Chapter 1

Biochemistry Boot Camp

SURVIVAL IN THE BIOCHEMISTRY LAB

TOPICS

Introduction

In this chapter, we discuss some fundamentals of laboratory science. This material is often overlooked by instructors who, correctly or incorrectly, assume that you have learned it well in other courses. It could be argued that the material presented here is the most important, however, due to the shear magnitude of the repetition. You will constantly be doing calculations involving units, concentrations, and dilutions. You will be presenting your data using tables and graphs. You will do hundreds of pipettings during a semester. If this material is not mastered, you will pay a heavy price all semester. Read this chapter thoroughly, even if you think you already know it. You may just find an informational jewel you had not expected.

1.1 Lab Safety

A lab can be a dangerous place if you are not careful. This potential danger comes from several sources. First, the very nature of the chemicals and equipment used may be hazardous. We try to minimize use of hazardous materials, but some modern techniques in biochemistry require that we use them. Hazardous chemicals are noted in the "Materials" section of the experiments. Some equipment may be dangerous due to moving parts, heat, or potential electric shock. Only by using the equipment correctly, as instructed, will you be sure that you are safe.

Second, glassware used in a lab is always considered dangerous because it breaks when dropped or mishandled. Flying glassware may come from anywhere, so you may not be the one who makes the mistake, but you may be the one who pays for it. Proper clothing and eye protection is the only sensible way to protect yourself against most common lab accidents.

Third, most accidents are caused by carelessness. A student who is mentally prepared to undertake the lab, has studied the material, and understands the procedures is much less likely to make a mistake that could injure someone. Simple mistakes like careless washing of glassware can be dangerous if water ends up on the floor and isn't cleaned up. Note and observe the following lab procedures.

Things You Should Do

1. Always use some form of protective eyewear. The most reliable are certified lab goggles that protect from the sides as well as the front. Contact lenses can be a problem in a lab because tears do not wash out things spilled in the eye when a contact lens is present. Eyewash stations are not efficient if you are wearing contacts. The American Chemical Society, however, has recently removed its recommendation against contact lenses in the lab. Evidence shows that contacts are not dangerous **IF** proper protection (goggles) is used.
2. Be aware of what chemicals you are using. Wear gloves when using toxic chemicals. Remember that, although you are not using a dangerous chemical, the student beside you may have spilled one right next to you.
3. Wear proper clothing in the lab. The lab is a good place for long sleeves, long pants, closed-toed shoes, and standard-lens glasses. It is a bad place for short sleeves, shorts, and sandals. Don't even think about going into a lab barefoot.
4. Familiarize yourself with the layout of the lab. Do you know where the fire extinguishers are? Where are the eyewash stations? Where is the first aid kit?
5. Label all reagents that you bring to your bench with tape.

Things You Should Never Do

1. Never eat, drink, or smoke in the lab. Although you may see more advanced scientists doing the first two in their labs, it is a bad idea in a teaching lab. There may be 200 students using that lab in a week, and the instructor cannot control what all of them are doing. If you need to drink often, then bring a water bottle but leave it outside. Nobody will mind it if you step outside to eat or drink, but bringing any food or beverage into the lab, even if sealed, is a potential danger.
2. Never use mouth suction on glass pipets to draw up a solution. Even if you think the solution is a harmless buffer, the pipet may be contaminated with something hazardous. Use pipet pumps and bulbs to draw up solutions.

3. Never work alone. Someone else should always be in lab with you when you are working.

Additional Lab Courtesy

Although lab etiquette is not strictly a safety issue, you should follow it as well. The following items will make your lab run more smoothly and lead to efficient transitions between lab sections.

1. Never stick your personal pipets into a community reagent bottle. If your pipet is dirty, you will contaminate the supply for the whole class. If you need 10 mL of a reagent and a 1-L bottle is in a community reagent area, use a small beaker and pour in about 10 mL. That way, if your beaker is dirty, you will have contaminated only your own supply.
2. Never take a community reagent back to your own bench. One of the most frustrating things that can happen in a lab is not finding something you need.
3. Don't use more of the chemical than you need. Students often take chemicals from the community area before they have the slightest clue about how much they need. They look at the size of the community reagent bottle and try to guess from there. Just because the reagent is in a 1-L bottle doesn't mean that you should take 100 mL of it. You might only need 2 mL of it. The 1-L bottle may be the total supply for all sections of the lab for the week.
4. Always clean up your lab area and any equipment and glassware that you used. The next class may need to use the same materials. The job is not over until the lab is clean and the equipment is ready for the next class.

1.2 Scientific Notation

You will probably see many numbers written in many different ways during the course of your lab. The idea is to accurately and clearly communicate numerical information, and nothing is necessarily wrong with any system of numbers that does that. However, some traditional ways of handling numbers are used in science. One of these is called scientific notation.

Any number in **strict** scientific notation starts with one nonzero digit followed by a decimal point and some other numbers. That is followed by an exponent that tells to what power to raise it. Thus, the number 623 would be written 6.23×10^2. The number 0.0456 would be written as 4.56×10^{-2}.

When not using strict scientific notation, avoid starting a number with just a decimal. Thus, rather than writing .453, write 0.453. When you label a test tube for storage, sometimes the ink fades. It always seems like the decimal point fades faster than the numbers. The beauty of using strict scientific notation is that a fading decimal point wouldn't matter because you would always know that the decimal point belonged after the first number.

TIP 1.1 Be careful when using exponents on your calculator. Most have an EXP or EE key. When you push that key, you are multiplying whatever is on the screen by 10 to the power that you are about to enter.

A common example of how this can be a problem is with the well-known constant for the dissociation of water, which is usually written as 10^{-14}. To divide the water constant by 0.2 to get an answer, you should press 1, followed by the EXP, key and press −14. What many students do is press 10 EXP, followed by −14. That is 10×10^{-14} which is 10^{-13} instead of 10^{-14}.

1.3 Significant Figures

When you take a measurement or do a calculation, you can only rely on a certain amount of the information that you get from it. Analysis of significant figures is our way of determining how reliable the numbers are. Nowadays, many students use calculators or computers for everything, often with humorous results.

If you have a standard ruler with inches on one side and centimeters on the other and ask me to measure the height of your 250-mL beaker, what will you think if I tell you it is 8.423587763 cm? You might say, "Thanks, Mr. Spock, but 8.4 cm is sufficient," but you will certainly see that I cannot measure something to that many decimal places when the smallest division on the ruler is 1 mm, or 0.1 cm. However, you might easily make a very similar mistake when you do a calculation. If you measure a cube to be 9.6 cm on a side and want to calculate the volume in cubic centimeters, you might multiply $9.6 \times 9.6 \times 9.6$ and report the volume as 884.736 cc. Students do that all the time without recognizing that they are as wrong as in the Mr. Spock example. If each side is measured to an accuracy of one decimal place, the answer cannot be given in three decimal places.

Definition of Significant Figures

So, what is a significant figure? That has to do with the accuracy and precision of the instrument being used to make the measurement. If you have a balance, you weigh out a sample of histidine, and the balance reads 1.473 g, then you have four significant figures. The last figure, 3, is probably at the limit of the machine and is an estimate. If you weigh the same sample again, it might read 1.472 or 1.474. This is like estimating between the closest marks on a ruler. Maybe a cruder balance reads 1.5 g, and you then have only two significant figures.

