

**MASSACHUSETTS INSTITUTE OF TECHNOLOGY**

Department of Chemistry  
5.310 Laboratory Chemistry

**EXPERIMENT #2**

**An Analysis of Charles River Water Samples For Quantification of  
Dissolved Oxygen and Phosphate Content<sup>1,2</sup>**



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<sup>1</sup> 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 May 2017.

<sup>2</sup> MIT across the Charles River. Photograph by [Anshul Nigam](#), on flickr. license [BY-NC-SA](#).

## **I. PURPOSE OF THE EXPERIMENT**

This is an integrated experiment, which combines techniques from Organic, Biological, Physical, and Analytical Chemistry, its purpose is to introduce students to:

- The colorimetric method as an analytical technique
- Spectrophotometric analysis
- Water sample analysis
- Quantitative techniques in volumetric analysis

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

- Learning the correct handling and operation of a UV-VIS spectrometer and performing calculations using Beer's Law
- Precise handling and measurement techniques for sampling water from the Charles River
- Making up solutions using serial dilution
- Using Microsoft Excel for providing graphical and numerical output analysis to prepare a calibration curve and perform an error analysis on the results

## **II. BACKGROUND**

In this experiment students will work with water samples, which they will obtain directly from the Charles River. 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.<sup>3</sup> 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

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<sup>3</sup> Charles River Watershed Association see <http://www.crwa.org/watershed.html>

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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.<sup>4</sup> 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.<sup>5</sup> Once DO gets down to levels less than around 2 mg/L then the dead fish floating in 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.

### III. SAFETY<sup>6</sup>

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.

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<sup>4</sup> A Beginner’s Guide to Water Management, University of Florida, Florida Lakewatch, Department of Fisheries & Aquatic Sciences, 1<sup>st</sup> ed., June, 2004, p. 5.

<sup>5</sup> Dissolved Oxygen, Water Quality with Vernier, Computer 5, Vernier Software & Technology, Beaverton, OR, p. 1.

<sup>6</sup> Various Material Safety Data Sheets: Malinckrodt Chemicals, J. T. Baker, Phillipsburg, NJ; Fischer Scientific, MSDS, Pittsburgh, PA

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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.
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. Alkalies, 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.

#### **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.

Available online as a PDF document: <https://fortress.wa.gov/ecy/publications/documents/94149.pdf>

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|---|--------------------------------------|
| • Measuring Mass and Volume             | <b>MHS</b> , Chapter 5, pp. 52-64    |
| • Pipets, Transfer of Liquids           |                                      |
| • Standard Reducing Agents              | <b>SWH</b> , Chapter 20, pp. 511-513 |
| • Standardizing Thiosulfate Solutions   | <b>SWH</b> , Chapter 20, pp. 513-514 |
| • Applications of Thiosulfate Solutions | <b>SWH</b> , Chapter 20, p. 514      |
| • Dissolved Oxygen                      | <b>SWH</b> , Chapter 23, pp. 681-682 |
| • Phosphate Ion Determination           | <b>SWH</b> , FIA, pp. 1059-1061      |
| • UV & VIS Spectroscopy                 | <b>MHS</b> , 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**<sup>7</sup>

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.<sup>8,9</sup> The procedure was first written up by a graduate student in 1888<sup>10</sup> and has since become the standard for determination of dissolved oxygen in sewage, streams, rivers, and various water systems.

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<sup>7</sup> 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, 7<sup>th</sup>. Edition, Prentice Hall, 1997, p. 386.

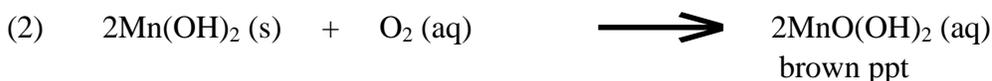
<sup>8</sup> Standard Methods for Examination of Water & Wastewater, 21<sup>st</sup> Ed., 2005, American Public Health Association & American Water Works Association, pp. 4-138 to 4-140.

