Experiment 2:

Liquid-Liquid Extraction – Separation of Organic Acid, Organic Base and Neutral Components of a Mixture

and

Recrystallization as a means of purification of organic solids

Purity, Purification and Physical Properties

Naturally occurring compounds are seldom found in a 'pure' form but more commonly as mixtures with a number of other compounds. Similarly, chemical reactions lead invariably to mixtures of products and unreacted starting materials. Clearly methods must be available, or devised, for the separation, isolation and purification of the molecules of interest. The most important methods that have evolved over a period of time are based primarily on **differences in physical properties** between compounds.

The various physical properties of compounds are, in large measure, determined by intermolecular attractive forces, either between the 'like' molecules of one compound or the 'unlike' molecules of two different compounds. This applies not only to the aggregate physical state of a compound (solid, liquid, and gas) but also to intrinsic properties such as density, vapor pressure, solubility, etc. The most commonly used methods of separating and purifying compounds are based on differences in only three particular physical properties:

VOLATILITY: the 'escaping tendency' of molecules from the liquid or solid state to the vapor state; 'vapor pressure' is its quantitative measure.

SOLUBILITY: the extent to which one compound (solid, liquid or gas) will 'dissolve' in a second compound (most commonly a liquid) to form a single, homogeneous phase.

ADSORPTION: the tendency of foreign molecules to be attracted and held in a mono-molecular layer on the surface of a solid; involves weak Van der Waals forces similar to those determining solubility.

Summarized below, are the four methods of separation and purification that are commonly used in the laboratory;

Method	Physical Property	Type of Material
Distillation	Volatility	Liquids
Recrystallization	Solubility	Solids
Extraction	Solubility	Solids or Liquids
Chromatography	Adsorption/Solubility	Solids or Liquids (or gases)

Although by definition a pure compound is one that is completely free of any impurity, in practice this is never possible if for no other reason that it is impossible to measure very small amounts of impurities. Compound A, for example, may be contaminated by 20%, 1%, one part in a thousand, or even one part in a million of a compound B. The purity required of a compound is often dependent on its intended use. In this laboratory course, compounds that are 99% pure are usually quite satisfactory reagents, but in specialized work, such as quantitative analysis, compounds of much higher purity, e.g. 99.999% may be required.

As mentioned above, the concept of purity implicitly assumes the ability to measure it. Thus, not only are methods of purification needed, but also **methods of determining purity**. The methods used are based on those physical properties.

For **liquids**, the common criterion of purity is the **boiling point**, but it suffers many disadvantages. For **solids**, the **melting point** is the physical constant commonly used. Chromatographic techniques and spectroscopic techniques also play an important and major role for this purpose, especially in research laboratories.

In the first experiment you learned the general technique of separation using absorption techniques, namely thin-layer and column chromatography and solvent extraction. This experiment will demonstrate **solvent extraction** as practice in the technique for the separation and isolation of the components of a mixture. As outlined briefly in the following discussion, the utility of this simple technique is enormously enhanced when used in conjunction with the acid/base properties of the compounds involved. Another problem commonly associated with the separation of mixtures is that the identity of one or more of its components will be unknown and must be determined experimentally. That identification may be as simple as determining the melting or boiling point. Typically, of course, much more information will be required. Various spectroscopies, especially Infrared (IR), Ultraviolet (UV) and Nuclear Magnetic Resonance (NMR) are particularly useful. In this experiment you will be introduced to the measurement of **Infrared spectra** and their use for structural elucidation. During the course of the year you will learn and use NMR spectroscopy, which is a much more powerful technique for structural elucidation of unknowns.

