EXERCISE 2 - Lab Procedures

⇒ **CAUTION: Potassium permanganate has a health hazard rating of 2 and a reactivity rating of 3. You must wear approved safety eyewear and gloves during this entire lab, avoid breathing dust, and notify your instructor immediately if any spills occur.**

⇒ **CAUTION: Potassium permanganate is a hazardous waste. You must discard any potassium permanganate solutions in the special waste containers provided.**

⇒ **If your Spec-20 spectrophotometer has not already been turned on, now is a good time to turn it on so that it will be warmed up by the time you are ready to use it.**

I. Prepare a solution of a specific molarity using a solid solute

1. The formula weight of potassium permanganate ($KMnO_4$) is 158.04 g/mole. Calculate the weight of $KMnO_4$ needed to prepare 80 mL of a 0.01 M solution.

   Weight of $KMnO_4$ = _____ (**Have your instructor check this calculation before proceeding.**)

2. Weigh out the calculated weight of $KMnO_4$ using a top-loading balance and a small weigh boat.

   NOTE: Free-flowing solids are removed from stock bottles by slowly pouring from the bottle or its lid into the weigh boat. Small quantities are best transferred by first pouring some of the solid into the lid of the stock bottle, then pouring from the lid into the weigh boat. Any excess can be poured from the lid back into the stock bottle. To avoid contamination, **never** place any objects into a stock bottle and **never** pour chemicals from the weigh boat or another container back into a stock bottle.

3. Pour approximately 40 mL of deionized water (dH_2O) into a clean 100 mL beaker, estimating the volume from the calibration marks on the side of the beaker. Place a stir bar into the beaker and set the beaker on a stir plate. Switch on the stir-plate and adjust the stirring rate so mixing is moderately fast but the solution does not splash.

4. Slowly pour the weighed $KMnO_4$ into the beaker and continue stirring until the $KMnO_4$ is completely dissolved. To transfer any $KMnO_4$left behind on the weigh boat into the beaker, use a plastic squeeze bottle of dH_2O.

5. Once the solute is completely dissolved, transfer the solution to a 100 mL graduated cylinder without transferring the magnetic stir bar: hold a large stir-bar against the outside of the beaker so that the stir-bar inside is held against one side. Slowly pour the solution into a 100 mL graduated cylinder while making sure the stir-bar does not fall out of the beaker.

6. Transfer 3 or 4 mL of dH_2O from a plastic squeeze bottle into the beaker, forcing the water all around the inside of the beaker to rinse any remaining solution to the bottom. Swirl the liquid for a few seconds, and then pour it into the graduated cylinder containing the $KMnO_4$ solution while preventing the stir bar from falling out of the beaker.

7. Rinse the beaker again with a few milliliters of dH_2O and pour the liquid into the graduated cylinder. Repeat, if necessary, in order to transfer all of the solute to the graduated cylinder, but be careful that the total volume of your solution does not exceed 80 mL.

8. Use the squeeze bottle or a Pasteur pipet to slowly add enough dH_2O to the graduated cylinder in order to bring the total volume of solution to 80 mL.

9. Stretch a piece of Parafilm® securely over the top of the graduated cylinder and mix the solution by tipping the graduated cylinder ***slowly*** upside down several times while holding the Parafilm® firmly in place.

10. Attach a piece of marking tape to the graduated cylinder. With a permanent marking pen, write the concentration and composition of the solution on the tape (i.e. 0.01 M $KMnO_4$).

II. Prepare a solution of a specific molarity using a previously prepared stock solution

1. Now, calculate the volume of 0.01 M $KMnO_4$ solution that should be diluted in order to make 25 mL of a 2 mM $KMnO_4$ solution.

   Volume of 0.01 M $KMnO_4$ solution needed = _____ (**Have your instructor check this calculation before proceeding.**)

2. Using an appropriate measuring device, transfer the required solution into a 25 mL graduated cylinder.

3. Add enough dH_2O to the graduated cylinder to bring the total volume to 25 mL. Stretch a piece of Parafilm® over the top of the graduated cylinder to make a tight seal and then mix the contents of the cylinder.

4. Attach a piece of marking tape to the graduated cylinder. With a permanent marking pen, write the concentration and composition of the solution on the tape (i.e. 2 mM $KMnO_4$).

