CHEM 108b lab

Chemical Reactivity in the Marine Environment

Professor Rachel Narehood Austin

Introduction

Students enrolled in CHEM 108b, *Chemical Reactivity in the Environment*, are also enrolled in the accompanying lab, which meets either Thursday or Friday afternoons from 1-4 p.m. or Thursday morning from 8:30-11. The practical goal of this laboratory is to construct, study, and maintain an ecosystem: a salt-water aquarium. This lab is based on a similar lab developed for advanced chemistry students by Professor K. Hughes when he was at Georgia Tech.

This ecosystem is isolated and incomplete - not part of a balanced, complete, self-sustaining system like those that exist in nature, having evolved over millions of years. Consequently its health will depend on our ability to manage it. With the exception of certain gases that the aquarium's water can exchange with the atmosphere, the only things added to or taken out of this ecosystem (the aquarium) will be those things that we add or take out, whether intentionally or unintentionally. In other words, this ecosystem is ours to manage, and if we fail to manage it properly the ecosystem will fail. To do this effectively, we must understand the variables (salinity, nitrate, phosphate, temperature, light, alkalinity, flora and fauna, dissolved oxygen, etc.), know how to sample and measure these variables, and how to adjust them if they are out of balance.

In the process of studying our aquarium we will observe how certain elements (C, N, P, S) cycle in the environment, which is the dominant theme in the course. I hope that by spending the entire semester on a single project you will have lots of opportunity to appreciate the connections between chemistry and the world. And I hope that you will learn a range of standard laboratory techniques that will be useful to you if you continue study in chemistry or a related science – in a context where the results of your work really matter. Finally, whether or not you choose to continue in science, you will learn problem-solving techniques that will be useful in whatever field you choose, and in other aspects of your life.

Lab structure

There are many different measurements and tasks, which have been broken down into three sets (shown below). Through the course of the semester, your group (3-4) students will have the chance to do each of these analyses. You can break down the responsibilities within your group in many different ways. However, your group will submit **one** report and receive **one** grade.

At a minimum, measurements should be taken once a week (during your assigned lab time). During times of rapid change, more frequent monitoring may be useful. Tank maintenance requires checking once a day. The results of your analyses, maintenance information, observations, etc. will be kept in the lab room. This will make it easy to summarize our results and monitor our ecosystem.

All measurements must be recorded in a lab notebook. Each member of the group must have his or her own notebook (permanently bound, no spiral bindings or removable pages). Data entries should be made directly in this notebook, in ink.

The three tasks are listed below:

Set A: Nitrogen cycle - ammonia, nitrite, nitrate

Set B: Minerals (SO_4^{2-}, PO_4^{3-}, Ca^{2+}, Mg^{2+})

Set C: Alkalinity, Salinity, Dissolved Oxygen

In addition, one group from the entire class will be responsible for tank maintenance.

Calendar:

Week 1: introduction, excel training course

Week 2: technical training, overview of tank and lab

Week 3: Research and preparation on your own. You must be completely ready for lab the following week (i.e. you must have all calculations done and know exactly how you are going to do your experiments). Each group must meet during this week to plan things out as a group.

Weeks 4-6: first rotation

Week 7: first written report due

Weeks 7-9: second rotation

Week 9: second written report due

Weeks 9-11: third rotation

Week 12: third written report due, final reports (oral)

The best source of information for developing appropriate analytical procedures is the book **Standard Methods for the Examination of Water and Wastewater**. A copy is on reserve in the library.

In addition, there are specific analytical procedures that have been adapted for CHEM 108b lab. They are available on the CHEM108b laboratory web page. There are additional resources on that page as well.

Lab Report Format:

Lab reports are required the week after your rotation through an assignment ends. These reports are a major component of your grade (90% of the lab grade). The lab reports should reflect the effort and input of all of the members. You may have the major responsibility for one section, or for researching and writing the introduction. Regardless, all group members are asked to sign off on the *completed* report, acknowledging that he or she has contributed to collecting the data and writing the report. Your signature is also a confirmation that you are satisfied with the final report. These reports will be graded on completeness, demonstrated chemical knowledge, and the clarity and precision of the writing. Your report should be a convincing professional assessment of your completed tasks.

Lab reports should contain

1. Abstract- brief statement that succinctly states what was measured, how it was measured, and what the results were (concentration, variability, observations, etc.) This should always be the

first page of your report. It must fit on that first page (along with the title of the report, group members, dates, etc.)

2. Introduction- provides some background information on the roles of the species reported (NO_3^- for example) in the marine ecosystem. Potential sources are included on the webpage; these sources should be acknowledged with footnotes or endnotes. Depending on how many species your group is analyzing, this section will be 1.5-3 pages.
3. Experimental Section- provides detailed information on how all the analyses were done.
4. Results- presents your results clearly. May include graphs ($[Cl^-]$ vs. time, for example) or summary tables. If you averaged data points or did other statistical manipulations (Q-tests), that should also be stated. An estimation of the experimental error of your results is also helpful.
5. Discussion and Conclusion- summarizes what was learned from the experiments and speculates about the chemistry. This section (and only this section) can include hypotheses about the relationships between your data and other chemical or physical processes occurring in the aquarium. For example, you could include a statement like "The decrease in dissolved oxygen from January 21 to January 28 is coincident with the appearance of a green algae bloom in the top of the tank" and go on to further develop the relationship between dissolved oxygen and algae. But be careful not to overstate your conclusions. Don't say "On February 3, a large increase in nitrate concentration killed four fish" but rather "the large increase in nitrate concentration coincided with the death of four fish". After all, you don't know the causality: Did the increase in nitrate cause the fish to die? Or did the fish die from some other reason and their death caused the nitrate levels to go up? Be careful and examine your logic.

Finally, the nitty-gritty details…

a. These reports will vary in length from 10-15 pages (double spaced, 10-12 pt type). That sounds like a lot, but you will find that this is reasonable. I'm not counting pages, but looking for completeness. A complete nine page report is much better than a sloppy, incomplete 14 page report.

b. The report should be written in the past tense and describe exactly what you did. Don't simply copy the experimental guidelines.

c. Figures, graphs and tables should be incorporated into the text of the report. If your word processing program does not allow this, you can attach the figures and tables in an appendix. Just be sure to number the figures and tables and refer to them by number in the text so I can find what you are referring to.

d. Chemical equations are required. You can write them in by hand, if necessary, but many word processing programs can handle simple equations. Use subscripts and superscripts properly in your text: SO_4^{2-} is much more readable than SO42-.

e. Attend to the writing. Make sure that the meanings you want to convey are clear. The Writing Workshop has staff proficient in scientific writing. Take advantage of their expertise! If different group members are writing different parts of the report, be careful to integrate them so that they read well and don't unnecessarily repeat or omit information.

f. Proofread, spellcheck, proofread, and proofread again. Spelling, grammar and punctuation do count.

I. Nitrogen Cycle

Nitrogen, an essential part of proteins, has a complex cycle in the marine environment. The majority of nitrogen exists as nitrogen gas (N_2) which is inert. Certain bacteria and blue-green algae can "fix" the nitrogen (put it into a useful form) and these fixed forms of nitrogen end up in plant proteins. Fish and other marine organisms consume these plants and convert the plant proteins into animal proteins. The proteins are then digested in the organism. The digested nitrogen is excreted in the form of ammonia (NH_3) which is in equilibrium with its ion ammonium (NH_4^+).

Unfortunately, ammonia is highly toxic to organisms even at very low concentrations (0.1-1 ppm) in the marine environment and can increase in concentration as pH changes. In order to rid the water of the ammonia, beneficial bacteria change the highly toxic ammonia into less toxic forms. *Nitrosomona* bacteria take the ammonia and break it down into nitrite (NO_2^-) by the following reaction:

$$NH_3 + 2\ O_2 + NADH \rightarrow NO_2^- + 2\ H_2O + NAD^+$$

The nitrite (desirable levels are less than 1 ppm) is then broken down by bacteria called *Nitrobacter* into a much less toxic and actually useful ion called nitrate (NO_3^-) by:

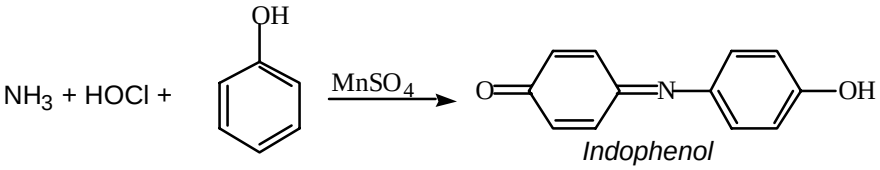
$$2\ NO_2^- + O_2 \rightarrow 2\ NO_3^-$$

Nitrate can then be used as a plant nutrient or can be directly reduced, by bacteria, into nitrogen gas and reenter the atmosphere. Nitrate levels less than 20 ppm are usually acceptable, less than 10 is better for the fish and invertebrates living in the aquarium (in general, the lower the better!).