BASIC PRINCIPLES

The technique of extraction, or, more accurately, solvent extraction, is probably the most widely used method for either the initial isolation of natural products from their source materials or the preliminary separation of products from reaction mixtures. In general terms, separation by extraction is based on the principle of phase distribution. This involves selective transfer of one or more components of a mixture to a second, immiscible phase in contact with it. In practice, the mixture is either solid or liquid and the second phase is always a liquid solvent. In an **ideal** case, the desired compound would be completely transferred to the new liquid phase, while all impurities or other components are left behind. More commonly, particularly with two immiscible liquid phases, the desired compound is distributed or partitioned between the two phases i.e. part of it remains in the original phase and part is transferred to the new phase. The partitioning is the consequence of differing relative solubility of the component(s) in the two immiscible solvents. (See the Appendix for a discussion) In those instances in which it is the impurities that are removed, leaving the desired compound in the original phase, the extraction process is more commonly referred to simply as "washing".

LIQUID-SOLID EXTRACTION

This simplest form of extraction is well known and practiced by most of us each day (or at least by those at Tim Horton's or Starbuck's) in brewing a cup of coffee or tea. A finely divided solid mixture is stirred, and usually warmed, with a suitable solvent to effect selective dissolution of one or more components of the solid. After mechanical separation of the liquid solution from undissolved solids by filtration, the resultant solution may be used for some purpose (drinks, drugs, etc.) or, if desired, the extracted components may be isolated, usually by evaporation of the solvent (instant coffee powder!).

The extraction of alkaloids from leaves and barks, flavoring extracts from seeds, perfume essences from flowers and sugar from sugar cane are typical examples of separations of this type. The solvents most commonly used for this purpose are water, various alcohols, ether, chloroform, methylene chloride (a.k.a dichloromethane), acetone, and benzene or other hydrocarbons.

LIQUID-LIQUID EXTRACTION OF ORGANIC ACIDS AND BASES

The extraction or separation of organic acids and organic bases is based on the observation that acids and bases can be interconverted from their uncharged (neutral) form

to a charged form upon treatment with strong acids or strong bases. In general, charged species (e.g. ions) are soluble in water and are insoluble in organic solvents (e.g. methylene chloride, diethyl ether). On the other hand, neutral, uncharged species tend to be water insoluble and soluble in organic solvents. Thus, if a charged species has its choice between water and an organic solvent, it will go into the water; a neutral species will choose an organic solvent over water.

Like all generalizations, there certainly are exceptions to this rule. For example, while sodium chloride dissolves in water, silver chloride does not.   There are quite a few ionic compounds that are water insoluble. However, there are very few ionic compounds that dissolve in organic solvents. Similarly, there are several neutral compounds that dissolve readily in water. Most of these are small polar molecules capable of hydrogen bonding to the water. Examples include sugar, methanol, ethanol, acetic acid and acetone.

There are very few types of organic compounds that are acids and the most common contain either the carboxylic acid group ($-CO_2H$) or the sulfonic acid group ($-SO_3H$). The one you will encounter in this experiment contain the carboxylic acid group and most of these are weak acids with a pKa ~ 5.   This consequence is that an excess of hydroxide will deprotonate the uncharged acid and convert it to its charged conjugate base form. Treatment of the charged conjugate base form with excess hydrochloric acid will protonate it and convert it back to the uncharged carboxylic acid form.

uncharged
organic soluble

charged
water soluble
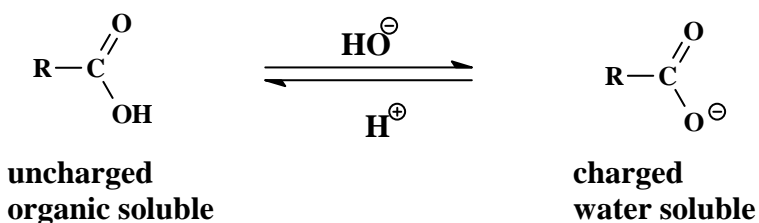
If you now recall the solubility behavior discussed above, this means that an organic acid can be moved back and forth from water to an organic solvent by treatment with excess hydrochloric acid or excess sodium hydroxide. When excess hydrochloric acid is used, the organic acid exists in the uncharged form and will select an organic solvent over water; with excess hydroxide, the organic acid exists in the charged form and will select water over an organic solvent.

