-Swallow-Richards

- The atomic absorption method and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) as analytical techniques
- UV-VIS Spectrophotometric analysis
- Fish, soil and water sample sampling methods and analysis
- Quantitative techniques used in the analysis and detection of essential and toxic elemental substances.

This experiment will also improve students' lab and computational techniques in the following areas:

- Learning the correct handling and operation of a Tri-Cell AA (Atomic Absorption) mercury analyzer and an ICP-MS mass spectrometer.

[3] https://en.wikipedia.org/wiki/Ellen_Swallow_Richards

[4] https://www.britannica.com/biography/Ellen-Swallow-Richards

- Precise handling and wet chemistry techniques for work up of a variety of fish, sediment, water and environmental samples.
- Using Microsoft Excel for providing graphical and numerical output analysis to prepare calibration curves and perform an error analysis on the results
- Learning about Microwave Digestion as a technique to prepare samples for analysis.

II. BACKGROUND

Part 1: Complete Elemental Analysis of Water in the Charles River including Determination of Dissolved Oxygen and Phosphate concentrations.

The Charles River extends itself in a serpentine path, some 80 miles through more than 20 cities and towns from its starting point in Hopkinton, Massachusetts.[5] The river ends at Boston Harbor, which opens to the Atlantic. Dozens of lakes and ponds are part of this river. The oxygen that dissolves in the river plays an important role in supporting all types of aquatic life and microbes. The dissolved oxygen depends on many factors including the surrounding air pressure and temperature. It is also influenced by its surroundings, which could deposit substances into the water. The three major sources of pollutants & organic matter that find their way into the river include municipalities (sewage & runoff), agriculture (fertilizers) and industrial (various). The organic matter, fertilizers and detergent waste provide material for explosive growth in vegetation and algae. The vegetation eventually dies and falls to the bottom of the river and combines with other organic matter to decompose through the action of various microorganisms and bacteria. Enormous amounts of oxygen are used up during the decay process. Various bacteria, as well as other organisms such as algae and fish use up the oxygen that makes its way into the water through the process of photosynthesis and diffusion from the surrounding atmosphere. There are many different levels of oxygen that have been discussed in terms of a satisfactory baseline number needed to support life and, from all accounts, that appears to be somewhere around 5.0 mg/L of dissolved oxygen. DO (Dissolved Oxygen) levels from 3.0 to 4.0 mg/L can create a stressful environment for fish.[6] This might lead to erratic movement or lack of interest in feeding. Levels of DO which are needed can vary from organism to organism with some fish species such as trout and small mouth bass requiring minimum levels of DO equal to 6.5 mg/L Other fish such as mud dwellers like catfish and carp can survive on as little as 2.0 to 3.0 mg/L of DO.[7] Once DO gets down to levels less than around 2 mg/L then the dead fish floating in

[5] Charles River Watershed Association

[6] A Beginner's Guide to Water Management, University of Florida, Florida Lakewatch, Department of Fisheries & Aquatic Sciences, 1st ed., June, 2004, p. 5.

[7] Dissolved Oxygen, Water Quality with Vernier, Computer 5, Vernier Software & Technology, Beaverton, OR, p. 1.

the Charles River "eutrophication" becomes a problem. On an ongoing basis, this experiment will measure the gaseous oxygen dissolved in the aqueous environment of the Charles River at various locations and under various seasonal conditions (temperature and pressure). The experiment will also study algae blooms fueled by phosphate via a colorimetric study to determine the phosphate concentrations present in the Charles River. Phosphates present in detergents are a nutrient for algae growth. Finally, an elemental analysis of the water will be performed with a state of the art Agilent 7900 ICP-MS system.

Part 2: Determination of Total Hg in Fish & Sediment Using a Direct Mercury Analyzer® (DMA-80 TRICELL).

This lab segment looks to assess the total Hg levels in fish and sediment using a Direct Mercury Analyzer® (DMA-80 TRICELL). Standard reference solutions will be used to validate the method. The method relies on the thermal decomposition of the fish & sediment, amalgamation of the mercury followed by detection by atomic absorption spectroscopy. The analysis of the samples is fast taking less than a few minutes.

Mercury is classified as a neurotoxin harmful to everyone but especially unborn fetuses and smaller children.[8] The mercury accumulates in our environment and surroundings. Mercury in the air is usually present as the elemental form of mercury in the vapor phase. Mercury in our environment can come from many natural sources including a burning forest fire, mining operations, a volcano, fossil fuels, forest fires, and garbage incinerator plants. By far the largest source of mercury in our environment would be from coal fired power plants which produce as much as 50 tons of mercury every year.[9]
As pH in surrounding waters drops, the heavy metals like mercury become more soluble and are absorbed more easily by aquatic life. When we eat a fish heavily contaminated with mercury, we end up with the mercury. Mercury in water is a serious global problem and it's not biodegradable, it has a tendency to accumulate. Once deposited into the ecosystem both land and water, the mercury is eventually converted to a very toxic organo-mercury compound called methyl mercury (CH_3Hg^+). This toxin gets absorbed into the vegetation, plants, crops and fish that we consume. Larger fish tend to have higher mercury levels. Fish such as tuna, swordfish, shark, and mackerel tend to accumulate the highest concentrations which can present health problems for certain segments of the population especially pregnant women and young children. Mercury also accumulates in humans much like it does in fish, the target of its toxicity is the central nervous system.

Driving in to MIT each day I subscribe to satellite radio and usually tune into the BBC news broadcast from London. I recently listened carefully as a group of Swedish

[8] Bose-O'Reilly S., McCarty K. M., Steckling N., Lettmeier B. Curr Probl Pediatr Adolesc Health Care. 2010 Sep; 40(8): 186–215.

[9] Pollution from FF Power Plants: http://en.wikipedia.org/ wiki/Fossil_fuel_power_plant

researchers had announced that rising temperatures could boost mercury levels in fish by as much as seven times current levels.[10] In the broadcast, Swedish scientists pointed out that with increasing storms, the heavy rainfalls will increase the amount of organic matter flowing into the waterways. This warm climate in effect would alter the food chain by increasing levels of methylmercury in zooplankton as much as seven times by the end of the century. These organisms are at the bottom of the food chain ladder and would pass this on up the chain.

[10] https://www.bbc.com/news/science-environment-38769697

III. SAFETY[11]

Students will handle a number of chemicals during this experiment, many of which must be treated with care in order to avoid harm. None of the chemicals listed here should be ingested or allowed to come in contact with your skin or eyes. The TAs will provide additional safety information and procedures during the weekly pre-laboratory lectures for this module.

1. **Sodium Azide:** Very hazardous in case of skin contact, eye contact, inhalation or ingestion. Severe over-exposure can result in death. Inflammation of the skin and eye is characterized by redness and itching.

2. **Sodium Hydroxide:** Very hazardous in case of skin contact (corrosive, irritant, permeator) of eye contact (irritant, corrosive) of ingestion and inhalation. Tissue damage is proportional to length of contact. Eye contact can result in corneal damage or blindness. Skin contact is characterized by inflammation and blistering. Severe over exposure can lead to lung damage, choking, unconsciousness and death.

3. **Chloroform:** Hazardous in case of skin contact, eye contact, ingestion and inhalation. Carcinogen.

4. **Sodium Thiosulfate Pentahydrate:** Hazardous in case of ingestion and inhalation. Slightly hazardous in case of skin contact and eye contact irritant and sensitizer.

5. **Potassium biiodate:** Slightly hazardous in case of ingestion or inhalation. Toxic to the lungs and mucous membranes. Potential eye, skin and respiratory tract irritation. Avoid breathing vapor or dust. Use adequate ventilation. Wash thoroughly after handling. This is an oxidizer so contact with combustible material could cause a fire.

6. **Potassium Iodide:** May cause respiratory tract, eye and skin irritation. Can cause digestive tract irritation with nausea, vomiting and diarrhea.

7. **Manganese II Sulfate Monohydrate:** Harmful if inhaled or swallowed. May cause eye, skin, and respiratory tract irritation. May cause lung damage. May cause central nervous system effects. Hygroscopic (absorbs moisture from the air). *Target Organs:* Central nervous system, lungs, and reproductive system.

[11] Various Material Safety Data Sheets: Malinckrodt Chemicals, J. T. Baker, Phillipsburg, NJ; Fischer Scientific, MSDS, Pittsburgh, PA

8. **Potassium Dihydrogen Phosphate:** May Cause Irritation to skin, eyes, and respiratory tract. May be harmful if swallowed or inhaled.

