BCH 372
Modern Concepts in Biochemistry Laboratory

Laboratory 1
Biochemistry Boot Camp

The purpose of this part of the laboratory session is to review some of the basic laboratory skills and calculations that will be needed for this semester's lab work. As part of this lab, you will learn to use micropipetters, graduated cylinders, and glass pipets correctly and to read laboratory balances accurately. You also will practice doing several types of biochemical calculations.

I. Pre-Lab Preparation

Before the lab, please read Chapter 1 in the lab manual **Experiments in Biochemistry: a hands-on approach, second edition,** by S. O. Farrell and L. E. Taylor. Some of the material in this chapter such as section 1.8 on Graphing will not be used in this lab, but will be important in later labs. You may also want to refer to **Supplement 1** on **Basic Biochemical Calculations** which is available as part of the lecture course (LSC 394 – Modern Concepts of Biochemistry) and has also been posted on the Blackboard site for this class. Also, be sure to purchase a **lab notebook** and bring it to the lab along with your calculator and other lab supplies. In conjunction with this lab, also read Chapter 1 in Boyer's book **Biochemistry Laboratory: modern theory and techniques**, **second edition**.

Please note that on pages 12-13 in Chapter 1, the authors describe a **dilution factor** in terms of the ratio of the final volume or concentration over the initial volume or concentration. For example, if 10 ml of a solution is diluted to a final volume of 100 ml, the dilutions factor is defined a:

$$D_f = 100 \text{ ml}/10 \text{ ml} = 10.$$

This is a somewhat unusual convention. In most laboratories and in the laboratory handouts to be used in this course, the more common convention of defining a dilution factor as **the ratio of the initial volume or concentration to the final volume or concentration** will be used. For example, diluting 10 ml of a solution to a final volume of 100 ml will be referred to as a 1/10 or 10^{-1} dilution.

II. Laboratory Procedures

A. Use of Micropipetters and Balances

1. Follow the general directions for **Part A** of Experiment 1 beginning on page 30 of the lab manual. To make it easier to see the liquid, you will use water that has been colored with red food coloring.

In using the micropipetters, be sure to depress the piston only down to the **first stop** when you are drawing up liquid into the plastic tip. Also, be sure to wipe off the outside of the plastic tip with a **Kim-Wipe** to remove excess liquid. To eject the liquid, depress the piston all the way down to the second stop.

Do some practice trials in which you determine the weight of ten (10) 100 µl aliquots (portions) of colored water as dispensed with a P-100 micropipetter. The easiest way to do this is to dispense the liquid into a plastic weighing dish, determine the weight, and then reset (tare) the balance to 0.000 g. Then do ten (10) trials with 30 µl and 10 µl volumes using the same pipetter. Record all of the weights in your laboratory notebook using the following headings. Then calculate the average, the % error, and the mean deviation as described on page 31 of the lab manual.

Please note: these headings are suggestions for setting up a data table in you lab notebook. Record the results directly in your lab notebook, not on this handout.

trial	100 µl	30 µl	10 µl
1	_____	_____	_____
2	_____	_____	_____
3	_____	_____	_____
4	_____	_____	_____
5	_____	_____	_____
6	_____	_____	_____
7	_____	_____	_____
8	_____	_____	_____
9	_____	_____	_____
10	_____	_____	_____

average	_____	_____	_____
% error	_____	_____	_____
mean deviation	_____	_____	_____

2. Follow the directions for **Part B** of Experiment on page 30 of the lab manual. After doing ten (10) trials in which you determine the weight of 1000 µl aliquots of water as dispensed with the P-1000 micropipetter, also do ten (10) trials with 500 µl, 250 µl, and 100 µl volumes using the same micropipetter. Then calculate the average, the % error, and the mean deviation as described on page 31 of the lab manual. Record all of the weights in your laboratory notebook using the following headings:

trial	1000 µl	500 µl	250 µl	100 µl
1	_____	_____	_____	_____
2	_____	_____	_____	_____
3	_____	_____	_____	_____
4	_____	_____	_____	_____
5	_____	_____	_____	_____
6	_____	_____	_____	_____
7	_____	_____	_____	_____
8	_____	_____	_____	_____
9	_____	_____	_____	_____
10	_____	_____	_____	_____
average	_____	_____	_____	_____
% error	_____	_____	_____	_____
mean deviation	_____	_____	_____	_____