Analogously an organic base, typically an amine (RNH_2, R_2NH or R_3N), can exist as either a charged or uncharged species depending upon the pH of the solution. However, the behavior is the opposite of an organic acid. In acidic solution, the base is protonated and exists as an ammonium salt and is charged. If the solution is made basic, the ammonium

salt is deprotonated and the base becomes uncharged.   Thus under acidic conditions, the charged base will prefer to dissolve in water and under basic conditions, the base will choose an organic solvent.

**charged
water soluble**   **uncharged
organic soluble**
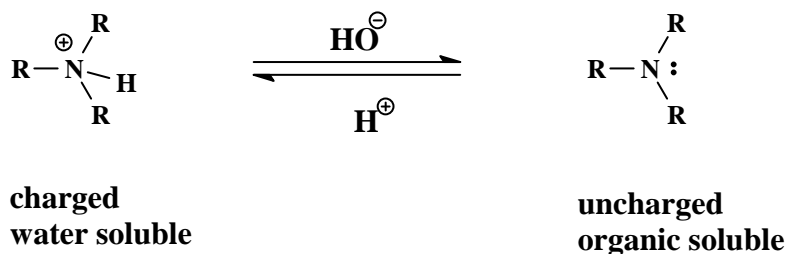
The great majority of organic compounds are neither acids nor bases and these 'neutral' organic compounds will not be protonated or deprotonated.   Consequently, these neutral compounds remain uncharged species that greatly prefer to dissolve in organic solvents in preference to water.

It is this differing behaviour between acids, bases and neutral organic compounds that forms the basis for their separation by extraction.   You will be given a mixture of an organic acid, an organic base and a neutral organic compound that you will separate by solvent extraction and tested appropriately to determine the identity of each component.

Apparatus and Method

Extraction is made possible since water and organic solvents are immiscible, which form two distinct layers (based on their specific density) in the separatory funnel. The glass bulb has a stopcock and stem at the bottom, as well as a plug at the top. The whole device is supported with a ring clamp covered in rubber tubing.

The solution is poured into the separatory funnel with the stopcock closed and a beaker under it just in case of a leak. A small amount of extracting solvent is added to the funnel (the flask should never be more than ¾ full) and with the upper opening and stopcock closed, the funnel is shaken with both hands. The opening should always be facing away from you into the fume hood away from others (Figure 1).
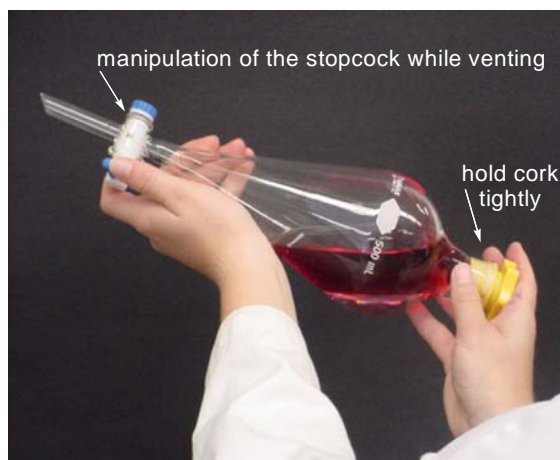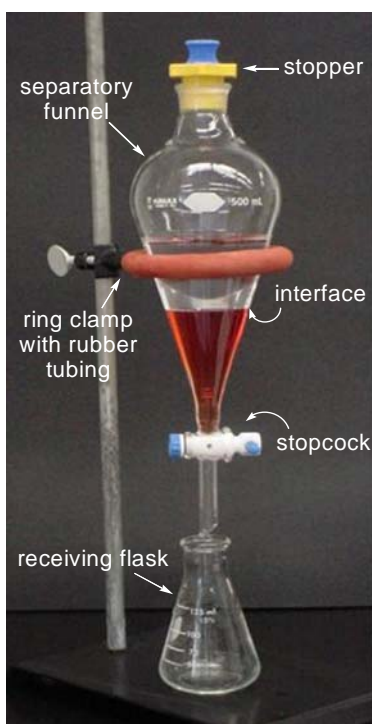

