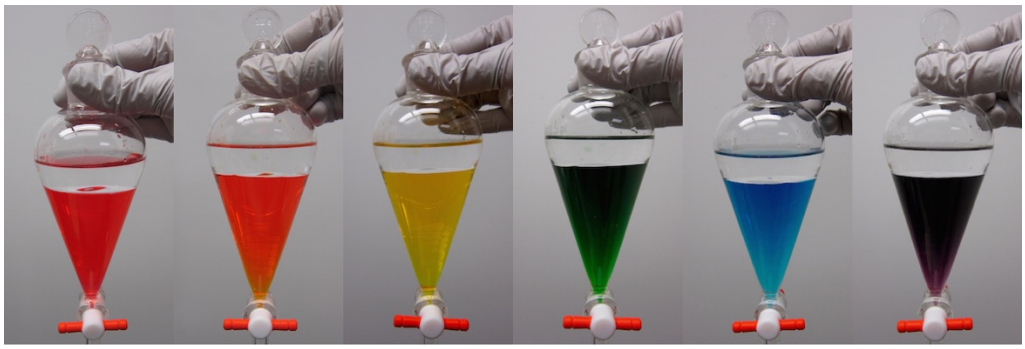
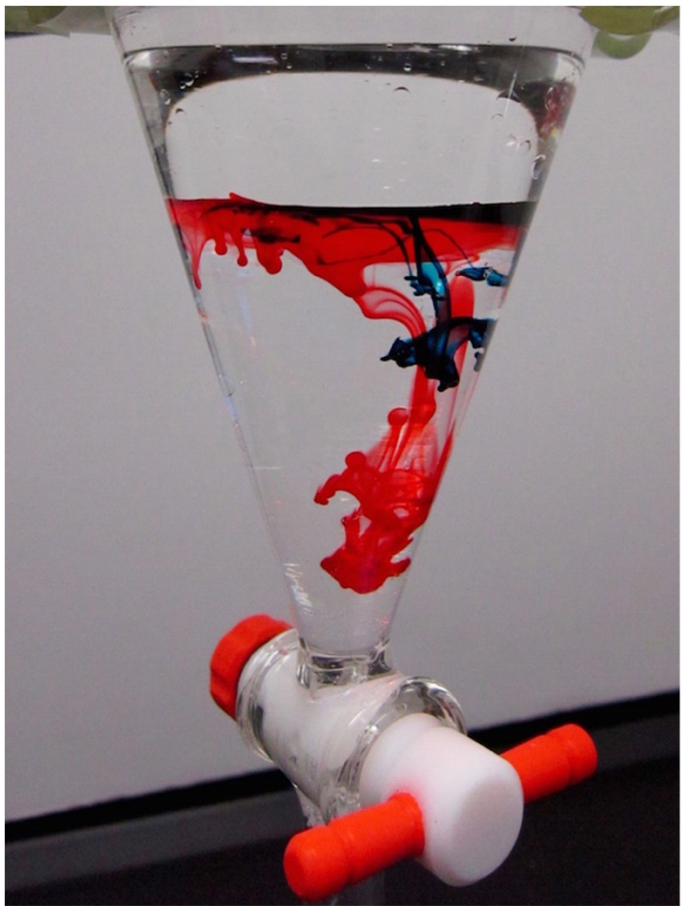
CHAPTER 4

EXTRACTION



Organic solvents and colored aqueous solutions in separatory funnels.



Food dyes dissolving in aqueous solution.

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4.1 OVERVIEW OF EXTRACTION

"Extraction" refers to transference of compound(s) from a solid or liquid into a different solvent or phase. When a tea bag is added to hot water, the compounds responsible for the flavor and color of tea are extracted from the grounds into the water (Figure 4.1a). Decaffeinated coffee is made by using solvents or supercritical carbon dioxide to extract the caffeine out of coffee beans. Bakers use the extract of vanilla, almond, orange, lemon, and peppermint in their dishes, essences that have been extracted from plant materials using alcohol (Figure 4.1b).

Figure 4.1: Examples of extraction: a) Tea, b) Baking extracts, c) Plant pigments extracted into water droplets after sprinklers hit a fallen leaf on the sidewalk.

In the chemistry lab, it is most common to use liquid-liquid extraction, a process that occurs in a separatory funnel (Figure 4.2). A solution containing dissolved components is placed in the funnel and an immiscible solvent is added, resulting in two layers that are shaken together. It is most common for one layer to be aqueous and the other an organic solvent. Components are "extracted" when they move from one layer to the other. The shape of the separatory funnel allows for efficient drainage and separation of the two layers.

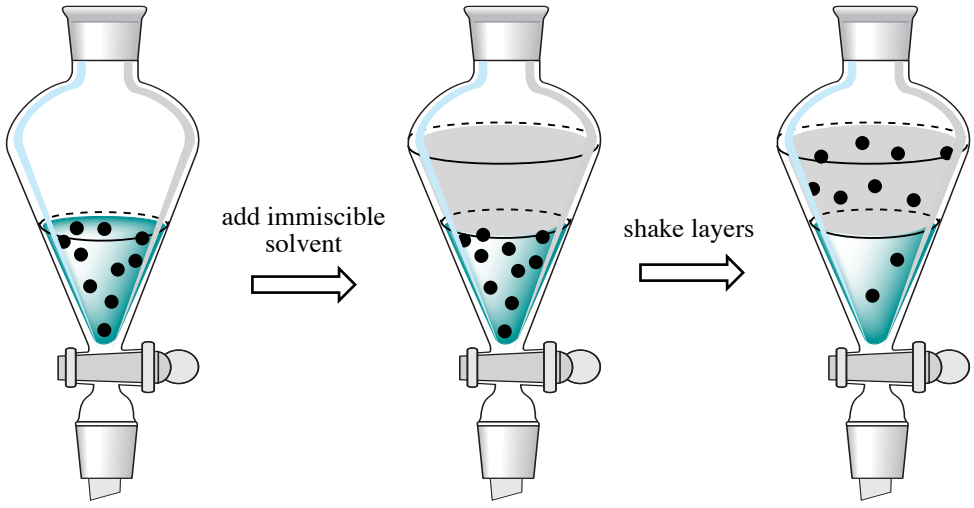

Figure 4.2: Schematic of extraction.

Compounds move from one liquid to another depending on their **relative solubility in each liquid**. A quick guide to solubility is the "like dissolves like" principle, meaning that nonpolar compounds should be readily extracted into nonpolar solvents (and vice versa). The compounds responsible for the taste and color of tea must be polar if they are readily extracted into hot water. When allowed to equilibrate between two liquids in a separatory funnel, the majority of a compound often ends up in the layer that it is more soluble.

4.2 USES OF EXTRACTION

There are several reasons to use extraction in the chemistry lab. It is a principal method for isolating compounds from plant materials. Extraction moves compounds from one liquid to another, so that they can be more easily manipulated or concentrated. It also enables the selective removal of components in a mixture.

