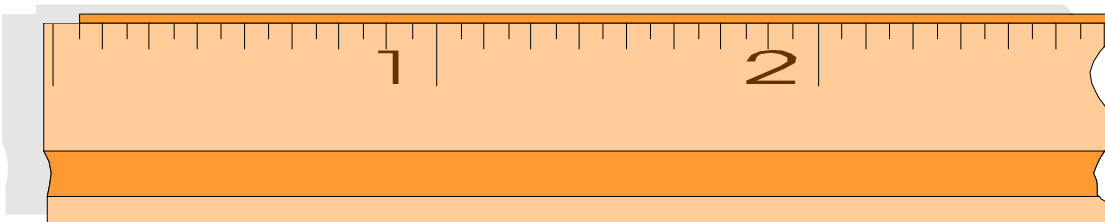


COC Biotechnology Program



Metric System, Dimensional Analysis, Volumetric Determination and Error

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- The metric system is a decimal system of measurement of such features as: length, mass, area, and other physical conditions of objects.
- Dimensional analysis is the conversion of one unit of measurement to a different unit of measurement (i.e. inches to centimeters.)
- The conversion is accomplished using equalities and proportions.
- Proper dimensional analysis uses conversion factors that are equal to one, the units cancel accordingly.
- Volumetric analysis is the transfer of a given amount of solution from one container to another with an emphasis on accuracy.
- Transfer of solution can be either on a large scale (L or ml) or on an extremely small scale (μl or pm)
- For small solution transfers (the most common in a biotechnology laboratory), a pipette or micropipette is used.
- Solution preparation involves conversion factors, volumetric transfer and familiarity with molar equations.
- These topics are further explored in supplementary reading and assignments.
- *The Molarity and Dilutions and Serial Dilutions exercises (at the back of this handout) should be completed after doing the Concentration Calculations and Expressions lab (due February 3/see syllabi)*

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I. Objectives:

1. To change one unit of measure to a different unit of measure using conversion factors and to become familiar with the metric system
2. Determine the percent error of volumetric transfers inherent in different volumetric devices.
3. Practice calibration, reading and solution transfer using serological and micropipettes.
4. Become proficient at calculating solution concentrations using a variety of methods (molarity, percent solution, serial dilution, pH calibration, etc.)

II. Background:

Accurate measurement is the cornerstone of modern science. It is important to understand that certain features of scientific measurement have become standard methods or practices that are accepted as being *the* way a certain process or procedure is done. One such standard in science is the use of the metric system, which is a decimal system of measurement of such features as: length, mass, area and other physical conditions of objects.

In the metric system, larger and smaller units are easily expressed by changes in the prefix of the name of the unit of measure. These prefixes reflect changes in multiples of 10, making all measurements easily converted by movement of the decimal point.

While there are many prefixes in the metric system the following table shows those most commonly used:

Conversion factor	Prefix	Symbol
1,000,000,000 = 1.0×10^9	giga	G
1,000,000 = 1.0×10^6	mega	M
1,000 = 1.0×10^3	kilo	K
100 = 1.0×10^2	hecto	H
1 = 1		
0.1 = 1.0×10^{-1}	deci	D
0.01 = 1.0×10^{-2}	centi	C
0.001 = 1.0×10^{-3}	milli	M
0.000001 = 1.0×10^{-6}	micro	μ
0.00000001 = 1.0×10^{-9}	nano	n

The prefixes mentioned above are attached to base values. These values define the type of measurement, i.e., length, mass, etc. By convention prefixes greater than one are often capitalized and those less than one are often lower case when used without the unit.

length = **meter (M)**
mass = **gram (g)**
liquid volume = **liter (l)**
temperature = **Kelvin/Celsius**

So a centimeter is 1/100th of a meter while a kilometer is 1000 meters, and a millimeter is 1/1000th of a meter. By simply changing the prefix, you are able to create measurements with vastly different units.

It is often necessary to convert one unit of measure to another, i.e. one that is either larger or smaller. This is done using **dimensional analysis**, a process that employs **conversion factors** as part of a methodical approach to the solution of the problem. Dimensional analysis requires that you define the units to be converted from and those to which this value will be converted. Determine the value relationship of each and then make up an appropriate conversion factor; for instance, if one wishes to determine the number of meters in 12.5 kilometers, the first step is to determine the correct conversion factor for kilometers to meters. Since there are 1000 meters in one kilometer the conversion factor would be:

$$\frac{1km}{1000m} \text{ Conversion factors can also be flipped, so... } \frac{1000m}{1km}$$

Note: The conversion factor does equal one, as any value over its equal does equal one!