<sup>9</sup> Alsterberg, G., Methods for the determination of elementary Oxygen dissolved in water in the presence of nitrite, *Biochem. Z.*, 159 (1925) 36.

<sup>10</sup> L. W. Winkler, *Berlin. Deut. Chem. Ges.*, 21 (1888) 2843.

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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:



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  $\text{Mn}(\text{OH})_3$  others have indicated that hydrated  $\text{MnO}_2$  could also be the brown color.<sup>11</sup> In the next part of the experiment with the addition of acid and KI in the alkaline potassium iodide azide solution the oxidized brown precipitate is acidified and goes back into solution simultaneously as the manganic ion is reduced back to manganous and elemental Iodine is generated via the oxidation of  $\text{I}^-$  in the acidic medium. The amount of iodine, which is generated, is proportional to the amount of oxygen, which is present in the original sample. The reaction for the acidification and reduction is as follows:



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:



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



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

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<sup>11</sup> 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**<sup>12</sup>

The TAs will prepare a solution of approximately 0.025XX M  $\text{Na}_2\text{S}_2\text{O}_3$  as follows: Mass out 6.205 g of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  and dissolve it in 800 mL of hot distilled water.<sup>13</sup> 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.<sup>14</sup>

### **Standardization of approximately 0.025XX M sodium thiosulfate solution with potassium bi-iodate solution**<sup>15</sup>

The TAs will distribute a dry weighing bottle to each student containing approximately 0.1 gram of reagent grade potassium bi-iodate  $\text{KH}(\text{IO}_3)_2$ , which has been previously dried in a 103-105 °C drying oven for 1.5 hours or overnight.<sup>16</sup>

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  $\text{KH}(\text{IO}_3)_2$  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  $\text{KH}(\text{IO}_3)_2$  from the weighing bottle which had been previously heated for at least 1.5

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<sup>12</sup> Adapted from: Standard Methods for Examination of Water & Wastewater, 21<sup>st</sup> Ed., 2005, American Public Health Association & American Water Works Association, pp. 4-138 to 4-140.

<sup>13</sup> Many textbooks recommend boiling the water beforehand to remove dissolved  $\text{CO}_2$

<sup>14</sup> 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.

<sup>15</sup> Adapted from: Standard Methods for Examination of Water & Wastewater, 21<sup>st</sup> Ed., 2005, American Public Health Association & American Water Works Association, pp. 4-138 to 4-140.

<sup>16</sup> 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.

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hours and has now cooled in your dessicator into 50 mL of warm distilled water<sup>17</sup> and dilute to 100 mL in a volumetric flask. The solution will be warm and can be titrated warm. Estimate all weights to  $\pm 0.1$  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<sup>18</sup> 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**<sup>19</sup> add 2.0 g of potassium iodide (KI) into 100 mL of distilled water then add a few drops of concentrated sulfuric acid (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. When the solution becomes a pale yellow color add 1.0 mL of the freshly prepared hot aqueous starch solution (15 to 20 drops), which changes the color of the solution from pale yellow to blue.<sup>20</sup> Continue the titration until the color changes from blue to colorless. Record the volume of the thiosulfate used from the buret. Disregard any change back to the blue color after the endpoint has been

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<sup>17</sup> 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.

<sup>18</sup> Nichols, Ind. Eng. Chem., Anal. Ed., 1 (1929) 215.

<sup>19</sup> Possible source of error in the titration would be the air oxidation of I<sup>-</sup> to I<sub>2</sub>. If the solution is allowed to stand too long before it is titrated, the oxidation will produce values that may be too high.

<sup>20</sup> 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.

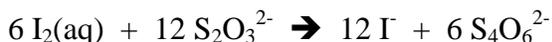
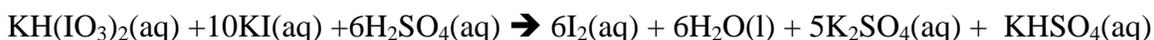
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reached.<sup>21</sup> Repeat the titration with additional samples. Volumes should agree to within 5%.