9. **Sulfuric Acid:** Extremely corrosive causes serious burns. Highly toxic. Harmful by inhalation, ingestion and through skin contact. Ingestion may be fatal. Skin contact can lead to extensive and severe burns. Chronic exposure may result in lung damage and possibly cancer.

10. **Ammonium Molybdate:** Harmful if swallowed. May cause irritation. Avoid breathing vapors, or dusts. Use with adequate ventilation. Avoid contact with eyes, skin, and clothes. Wash thoroughly after handling. Keep container closed. Hazardous decomposition products: ammonia, nitrogen oxides, and toxic fumes.

11. **Ascorbic Acid (Vitamin C):** Pleasant, sharp acidic taste. Stable in air when dry. Aqueous solutions are rapidly oxidized by air. Alkalis, iron, and copper accelerate the reaction. Used as antimicrobial and antioxidant in foodstuffs. Not considered toxic except in immense quantities.

12. **Potassium Antimonyl-Tartrate:** Very toxic by inhalation, ingestion or contact with skin. Work under hood with good ventilation use gloves and goggles.

13. **Nitric Acid:** May be fatal if inhaled. Can cause severe skin and eye burns. Strong oxidizer contact with other organic materials can cause fire. Direct contact in eye could cause blindness. Deep penetrating skin ulcers have been reported. Severe even permanent damage to the digestive tract. Exposure to vapor or mist can cause a host of problems including chemical burns to respiratory tract, chemical pneumonitis and pulmonary edema.

14. **Hydrochloric Acid:** Very hazardous in case of skin contact, corrosive irritant. Lung sensitizer if inhaled. Liquid and spray may produce tissue damage, inflammation irritation to eyes, blistering type burns on skin, irritation to the respiratory tract.

IV. INTRODUCTION

General References – These are provided to improve your understanding of the techniques and afford practical hints that may help you avoid mistakes that may prove costly in terms of laboratory time.

Joy Michaud, A Citizens Guide to Understanding and Monitoring Lakes and Streams, (1994) Washington State Department of Ecology, Chapter 4, pp. 45-58.

• Measuring Mass and Volume	**MHS**, Chapter 5, pp. 52-64
• Pipets, Transfer of Liquids	
• Standard Reducing Agents	**SWH**, Chapter 20, pp. 511-513
• Standardizing Thiosulfate Solutions	**SWH**, Chapter 20, pp. 513-514
• Applications of Thiosulfate Solutions	**SWH**, Chapter 20, p. 514
• Dissolved Oxygen	**SWH**, Chapter 23, pp. 681-682
• Phosphate Ion Determination	**SWH**, FIA, pp. 1059-1061
• UV & VIS Spectroscopy	**MHS**, Chapter 25, pp. 465-475

Required Videos: Digital Laboratory Techniques Manual

- #1 Volumetric Techniques
- #2 Titration
- #7 Filtration
- #11 Balance
- #13 Automatic Pipet

PART I: DISSOLVED OXYGEN (DO) DETERMINATION[12]

This experiment will sketch out the procedure for determining the dissolved oxygen (DO) levels in water samples obtained from the Charles River. This experiment uses the azide modification of the iodometric Winkler titration method.[13,14] The procedure was first written up by a graduate student in 1888[15] and has since become the standard for determination of dissolved oxygen in sewage, streams, rivers, and various water systems.

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[12] Equations for reactions in this section adopted & modified from: John H. Nelson & Kenneth C. Kemp, Laboratory Experiments, *Analysis of Water for Dissolved Oxygen*, Chemistry the Central Science, 7th. Edition, Prentice Hall, 1997, p. 386.
[13] Standard Methods for Examination of Water & Wastewater, 21st Ed., 2005, American Public Health Association & American Water Works Association, pp. 4-138 to 4-140.
[14] Alsterberg, G., Methods for the determination of elementary Oxygen dissolved in water in the presence of nitrite, *Biochem. Z.*, 159 (1925) 36.
[15] L. W. Winkler, *Berlin. Deut. Chem. Ges.*, 21 (1888) 2843.

The experimental method is based on the oxidation of Manganese (II) from a manganous sulphate solution to a higher trivalent/tetravalent oxidation state. The resulting oxidation in the presence of base uses oxygen as the oxidizing agent and results initially in the formation of a white precipitate and later in the formation of a brown precipitate. The reaction scheme for the initial oxidation, which involves the addition of manganous sulfate solution and alkaline base, is as follows:

(1) $Mn(SO_4)\,(aq) + 2KOH\ aq. \longrightarrow Mn(OH)_2\,(s) + K_2SO_4$
white ppt

(2) $2Mn(OH)_2\,(s) + O_2\,(aq) \longrightarrow 2MnO(OH)_2\,(aq)$
brown ppt

There are different perspectives in the literature as to how exactly the oxidized manganese brown ppt should be represented. Some have established it as trivalent manganese in the form of $Mn(OH)_3$ others have indicated that hydrated MnO_2 could also be the brown color.[16] With the addition of acid and KI the oxidized brown precipitate is acidified and goes back into solution. Simultaneously, the manganic ion is reduced back to manganous and elemental Iodine is generated via the oxidation of I^- in the acidic medium. The amount of iodine, which is generated, is proportional to the amount of oxygen, present in the original sample. The reaction for the acidification and reduction is as follows:

(3) $MnO(OH)_2\,(s) + 2H_2SO_4\,(aq) \longrightarrow Mn(SO_4)_2\,(aq) + 3H_2O\,(l)$

(4) $Mn(SO_4)_2\,(aq) + 2KI\,(aq) \longrightarrow MnSO_4\,(aq) + K_2SO_4\,(aq) + I_2\,(aq)$

In the final stage of the azide modification of the (Winkler) titration sodium thiosulfate is added. The sodium thiosulfate reacts with elemental iodine to produce sodium iodide. At the moment that all of the elemental Iodine has been converted the solution turns from yellow to clear. A starch indicator is used to capture the dramatic color change at the endpoint. The reaction is as follows:

(5) $2Na_2S_2O_3\,(aq) + I_2\,(aq) \longrightarrow Na_2S_4O_6\,(aq) + 2NaI\,(aq)$

The net overall ionic equation for the scheme presented is as follows:

(6) $O_2\,(aq) + 4S_2O_3^{2-} + 4H^+ \longrightarrow 2S_4O_6^{2-}\,(aq) + 2H_2O\,(l)$

From the net reaction we can readily see that 4 moles of thiosulfate are required for each mole of oxygen.

[16] C. Numako and I. Nakai, Physica B, 208/209 (1995) 387-388.

Day #1: Standardization of Sodium Thiosulfate Solution

TAs Preparation of 0.025XX M sodium thiosulfate solution[17]

The TAs will prepare a solution of approximately 0.025XX M $Na_2S_2O_3$ as follows: Mass out 6.205 g of $Na_2S_2O_3$. $5H_2O$ and dissolve it in 800 mL of hot distilled water.[18] Add 1.5 mL of 6N NaOH to slow down bacterial decomposition and dilute the solution to 1 Liter in a volumetric flask. The solution bottle should be closed and stoppered immediately. Store the solution in the refrigerator until ready to use. Mix the dilute sodium thiosulfate solution very thoroughly by vigorous shaking with repeated inversions for several minutes each time the solution is used.[19]

Standardization of approximately 0.025XX M sodium thiosulfate solution with potassium bi-iodate solution[20]

The TAs will distribute a dry weighing bottle to each student containing approximately 0.1 gram of reagent grade potassium bi-iodate $KH(IO_3)_2$, which has been previously dried in a 103-105 ^{0}C drying oven for 1.5 hours or overnight.[21]

At the start of the lab, each student should remove the weighing bottle from the oven and let it cool in a small desiccator charged with calcium chloride. Leave the stopper off the weighing bottle until the first time the desiccator is opened up after the $KH(IO_3)_2$ has cooled. Observe the precautions involved in the use of desiccators mentioned in Chapter 6 of the Techniques Manual.

For the standard potassium bi-iodate solution, 0.0021 M: mass out 0.0818 g of dry $KH(IO_3)_2$ from the weighing bottle which had been previously heated for at least 1.5

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[17] Adapted from: Standard Methods for Examination of Water & Wastewater, 21st Ed., 2005, American Public Health Association & American Water Works Association, pp. 4-138 to 4-140.