The actual dimensional analysis is:

$$12.5 \text{ km } \frac{(1000 \text{ m})}{(1 \text{ km})} = 12,5000$$

As a general rule for dimensional analysis always put the unit you start in on the bottom of the next conversion factor. Just as in a typical math equation you can now cross cancel the units on the top and bottom (In this case you could cancel out “the unit you are in”):

$$\frac{\text{The unit you are in}}{\text{The unit you are in}} \times \frac{\text{the unit you want to convert to}}{\text{The unit you are in}}$$

THERE ARE TWO GOLDEN RULES OF Dimensional Analysis:

1. **Make sure that the conversion factors are valid** (example: 1 inch = 2.540cm).
2. **Ensure that the units cancel.** As you proceed, the respective numerators and denominators should cancel nicely. This idea is especially crucial in multi-unit conversions (example problem set 2.)

When it comes to general dimensional analysis, there are three types to be concerned with.

1. **Single unit conversion:** As the name implies, these are the simplest of the conversions. The above is an example and here are a few more:

With the knowledge that: * 1 gallon = 3.85 liters, 1 ounce =28 grams, 1 meter =39 inches

Lets try some:

How many liters in 3.5 gallons?

How many grams in 2.6 ounces?

$$3.5 \text{ gallons } \frac{(3.85 \text{ liters})}{(1 \text{ gallon})} = 13.48 \text{ liters}$$

$$2.6 \text{ ounces } \frac{(28 \text{ grams})}{(1 \text{ ounce})} = 72.0 \text{ grams}$$

Notice that both golden rules of dimensional analysis are being adhered to! Make sure you clearly understand these examples before moving on to example set 2, as they will get a little more complex.

2. Multiple unit conversions: While this sounds more intimidating, it is really just a series of single unit conversions put back to back. The golden rules are especially crucial here as your chances of messing up are compounded by the multiple conversions. Also, multiple unit conversions help you by not requiring you to memorize every possible conversion factor. Instead you can use a series of more familiar conversion factors and link them together. Let's try the example mentioned below.

How many micro-liters in a 2.5 pints?

Start by verifying the accuracy of the simple conversion units.

$$1 \text{ pint} = 0.5 \text{ quarts} \qquad 1 \text{ quart} = 0.962 \text{ liters} \qquad 1 \text{ liter} = 10^6 \text{ micro-liters}$$

Please note, these conversion factors are either common knowledge, or can be calculated by knowledge of previously given conversion factors and/or the metric conversions.

There are many routes to this answer, but for brevity, the shortest has been provided.

$$2.5 \text{ pints} \left(\frac{0.5 \text{ quarts}}{1 \text{ pint}} \right) \left(\frac{0.962 \text{ liters}}{1 \text{ quart}} \right) \left(\frac{1 \times 10^6 \text{ microliters}}{1 \text{ liter}} \right) = 1.203 \times 10^7 \text{ microliters}$$

Notice that the units all cancel and that each individual conversion is valid.

3. Multi-dimensional analysis: This level of analysis is again just a more complex form of dimensional analysis. The golden rules still apply; you just need to ensure that the conversion factors are valid. Also, some of the conversion factors are more sophisticated in the fact that they can be used to convert one unit of measure to another. The reason for this is that the metric system has been designed to allow this. For instance, 1 ml of water weighs one gram and takes up 1 cubic centimeter of space ($1 \text{ ml} = 1 \text{ cm}^3$). Using this conversion factor we can go from liquid volume (ml) to the volume of a solid (cm^3). These observations are contingent on a few realities. For instance, the water must be pure, at about room temperature and at atmospheric conditions.

Despite the qualifications, these conversion factors can be very powerful. If you use them, just ensure that the conditions of the solution do not deviate substantially from the above mentioned conditions (e.g. not too warm, concentrated (i.e. too salty, etc.))