Ammonia (NH_3) Determination

Chemistry

The concentration of the highly toxic ammonia can be detected and measured by a color change when in the presence of sodium hypochlorate (NaOCl) from household bleach and phenol



Indophenol

(C_6H_5OH).

The concentration of ammonia is calculated by measuring the absorbance of the solution at 630 nm. Indophenol (product of the reaction between ammonia, hypochlorite, and phenol) absorbs light at 630 nm and the amount of light absorbed is proportional to its concentration by Beer's Law: You can review the background on Beer's Law in the General Techniques portion of the lab manual.

We strongly recommend that you carefully read the method in the handbook on reserve. Students have had problems with this assay!!

Reagents

Manganous sulfate catalyst: dissolve 25 mg $MnSO_4 \cdot H_2O$ in 50 mL of distilled water. Store in a small bottle or indicator bottle.

NaOCl solution: to 10 mL of distilled water, add 2.5 mL of commercial bleach (NaOCl). Prepare this reagent in the fume hood! Adjust the pH to 6.5-7.0 using 1 M HCl (6-10 drops, use pH paper to test. The bleach will quickly change the color of the pH paper, so look at the initial color.) Make this solution fresh each week.

Phenol solution: dissolve 0.25 g of NaOH and 1 g of phenol in 10 mL of distilled water. Handle phenol with extreme caution and prepare in the fume hood! Make this reagent fresh each week.

Keep all ammonia stock and standard solutions tightly stoppered.

Stock[1] ammonia solution: dissolve 0.382 g ammonium chloride (NH_4Cl) in 1000 mL volumetric flask filled with distilled water. This solution is 122 ppm NH_3. Store in a tightly capped bottle.

Seawater blank: obtain from coworker, or see nitrate analysis.

WASTE: phenol waste goes in organic waste container, ammonia solution goes in aqueous waste (put ppm of solution and total volume of solution), any excess catalyst can go in aqueous waste (again report concentration of $MnSO_4$ and total volume of liquid added to the waste), bleach solution can be poured down the drain.

Procedure

1. Standard

Before employing any analytical method to determine the concentration of an unknown sample, one should always try the method with solutions or standards of known concentrations. Using the procedure outlined below, determine the accuracy and precision of the assay using standard ammonia solutions of several known concentrations. These will constitute your Beer's Law plot.

Example of a standard ammonia solution: dilute 1 mL (using a transfer pipet or pipetter) of the stock solution (122 ppm NH_3) to 250 mL in a volumetric flask with distilled water to get a 0.49 ppm solution. A set of standard solutions can be made in this way with concentrations ranging from 0.1 to 1.00 ppm NH_3.

To a 10 mL sample of an ammonia standard, add 1 drop of the $MnSO_4$ catalyst and place on a magnetic stirrer. Turn the stirrer on a slow stir setting. Once stirring, add 0.5 mL of NaOCl solution and immediately add 0.6 mL of phenol solution. Let the solution stir for a few minutes to get the most intense color. Transfer the solution to a clean cuvette and measure the absorbance at 630 nm. (Do not forget to blank the instrument with seawater beforehand.) It is important to remember that readings over 1 for absorbance are not reliable; thus solutions must be diluted until the readings are between 0 and 1. It is a good idea to do a quick plot of your standards (absorbance on the y-axis, concentration on the x-axis). The data points should be linear. The slope of the line equals εb.

2. Sample

[1] The term “stock solution” or “standard solution” means something quite specific in chemistry. This solution should be made as precisely as possible so that the actually concentration in the solution matches the intended concentration. For different types of analyzes the available precision will vary. In this course, ”stock” or “standard” solutions should always be made with the analytic techniques introduced – analytical balances, pipets, and volumetric flasks.

Obtain a 10 mL sample of aquarium water. Treat as above. Calculate the concentration of ammonia in the sample by using Beer's law. Report the concentration in ppm NH_3.

Nitrite Determination

Chemistry

The concentration of nitrite can be determined through the formation of a reddish purple azo dye produced at a low pH. An azo dye is a chromophore (colored compound) that has a nitrogen-nitrogen triple bond. The azo dye used in this assay is formed by combining sulfanilamide and nitrite in the water. This diazoated sulfanilamide then reacts with N-(1-naphthyl)-ethylenediamine dihydrochloride (NED-dihydrochloride) to form the azo dye.

Once the dye has been formed, its absorbance can be measured at 543 nm and compared to the absorbances of standard nitrite solutions. Again, the success of this assay depends on the meticulous preparation of standard solutions. See the instructor for help with volumetric glassware.

Reagents

Color reagent: to 80 mL of distilled water, add 1.5 mL of 85% phosphoric acid and 1 g of sulfanilamide. After dissolving the sulfanilamide, add 0.1 g of NED dihydrochloride and dilute to 100 mL with distilled water (stable for 1 month, store in a small bottle).

Stock nitrite solution: add 0.1875 g of $NaNO_2$ to a small amount of distilled water in a 500 mL volumetric flask. Dilute to the mark, mix well. This solution will be 250 ppm nitrite).

Intermediate nitrite solution: dilute 5.00 mL stock nitrite solution to 100 mL in a volumetric flask with distilled water. Mix thoroughly. This solution will be 12.5 ppm nitrite.

Standard nitrite solution: use *intermediate* nitrite solution to make 4-6 standard solutions between 0.100 and 1.00 ppm that are 50 mL in volume using volumetric glassware.

Seawater blank: obtain from coworker, or see nitrate analysis.

WASTE: All solutions can go into aqueous waste. Record both the concentration of the solution and the total volume.

Procedure

1. Standards

Dilute 25 mL of a standard solution to 100 mL with distilled water. Transfer 50 mL of the sample to an Erlenmeyer flask and add 2.0 mL of the color reagent. Mix the solution for 10 minutes. Meanwhile, set a spectrophotometer to 543 nm and blank (set to 0.00 absorbance) with seawater.

Measure the absorbances of your 0.1, 0.2, 0.5, and 1.0 ppm standard solutions (don't forget to blank each one!) Make a plot of absorbance versus the concentration (Beer's Law plot, $A = \varepsilon bc$). The slope of the plot should be the extinction coefficient.

2. Sample

Withdraw a sample of aquarium water. Treat as above. Use the extinction coefficient determined by the standard solutions to calculate the concentration of nitrate in the aquarium water. Report the concentration in ppm nitrite.

Nitrate Determination

Chemistry

Solutions containing nitrates absorb UV light at 220 nm. Thus, the concentration of nitrates in a water sample can be calculated by comparing the sample's absorption to that of a known nitrate concentration. However, dissolved organic materials can also absorb at 220 nm (thus skewing the nitrate value.) A correction can be calculated by using a second absorption value at 275 nm. At this wavelength, nitrates do not absorb, but dissolved organics do.

The nitrate levels in our aquarium can vary significantly, from 1 ppm to over 20 ppm. You may want to check the aquarium log or do an initial test with the nitrate "test kit" to determine the approximate nitrate level before constructing your calibration curve. For example, if the approximate nitrate concentration is 15 ppm, the calibration curve should be in the range of 5-25 ppm. If the nitrate level is around 5 ppm, a calibration curve of 1-10 ppm will give you more accurate results.

If the nitrate level in the tank is extremely high (more than 40 ppm) you may need to dilute the tank water sample to get an accurate analysis. Consult with an instructor about your strategy.