[18] Many textbooks recommend boiling the water beforehand to remove dissolved CO_2

[19] It is always important to mix a standard solution very thoroughly and it is surprising how much mixing is necessary. Unless this is done, significant differences in concentration can persist and cause lack of agreement in subsequent titrations. In the present instance, the sodium thiosulfate solution needs to be dispersed throughout the solution by repeatedly inverting the bottle and shaking vigorously. This procedure should be repeated every time you prepare to take out additional sodium thiosulfate.

[20] Adapted from: Standard Methods for Examination of Water & Wastewater, 21st Ed., 2005, American Public Health Association & American Water Works Association, pp. 4-138 to 4-140.

[21] Solid potassium bi-iodate is available in high purity and is of a high enough molecular weight making it an excellent candidate as a primary standard for alkalimetry where starch is used as an indicator. The purpose of the drying period is to remove superficial moisture.

hours and has now cooled in your desiccator into 50 mL of warm distilled water[22] and dilute to 100 mL in a volumetric flask. The solution will be warm and can be titrated warm. Estimate all weights to ±0.1 mg (0.0001 g) and record all data immediately in your lab notebook.

For the preparation of an aqueous starch solution: Dissolve 0.5 g of soluble starch and 0.05 g salicylic acid preservative[23] by adding a few mL of distilled water to make a paste and dissolve in 25 mL of hot distilled water. The starch solution should be prepared fresh on the day you are going to use it. Keep it warm on hot plate & add hot to your solution.

For the standardization titration: Take 100 mL of freshly mixed thiosulfate solution 0.025XX M and pour it into a beaker and keep it stirred and well mixed. The stock solution must be mixed several times before you draw off the 100 mL. Sodium thiosulfate solutions have a tendency to come out of solution when sitting for a period of time and are described as being perishable. Obtain a 50 mL burette and use a few mL of the thiosulfate solution to clean the burette. Then fill the burette with the freshly mixed thiosulfate solution letting it run down the sides slowly to avoid any bubbles forming on the inside of the burette. If you do get a bubble, tap the burette lightly on the lab bench or flick the burette with your finger to drive any bubbles to the surface. Make sure the tip of the burette is filled with thiosulfate solution and not air. For the standardization titration prepare three separate 250 mL Erlenmeyer flasks. **Prior to the start of each titration[24]** add 2.0 g of potassium iodide (KI) into 100 mL of distilled water then add a few drops of concentrated sulfuric acid using dropper bottles supplied by the TA (DO NOT ADD SULFURIC ACID DIRECTLY TO KI AS I_2(g) WOULD ESCAPE). Pipette out and add 25.0 mL of the warm potassium bi-iodate solution then add 75 mL of distilled water for a total volume of approximately 200 mL. Immediately start the titration and titrate the liberated iodine in each flask with the thiosulfate titrant, stirring constantly. Place a sheet of white paper under the flask on the stir plate to help see the colors clearly. When the solution becomes pale yellow, add 1.0 mL of the freshly prepared hot (bubbling) aqueous starch solution (15 to 20 drops about one squirt from a 9" glass Pasteur pipette), which changes the color of the solution from pale yellow to blue.[25] Continue the titration until

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[22] Dissolve the $KH(IO_3)_2$ sample by swirling in 50 mL of warm water. Warming may be necessary as it is essential that the sample dissolve completely. Even a few small particles remaining can cause a serious titration error.

[23] Nichols, Ind. Eng. Chem., Anal. Ed., 1 (1929) 215.

[24] Possible source of error in the titration would be the air oxidation of I^- to I_2. If the solution is allowed to stand too long before it is titrated, the oxidation will produce values that may be too high.

[25] The starch solution must be added just before the endpoint is reached, that is at the point when the deep yellow color of the iodine solution turns to pale yellow or light amber. If the starch solution was added earlier the dark blue-black starch-iodide complex that forms would make it difficult to find the endpoint as the color change comes almost instantaneously so it is easier to watch for the approach of the endpoint with the yellow solution changing to a pale-yellow color and then adding the aqueous starch solution.

the color changes from blue to colorless. Record the volume of the thiosulfate used from the burette. Disregard any change back to the blue color after the endpoint has been reached.[26] Repeat the titration with additional samples. Volumes should agree to within 5%.

Each time you fill the burette with fresh solution, rinse the burette 3 times with 2mL of the new solution. Discard each wash into the appropriate waste container. Tilt the burette to allow the entire inner surface of the burette to come into contact with the liquid. After rinsing out the burette, fill it with the $Na_2S_2O_3$ solution. Expel air bubbles trapped below the stopcock by fully opening the stopcock a second or two. If this is unsuccessful, see your TA for additional advice.

The titration may be carried out rapidly at first, but the endpoint should be approached carefully. The endpoint should be sharp and easily located to within a fraction of a drop. The endpoint is taken as the first distinct colorless solution that persists for 10 seconds or more after thorough mixing. The color is not permanent and may fade back to the blue in a matter of minutes, which should be disregarded.

Make all burette readings by estimating to the nearest 0.01 mL, allowing time for drainage. The tendency of liquids to stick to the walls of the burette can be diminished by draining the burette gradually. A slowly drained burette will provide greater reproducibility of results. Run a sufficient number of titrations to assure a precise and presumably accurate standardization. Record the final burette readings from each trial and subtract from the initial readings on the burette to quantify the amount in mL of thiosulfate used. The standardization titration should be repeatable to within 5%. The balanced equations for the standardization reactions are as follows:

$$KH(IO_3)_2(aq) + 10KI(aq) + 6H_2SO_4(aq) \rightarrow 6I_2(aq) + 6H_2O(l) + 5K_2SO_4(aq) + KHSO_4(aq)$$

$$6\ I_2(aq) + 12\ S_2O_3^{2-} \rightarrow 12\ I^- + 6\ S_4O_6^{2-}$$

From the equations you now have the stoichiometry of the reactions and should now be able to calculate the Molarity of the Thiosulfate solution.

If three titrations do not result in the desired precision, it will be necessary to conduct additional titrations. With your notebook pages turned in at the end of the day, include a table giving the calculated molarity of the $Na_2S_2O_3$ from each titration, calculate the

[26] The starch endpoint is taken as the first distinct change from blue to colorless that persists for 10 seconds or more after thorough mixing. The color may not be permanent but may change back to blue in a matter of minutes or less. This should be disregarded and the endpoint should be taken as the first point at which the blue starch-iodide complex disappears resulting in a colorless solution. The student should think of some reasons for the possible return of the blue color and perhaps substantiate the reasoning with some chemical equations.

average, standard deviation and the 95% confidence limits of the mean. No error propagation necessary for the thiosulfate standardization calculations.

Record your calculated molarity for each titration on your TAs class data sheet before leaving the lab for the day. The TAs will average the results from the entire team and present each team member with the team average.

Day #2: Determination of Dissolved Oxygen Levels (DO) in the Charles River

Field Trip to Collect Water Samples from the Charles River—Titrate the Samples with Standardized Sodium Thiosulfate Solution

TAs Prepare Manganous Sulfate Solution and Alkali-Iodide-Azide Reagent[27]

For the Manganous Sulfate solution, dissolve 364 g of $MnSO_4 \times H_2O$ into distilled water, filter and dilute to a volume of 1 Liter. The $MnSO_4$ solution should not give a color with starch when added to an acidified potassium iodide (KI) solution.

For the alkaline iodide-azide reagent dissolve 700 g of KOH and 150 g KI in distilled water and dilute to 1 Liter. Then, add a solution consisting of 10 g NaN_3 dissolved in 40 mL of distilled water.

Collection of Samples from the Charles River[28]

Follow the instructions of the TA for the time to meet at the pre-determined collection site on the Charles River. Collect the samples to be tested into special 300 mL BOD bottles taking precautions not to introduce air bubbles into the sample collection bottles. Hold the special designed water sampling device snapping the bottle in place then holding the device with BOD bottle approximately one arm length under water and allow the collection bottle to fill slowly with no air bubbles. Once filled, carefully raise the device and insert the glass stopper making sure that no air bubbles are present in your sample especially below the glass stoppered neck area. If you see any air bubbles you should discard the sample and start over again. Once you have collected your samples, carry the samples back to the laboratory for the workup and titration procedure, which follows.