How many microliters are in 1.6 lbs of water?

$$(1 \text{ lb} = 454 \text{ grams}) \quad (1 \text{ gram} = 1 \text{ milliliter})$$

$$1.6 \text{ lbs} \left(\frac{454 \text{ grams}}{1 \text{ lb}} \right) \left(\frac{1 \text{ milliliter}}{1 \text{ gram}} \right) \left(\frac{1000 \mu\text{l}}{1 \text{ ml}} \right) = 7.26 \times 10^5 \mu\text{l}$$

Ready for a really scary one? Try this one on for size. A cube of water, 0.6 meters on side is filled with salty water of a density of 1.045 grams/ milliliter. What is the weight of this cube in ounces? Come see me if you want to check your answer, need hints, etc...

Metric Conversions Worksheet

A. Which metric unit and prefix would be used to measure the following (if no prefix is used write "none")

length = _____ inches = _____

weight = _____ pounds = _____

temperature = _____ ounces = _____

liquid volume = _____ feet = _____

yards = _____ gallons = _____

pints = _____ miles = _____

B. Circle the larger unit of measurement.

m or cm dl or l kg or μg

μm or m ml or μl mg or g

μm or cm μl or cl μg or mg

dm or m l or ml g or kg

C. Write the two golden rules of dimensional analysis:

1.

2.

D. Complete the following relationships. Show the dimensional analysis.

1 m = _____ cm

10 μl = _____ ml

4 mg = _____ g

0.5 m = _____ mm

6.13 l = _____ ml

115 cm = _____ m

7.57 g = _____ μg

2.3 ml = _____ μl

E. Answer the following questions using dimensional analysis.

1. What is your height in m? cm?
2. What is your ideal weight in g? kg?
3. At what temperature does water boil in Celsius? In Kelvin?
4. How far is COC from your home in km? m?
5. What is the average speed you drive to COC in km/hr? m/hr?
6. How much coffee/tea do you drink in a day in μl ? ml?
7. What is room temperature in Celsius?
8. How big of a steak (or tofu patty if you are a vegetarian) do you like to eat in g? In mg?
9. What is your body temperature in Celsius?
10. What is the length of your hand in mm? km?

B. During the course of the semester you will be working with very small volumes. To prepare you for this, do the following conversions for liquid measurements and indicate which instrument you would use to measure each volume (choose from micro-pipette, transfer pipet, serological pipette or volumetric glass ware). Take a minute to review the actual equipment to help you with your decisions. For most answers, there is one best choice, with some latitude.

	<u>Volume</u>	<u>Instrument</u>
1.	$10 \mu\text{l} = ?\text{ml}$	
2.	$250 \mu\text{l} = ?\text{ml}$	
3.	$514 \mu\text{l} = ?\text{ml}$	
4.	$25 \mu\text{l} = ?\text{ml}$	
5.	$4 \mu\text{l} = ?\text{ml}$	
6.	$3 \text{L} = ?\text{ml}$	
7.	$4 \mu\text{l} = ?\text{ml}$	
8.	$1.2 \text{ml} = ? \mu\text{l}$	
9.	$150 \text{L} = ? \mu\text{l}$	
10.	$.08 \text{ml} = ? \mu\text{l}$	
11.	$20 \text{ml} = ? \mu\text{l}$	
12.	$12 \text{ml} = ? \mu\text{l}$	

What is Avogadro's Number

What is used for?

Molecular Weights and Formula Weights

Write the molecular weights for the following elements.

Calcium	Oxygen
Carbon	Magnesium
Sulfur	Potassium
Helium	Phosphorous
Iron	Hydrogen
Sodium	Chloride

Calculate the formula weights for the following compounds. (Show your work.)

Water

Carbon Dioxide

Sodium Chloride

Sodium Hydroxide

Hydrochloric Acid

H_2SO_4

Na_2HPO_4

KH_2PO_4

MgCl_2

NaHCO_3

Solutions

Calculate how many grams of a compound would be needed to make the following solutions. NOTE: Depending on your comfort with this material, you may want to reference the lab on Concentration Expressions and Calculations.

1 L of 10% Sodium Chloride

1 L of 28% Calcium

50ml of 3% Tris-Cl

20ml of 0.02% Phenol Red

50ml of 0.9% Agarose gel

50ml of 0.3% Agarose gel

Calculate how many grams of a compound would be needed to make the following solutions. NOTE: Depending on your comfort with this material, you may want to reference the lab on Concentration Expressions and Calculations.