Figure 1: The Separatory Funnel

Shaking the flask is important since it maximizes the surface area of each component and allows intimate mixing. Every couple seconds the stopcock is slowly opened while pointing up to "vent", which allows built-up pressure to be released. The flask is put back on the ring clamp (with the stopcock closed!) and the top plug removed. The two layers are allowed to settle and the bottom layer is removed at a slow rate into a labeled flask. Just before the last couple drops of the lower layer are collected, swirl the funnel to collect any compound that may have been on the walls. ***Collect any last amounts of the lower layer and remove the top layer by pouring it out from the top***. (bottom layer always out of the bottom, top layer always out of the top)

Never discard any layer until you are certain you do not need it. If you are unable to identify which is the organic or aqueous, drop a small amount of water into one and if it dissolves it is aqueous. Normally, three extractions are performed to have an efficient extraction. To remove any last races of water in organic solvent, anhydrous magnesium or sodium sulfate is used before removing solvent by rotary evaporation.

Experimental Procedure

DO ALL PROCEDURES IN THE FUMEHOOD!

DO NOT WORK OUTSIDE OF THE HOOD, except for the melting points.

You will be using a lot of different solutions and flasks, so LABEL THEM APPROPRIATELY and be organized

When a procedure mentions to use water, use deionized water (from blue plastic pipes)
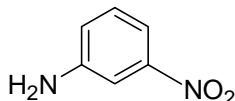
BEFORE COMING TO THE LABORATORY COMPLETE THE FLOW CHART THAT APPEARS AT THE END OF THIS PROCEDURE. IT WILL HELP CLARIFY THE VARIOUS STEPS AND WILL BE A USEFUL GUIDE DURING THE EXPERIMENT.
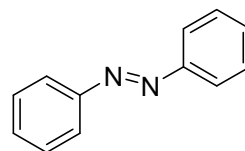
Step A: Dissolve the mixture

- You will be given approximately 1.0 g mixture that contains equal portions of benzoic acid, azobenzene, and 3-nitroaniline. Weigh the mixture to <u>obtain an accurate mass</u> and then dissolve it in ~25 mL of dichloromethane in a 50 mL Erlenmeyer flask.
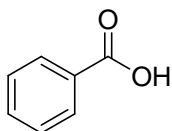
Benzoic Acid (mp 122.4 ^{o}C)   3-nitroaniline (mp 114 ^{o}C)   Azobenzene (mp 69 ^{o}C)

Step B: Extraction of the Organic Base

- Place the dissolved mixture into a separatory funnel. Make sure the stopcock is closed. Proper technique is to be sure a large beaker is placed below the sepratory funnel in case of any spillage or leaking from the stop-cock. Add ~15 mL of 3 M hydrochloric acid to the dichloromethane solution in a separatory funnel. Invert the funnel with the

stopcock closed and top corked. Point the flask into the fume hood and shake gently, while regularly venting. Remove the two layers into labeled, clean 250 mL Erlenmeyer flasks. The lower organic layer is replaced back into the funnel, where it is extracted two more times with additional 3 M HCl (~15 mL portions). All aqueous layers are combined in a 250 mL Erlenmeyer flask and set this flask aside until **STEP E**. The organic layer is carried forward to **Step C.**

Step C: Extraction of the Organic Acid

- To the dichloromethane solution (you may want to add a bit more dichloromethane), add ~15 mL of 3 M sodium hydroxide in a separatory funnel and extract the two layers. The lower organic layer is washed two more times with additional 3 M NaOH (~15 mL portions). Both layers are collected in clean and labeled flasks. All aqueous layers are combined in a 250 mL Erlenmeyer flask and set this flask aside until **STEP F**. The organic layer is carried forward to **STEP D**.

Step D: Extraction of the Neutral Organic

- Lastly, wash the dichloromethane solution in a separatory funnel 3 times with ~10 mL distilled water. The combined upper aqueous layers may be properly discarded.
- Place the dichloromethane solution in an Erlenmeyer flask (you may have to add more dichloromethane). Add an appropriate amount of anhydrous sodium sulfate to this solution (until you see the snow globe effect) and allow it to dry for ~5 min.   Gravity filter the solution through a fluted filter paper into another _pre-weighed_ dry Erlenmeyer flask containing a boiling stone.
- Evaporate the dichloromethane solution (until less than 1 mL remains) on a hot plate (on a low setting) with one boiling stone. Allow the solution to cool to room temperature and then put in an ice bath.
- Weigh the flask and crystals to determine the mass of product. Record the colour and melting point of the product. Use this is **Step G**.

