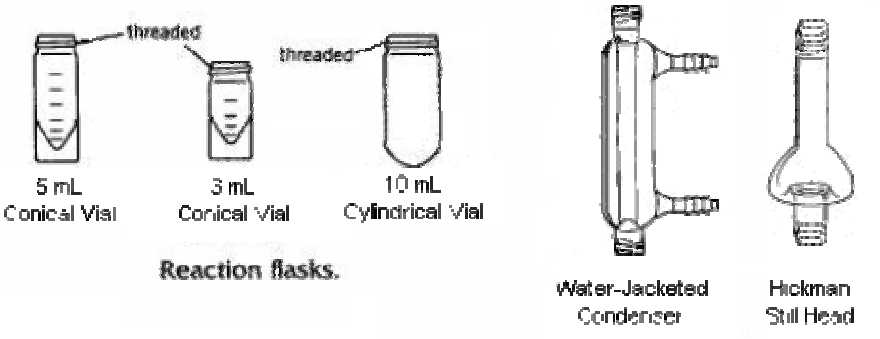
Microscale Laboratory Techniques

Traditionally, experiments in organic chemistry are carried out on a **macroscale** level, employing quantities of chemicals on the order of 5-100 g, using glassware designed to contain between 25 and 500 mL of liquids. For quantities of materials in the 0.005-0.5 gram range, one employs different, "**microscale**" techniques and equipment in order to carry out the various standard organic laboratory operations. In the following, the student is introduced to the special equipment used in microscale experiments, as well as the somewhat different methods which are used.

Basic Equipment

The glassware used for microscale experiments is in your locker. You will make sure it is all there during check-in and it must still be there during check-out . The student will be charged for missing items.
Some of the contents of the microscale kit are illustrated in the drawing below.



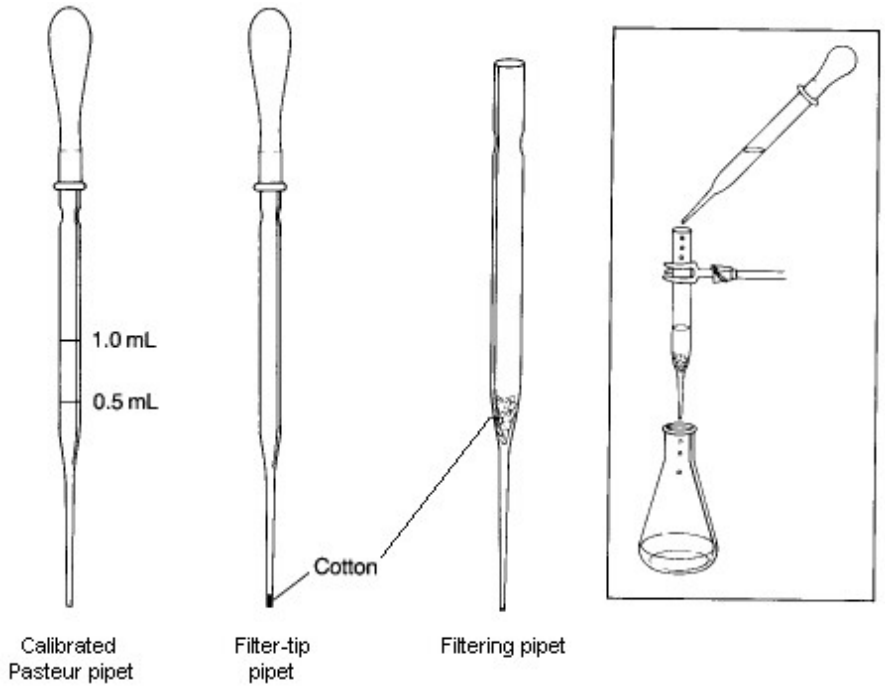
The conical vial is used as a reaction vessel, for extractions, and as a storage container. Its flat base allows it to stand upright on the laboratory bench. The interior of the vial tapers to a narrow bottom, making it possible to withdraw liquids completely from the vial using a disposable Pasteur pipet. The vial has a screw cap which tightens by means of threads cast into the top of the vial. These threads also allow attachment of various other pieces such as a condenser or distillation head, using the double-caps provided in the kit.

