

Lab I - Introduction and Laboratory Skills

Purpose: Introduction of students to biochemistry laboratory, review of syllabus and safety requirements for the course, and needed laboratory skills.

Key Concepts/Terms:

Syllabus	Pipetting devices	Graphing Data
Lab Coats	Emergency Equipment	Metric System
Goggles	Safety Hoods	Dilutions
Gloves	Chemical/Waste disposal	Buffers
Pipets		Significant Figures

Laboratory Skills:

- (i) Review of Department safety procedures.
- (ii) How to present data in a graph.
- (iii) How to use the metric system.
- (iv) Pipets
- (v) How to make dilutions.
- (vi) How to prepare buffers.
- (vii) Significant Figures

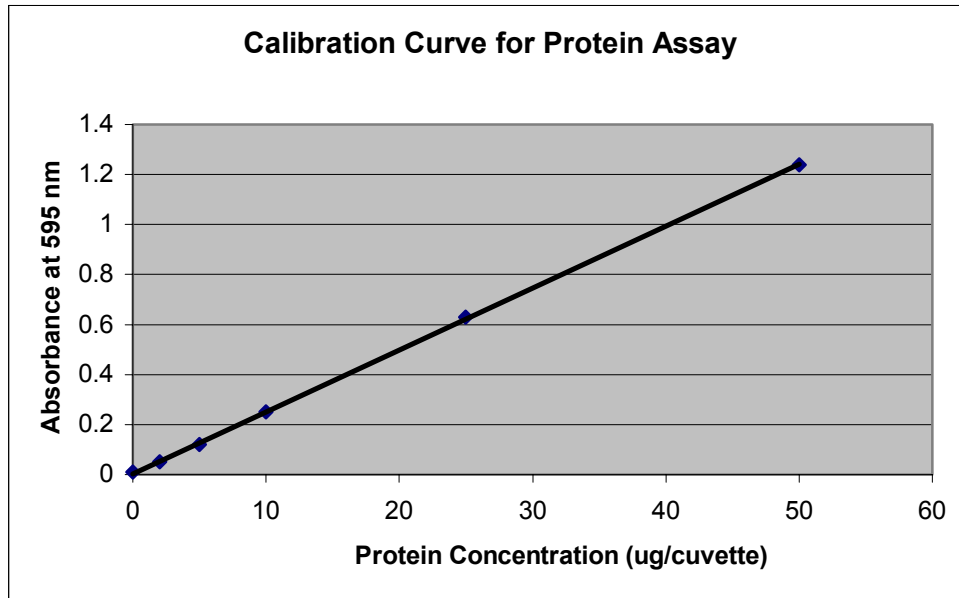
Safety:

While working the Biochemistry Laboratory you will be required to follow the Laboratory Rules for the Department of Biological Sciences. Some of the labs require the use of chemicals that can be hazardous to your health if safety precautions are not taken. Lab coats and gloves are required for work in the lab. At times you will be asked to wear goggles as well. Food and beverages are not allowed in the lab.

Graphing Data:

During this course you will be asked to summarize the data from your experiments in the form of a graph. Graphs can be drawn using graph paper or a computer program such as Microsoft Excel. Using the data shown below, a graph was made using Microsoft Excel:

Concentration, $\mu\text{g}/\text{cuvette}$	Absorbance, 595 nm
0	0.01
2	0.05
5	0.12
10	0.25
25	0.63
50	1.24



Notice that the graph contains a title and labels for both of the axes. Since this is a linear graph, each of the axes is divided into equal intervals. The data has been plotted on the graph, and the line best fitting the data drawn in. This graph represents a calibration curve for a protein assay similar to one you will be doing in the next couple of labs.

The Metric System:

Measurement in the scientific world is done using the metric system. To work in the lab, you must be comfortable with the metric system and understand how to manipulate it. The relationships between the units used in our labs are as follows:

Volume: 1 liter = 1000ml 1ml = 1000 μ l

To calculate 0.12liters = ? μ l: $0.12 \text{ liters} \times 1000\text{ml/liter} \times 1000\mu\text{l/ml} = 1.2 \times 10^5 \mu\text{l}$.

