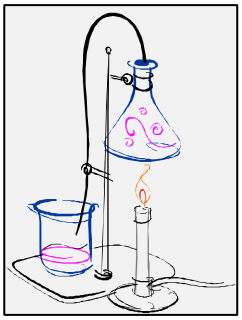


RESEARCH IN THE LABORATORY

Step out of the science hallway into a room with beakers on the benches, machines that go whiz-bang, and safety shower in the corner. You will immediately recognize that you are in a lab! A science lab is more than a place with ritualized protocols and specialized – often expensive – equipment. It is a place with its own kind of culture and organization, camaraderie and dynamic unique flavor.

Scientists Make a Team Effort!

In a research lab everyone plays an important role. A well-functioning lab depends on each person doing his or her part to contribute. Scientists carry out the work to set up, maintain, and collect data for their own experiments. They also clean up after each experiment by washing, storing, and disposing of their materials properly. In other words, when carrying out research, scientists take ownership of and responsibility for their own projects.

Keeping it Clean:

Many materials are shared among the entire lab. It takes a group effort to keep the shelves stocked and the lab clean! Some ways you can help your entire class while carrying out your research include:

- ✓ Returning shared materials and resources to their storage place when you are done with them.
- ✓ Putting a note on the board or telling your teacher if some materials are running low.
- ✓ Taking off your lab gloves when opening doors or using the computer in order to avoid getting chemicals, soil, or other materials on surfaces others will touch.
- ✓ Keeping your own lab bench area clean and organized.
- ✓ Cleaning up any messes you make in shared parts of the lab before you leave!

Sharing Makes Good Science:

Taking responsibility for your research can make science sound like solitary work, but scientists working in same lab often share to help each other get their work done. They may:

- ✓ Share expertise by mentoring newer researchers.
- ✓ Share techniques and troubleshooting tips with others using the same methods.
- ✓ Share lab equipment – even with scientists outside the lab if the equipment is expensive!
- ✓ Run others' samples with their own on a shared piece of equipment to save time.
- ✓ Share results and ideas with each other to move forward on a project.

Make Safety Part of the Plan:

Before you begin any experiment, remember to think about safety issues. Always identify the specific materials, chemicals, equipment, and plants you will be using before you start. It is your responsibility to use these items with care during your work. Sharing information is expected in a science lab, and it can also be important for safety. Don't be afraid to ask questions! If you don't know how to use a piece of equipment or are unsure of what a particular chemical is, it is better to find out by asking than by using these items in a risky way.

In each **PlantingScience** protocol, safety considerations are written in **highlighted red text**. In addition, general safety considerations for any module that involves working in the lab, handling plants, or handling chemicals are described in ***Investigating Plants Safely***.

The Goal: Carrying Out Good Science!

Everyone in a science lab has the same goals of carrying out careful experiments and collecting data as accurately and as precisely as possible. In addition to working harmoniously with others in the lab and keeping the lab safe, you will always be responsible for your own project.

In carrying out your project, remember to:

- ✓ Respect the rules of the lab.
- ✓ Get any training you need before you start.
- ✓ Keep an up-to-date lab notebook.
- ✓ Be honest about your data – all scientists make mistakes!
- ✓ Respect your labmates' work.

Measuring Materials:

Weighing Solid Materials:

Suppose your team wants to test the effect of different amounts of fertilizer pellets on plant growth during ***The Wonder of Seeds***. You could count out individual fertilizer pellets, but it would better to know the total mass of fertilizer you are adding to each treatment. How would you do this?

For solids in most experiments, you will only need to measure mass. Digital balances can measure the mass of fertilizer pellets, powdered chemicals, soil, and plant parts. Balances vary in their precision, however – some will round the measurements to the nearest gram, while others can measure a tenth of a milligram! The more precise a balance is, the more important it is to use it with care. Avoid jostling a balance while measuring, and be sure to clean up any trace materials before and after you use it.

You can transfer your materials to the balance using a **scoopula**, a *small spoon-like tool designed to resist corrosion by lab chemicals*. A kitchen spoon may also work, but scoopulas are often more durable. A piece of **weighing paper** or a disposable **weighing boat** can hold the materials you are weighing and make it easier to transfer them to their final container. Remember to **tare** the balance (i.e., *set it to zero*) after putting any container onto the weighing pan. This way, you won't have to subtract off the mass of the paper or boat to accurately figure out how much material you have.