Handling of Liquids

Since one rarely works with volumes larger than 2-3 mL, graduated cylinders are rarely used in microscale experiments. Instead, one uses smaller scale volumetric devices such as syringes, automatic pipets, and calibrated disposable Pasteur pipets.

Syringes are especially useful when anhydrous conditions must be maintained during an experiment. The needle can be inserted through a rubber septum sealing the reaction vessel, and the liquid added to the reaction mixture. We use plastic (polyethylene) syringes which, although they are called "disposable", can be cleaned and re-used. To fill the syringe, insert the needle into the liquid and draw in the required volume. Withdraw the syringe and pull the barrel back ever so slightly to draw any liquid remaining in the needle into the syringe.

Disposable Pasteur pipets are used for dispensing small quantities of liquids, as filtration devices, and as columns for small-scale column chromatography. Although they are considered disposable, you should be able to clean them for reuse as long as the tip remains unchipped.
Pasteur pipets may be calibrated for use in operations where the volume does not need to be known precisely, such as for measurement of solvents need for extraction and for washing a solid obtained following crystallization. To calibrate a Pasteur pipet, weigh 0.5 g (0.5 mL) of water into a small test tube on a balance. Attach a rubber bulb to a short Pasteur pipet. Squeeze the rubber bulb before inserting the tip of the pipet into the water. Try to control how much you depress the bulb so that, when the pipet is placed into the water and the bulb is completely released, only the desired amount of liquid is drawn into the pipet. When the water has been drawn up, place a mark with an indelible marking pen at the position of the meniscus. A more durable mark can be made by scoring the pipet with a file. Repeat this procedure with 1.0 g of water, and make a 1-mL mark on the same pipet.



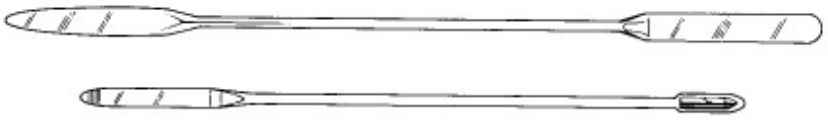
A **Filtering Pipet** is used to remove solid impurities from a liquid with a volume less than 10-mL. To prepare it, a small piece of cotton is inserted into the top of a Pasteur pipet and pushed down to the beginning of the lower constriction in the pipet. It is important that enough cotton is used to collect all the solid being filtered; however, the amount used should not be so large that the flow rate throught the pipet is significantly restricted. The cotton plug can be pushed down with a long thin object such as a glass stirring rod or a wooden applicator stick. In some cases, such as when filtering a strongly acidic mixture or when performing a very rapid filtration, it may be better to use glass wool in place of the cotton, even though it is not quite as good as a filtering aid. To conduct a filtration, the filtering pipet is clamped so that the filtrate will drain into an appropriate container. The mixture to be filtered is transferred to the filtering pipet with another Pasteur pipet. If the volume of the mixture being filtered is less than 1-2 mL, you should rinse the filter and plug with a small amount of solvent after the last of the filtrate has passed through the filter. If desired, the rate of filtration can be increased by genly applying pressure to the top of the pipet using a pipet bulb.

A **Filter-tip Pipet** is useful for transferring volatile solvents during extractions and in filtering very small amounts of solid impurities from solutions. It is made by loosely shaping a tiny piece of cotton into a ball, and

pushing it to the bottom of the pipet using a wire with a diameter slightly smaller than the inside diameter of the narrow end of the pipet. If it is difficult to push the cotton into the tip, you've probably used too much cotton. To use the filter-tip pipet, simply draw the mixture to be filtered into the pipet using a pipet bulb and then expelling it. With this procedure, small amounts of solid will be captured by the cotton.

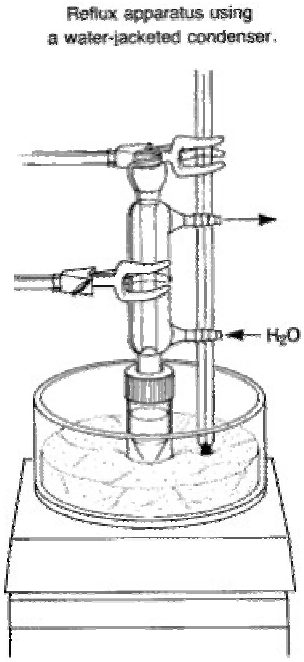
Handling of Solids

Microscale experiments involve quantities on the order of 200-300 mg at most, and it is thus important to be able to weigh solid substances to the nearest milligram. This requires use of a sensitive top-loading balance protected against drafts with a shield, or an analytical balance.
All weighings must be made into a previously weighed ("**tared**") container. The tare weight is then subtracted from the total weight of container plus sample to give the weight of the sample.
Solid samples are manipulated using **microspatulas** similar to those shown below. The larger style is more useful when relatively large quantities of solid must be dispensed.