To calculate 3410 μ l = ? liters: $3410 \mu\text{l} \times 1 \text{ ml}/1000 \mu\text{l} \times 1 \text{ liter}/1000\text{ml} = 3.410 \times 10^{-3} \text{ liters}$.

Weight: 1 gram = 1000mg 1 mg = 1000 μ g 1 μ g = 1000 ng 1 ng = 1000 pg

To calculate 26.2 mg = ? pg: $26.2 \text{ mg} \times 1000 \mu\text{g/mg} \times 1000 \text{ ng}/\mu\text{g} \times 1000 \text{ pg/ng} = 2.62 \times 10^{10} \text{ pg}$.

To calculate 57 μ g = ? g: $57 \mu\text{g} \times 1 \text{ mg}/1000 \mu\text{g} \times 1 \text{ gram}/1000 \text{ mg} = 5.7 \times 10^{-5} \text{ grams}$.

Molarity: 1 mole = 1000 mmole 1 mmole = 1 μ mole

The same logic applies to convert between the units. Sometimes, you will need to convert more than one unit.

15 μ g/ml = ? g/liter: $\frac{15 \mu\text{g} \times 1 \text{ mg}/1000 \mu\text{g} \times 1 \text{ gram}/1000 \text{ mg}}{1 \text{ ml} \times 1 \text{ liter}/1000 \text{ ml}} = 1.5 \times 10^{-2} \text{ g/liter}$.

Pipets:

We use two different types of pipets in the biochemistry lab, Gilson Pipetman and glass serological pipets. The Gilson Pipetman are adjustable mechanical pipets that come in three sizes, P20, P200, and P1000.



The P20 has a range of 5-20 μl ; the P200 has a range of 20-200 μl ; and the P1000 has a range of 200-1000 μl . The P20 and P200 use the yellow tips, and the P1000 uses the blue tips. When you push the plunger on these pipets, there are two "stop" positions. To take up solution, you push the plunger to the first "stop" position and draw the solution into the pipet tip. To expel solution, you push the plunger all the way to the second "stop" position to ensure that all the solution is pushed out of the pipet tip. The volume of each of the Gilson pipets is set by turning the black knob to get the desired volume in the window. The formats are slightly different for each pipet; therefore practice pipetting specific volumes before using the pipets in your experiment.

Glass serological pipets have calibrated volume markings along their length. Three sizes are commonly used in the lab, 1 ml, 5 ml and 10 ml. Pipet pumps or bulbs are fitted on the tops of the glass pipets to draw and expel solutions. Mouth pipeting is never allowed in the lab. The volume in a glass pipet is determined by viewing the bottom of the meniscus of the solution in the pipet.

A new glass pipet or pipet tip is usually used for each different solution pipeted to avoid cross contamination of experiments.

Dilutions:

A dilution is the process of combining measured volumes of a concentrated solution of analyte with a buffer to make less concentrated solutions. It is a process repeated constantly in the laboratory. There are several ways to make dilutions.

Dilution Factors

Most people are using this dilution method when they make up orange juice from the frozen product. One can of orange juice is diluted with four cans of water. One can of concentrated juice is diluted to a final volume of 5 cans by adding 4 cans of water. This is a 1:5 (1 to 5; or 1/5) dilution. In this lab, 1:5 always means 1 volume of concentrated material diluted to a final volume of 5. The table below gives some examples:

<u>Dilution</u>	<u>Vol Concentrate</u>	<u>Vol buffer</u>	<u>Final Vol</u>
1:2	1 ml	1 ml	2 ml
1:3	1 ml	2 ml	3 ml
1:3	3 ml	6 ml	9 ml - This is a 3:9 dilution, but that = 1:3
1:4	1 ml	3 ml	4 ml
1:100	1 ml	99 ml	100 ml
1:1.5	1 ml	0.5 ml	1.5 ml

In the example below, You have a protein solution (a stock solution) at 2 mg/ml. You need to make the following levels: 400 µg /ml, 100 µg /ml, 20 µg /ml, 5 µg /ml and 1 µg /ml. You need 50 ml of each concentration. The first thing to do is to get everything into the same units: 2 mg/ml x 1000 µg/mg = 2000 µg/ml. Then it helps to put everything into a table.