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<sub>2</sub>S<sub>2</sub>O<sub>3</sub> 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 buret readings from each trial and subtract from the initial readings on the buret 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:



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<sub>2</sub>S<sub>2</sub>O<sub>3</sub> from each titration, calculate the average, standard deviation and the 95% confidence limits of the mean. No error propagation necessary for the thiosulfate standardization calculations.

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<sup>21</sup> 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.

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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: Collect (DO) Water Samples from the Charles River—Titrate the Samples with Standardized Sodium Thiosulfate Solution**

#### **TAs Prepare Manganous Sulfate Solution and Alkali-Iodide-Azide Reagent<sup>22</sup>**

For the Manganous Sulfate solution, dissolve 364 g of  $\text{MnSO}_4 \times \text{H}_2\text{O}$  into distilled water, filter and dilute to a volume of 1 Liter. The  $\text{MnSO}_4$  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  $\text{NaN}_3$  dissolved in 40 mL of distilled water.

#### **Collection of Samples from the Charles River<sup>23</sup>**

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.

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<sup>22</sup> Adapted from: Standard Methods for Examination of Water & Wastewater, 21<sup>st</sup> Ed., 2005, American Public Health Association & American Water Works Association, pp. 4-138 to 4-140.

<sup>23</sup> Collection protocol adapted from: Joy Michaud, A Citizens Guide to Understanding & Monitoring Lakes and Streams (1994) Washington State Department of Ecology, 45-51.

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- 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.
- 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.
- Carefully remove the stopper and add 2 mL of concentrated sulfuric acid (or about 28 drops from a Pasteur pipette) to the surface of the 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  $\text{H}_2\text{SO}_4$ , you may see an air bubble in your sample, which is fine at this point.
- 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  $\text{MnSO}_4$  solution and alkali-iodide-azide reagents into the 300 mL collection bottle, titrate  $200 \times 300 / (300 - 4) = 203$  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. **An easier option here is to titrate exactly 200 mL then multiply your result by a correction factor 203/200.** 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 and in 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.

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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.<sup>24,25</sup> 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.<sup>26,27</sup> After determining your measured DO concentration you will calculate the % saturation of your sample.

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<sup>24</sup> 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.

<sup>25</sup> Dissolved Oxygen, Water Quality With Calculators, Texas Instruments, 2006, p. 2.

<sup>26</sup> 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

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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.

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

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<sup>28</sup>. This formula has been reported to work well for temperatures between 0<sup>0</sup>C and 50<sup>0</sup>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.<sup>29</sup>

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forming gas bubbles in the body cavities of fish. The bubbles can block the blood flow to cells causing cell death.

<sup>27</sup> Dissolved Oxygen, Water Quality With Calculators, Texas Instruments, 2006, p. 2.

<sup>28</sup> W. D. Hatfield, A Nomograph for Dissolved Oxygen Saturation in Water, *Sewage Works Journal*, Vol. 13, No. 3 (May, 1941), pp. 557-560.

<sup>29</sup> *Standard Methods for the Examination of Water and Wastewater*, 12<sup>th</sup> ed., American Public Health Association, 1965, pp. 408-410.

EXPERIMENT #2: *Charles River Water Analysis*

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

$$0^{\circ}\text{C} < T < 30^{\circ}\text{C}$$

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

$$30^{\circ}\text{C} < T < 50^{\circ}\text{C}$$

where P is the barometric pressure at the collection site in mm Hg, T is temperature of water in  $^{\circ}\text{C}$ , 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:

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

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.