The number and position of zeros is often confusing to students with regards to significant figures. This is another reason to use scientific notation because there is no ambiguity when using it. For example, how many significant figures are in 0.0456? The answer is three. Zeros before the first nonzero digit have nothing to do with the accuracy; rather, they mark the place of the decimal point in scientific nomenclature. In scientific format, this number is 4.56×10^{-2}. How many significant figures are in 0.045600? In this case, there are five. The zeros following the 6 are significant and tell you about the accuracy of the measurement. In scientific notation, this number is 4.5600×10^{-2}. In scientific notation, all zeros are significant. The real ambiguity comes when you write a number like 3200. How many significant figures are in 3200? You can't really tell. You could mean 3.2×10^2, 3.20×10^2, or 3.200×10^2, which would be two, three, or four significant figures, respectively.

Significant Figures in Calculations

There are a couple of simple rules for using significant figures in calculations.

Multiplication and Division When you multiply numbers, the answer will have the same number of significant figures as the number with the fewest. For example, if you want to calculate the volume of a cube by multiplying the length of the sides, it might look like this:

$$(3.4\,\text{cm}) \times (56.8\,\text{cm}) \times (2.435\,\text{cm}) = 470.2472 \qquad \text{(on our calculator)}$$

How many significant figures can you claim in your answer? The answer is two because the first number you multiplied has only two significant figures. So the answer is $4.7 \times 10^2\,\text{cm}^3$. Division is done exactly the same way.

Addition and Subtraction Addition and subtraction are a little different. When you add strings of numbers, look at the number of decimal places to determine the accuracy of the measurement. The final answer cannot be more accurate than the least accurate number added. For example, if you are adding volumes to get a total, it might look like this:

$$(22.4\,\text{mL}) + (3.5 \times 10^2\,\text{mL}) + (0.543\,\text{mL}) = 372.943\,\text{mL}$$

How many significant figures can you claim? In this case, we can't really look at the significant figures in each term; rather, we look at decimal places. The figure with the fewest decimal places is 350. Therefore, our answer cannot go into tenths and hundredths. The true answer is 373 mL. Notice that the answer has three significant figures whereas one of the numbers added has only two.

1.4 Statistics and Scientific Measurements

As a scientist, you will deal with a great many numbers. We use statistics to help us get more meaning out of the numbers. An example any student can relate to would be test scores. If your friend tells you, "Hey, I got a 40 on the last exam," you would not immediately know whether you should be happy for him or not. How happy you would be might depend on several issues, including the total possible points for the exam, the average score on the exam, the standard deviation, and your score on the exam. You might also need to know whether you could really believe that number. Was the score added correctly? Was it a subjective or an objective score? To truly understand how that exam score relates to your friend's ability in that subject, you also have to know whether it is likely that he would score the same again on a similar exam or is that score overly high or low for some reason. When making measurements during experiments, we need to draw meaning from the numbers we see, and we often use statistics for this purpose.

If 200 students take the first exam in a class, we may list their scores as $x_1 = 85$, $x_2 = 64$, $x_3 = 98, \ldots, x_{200} = 12$. An individual number is usually designated x_i. What can we tell from these data? Students will want to know what their score means. If the professor is using a straight-scale grading system where 90–100% is an A, 80–89% is a B, and so on, then the first student (x_1) knows that she got a B. The third student knows that he got an A. The last student (x_{200}) knows that she should consider dropping the class. If the professor is using a grading curve, then students need to know some statistics to figure out how they are doing in the course.

There are two basic types of statistical measures. The first is a **measure of central tendency.** The one most often seen is the **average,** or **arithmetic mean** (x_{avg}). The average is calculated by adding all the numbers and dividing by the number of scores.

$$\textbf{arithmetic mean } (x_{avg}) = \frac{x_1 + x_2 + x_3 + \cdots + x_n}{n} = \frac{\Sigma x_i}{n}$$

Therefore, we would add the scores for all the students and divide by 200 to get the average on the first exam. If the average turns out to be 85, then the student with a 98 will feel pretty good. The student with an 85 would know that she is at the middle ground. On the other hand, if the average is only 40, then even the student scoring in the 60s will know that he has done better than most. However, to calculate their current grades on a curve, students need more information.

A second type of statistical measure is a **measure of dispersion.** It usually measures the variations in the values compared to the mean. Often such a measure is necessary to truly understand the data presented. If I am the professor of the class taking the exam and I see that, of my 200 students, 180 of them scored an 85 on the exam, with a few being higher and a few lower so that the average was still an 85, I will know that I have a very homogeneous class. Everybody did about the same with just a few exceptions. If, on the other hand, the scores are scattered all over with many students scoring below 40 and many above 90, I will know that the class is heterogeneous. I would adapt my teaching strategy differently to the two classes. When grades are assigned on a curve, it is always the mean and some measure of dispersion that determines the grade.

There are many different measurements of dispersion, and the exact reason for using one or another is beyond the scope of this text. Some of the more common ones are presented next.

$$\textbf{mean deviation} = \frac{\Sigma |x_i - x_{avg}|}{n}$$

where n is the number of samples measured and the $| \ |$ means the absolute value of the difference between the individual value and the mean.

$$\textbf{percent deviation} = \frac{\textbf{mean deviation}}{\textbf{mean}} \times 100$$

$$\textbf{variance} = \sigma^2 = \frac{\Sigma(x_i - x_{avg})^2}{n - 1}$$

$$\textbf{standard deviation} = \sigma = \sqrt{\sigma^2}$$

The mean plus the standard deviation are the two most common statistical parameters. Students taking the exam will want to know their score in relation to the mean and the σ (sigma) because if the class is graded on a strict bell curve, it usually takes a score of the mean $+1\sigma$ to give a grade of B and mean $+2\sigma$ to give a grade of A. Most students' calculators will determine mean and standard deviation for sets of data.

Errors in Experiments

You will often read about errors and error analysis in the data reported for an experiment. There are many sources of error in an experiment, and the term is often used as a catchall to explain numbers that are not perfect. It is important to realize that statistical error may not mean the same thing as the errors that you are more familiar with. When you think of an error, you think of something like multiplying 6 times 9 and getting 42. When scientists think of error, they more often think of differences in members of a population. For example, if you measure the activity of the enzyme lactate dehydrogenase from the serum of ten different people and express it as enzyme units per milliliter of blood serum, you will not get the same number twice. You might have a range of values from 0.1 unit/mL to 26 units/mL. You could calculate the average and standard deviation and report your findings as

$$18.3 \pm 5.3$$

That is error analysis, but the error does not imply that you made a mistake in your work. This is biological error and simply reflects the individual variation in a population. You could have reported the values as mean ± variance or mean ± mean deviation. Each way would have given you different numbers.

If you attempt to pipet 1 mL with a Pipetman and you actually pipet 0.7 mL, there is error in the process. Is it caused by your inability to pipet correctly? Is the Pipetman miscalibrated? In this case, there is a *true* value that is known, so it is easier to determine the source of error. When sampling enzymes in the sera of biological organisms, it is more difficult to know the true value.

Accuracy versus Precision

A measurement is **accurate** if it gives the true value. If you attempt to pipet 1 mL of water, which should weigh 1 g, and the balance reads 1 g after you dispense the solution, your pipetting is accurate. The arithmetic mean is the usual grounds for accuracy.

Measurements are **precise** if the same measurement can be made again and again. For example, if you try to dispense the 1 mL with the pipet ten times and you dispense 0.7 mL ten times in a row, your pipetting was very precise but inaccurate. This usually draws attention for the problem away from you and onto the equipment being used. Measurements of dispersion are the usual criteria for precision.