Step E: Isolation of the Organic BASE

- _To be sure you have he right flask, check the pH using litmus paper. It should be acidic to start; you will neutralize it by adding base._
- Neutralize the combined acidic aqueous extracts (containing the organic BASE) by adding 6 M NaOH dropwise (with swirling) until the solution is alkaline. This may be

monitored by using litmus paper. The litmus paper will change from red to blue. **Do not add too much base!** Notice the color change! (What is going on?)

- Cool the flask in an ice bath for 10 minutes and collect the solid precipitate by vacuum filtration using a Buchner funnel. Wash with 2 mL of cold distilled water.

- Dry and weigh the crystals. Record the colour of the product and determine its melting point.

- The dried filtrate should be recrystallized from a suitable solvent until pure (constant melting point) and kept for identification. It should be weighed before and after recrystallization to determine the purification yield as well as the overall yield. Use the melting point for identification.

- Obtain an IR spectrum of the dried, recrystallized product. (second lab day). The TAs will assist with collection of the spectra.

**Step F: Isolation of the Organic ACID**

- *To be sure you have the right flask, check the pH using litmus paper. It should be basic to start; you will neutralize it by adding acid.*

- While on ice, neutralize the combined basic aqueous extracts (containing the organic ACID) by adding 6 M HCl dropwise (with swirling) until the solution is acidic. This may be monitored by using litmus paper. The litmus paper will change from blue to red.

- Continue to cool the flask in an ice bath for 10 minutes and collect the solid precipitate by vacuum filtration using a clean Buchner funnel. Wash with 2 mL of cold distilled water.

- Dry and weigh the crystals. Record the colour of the product and determine its melting point.

- The dried filtrate should be recrystallized from a suitable solvent until pure (constant melting point) and kept for identification. It should be weighed before and after recrystallization to determine the purification yield as well as the overall yield. Use the melting point for identification.

- Obtain an IR spectrum of the dried, recrystallized product. (second lab day)

Step G:

- The neutral component will be impure (why? - think about the extraction process; is it 100% effective? Read the appendix) To purify this component, you will recrystallize it from an appropriate solvent. (details to be given)
- Obtain an IR spectrum of the dried, recrystallized product. (second lab day)

*Waste: Combine all the aqueous layers throughout the experiment and adjust the solution to neutral using the acid or base provided before pouring down the sink with lots of water. The neutral organic flask is rinsed with acetone in the organic waste container.