Azide – Winkler Method Workup and Titration Procedure

1. Carefully remove the stopper from the 300 mL BOD collection bottle avoiding aeration of the sample. Using a calibrated pipette, add just below the surface of the liquid 2 mL of 2.15M manganous sulfate solution, which has been prepared by the TAs. Pipette the solution in slowly to avoid any introduction of air into your collection bottle.

2. Carefully repeat the above procedure again adding just below the surface of the liquid 2 mL of alkaline-iodide-azide reagent, which has been previously prepared by the TAs.

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[27] Adapted from: Standard Methods for Examination of Water & Wastewater, 21st Ed., 2005, American Public Health Association & American Water Works Association, pp. 4-138 to 4-140.

[28] Collection protocol adapted from: Joy Michaud, A Citizens Guide to Understanding & Monitoring Lakes and Streams (1994) Washington State Department of Ecology, 45-51.

3. Stopper the collection bottle being careful not to introduce any air into the collection flask and noting that the collection flask now contains an excess of liquid. Holding the stopper securely, invert the bottle several times to mix the sample. Check for air bubbles discarding the sample and starting over if any is seen. If oxygen is present in your collection flask you will see a milky precipitate form initially which quickly turns a yellowish-brown color. When the precipitate has settled invert the sample container again allowing the precipitate to thoroughly mix with the sample and then settle out to the bottom again. Do this approximately three times.

4. Carefully remove the stopper and add 2 mL of concentrated sulfuric acid (or about 28 drops from the dropper bottles supplied by the TA) to the surface of the liquid sample, just letting it gently run down the inside mouth of the collection flask. Carefully stopper and wipe off the top of the flask to remove any trace of acid then invert the bottle and continue mixing thoroughly until the precipitate has dissolved. This may take about 30 minutes or so. If it does not dissolve add another 1-2 mL of acid. The sample is now technically fixed and can be stored in a cool dark place for several hours. After addition of H_2SO_4, you may see an air bubble in your sample, which is fine at this point.

5. Titrate a volume representing 200 mL original sample after correcting for sample loss by displacement with reagents. Since we have added 4 mL (2 mL each) of $MnSO_4$ solution and alkali-iodide-azide reagents into the 300 mL collection bottle, titrate 200 x 300/ (300-4) = 203 mL. Pour 203 mL of the sample from the collection bottle into a 250 mL Erlenmeyer flask. Use a volumetric flask to measure out 200 mL of the solution for the titration. Use a 10 mL graduated cylinder to measure out the final 3 mL of volume until the 203 mL volume is achieved. Pour the 203 mL into a 250 mL Erlenmeyer flask, insert a stir bar into the flask, and get a good stir rate creating a vortex in the liquid then immediately start the titration. Titrate the sample with the standardized thiosulfate solution with constant stirring until a pale yellowish color develops record the amount of titrant used. Add 1 mL approximately 20 drops of 1% starch indicator solution and continue the titration until the solution turns colorless for the first time. Approach the endpoint carefully: as it only takes one drop of titrant to change the color from blue to colorless. Ignore the return of the blue color with time after the first colorless endpoint has been reached. Record the volume of titrant used. Each pair of students should do three titrations.

Calculate the dissolved oxygen content of your samples in mg/L (ppm).
For the dissolved oxygen (DO) determination error analysis:
Calculate the error propagation for the DO concentrations for each trial. For the error in the thiosulfate concentration, use the standard deviation provided by your TA associated with the class average if that is not available use your own thiosulfate data.
For the DO concentrations, calculate the average, standard deviation, and the 95% confidence interval.

What interferences could have affected your DO calculations using the modified Winkler titration procedure? Do an error analysis on your sample results. Calculate the saturation

level (SL) for your water samples. Do your results indicate that the Charles River water will support aquatic life?

Dissolved Oxygen % Saturation and Measuring Temperature of Water

Not only pollutants that enter the river effect dissolved Oxygen levels in river water; they are also affected by Temperature and atmospheric Pressure. For example, the lower the temperature, the more oxygen that can dissolve in the water. As the water warms up, the saturation level of DO will drop. You will need to measure the temperature and pH of the water at the collection site. The best way to measure temperature is to simply insert the thermometer directly into the Charles River. This should be done immediately at the time and place you collect the sample. Simply lower the thermometer tip a few inches below the water surface or place the thermometer into the sample container and allow the thermometer time to equilibrate with the collected water in your container. For the pH we will have a calibrated pH meter on hand and will read the pH directly from the meter. The meters will be calibrated in the lab using two buffers pH 7 and pH 10.

Calculate the temperature and pH of your water sample and discuss why they are important in terms of their variation and impact on pollution. Relate the values to your measured DO level.

The actual dissolved oxygen that we calculate in our experiment is in units of mg / L and represents the amount of oxygen gas dissolved in one liter of river water. Dissolved oxygen concentrations can range from 0 to upwards of 15 mg/L. As we look at the water quality of the Charles River, it might be useful to have another way to express it other than in the units of mg / L. Frequently, when talking about DO concentrations, the term % saturation is used. The saturation level of DO (SLDO) represents the theoretical amount that the river could potentially hold based on conditions of temperature, atmospheric pressure and altitude. As a general rule % saturation levels less than 60% are not good and represent unacceptable DO levels.[29,30] Levels between 60 to 70% are considered to be Satisfactory, and those between 70% and 90% Very Good, % saturations of 90% to 100% are generally viewed as being Excellent. Levels above 100% indicate supersaturation.[31,32] After determining your measured DO concentration you will calculate the % saturation of your sample.

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[29] Poor SLDO % numbers could indicate a high period of decomposition with bacteria using up the available DO or could occur during a period of higher temperatures.
[30] Dissolved Oxygen, Water Quality with Calculators, Texas Instruments, 2006, p. 2.
[31] Lower supersaturated levels are probably not of major concern as water can go in and out of a supersaturated level of DO over short periods of time. However, any prolonged supersaturated levels of DO should be cause for concern as it can have the effect of forming gas bubbles in the body cavities of fish. The bubbles can block the blood flow to cells causing cell death.
[32] Dissolved Oxygen, Water Quality with Calculators, Texas Instruments, 2006, p. 2.

There are several methods for determining the saturation level of the dissolved oxygen (SLDO) in the Charles River. Knowing the DO and the SLDO we can calculate the actual % Saturation Level of DO that is the ratio of the measured DO in ppm divided by the SLDO in ppm.

$$\% \ SL = \frac{Actual \ DO \ in \ ppm}{SLDO \ in \ ppm} \ X \ 100$$

Since the % saturation depends on both the temperature and pressure (elevation) a pressure correction factor should be included. In Appendix I there is a DO Pressure correction chart. Simply find the correct barometric Pressure and take the pressure correction factor and multiply it times the DO concentration that you have measured. This becomes your pressure corrected DO concentration. Because the Charles River is at sea level we do not have to worry about a major elevation Pressure correction. With the corrected DO measurement in hand; you can use the nomograph chart in Appendix II as a quick solution to determine the % saturation level for the Charles River. Simply find the corrected DO measurement on the bottom scale, mark off the corresponding temperature of the river water in degrees Celsius on the top scale, and connect the two marks with a straight line. The point where the line crosses the % saturation axis for your water sample is known as the % saturation level.

An even better way would be to calculate the saturation level of dissolved oxygen (SLDO) directly taking vapor pressure and temperature into account making use of a simple empirical formula derived from Henry's Law[33]. This formula has been reported to work well for temperatures between 0^0C and 50 ^{0}C, and allows us to calculate the amount of oxygen that theoretically could be present in oxygen-saturated water. The formulas apply to oxygen in distilled water:[34]

$$ppm \ \ dissolved \ \ oxygen = \frac{(P - p) \ \ X \ \ 0.678}{35 \ + \ T} = SLDO$$

[33] W. D. Hatfield, A Nomograph for Dissolved Oxygen Saturation in Water, *Sewage Works Journal*, Vol. 13, No. 3 (May, 1941), pp. 557-560.
[34] *Standard Methods for the Examination of Water and Wastewater*, 12th ed., American Public Health Association, 1965, pp. 408-410.

$0^0C < T < 30^0C$

$$ppm \quad dissolved \quad oxygen = \frac{(P - p) \quad X \quad 0.827}{49 + T} = SLDO$$

$30^0C < T < 50^0C$

where P is the barometric pressure at the collection site in mm Hg, T is temperature of water in ^0C, and p is the aqueous vapor pressure in mm Hg. To calculate p the vapor pressure of water in the air you can use the following equation:

$$p_{water \quad vapour} = e^{\left(20.386 - \frac{5132}{T} \right)}$$

where P= vapor pressure in (mm Hg) and T= temperature of air in Kelvin (K).