## **V. EXPERIMENTAL BACKGROUND FOR COLORIMETRIC ORTHOPHOSPHATE (PO<sub>4</sub><sup>3-</sup>) DETERMINATION**<sup>30,31</sup>

Natural waters contain a combination of phosphorous compounds including soluble inorganic orthophosphates (PO<sub>4</sub>)<sup>3-</sup>, dissolved larger types of phosphorous compounds called polyphosphates (P<sub>2</sub>O<sub>7</sub>)<sup>4-</sup> and (P<sub>3</sub>O<sub>10</sub>)<sup>5-</sup>, 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.<sup>32</sup>

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<sub>4</sub><sup>3-</sup>) as the UV-VIS colorimetric analysis of phosphorous only works for

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<sup>30</sup> 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.

<sup>31</sup> 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.

<sup>32</sup> Seth Jaffe, EPA Issues Construction Stormwater Rule—First National Standards with Numeric Limits, *Law & The Environment*, Published by Foley Hoag LLP, 2009.

## EXPERIMENT #2: *Charles River Water Analysis*

orthophosphates, the soluble inorganic form of phosphorous. The exact phosphate ions 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.<sup>33</sup> 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:

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<sup>33</sup> 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.

## EXPERIMENT #2: *Charles River Water Analysis*

$$\% T = I / I_0 = 10^{-A} \quad A = \epsilon c l$$

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,  $\epsilon$ , called the molar absorptivity coefficient ( $L \text{ mol}^{-1} \text{ cm}^{-1}$ ) 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, ( $\epsilon l$ ). 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**<sup>34</sup>

**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  $\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \times 4\text{H}_2\text{O}$  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°C. 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  $\text{C}_8\text{H}_4\text{K}_2\text{O}_{12}\text{Sb}_2 \times 3\text{H}_2\text{O}$  in 500 mL of Milli-Q water. The solution can be stored at room temperature and should be stable for the entire semester.

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<sup>34</sup> 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 Phosphorus. *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, 20<sup>th</sup> ed. American Public Health Association, American Waterworks Association, Water Environment Federation, 4500-P Phosphorous and 4500-P E, Ascorbic Acid Method.

## EXPERIMENT #2: *Charles River Water Analysis*

**Ascorbic Acid**<sup>35</sup> 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 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.<sup>36</sup> 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  $\text{KH}_2\text{PO}_4$  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.

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<sup>35</sup> 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.

<sup>36</sup> 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.

## EXPERIMENT #2: Charles River Water Analysis

Transfer these solutions to amber glass stock bottles and add 1 mL of chloroform to each.<sup>37</sup>

### **Student Preparation of the Phosphate Working Standard Stock Solution**

Using a biological 1.00 mL adjustable pipette transfer 1.0 mL from the TAs Primary Standard Solution ( $1 \times 10^{-3} \text{M}$ ) to a 100 mL volumetric flask previously rinsed with 10% HCl solution and several times with Milli-Q water. Bring the 100 mL flask to the mark with Milli-Q water resulting in a  $1 \times 10^{-5} \text{M}$  working solution.

### **Student Preparation of Diluted Phosphate Standards from Stock Working Solution**

Set up on the lab bench 12 50 mL beakers. 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  $\text{KH}_2\text{PO}_4$  stock working standard solution as illustrated below:

Volume of $\text{KH}_2\text{PO}_4$ Stock	Volume of Milli-Q $\text{H}_2\text{O}$ to Add	Final $\text{PO}_4^{3-}$ Concentration
0.00 mL	10.00 mL	A- 0.00 $\mu\text{M}$
0.50 mL	9.50 mL	B- 0.50 $\mu\text{M}$
1.00 mL	9.00 mL	C- 1.00 $\mu\text{M}$
2.00 mL	8.00 mL	D- 2.00 $\mu\text{M}$
4.00 mL	6.00 mL	E- 4.00 $\mu\text{M}$
6.00 mL	4.00 mL	F- 6.00 $\mu\text{M}$
8.00 mL	2.00 mL	G- 8.00 $\mu\text{M}$

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  $\mu\text{M}$  standard will also serve as the blank for the experiment.