4.2.A EXTRACTING NATURAL COMPOUNDS

Fruit and plant leaves are primarily composed of cellulose and water, but also contain "essential oils." a greasy mixture of compounds that capture the "essence" of the plant material's smell and taste. Orange oil is roughly 95% limonene (Figure 4.3b), and due to its nonpolar structure, can be extracted from its rind into an organic solvent like hexanes or dichloromethane (Figure 4.3a). The oil can then be concentrated and used to flavor or scent foods, cleaning supplies, and candles.

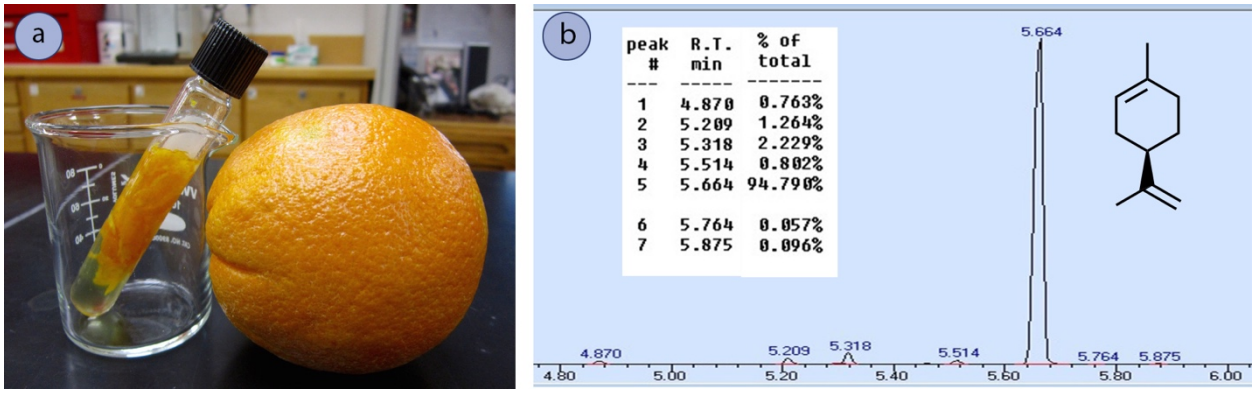

Figure 4.3: a) Orange rind extracted into dichloromethane, b) GC spectrum of orange oil.

In the chemistry lab, essential oils are often extracted from their source using solvents, and analyzed using gas chromatography or spectroscopy.

4.2.B TRANSFERRING COMPOUNDS FROM LAYERS

Another method for extracting essential oils from fragrant plant materials is through steam distillation (Figure 4.4b). This process often results in the lovely smelling compounds suspended in the aqueous distillate (Figure 4.4c). In order to concentrate the oil, the aqueous suspension is often extracted with a low-boiling organic solvent (Figure 4.4d), which can then be easily removed from the oil.

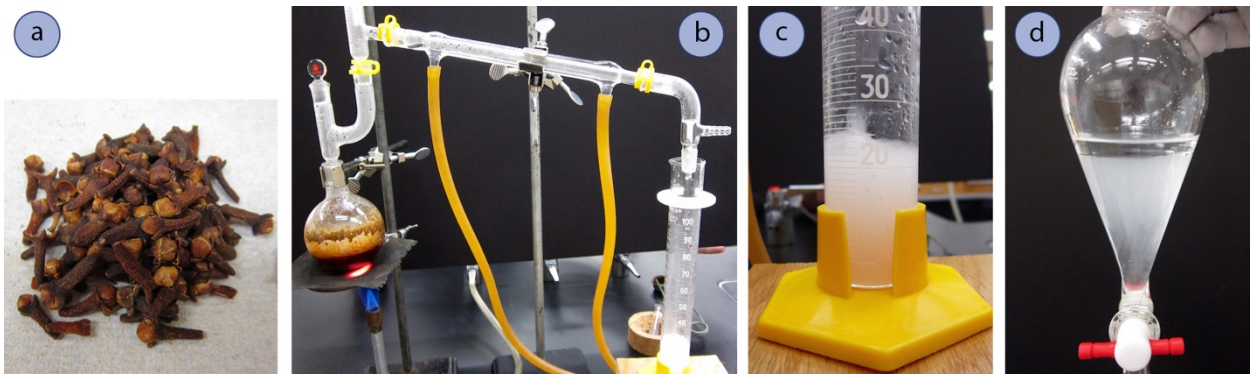

Figure 4.4: a) Whole cloves, b) Steam distillation of cloves, c) Milky distillate composed of oil and water, d) Using extraction to separate the oil from the water.

4.2.C SELECTIVE REMOVAL OF COMPONENTS

When conducting an experiment that synthesizes a chemical product, a reaction is often complete whenever stirring or heating is ceased. And yet, there are always more steps in the procedure! What commonly happens directly afterwards is to "**work-up**" the reaction in some way. A work-up refers to methods aimed at isolating the product from the reaction mixture, and often begins by using a separatory funnel and extractions.

For example, imagine that acetic acid and isopentanol have been heated in the presence of an acid catalyst for one hour (Figure 4.5) in order to make isopentyl acetate, an ester that smells of bananas (see reaction scheme in Figure 4.6). After the one-hour time period, there is unfortunately not *just* the banana-smelling ester in the flask. The flask will also contain byproducts (the water in this case), leftover starting materials if the reaction is incomplete, as well as any catalysts used (H_2SO_4 in this case). In this example, there could be *five* compounds in the reaction flask after heating is ceased (Figure 4.7)!

Figure 4.5: Reaction to make isopentyl acetate.

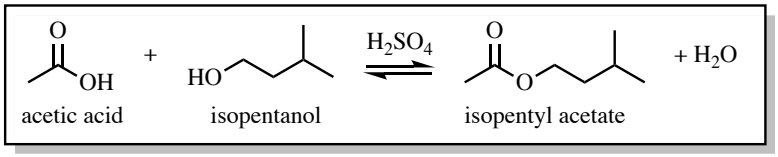

Figure 4.6: Reaction scheme to produce isopentyl acetate.

When "working up" this reaction, the resulting mixture is often poured into a separatory funnel along with some water and organic solvent. This produces two layers in the separatory funnel: an aqueous layer and an organic layer.

After shaking this heterogeneous mixture, the compounds distribute themselves based on their solubility. Compounds that have high water solubility favor the aqueous layer while less polar compounds favor the organic layer. In this example, the acid catalyst and residual carboxylic acid or alcohol would likely be drawn into the water layer. The ester would have a greater affinity for the organic layer than the aqueous layer, causing it to be isolated from the other components in the reaction mixture (Figure 4.7).