You can now take your corrected DO concentration and divide it by the SLDO, then, multiplying this by 100 gives you your % saturation.

Calculate the % saturation for your sample and from the % oxygen saturation level determine if there is a deficit or surplus of oxygen present. Explain your reasoning behind the deficit or surplus in the context of what it means in terms of respiration and aquatic life. Comment on any errors that could have caused any discrepancies in your calculated % saturation. No error propagation necessary for the SLDO and %SL results.

EXPERIMENTAL BACKGROUND FOR COLORIMETRIC ORTHOPHOSPHATE (PO_4^{3-}) DETERMINATION[35,36]

Natural waters contain a combination of phosphorous compounds including soluble inorganic orthophosphates $(PO_4)^{3-}$, dissolved larger types of phosphorous compounds called polyphosphates $(P_2O_7)^{4-}$ and $(P_3O_{10})^{5-}$, and phosphorous that is attached to organic matter. The exact form of the phosphate depends to some extent on the pH. The polyphosphates can all be hydrolyzed into the simpler soluble reactive form of orthophosphate. Phosphate is the principal nutrient responsible for algae growth in inland environments. One of the top problems facing our rivers is eutrophication caused directly by the excessive amounts of nutrients getting into our waterway systems. It can kill our fish and aquatic organisms, produce nasty odors along the shoreline, and impose limitations on our recreational swimming, fishing and boating. Most algae growth in rivers is a direct result of increased phosphorous dumping from municipal wastewater treatment plants, agricultural run-off, and industrial sources of pollutants. Leaves and grass clippings can be another source of phosphorous release into our rivers. The leaves and grass clippings end up along the shoreline and in gutters and are summarily washed into the river. Cutting the grass along the Charles looks quite innocent yet the clippings can have a major impact on the phosphate levels in the river. Soil erosion is another big contributor of phosphates during wind and rainstorms; the soil particles falling into the river carry with them their attached soil-bound phosphates. Presently there really are no strict regulations only a list of suggested recommendations from the EPA. Although this is changing, in November, 2009 the EPA established its first national standards containing numeric limitations on stormwater discharges.[37]

Phosphorous quantitation requires the conversion of the various forms of phosphorous into soluble reactive orthophosphates followed by colorimetric determination of the soluble dissolved phosphate. Samples must be collected in acid washed bottles and pre-treatment involves filtering off any suspended matter or particles. The larger solid phosphates must first be broken down into detectable orthophosphates (PO_4^{3-}) as the UV-VIS colorimetric analysis of phosphorous only works for orthophosphates, the soluble inorganic form of phosphorous. The exact phosphate ions

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[35] The background discussion on pH including ranges was adapted from: Joy Michaud, A Citizens Guide to Understanding & Monitoring Lakes and Streams (1994) Washington State Department of Ecology, p. 32.

[36] Background information adapted from the following however, the vanadomolybdate method used in this lab is not suitable for concentrations normally found in river water. The method used in this lab appears suitable for calculating the total phosphorous levels for something like raw sewage where the concentrations of phosphorous are high but not river water or fresh water lakes which require a more sensitive method: John H. Nelson & Kenneth C. Kemp, *Chemistry the Central Science*, Seventh Edition, Prentice Hall, (1997), pp. 377 to 383.

[37] Seth Jaffe, EPA Issues Construction Stormwater Rule—First National Standards with Numeric Limits, *Law & The Environment*, Published by Foley Hoag LLP, 2009.

that are usually present in the river will run the gambit, the structure heavily dependent on pH, although orthophosphate is the principal form found in natural waters. pH is an important parameter for most natural waters. The river will generally show a variable pH range somewhere between 6.5 and 8.5. The larger the amount of phosphate pollution, the greater the pH. This makes sense as phosphate pollution is usually equated with increased activities such as photosynthesis and a loss of H^+ ions resulting in an increase in pH. pH is generally higher during the daytime and periods of dense algae blooms and growth in the springtime. The pH of the river can also be influenced directly by discharges of municipal and industrial waste into the river. Natural rivers contain buffers to absorb sudden changes that might cause a drastic increase or decrease in pH. The natural buffers allow the pH to change slowly over time. As part of this lab we will take temperature and pH readings during our collection at the site.

To analyze the filtered river water for the presence of orthophosphate we will use a modified Molybdate Blue method that was proposed by Strickland and Parsons for Seawater in 1968.[38] This involves treating the sampled water with a color developing mixture of chemicals consisting of ammonium molybdate, sulfuric acid, ascorbic acid, and potassium antimonyl-tartrate, which reacts with soluble phosphate to form a phosphomolybdic acid. The phosphomolybdic acid is then subsequently reduced by the ascorbic acid to a blue complex:

Phosphate + Molybdate $\rightarrow$ Phosphomolybdic Acid

Phosphomolybdic Acid + Ascorbic Acid $\rightarrow$ Reduced Phosphomolybdate complex

The reduced phosphomolybdate complex can be observed at 880 nm in the near IR region using a UV-VIS spectrometer. The technique is based on the measurement of the orthophosphate, which is the soluble form of phosphorus present. Digestion of both dissolved organic as well as polyphosphate phosphorous compounds is important for determining the total P present which is sometimes referred to as phosphate or orthophosphate. It's this soluble form of phosphate that makes itself available to organisms for growth. The concentration is assessed by the reduced molybdate-ascorbic acid complex absorbance at 880 nm. The intensity of the blue color is proportional to the concentration of phosphate present in solution. It has been shown that in dilute acidic solutions with an excess of molybdate present, Beers law is obeyed with respect to orthophosphate.

According to the Lambert-Beer law, the amount of light transmitted by an absorbing sample is given by the following equations:

[38] Strickland, J.D.H., and Parsons, T.R. (1968). Determination of Reactive Phosphorous. *A Practical Handbook of Seawater Analysis*, Fisheries Research Board of Canada, 167, 49-56.

$$\% T = I / I_o = 10^{-A} \qquad A = \varepsilon c l$$

Where, the absorbance A is proportional to the concentration (c, in mol/L) of the solute, the length of the path the light travels through the sample (l, in cm), and the constant of proportionality, ε, called the molar absorptivity coefficient (L mol^{-1} cm^{-1}) or molar extinction coefficient. Once the Beer-Lambert law is confirmed, a plot of absorbance v. concentration will give a straight line, the slope of the line is the molar absorptivity, (εxl). Aqueous solutions of the blue complex show absorption of light at 880 nm. The intensity of the blue color at 880 nm is directly proportional to the phosphate concentration in the solution. The solutions are analyzed with a UV-VIS spectrometer and the concentration of the orthophosphate ion is determined from a calibration curve.

Day #3: Preparation of Phosphate Calibration Curve and Analysis of Charles River Water Samples for Quantitation of Orthophosphate

TAs Prepare Color Developing Solutions[39]

Prepare a 2.6M Sulfuric Acid solution by pouring 140 mL of concentrated sulfuric acid into approximately 200 mL of Milli-Q water in a one Liter volumetric flask. Dilute to 1 Liter volume with Milli-Q water. Transfer into glass storage bottles this solution should be stable for months. *Sulfuric acid is extremely corrosive and can cause severe burns. This operation should be conducted in the hood with proper gloves and goggles worn at all times. Always add the acid to water never the reverse.*

Ammonium Molybdate solution is prepared by dissolving 40 grams of Ammonium Molybdate tetrahydrate $NH_4)_6Mo_7O_{24}x4H_2O$ into approximately 0.5 Liters of Milli-Q water in a 1 Liter volumetric flask, dilute to 1 Liter with Milli-Q water and transfer to dark amber bottles. Store the solution in the refrigerator at 4^0C. Generally, this solution will be stable if stored properly however, any evidence of a ppt could be an indication that the solution is breaking down and should be freshly prepared.

Potassium Antimonyl-Tartrate solution is prepared by dissolving 0.680 grams of $C_8H_4K_2O_{12}Sb_2$ x $3H_2O$ in 500 mL of Milli-Q water. The solution can be stored at room temperature and should be stable for the entire semester.