<u>Final Concentration</u>	<u>Calculate Dilution</u>	<u>Dilution</u>	<u>Vol stock</u>	<u>Vol buffer</u>	<u>Final Vol</u>
400 µg /ml	400/2000=1/5	1:5	10 ml	40 ml	50 ml
100 µg /ml	100/2000=1/20	1:20	2.5 ml	47.5 ml	50 ml
20 µg /ml	20/2000=1/100	1:100	0.65 ml	64.35 ml	65 ml
5 µg /ml	5/2000=1/400	1:400	0.125 ml	49.875 ml	50 ml
1 µg /ml	1/2000=1/2000	1:2000	0.025 ml	49.975 ml	50 ml

The 5 µg /ml and the 1 µg /ml solutions have large dilutions and volumes of the stock too small to pipet accurately. Sometimes it is helpful to use a second less concentrated stock solution. Suppose we made an extra quantity of the 20 µg /ml level and used that to make these solutions. How much extra beyond 50 ml do we need?

<u>Concentration</u>	<u>Calc Dilution</u>	<u>Dilution</u>	<u>Vol stock</u>	<u>Vol buffer</u>	<u>Final Vol</u>
5 µg /ml	5/20=1/4	1:4	12.5 ml	37.5 ml	50 ml
1 µg /ml	1/20=1/20	1:20	2.5 ml	47.5 ml	50 ml

We need an extra 15 ml of the 20 µg /ml level, so the quantities for that concentration have been adjusted in the table above.

Serial Dilutions

In the section above we used one or two concentrated solutions to make up all the dilutions. Each dilution is made independently (except for the levels made from the 20 µg /ml level) from another dilution. If an error is made it only affects one dilution. When serial dilutions are done, each dilution is made from a more concentrated dilution. A common use of serial dilutions is to create a set of dilutions each half the concentration of the previous level.

<u>Initial Concentration</u>	<u>Vol solution</u>	<u>Vol buffer</u>	<u>Final Concentration</u>
100 mg/ml	5 ml of 100 mg/ml	5 ml	50 mg/ml
50 mg/ml	5 ml of 50 mg/ml	5 ml	25 mg/ml
25 mg/ml	5 ml of 25 mg/ml	5 ml	12.5 mg/ml
12.5 mg/ml	5 ml of 12.5 mg/ml	5 ml	6.25 mg/ml
6.25 mg/ml	5 ml of 6.25 mg/ml	5 ml	3.125 mg/ml

The advantage of this method is ease of pipeting. The disadvantage is that an error in one dilution causes errors for all the remaining dilutions.

Dilutions Using $V_1C_1=V_2C_2$ Method

In some cases you will have a limited quantity of an expensive material. You will need to prepare a diluted sample of this material and use the minimum amount. You have an expensive protein that you have purchased. You have 100 µl at a concentration of 7.22 mg/ml. From this material, you need to make 4 ml of a 50 µg/ml solution. To determine the amount of the expensive protein you need, use the formula:

$V_1C_1=V_2C_2$ where V = volume and C = concentration.

The first thing to do is to get everything in units of mg/ml for concentration and ml for volume. Then we have:

$V_1 \times 7.22 \text{ mg/ml} = 4 \text{ ml} \times 0.05 \text{ mg/ml}$, where V_1 = the unknown volume of expensive protein

We rearrange terms so that $V_1 = \frac{4 \text{ ml} \times 0.05 \text{ mg/ml}}{7.22 \text{ mg/ml}}$

Then $V_1 = 0.028 \text{ ml}$ of expensive protein. To make the dilution, you would take 0.028 ml (28 µl) of the expensive protein solution and add it to 3.972 ml buffer to get a final volume of 4 ml.

Preparation of Buffers:

Preparation of buffers and other chemical formulations is an essential part of laboratory work. Buffer formulations can be given as molar solutions or percent solutions.

There are some general guidelines for the preparation of buffers. If water is the solvent, prepare buffers with fresh double-distilled or deionized water. Remove powders from their parent containers by shaking the container or with a disposable spatula. Using the same spatula with

different chemicals can result in cross contamination. Wear gloves when handling chemicals and observe safety precautions for handling hazardous chemicals.