Reagents:

Stock nitrate solution: Potassium nitrate (KNO_3) has been dried in an oven at 105°C for 24 hours. It is stored in a small dessicator. Dissolve 0.0408 g in 250 mL of distilled water in a volumetric flask (use the analytical balance to mass out the potassium nitrate). This solution is 100 ppm NO_3^-. *Don't forget to return the KNO_3 to the dessicator when you are done!*

Seawater: Tare a large beaker on a balance. Add 50.0 g distilled water to the beaker. Add small portions of sea salt to the water until you reach the desired weight:
*50.0 g * specific gravity = desired weight of seawater. (for example, if the specific gravity of the tank is 1.020 that day, your desired weight is 50.0 * 1.020 = 51.0 g.)*
Mix well, use as your spectrometry blank. You can share your leftover seawater with the several other people in your lab section (NH_3, NO_2^-, PO_4^{3-}) who need a few mL for their analyses. Any unused seawater can be disposed of down the drain.

WASTE: All waste can go in aqueous waste. Put concentration of all ions and total volume placed in waste.

Procedure

1. Standards

Prepare nitrate calibration standards (using volumetric methods) (50 mL each) in the appropriate range. Use distilled water to dilute. Treat each standard with 1 mL of 1 M (or 1 N) HCl. Continue with spectometry as below.

2. Sample

Draw a water sample from the tank and filter to remove particulates. (if you need to dilute your aquarium water sample, use distilled water and do it here.) Pipet 50 mL into a beaker, add 1 mL of 1 M HCl. Mix well, transfer your sample to a square UV-Vis cuvette.

You will need to measure the absorbance at two different wavelengths (using UV-Vis in Dana 312). Set the spectrometer to 220 nm and blank with seawater. Measure the absorbance of your standards and the sample. (If the absorbance is about 1.0, re-blank and measure again to be sure you have a reliable number.) Now reset the spectrometer to 275 nm. Rezero with seawater. Measure the absorbance of your standards and the sample.

For the sample and standards, subtract two times the absorbance reading at 275 nm from the reading of 220 nm to obtain the absorption due to NO_3^-. Construct a standard curve by plotting the absorbance due to NO_3^- against the NO_3^- concentration of the standard. Using Beer's law, determine the NO_3^- concentration of the sample from the standard curve. Report the concentration of nitrate in the aquarium water in ppm.

Absorbance due to nitrate = A at 220 nm - 2 x A at 275 nm

In addition, consult instructor and be prepared to use the ion chromatograph to do nitrate analyses as well. Detailed instructions will be provided but a calibration curve with a blank will still be required.

II. Minerals

Minerals are critical trace elements for marine life. These minerals are insoluble ionic compounds containing many different cations and anions. They enter the seawater by the erosion and weathering of rocks, dirt, and shells. We will be talking about only four of these ions: phosphate, sulfate, calcium and magnesium.

Phosphorus is a very important element for the marine environment. Phosphorous (as phosphate) is used to make bones, teeth, and shells. The primary molecule that organisms use to store energy, ATP, is made of an adenosine molecule and three phosphate (PO_4^{3-}) groups (hence Adenosine TriPhosphate.) In deoxyribonucleic acid, DNA, the backbones of the double helix are alternating deoxyribose sugars and phosphate groups. Phosphate must be tightly controlled, however, because levels that are too high can cause uncontrolled algae growth (alga blooms). Very high levels of phosphate also interfere with shell growth of live corals and other invertebrates. Acceptable levels of phosphate are between 1.0 and 3.0 ppm.

Sulfates (SO_4^{2-}) are major parts of seawater and change concentrations with salinity. Sulfate helps to buffer seawater from abrupt changes in pH. Sulfate is also used as a source of biological sulfur compounds like thiols and some amino acids. Sulfur is also important in the active sites of many enzymes such as the nitrogen-fixing nitrogenase enzyme that contains a Mo_6S_8 core. However, some sulfur-containing compounds are toxic to the marine environment. In particular hydrogen sulfide (H_2S, characteristic smell of rotten eggs) is toxic even at low concentrations. A healthy aquarium (or seawater) typically has sulfate levels of about 2700 ppm sulfate, or 2.7 parts per thousand.

The cations Mg^{2+} and Ca^{2+} are present along with sulfate and phosphate. Most magnesium and calcium salts (calcium carbonate, magnesium phosphate, etc.) are only slightly soluble in water. The "sand" at the bottom of our aquarium is actually crushed coral, composed primarily of calcium carbonate. This provides a very low, but steady, concentration of calcium to the aquarium. Calcium is essential for invertebrate growth. Calcium concentrations of 400 - 450 ppm are desirable. Magnesium concentrations are usually around 1350 ppm (1.35 parts per thousand).

Several different methods will be used to determine the concentrations of sulfate, phosphate, calcium, and magnesium in the marine aquarium. A colorimetric approach will be used to determine the concentration of phosphates. In it, phosphates will react with ammonium molybdate and stannous chloride to form an intense blue color. Its absorbance will be measured on a spectrophotometer and compared to standard phosphate solutions. Sulfate will be determined gravimetrically by precipitating barium sulfate. This precipitate can then be dried and weighed to determine the amount of sulfates contained in seawater. Calcium and magnesium determinations are by titration with EDTA, a complexing agent.

Phosphate Determination

Chemistry

Phosphate and ammonium molybdate will react *under acidic conditions* to form phosphomolybdate:

$$PO_4^{3-} + (NH_4)_6Mo_7O_{24} \rightarrow [Mo_{12}O_{36}(PO_4)]^{3-} + 3\ NH_4^{+} \text{ (unbalanced)}$$

In phosphomolybdate, the molybdenum is present as Mo(VI). When it is reacted with stannous chloride ($SnCl_2$), some of the molybdenum atoms are reduced to Mo(V). This molecule has a distinct blue color. When its absorbance is measured with a spectrophotometer, it can be compared to absorbances of known phosphate concentrations and the sample's concentration can be calculated using Beer's law.

Reagents

Concentrated HCl (available in acid/base hood). Obtain 5-10 mL conc HCl in a beaker or indicator bottle. *Wear gloves when working with concentrated acids.*

Ammonium molybdate solution: dissolve 2.5 g of $(NH_4)_6Mo_7O_{24}$ in 17.5 mL distilled water. Meanwhile, dilute 28 mL of concentrated H_2SO_4 in 40 mL of distilled water. When the acid mixture has cooled down, combine it with the molybdate and dilute the entire solution to 100 mL with distilled water. *Use extreme caution when using concentrated H_2SO_4.* Store in a small bottle.

Stannous chloride solution: dissolve 0.50 g of $SnCl_2 \cdot 2H_2O$ in 20 mL of glycerol. Warm the glycerol on a hot plate to speed up the process. Store in a small bottle.

Stock phosphate solution: dissolve 46 mg K_2HPO_4 in a 500 mL volumetric flask with distilled water. *Use the analytical balance to mass out this small amount. Use your best technique to get an accurate mass.* This solution is 50 ppm PO_4^{3-}.

Seawater blank: obtain from coworkers, or see nitrate analysis (Set A) for procedure.

WASTE: All waste can be placed in aqueous waste. Calculate concentrations of ammonium molybdate, phophate, and tin chloride and include that information on waste disposal sheet along with total volume of material disposed.

Procedure

1. Standards

Prepare a series of standards (100 mL – use volumetric flasks) with concentrations between 0.5 and 5 ppm PO_4^{3-} by dilution of the stock solution. Mix well, then pour the entire volume into an Erlenmeyer. Using pH paper, adjust the pH with 0.1 M HCl (added dropwise) until the pH is slightly acidic (between 6.0 and 6.5). Add 4.0 mL of molybdate reagent and, after a thorough mixing, add 15-17 drops of the stannous chloride reagent. Start timing and mix well. Set the spectrophotometer at 690 nm and blank it (set to 0.00 absorbance) with seawater. *Exactly* ten minutes after adding the stannous chloride solution, measure the absorbance reading for the sample. Construct a calibration curve by plotting absorbance against the PO_4^{3-} concentration of the standards. It is very important that your timing be consistent for all of your standards and your sample.

2. Sample

Pipet 100 mL aquarium water into an Erlenmeyer flask. Adjust the pH with HCl (you may find that 1 M HCl is more efficient than 0.1 M for the aquarium water). The aquarium water sample may require more acid than the standards because the aquarium water is very well buffered. Continue with addition of reagents and spectrometry as above. During weeks two and three of your rotation, repeat one of the standards as well as your aquarium water sample. Using Beer's law, determine the PO_4^{3-} concentration of the sample from the standard curve.