Ascorbic Acid[40] solution is prepared on the day of the lab and must be used the same day. Dissolve 27.0 grams of ascorbic acid in approximately 200 mL of Milli-Q water in a 500 mL volumetric flask. Dilute to 500 mL with Milli-Q water. This solution is stable only for the duration of the laboratory and should be discarded at the close of the lab.

TAs should set up four burette dispensing stations for each of the above solutions under the hood. The solutions should be clearly labeled.

TAs Prepare 10% HCl Solution

TAs prepare a 10% HCl solution from stock and treat BOD bottles, beakers and volumetric flasks that your students will use for the lab about 1 hour prior to the start of the lab. All treated BOD bottles and beakers should be triply rinsed with Milli-Q water

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[39] Solutions for the color-developing reagent were modified and adapted from the following: Strickland, J.D.H., and Parsons, T.R. (1968). Determination of Reactive Phosphorous. *A Practical Handbook of Seawater Analysis*, Fisheries Research Board of Canada, 167, 49-56; MIT Open Courseware, Earth, Atmospheric & Planetary Sciences, 12.097 *Chemical Investigations of Boston Harbor*, Lab 3: Determination of Phosphate, January (IAP) 2006.; Standard Methods for the Examination of Water and Wastewater, 20th ed. American Public Health Association, American Waterworks Association, Water Environment Federation, 4500-P Phosphorous and 4500-P E, Ascorbic Acid Method.

[40] Ascorbic acid is aerobically oxidized in air reacting with the oxygen in solution and therefore should only be prepared and used at the time of the lab.

and placed into the racks to dry. Racks should be brought out into the lab for students to pick up glassware that they will need at the start of lab and all glassware should be rinsed out with distilled water by students and returned to the racks at the close of lab.

Preparation of Sample to be analyzed

Students will not go to the river until after the preparation of the Phosphate standards in this lab. Once at the river please obtain samples at the designated sampling site. The samples will be collected in 300 mL BOD bottles that have been rinsed with a 10% dilute HCl solution and finally rinsed several times with Milli-Q water.[41] Bottles are then air dried on a rack in preparation for the lab. Students will collect water samples as directed by the TAs making sure that no trapped air enters the collection bottle. Upon returning to the lab allow the water samples to sit on the lab bench for five minutes undisturbed letting the turbidity and solids settle out. Samples should be analyzed immediately before coming to room temperature. Once allowed to settle take out 50.0 mL of the collected sample and pipette 10.0 mL into five separate small beakers. These will represent the unknown samples. If these end up being too concentrated or fall outside of the standard curve below, you will need to dilute accordingly.

TAs Preparation of Primary Standard Solution

Prepare a stock solution by taking out 1.0 mL of KH_2PO_4 out of a reagent grade 1.0 M potassium phosphate monobasic solution and dilute with approximately 200 mL of Milli-Q water into a 1 Liter volumetric flask. Then dilute with Milli-Q water to 1 liter. Transfer these solutions to amber glass stock bottles and add 1 mL of chloroform to each.[42]

Student Preparation of the Phosphate Working Standard Stock Solution

Using a glass volumetric pipette transfer 1.0 mL from the TAs Primary Standard Solution ($1x\ 10^{-3}$M make sure TA has poured this into an acid washed beaker never pipette directly into a stock bottle) to a 100 mL volumetric flask previously rinsed with

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[41] Procedure for cleaning collection bottles and glassware has been modified slightly and adapted from: MIT Open Courseware, Earth, Atmospheric & Planetary Sciences, 12.097 *Chemical Investigations of Boston Harbor*, Lab 3: Determination of Phosphate, January (IAP) 2006.

[42] The chloroform will appear as a bubble at the bottom of the bottle. It does not mix with the aqueous solution and is a toxin that keeps mold and bacteria from growing in the standard solution. It slowly evaporates over time and can be replenished if the bubble is no longer visible. The solution is stable for about four months. This has been adapted from: MIT Open Courseware, Earth, Atmospheric & Planetary Sciences, 12.097 *Chemical Investigations of Boston Harbor*, Lab 3: Determination of Phosphate, January (IAP) 2006.

10% HCl solution and several times with Milli-Q water. Fill the 100 mL volumetric flask to the mark with Milli-Q water resulting in a $1x10^{-5}$M working solution.

Student Preparation of Diluted Phosphate Standards from Stock Working Solution

Set up on the lab bench 12 50 mL beakers (supplied by TA). Place a few sheets of white paper under the beakers for labeling. It's easier to mix the solutions with gentle swirling in the beakers as opposed to test tubes, which may be difficult to mix uniformly. The beakers should have been previously washed with 10% dilute HCl solution and then rinsed several times with Milli-Q water and allowed to dry. Label each beakers position on the sheets of white paper. Prepare a fresh set of Phosphate standards by diluting the KH_2PO_4 stock working standard solution as illustrated below:

Volume of KH_2PO_4 Stock	Volume of Milli-Q H_2O to Add	Final PO_4^{3-} Concentration
0.00 mL	10.00 mL	A- 0.00 μM
0.50 mL	9.50 mL	B- 0.50 μM
1.00 mL	9.00 mL	C- 1.00 μM
2.00 mL	8.00 mL	D- 2.00 μM
4.00 mL	6.00 mL	E- 4.00 μM
6.00 mL	4.00 mL	F- 6.00 μM
8.00 mL	2.00 mL	G- 8.00 μM

Pipette the correct aliquots of each standard + Milli-Q Water for a total volume of 10.00 mL into the first seven beakers, pipette 10.00 mL of the unknown samples from the BOD collection bottle into the next five beakers. Your 0.00 μM standard will also serve as the blank for the experiment. **Use only glass 10.0 mL glass volumetric pipettes previously cleaned with 10% HCl & triple rinsed with Milli-Q water, no digital pipettes should be used here as these could contaminate the samples resulting in your having to repeat the lab.**

Student Prepares Color Developing Reagent[43]

Take a small 50 mL Erlenmeyer flask (previously cleaned with 10% HCl and triple rinsed with Milli-Q water) to the hood area and add to the clean flask the following specified volumes of reagents in the following order (This solution should be obtained just prior to when you are going to use it) TAs will have these solutions set up in labeled burettes under the hood:

Reagent	Volume	
Ammonium Molybdate	5.0	mL
Sulfuric Acid	12.50	mL
Ascorbic Acid	5.0	mL
Potassium Antimonyl-Tartrate	2.5	mL
Total Volume	25.0	mL

Student Prepares the Samples with Addition of Color Developing Reagent

Before adding the color-developing reagent to your samples check with the TA to make sure a UV will be available for your run. UVs will be assigned to each team when they have shown the TA that they have the color developer ready to add to their samples. Now using a clean automatic pipette with a disposable tip Pipette 1 mL of the color developing solution into each of the 12 beakers including the blank. Flick the tubes gently or swirl the beakers carefully allowing the samples and reagents to mix thoroughly. Allow the solutions to sit for at least 20 minutes to fully develop the color then run the solutions in the UV. The solutions should be good as long as they are run within an hour after adding the color-developing reagent. Set up twelve 4.0 ml cuvettes for spectrophotometric analysis and fill each cuvette with the twelve prepared samples swirling the beakers and pouring directly into the UV cuvettes. Measure the absorbance of each solution at 880 nm following the UV-VIS instructions in the appendix attached to this experiment. Cuvettes should be placed into the UV spectrometer with the arrows on the cuvettee pointing in the direction the light beam is traveling. If the absorption of your unknowns does not fall within the range of your calibrated standard, prepare either a more dilute or more concentrated sample. We will be using an automatic cell changer and recording the absorbance readings in one run as prompted by the computer.

[43] Volumes were modified however, the order of addition and quantitation was adapted from: MIT Open Courseware, Earth, Atmospheric & Planetary Sciences, 12.097 *Chemical Investigations of Boston Harbor*, Lab 3: Determination of Phosphate, January (IAP) 2006.

Phosphate Analysis of Data

Measure the absorbance of five unknowns and the standards. Using Microsoft Excel plot the concentration of your standards on the x-axis in μM versus the absorbance. Make sure to take the blank into account and include the zero point on your graph. Eliminate any outliers using statistical analysis of the five unknown results.

Find the regression line for your standard curve and choose a linear fit with the equation and R^2 value displayed on your graph.

Include a copy of your graph in the Appendix of your lab report.

Use the LINEST function in the worksheet of Excel to calculate the errors of your slope intercept and y values.