Another parameter that can be measured in the case of pipetting is the **percent (%) error.** If you are trying to measure a known quantity, such as 1 mL of water, and you expect it to weigh 1 g, the % error will give you a relative estimate of the error of your pipet or your pipetting technique.

$$\textbf{\% error} = \frac{|x_{avg} - x_{true}|}{x_{true}} \times \textbf{100}$$

So, in our pipetting example, if your average had been 0.7 g when it was supposed to be 1 g, the % error would be calculated as follows:

$$\% \text{ error} = \frac{|0.7\text{ g} - 1.0\text{ g}|}{1.0\text{ g}} \times 100 = 30\%$$

1.5 Units

The international system of measurements is known as the SI (from *Système International d'Unités*) and is based on the MKS (meter–kilogram–second) system. The base units for SI are given in Table 1.1.

Many other units are derived from SI units, such as the joule (m^2kg/s^2), the unit of energy. Some non-SI units are also frequently used, such as degrees in Celsius (°C), pressure in atmospheres, etc. The common volume liter is not a SI unit because volume is measured in cubic meters. A liter is actually a cubic decimeter.

TABLE 1.1 *Basic SI Units*

Length	meter	m
Mass	kilogram	kg
Time	second	s
Temperature	Kelvin	K
Electric current	ampere	A
Amount	mole	mol
Radioactivity	Becquerel	Bq

TABLE 1.2 *Prefixes for Multiple Units*

Quantity	*Prefix*	*Abbreviation*
10^{12}	tera	T
10^{9}	giga	G
10^{6}	mega	M
10^{3}	kilo	k
10^{-1}	deci	d
10^{-2}	centi	c
10^{-3}	milli	m
10^{-6}	micro	μ
10^{-9}	nano	n
10^{-12}	pico	p
10^{-15}	femto	f
10^{-18}	atto	a

Multiples of these units are most frequently used in biochemistry, and it is important that you know these. Table 1.2 gives the most common multiples. With these multiples, we arrive at units such as millimeters (mm), micromoles (μmol), etc.

Very, very few numbers in biochemistry exist without units. If you calculate the volume of something to be 12.34 cm^3, your answer will be wrong if you just report the number. It will be just as wrong as if you got the number wrong. It may seem like a small point to forget a unit, but think about the situation in which a physician tells a nurse to prepare a hypodermic syringe with 10 of phenobarbital. What will the nurse prepare? Ten what? Ten milliliters? Ten milligrams? If it is 10 mL, then 10 mL of what concentration? Is the drug absolutely pure or diluted? Without units, the nurse wouldn't know what to prepare.

1.6 Concentration of Solutions

Every experiment you do will use at least one solution of a chemical. When chemicals are used as liquids, they can be either pure liquids or solutions. An example of a pure liquid is absolute ethanol. The chemical is a liquid, but every molecule in the bottle is ethanol with nothing mixed in. A **solution** contains a chemical dissolved in another liquid. When this is the case, you must not only know what the chemical of interest is but also what it is dissolved in and how much of it is dissolved. The chemical in the smaller quantity is called the **solute;** the liquid it is dissolved in is called the **solvent.** When calculating the amount of the solute dissolved in the solvent, you have determined the concentration. The concentration is very important to the biological or chemical function of a liquid. A good example is making a glass of Gatorade from powder. If you follow the directions,

TIP 1.2 When you calculate concentrations, the important thing is the final volume of the solution, not necessarily the amount you add. If you have 100 g of a solid chemical and add 1 L of water, the final volume will be more than 1 L. All calculations based on concentration assume that you have correctly calculated the final volume *after* mixing the solute with the solvent.

you would put one scoop into an 8-oz container of water. If you instead mix three scoops with 8 oz of water, you will still have Gatorade, but its concentration will be totally wrong. It will taste terrible and will not empty from your stomach properly. You might actually become dehydrated during your workout by using it.

Definition of Concentration

A **concentration** is always the ratio of an amount of a chemical divided by the total volume. Distinguishing values that are concentrations from those that are not, which we will call **amounts,** is important. Remember that amounts are additive, but concentrations are not. For example, if we put 1 g of salt in a beaker, that is an amount. If we bring the volume up to 1 L with water, then we have a 1-g/L solution, which is a concentration. If we have 1 millimole (mmol) of salt in a beaker, that is an amount. If we bring the volume up to 1 liter with water, then we have a 1 mM (millimolar) solution, which is a concentration.

If we have 1 g of salt in one beaker and 1 g of salt in another beaker and we add them together, we will know that we have 2 g of salt. **Amounts are always additive.** If we have a solution that is 1 g/L and we add it to a solution that is 2 g/L, we do *not* get a solution that is 3 g/L. In fact, the solution would have a concentration between 1 and 2 g/L based on the volumes that we added. **Concentrations are not additive.**

Percent Solutions

Solutions based on percent are the easiest to calculate because they do not depend on a knowledge of the molecular weight. Your instructor can give you a tube with an unknown white powder and tell you to make up a 1% w/v solution in water, and you can do it without knowing anything about the white powder.

5% sucrose
5gm
how to make

> **% w/v** means percent weight to volume and has units of grams/100 mL. Therefore, a 1% w/v solution has 1g of solute in a total of 100 mL of solution.
>
> **% v/v** means percent volume to volume and has units of milliliters/100 mL. Therefore, a 1% v/v solution of ethanol has 1 mL of pure ethanol in 100 mL of total solution.

You can assume that, unless told otherwise, the solvent is water for any solution.

TIP 1.3 Derivatives of molar solutions, such as mM, μM, and nM, still refer to an amount divided by liters of solution. Many students mistakenly think that a 1-mM solution means 1 mmol in 1 mL. What it actually means is 1 mmol in 1 L!

Molar Solutions

The most common types of solutions are molar solutions. A 1-M solution means 1 mol of solute in a total volume of 1 L. A 1-mM solution has 1 mmol (10^{-3} mol) in a total of 1 L of solution. A 1-μM solution has 1 μmole (10^{-6}) of solute in a total of 1 L of solution.

Remember that moles are calculated by dividing grams by the formula weight in grams per mole. For example, if we have 13 g of compound X and its formula weight is 39 g/mol, it means that 1 mol of compound X weighs 39 g. If we have 13 grams of it, we calculate moles thusly:

$$13\text{ g} \div 39\text{ g/mol} = 0.33\text{ mol}$$

If we then take the 13 g and bring it to 0.5 L with water, our molar concentration will be

$$0.33\text{ mol} \div 0.50\text{ L} = 0.666\text{ mol/L} = 0.67\text{ M}$$

1.7 Dilutions

10 fold dilution

100 fold dilution

Dilutions seem to be one of the hardest concepts for most beginning biochemistry students. Even after chanting the mantra **"Dilutions are easy, dilutions are fun, dilutions make sense,"** students still struggle. Doing problems is the only way to really understand dilutions. A book such as Irwin Segel's *Biochemical Calculations* can also be a big help.

When doing a dilution, you always start with a concentration of something and add more solvent to it, thereby lowering the concentration. *Think about that!* If you do your calculations and determine that the concentration increased, you have made a mistake. Check your work each time by making sure that your concentration did in fact decrease with your dilution.

There are two simple ways of doing all dilution problems: the C_1V_1 method and the dilution factor method. Both methods are essentially the same if you understand math but also have subtle differences, which you may or may not appreciate.

C_1V_1 Method

This method uses the formula

$$C_1V_1 = C_2V_2$$

and is good for those of you who are less comfortable with math. It is very reproducible and always works, once you know how to define the variables.

The disadvantage is that it is slower and you generate worthless intermediates if you have multiple dilutions. It is, however, a good starting point.

Practice Session 1.1

We have a sodium phosphate buffer at a concentration of 0.2 M (mol/L). Take 10 mL of the buffer and add it to 90 mL of water. What is the final concentration after the dilution?