Microspatulas

Carrying out Reactions

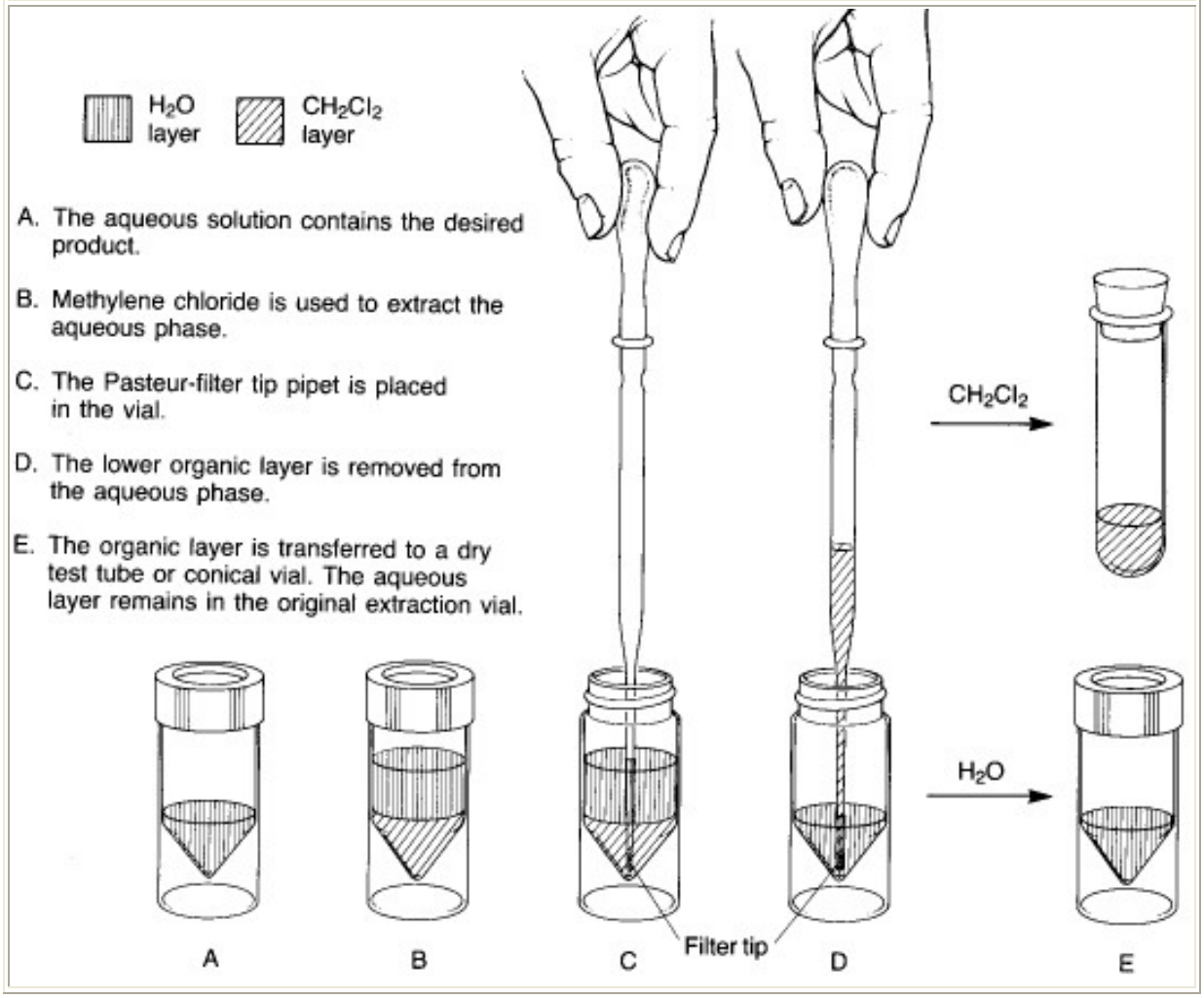


A typical assembly for heating a reaction mixture under reflux is shown at the left. While an air condenser is adequate for most applications (no water passing through the condenser), a water-jacketed condenser is supplied, for cases where the solvent is very volatile or where the ambient air temperature is very high. A "spin vane" might also be included for magnetic stirring of the reaction mixture - this is a triangular device coated with teflon, which is shaped to fit the bottom of the conical flask.
Note that the apparatus is clamped at the condenser rather than at the flask, as one would do for a macroscale experiment using conventional ground-glass joint glassware. The apparatus can be clamped in this way because of the screw-cap connection between the condenser and reaction vial, which prevents the connection from falling apart.
Heating is provided by a sandbath atop a magnetic stirrer/heater. A thermometer should be clamped in contact with the sand so as to allow monitoring of the bath temperature. The bath contains