Judging Liquid Volume:

You may have noticed that when a liquid is placed in a container, especially one with a circular opening and narrow diameter, the *surface of the liquid will form a crescent-shaped curve*. This curve is called a **meniscus**. In water or aqueous solutions, the meniscus is concave; in other liquids, the meniscus can be flatter (e.g., ethanol) or even convex (e.g., mercury). Regardless of shape, the central part of the meniscus is used to accurately measure a liquid's volume. To do so, liquid is added until the central part of the meniscus exactly lines up with a calibration line on the glassware (Figure 1).

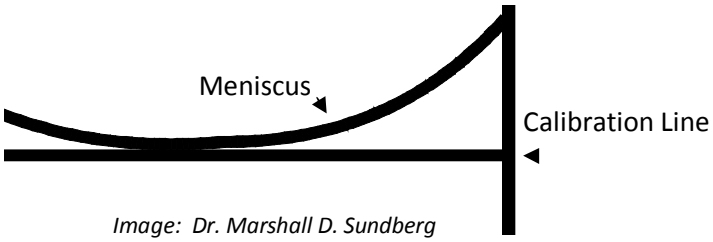


Image: Dr. Marshall D. Sundberg

Figure 1. Measure the volume of an aqueous solution at the bottom of its meniscus.

Large Volumes of Liquid:

In ***The Power of Sunlight***, suppose your team decides to test the effects of multiple concentrations of baking soda on leaf floatation time. Calculating concentration is described in detail below, but an important part of creating known concentrations of a substance is using a known volume of liquid. How might you make sure that all of your treatments are using equal volumes of water?

If you wanted to, you could weigh out the water on a balance, but graduated cylinders can more easily measure liquid volumes greater than 10 mL. Choose a graduated cylinder with a maximum volume as close as possible to that you want to measure to ensure accuracy. Fill it to nearly the volume you want by pouring liquid into the cylinder from a tap, beaker, or another vessel. Stop before the liquid reaches the appropriate graduated line on the side of the cylinder. Carefully add the last bit with a squirt bottle by touching the bottle's tip to the side of the graduated cylinder. Letting liquid run down the inside of the cylinder prevents bubbles, which can make it hard to read the meniscus. Slowly add liquid until the bottom of the meniscus exactly matches the calibration line you want. Pour the measured liquid slowly to any new vessel, so that you don't lose any of what you so carefully measured!

Small Volumes of Liquid:

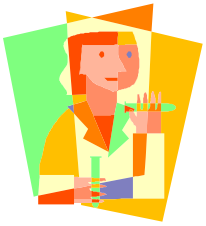
Graduated cylinders are helpful for large liquid volumes, but what if you are trying to measure 1.0 mL volumes of an algal culture into a nutrient medium for each treatment of an experiment testing the effects of light color in ***The Power of Sunlight***?

A **pipette** is an *apparatus for measuring small volumes of liquid*. The larger types of pipettes are long, thin, glass or plastic tubes having pointed tips at one end to help deliver liquid. For this kind of pipette, a squeezable rubber bulb or other suction apparatus can be attached to the other end. Suction is then used to fill the pipette. While it might be tempting, you should **never** fill a pipette using mouth suction!

Several larger types of pipettes are available. **Volumetric pipettes** are designed to *deliver only one volume precisely*. **Measuring pipettes** are *graduated, but the lines stop at a baseline before the pipette begins to narrow*, so you can use them to measure and deliver many possible liquid volumes. Finally, **Serological pipettes** are similar to measuring pipettes, except that they lack a base mark at the tip. They are *graduated to deliver the full volume measured* instead of stopping at a second graduation mark.

The other major category of pipettes are **micropipettors**, *hand-held instruments that can be fit with disposable, plastic tips to measure volumes in microliters* (1000 µL = 1 mL). Experiments using microscopic organisms or low concentrations of a nutrient often require volumes less than a single mL, and micropipettors are as easy to operate as their larger cousins. Many online videos are available to help you learn how to correctly use these tools – see the **Additional Resources** section for examples!