3. In the same way, do ten (10) trials in which you determine the weight of 2 µl and 10 µl aliquots as dispensed with a P-10 micropipetter. Record all of the weights in your laboratory notebook. Use the following headings:

trial	2 µl	10 µl
1	_____	_____
2	_____	_____
3	_____	_____
4	_____	_____
5	_____	_____
6	_____	_____
7	_____	_____
8	_____	_____
9	_____	_____
10	_____	_____
average	_____	_____
% error	_____	_____
mean deviation	_____	_____

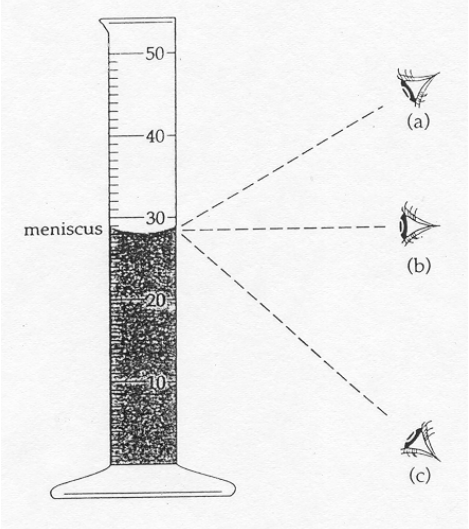
4. Look closely at the resulting data. How accurate are the micropipetters? How does this accuracy vary with the volume transferred with each size of the instrument?

B. Use of Graduated Cylinders and Pipets

While micropipetters are extremely useful for transferring small volumes of liquid, there are times when it is necessary to transfer a volume of liquid greater than 1.0 ml (1000 µl). Graduated cylinders are commonly used for volumes of 10-1000 ml and glass pipets are used for volumes of 1-25 ml. The purpose of this part of the experiment is to learn to use these materials in the same way as you learned to use micropipetters.

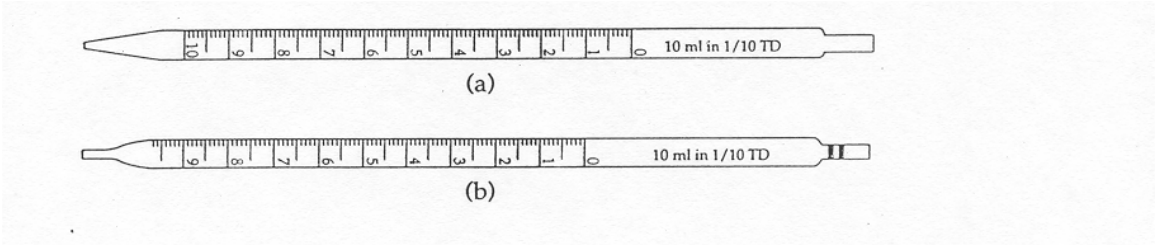
1. Graduated Cylinders

For relatively large volumes (greater than 10 ml), graduated cylinders are most often used. A graduated cylinder has markings on the side of the glass or plastic which indicate the volume. Depending on the total capacity of the cylinder, the meaning of the markings will vary. For a 10 ml, 25 ml, or 50 ml cylinder, they are most often 1 ml apart. For a 250 ml or 500 ml cylinder, they are most often 10 ml apart. One of the problems with most cylinders is that an aqueous solution (that is, one made up in water) often shows a pronounced meniscus or curvature when it is placed in the cylinder. This is due to the binding of water molecules to one another and to the sides of the cylinder. As shown in figure on the next page, the volume in a cylinder is read most accurately by looking at it straight on and reading the volume at the bottom of the meniscus. A graduated cylinder can be used either to measure the volume of unknown solution or to measure out a certain volume of solution and then to transfer it to another container.