Draw the appropriate chemical structure (acid, base, neutral, conjugate base, conjugate acid) in the appropriate box in the following flow chart.
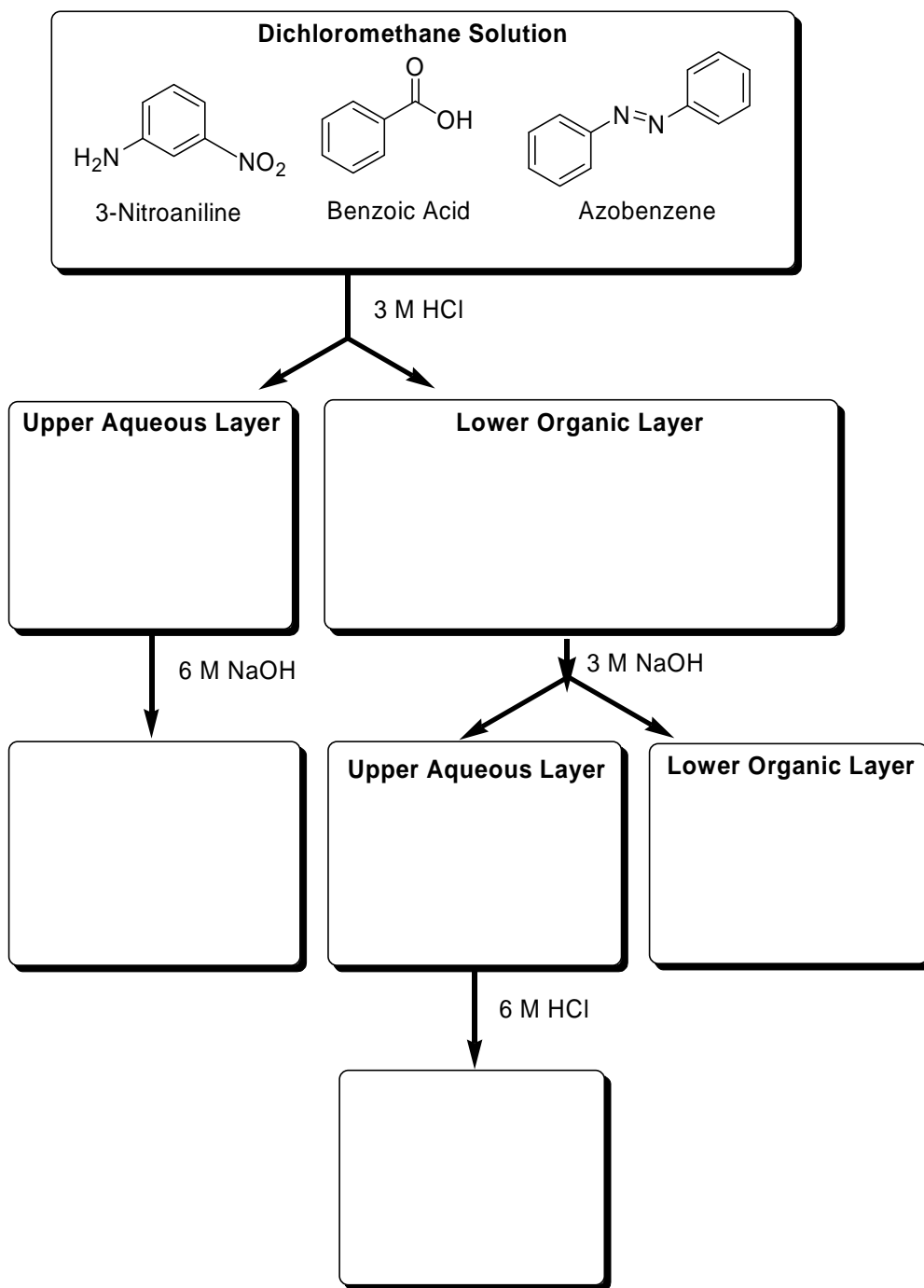
Appendix 1: Solvent Partitioning in Liquid-Liquid Extractions

In organic chemistry, most commonly one of the solvents is organic and the other is aqueous. Inorganic compounds can usually be separated from organic compounds in this way; the former dissolve in the aqueous phase and the latter in the organic solvent. In such cases, a single extraction may be sufficient to effect satisfactory separation. However, many organic compounds (particularly oxygen – or nitrogen – containing compounds, such as aldehydes, alcohols, esters, and amines, which can form hydrogen bonds) are partially soluble in water. They distribute themselves between the aqueous phase (w, for water) and the organic solvent (o) in proportion to their relative solubilities (S) in the two solvents. In this sense the extraction can be considered a competition between two immiscible liquids for the solute, with the solute partitioning between these two liquids.

The ratio of the concentrations of a substance in the two solvents (C_o /C_w) at equilibrium is called its distribution coefficient, K_D, and can be expressed mathematically:

$K_D = S_o/S_w$ = [A] in organic solvent/[A] in water = C_o/C_w

For example, suppose the solubility of compound A is 0.60 g/100 mL in ether and 0.12 g/100 mL in water. K_D is then 0.60/0.12 = 5.