First, to use the formula, you need three of the four variables:

C_1 = initial concentration, which in this case is 0.2 M

V_1 = initial volume, which is 10 mL

C_2 = final concentration, which is what we want to know

V_2 = final volume. **This is a potential danger point!** The final volume is 100 mL. Remember that the volume of solvent added (that is, 90 mL) is relevant only in that it usually gives us the total volume. Sometimes 10 mL + 90 mL will not equal 100 mL, but for this example we assume that it does. For the calculation, you always use the total volume, so

$$C_1V_1 = C_2V_2$$

$$(0.2\,\text{M})(10\,\text{mL}) = C_2(100\,\text{mL})$$

$$C_2 = \frac{(0.2\,\text{M})(10\,\text{mL})}{100\,\text{mL}} = 0.02\,\text{M}$$

Dilution Factor Method

The dilution factor method is faster and better for multiple dilutions. We define a dilution factor to be the final volume divided by the initial volume. This is just a convention, but we use it consistently throughout this book. Therefore, in our previous example, the dilution factor is 100 mL/10 mL = 10, or equally said, a 10-to-1 dilution.

Now that we have the dilution factor, what do we do with it? Well, there are really only a couple of things we can do. We can either multiply it by C_1 or divide it into C_1. One possibility gives an answer greater than what we started with, which we know can't be right. Therefore,

$$C_2 = \frac{C_1}{D_f}$$

$$C_2 = \frac{0.2\,\text{M}}{10} = 0.02\,\text{M}$$

Can you see that the two methods really are the same thing?

The reason that so many students get answers that indicate the concentration increased with dilution is because of nomenclature. Many books refer to a dilution as a 1 to 10, or 0.1. They get that by defining a dilution factor as initial volume over final volume. There is nothing wrong with doing that, but you will have to then multiply your initial concentration by your dilution factor. Any way you do it that is consistent should work for you, but we will always be consistent and do it the way described.

Multiple Dilutions

When you have multiple dilutions, using the dilution factor method is more efficient. If, for example, we make a dilution three times, calculate the final concentration thusly:

$$D_{f_1} = \frac{100 \text{ mL}}{10 \text{ mL}} = 10$$

$$D_{f_2} = \frac{50 \text{ mL}}{2 \text{ mL}} = 25$$

$$D_{f_3} = \frac{90 \text{ mL}}{30 \text{ mL}} = 3$$

$$D_{f_{\text{total}}} = 10 \times 25 \times 3 = 750 \text{ to } 1$$

If we start with the same 0.2-M solution, calculate the new concentration as follows:

$$C_{\text{final}} = \frac{C_1}{D_{f_{\text{total}}}} = \frac{0.2 \text{ M}}{750} = 0.00027 \text{ M}$$

$$= 0.27 \text{ mM}$$

$$= 270 \ \mu\text{M}$$

The final conversion from molar to millimolar to micromolar will eventually be simple for you. Refamiliarize yourself with the metric system so that you can do such conversions effortlessly.

1.8 Graphing

Many experiments in this book will require you to make graphs as you analyze your data, so we will spend some time reviewing graphing basics. The first part assumes that you will be drawing the graph by hand instead of using a computer program. Understanding these concepts before trying to make a computer do this for you is important.

Drawing Graphs by Hand

A graph is a plot of two quantities. One is something that you control and is called the **independent variable;** usually it goes on the *x* axis. The other is the quantity that changes as you change the independent variable. It is called the **dependent variable** and goes on the *y* axis. For instance, if you put varying quantities of protein in a series of test tubes and then measure the absorbance, the variable you control is the quantity of protein, so it goes on the x axis. The absorbance is what changes as you change the protein quantity, so it goes on the y axis. By convention, "plot p versus q" means that p is on the y axis. **Always use graph paper!** A graph scribbled on binder paper is *not* a graph.

Units and Exponents Sometimes it is challenging to figure out what values to plot. Let's say we did a protein determination and recorded the adjacent data.

Quantity of Protein	*Absorbance*
0.001 mg	0.05
0.003 mg	0.16
0.005 mg	0.225
0.007 mg	0.33
0.008 mg	0.375

It would be particularly messy to plot those values in that form due to the number of zeros. The best thing to do is to change the units so that easier numbers can be plotted. The easiest way is to plot micrograms (µg) instead of milligrams (mg). That makes nice numbers like 1, 3, 5, 7, and 8 (Figure 1.1). Another way is to use exponents. Most science students make a mistake with the usage of exponents because most graphs that they have seen have not been done with standard scientific nomenclature. Notice that the x axis is labeled mg • 10^3, *not* • 10^{-3}. For scientific writing, the exponent indicates what you have already done to the number to put it on the graph with that scale, not what you expect the reader to do. In other words, the first value was 0.001 mg. To get rid of all of the zeros, we multiplied by 1000 to give a final value of 1. Therefore, on the axis we indicate that the value represents the milligrams of protein multiplied by 10^3.

Drawing the Line To make a graph, you must know something about the system and what you expect to see. When we do a protein assay, for example, we expect to get a straight line, at least to a certain limit of protein in the tube.

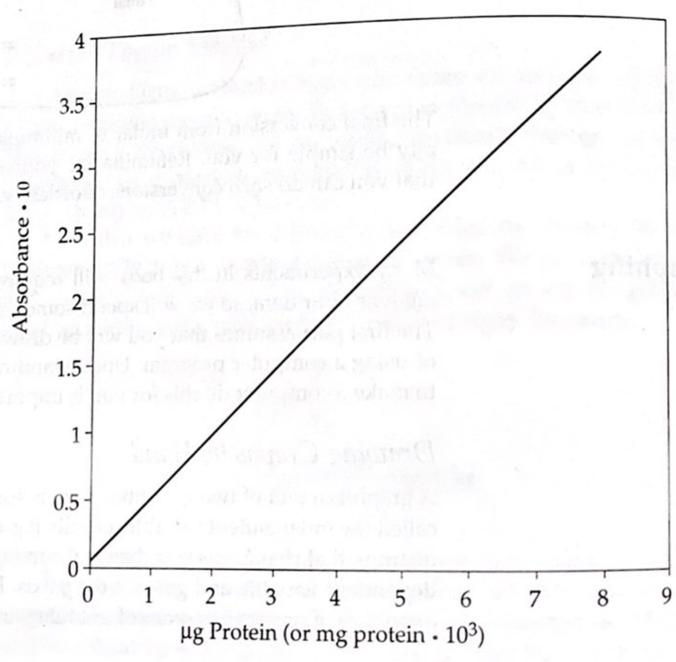


FIGURE 1.1 *A proper linear graph*

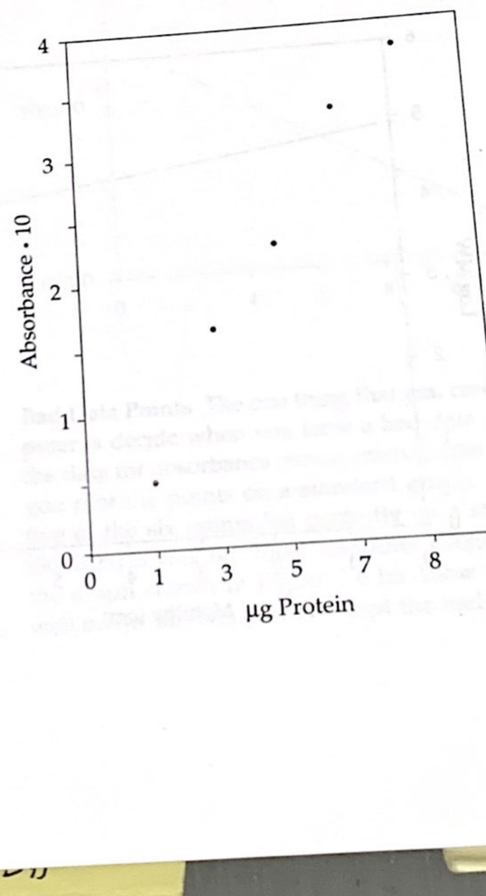
Therefore, we try to draw the best straight line that we can through the points. You can do this by using a computer program to draw the line for you (discussed later), by using a calculator with a statistics function to tell you the slope of the line, or by visually putting down the line that goes through the most points. In Figure 1.1, it is a mistake to just "connect the dots," thereby giving you a zigzaggy line.