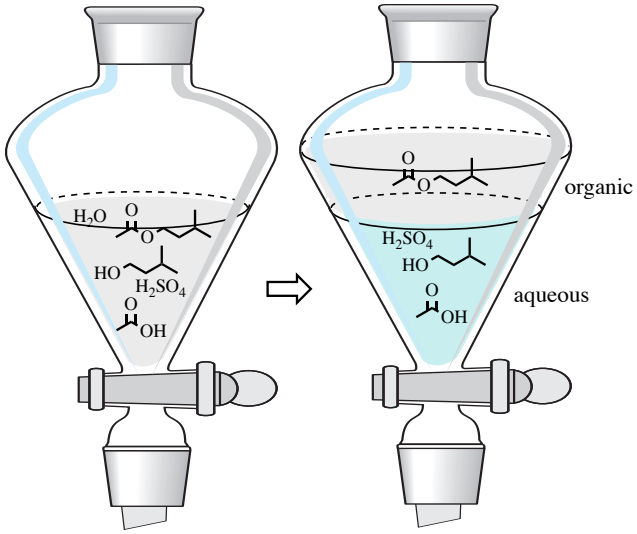

Figure 4.7: Extraction using water and an organic solvent to isolate isopentyl acetate from the reaction mixture.

In this example, it is possible that small amounts of alcohol are also drawn into the organic layer, but they could likely be removed with a water "**wash**." In a wash, the desired compound (*e.g.* the isopentyl acetate), remains in its current layer of the separatory funnel (in this example the organic layer), and unwanted compounds are removed, or "washed away" into another layer (*e.g.* the aqueous layer). A wash is different than an extraction, because in an extraction the desired compound *moves* from its current location (*i.e.* moves from an aqueous layer to an organic layer), while in washing the desired compound stays in its current layer.

4.3 WHICH LAYER IS WHICH?

4.3.A DENSITY

It is essential that you know whether the aqueous layer is above or below the organic layer in the separatory funnel, as it dictates which layer is kept and which is eventually discarded.

Two immiscible solvents will stack atop one another based on differences in density. The solution with the lower density will rest on top, and the denser solution will rest on the bottom.

Most non-halogenated organic solvents have densities less than 1 g/mL, so will float atop an aqueous solution (if they are immiscible). A notable exception is that halogenated solvents are denser than water (have densities > 1 g/mL), and so will instead sink below aqueous solutions (Table 4.1 and Figure 4.8).

Solvent	Density (g/mL)
Pentane	0.626
Petroleum Ether (mixture of C_5-C_6 hydrocarbons)	0.653
Hexanes (mixture of C_6 hydrocarbons)	0.655
Diethyl ether	0.713
Ethyl acetate	0.902
Water	0.998
Dichloromethane (CH_2Cl_2)	1.33
Chloroform ($CHCl_3$)	1.49

Table 4.1: Density of common solvents at room temperature.[1]

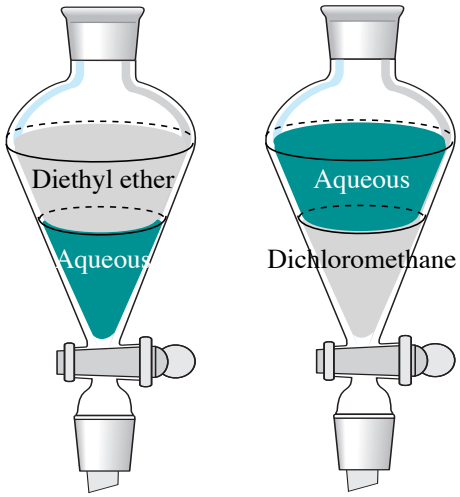

Figure 4.8: Relative position of aqueous and organic layers. Most organic solvents are on top, except for halogenated solvents, which are typically on bottom.

Many solutions used in separatory funnels are fairly dilute, so the density of the solution is approximately the same as the density of the solvent. For example, if mixing diethyl ether and a 10% NaOH *(aq)* solution in a separatory funnel, knowledge of the exact density of the 10% NaOH solution is not necessary. A 10% NaOH *(aq)* solution is 90% water (by mass), meaning the density should be fairly close to the density of water (approximately 1 g/mL). The actual density of a 10% NaOH *(aq)* solution is 1.1089 g/mL, a value only slightly greater than the density of water. The diethyl ether will be the top layer in this situation.

There are times, however, when so many solute particles are dissolved that a *solution's* density is much greater than the solvent density. For example, a saturated NaCl *(aq)* solution has a density around 1.2 g/mL (significantly greater than the density of water), and can cause separation problems with solvents of similar densities like dichloromethane.

[1] The solvents listed in Table 4.1 are pure compounds except for petroleum ether and hexanes. "Petroleum Ether" contains pentane, 2-methylbutane, 2,2-dimethylpropane, *n*-hexane, 2-methylpentane, 3-methylpentane, 2,2-dimethylbutane, and 2,3-dimethylbutane. "Hexanes" contains 2-methylpentane, 3-methylpentane, *n*-hexane and methylcyclopentane.

4.3.B HOW TO DETERMINE THE AQUEOUS LAYER

Solvent densities may be used to predict which layer is organic and which is aqueous in a separatory funnel, but there are other methods that can be useful in this determination. If unsure which layer is aqueous and which layer is organic, do one of the following things:

1. Add a bit of water from a squirt bottle to the separatory funnel (Figure 4.9a) and watch where the water droplets go.

   If the top layer is aqueous, the water droplets should mix with the top layer, and they will look as if they disappear. If the bottom layer is aqueous, the water droplets will fall through the top layer to mix with the bottom layer (as indicated by an arrow in Figures 4.9 b+c). If it is difficult to track where the water droplets go, also keep track of the volume of the layers: whichever layer increases with the addition of water is the aqueous layer.

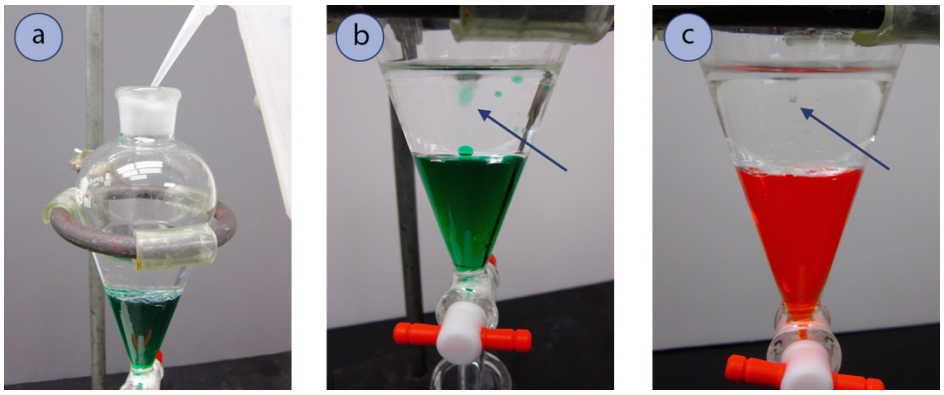

Figure 4.9: a) Adding water from a squirt bottle to determine which layer is aqueous, b) Water colored with green food dye is dropped into the funnel and falls to the bottom layer (aqueous), c) Water falling to the bottom layer, as indicated by the arrow.