Sulfate Determination

Chemistry

In the presence of hydrochloric acid (HCl) and barium chloride ($BaCl_2$), sulfate (SO_4^{2-}) precipitates to form barium sulfate ($BaSO_4$):

$$SO_4^{2-}(aq) + Ba^{2+}(aq) \rightarrow BaSO_4(s)$$

This process is carried out near the boiling point of water and "digested" by heating to create large particles of $BaSO_4$. The solution is filtered and the precipitate is rinsed with water to rid the precipitate of chloride ions. (Chloride has the tendency to react with barium and reform $BaCl_2$. Therefore residual chloride must be removed from the precipitate to give an accurate determination of sulfate in the seawater. Silver ions are used to test for chloride ions in the filtrate.) Once all of the chloride ions are flushed out, the precipitate is dried and weighed and used to calculate the amount of sulfate in the water.

Reagents

Barium chloride solution: dissolve 2 g of $BaCl_2 \cdot 2H_2O$ in 20 mL of distilled water. Filter through filter paper before use. Store in a small bottle.

Silver nitrate-nitric acid solution: dissolve 0.85 g $AgNO_3$ and 0.5 mL of concentrated HNO_3 in 50 mL of distilled water. *Wear gloves when working with any silver solution. The silver ion is photoactive. Any silver that gets on your hands will turn brown when exposed to sunlight. This is harmless, but unsightly.*

Procedure

Draw 20 mL of water from the aquarium and dilute to 250 mL with distilled water. *Caution: you may find that it is not a good idea to dilute the sea water - try it both ways and see which works best.* Adjust the pH to 4.5-5 with 0.5 M HCl and then add an additional 2 mL of 0.5 M HCl. Heat this

solution to just below boiling while warming the barium chloride solution. While stirring the sample on the hotplate, add (dropwise) warm barium chloride solution to your warm sample. A white precipitate of $BaSO_4$ will form. Add barium chloride until no additional precipitate forms, then add 2 mL more. Rinse the walls of the beaker with distilled water and cover with a watch glass.

Warm on a hot plate for 30 minutes. You don't want the solution to boil, so keep the hot plate set on low and remove the solution from the hot plate if it gets too hot. After 30 min, remove from heat and let the precipitate settle. (During this time you can mass the fritted funnel and warm some distilled water.)

Collect the precipitate in a fritted glass funnel using vacuum filtration. Wash the precipitate with several small portions (5-10 mL each) of hot distilled water. Stop the vacuum and empty your filter flask into the Ba/Ag waste. Reassemble your filtration setup with a clean filter flask.

We use the simple precipitation reaction shown below to test for chloride. When silver ions are added to a solution with chloride ions, silver chloride immediately forms as a white cloud.

$$Ag^+ (aq) + Cl^- (aq) \rightarrow AgCl (s)$$

Use a clean filter flask to collect the filtrate. A few mL of filtrate are transferred to a small test tube with a Pasteur pipet and tested by adding 1-3 drops of silver nitrate. If the solution is cloudy, chloride is still present. The precipitate should be washed 2-3 times with small amounts of warm distilled water. The filter flask is then rinsed out and the operation repeated. If the solution does not turn cloudy, you can proceed with the drying operation.

Place the fritted funnel containing your sample in a small beaker, label it with your name and lab section, and place it in the oven to dry (at least overnight). *Dispose of your aqueous waste carefully in the designated Ba/Ag waster container.*

Let the dried sample cool, then mass the funnel + barium sulfate. The mass of barium sulfate can be related to the concentration of sulfate in the original water sample through simple stoichiometry calculations.

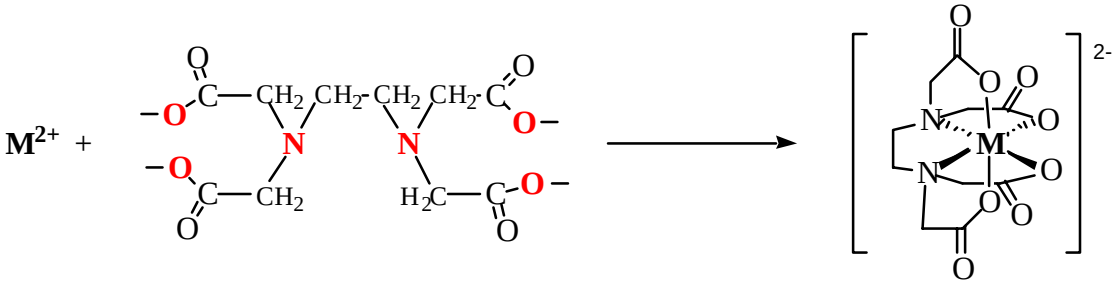
To clean the fritted funnel, use a small spatula or rubber policeman to *gently* scrape the white precipitate out into a waste beaker. The fritted glass is fragile, so be careful! Then use distilled water to rinse any remaining solid into the beaker as well. *Dispose of the solid barium sulfate and any washings in the Ba/Ag waste jar.* Return the funnel to the stockroom.

Calcium and Magnesium Determinations

Chemistry

Water hardness is caused by dissolved cations, notably Ca^{2+} and Mg^{2+}. Most analyses for water hardness assume that all of the cations are calcium. In the case of seawater, most of the hardness is actually magnesium. So we'll have to do two titrations to determine both calcium and magnesium in our aquarium water.

Your titrant for these analyses is EDTA (ethylenediaminetetraacetate). This remarkable ligand uses



EDTA
(atoms that bind to the metal are shown in red)

M^{2+}-EDTA complex
(carbons not shown for clarity)

the two nitrogens and four oxygens to wrap around metal ions. (This process is so effective that "chelate therapy" is often used for metal overdoses and metal poisoning.)

Editing note: atoms that bind have asterisks next to them – they are not red as the manual suggests.

EDTA chelates both Mg^{2+} and Ca^{2+}, as well as many other metals. In the first titration below, the solution is buffered to keep both the magnesium and calcium ions in solution. You get the *total hardness* (Ca + Mg) from this titration. For the second titration, the solution is made strongly basic. Under these conditions, all the magnesium ions will react with hydroxide and form a white precipitate of $Mg(OH)_2$.

$$Mg^{2+}(aq) + 2OH^-(aq) \rightarrow Mg(OH)_2(s)$$

The calcium remains in solution and is titrated as before. A slightly different indicator is used. The "Calcon" indicator is specific for the Ca^{2+}-EDTA complex.

Reagents

0.0100 M EDTA solution: place 0.931 g of reagent grade EDTA in a 250 mL volumetric flask. Add about 200 mL distilled water and 2-3 mL 6 M NaOH. Shake until all the solid is dissolved, then add more distilled water to the 250.00 mL mark. Store in PET bottles, good for about a month. 1 mL = 400.8 μg Ca.

pH 10 ammoniacal buffer: *Working in the hood,* dissolve 1.69 g NH_4Cl in 14.3 mL conc. NH_4OH. Dilute to 25.0 mL with distilled water. Store in a tightly capped bottle.

NaOH, 2 M: already prepared

0.1% Eriochrome Black T indicator: This will need to be prepared by Thursday am group for use through out the three week rotation. It must be made every three weeks. Dissolve 0.05 g of Erichrome Black T in 0.5 mL of ammonical buffer and dilute to 50 mL with distilled water (use volumetric glassware)

0.1% Calcon indicator : This will need to be prepared by Thursday am group for use through out the three week rotation. It must be made every three weeks. Dissolve 0.05 g of the calcon indicator in 50 mL of methanol. This material must be disposed of in organic waste.

Procedure

1. Total Hardness (Ca + Mg) titration

Obtain a water sample from the aquarium. Pipet 2.00 mL seawater into a 250 mL Erlenmeyer and add approximately 50 mL of distilled water. Add 2 mL of ammoniacal buffer. Add Eriochrome Black T solution indicator until the solution is a light pink (3-5 drops). Titrate with the EDTA solution until the solution changes from violet to sky blue (You may need to practice this endpoint). Record the volume used. Repeat two to three times to get at least two consistent readings. Calculate the "total hardness" by multiplying the volume EDTA x 400.8 μg Ca/mL EDTA. Divide by the volume of your water sample to get "total hardness as Ca" in ppm.

2. Calcium-only titration

Pipet a 10.00 mL sample into a 250 mL Erlenmeyer and add 50 mL distilled water. While stirring, add NaOH (5 mL). Add 3-5 drops Calcon indicator until the solution is light pink (3-5 drops). Titrate the solution with EDTA until clear and record the volume. Repeat two to three times to get at least two consistent readings.