For weighing mg quantities of chemicals use an analytical balance. For larger quantities a pan balance is acceptable. To make a buffer, add ~3/4 volume of liquid components to a beaker, then add the weighed ingredients. Mix to dissolve using a clean stirbar. When all the ingredients have dissolved, check and adjust the pH. Remove the stirbar with a magnet from outside the container. Carefully add water to the final volume needed by pouring the buffer into a graduate cylinder or volumetric flask. Determine the volume by looking at the bottom of the meniscus. Add the stirbar to the solution to complete mixing. Place buffer in a clean container, seal and label with name of buffer, date prepared and name of who made it. Depending on the buffer constituents, buffers can be filtered or sterilized for long term storage.

Molar Solutions (Unit = M = moles/liter)

A 1.0 Molar (1.0 M) solution is equivalent to 1 formula weight (g/mole) of chemical dissolved in 1 enough water to make 1 liter. The formula weight (FW) is given on the label of a chemical bottle, or use molecular weight (MW) if formula weight is not given.

Here are some examples:

1. Prepare 2 liters of a 0.2 M (0.2 mole/liter) solution of a chemical having a FW of 154.2 g/mole. How many grams of the chemical do I need to use?

?grams = 2 liters x 0.2 mole/liter x 154.2 g/mole = 61.68 g of chemical. Thus, add 61.8 grams to about 1.5 liters of water to dissolve, then make up the final volume to 2.0 liters with distilled water.

2. You have a 5 mg/ml solution of a protein with a molecular weight of 66,000. How many moles/liter protein are there in your solution?

5 mg/ml x (1 gram/1000mg) x (1000ml/ 1 liter) = 5 gram/liter solution of protein

5 g = 1 liter x ?mole/liter x 66,000 g/mole. Rearranging the equation gives you

$$? \text{ mole/liter} = \frac{5 \text{ g}}{1 \text{ liter} \times 66,000 \text{ g/mol}} = 7.6 \times 10^{-5} \text{ mole/liter}$$

Percent Solutions

Many chemical solutions are mixed as percent concentrations. When working with a dry chemical, the percent is calculated as grams dry chemical in 100 ml. This is known as a weight/volume or W/V formula. For example, a 0.9% NaCl solution is 0.9 g NaCl in 100 ml water. When the formula includes a liquid chemical, then the percent solution is volume added chemical in ml made up to 100 ml with solvent. A 10% acetic acid solution in water is 10 ml glacial acetic acid added to 90 ml water.

Significant Figures:

Significant figures provide a way to express the degree of accuracy in experimental data. Data should be recorded with as many digits as can be accurately measured. For example, an absorbance measurement in one part of the range is 0.67, but is 1.2 in the upper part of the range. A second decimal should not be added to the 1.2, since it is not reflected in the accuracy of the instrument. The number of significant figures is the number of nonzero values in a measurement. In the absorbance example 0.67 and 1.2 both contain two significant figures, although there is a difference in the number of decimals.

If calculations are performed on measurements having differing numbers of significant digits, the answer contains the smallest number of significant digits.

We have 5.15 ml of a solution containing 1.973g/ml protein. How much total protein do we have? The answer is $5.15 \text{ ml} \times 1.973 \text{ g/ml} = 10.16095 \text{ g}$. Since the smallest number of significant figures is three, the answer is rounded to 10.2 g.

We have five results from an experiment: 2.6%, 2.4%, 2.5%, 2.3%, 2.4%. The average of these five measurements is 2.44%. Since the data contain two significant figures, the mean value is rounded to 2.4%.

While doing multiple calculations on the same data set, rounding should be done for the final answer.

References:

Resource Materials for Biology Core Courses at <http://abacus.bates.edu>.

Lab FAQ's at <http://biochem.roche.com>.

Robyt, John F and Bernard J. White. 1987. Biochemical Techniques Theory and Practice. Brooks/Cole Publishing Company, Monterey, CA: 1-3, 21-25.

Revised 5/02 jcm