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<sup>37</sup> 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.

### **Student Prepares Color Developing Reagent**<sup>38</sup>

Take a small Erlenmeyer flask 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:

<u>Reagent</u>	<u>Volume</u>
Ammonium Molybdate	5.0 mL
Sulfuric Acid	12.50 mL
Ascorbic Acid	5.0 mL
<u>Potassium Antimonyl-Tartrate</u>	<u>2.5 mL</u>
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. Measure the absorbance of each solution at 880 nm following the UV-VIS instructions in the appendix attached to this experiment. 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.

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<sup>38</sup> 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.

## EXPERIMENT #2: *Charles River Water Analysis*

### **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  $\mu\text{M}$  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  $(\text{PO}_4)^{3-}$

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.

## **UV-Vis Operation Guide<sup>39</sup>**

### ***Guidelines for measuring a UV-Vis spectrum using the Perkin Elmer Lambda 35 UV/VIS Spectrometer for the Orthophosphate Determination with Automatic Cell Feeder***

1. Turn on computer
2. Insert your personal jump drive into the top USB port on the front of computer
3. On the main screen double click on the Perkin Elmer UV WinLab icon top right corner of the screen
4. At Perkin Elmer login message User name must say student or students click OK and you will see the Base Methods appear in the left hand part of the screen
5. Left click on Phosphate Method Module you are running. Sample info window then loads. Under sample ID identify your samples will be indentified. Samples box at top of the screen should read 0. Do not change the cell or carousel numbers. Press Start to do an air blank with nothing in the machine.
6. Message appears to remove sample. Open door and place your distilled water/buffer blank in position 1 (extreme lower left port on cell changer) this will be scanned as a blank to produce a spectral background. Never touch the clear sides of the cuvette since it will always be in the path of the light beam. Keep the exterior of the cuvette clean and dry before inserting it. Close the door and say OK to the message.
7. After about a minute another message appears asking you to insert your seven standards into cells 2 thru 8 respectively depending you can run up to seven standards/samples simultaneously. Open the door; take your zero standard out of the position 1 and place it in position 2 then insert the other standards in correct order. Close the door and say OK. Machine begins to scan samples and overlay the graphs one at a time. Once the standards/samples have been run it will ask you to load the five samples in cells 2 thru 6 follow the instructions then say OK.
8. Message then appears that all samples in the table have been run say OK. Calibration message appears answer it OK.
9. Now on the left side of screen click on the word Output then go up to the top and click on file then select report. Say OK to Print and it will print your graph and or your list of absorbances v. concentration values. We are using a linear least squares best-fit graph for the method.
10. Now Save Data to your jump drive which you inserted previously, go to File then select Save Results then choose As a New Task you will then name your file and save task message. The computer creates a new folder by that name on your removable jump drive
11. To safely remove your hardware device from the computer look for the green icon on the lower bottom right side called Safely Remove Hardware Icon click it and a Safely remove hardware message opens select the task and click Stop a hardware device window opens select your device from the menu and say OK. Message appears at bottom of screen that it is safe to remove Hardware. You can now pull out your USB device.

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<sup>39</sup> Written by John J. Dolhun