To illustrate how the distribution coefficient K_D can be used, let us calculate the amount of A that is removed from a solution containing 80 mg of A in 80 mL of water by extracting with 150 mL of ether. If we let *x* be the number of milligrams of A extracted into the ether layer, then (80 - *x*) represents the milligrams of A remaining in the water. The equation for K_D is, therefore,

$K_D = C_o/C_w = 5 = (x/150)/((80-x)/80)$

Solving for *x*, we find that 72.3 mg of A will be extracted by the ether and, consequently, that 7.7 mg of A (80 – *x*) will remain in the water.

It is easy to show that if we had extracted A twice with 75 mL of ether instead of once with 150 mL of ether, we would have removed 65.9 + 11.6 mg = 77.5 mg of A from the water. In general, performing several extractions using smaller volumes of solvent is more efficient than performing a single extraction using a larger volume of solvent.

Practical Considerations

The selection of the appropriate extraction solvent is a key to the success of the technique of isolating and purifying compounds. An extraction solvent:

1. must not react in a chemically irreversible way with the components of the mixture.

2. must be immiscible, or nearly so, with the original solution

3. must readily dissolve the substance to be extracted

4. should extract only the desired substance or as small an amount as possible of any other substance present (related to the relative K_D's)

5. should be easily separated from the desired solute after extraction. This last requirement can be met if the solvent is low-boiling and easily removed by distillation or evaporation.

Common organic solvents that fulfill these requirements include many hydrocarbons and their chloro derivatives, such as benzene, petroleum ether (not a "true" ether, but rather a mixture of low-boiling alkanes), dichloromethane, chloroform, and carbon tetrachloride. Another common solvent is diethyl ether (a "true" ether and usually referred to as just ether, although this is incorrect). Diethyl ether is highly flammable and is slightly water-soluble (about 7 g/100 mL) but since most organic compounds are highly soluble in it and because of its low boiling point (35° C), ether is frequently used despite its drawbacks and its cost. Remember that all organic solvents are potentially harmful, but they can be used safely if we carry out operations in an efficient fume hood and take care to avoid getting them on the skin.

The above discussion focused on partitioning of one substance between two immiscible solvents. The same principles apply if one has a mixture of two or more compounds

Appendix 2: Recrystallization

Impure crystalline substances can be purified by recrystallization from a suitable solvent or solvent mixture. This process depends on the fact that most compounds are more soluble in hot solvents than in cold ones and on the fact that the impurities present have solubilities different from those of the desired compounds. The procedure involves:

1. Dissolving the impure material in a <u>minimum</u> amount of boiling solvent,

2. Filtering the hot solution to remove insoluble impurities,

3. Allowing the solution to cool and to deposit crystals of the compound,

4. Filtering the crystals from the solution (called the mother liquor),

5. Washing the crystals with a <u>little cold solvent</u> to remove the mother liquor, and

6. Drying the crystals to remove the last traces of solvent.

General Concepts:

If recrystallization is to be effective, the solvent must be properly selected. A good recrystallization solvent should:

1. dissolve a moderate quantity of the substance being purified at an elevated temperature, but only a small quantity at low temperatures,

2. not react with the substance being purified,

3. dissolve impurities readily at a low temperature or not dissolve them at all, and

4. be readily removable from the purified product. This last requirement usually means that the solvent should have a fairly low boiling point and should evaporate readily. If a single solvent cannot be found that meets all these requirements, it is possible to use a mixture of two solvents.

Solvents suitable for recrystallizing a known compound are generally reported in the chemical literature. If none is reported, or if the substance is a new compound, several solvents can be tested in the following way. Place about 10 mg (a small spatula tip-full) of the substance to be purified in each of several 10 × 75 mm test tubes, and add about 0.25

mL of a different solvent to each. Then observe the solubility of the sample in each solvent, when cold and when heated. Also note whether abundant, well-formed crystals are produced as the hot solution cools.

To obtain a good recovery of purified material, it is best to avoid using unnecessarily large volumes of solvent. Dissolving the substance in the smallest possible amount of hot solvent minimizes the amount of pure material lost by retention in the mother liquors. In practice, 3-5% more solvent than the minimum required is used so that the hot solution will not be quite saturated. This helps to prevent separation of the crystals and clogging of the filter paper during filtration of the hot solution.