When the relationship is expected to be linear, the best line is determined by **linear regression.** This is a mathematical process in which the line is placed to minimize the area between the points and the line. This is also called the **least-squares method.** Calculators and computers can do this easily, but your eyes cannot.

Constant Scale It is also important to remember that the scale must be constant. If you define 1 cm on the graph to be 1 µg as in Figure 1.1, then that scale must be maintained. A common mistake is to change the scale to fit your data instead of plotting your data on a given scale. This mistake is shown in Figure 1.2. This gives a very ugly line because the proper distance between the points is not maintained.

Use the Whole Graph Figures 1.1 and 1.2 are actually a bit small. It is generally best to use as much of the graph as possible. Also, the closer the line is to a 45° angle, the more accurate you will be when interpolating an unknown from it.

FIGURE 1.2 *An improper graph*



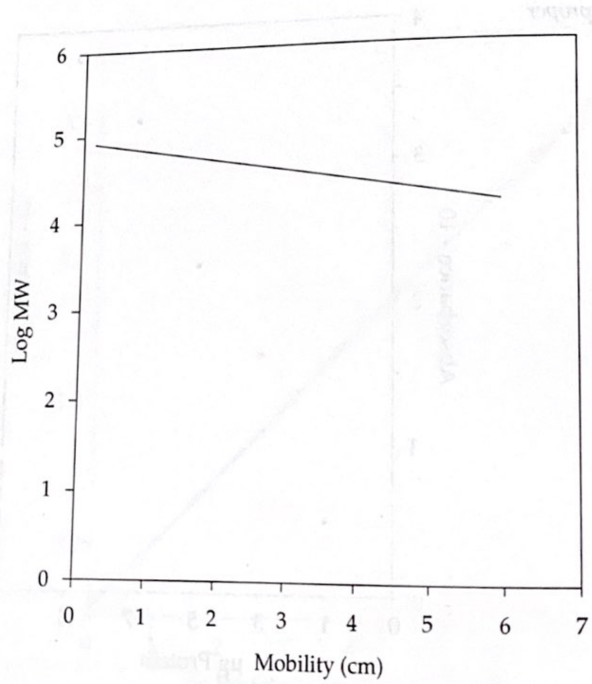
Must Zero Be a Point on the Graph? Most people naturally put the origin (0, 0) on the graph. Most of the time, that is correct, but there are times when it is inappropriate. A common reason not to include zero is when you are graphing log molecular weight (MW) versus mobility. This is done after doing an experiment with gel electrophoresis or gel filtration. For example, let's plot the log of MW versus mobility for the adjacent values.

MW	Log MW	Mobility (cm)
15,000	4.18	6
40,000	4.60	2.9
66,000	4.82	1
90,000	4.95	0.1

If we start the graph at the origin, we get a graph that looks like Figure 1.3. This graph is very compressed on the y axis and does not use much of the graph paper. There would be huge error if we tried to interpolate within this graph. For this type of graph, it is much better to start the graph at 4 on the y axis and go until 5, as in Figure 1.4.

Log Scales You will make many graphs using a log scale such as in Figure 1.4. However, there is a much easier way to plot such data. Buy some semilog paper, which does the calculations for you. Log paper is divided into cycles. A cycle is for data points that are all within one power of 10. The data from the preceding example fit onto one cycle because 15,000 – 90,000 are all within one power of 10. If we want to plot the log of 200,000, then we would need a second cycle. With log paper, just plot the numbers 15,000, 40,000, 66,000, 90,000, and 200,000 directly on the graph on the y axis and the paper takes the log for you. **Do not take the log and try to plot 4.18, 4.60, . . . on log paper.** Figure 1.5 shows how these data are plotted on two-cycle semilog paper.

FIGURE 1.3 *An unacceptable log-scale graph*



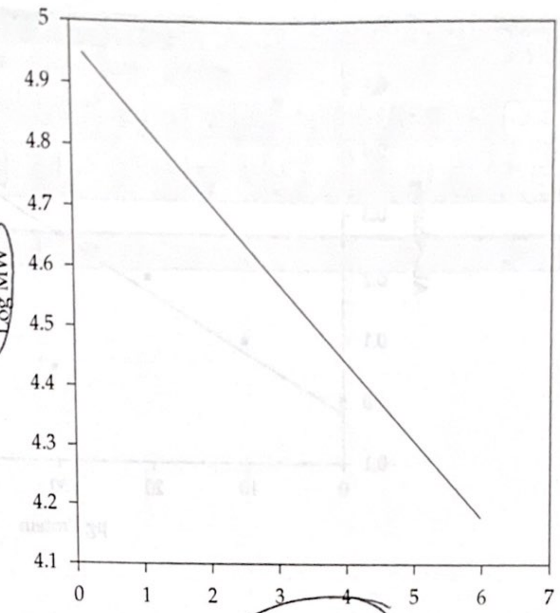


FIGURE 1.4 *A proper log-scale graph*

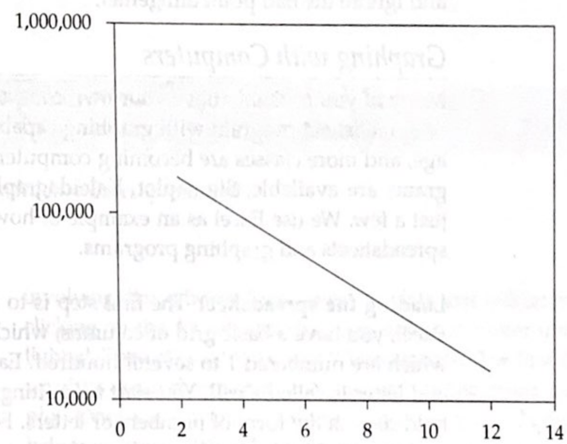


FIGURE 1.5 *Two-cycle semi-log graph*

TABLE 1.3 *Absorbance versus Micrograms of Protein*

μg Protein	*Absorbance*
0	0
10	0.10
20	0.20
30	0.05
40	0.40
50	0.50

Bad Data Points The one thing that you can do manually better than a computer is decide when you have a bad data point. Let's say we want to plot the data for absorbance versus micrograms of protein shown in Table 1.3. If you plot the points on a standard graph, you will most likely notice that five of the six points fall perfectly on a straight line. However, one point (30, 0.05) is way off. Most computer programs default values would draw the graph shown in Figure 1.6 for these data. As you can see, the line is well below all data points except the bad one. Most of us would choose to

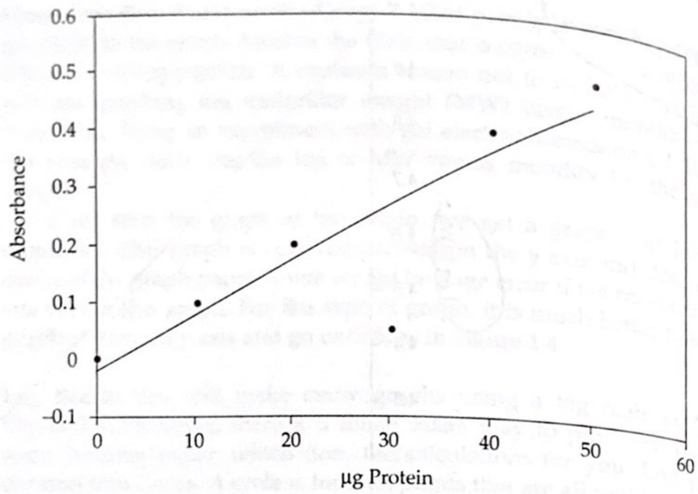


FIGURE 1.6 *Linear regression for a graph with a bad data point*

redo the 30-μg tube rather than report data of this caliber. If that were not possible, we would probably draw the line through the five perfect points and ignore the bad point altogether.