Calculate the "Ca" by multiplying the volume EDTA x 400.8 μg Ca/mL EDTA. Divide by the 10.00 mL of your water sample to get Ca in ppm.

Calculate the Mg by subtracting the Ca in ppm from total hardness in ppm and multiply by the ratio of atomic masses to determine the concentration of Mg present.

ppm Mg = (ppm total hardness as Ca – ppm Ca) • (24.31 g Mg/40.08 g Ca)

III. Salinity, Alkalinity, Dissolved Oxygen concentration

Dissolved Oxygen Determination

Reactions between oxygen and biotic and abiotic chemicals have large negative Gibb's Free energies (ΔG's). It is hard to chart one all-telling cycle for this essential element. Oxygen represents 21% of the gases in the atmosphere but in the marine environment, its concentration is only 4-8 ppm. Like nitrogen, oxygen can be exchanged between the atmosphere and the seawater at the surface. As you may have guessed, oxygen is essential for many biological processes. Aerobic organisms (like fish, snails and algae) all need a supply of oxygen for respiration, metabolism, and oxidation; thus oxygen concentration can effect the marine environment dramatically. Oxygen concentration depends on many factors; including the salinity of the water, its temperature and its depth.

Chemistry

The iodometric determination of dissolved oxygen depends on a color change during a titration. First, a manganese solution is added to the water. The dissolved oxygen (DO) quickly oxidizes Mn^{2+} to Mn^{3+}.

$$4\ Mn(OH)_2 + O_2 + 2\ H_2O \rightarrow 4\ Mn(OH)_3$$

Next, iodide (I^-) is added and the solution is made acidic. Mn(III) is reduced to Mn(II) while iodide is oxidized to iodine (I_2).

$$2\ Mn(OH)_3 + 2\ I^- + 6\ H^+ \rightarrow 2\ Mn^{2+} + I_2 + 6\ H_2O$$

The iodine combines with starch to form a dark blue complex. This complex is then titrated with sodium thiosulfate. The thiosulfate converts the iodine back into iodide. Thus, the starch-iodine complex breaks down and the solution turns clear.

$$\text{Starch-}I_2 + 2\ S_2O_3^{2-} \rightarrow 2\ I^- + S_4O_6^{2-}$$

(blue color) (colorless)

The point at which the solution turns from dark blue to clear tells you how much thiosulfate is used. The volume of thiosulfate is then used to calculate the DO concentration in the water. You will use the stoichiometry of each of the three reactions above to calculate the DO.

Reagents (Use volumetric glassware)

$MnSO_4$ solution: make a 2.15 M $MnSO_4 \cdot H_2O$ solution in 10 mL of distilled water. Filter to rid solution of particulates. Store in a small bottle.

Alkali-iodide-Azide solution: dissolve 5 g of NaOH and 1.3 g NaI in 5 mL of distilled water. Add 0.1 g of NaN_3 and dilute to 10 mL. Store in a small bottle.

Starch indicator: prepared for you.

Standard sodium thiosulfate solution: dissolve 1.55 g of $Na_2S_2O_3 \cdot 5\ H_2O$ in distilled water; add 0.3 mL of 6 M NaOH and dilute to 250 mL in a volumetric flask. Store in a tightly capped bottle.

Waste: All waste can be disposed of in aqueous waste. Calculate concentration of Mn^{2+}, SO_4^{2-}, Na^+, I^-, S_2O_3 and include total volume of all solutions disposed of.

Procedure

In this lab, we will be using special dissolved oxygen bottles. These bottles are designed to minimize the amount of atmospheric oxygen introduced into a water sample. The bottles can be checked out of the chemistry stockroom. When you are adding reagents, use a pipet and make sure the pipet tip is below the surface of the water so no air bubbles are introduced. (Check out several 1 mL pipets so you'll have a clean pipet for each reagent.) The bottle will overflow, so you will want to have a large beaker or dish around the DO bottle to catch the drips. Minimize the time the solution is exposed to the atmosphere by planning ahead and having all of your reagents ready to add to the bottle.

To obtain a sample of aquarium water, start a siphon and place the tube in the dissolved oxygen bottle. Let the bottle fill and overflow for a minute. With the bottle absolutely full, place the ground glass stopper in the bottle. The bottle should then have a bead of water sealing the sample water from the atmosphere.

Back at your bench, add 1 mL of the $MnSO_4$ solution and 1 mL of alkali-iodide-azide solution. Stopper tightly and mix by inverting the bottle several times. Let the bottle sit for 2-3 minutes, then add 1.0 mL of concentrated sulfuric acid. Restopper the flask and invert many times.

With a large pipet, transfer 200 mL from the dissolved oxygen bottle into a 500 mL Erlenmeyer flask. Add a stir bar and stir slowly with a mechanical stirrer. Add starch solution until the solution turns a deep blue (typically, 3-4 drops). Fill a buret with the thiosulfate solution and carefully read the initial volume. Add thiosulfate solution dropwise until the sample turns clear. Record the amount of thiosulfate used.

Alkalinity Determination

Alkalinity is determined as the amount of acid required to neutralize all of the base in one liter of water. The most important bases in seawater are carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) ions. Alkalinity is a measure of the water's ability to buffer itself from the harmful effects of acids in the water. The carbonate and bicarbonate ions in the water act as "banks" which can take up excess acids (CO_2 from respiration, organic acids from metabolism and decay) and maintains the pH at about 8.5 for seawater.

Chemistry

For this module, potentiometric titration will be used. In it, an acid will be titrated into a sample of the aquarium water. As long as appreciable amounts of carbonate and bicarbonate are present, the pH

will remain around 7.5 to 8.5. When the carbonate and bicarbonate ions are "used up" the pH will change quickly and dramatically. The end point of the titration is reached when the pH reaches 4.0. Thus, the amount of acid used in the titration can be used to calculate the alkalinity of the water. (We are going to assume that all of the "alkalinity" (CO_3^{2-}, HCO_3^-, OH^-, $H_2PO_4^{--}$, etc.) is actually present just as CO_3^{2-}. By convention, the total alkalinity is reported as ppm $CaCO_3$)

$$CO_3^{2-}\ (aq) + 2\ H^+\ (aq) + \rightarrow\ [H_2CO_3]\ (aq) \rightarrow H_2O + CO_2\ (aq)$$

Reagents

Standard hydrochloric acid solution (0.1 M HCl). To make it, dilute 8.3 mL (measure with a pipet) of concentrated HCl to 1.00 L (volumetric flask) with distilled water.

Procedure

First, set the pH meter to 7.00 (neutral) with a standard pH 7 buffer. Draw off a sample of aquarium water, and carefully pipet 200 mL into a large Erlenmeyer flask. Record its pH. Fill a buret with the standard acid solution and titrate, with the electrode in the water, until the pH reaches 4. Record the volume titrant added and the pH for several data points during the titration. Record the amount of acid used. Use the stoichiometry of the titration to determine the total alkalinity (expressed as ppm $CaCO_3$) in the aquarium water. Repeat to get 2-3 consistent readings.

Salinity/Chloride Determination

Salinity refers to the amount of dissolved solids in water. In seawater, about 98-99% of these solids are salts. Of the anions, chloride makes up the highest percentage, 55.04%, an approximate concentration of 19000 ppm, 19.0 parts per thousand. The salinity of the sea is essentially constant due to the continual leaching of salts from rocks and soil. Changes in salinity of the sea can stress organisms; thus, a stable salinity is needed for ocean organisms. To determine the concentration of chloride, we will do a titration with a Ag^+ solution.

Chemistry

By titrating with a silver nitrate solution ($AgNO_3$), chloride will precipitate from the solution as silver chloride:

$$Ag^+\ (aq) + Cl^-\ (aq) \rightarrow AgCl\ (s)$$

white solid

Once all of the chloride has precipitated, the silver nitrate will start to react with the potassium chromate and form the orange precipitate silver chromate:

$$2\ Ag^+\ (aq) + CrO_4^{2-}\ (aq) \rightarrow Ag_2CrO_4\ (s)$$

red solid

The silver chromate (orange) will only persist when all of the silver chloride has been precipitated. Thus, we will know when the endpoint of the titration occurs because the solution will turn red, indicating that all of the chloride is out of solution. With the volume of silver nitrate used to precipitate all of the chloride, one can determine the concentration of chloride (salinity) of the original water.

Reagents

Potassium chromate indicator solution: dissolve 0.5 g K_2CrO_4 in 10 mL of distilled water. Store in an indicator bottle.