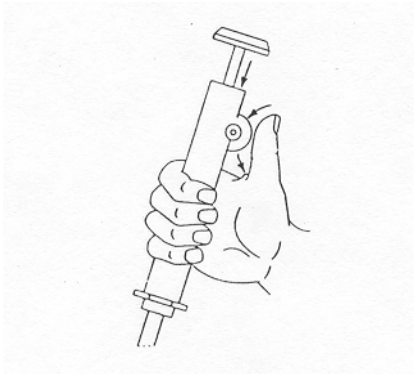


2. Pipets

For intermediate volumes (between 1 ml and 25 ml), pipets are most often used. A pipet is a calibrated piece of glass tubing with markings along the side. Again, depending on the total capacity of the pipet, the meaning of the markings will vary. For a 1 ml pipet, the markings may be either 0.1 ml or 0.01 ml apart. For a 5 ml or 10 ml pipet, the markings are usually 0.1 ml apart. Each pipet usually is marked at the top so that the gradations are clear: it will say 5 in 1/10 ml or 1 in 1/100 ml. As with graduated cylinders, there is often a pronounced meniscus with aqueous solutions. A certain volume of liquid may be transferred from one container to another by drawing the liquid up into the pipet and then allowing a volume of liquid between two specific markings to flow out. Mohr or measuring pipets are calibrated only to a point near the bottom of the glass tube. A specific volume is delivered by measuring the amount of liquid between two markings. Serological pipets are calibrated all the way to the tip and so must be "blown out" in order to deliver all of their volume. This is illustrated in the following figure.



To use a pipet, it is necessary to draw liquid up into it. In the past, this was often done by sucking on the end of the tube with your mouth (as with a straw) and covering the end with your index finger. However, this type of mouth pipeting is no longer considered safe and various types of bulbs, pipet aids, or propipets are used instead. MOUTH PIPETTING IS NOT ALLOWED IN THE COURSE. In this class, plastic pipet pumps will be used as shown in the following figure.



A green pump should be used with 5 ml or 10 ml pipets; a blue pump should be used with 1 ml pipets. To use this type of pump, insert the top end of the pipet into the pump and twist it so that it seals against the plastic. Then rotate the plastic wheel to draw the liquid up into the pipet. To let liquid out of the pipet, rotate the wheel in the opposite direction. Pipets are most often used to transfer a certain volume of liquid from one container to another, although with care they also can be used to measure the volume of a solution.

C. Measuring Volumes with a Graduated Cylinder and Pipets

1. Take a 100 or 150 ml beaker and fill it about half fill with the red colored liquid in a flask at your station. The liquid again is just water with some food coloring added to it so you can see the liquid easily. Note that while the stock flask or the beaker may have markings on their sides, these values are only approximate and are never to be used for measurement. Pour the liquid into a 100 ml graduated cylinder. Look at the meniscus, measure the volume, and record the results below.

   volume of red liquid: _____ ml

2. To test the accuracy of this measurement, place a clean dry beaker on the top-loading balance and reset it to 0.00 g. Pour the liquid into the beaker and measure the weight in grams. The density of pure water is 1.00, so 1.0 ml = 1.0 g. Record the weight of liquid below:

weight of red liquid: _____ g

Note that at one atmosphere of pressure, the terms weight and mass can be used interchangeably.

3. Now fit a 10 ml serological pipet with a green pipet pump. Draw some of the liquid up into the pipet and look at it carefully. Rotate the wheel and then transfer 10.0 ml of this liquid back into the original container.

4. Repeat this process with volumes of 6.7, 5.8, 4.2, and 3.1 ml. Note that you use the markings anywhere along the pipet to transfer a certain volume. You can also read the pipet from the top down or from the bottom up.

5. Once you have figured out how to read the markings correctly, place a clean dry beaker on the top-loading balance and reset it to 0.00. Using the pipet, transfer 7.3 ml of liquid to the beaker and measure the weight in g. The transfer will be most accurate if you touch the tip of the pipet to the glass and allow the liquid to run into the container. Repeat the process four more times and record your values in your lab notebook using the following headings. Then calculate with average (mean), the % error, and the mean deviation as described in section 1.4 and on pages 31-32 of the lab manual.

trial	7.3 ml
1	_____
2	_____
3	_____
4	_____
5	_____
average	_____
% error	_____
mean deviation	_____

6. After completing these exercises, answer the following questions from **Part C** on pages 32-33 of the lab manual: # 1, 2, 4, 5, 6, 7, and 8. Enter the answers in your lab notebook.

D. Solution Calculations

1. Working with your lab partner(s), complete the calculations in the Additional Problem Set on page 35 of the lab manual.

2. Record the complete answers in your lab notebook for future reference and for the Datum Sheet.