1 L of 2M NaOH

1L of 0.5M HCl

250ml of 5M NaCl

30ml of 2M KCl

1 L of 15mM Na₂CO₃

1 L of 71mM NaCl

500ml of 3.1mM NaN₃

100ml of 35mM NaHCO₃

III. SOP:

A. Volumetric Determination and Error

During many procedures, scientists and technicians often must transfer a given amount of solution from one container to another. Accuracy and time management are often essential, so sophisticated machines have been developed to facilitate quick and accurate transfer. A serological pipette is usually a glass or plastic cylinder with graduations for measuring liquid. Common pipettes can be purchased that measure volumes from .001 ml up to 25 mls, and the accuracy of the pipette is related to the size of its smallest unit of measure. Transfer pipettes are made of plastic, and have the bulb fused to the column. The column usually has demarcations on it that allow for measurement as low as 10 μl (although they are often not as accurate as serological pipettes.) A micropipette is a calibrated device that can measure liquid down to fractions of a microliter and typically has a range from 1 ml to 0.01 μl . For the following exercise you will be using a serological pipette.

Use a 5 ml serological pipette and a 100 ml beaker.

Before you begin, weigh the empty beaker and record your results below.

Now take the pipette and transfer the following volumes of water to the beaker:

5.0, 4.5, 4.3, 4.2, 4.0, 3.5, 3.3, 3.2, 3.0, 2.0, 1.5, 1.0, 0.5 ml

These volumes should be transferred to the beaker individually. The total volume of the 13 combined volumes should be 40 ml. How close you came to the theoretical result can be defined on the basis of the total weight of the water in your beaker. The density of water at 23° C (room temperature) is 0.9976 gm/ml.

B. Use dimensional analysis to determine the theoretical weight of 40 mls of water.

Weight of beaker and water = _____

Weight of empty beaker = _____

Weight of water (experimental) = _____

Calculated weight of water (theoretical) = _____

Deviation from expected = _____

To determine the amount of your error (your deviation, as expressed as a percent of the expected value), you *subtract the expected or theoretical weight of the water from*

the weight of the water that you actually transferred (experimental value). This first step gives you information as to how much weight your sample was over or under (deviation from) the expected weight.

To find how acceptable this deviation is, you simply *divide the value above by the theoretical value* to find out what fraction of the expected your deviation represented. Normally error is expressed in percent and to convert your value to a percent, simply multiply by 100.

While this sounds confusing, the equation below illustrates the calculation:

$$\% \text{ Error} = \frac{\text{Experimental value} - \text{theoretical value}}{\text{Theoretical value}} \times 100\%$$

Here is the above equation expressed in terms of our specific experiment

$$\% \text{ Error} = \frac{\text{Measured wt. of H}_2\text{O} - \text{calculated wt. of H}_2\text{O}}{\text{Calculated wt. of \% H}_2\text{O}}$$

$$\% \text{ Error} = \frac{\hspace{10em}}{\hspace{10em}} \times 100\%$$

$$\% \text{ Error from your experiment} \quad \frac{\hspace{10em}}{\hspace{10em}}$$

A deviation of 1-2 % is not unusual. If 5 % is seen, practice your technique again. Note the percent error is expressed as an absolute number, (no negative percents please!)

How would you explain the error in your experiment? List at least three possible causes.

1.

2.

3.

C. Micropipette Practice: (NOTE: Read this hand out completely prior to starting work with the micropipette).

1. Getting familiar with the micropipette:

A. The pipette used in this experiment measures from 2 to 20 microliters. (the volumes measured are very small since there are one million microliters in a liter.) Two other common micropipettes measure from 2-20 μl and 100-1000 μl

B. Try to visualize these volumes (you will note that this is difficult as these are very small volumes, and on the pipette scale at first glance they can look identical.) Note the diagram listed (of the scale panel for a 2-20 μl pipet) below to become familiar with the approximate volumes as they appear on the pipette.

NOTE...(XXXX) If the pipette were to be forced to this volume (0.2 ul) it would destroy the device (and this is clearly outside of the 2-20 ul range for the device)

20.0ul ---→	2	2.6ul---→	0	0.2 ul ten	0
	0		2	ones	0
Red decimal line	0		6	tenth	2XXXX

Become familiar with the function of the volume adjuster (the black dial in the handle of the device, just below the colored button you depress to operate the device.)