Making Chemical Solutions:



How much salt do you need to make a solution to soak celery in for ***The Celery Challenge***? What is the best concentration of sodium bicarbonate to use for the leaf disc floatation tests in ***The Power of Sunlight***? How can you make a liquid fertilizer with different amounts of phosphorus to grow plants during ***The Wonder of Seeds***? Chemical solutions show up everywhere in biology, and understanding how to make them correctly will broaden the range of possible experiments you can try out! A **solution** is a *mixture of one chemical dissolved in another*. The *chemical that does the dissolving* is called the **solvent** and the *chemical that gets dissolved* is the **solute**.

Concentration of Solutions:

Solution concentration is usually described as either **percent** (%) or **molarity** (**M**). Percent literally means *parts per hundred*. However, there are different ways of making solutions using a percentage. Since each approach gives a slightly different number of solute molecules per unit volume, it is important to always indicate the **basis** used: weight-to-weight (w/w), weight-to-volume (w/v), or volume-to-volume (v/v).

Suppose we want to make a 10% solution of table salt (sodium chloride) in water as a soaking solution during ***The Celery Challenge***. In the *weight-to-weight method* (w/w), 1.0 g of sodium chloride (the solute) is dissolved in 9.0 g of water (the solvent), for a total of 10.0 g. More commonly, either the *weight-to-volume* or *volume-to-volume* method is employed. In a 10% weight-to-volume (w/v) solution, 1.0 g of solute is brought to a volume of 10 mL by adding solvent. The final mass of a 10% (w/v) saltwater solution, though, will be different from that of the 10% (w/w) saltwater solution.

The third method only applies when mixing two liquids. A good example of a 10% (v/v) solution is the 10% bleach solution suggested for sterilizing seeds before planting them in ***The Wonder of Seeds***. To prepare a 10% (v/v) bleach solution, 1.0 mL of bleach is added to 9.0 mL of water. This produces 10.0 mL of final solution, one tenth of which is the bleach solute.

Solution concentration is measured in percent for a wide range of everyday purposes, but it used less often in the laboratory. Instead, scientists more often prepare solutions on a **molar** basis. This is done by *determining the number of moles of the solute desired in a given solution volume*. A 1 **M** solution contains one mole of solute in every liter of solution volume. The number of moles is calculated in grams based on the molar mass of the compound. For instance, the mass of one mole of sodium chloride molecules is about 58.4 g. To prepare a 1 **M** NaCl solution, then, one would weigh out 58.4 g of NaCl solid crystals, then dissolve them in water to bring the final volume to 1 L.

> ***Thought Exercise:*** What is the percent (w/v) of a 1 **M** NaCl solution? Is the percent (w/v) of a 1 **M** KCl solution the same or different? Why or why not?

Dilutions and Serial Dilutions:

Diluting a solution is *making its solute less concentrated by adding more solvent*. For example, when we add more hot tea to a mug containing tea, lemon, and sugar, the lemon and sugar will become more dilute. In the laboratory, it is usually important to know the concentration you want for a diluted solution. This is done by carefully measuring a specific volume of stock solution with a known concentration and adding it to a known amount of solvent.

Suppose you are testing the bending of celery stalks in several different concentrations of saltwater for ***The Celery Challenge***. One of your teammates has already made up a 1 **M** NaCl solution, but you want to test 0.5 **M** and 0.3 **M** solutions as well. The following formula can be used to determine how much water to add to the 1 **M** stock solution to make the other two solutions:

$$v_1 M_1 = v_2 M_2,$$

where v_1 and v_2 are the volumes and M_1 and M_2 are the molarities of the stock and diluted solutions, respectively. For example, if you wanted to make 100 mL of a 0.5 **M** NaCl solution in water from the 1 **M** stock:

v_1 x 1 **M** NaCl = 100 mL x 0.5 **M** NaCl
v_1 = 100 mL x 0.5 **M** NaCl/1 **M** NaCl = 50 mL.

Therefore you would measure out 50 mL of the 1 **M** stock solution. Since you want a final volume of 100 mL, 100 mL – 50 mL = 50 mL of water should be added to produce the 0.5 **M** NaCl solution.

> ***Thought Exercise:*** After making up the 0.5 **M** saltwater solution, you have 150 mL left of the 1 **M** stock. You decide to save 100 mL of this for testing celery bending. How much 0.3 **M** solution could you make from the remaining 50 mL? How much water should you add?