2. Consider relative volumes of aqueous and organic solvents, based on quantities used in the experiment.

   Figure 4.10a shows a 125 mL separatory funnel containing 10 mL hexane and 100 mL water (tinted with blue dye). If these were the quantities used in an experiment, the aqueous layer would have to be the lower layer as it is so much larger. Although unequivocal in this case, it is important to know that the odd shape of the separatory funnel may cause you to misjudge volumes. A separatory funnel with *equal* volumes of aqueous and organic layers is shown in Figure 4.10b, although the layers rise to different heights in the funnel.

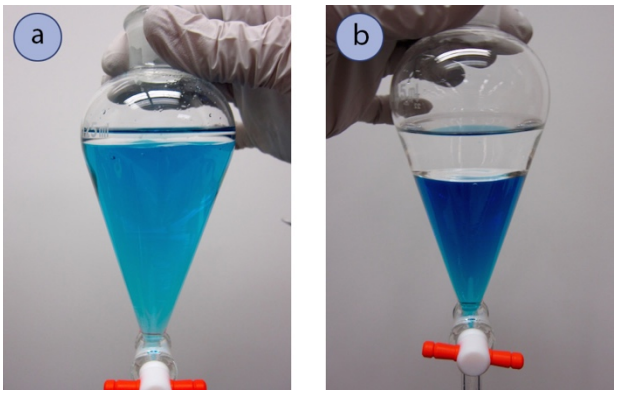

Figure 4.10: a) 10 mL organic solvent (hexanes) with 100 mL water (colored with blue dye) in a 125 mL separatory funnel, a) 40 mL each of organic solvent (ethyl acetate), and water (colored with blue dye).

4.4 EXTRACTION THEORY

4.4.A PARTITION / DISTRIBUTION COEFFICIENT (*K*)

When a solution is placed in a separatory funnel and shaken with an immiscible solvent, solutes often dissolve in *part* into both layers. The components are said to "partition" between the two layers, or "distribute themselves" between the two layers. When equilibrium has established, the ratio of concentration of solute in each layer is constant for each system, and this can be represented by a value *K* (called the **partition coefficient** or **distribution coefficient**).

$$K = \frac{\text{Molarity in organic phase}}{\text{Molarity in aqueous phase}}$$

For example, morphine has a partition coefficient of roughly 6 in ethyl acetate and water.[2] If dark circles represent morphine molecules, 1.00 g of morphine would distribute itself as shown in Figure 4.11.

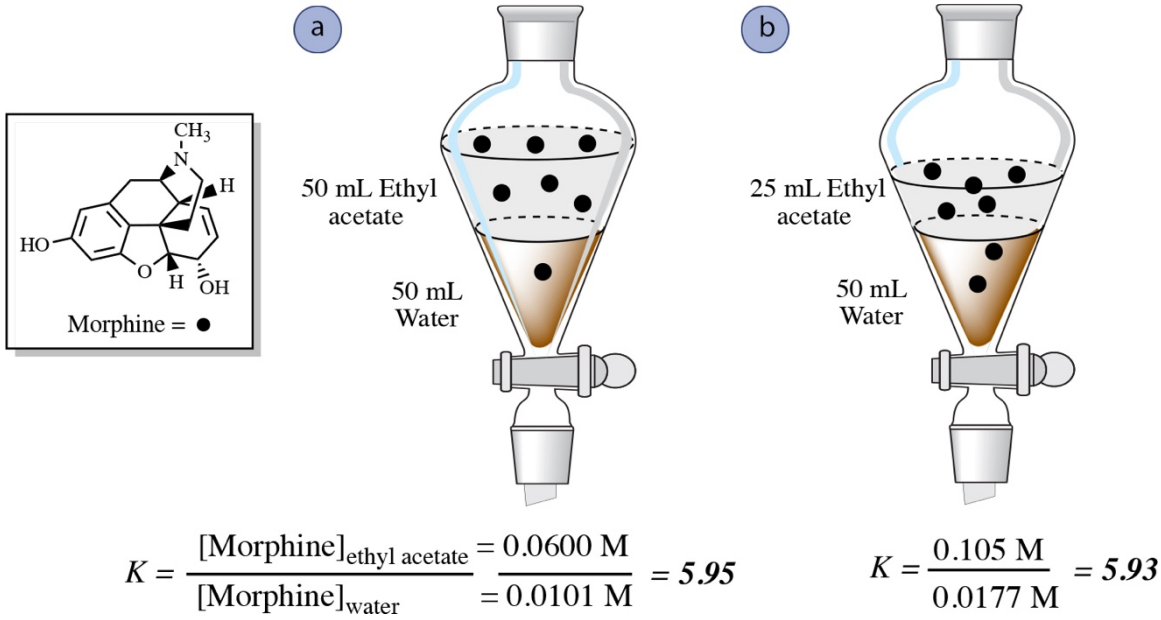

Figure 4.11: Distribution of morphine in ethyl acetate and water.

Note that with equal volumes of organic and aqueous phases, the partition coefficient represents the ratio of particles in each layer (Figure 4.11a). When using equal volumes, a *K* of ~6 means there will be six times as many morphine molecules in the organic layer as there are in the water layer. The particulate ratio is not as simple when the layer volumes are different, but the ratio of *concentrations* always equals the *K* (Figure 4.11b).

The partition coefficients reflect the solubility of a compound in the organic and aqueous layers, and so is dependent on the solvent system used. For example, morphine has a *K* of roughly 2 in petroleum ether and water, and a *K* of roughly 0.33 in diethyl ether and water.[2] When the *K* is less than one, it means the compound partitions into the aqueous layer more than the organic layer.

[2] The partition coefficients were approximated using solubility data found in: A. Seidell, *Solubilities of Inorganic and Organic Substances*, D. Van Nostrand Company, **1907**.

4.4.B CHOOSING A SOLVENT WITH SOLUBILITY DATA

The partition coefficient K is the ratio of the compound's concentration in the organic layer compared to the aqueous layer. Actual partition coefficients are experimental, but can be estimated by using solubility data.

$$K = \frac{\text{Molarity in organic phase}}{\text{Molarity in aqueous phase}} \qquad K \sim \frac{\text{Solubility in organic phase}}{\text{Solubility in aqueous phase}}$$

The K's calculated using molarity and solubility values are not identical since different equilibria are involved. The true K represents the equilibrium between aqueous and organic solutions, while solubility data represent the equilibrium between a saturated solution and the solid phase. The two systems are related however, and K's derived from solubility data should be similar to actual K's.

Solubility data can therefore be used to choose an appropriate solvent for an extraction. For example, imagine that caffeine (Figure 4.12) is intended to be extracted from tea grounds into boiling water, then later extracted into an organic solvent. Solubility data for caffeine is shown in Table 4.2.