Graphing with Computers

Many of you probably have your own computer with a graphing program or spreadsheet program with graphing capability. We live in the computer age, and more classes are becoming computer driven. Many graphing programs are available; Sigmaplot, Kaleidagraph, Quattropro, and Excel are just a few. We use Excel as an example of how you can analyze data using spreadsheets and graphing programs.

Loading the spreadsheet The first step is to set up the spreadsheet. With Excel, you have a basic grid of columns, which are labeled A–Z, and rows, which are numbered 1 to several hundred. Each combination of a number and letter is called a **cell.** You start by putting data into the cells. Cells can hold data in the form of numbers or letters. For most applications that we are interested in, we put in numbers. If you want to plot absorbance versus micrograms of protein for the Bradford protein assay, you might have data that look like that shown in Table 1.4.

TABLE 1.4 *Absorbance versus Micrograms of Protein for Bradford Assay*

μg Protein	*Absorbance*
0	0
10	0.10
20	0.21
30	0.29
40	0.40
50	0.52

When you load this into Excel, you can put the column labels (μg Protein and Absorbance) into the spreadsheet, but that is not necessary when planning to use the data to make a graph. The program will "talk" you through creation of the graph, including labeling the axes. It is best to put the values that will be on the *x* axis into the left-hand column because it simplifies the graphing process. Figure 1.7 shows what the spreadsheet looks like.

Creating the Graph Once you have loaded the data into the spreadsheet, you are ready to create the graph. Excel provides a user-friendly process

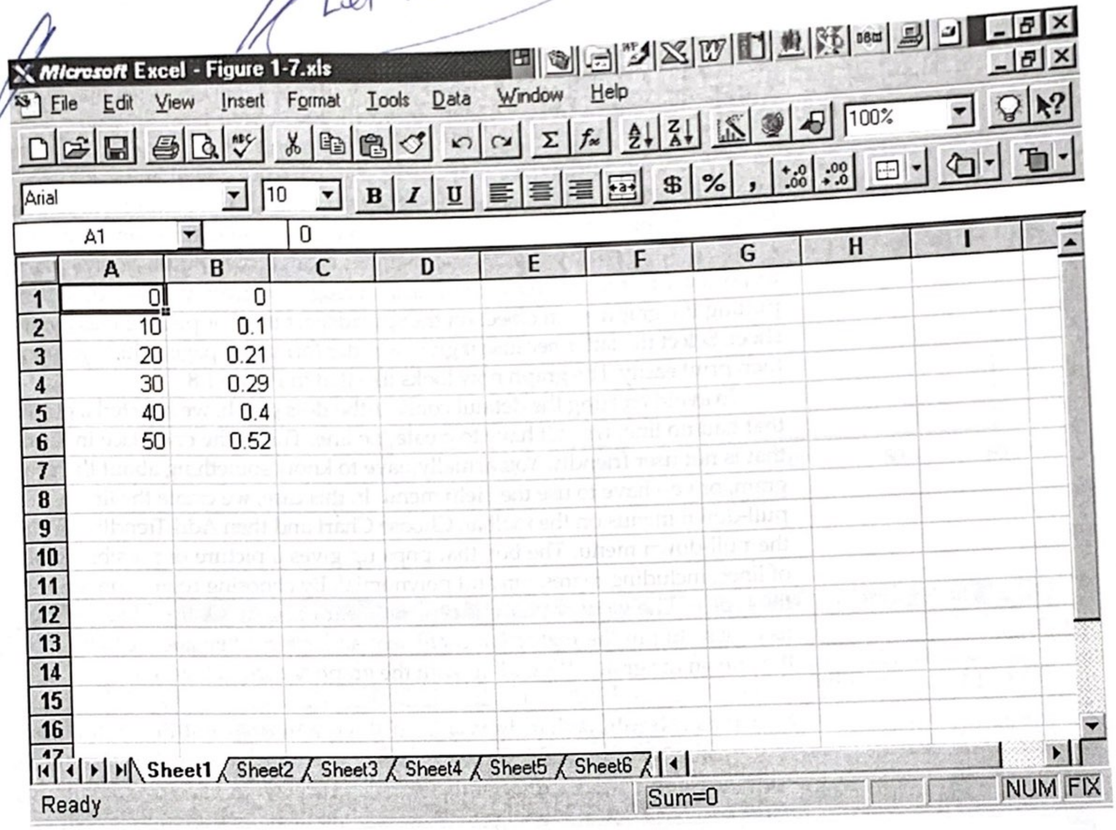


FIGURE 1.7 *Graphing data loaded into Excel spreadsheet*

involving dialog boxes. First, select the data that will be put into the graph by clicking on the A1 cell and dragging down and over until all data are highlighted. Then click on the Chart Wizard button. The first dialog box that pops up gives you a choice of chart types, such as bar graph, pie graph, and scatter plot. Once you choose a basic type, it gives you subchoices and pictures to help you choose. We will choose to do the XY scatter plot, which is normally what you will want to do. *Here is a potential danger point of using a computer to make a graph:* The default value for many computer programs has straight lines connecting the points (the dreaded connect-the-dot graph). This is usually wrong in biochemistry. What you want is a best-fit line of some type; in our case, it is a linear regression line. At this point in the process, choose the XY scatter plot that does not attempt to put any line over the points; the line will be put in later. With whatever program you are using, you must learn how to make the program give you the type of graph you want. Don't rely on the default values. With Excel, click on Next to continue the dialog. The next box that pops up (box two of four) asks about chart source data. If you have correctly selected the data from the columns you want, go on to the next box by clicking on Next. This brings us to box three of four in the dialog process. This is the chart-formatting step in which you decide how the graph is to look. Select the Title tab and then add in the graph title and the title of both axes. Select the Gridline tab that allows you to decide how many gridlines, if any, will appear on the graph. At each step, a minigraph picture shows you how the graph currently looks, so you can continue to make changes until it looks the way you want. Anytime that you change your mind, click on Back to go back to the last box. The fourth dialog box gives you the option of putting the graph as an object on the spreadsheet itself or putting it as a new sheet. Select the latter because it gives a better formatted page, which you can then print easily. The graph now looks like that in Figure 1.8.

To avoid creating the default connect-the-dots graph, we selected a graph that had no line; we still have to create the line. This is the one place in Excel that is not user friendly. You actually have to know something about the program, or you have to use the Help menu. In this case, we create the line using pull-down menus on the toolbar. Choose Chart and then Add Trendline from the pull-down menu. The box that pops up gives a picture of possible types of lines, including regression and polynomial. By choosing regression, we get the type of line we want for this type of linear data. An Options tab also lets us choose to put the regression coefficient and the mathematical equation of the line on the graph. We end up with the graph shown in Figure 1.9.