- With the number display facing you:
- -----> increases volume
- <----- decreases volume

2. Memorize these “pipette nevers”

- Never rotate volume adjuster below 2 or above 20 microliters (or outside the range, or the pipette may jam.)
- Never use without a tip in place.
- Never lay the pipette down with fluid in it.
- Never let the pipette button (the one with the range printed on it) snap back.

3. Become familiar with how to add and eject a tip.

Insert pipette tip into disposable tip and twist to snugly fit in place. Press the ejector button to remove the tip. Try this until you are very comfortable with the process.

- Loading and emptying: The yellow button has two stops. The first stop is for filling and dispensing the sample. The second stop is to "blow out" the last little bit of the sample. Find these stops and make sure you are very familiar with these stops.

Loading:

- Depress button to first stop. Dip pipette tip into sample and draw fluid up by gradually releasing button. Make sure you keep the tip submerged in the fluid during this step.
- Remove the pipette tip from the sample and examine the sample. It should extend all the way to the pipette tip. There should be no air at the tip or anywhere along the length of the fluid.
- Make certain that you understand the importance of depressing the button to the first stop for filling. If you depress to the second stop, you will get 5 extra microliters of sample (often a catastrophic amount!)

B. Emptying:

- Take a clean microfuge tube and touch the pipette tip to the bottom of the microfuge tube.
- Depress the button to the first stop to expel the sample.
- Depress the button to the second stop to "blow out." Try stirring the sample while the button is still depressed (to aid in delivery.)
- Keep the button down while removing the pipette from the sample
- Gently release the button.

Practice Exercise:

1. Using the red practice solution, pipette the following amounts into a clean tube:
 - 5 microliters, 3 microliters, 2 microliters
2. To check that your measurements were accurate, set the pipette to 10 microliters and withdraw all the solution.
 - Is the tip filled all the way to the end? There should be no residue left in the tube.
 - If there is fluid left in the tube, or if there is an air space in the tip, your technique could use some improvement.

Gel loading: See the instructor for sample gels to practice loading gel wells. Put water in the Petri dish to more accurately simulate loading a real electrophoresis gel.

Molarity and Dilutions: NOTE: Depending on your comfort with this material, you may want to reference the lab on Concentration Expressions and Calculations to help with your calculations. The actual lab/handout itself should be completed by the fourth lab meeting on (February 3).

Protocol

Calculate the amount of NaHCO_3 (in grams) needed to make 10mls of solutions with the following molarities:

2.5 M	10 mM	10 μM
1.0 M	1 mM	1 μM
0.1 M	0.1 mM	

- Make 10 mls each of the above solutions in 50 ml centrifuge tubes (check to see if the pH meter probe will fit into the tube prior to making solutions.) Please note, it is not an oversight that some of the solutions reagents can not be weighed using our scales. If this is not possible, do not attempt to prepare the solution. Move on to the serial dilution part and compare the pH of the solutions that can be prepared.
- Record the pH of each of the solutions.
- Calculate how the same solutions could be generated by using the process of serial dilution.
- Now generate a new set of solutions using serial dilution. For the first solution in the series, start with the solution that fully solubilizes (e.g. dissolves completely)
- Record the pH of these solutions.
- Create a table and record the pH of the different solutions as prepared by both direct molar measurements and serial dilution.

Questions

Which process of generating the set of solutions was least time consuming?

Is there any difference in the pH values when the two sets are compared. Look at your table, and look for inconsistencies in the data.

Which process was the most accurate? Why?

Serial Dilutions: NOTE: Depending on your comfort with this material, you may want to reference the lab on Concentration Expressions and Calculations to help with your calculations. The actual lab handout itself should be completed by the fourth lab meeting on (February 3).

Serial dilutions are dilutions made in a series that for the most part have the same dilution factor (how is that for elucidating the obvious!) For the following problems show the serial dilutions you would make to arrive at the final concentration. Assume that the original stock solution is a 1 molar concentration and you want 50 mls of the final solution. FYI, dilutions rarely exceed 1:100 as this may contribute to substantial measurement errors. In the dilution series, state each solution's amount and molarity.
0.05 M glucose (hint, start off with 2.5 mls of 1 M stock glucose)

1 μ M NaCl

10 nM Phosphate Buffered Saline

0.15 mM albumin

The previous lab protocol can be reproduced for educational purposes only. It has been developed by Jim Wolf, and/or those individuals or agencies mentioned in the references (when present).