Solvent	1 g caffeine dissolves in (25 °C)
Water	46 mL
Diethyl Ether	530 mL
Benzene	100 mL
Chloroform	5.5 mL

Table 4.2: Solubility of caffeine in different solvents.[3]

Figure 4.12: Caffeine.

Both diethyl ether and benzene at first glance appear to be poor choices for extraction because caffeine is more soluble in water than in either solvent (if a gram of caffeine dissolves in 46 mL water, but 100 mL of benzene, caffeine is more soluble in water). When extracting with either of these solvents, the K would be less than one (see calculation below) and it would be an "uphill battle" to draw out the caffeine from the water. However, caffeine is more soluble in chloroform than water, so chloroform would be the best choice of the solvents shown in terms of the maximum extraction of caffeine.

$$K_{\text{benzene}} \sim \frac{\left(\frac{1\text{ g caffeine}}{100\text{ mL benzene}}\right)}{\left(\frac{1\text{ g caffeine}}{46\text{ mL water}}\right)} \sim 0.46 \qquad K_{\text{chloroform}} \sim \frac{\left(\frac{1\text{ g caffeine}}{5.5\text{ mL chloroform}}\right)}{\left(\frac{1\text{ g caffeine}}{46\text{ mL water}}\right)} \sim 8.4$$

Another consideration when choosing a solvent for extraction is toxicity: chloroform is carcinogenic and therefore is probably *not* be the best option despite its excellent solvation ability. A further consideration is the solubility of other components present in a mixture. If the goal is to extract caffeine preferentially and leave behind other components in the tea, one solvent may be more selective in this regard.

[3] From: *The Merck Index*, 12th edition, Merck Research Laboratories, **1996**.

4.4.C QUANTITATING SINGLE EXTRACTION

Hyoscyamine is an alkaloid from a plant in the nightshade family (Figure 4.13a), and is used medicinally to provide relief for a variety of gastrointestinal disorders. Its solubility data is shown in Figure 4.13b.

Imagine that a nearly saturated solution of **0.50 g hyoscyamine in 150 mL water is to be extracted into 150 mL diethyl ether**. How much hyoscyamine would be extracted into the diethyl ether layer in this process?

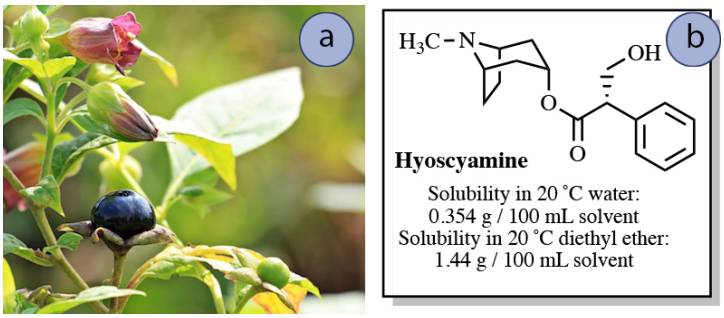

Figure 4.13: a) Deadly nightshade plant (photo public domain from pixabay.com), b) Solubility data for hyoscyamine (Ref. 2).

This quantity can be approximated using the solubility data. Taking the ratio of the compound's solubility in diethyl ether compared to water gives an approximate K of 4.

$$K \sim \frac{\text{organic solubility}}{\text{water solubility}} \sim \frac{(1.44 \text{ g hyoscyamine / 100 mL diethyl ether})}{(0.354 \text{ g hyoscyamine / 100 mL water})} \sim \mathbf{4.07} \text{ (approximate } K\text{)}$$

If *"x"* is the gram quantity of hyoscyamine extracted into the diethyl ether layer, then "0.50 g – x" would remain in the aqueous layer after equilibrium is established. Knowing the value of K, the value of x can be solved for using the equation below.

$$4.07 = \frac{\left(\frac{x}{150 \text{ mL ether}}\right)}{\left(\frac{0.50 \text{ g - } x}{150 \text{ mL water}}\right)} \quad \text{After solving the algebra, } x = \mathbf{0.40 \text{ g}}$$

This result means that 0.40 g of the original 0.50 g of hyoscyamine is extracted into the diethyl ether using a single extraction. This process is summarized in Figure 4.14.

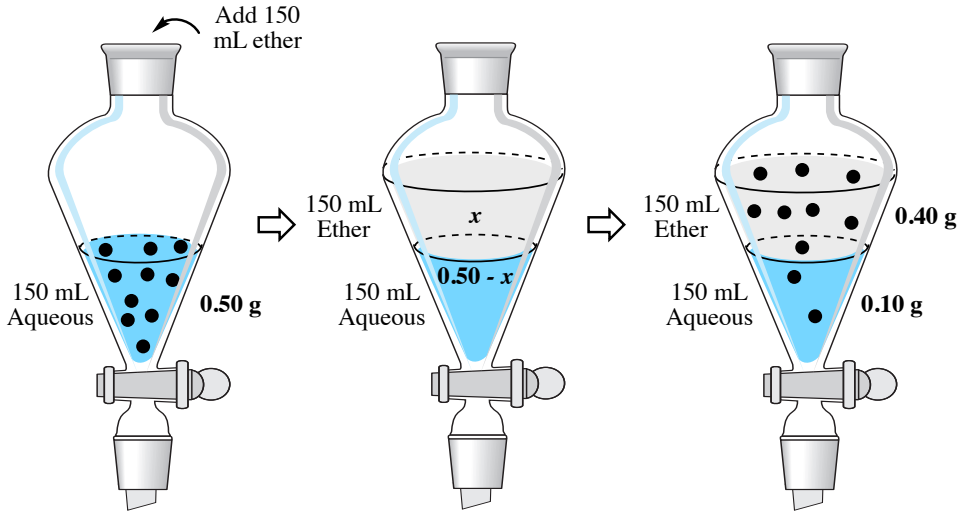

Figure 4.14: Single extraction of hyoscyamine ($K \sim 4$) from water into diethyl ether.

In this example, a single extraction resulted in extraction of 80% of the hyoscyamine (100% × 0.40 g / 0.50 g) from the aqueous layer into the organic layer. The partitioning of the compound between the two layers caused the sample to be incompletely extracted.

4.4.D MULTIPLE EXTRACTIONS

4.4.D.1 OVERVIEW OF MULTIPLE EXTRACTION

Depending on the partition coefficient for a compound in a solvent, a single extraction may be all that is needed to effectively extract a compound. However, more often than not a procedure calls for a solution to be extracted multiple times in order to isolate a desired compound, as this method is more efficient than a single extraction (see journal article in Figure 4.15b for an example of where this process is used).