III. Prepare several solutions using the parallel dilution technique

1. Calculate the amount of 0.01M $KMnO_4$ solution and the amount of dH_2O needed to make 10 mL each of the following solutions:

	Solution	**Volume of 0.01 M $KMnO_4$ Solution Needed**	**Volume of dH_2O needed**
1	1.0 mM $KMnO_4$		
2	0.6 mM $KMnO_4$		
3	0.4 mM $KMnO_4$		
4	0.2 mM $KMnO_4$		
5	100 μM $KMnO_4$		
6	50 μM $KMnO_4$		
7	20 μM $KMnO_4$		

   ➔ **Have your instructor check your calculations before proceeding.**

2. Use tape to label seven 20 mL test tubes with the final concentrations of the solutions being prepared, and then place the tubes in a test tube rack.

3. To make your first solution, use an appropriate measuring device to transfer the required amount of 0.01M $KMnO_4$ solution into a 10 mL graduated cylinder. Make sure you use the 0.01M $KMnO_4$ solution in the 100 mL graduated cylinder not the 2 mM $KMnO_4$ solution in the 25 mL graduated cylinder.

4. Add enough dH_2O to the graduated cylinder to bring the total volume to 10 mL.

5. Stretch a piece of Parafilm® over the top of the graduated cylinder to make a tight seal and then mix the contents of the cylinder.

6. Pour the contents of the cylinder into the tube labeled with the $KMnO_4$ concentration that you just prepared.

7. Clean your 10 mL graduated cylinder with dH_2O and prepare the rest of the dilutions in the same way.

8. Place the tubes with the 7 parallel dilutions into a test tube rack in order of decreasing concentration and label the rack "parallel dilutions."

9. Look at the 7 test tubes. Can you tell if your dilutions were made correctly? Well, there should be the same volume of liquid in each tube, and the color should get lighter as the solutions become more dilute, but other than that there is no way to tell if your dilutions were made correctly simply by looking at them. Therefore, it is extremely important that all calculations and procedures for preparing your dilutions are done carefully and accurately. **Any mistakes will be difficult to detect, and incorrectly prepared dilutions can invalidate the results of many long hours of lab work!**

IV. Prepare several solutions using the serial dilution technique

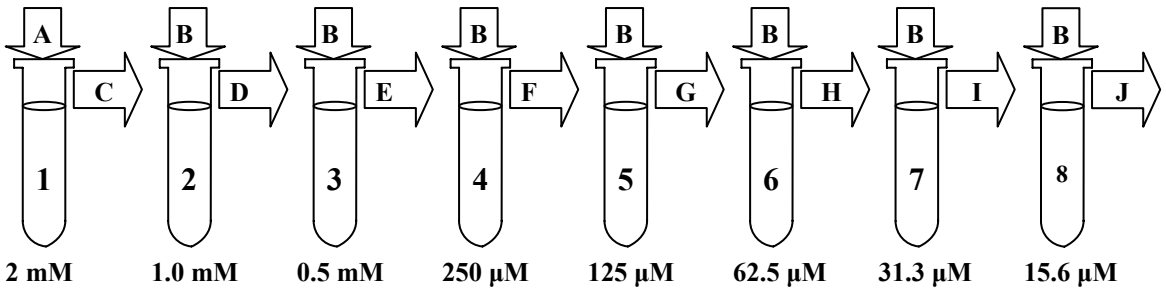
Starting with your solution of 2 mM $KMnO_4$, you will make 6 mL of each of the following concentrations of $KMnO_4$: 1.0 mM, 0.5 mM, 250 µM, 125 µM, 62.5 µM , 31.3 µM , and 15.6 µM.

What is df for this dilution series? _____

What is V_2 for this dilution series? _____

What is V_1 for this dilution series? _____

Label the arrows in the diagram below to show how you would make the required solutions. Above each vertical arrow, write down the volume and type of liquid you will place in the tube (e.g. 13 mL of stock solution or 9 mL of dH_2O.) Below each horizontal arrow, write down how much solution you will transfer from one tube to the next (e.g. 3 mL):



➔ **Have your instructor check your calculations and diagram before you proceed.**

Now, use tape to label eight 20 mL test tubes–the first tube with the molarity of your stock solution and the remaining tubes with the final concentrations of the dilutions being prepared. Place the tubes in a test tube rack.