One quick way of generating known concentrations of substances, or even known population densities of microorganisms, is by making **serial dilutions**. Serial dilution is *the creation of a series of solutions with concentrations that differ by a constant known amount*. For example, instead of preparing solutions of 1 **M**, 0.5 **M**, and 3 **M** NaCl in water for celery bending tests, you could make each solution

one-fourth as strong as the next higher concentration: 1 **M**, 0.25 **M** and 0.0625 **M** NaCl in water. One way to make a dilution series is by adding a set volume of the previous, more concentrated solution to a set amount of solvent, and repeating this procedure for each new solution you prepare. In the above example, you could make up the 0.25 **M** NaCl solution by adding 25 mL of 1 **M** stock to 75 mL of water, so that the resulting solution is one fourth as concentrated as the original stock. Adding 25 mL of this one-fourth diluted solution to another 75 mL of water produces a second one-fourth dilution, so that this second dilution is now 1/16 (1/4 x 1/4) the concentration of the original solution. Since small errors made at one dilution will be magnified in all later dilutions, you will want to be extremely careful in measuring the volumes for a dilution series!

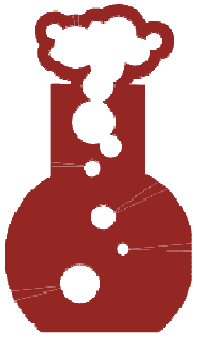
Acids and Bases:

You may recall that different types of plants respond differently to acidic or basic conditions. Maybe you have already thought about investigating the difference between acidic and basic soil conditions for an experiment in *The Wonder of Seeds*. What are acids and bases, and how do we measure how strong they are? After all, we want to see if the plants grow differently, not kill them!

A substance's pH can tell you whether it is an acid or a base. The pH of pure water, which is considered neutral, is 7. **Acids** are chemical compounds having a pH lower than 7, while **bases** have a pH higher than 7. An example of a typical acid might be sulfuric acid (H_2SO_4), while a typical base might be potassium hydroxide (KOH). Acids in our food, such as vinegar (acetic acid), often taste sour. Bases often feel soapy or slippery based on how they interact with our skin.

You might have noticed that the acids above would produce H^+ (hydrogen ion, or proton) in aqueous solution, while the example base would produce OH^- (hydroxide ion). Based on this, it may be little surprise that acids and bases chemically react with each other. When the number of protons an acid adds to a solution is equal to the number of hydroxide ions a base adds, the resulting mixture will react, eventually producing a neutral pH. For example, if we mixed 1 mol each of hydrochloric acid and sodium hydroxide in water, we would see the reaction:

$$HCl + NaOH \rightarrow H^+ + Cl^- + Na^+ + OH^- \rightarrow NaCl + H_2O.$$



That is, the acid and the base would react to form salt water with a neutral pH. Despite this fact, **it can be extremely risky to mix acids with bases**. Acid-base reactions can produce extreme heat or large amounts of gases, either of which may be sudden and violent enough to shatter lab glassware. Unless such a pairing is explicitly described in a lab protocol, you should never mix an acid and a base.

Even adding a neutral liquid to a strong acid or base can produce a strong reaction. The safest approach when mixing such solutions is to **add small amounts of the concentrated acid or base to the more neutral liquid** while stirring the liquid and wearing protective clothing. By limiting the amount of acid or base entering the liquid, the overall reaction will be smaller, and the heat or gas buildup is less extreme.

Gently holding the container the solution is in can also help you monitor any reaction. If the container gets too warm, wait a moment to let the chemicals react before adding more acid or base.

How Strong is My Acid or Base?

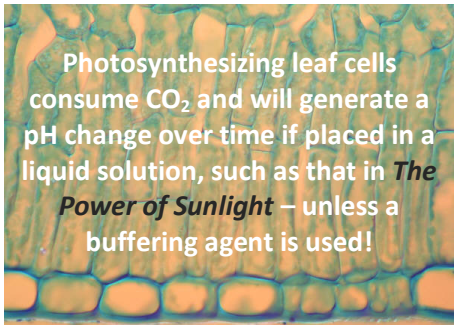
Even in a solution of pure water, a few H_2O molecules are always broken into protons and hydroxide ions. Pure water is considered neutral, because one water molecule produces one acidic hydrogen ion and one basic hydroxyl ion when it breaks apart. However, free protons are acidic, so **pH**, or *the negative logarithm of the number of free protons in a solution*, is a quantitative measure of a solution's acidity. In one liter of pure water, one in every ten million water molecules is broken into ions, and so the concentration of protons is about 1/10,000,000. The pH for pure water is therefore $-\log(10^{-7})$, or 7.