Silver nitrate titrant: dissolve 0.24 g of $AgNO_3$ in 100 mL of distilled water (use the analytical balance and a volumetric flask). Store in a brown bottle to prevent photoreduction. This solution is 500 ppm Ag^+, 0.0141 M Ag^+

Procedure

Draw off a sample of aquarium water. Pipet a small sample (between 1.00 and 2.00 mL, you can see what volume works best for you) into a 125 mL Erlenmeyer flask. Add 10 mL distilled water. Add 1.0 mL (20 drops) of potassium chromate indicator to the water. Place on a magnetic stirrer and stir slowly. Fill a 50 mL buret with the silver nitrate solution. Titrate with the silver nitrate (dropwise) until an orangish-red endpoint is observed. The initial solution will be yellow; the end point is orange. Record the amount of silver nitrate used. Repeat the titration to get at least two runs that give consistent results (try to be consistent about the color of the solution at the endpoint). Report your result in ppm chloride. *Dispose of your waste in the barium/silver waste bottle.*

IV. Tank Monitoring and Maintenance

Between the three lab sections, several students are charged with monitoring the tank once a day M-F, around 3 P.M. You will want to sign up for the monitoring dates that are most convenient for you. You can add more if you like! Each day, you should check the temperature, pH and specific gravity of the tank. Wipe down the front window of the tank with a damp paper towel to clean up any salt water spills. Also note any important observations (fish/invertebrate behavior, water cloudiness etc.). Lorna usually feeds the fish about 3, so this is a good time to count noses and make sure all the fish and invertebrates are doing well. We also have some test kits if you want to use these imprecise tools for a "quick and dirty" determination of ammonia, nitrite, nitrate, and calcium.

SHOW TANK MAINTENANCE

DAILY

1. Check water levels in show tank (about 1 cm from top) and sump (the water level in the sump should be between the indicated marks. *If the level is below the lower mark ADJUST by using the directions for "water replacement."*).

2. Assure that water is flowing from the tank to the pump by observing that water trickles through the blue spheres in the sump (in the left lower chamber).
3. Check appearance of vertebrates and invertebrates. Notify instructor or A.I. if anything is dead or if fish have any spots on them.
4. Brush the scum off of the top of the canister of protein skimmer with medium white brush. Rinse brush.
5. Feed sparingly once a day in the morning. The fish should eat all of the flake food within one minute. Periodically change the food: use 3 large green flakes directly into tank, or 1/4 cube of frozen food (suspend and thaw frozen food in a small beaker with about 20 mL tank water and dissolve into chunks. Pour into tank. Rinse beaker well with hot water.)
6. Measure and record temperature (74-78^{o}F), SPGR(1.022-1.025), and pH(8.0-8.5). Rinse SPGR meter with distilled water, and the beaker for pH with hot water. Include Name, date, and time with your records.
7. Make up Calcium Hydroxide solution for next day:
    g. Fill 4-L graduated cylinder to the 4-L mark with distilled water.
    h. Place cylinder with stir bar on stirrer, adjust to medium stir.
    i. Add 1/2 Tsp. Calcium Hydroxide, cover with watch glass, stir for 3 hours.
    j. Turn off stirrer after 3 hours and let sit overnight
    k. Next day, use the filter funnels with paper to filter the solution, then add to right-hand chamber of sump being careful not to disturb sediment in sump.
    l. Prepare so that solution is added on Mon-Wed-Fri basis in the mornings before the lights come on.

WEEKLY

1. Feed the anemone once per week. Use a tiny piece of frozen haddock (about the size of a pea). Suspend in a small beaker with 5 mL of tank water, thaw, and mash by continual aspiration and expelling in a 10 mL syringe. With the syringe, gently dispense the mixture onto the anemone's tentacles. Rinse syringe and beaker well with hot water.
2. **Ten Gallon Water Change and Cleaning.** Usually Wednesday. Push light box to rear and fasten with chains. Return light box when through.

Cleaning Sides:

1. Wash hands and arms with soap and lots of water to remove any lotions from skin prior to entering the tank. Be aware that the damsels are aggressive and will peck you. Be aware that the invertebrate specimens on the live rock are delicate, please be careful and do not disturb them when cleaning the tank.
2. Wipe all sides with soft sponge. Disturb a bit of sand around the edges.
3. Scrub sides with Dobie sponge (use only approved sponge for this). Be aware that when scrubbing the tank you should not be scratching the surface of the tank.
4. Remove coralline algae (red spots) with credit card.

Vacuuming Sand:

1. With vacuum device, create siphon and vacuum sand by drawing up then releasing. Be careful to avoid displacing snails and upright them if necessary. Snails cannot upright themselves and will be eaten by hermit crabs if left upside down.

2. Siphon water into 5 gallon white bucket. After about 2 gallons are removed, the pump will make a straining sound. Unplug the pump at this point. Water level in sump should rise, but not flood over sides.
3. Remove 10 gallons of water total. Discard water and rinse buckets thoroughly. Clean out vacuum device as well.

Cleaning Filter Accessories and Skimmer Canister:

1. Remove U siphon tube and scrub with white brush.
2. Remove Filter (sponge). Clean both top and bottom and rinse with distilled water. Replace. **CAUTION:** Be aware that macroalgae might be in the clear box. These are here so that they can grow, protected from the fish, and later be added, in small amounts, to the tank as food for the fish. If the algae are present, move them to the black box(just inside of the tank) temporarily, so that they do not get sucked down the tube into the pump. Be sure to place the algae back into the clear box, AFTER you replace the filter sponge.
3. Unplug skimmer. Remove top of skimmer (canister). Empty waste and scrub inside of canister and top of canister with white brush. Replace. Plug in skimmer. Assure that skimmer pump comes back on (foam bubbles should form in the inner tube to the canister).
4. Clean both sides of filter containers with brush, credit card, etc.

Water Replacement:

1. Add one and one half beaker full of salt to a five gallon white bucket.
2. Add DISTILLED WATER until nearly full.
3. Mix in salt with cupped hand and arm.
4. Check SPGR and adjust accordingly (about 1.023 is appropriate).
5. Repeat steps 1-4 for a ten-gallon water change.
6. Add water to tank slowly, being careful not to disturb sand and invertebrates. Save about a gallon of water incase level must be adjusted (below).
7. Replace siphon as follows:
    a. Fill both filter boxes with tank water.
    b. Fill siphon U tube with tank water and loosely cork both ends with stoppers.
    c. Invert U tube so that both boxes are connected.
    d. Carefully remove stoppers. Push black box down on tank side to assure continuous water flow.
    e. Plug in pump. Water level in sump should begin to drop. If not, SLOWLY raise the black box making sure there is enough water in the box to keep siphon going. See “adjusting water levels” below for more details.
8. Add One Pasteur Pipet squirt of iodine to the clear filter box (left side of tank).
9. Wipe down outside of tank with cloth.

Adjusting Water Levels:

1. The water level of the tank should be about 1 cm from the top. The water level in the sump should be between the indicated marks.

2. Remember that pushing the black box DOWN in the tank, makes the water level in the sump go UP. If there is now more water in the sump, there is less water in the tank, so the tank level decreases.
3. Raising the black box makes the water level in the sump go down, thereby increasing the level of water in the tank.
4. Whenever you reposition the black box, do it in small increments so that you always maintain siphon and that you do not overfill the sump.
5. Final adjustment of the tank level is determined by the sump level. Add water to the show tank until water in the sump is at the "high" black mark, adjustment is gradual as water needs to trickle through the filter.

3. Automatic Feeder:

1. Set this up on Friday afternoons, or for holiday coverage.

General Technical Comments

Units of Concentration

Molarity (moles per liter) is commonly used for a unit of concentration. But molarity is not that useful for the low concentrations of ions present in seawater. A more useful concentration unit is parts per million (ppm).

Parts per million is a weight/weight ratio. A concentration of 1ppm is 1 g/1000000 g or 1 mg/1000 g. A convenient approximation is mg solute/1 L solution. This is a good approximation as long as the density of the solution is very close to the density of pure water.