Find the average and standard deviation of the five-absorbance measurements for your sample. Also, calculate the 95% confidence interval for your answer. For the sample, find the concentration of phosphate from the regression line that you calculated.

No error propagation for this part of the lab.

Report the final concentration as mg/L or ppm for total phosphorous (P) and $(PO4)^{3-}$

Compare your phosphate concentrations with others in the literature and comment on what your numbers mean in terms of pollution and how they might relate to your DO measurements.

Day #4: Obtain Fish and Surface Sediment Samples from the Charles River for Determination of Mercury Using a DMA-80 Mercury Analyzer

We will proceed to the site and collect samples along the Charles River directly abutting Storrow Drive. Sediment samples will be collected using Environmental Protection Agency Guidelines hyperlinked here EPA Guidelines.

All sediment samples will be collected in special En Core sample containers in addition to 2 oz sediment jars with septum lids. Samples will be photographed, labeled, bagged and stored in an ice chest for work-up at MIT in the Undergraduate Chemistry Teaching Laboratory.

Work areas should be wiped down with Milli-Q water prior to analysis. Metal free apparatus is preferable for analysis. Gloves must be clean and changed constantly to prevent contamination.

This lab segment looks to assess the total Hg quantification in fish & sediment using a Direct Mercury Analyzer® (DMA-80 TRICELL). Standard reference solutions will be used to validate the method. The method relies on the thermal decomposition of the fish & sediment, amalgamation of the mercury followed by detection by atomic absorption spectroscopy. The analysis of the samples is fast taking less than a few minutes. The EPA Method that we will adopt is EPA-7473.

Experimental Background for Determination of Mercury Levels in Fish & Sediment

A variety of native fish samples and sediment from the Charles River, will be analyzed using a Milestone Tricell DMA-80 mercury analyzer. Fish and sediment samples will be weighed, loaded into nickel boats, and placed in an autosampler. Once the program starts, the nickel boats will be loaded by the autosampler into a furnace, the sample will be dried, ashed, and carried by a stream of O2 gas until it enters a catalyst tube. Only the mercury is allowed to pass out of the catalyst tube and is sent via a stream of molecular oxygen into a second furnace where it is absorbed onto a gold amalgamator. Once all of the mercury from the sample has been processed and is absorbed onto the amalgam, the amalgam is heated to a high temperature allowing for the release of all of the mercury into an aluminum cuvette block where the concentration is determined based on atomic absorption. The analysis is fast taking less than 5 minutes for a sample. The system uses a mercury lamp for the excitation source which excludes any species that do not absorb at that specific wavelength. Any nonmercury components present in the analysis remain trapped in the catalyst tube.

Safety Hazard: Students must wear gloves when handling the fish tissues provided. The exhaust from the instrument is passed through a carbon filter at the rear of the instrument and vented at all times during the operation.

Sample Measurement with Milestone DMA-80

1. Switch on the DMA-80 System and perform the login. The DMA-80 system requires about 10-15 minutes of pre-heating time before measurement can be started.
2. Software opens up with login page measuring/sample. The last file used by the login user is loaded. If needed, create a new file. Create at least one new sample line. Select the operating mode automatic or simple.
3. Select the correct calibration under Measurement/Links.
4. Select the Method under Measurement/Links.
5. Carry out a blank measurement to make sure that the system is free from Hg contamination. (It is recommended to run a number of blank values before starting any measurements) For the blanks, you must enter a weight different from 0.0000 so entering 1.0000 will work and enable the measurement. The weight does not matter as the concentration is not substantial for the blank value. When running the "blanks" use air only no weighing boats. This insures any Hg contamination is coming from the instrument not the weigh boats. Use "BV" Method for automatic blanking.
6. Check calibration of the system measuring a reference sample. At the end, the signal curve can be tested in the Measurement/Signal menu. The reference sample is used to check the calibration curve. Measure a reference sample with known Hg content, this will keep the calibration under control. Open a measuring file. Make a blank run to check for Hg contamination. Now, create a reference measurement. Carry out multiple determinations to make sure the result is correct. Define the sample in the status column of "Results" table as "Reference Sample." The column concentration lights up and you can input the concentration of the reference sample. Enter concentration and weight of sample. Insert the reference sample into the autosampler in a perfectly clean boat. Start the measurement. The sample size should be selected, so that the mercury amount is included in the normal range. (i.e. 10ng: a solution with 0.1 mg/kg Hg: scale of 0.1g). After measuring the reference sample, the result is automatically compared to the set limit values and, in case of exceedance of the maximum deviation, the specific action is taken. Reference measurements are documented in a separate file.
7. Add the data necessary for the measurement. Measurement cannot commence until a weight is placed in the que for all data records and samples in the run.
8. Students enter the sample weight, then, place the sample in the Autosampler and start measurement.
9. If samples vary greatly with different Hg values, carry out another blank measurement after any samples are ran with high Hg values. This can be carried out automatically by the help of blank value cycles.

In this segment of the lab we will follow EPA Guidelines established in Method 7473

Day 5 Complete Elemental Analysis of Charles River Surface Water Using Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Preparation of Charles River Water Samples for Analysis by ICP-MS.
For this portion of the lab follow the guidelines in EPA Method 200.8, Revision 5.4. A hyperlink has been provided to the method. Guidelines for operation of the ICP-MS will be provided on site in the laboratory.

The method provides detailed step by step procedure for determination of dissolved elements in Surface Water. Some of the elements we will be looking for include the following: Aluminum (Al), Antimony (Sb), Arsenic (As), Barium (Ba), Beryllium (Be), Bismuth (Bi), Boron (B), Cadmium (Cd), Calcium (Ca), Chromium (Cr), Cobalt (Co), Copper (Cu), Iron (Fe), Lead (Pb), Magnesium (Mg), Manganese (Mn), Molybdenum (Mo), Nickel (Ni), Potassium (K), Rubidium (Rb), Selenium (Se), Silver (Ag), Sodium (Na), Strontium (Sr), Tellurium (Te), Thallium (Tl), Vanadium (V), and Zinc (Zn).

Procedure for Collecting the Water:

1. Students can work in groups of approximately 5-10 for this part of the experiment. Take a clean 500 mL wide mouth polypropylene plastic bottle to the river. Fill it 3X with river water and discard the rinsing's. Finally, fill it nearly to the top with river water and cap it.

2. On returning to lab, the sample must be filtered, each Team should obtain from the stockroom:

   1. Clean disposable plastic filter 2. A disposable vacuum filtration apparatus with a 0.45μm membrane filter 3. A 250 mL small mouth polypropylene bottle. 4. A portable pH meter 5. A tube of high purity nitric acid (1+1) (provided by the TA).

3. First, with the lab vacuum connected to the disposable filtration system, pour about 20-40 mL of the river water out of the wide mouth 500 mL container into the vacuum membrane filter system with the vacuum on, gently swirl to rinse out the filtration container collection flask. Shut off the vacuum, pull off the side vacuum arm revealing a pour spout, and pour the collected water from the pour spout into the sink. Reconnect the side arm of the filtration system, turn on the vacuum, and pour the remaining river water through the membrane filter but stop filling before the liquid reaches the vacuum connection line. Connect a clean disposable funnel to a new 250 mL small mouth polypropylene container. Shut off the vacuum and disconnect the side arm from the filtration flask. Pour the clean collected filtered river water into the new 250 mL small mouthed container. Repeat the process filtering the remaining river water about 3 more times until

you have nearly filled the 250 mL small mouthed bottle with filtered river water approximately 250 mL.

4. Immediately add 3.0 mL of high purity nitric acid (1+1) to the 250 mL small mouthed collection flask with the filtered river water using a digital pipettor. This should adjust the pH < 2. Measure the pH as directed in step 5. If the pH is >2 add another 1.0 mL of high purity nitric acid (1+1).

5. Pour about 10 mL of the acidified filtered river water into a 20 mL scintillation vial and measure the pH with a calibrated pH meter. The pH should verify less than 2.0. Record the pH in your notebooks and discard the pH tested water into a labeled River hazardous waste container.

6. Call your Instructor over and pour 10 mL of sample into a special turbidity sampling vial. Calibrate the turbidity meter and record the turbidity of the filtered sample of river water in your lab notebooks. The turbidity should be less than 1 NTU for ICP-MS analysis.