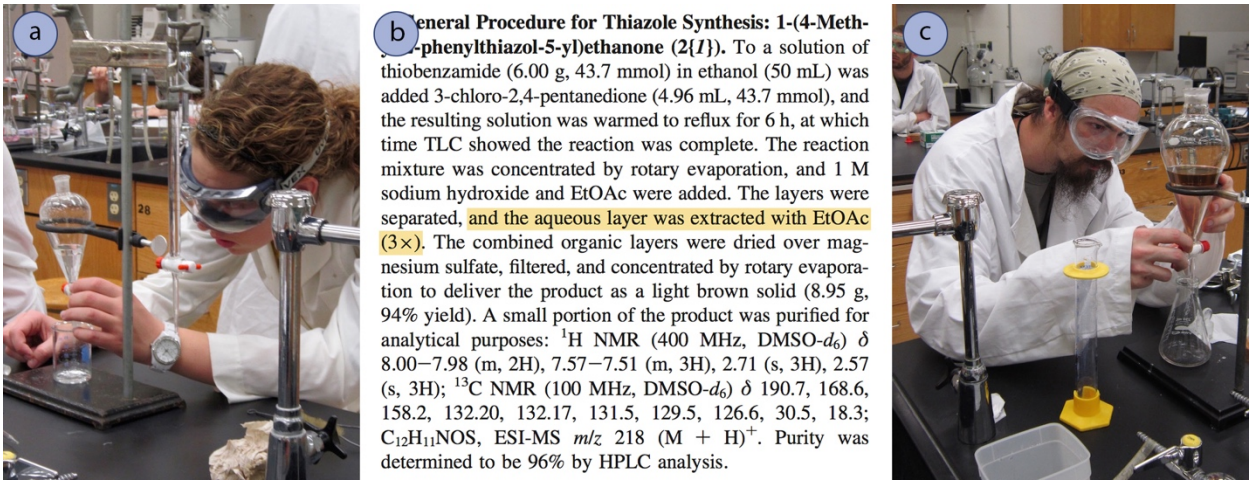

Figure 4.15: a+c) Students using a separatory funnel, b) Journal article from: K.A. Milinkevich, M.J. Kurth, *J. Comb. Chem.* **2008**, *10,* 521–525.

In a multiple extraction procedure, a quantity of solvent is used to extract one layer (often the aqueous layer) multiple times in succession. The extraction is repeated two to three times, or perhaps more times if the compound has a low partition coefficient in the organic solvent. Figure 4.16 shows a diagram of an aqueous solution being extracted twice with diethyl ether. Diethyl ether has a density less than 1 g/mL, so is the top organic layer in the funnel.

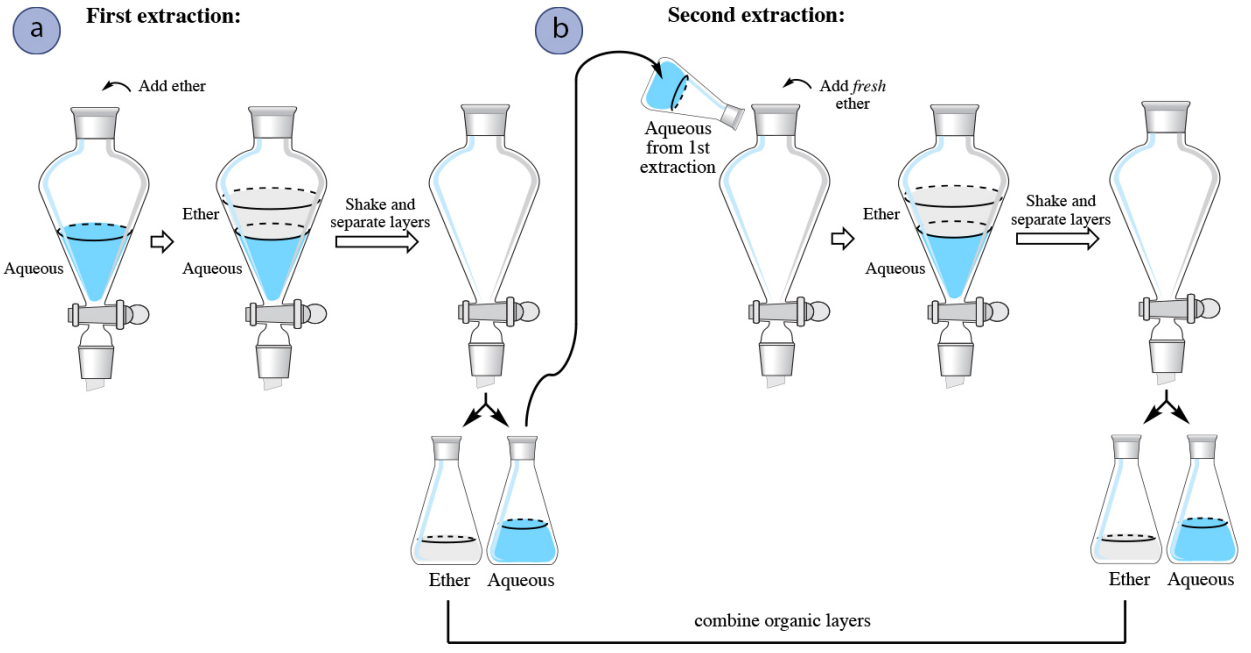

Figure 4.16: Multiple Extractions of an aqueous layer when the organic layer is on the top: a) First extraction, b) Second extraction.

In a multiple extraction of an aqueous layer, the first extraction is procedurally identical to a single extraction. In the second extraction, *the aqueous layer* from the first extraction is returned to the separatory funnel (Figure 4.16b), with the goal of extracting additional compound. Since the organic layer from the first extraction had already reached equilibrium with the aqueous layer, it would do little good to return it to the separatory funnel and expose it to the aqueous layer again. Instead, *fresh* diethyl ether is added to the aqueous layer, since it has the potential to extract more compound.

The process is often repeated with a third extraction (not shown in Figure 4.16), with the aqueous layer from the second extraction being returned to the separatory funnel, followed by another portion of fresh organic solvent. In multiple extractions, the organic layers are combined together, as the goal is to extract the compound into the organic solvent.

When an aqueous solution is extracted with an organic solvent that is denser than water (for example dichloromethane, CH_2Cl_2), the only procedural difference is that there is no need to ever drain the aqueous layer from the separatory funnel. After draining the organic layer from the first extraction, fresh solvent can be added to the aqueous layer remaining in the funnel to begin the second extraction (Figure 4.17b).