Nonlinear Graphs What do you do if the graph does not depict a linear relationship? Making such a graph starts out the same way, but then you use the Chart Wizard differently to customize your graph. A common example of this is analyzing an enzyme kinetics experiment, such as in Chapter 8. When enzyme velocity is plotted versus substrate concentration, a hyperbolic relationship is seen. Figure 1.10 shows typical enzyme

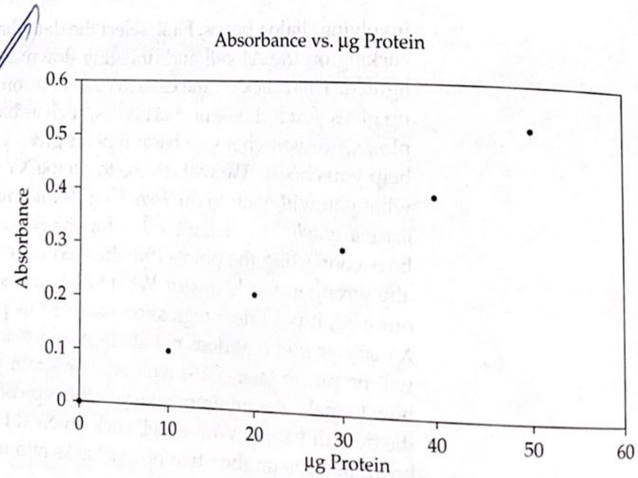
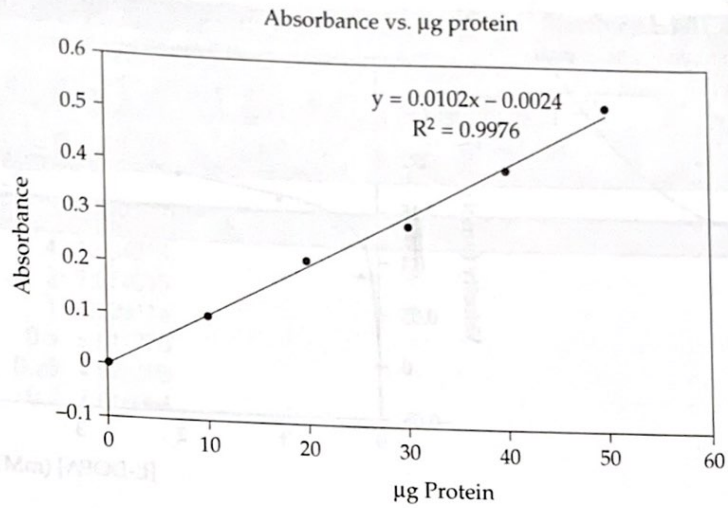
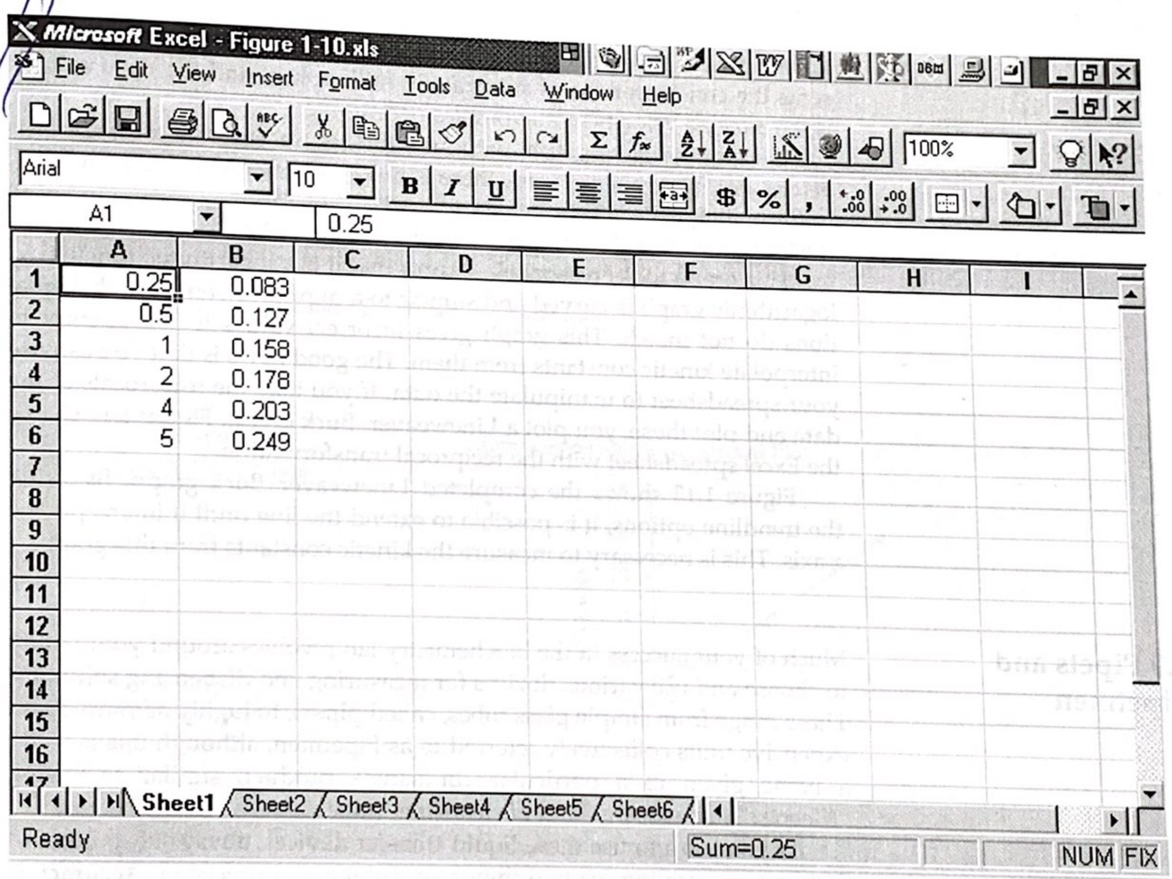


FIGURE 1.8 *Absorbance versus µg protein from the Excel graphing demonstration*

FIGURE 1.9 *Customized graph of data presented in Figure 1.7*





	A	B
1	0.25	0.083
2	0.5	0.127
3	1	0.158
4	2	0.178
5	4	0.203
6	5	0.249

FIGURE 1.10 *Enzyme kinetic data loaded into Excel spreadsheet*

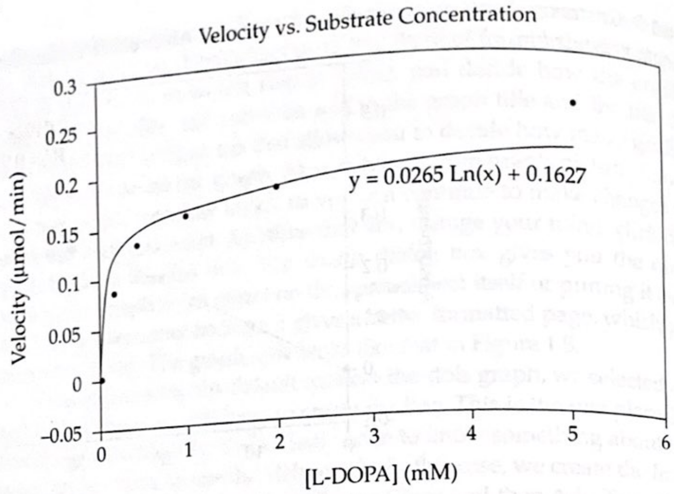


FIGURE 1.11 *Logarithmic graph of enzyme kinetic data* (incorrect)

kinetic data as they appear in an Excel spreadsheet. The left column represents the concentrations of substrate in millimolars, and the right column is the enzyme velocity in micromoles per minute.