7. Pour about 10 mL of sample into a 20 mL high density polyethylene (HDPE) plastic sampling test tube. fill each polyethylene (HDPE) tube approximately half full with the filtered acidified river water. Volume is not important as we have not diluted the river samples.) (Obtain these tubes from the stockroom). Prepare one for each student in the group.

8. Obtain a 50 mL graduate polypropylene tube from the stockroom. Pour exactly 5 mL of the acidified filtered river water into the graduated tube. The volume must be exact perfectly on the 5 mL line. Fill the tube up to the 50 mL mark with 1% high purity nitric acid which you simply pour into the tube until you are within 2-3 mL of the 50 mL line. Then, use a plastic polypropylene disposable pipettor to drip 1% HNO_3 acid solution up to the 50 mL line on the tube. Mark this tube 10:1 dilution on the cap and side of the tube.

9. Each student takes a tube of water to the ICP-MS auto sampler place it into the que and record the sample position and name for the computer run as directed by the TA. Each Team brings one tube of the 10:1 diluted river water sample to the ICP-MS auto sampler.

10. Once all the samples are loaded into the ICP-MS discard the previously filtered acidified river water into a hazardous waste container. The wide mouth and narrow mouth 500 and 250 mL plastic bottles, the plastic funnel, should be rinsed out with DI water and turned in to the stockroom. Tear the white paper membrane filter out of the filtration flask and dispose of it in the black solid waste container by the analytical balances. The filtration system itself should be washed out with DI water and turned in to the stockroom.

CARY 60 UV-VIS__5.310
OPERATING INSTRUCTIONS

Guidelines for measuring a UV-Vis spectrum using the Agilent UV/VIS Spectrometer for the Orthophosphate Determination with Automatic Cell Feeder

1. Turn on UV-VIS then Turn on computer
2. Logon USER: student PASSWORD: student
3. Click Method on Screen either Charles River Phosphate Determination, Catalase Protein Concentration or Catalase Iron Quantitation depending on the experiment you are doing.
4. Wait until the zero is highlighted in left margin.
5. Click ZERO button in left column.
6. CELL LOADING GUIDE appears. Open cell changer drawer and place Zero Blank into Position 1 as shown in cell loading guide on screen.
6. Close cell changer drawer on UV VIS.
7. Click OK on Cell Loading Guide machine now scans blank which is also your zero standard.
8. Remove the zero blank.
9. Press START on computer located at top.
10. Standard Sample Selection Guide appears standards and unknowns should be listed under selected for analysis.
11. Click OK on Standards / Sample selection guide.
12. CELL LOADING GUIDE appears load standards and unknowns as per loading guide. Load standards from low to high concentration then load samples into positions 8 to 12.
13. Close the UV lid and click OK on Cell Loading guide.
14. Click PRINT Button bottom left to print data.
15. Print to local laser printer default or select Microsoft Print to PDF and click OK

Insert personal USB and browse to USB and list a file name and save to USB

16. If calibration fails, you will get a warning click OK. Click OK again to continue with your analysis. You will see no results for your unknowns in the concentrations. You can click RECALCULATE and the standards pop up with an option to ELIMINATE a particular bad standard. Double click the YES next to the standards you want to eliminate and it turns to a NO, you can also change the minimum R2 value to a lower number this will permit the calculations of the curve. Then click OK.

APPENDIX I

Pressure Correction Factors for DO Measurements[44,45]

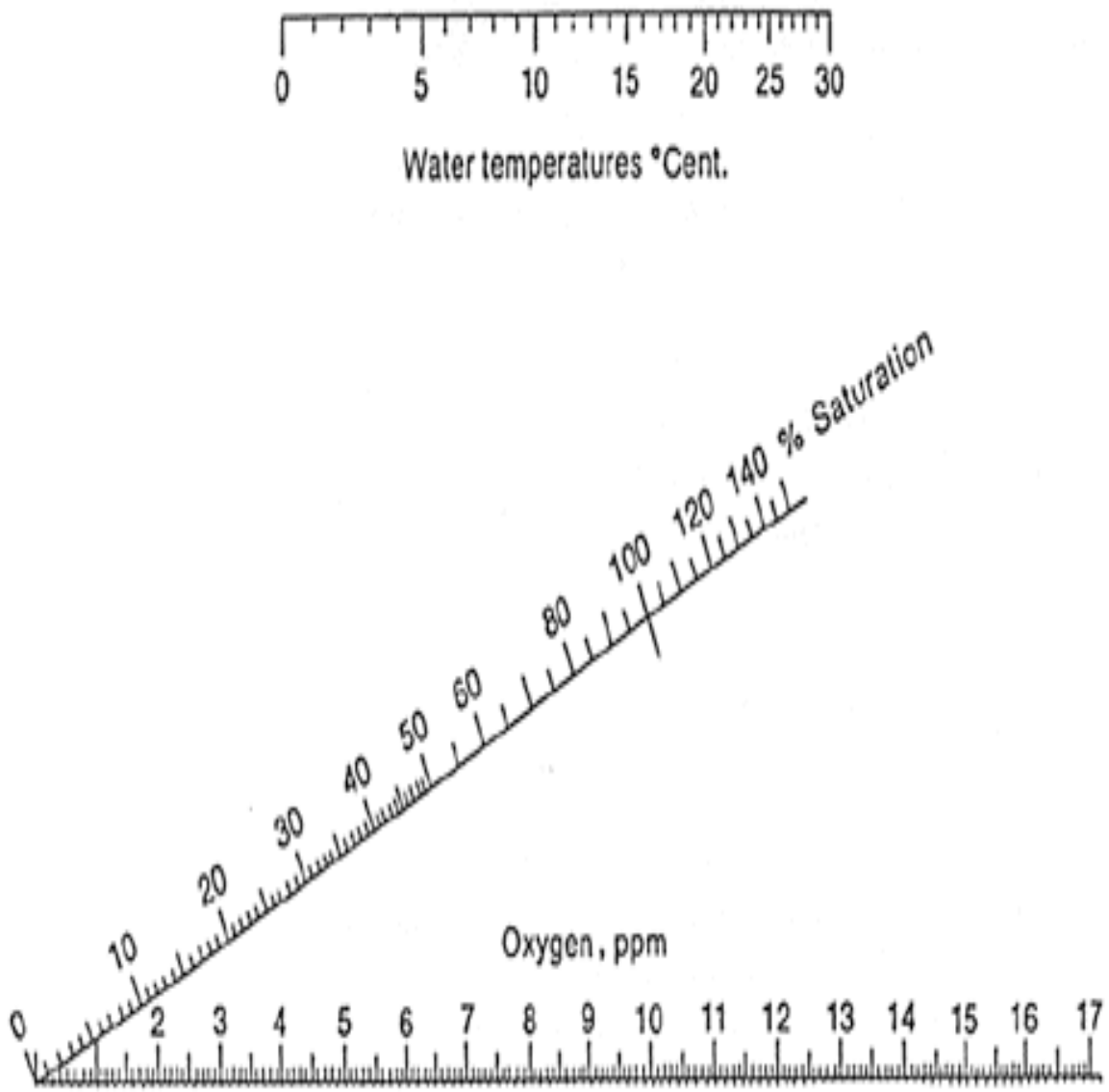
Atmospheric Pressure Torr	Equivalent Altitude (ft)	Correction Factor
760	0	1.00
745	542	0.98
730	1094	0.96
714	1688	0.94
699	2274	0.92
684	2864	0.90
669	3466	0.88
654	4082	0.86
638	4756	0.84
623	5403	0.82
608	6065	0.80
593	6744	0.78
578	7440	0.76
562	8204	0.74
547	8939	0.72
532	9694	0.70
517	10472	0.68

[44] Source: Derived from "Standard Methods for Examination of Water and Wastewater" and verified with Department of Fisheries and Aquatic Sciences, Institute of Food and Agricultural Sciences, *A Beginner's Guide to Water Management—Oxygen and Temperature,* University of Florida, Gainesville, Florida.

[45] After finding your measured value of DO in mg / L from the Charles River sample you can apply a correction factor to your measurement. Simply take your measured value and multiply it by the correction factor closest to the barometric pressure. This then becomes your corrected DO concentration.

APPENDIX II

Nomograph for determining % Saturation of DO at a given Temperature.[46]



[46] Once you have found your Pressure corrected DO concentration simply mark it on the bottom scale, then mark the corresponding temperature of the water on the upper scale. Use a ruler to connect the two points with a straight line. Read off your % saturation of DO at the intersection of your line on the middle scale.

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