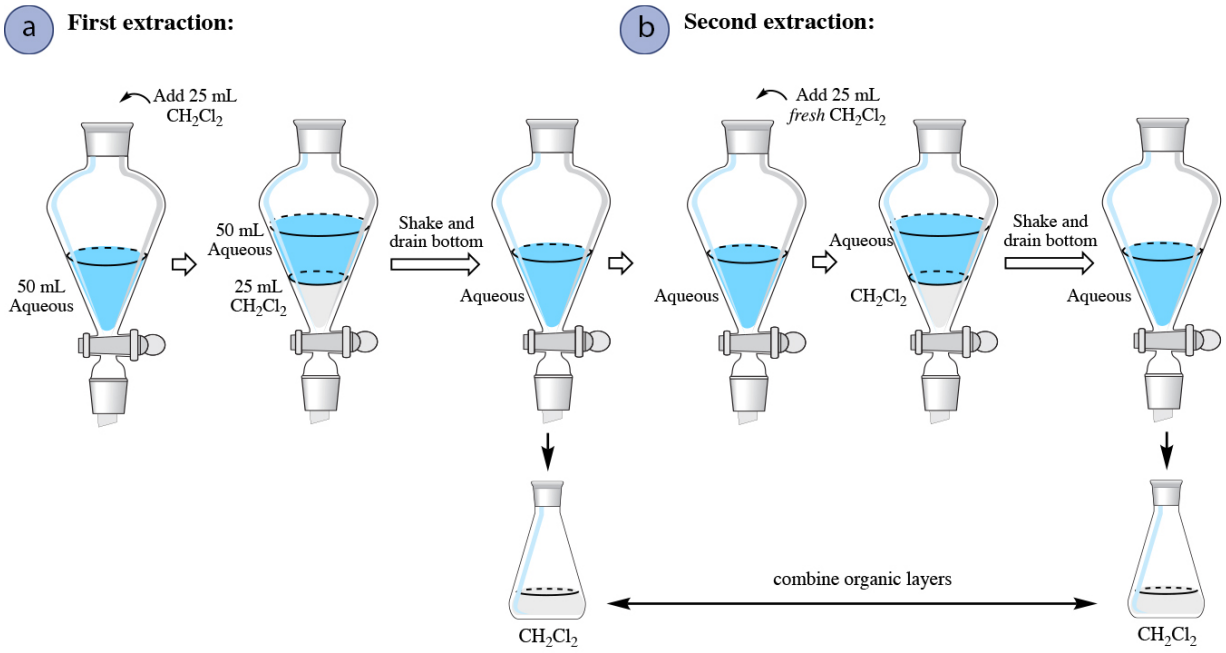

Figure 4.17: Multiple Extractions of an aqueous layer when the organic layer is on the bottom: a) First extraction, b) Second extraction.

4.4.D.2 QUANTITATING MULTIPLE EXTRACTION

To demonstrate the effectiveness of a multiple extraction, let's return to the problem from the single extraction section, where a solution of **0.50 g hyoscyamine in 150 mL water is to be extracted** into diethyl ether. Instead of using one 150 mL portion, let's instead split the solvent into **three 50 mL portions of diethyl ether**. How much hyoscyamine would be extracted with this method?

In the previous section, solubility data was used to estimate the partition coefficient *K,* and it was found to be 4.07. As before, we can assign the quantity of hyoscyamine extracted into the diethyl ether the value "*x,*" which would leave "0.50 g – *x*" remaining in the aqueous layer of the first extraction. Using *K*, the calculation is identical to the previous discussion, differing only in the smaller volume of the organic layer (50 mL instead of 150 mL).

$$4.07 = \frac{\left(\frac{x}{50\ mL\ ether}\right)}{\left(\frac{0.50\ g - x}{150\ mL\ water}\right)}$$

After solving the algebra, $x = 0.29$ g

This result means that 0.29 g is extracted into the diethyl ether in the first extraction and 0.21 g remains in the aqueous layer (0.50 g – 0.29 g). As the aqueous layer is returned to the separatory funnel, the residual 0.21 g is the quantity to be further extracted, which alters the calculation for the second extraction by replacing the 0.50 g value.

$$4.07 = \frac{\left(\frac{x}{50\ mL\ ether}\right)}{\left(\frac{0.21\ g - x}{150\ mL\ water}\right)}$$

After solving the algebra, $x = 0.12$ g

This result means that 0.12 g is extracted into the diethyl ether in the second extraction and 0.09 g remains in the aqueous layer (0.21 g – 0.12 g). The calculation for the third extraction is as follows:

$$4.07 = \frac{\left(\frac{x}{50\ mL\ ether}\right)}{\left(\frac{0.09\ g - x}{150\ mL\ water}\right)}$$

After solving the algebra, $x = 0.05$ g

This result means 0.04 g remains in the aqueous layer (0.09 g – 0.05 g) after the third extraction. The results of the calculations in this section are summarized in Figure 4.18.

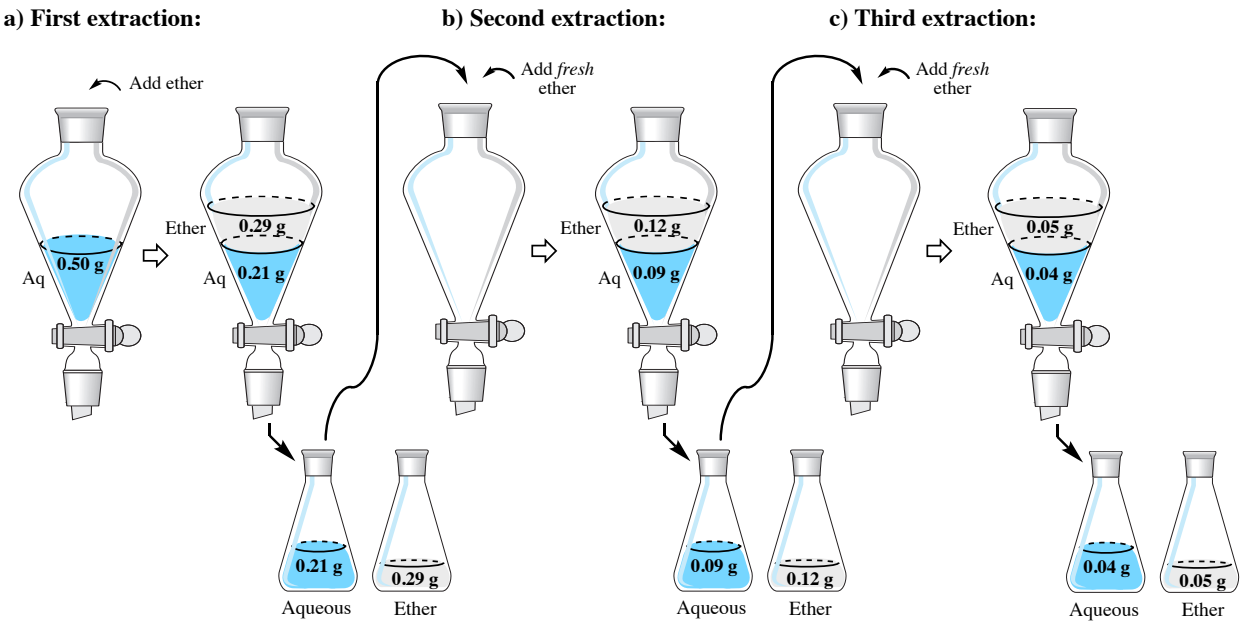

Figure 4.18: Multiple Extractions of hyoscyamine ($K \sim 4$) from water into diethyl ether.

