Introduction

Chemists use two general methods to measure exact quantities of chemical reagents, mass determination by using chemical balances and volume determination of liquids and solutions by using calibrated glassware.

Volumes can be crudely determined using the calibration markings on some beakers and flasks. More precise volume measurements can be done with a graduated cylinder. The most precise volume measurements are done with pipets, burets, and volumetric flasks.

Cleaning Glassware

Good volumetric technique requires clean glassware. The standard test for cleanliness of volumetric flasks, pipets and burets is to fill them with liquid and then let them drain. If droplets of liquid cling to the inner walls, the glass is not clean enough. Usually cleaning the glassware with hot tap water containing detergent (using a brush for volumetric flask necks and burets) followed by several rinses with water will adequately clean the glassware. Pipets are often difficult to clean by this method and may require a special cleaning solution. Consult your instructor if you cannot get your volumetric glassware clean with water and detergent.

Pipets

A basic safety rule in the use of pipets is never draw liquid into a pipet with your mouth. Always use the rubber pipet bulb for this purpose.

- Learn to handle the pipet bulb with your non-dominant hand thus leaving your dominant hand free to handle the pipet. Use the index finger of your dominant hand on the top of the pipet to control the position of the meniscus.
- Two kinds of pipets are used in this course, transfer pipets and graduated pipets.
• The transfer pipet has a single calibration mark etched on its stem. When filled to this mark the pipet will deliver its rated volume if it is allowed to drain freely. There will be a small residual volume of liquid remaining in the tip after the draining has ceased. Before using the transfer pipet check the delivery tip for possible breakage. A broken tip may slightly alter the volume delivered. Handle pipets with great care to avoid bumping the tip against a hard surface.

• Prior to pipetting a solution, rinse the pipet with several small portions of the solution. Do this by placing about 15 mL of the solution in a clean, dry 50 mL beaker. Before using the bulb make sure there is no liquid inside of it.

• Draw a few mL of solution into the pipet by gently holding together the pipet bulb and the upper end of the pipet (do not force the pipet bulb onto the end of the pipet), squeezing the air out of the bulb, immersing the pipet tip into the liquid in the beaker, and carefully releasing the bulb just enough to draw the desired quantity into the pipet.

• Prevent liquid from entering the bulb, which will easily happen if the pipet tip is removed from the solution while there is still suction in the bulb.

• Quickly remove the bulb and cover the upper end of the pipet with the index finger of your dominant hand to control the liquid flow. If your finger is too dry it will be difficult to maintain a satisfactory seal, but if it is too moist you will have trouble with precision control of the flow rate. For a well-conditioned index finger, try rubbing it in your palm. Another method of conditioning the index finger is to wet it with water and dry it with a paper towel.

• Roll the pipet back and forth in a horizontal position to wet thoroughly the entire inside surface by partially releasing the index finger seal as necessary. Then drain the pipet through the tip. Repeat the rinse two more times. Dry the outside of the tip with tissue, and discard the excess rinse solution in the beaker.

• **Without** further rinsing of the beaker, add fresh solution to the beaker. Using the rubber pipet bulb in the manner described above, fill the pipet to several centimeters above the calibration mark.

• Quickly remove the bulb, cover the stem end with your *index* finger as described above, and then carefully allow the bottom of the meniscus to drop to the calibration mark. To reduce parallax put the meniscus at the same level as your eye so that the front and back sides of the etched line coincide.

• To deliver the desired volume hold the pipet in a vertical position, and drain the solution into the container with the tip of the pipet touching an inner wall of the container. Keep the pipet tip in contact with the container wall for about 15 seconds after emptying. Do not blow or shake out the small amount of liquid remaining in the tip; this has been included in the calibration.

Note: Whereas the transfer pipet can be used only to deliver a fixed volume of liquid, the graduated pipet will deliver varying amounts. Most of the techniques for using the graduated pipet are similar to those for the transfer pipet with the exception that for most graduated pipets one does not deliver a measured volume by allowing the graduated pipet to completely drain. Its use requires an initial meniscus reading and a final meniscus reading; the volume delivered is the difference between the two readings as is the case with a buret or a graduated cylinder. Some graduated pipets can be filled to the desired level and then drained completely. Check the markings carefully to determine the type of graduated pipet you are using.
Micropipette

Micropipettes (or micropipets) have been calibrated with high precision and accuracy in order to quickly and easily transfer specific volumes of solution. The more common micropipette has the ability to dispense variable amounts of solution (for instance, a P20 micropipette can dispense between 2.0 and 20.0 μL of solution). In this course, however, it is more common to use single-volume micropipettes, which have the brand name: Lil’pet. These Lil’pet micropipettes dispense only the calibrated amount of solution (10 μL in this case). The most notable difference between the two types of micropipettes (beyond variable or static volume measurements) is that the Lil’pets do not have a second plunger for ejecting the tip. This is done manually on the Lil’pet (with a gloved hand).