Performing your serial dilutions:

⇒ **NOTE: The letters below correspond to the letters on the arrows in the diagram above.**

A. Using an appropriate measuring device, place the correct amount of 2 mM $KMnO_4$ ($V_1 + V_2$) into tube 1.

IMPORTANT: Make sure you use the 2 mM $KMnO_4$ from your 25 mL graduated cylinder and not the 0.01M $KMnO_4$ from your 100 mL graduated cylinder.

B. Using an appropriate measuring device, place the correct amount of solvent (V_2) into the remaining tubes.

IMPORTANT: When making a dilution series, make sure you use a clean measuring device each time you transfer a solution with a different solute concentration.

C. Transfer the correct amount of solution (V_1) from tube 1 to tube 2 and then thoroughly mix the contents of tube 2 with a Vortex mixer.

D. Transfer the correct amount of solution (V_1) from tube 2 to tube 3 and then thoroughly mix the contents of tube 3 with a Vortex mixer.

E. Transfer the correct amount of solution (V_1) from tube 3 to tube 4 and then thoroughly mix the contents of tube 4 with a Vortex mixer.

F. Transfer the correct amount of solution (V_1) from tube 4 to tube 5 and then thoroughly mix the contents of tube 5 with a Vortex mixer.

G. Transfer the correct amount of solution (V_1) from tube 5 to tube 6 and then thoroughly mix the contents of tube 6 with a Vortex mixer.

H. Transfer the correct amount of solution (V_1) from tube 6 to tube 7 and then thoroughly mix the contents of tube 7 with a Vortex mixer.

I. Transfer the correct amount of solution (V_1) from tube 7 to tube 8 and then thoroughly mix the contents of tube 8 with a Vortex mixer.

J. Remove the correct amount of solution (V_1) from tube 8 and discard it.

Finally, place the 8 tubes with your serial dilutions into a separate test tube rack in order of decreasing concentration and label the rack "serial dilutions."

V. Spectrophotometer: Absorption spectrum for $KMnO_4$

Make sure that your Spec-20 spectrophotometer has been turned on for at least 5 minutes to warm up.

In this section, **y**ou will be determining the **absorption spectrum** for a $KMnO_4$ solution. An absorption spectrum shows you how much light is absorbed by the solution at various wavelengths. In this experiment, you will measure the absorbance of light by the $KMnO_4$ solution at wavelengths ranging from 480nm to 580nm. The wavelength which gives you the highest absorbance is called the **wavelength maximum** for the solution.

1. Label a Spec-20 cuvette "B" with a small piece of label tape placed near the top of the cuvette. (Never write on Spec-20 cuvettes!) Fill the cuvette with dH_2O. This will be your zero standard or **blank**.

2. **Appendix D** at the back of this manual contains instructions on how to use the Spec-20 spectrophotometers. Remove Appendix D from your manual and keep it handy for reference while you are using the machine.

3. Determine whether you will be using an **ANALOG** Spec-20 (has a meter with a needle that moves across a printed scale) or a **DIGITAL** Spec-20 (has an LED readout). Read the section of Appendix D that corresponds to the type of the Spec-20 you will be using. Regardless of the type of Spec-20 used, you must perform 3 steps every time you change the wavelength of light used, in this order:

   a) adjust to the correct wavelength (using the knob on top of the Spec-20)

   b) BEFORE placing any cuvette in the instrument, adjust the **% transmittance** to zero (using the **left** knob on the front of the Spec-20)

   c) AFTER placing the blank cuvette (labeled "B") in the instrument, adjust the **absorbance** to zero (using the **right** knob on the front of the Spec-20)

4. Set the wavelength of your Spec-20 to 480 nm and adjust the filter if necessary. Now, calibrate the Spec-20 using the instructions in Appendix D or the instructions that are printed on the instrument. Remember, the Spec-20 must be calibrated at 2 points every time you set a new wavelength: the **% transmittance** must be set to zero when the holder is empty, and the **absorbance** must be set to zero when the blank is in the holder. **Ask your instructor to check your calibration of the Spec-20 before you take any measurements.**

5. Select one of your parallel dilutions to use for determining the absorption spectrum of $KMnO_4$. Choose a sample that has a distinct color, but that is light enough that you can see through it. **Ask your instructor to check your selection.**

6. Draw a data table in your lab notebook to record the absorbance, by the selected solution, of light at the following wavelengths: 480 nm, 500 nm, 520 nm, 540 nm, 560 nm, and 580 nm.