Traces of coloring matter or resinous impurities can sometimes be removed with selective absorbents, such as finely divided charcoal. To do this, add a small amount of decolorizing charcoal to the warm solution before filtering it. (*Do not add decolorizing charcoal to a hot solution. If a solution is at or near its boiling point, the addition of finely divided charcoal (which acts as thousands of boiling chips) will cause rapid boil over.) Avoid using excess decolorizing agent, however, because it may also adsorb appreciable amounts of the substance being purified.

Some substances readily form supersaturated solutions, and crystallization may not occur spontaneously when the hot solution is cooled. In such situations, it is sometimes possible to initiate crystallization by scratching the walls of the vessel beneath the surface of the solution with a stirring rod. The best way to induce crystallization is to "seed" the cold solution with one or two crystals of the substance being purified. It is necessary to filter the solution while it is still hot, otherwise the solution will cool rapidly and solids will form prematurely.

Glass rod

Ring support

Short-stemmed funnel
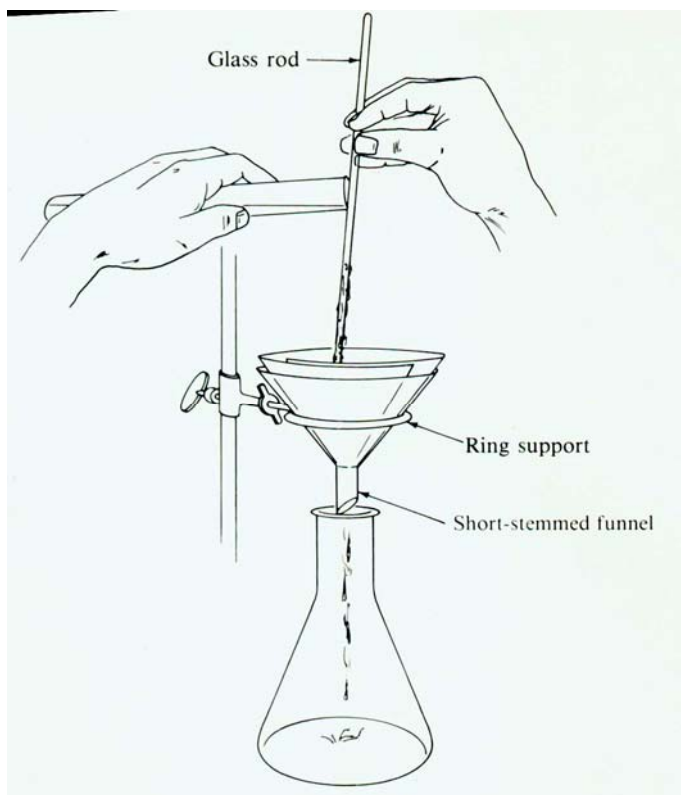
Apparatus for gravity filtration – when done hot, a fluted filter paper should be used – your TAs will show you how.

Apparatus for Hot Filtration and Vacuum Filtration

To remove insoluble impurities and decolorizing charcoal, it is necessary to filter the solution while it is hot using a fluted filter paper. (Figure 2)   Vacuum filtration is generally used to remove soluble impurities and solvent from the crystals of the purified substance. A Hirsch (or Buchner) funnel is fitted to a filter flask with a rubber adapter. A disk of filter paper just large enough to cover all the holes in the funnel is placed in the funnel and moistened with some of the solvent used in the recrystallization. The filter flask is then connected to the aspirator by thick-walled rubber tubing through a water trap, and a vacuum is applied. (set-ups are shown below). Hirsch funnels are generally used for smaller quantities and Buchner funnels for larger quantities. When the filter paper is drawn tightly to the funnel, the solution and crystals are transferred to the funnel. The solution passes through the paper, while the crystals deposit on the paper.

Set-us for vacuum filtration: (left: using Buchner funnel, right: using Hirsch) Far right is shown the Hirsch Funnel (left) and Buchner Funnel (right), respectively.