slightly more than 1 cm of sand - it is important to have enough to ensure good thermal contact with the reaction vial, but not so much that it is difficult to see the contents.

Extractions

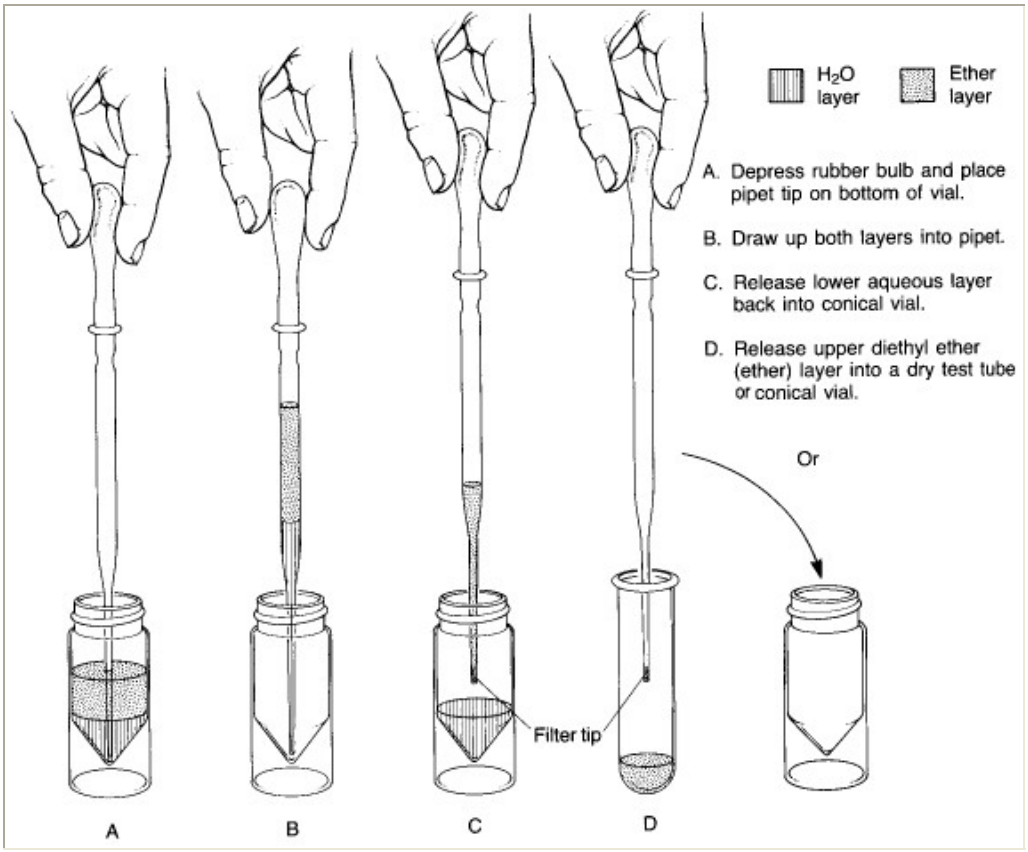
In microscale experiments, the conical reaction vial is the glassware item used for extractions. The two immiscible liquid layers are placed in the vial, and the top is sealed with a cap and a Teflon insert (with the Teflon side toward the inside of the vial). The vial is shaken to provide thorough mixing between the two liquid phases. As the shaking continues, the vial is vented periodically by loosening the cap and then tightening it again. After about 5-10 seconds of shaking, the cap is loosened to vent the vial, retightened, and the vial is allowed to stand upright in a beaker until the two liquid layers separate completely.
Two basic procedures are possible, depending on whether the solvent being used to extract the desired product is heavier or lighter than water. **Method A** is employed for extractions where the lower layer is a heavy solvent such as **dichloromethane**:

Method A - solvent heavier than water



Method B is employed for extraction with a solvent which is lighter than water, such as **diethyl ether**. Note that in this technique, one draws both phases into the pipet and then returns the heavy (aqueous) phase to the conical vial. Ether is so volatile that it is often difficult to hold it in the pipet. Use of a filter-tip pipet for this procedure will help prevent the volatile organic layer from squirting out in an uncontrolled way.

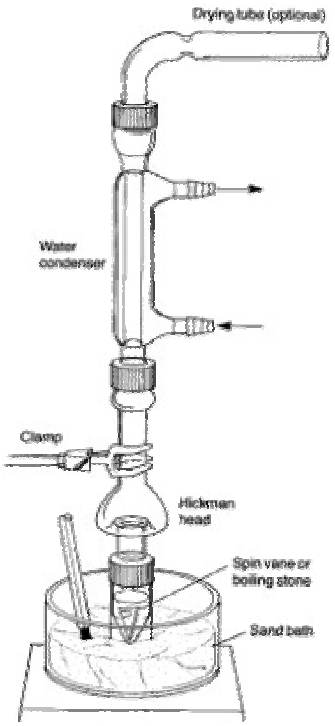
Method B - solvent lighter than water



Recrystallizations

Recrystallizations can be carried out using a conical reaction vial and conventional vacuum filtration to collect the crystals on a small filter paper.
In recrystallizations with a conical reaction vial, the conical vial simply takes the place of the Ernlenmeyer flask used for macroscale recrystallizations. The isolation of the crystals can be done in a number of ways depending on their form:
(i) Once crystallization is complete, the mother liquors and crystals are vacuum-filtered through a small Hirsch funnel. Most commonly, the material is transferred to the filter by pouring, using a microspatula to help transfer the crystals from the vial to the filter. In cases where the crystals are fairly small and fluffy, it may be more convenient to draw the entire mixture of crystals + mother liquors into a Pasteur pipet and transfer them to the Hirsch funnel that way.
(ii) If the crystals adhere to the side of the flask, then filtration is unnecessary. Simply use a filter-tip pipet to remove the mother liquors and transfer them to another flask. Fresh, cold solvent is added to wash the crystals, and this is then removed with the pipet in the same way. The crystals are then dried using a very light stream of air or nitrogen, but care must be taken to ensure that the stream is light enough that the crystals don't get blown out of the vial.

Distillation



The key to successful microscale distillations is in avoiding long distillation paths, since this is the main factor leading to loss of material during distillation. Short-path microscale distillations are carried out using the **Hickman distillation head** as the receiving device for the distilled liquid. Two types of Hickman head, 'ported' and 'unported', are shown in the figure below. The complete apparatus consists of a flask or vial containing the liquid and a magnetic spin vane or boiling stone, attached to the bottom joint of the Hickman head. If desired, a condenser is attached to the top joint. A thermometer can be suspended down the middle in order to record the distilling temperature, with the bottom of the thermometer in the lower part of the Hickman head just below the circular well. The vapours of the heated liquid rise upward and are cooled and condensed on either the inside walls of the Hickman head or on the walls of the condenser. As liquid drains downward, it collects in the circular well at the bottom of the still. The well can contain as much as 2-mL of liquid.

Collection of fractions is easiest with the ported Hickman head; the port is opened and the liquid in the well removed with a Pasteur pipet (see 'C'). With the unported head, the liquid is drawn out from the top with a Pasteur pipet (see 'A'). If a condenser or internal thermometer is used, the distilling apparatus must be partially disassembled in order to do this. In some stills the inner diameter of the head is so small that it is difficult to reach in at an angle with the pipet and make contact with the liquid. This problem may be remedied by bending the tip of the pipet slightly in a flame.
Once removed, the liquid is transferred to a small vial and capped with a Teflon-sealed cap.