7. Pour the selected solution into a clean, dry cuvette and place the cuvette into the Spec-20 holder.

   IMPORTANT: Before you place a cuvette into the Spec-20 holder, always wipe the outside of the cuvette with a Kimwipe to remove any fingerprints or dust. Also, make sure the line on the cuvette is lined up with the line on the holder.

8. Read the absorbance at 480nm and enter the result into the data table in your lab notebook.

   IMPORTANT: Make sure you measure absorbance and not % transmittance.

9. Remove the cuvette from the Spec-20. Change the wavelength to 500nm and re-calibrate the Spec-20. Remember, you must re-calibrated the Spec-20 at 2 points every time you set a new wavelength: the **% transmittance** must be set to zero when the holder is empty, and the **absorbance** must be set to zero when the blank is in the holder.

10. Place the same solution that you used to measure absorbance at 480 nm back into the Spec-20. Read the absorbance at 500 nm and enter the result into the data table in your lab notebook.

11. Continue in this way until you have measured the absorbance of this same solution at all of the wavelengths listed in the data table of your lab notebook.

12. When you have completed your table for all measurements between 480 nm and 580 nm, examine your data. What is the wavelength maximum for your $KMnO_4$ solution? _____

13. Set your Spec-20 to the wavelength maximum for $KMnO_4$. In the next section, you will use this wavelength to measure the absorbance of **all** your diluted $KMnO_4$ solutions.

VI. Spectrophotometer: Effects of concentration on absorbance

In this part of the lab, you will use the wavelength maximum determined in part V to measure the absorbance of all of the parallel and serial dilutions of $KMnO_4$ that you prepared in parts III and IV. Because you will be using a single wavelength, you do not have to re-calibrate the Spec-20 between each measurement. However, you should check the calibration every 15-20 minutes to make sure your Spec-20 hasn't "drifted."

1. Re-calibrate the Spec-20 with the wavelength set at the wavelength maximum that you determined for $KMnO_4$. (If you did not do part V of this lab, set the spectrophotometer at 540 nm to measure absorbance in this section.)

2. Measure the absorbance at the wavelength maximum for each solution that you made using the parallel dilution method. Record the absorbance of each sample in your lab notebook using a clearly labeled data table.

3. Measure the absorbance at the wavelength maximum for each solution that you made using the serial dilution method. Record the absorbance of each sample in your lab notebook using a separate data table.

4. Before you discard of your solutions, ask your instructor to check your absorbance measurements for plausibility.

VII. Determine the concentration of an unknown $KMnO_4$ solution

1. You will be given a solution with a concentration of $KMnO_4$ that is unknown to you. Measure the absorbance of this solution using the wavelength maximum for $KMnO_4$ that you determined in part V. Record the results in your lab notebook.

Clean up

Discard the $KMnO_4$ solutions: Make sure you discard all $KMnO_4$ solutions in the waste containers provided; do not dump them into the sink.

Clean the Spec-20 cuvettes: Spec-20 cuvettes are not ordinary test tubes. They are expensive, and great care must be taken to avoid scratching them. Scratches interfere with the passage of light through the tube and that can lead to inaccurate results. Rinse them only---do not use test tube brushes on cuvettes. Rinse the cuvettes thoroughly with tap water and then with dH_2O. Ask your instructor where they should be left to dry.

➔ ***Never use brushes or abrasives on Spec-20 cuvettes!***

Wash all regular glassware:

1. Pour contents into an approved container (Ask your instructor if you are unsure of how to discard a particular chemical.)
2. Rinse with tap water.
3. Scrub with soapy water and a test tube brush.
4. Rinse 3 times with tap water.
5. Rinse 3 times with distilled water.
6. Store inverted over absorbent towels until dry.

Wipe off your work space with a damp paper towel. You have been working with a purple dye. Don't be responsible for dyeing students purple in the next class! Or their belongings!

All instruments should be turned off and unplugged. Make sure everything that you have used is clean, put away, or discarded. Ask your instructor to check your work area before you leave.