Conversions use the molar mass of the molecule or ion. For example, a solution that is 0.00046 M acetic acid (or 0.46 mM) can be converted to ppm using the following strategy:

$$\frac{0.00046 \text{ mol}}{1 \text{ L solution}} \times \frac{60.05 \text{ g}}{\text{mol}} \times \frac{1000 \text{ mg}}{\text{g}} = \frac{27.6 \text{ mg}}{\text{L solution}} = 27.6 \text{ ppm acetic acid}$$

Occasionally you might need to consider the formula of a salt. For example, what is the ppm NO_3^- in 0.0012 M $Ca(NO_3)_2$?

$$\frac{0.0012 \text{ mole } Ca(NO_3)_2}{\text{L solution}} \times \frac{2 \text{ mol } NO_3^-}{\text{mol } Ca(NO_3)_2} \times \frac{62.01 \text{ g}}{\text{mol } NO_3^-} \times \frac{1000 \text{ mg}}{\text{g}} = \frac{149 \text{ mg } NO_3^-}{\text{L}} = 149 \text{ ppm nitrate}$$

Dilutions

To calculate dilutions, use the following

$$\text{dilution formula : } c_1V_1 = c_2V_2, \text{ or } c_2 = \frac{c_1V_1}{V_2}$$

For example, what is the concentration of a solution made by diluting 5.0 mL of 87.3 ppm Cl^- to a total volume of 25 mL?

$$c_2 = \frac{87.3 \text{ ppm} \times 5.00 \text{ mL}}{25.0 \text{ mL}} = 17.5 \text{ ppm } Cl^-$$

Colorimetric Techniques (Beer's Law Plots)

Several of these analyses use a colorimetric analysis. Often these involve making a colored derivative and using a spectrometer (like a Spec-20) to measure the intensity of the color. Most compounds obey Beer's Law:

$$A = \varepsilon bc$$

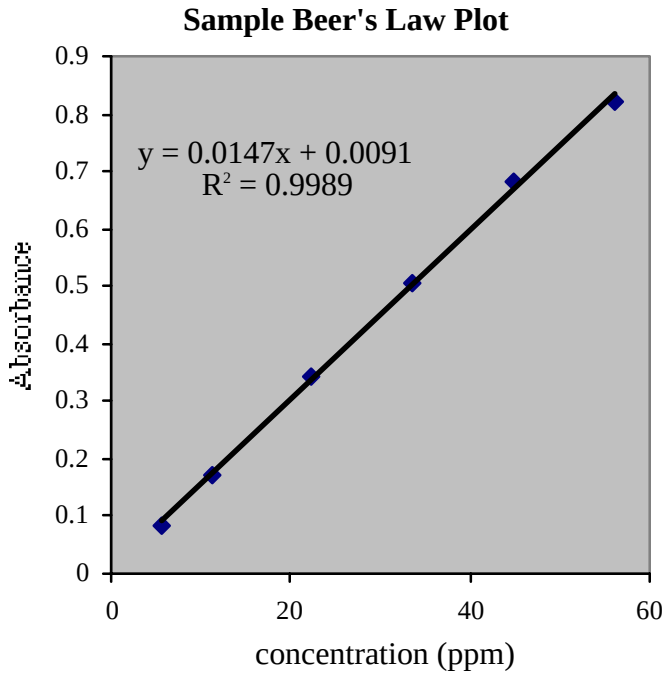
where A is the absorbance, b is the path length (cm) and ε (the Greek letter epsilon) is the extinction coefficient (in $ppm^{-1}cm^{-1}$). The concentration (c) will be in units of ppm. Simply put, since b and ε are constants, the intensity of the color (absorbance) is proportional to the concentration of the analyte.

To determine the value of the constant ε, you need to prepare a set of standards. These carefully prepared standards have known concentrations. Each of these standards is treated exactly the same way you treat the aquarium water sample. You may need to add reagents, adjust the pH, or let the solution stir. Set the spectrometer to the wavelength stated. Blank the spectrometer with a sample of seawater, then measure the absorbance of the sample. (absorbances between 0.1 and 1.0 are considered most reliable) The graph of absorbance vs. concentration is called a Beer's Law plot. The slope of this plot is εb. The path length, b, is usually 1.00 cm. Consider this example.

A stock solution was prepared to be 280 ppm. The standard solutions were made at concentrations from 5-50 ppm. Using a volumetric pipet, the desired amount of stock is transferred to a volumetric flask. The solution is then diluted to the mark of the volumetric flask.

#	Preparation	ppm	Abs
1	1.00 mL stock, diluted to 50 mL	5.6	0.085
2	2.00 mL stock, diluted to 50 mL	11.2	0.172

3	4.00 mL stock, diluted to 50 mL	22.4	0.345
4	6.00 mL stock, diluted to 50 mL	33.6	0.507
5	8.00 mL stock, diluted to 50 mL	44.8	0.682
6	10.00 mL stock, diluted to 50 mL	56.0	0.820



Note that the linear regression line is a good fit to the points, and that the intercept is near zero. The slope of the line (0.0147 ppm^{-1}) equals εb. With b = 1 cm^{-1}, ε = 0.0147 ppm^{-1} cm^{-1}.

Use the standard Beer's Law plot to determine the concentration for any absorbance in this range. So if the absorbance of the seawater sample is 0.426, the calculated concentration is c = 0.426/(0.0147 ppm^{-1} cm^{-1}•1 cm) + 0.0091ppm = 29.0 ppm.

Titrations

Titrations are a general technique for determining the concentration of a sample. Success with a titration depends on meticulous technique and care. Keep in mind that even the best lab results need to be interpreted correctly. You may find it easiest to do a quick titration just to get a rough estimate of how the titration will proceed.

Preparing the buret

- Checkout a clean buret. (Burets are expensive. Handle them with care.)
- Check to make sure the stopcock is seated tightly. Close the stopcock.
- Rinse the walls of the buret with a small amount of the titrant. Discard this in a waste beaker.
- Place the buret into a sable buret clamp. You may need to add a paper shim so the clamp holds the buret tightly.

- Using a funnel, fill up the buret with the titrant. Open the stopcock and let several milliliters of the titrant drain out of the buret into a waste beaker. Examine the tip carefully to make sure that any bubbles have been expelled.
- Refill the buret, if necessary. The initial volume should be somewhere near 0.00, but it doesn't have to be exactly on zero. Write the initial volume in your lab notebook.

Preparing the Analyte Solution (Unknown)

- Pipet a known amount of your analyte solution into an Erlenmeyer flask. The flask must be rigorously clean. As a general rule, choose an Erlenmeyer flask that will be no more than half-full *after* the titration.
- Add a stir bar (if you are using a mechanical stir plate). A piece of white paper under the flask may help you see a faint color change at the end point.
- Add the indicator and begin stirring slowly on the stir plate. You don't want to spatter solution outside the flask.
- Place the analyte flask under the buret tip so the drops of titrant fall directly into the solution.

Titrating

- Double check the initial volume. Make sure this is written in your lab notebook.
- Begin adding the titrant. It helps if you know approximately the volume required to reach the endpoint (See below). If you estimate your required volume to be 20 mL, you can add the titrant quite quickly at first, then slow down as the estimated volume is approached. In this example, I'd add the first 15 mL quickly, then next 3-4 mL several drops at a time, and the last 1-2 mL dropwise.
- As you near the endpoint, rinse the sides of the flask with a small amount of distilled water. This should wash down any titrant or analyte that may have splashed up.
- With many indicators, there will be a color change near where the titrant drops are entering the solution. This usually indicates that the endpoint is approaching. As you get very close, you may see a color change that then fades. Your endpoint occurs when the color change is permanent (lasts for at least a minute before fading).
- If in doubt about an endpoint, take a volume reading. Write it in your notebook. Then add another drop. Decide which is the better endpoint, then write your final volume in your notebook.

Strategy

- Usually you want three to four good titrations in an analysis. Calculate the result for each run individually, then average the results.
- As mentioned above, your titrations will go faster if you know approximately the volume required. It often helps to do a "quick and dirty" run as your first titration. No attempt is made to reach a perfect endpoint. You just want a rough idea of the required volume. Label this run in your notebook and don't include it in your averages.
- See the examples at the end of the manual for more help with titrations.

Waste Handling

Bates College Department of Chemistry has recently implemented new waste handling policies designed to bring us into full compliance with all EPA and OSHA guidelines. It is essential that these policies are followed. If you have any questions about them, please ask.