Never point a micropipet upward, or even sideways! Gravity should always be pulling the solution toward the floor to avoid destroying the micropipette. The MicroLab boxes have a spot for holding the Lil’pet with the tip pointing downward, or you can hang it from a ringstand.

• **Check to ensure that the plunger top is screwed on properly.** The Lil’pet has a pink plunger top that can slowly become loose. It is screwed on to the plunger itself. The plunger turns, as well, so look to see if the plunger (grey rod) and the plunger top (pink piece your thumb touches) turn together.

• **Load a sterile/new tip.** The tips for the Lil’pet micropipettes are clear (the same used for P200 and smaller common micropipettes). The tip should be changed whenever the solution being transferred is changed (new solution, new tip).

• The plunger will stop at two different positions as it is depressed.
  First stop: This is the Load Volume. Stop at this position when putting solution into the micropipette.
  Second stop: This is the Expelling Point. Depress the plunger to this point only when expelling the last drop from the tip.

• The tip of the micropipette should be placed **just under the surface of the solution** when loading. Always look at the tip to ensure that when it is loaded it has solution all the way down to the bottom (pointed end) of the tip. No air should be between the tip and the solution.

• **Go slowly.** In both loading the micropipette and in dispensing the solution, slow movements avoid inaccurate volumes. The goal is to deliver the same amount every time, so be systematic about the pressure and rate of depressing the plunger.

**Proper order of using the Lil’pet micropipette:**

1. Place the tip just below the solution's surface.
2. Depress the plunger slowly until it reaches the **first stop**.
3. *Slowly* release the plunger keeping the tip just below the solution’s surface.

4. Lift the micropipette slowly, keeping the tip *pointing down*.

5. Hover the tip just barely above (or just barely touching) the surface of the solution it will be dispensed into. *Note: it is common to dispense solutions by touching the tip to the side of the vial, however when using MicroLab vials, it is difficult to properly mix in solutions that are not dispensed directly into the solution.*

6. Depress the plunger slowly to the *first stop*.

7. *Wait one second.*

8. Continue depressing the plunger to the *second stop*, expelling all of the liquid.

9. *DO NOT RELEASE THE PLUNGER!* withdraw the tip from the solution without releasing the plunger. The tip should be empty of any solution.

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**Buret**

Before use, the buret should be cleaned, filled with water and checked for leaks. If clean and leak-free, empty the water from the buret.

- Rinse the buret with the solution to be used by pouring 3 or 4 mL of the solution into the buret via a short-stem funnel. Rinse the entire inner surface of the buret with the solution by tipping the barrel toward horizontal and rotating. Return the buret to upright and discard the solution through the tip so that it is rinsed as well. Repeat the rinse two more times using a new 3 to 4 mL portion of the solution each time.

- After rinsing is completed, secure the buret in the upright position by means of a buret clamp.

- Close the stopcock and, using the short-stem funnel, fill the buret to above the 0 mark.

- Remove any air bubbles that are trapped in the tip-stopcock region by opening the stopcock briefly.

- Allow the solution to be delivered through the tip until *below* the 0 mark, and remove the drop of liquid clinging to the tip by touching it to a beaker wall.

- Take an initial buret reading by recording the position of the bottom of the meniscus.

- Do not try to make the buret read 0.00 mL. This wastes time and biases your results. When one attempts to adjust the initial buret reading to 0.00 mL, the tendency to read anything from -0.02 mL to +0.02 mL as 0.00 mL is very strong. This introduces unacceptable error into the measurements.

- Use the white card with black tape which your instructor will provide for you. Hold the card behind the buret to enhance the sharpness of the meniscus. It is possible to estimate the mL reading to the second decimal place. This interpolation requires estimation between two calibration marks that are 0.1 mL apart. Be aware that numbers
increase in a downward direction. When using a buret always read the volume to the nearest 0.01 mL. A typical initial buret reading might be 0.73 mL.