Once the original graph, without a line, is created, add a trendline as before. For hyperbolic graphs, there is no correct menu choice with Excel, unfortunately. The best you can do is select Logarithmic when you get to the trendline. This gives the graph shown in Figure 1.11.

This graph looks reasonable, except that it is a logarithmic function. A logarithmic graph is curved and similar to a hyperbolic one, but the equations do not match. This graph gives incorrect values if you attempt to interpolate kinetic constants from them. The good news is that you can use your spreadsheet to manipulate the data. If you take the reciprocals of the data and plot those, you plot a Lineweaver–Burk graph. Figure 1.12 shows the Excel spreadsheet with the reciprocal transformation.

Figure 1.13 shows the completed Lineweaver–Burk graph. By using the trendline options, it is possible to extend the line until it intercepts the x axis. This is necessary to measure the kinetic constants from this graph.

1.9 Pipets and Pipetmen

Much of your success in the biochemistry lab revolves around your ability to choose and use various devices for measuring and dispensing solutions. These range from simple glass tubes, called pipets, to highly advanced and expensive units collectively referred to as Pipetmen, although this is really a name given to a particular company's product, similar to saying "Kleenex" to mean any kind of cleansing tissue.

As you learn to use these liquid-transfer devices, always keep in mind that you are striving for two things—accuracy and precision. **Accuracy** is the relation between the volume you dispense and the volume you wanted

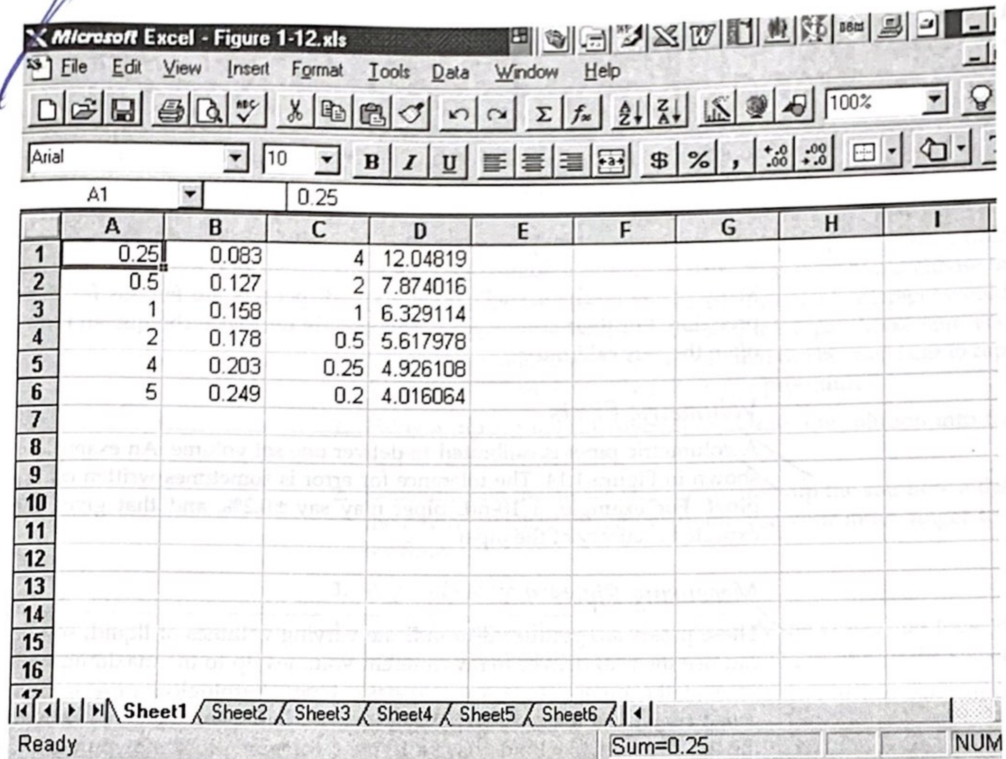


FIGURE 1.12 *Excel spreadsheet for linear transformation*

FIGURE 1.13 *Reciprocal graph of enzyme kinetic data*

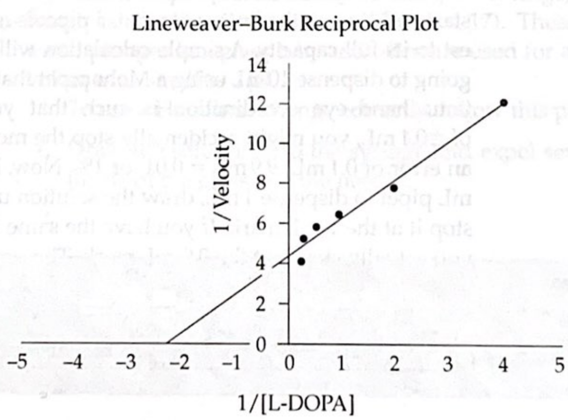


FIGURE 1.14 *Volumetric pipet*

to dispense. If you have a pipet and are trying to dispense 100 μL but actually dispense 70 μL, the volume was very inaccurate. **Precision** has to do with reproducibility. If you are to dispense 100 μL into each of five tubes and what you actually dispense is 70.0 μL, 69.9 μL, 70.2 μL, 70.1 μL, and 69.8 μL; then the volumes were very inaccurate but were very precise. Many of the mechanized Pipetmen-type dispensers are famous for their precision, but their accuracy depends greatly on *your* technique and how often they are calibrated.

Volumetric Pipets

A volumetric pipet is calibrated to deliver one set volume. An example is shown in Figure 1.14. The tolerance for error is sometimes written on the pipet. For example, a 10-mL pipet may say ±0.2%, and that gives the expected accuracy of the pipet.

Measuring Pipets

These pipets are graduated to indicate varying volumes of liquid, so you can use them to deliver many different volumes up to the maximum volume of the pipet. There are two basic types. **Serological pipets,** often called **blowout pipets,** are graduated to include the volume all the way to the tip (Figure 1.15). If you have a 10-mL serological pipet and you want to deliver 10 mL, bring the volume all the way up to the zero line and then expel all the liquid. **Mohr pipets** are not graduated to the tip; to dispense the same 10 mL, let the meniscus run down from the 0 line and then stop it at the 10 line (Figure 1.16).

Because your accuracy depends on how well you can get the meniscus to start and stop on the lines you want, a pipet is most accurate when used closest to its full capacity. A simple calculation will verify this. Suppose you are going to dispense 10 mL using a Mohr pipet that has graduations of 0.1 mL. If your hand–eye coordination is such that you routinely have an error of ±0.1 mL, you might accidentally stop the meniscus on the 9.9 line. That is an error of 0.1 mL/9.9 mL = 0.01, or 1%. Now, if you try to use the same 10-mL pipet to dispense 1 mL, draw the solution up to the 0 line and attempt to stop it at the 1-mL mark. If you have the same inaccuracy in your technique, you actually stop it at the 0.9-mL mark. The error is 0.1 mL/0.9 mL = 0.11, or

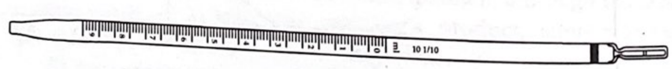


FIGURE 1.15 *Serological, or blowout, pipet*

FIGURE 1.16 *Mohr pipet*