Thought Exercise: Suppose you've measured the pH of a fertilizer solution and found that it is 3. How does the concentration of protons in this solution compare to that in pure water? Is it realistic to try to increase the pH by

It might sound difficult to count the number of protons in a solution, but you can easily measure the pH of a solution. The simplest way to measure pH is to use **indicator paper**, a *type of paper that contains an indicator dye that changes color depending on the pH of the solution*. Comparing its color to a standardized chart tells you the pH of the solution, but the chart usually only allows pH measurements to the nearest 1 or 0.5 units. A **pH meter** is a more precise way to measure pH, and you can usually get measurements to tenths or hundredths of a unit. A pH meter has an electrode which must always be kept moist, so this part is usually kept covered or immersed in a buffer. You also need to calibrate a pH meter before using it. If you would like to learn more about how to measure pH in liquids for your experiments, see "Monitoring pH to Assess Photosynthesis & Respiration of Aquatic Plants" in the ***Power of Sunlight Toolkit***.

Buffers:

A **buffer** is a *solution with two or more solutes that, when combined, tend to resist pH change*, producing a stable solution pH under a wide range of conditions. Most cells thrive at pH 6-8, but certain compounds may move a solution's pH out of this range. This makes buffers helpful for maintaining conditions favorable for life during experiments. No single buffer can be used for all possible experiments, so scientists have developed many types of buffers for different uses. You can usually figure out which one best fits your experiment by reading a little bit about it. "Preparation of Citrate-Phosphate Buffer for Maintaining pH" in the ***Power of Sunlight Toolkit***, for example, describes a buffer that helps prevent pH changes during photosynthesis trials.



Photosynthesizing leaf cells consume CO_2 and will generate a pH change over time if placed in a liquid solution, such as that in *The Power of Sunlight* – unless a buffering agent is used!

Additional Resources:

Videos:

Colorful Chemistry of Acids and Bases, by MITK12Videos. Tyler, an MIT student, shows how to tell acids, bases, and neutral solutions apart with an indicator dye you can make from a plant!
http://www.youtube.com/watch?v=Ko5iDMYzwWE

How to Pipette: Lab Survival Skills, by ThePenguinProf. This video provides a basic introduction to using pipettes and a helpful, detailed description of how to correctly use micropipettes.
http://www.youtube.com/watch?v=y-OHnnhWCdo

Molarity – Molality, by Brightstorm. Learn about five different ways to quantitatively express the concentration of a solution. Drawings and math help show how to solve dilution equations.
http://www.youtube.com/watch?v=0y8orxgqiK4

Scientists at Work in Laboratory, by Vid010. This short video can give you an idea of how a research biology laboratory can look and what scientists do there.
http://www.youtube.com/watch?v=tao0Aes5bko

Using a Graduated Pipette, by Mattocks4. Three students each show the proper technique for measuring liquids with a graduated pipette when using a squeeze bulb for suction.
http://www.youtube.com/watch?v=3E0rHqxExnY

Web Pages:

Chapter 17: Buffers, by Raymond Chang. This supplemental, interactive animation to the textbook Essential Chemistry, 2/e describes and shows how a buffer system can help a solution resist pH changes.
http://www.mhhe.com/physsci/chemistry/essentialchemistry/flash/buffer12.swf

pH, by John Kyrk. This website, developed by a scientific illustrator, shows how pH works at the molecular level and includes a sliding scale with examples of substances that have different pH values.
http://www.johnkyrk.com/pH.html

Recipe: Phosphate Buffered Saline, by Cold Spring Harbor Protocols. This page gives a recipe for a buffer commonly used in cell biology experiments that will have a pH of about 7.4.
http://cshprotocols.cshlp.org/content/2006/1/pdb.rec8247

Books and Articles:

Ruzin, S.E. 1999. Appendix II: Buffers. *Plant Microtechnique and Microscopy*. Oxford, England: Oxford University Press. 336 pp.