- After the initial reading has been recorded in your notebook, place the titration flask beneath the tip and proceed with the titration as described in the section below entitled Titration.

- The volume delivered by the buret is determined by the difference between the initial and final readings. For example:

  - Final: 31.78 mL
  - Initial: 0.52 mL
  - Volume Delivered: 31.26 mL

  When using a buret to measure the volume of a liquid (or of a gas displacing a liquid) always record the initial and final buret readings in your notebook.

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**Volumetric flasks**

Figure PAGENUM Volumetric flask with calibration mark and meniscus shown.

Volumetric flasks are available in sizes ranging from 1 to 5000 mL and are designed to contain the nominal volume. To prepare a solution of a precisely known volume, a volumetric flask is used. The flask has a circular etching around the neck which marks the level to which it should be filled. See Figure PAGENUM.

To use a volumetric flask a carefully measured solution or solid is quantitatively transferred (see next section) to the volumetric flask using the methods described above.

  - Enough water is added to fill the lower part of the flask about 3/4 full. This mixture is swirled to completely dissolve the sample and to make the resulting solution homogeneous. Then more water is added until the level is about one cm below the mark.

  - With stopper held firmly in place thoroughly mix the contents of the flask by repeatedly inverting the flask and returning to the upright position. Allow the liquid to drain back down the flask neck after shaking.

  - Add the last portion of solvent (usually RO water) dropwise with a dropping pipet to bring the bottom of the meniscus exactly to the mark (Figure PAGENUM). When observing the meniscus level take care to put your eye at the same level as the meniscus to avoid parallax. Hold a white card or meniscus enhancer behind the flask neck to accentuate the sharpness of the meniscus.

  - Finally, the stoppered flask is inverted with shaking about 20 more times to guarantee that the solution is homogeneous. This part of the mixing process is time-consuming and monotonous, but it is absolutely essential that the final solution is completely homogeneous before any of the solution is used or transferred to another container.
Note: Solutions are generally not stored in volumetric flasks because the solute tends to stratify upon sitting and it is difficult to mix the solution in the flask; consequently concentration inhomogeneities develop which are not easily eliminated. Also volumetric flasks are expensive and consequently are in short supply.

After the standard solution has been prepared it should be transferred to a clean, dry, stoppered, labeled bottle.

If the bottle to which the solution is to be transferred is clean but not dry, it can be rinsed with 3 small portions of the solution from the volumetric flask in much the same way that a buret is rinsed before being filled with a solution. The small rinse portions are discarded and the remainder of the solution is transferred to the bottle, which is stoppered, labeled and stored for future use.

Quantitative Transfer

In analytical procedures it is frequently necessary to transfer a solid or solution from one container to another without appreciable loss. For example, if a solution is simply poured from one beaker to another, it is likely that a drop or two will run down the outside of the beaker when the pouring is completed.

- To avoid this, the solution is poured down a stirring rod that is held in contact with the lip of the beaker and directed into the vessel into which the solution is being transferred. The original beaker still contains some of the solution since drainage is never complete.

- The next step is to rinse down the sides of the beaker with water from a wash bottle.

- The pouring is repeated, again using the stirring rod.

- After at least three such rinses, the transfer may be considered complete.

- If a solution is to be transferred to a volumetric flask or another small necked container, a funnel must be used. After the solution and rinses have been poured through the funnel into the small opening of the volumetric flask, a wash bottle is used to rinse the inside of the funnel. As the funnel is withdrawn from the flask its outside stem is also rinsed to be sure that none of the transferred solution is clinging to it.

- If a dry crystalline solid in a beaker is to be quantitatively transferred to a volumetric flask, it is necessary to use a funnel. However, if the dry solid is poured into the funnel, some crystals are likely to bounce and be lost. Add a little water to the solid in the beaker and then use the same technique as described above for transferring a solution to a volumetric flask. It is not necessary to dissolve the solid completely before the transfer is made. After pouring the slurry of crystals and water into the funnel, hold the beaker in the pouring position with the stirring rod in place and, with water from a wash bottle, flush the undissolved solid into the funnel. Follow this by several small rinses, always using the stirring rod when pouring into the funnel. Then rinse the funnel as above as it is withdrawn from the flask. Take care to use sufficiently small rinses so that the transfer is completed before the volumetric flask is 3/4 filled.