All hazardous waste must be stored in appropriate, sealed containers. Each container must be labeled with the words "Hazardous waste" and must be stored in a secondary waste containment vessel. Because much of the hazardous wastes we generate are volatile, waste accumulation areas are located in the fume hoods in each research and teaching lab. Generally, hazardous waste is separated into three types. These are: organic halogenated waste (any organic waste with any halogenated solvent. If you generate waste that has both methylene chloride and hexanes, for example, it must be placed in this container); organic non-halogenated waste; aqueous waste. Incompatible wastes such as strong acids and bases or strong oxidizing and reducing agents may not be stored together. Please ask if you have any questions about whether two wastes are incompatible or not. A record must be kept of everything that is placed in the hazardous waste container. These logs must be quite specific as to what is being placed in the container. Simply saying "aqueous waste" is not sufficient.

Here are a couple of common violations to be aware of. Liquid wastes must be kept capped. The fines for failure to cap wastes are quite high so remember to put the top back on the waste container after you use it. Liquid wastes need to be in the primary waste container and not spilled into the secondary container. Records need to be well maintained. Secondary containment needs to be in place at all times. Acetone is considered a hazardous waste and must be disposed of in hazardous waste containers.

Use of Adjustable Pipettes (guidelines modified from Professor Lawson, CHEM 321 lab manual)

Adjustable pipets are ideal for repetitively pipetting small volumes. They are generally less accurate than a high quality glass syringe, but they deliver small volumes with great reliability (if they are properly handled and maintained).

On the surface there may not appear to be any tricks for successful pipetting. In this laboratory, three commonly employed sizes, as determined by volume range, are available to you: 0 to 20 microliter (P-20), 20 to 200 microliter (P-200), and 200 to 1000 microliter (P-1000). These pipettes are used with disposable plastic tips. Each pipette should only be used to pipette the volumes that exist in the range for which they are calibrated. The volume to be pipetted is set by adjusting the dial in the handle of each pipette. To insure reproducibility, the desired volume setting should be approached by dialing down to the setting rather than dialing up. The last number in the P-20 setting is red and represents tenths of a microliter. The last number in the P-200 setting represents single microliter. The last number in the P-1000 setting represents ten microliter units. The first number in the P-1000 setting is red and represents milliliter units. The plunger of each adjustable pipette is thumb operated and has two positions that can be felt as the plunger is depressed. The first position is used to draw the set volume of liquid into the pipette tip. Depressing the plunger all the way to the second position forces the liquid out of the tip with a slug of air. The used pipette tip can be ejected with the ejector button, which is also present in the handle.

The procedure for accurate pipetting is as follows. A tip is placed on the pipette by inserting the end of the pipette firmly into the tip. The plunger is then depressed to the first position, and the

tip is inserted into the liquid to be transferred. The plunger is then released with a smooth, even motion to draw the liquid up into the tip. For non-viscous aqueous solutions or solutions which do not contain protein, lipids, or detergents, the liquid should be forced back out of the tip (back into the stock liquid) by depressing the plunger to the second position while holding the tip against the wall of the container. This procedure wets the inside of the tip. (Note: viscous solutions or solutions containing the molecules mentioned above should <u>not</u> be pipetted with a wetted pipette tip.) The plunger is then again depressed to the first position, the tip inserted into the liquid, and the solution is drawn back into the tip. The tip is then placed in the tube or container into which the liquid is to be transferred. It is best to place the tip near the bottom of the container, against a wall. The contents of the tip can then be smoothly ejected down the wall of the container. The used tip should then be disposed of. A fresh tip should be used for each pipetting operation. This prevents cross-contamination of the stock solutions and the individual sample solutions. The only time it is allowable to reuse a tip is when the same aqueous, non-viscous solution is being transferred to clean, dry tubes or containers. This may seem wasteful, but it is actually much more economical than having to repeat entire experiments because of cross-contamination.

Solving Quadratic (and Cubic) Equations

In the next several weeks, we will often have to solve mathematical problems like this

$$\frac{4x^2}{0.20 - x} = 3.6 \bullet 10^{-8}$$

There are two major strategies for solving problems like this.

m. Use the quadratic formula

n. Use the method of successive approximations

Quadratic Formula-this method is exact and mathematically rigorous. The algebra is straightforward, although it is easy to make a careless mistake. Always check your answers when you are done! First you rearrange your problem so that it is in the form

$$ax^2 + bx + c = 0$$

Then you apply the quadratic formula

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

Notice that the quadratic formula will always give you two "roots"—two values of x. In our chemistry problems, one of these roots will be clearly spurious and not make any sense in the context of the problem. For example, you can't have a negative concentration, or a concentration of 20000 M! Let's solve the problem above using the quadratic formula.

$$\frac{4x^2}{0.20 - x} = 3.6 \bullet 10^{-8}$$

$$4x^2 = 3.6 \bullet 10^{-8}(0.20 - x)$$

$$4x^2 = 7.2 \bullet 10^{-9} - 3.6 \bullet 10^{-8}x$$

$$4x^2 + 3.6 \bullet 10^{-8}x - 7.2 \bullet 10^{-9} = 0 \qquad a = 4, b = 3.6 \bullet 10^{-8}, c = 7.2 \bullet 10^{-9}$$

$$x = \frac{-3.6 \bullet 10^{-8} \pm \sqrt{\left(3.6 \bullet 10^{-8}\right)^2 - 4(4)\left(7.2 \bullet 10^{-9}\right)}}{2(4)}$$

$$x = \frac{-3.6 \bullet 10^{-8} \pm 3.39 \bullet 10^{-4}}{8}$$

$$x = 4.24 \bullet 10^{-5} \text{ or } \quad x = -4.24 \bullet 10^{-5}$$

And check the results to make sure they are correct.

Approximation- this method is not exact, but it is often "good enough" for chemistry problems. We will usually be assuming that the value of x is small. First you guess at a value for x (often relative to some constant), then solve for x. Once you get a value, you have to check your assumptions. You may have to repeat this approximation (successive approximations) until you get a value for x consistent with your approximation.

$\frac{4x^2}{0.20 - x} = 3.6 \bullet 10^{-8}$ first approximation : x is much smaller than 0.20. So 0.20 - x $\approx$ 0.20

$$\frac{4x^2}{0.20} = 3.6 \bullet 10^{-8}$$

$$x^2 = \frac{3.6 \bullet 10^{-8}(0.20)}{4}$$

$$x = \sqrt{1.8 \bullet 10^{-9}}$$

$x = 4.24 \bullet 10^{-5}$ Check the approximation : 0.20 - 0.0000424 $\approx$ 0.20 (yes!)

For this example, our first approximation worked, so we are done.

Solve the following quadratics (use either method, or both!)

$$\frac{x^2}{0.10 - x} = 4.9 \bullet 10^{-10}$$

$$\frac{x^2}{0.050 - x} = 3.5 \bullet 10^{-4}$$

Just for kicks, let's try a cubic equation as well (use approximation method)

$$x(0.10 + 2x)^2 = 7.4 \bullet 10^{-11}$$

Sample Titration Calculations

A titration calculation is one example of a stoiciometry calculation. Instead of grams and molar masses, we have volumes and molarities.

a. Jill titrated an HCL solution (unknown concentration) with NaOH. It took 14.73 mL of a 0.107 M NaOH solution to titrate a 15.00 mL sample. What is the concentration of HCl?

b. Suppose Nate was titrating a sample of H_3PO_4 with the same NaOH solution. In his case, it took 42.07 mL of 0.107 M NaOH to titrate 15.00 mL of H_3PO_4. What is the concentration of H_3PO_4?

$H_3PO_4 + 3NaOH \rightarrow 3H_2O + Na_3PO_4$ (note the 1:3 stoichiometry.).

c. You are titrating a sample of acetic acid (HCH_3CO_2, a monoprotic acid). It takes you 12.88 mL of 0.107 M NaOH to titrate 15.00 mL of your sample. What is the concentration of acid?

d. Janice is doing a salinity titration for Cl^-. The balanced equation is shown below. It took 23.66 mL of 500 ppm Ag^+ to titrate a 10.00 mL sample. What is the concentration of Cl^- in the solution in M and ppm?

$Ag^+ + Cl^- \rightarrow AgCl(s)$

Parts per million is a weight ration, not a mole ration. To correctly calculate the concentration, we have to convert the ppm Ag^+ to mole Ag^+/L, do the titration calculation, and then convert back. We cannot simply use ppm!

e. You are doing another salinity titration with the same Ag^+ reagent. It took 14.72 mL of 500 ppm Ag^+ to titrate a 10.00 mL sample. What is the concentration of Cl^- (M and ppm) in your sample?