## APPENDIX I

### Pressure Correction Factors for DO Measurements<sup>40,41</sup>

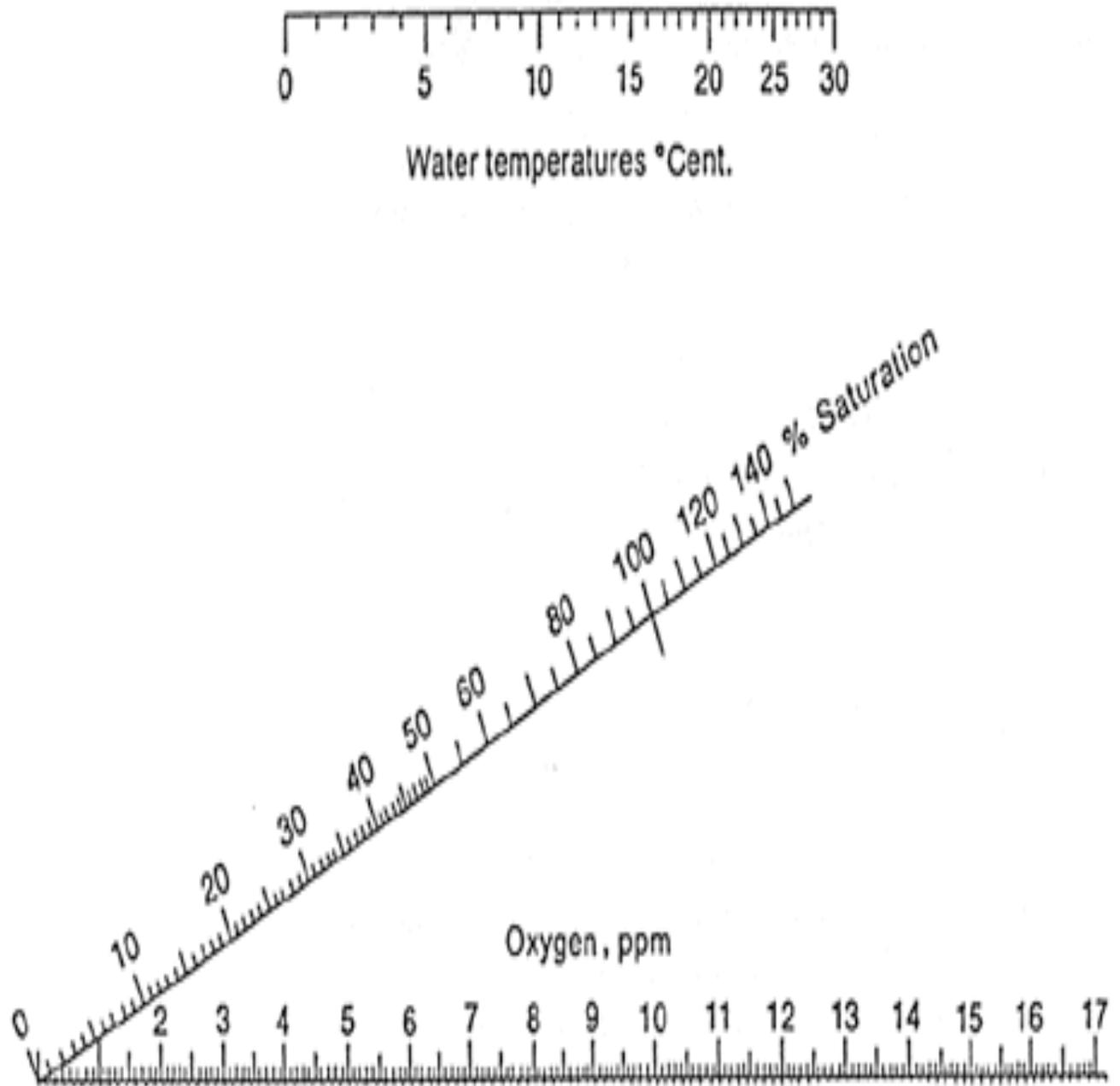
Atmospheric Pressure Torr	Equivalent Altitude (ft)	Correction Factor
760	0	1.00
745	542	0.98
730	1094	0.96
714	1688	0.94
699	2274	0.92
684	2864	0.90
669	3466	0.88
654	4082	0.86
638	4756	0.84
623	5403	0.82
608	6065	0.80
593	6744	0.78
578	7440	0.76
562	8204	0.74
547	8939	0.72
532	9694	0.70
517	10472	0.68

<sup>40</sup> 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.

<sup>41</sup> 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.<sup>42</sup>



<sup>42</sup> 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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