If the 50 mL diethyl ether extracts are combined in this example (Figure 4.19), there would be a total of 0.46 g of hyoscyamine in the combined organic extracts. Of the 0.50 g of hyoscyamine in the original aqueous layer, **92%** of the material is extracted into the organic layer (100% × 0.46 g / 0.50 g). This is a greater quantity than was obtained using a single extraction of 150 mL diethyl ether, which resulted in only 0.40 g of hyoscyamine extracted (**80%**).

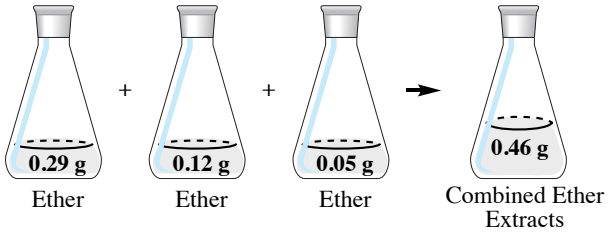

Figure 4.19: Combined organic layers containing hyoscyamine from multiple extractions into diethyl ether.

These calculations demonstrate that **using multiple portions of a solvent maximizes the extractive power of the solvent**. In general, three extractions are the optimal compromise between expended effort and maximizing the recovery of material.

4.5 STEP-BY-STEP PROCEDURES

4.5.A SINGLE EXTRACTION

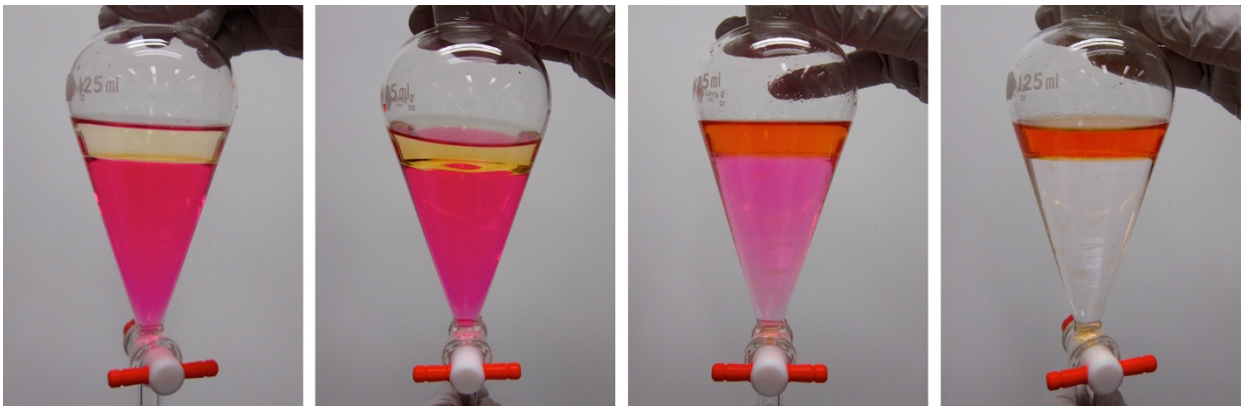

Figure 4.20: Progress of the extraction of methyl red (the colored compound) from the acidic aqueous layer (bottom) into the organic layer (top). The inversions were done very slowly in order to see the extraction stepwise. With even gentle mixing, the methyl red extracts rapidly.

The pictures in this section show a single extraction of methyl red (colored compound, Figure 4.21) from an aqueous solution (bottom layer) into 25 mL of ethyl acetate (top layer). The aqueous solution originally has a pink color, as the methyl red appears red in acidic solution (the aqueous solution was made from 50 mL water, 5 drops of 0.1 *M* HCl and 5 drops of 1% methyl red indicator solution). The methyl red has a large partition coefficient and is extracted from the aqueous layer into the ethyl acetate in this process.

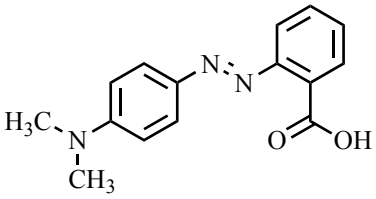

Figure 4.21: Structure of methyl red in highly acidic solution.

Figure 4.22: Organic chemistry students using separatory funnels.

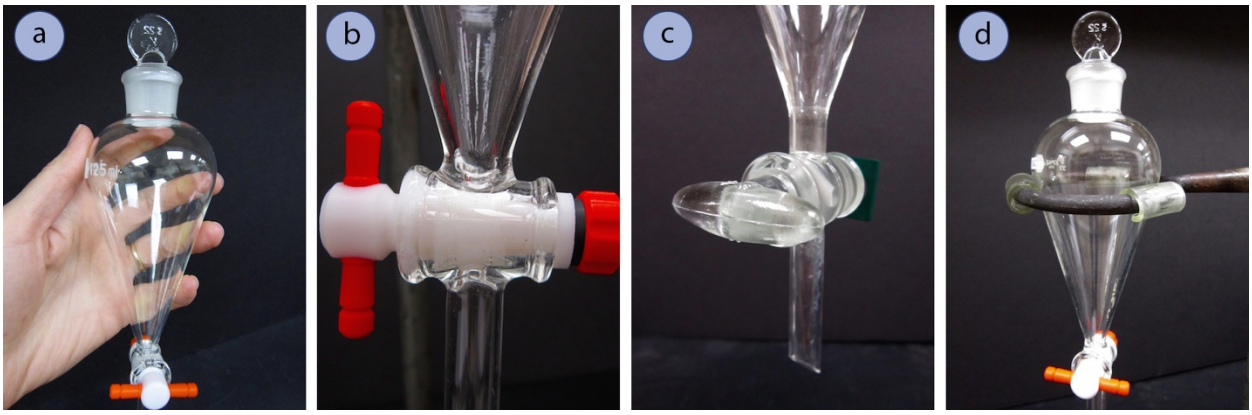

Figure 4.23: a) Separatory funnel, b) Correct order of stopcock components, c) Glass stopcock, d) Funnel in cushioned ring clamp.

Prepare the Setup

1. Obtain a separatory funnel (Figure 4.23a).

    a. If the separatory funnel has a Teflon stopcock, reassemble the stopcock if it was taken apart to dry, placing the parts in the appropriate order (Figure 4.23b). Be sure that the Teflon stopcock is moderately tight so that it can still easily turn, but is not so loose that liquid can seep around the joint.

    b. If using a glass stopcock (Figure 4.23c), it likely needs no further preparation. There should be a very thin layer of grease used to seal the stopcock and prevent freezing. If both glass and Teflon stopcocks are available, Teflon is a better choice as there is always a possibility that solvent can dissolve the grease used with glass stopcocks and contaminate the sample.

    c. Also obtain a stopper (Teflon or ground glass) that fits well in the top joint of the funnel (Figure 4.23a).

2. Place the separatory funnel in a ring clamp attached to a ring stand or latticework. The funnels are easy to break, so cushion the funnel in the metal clamp using pieces of slit rubber or plastic tubing (Figure 4.23d).

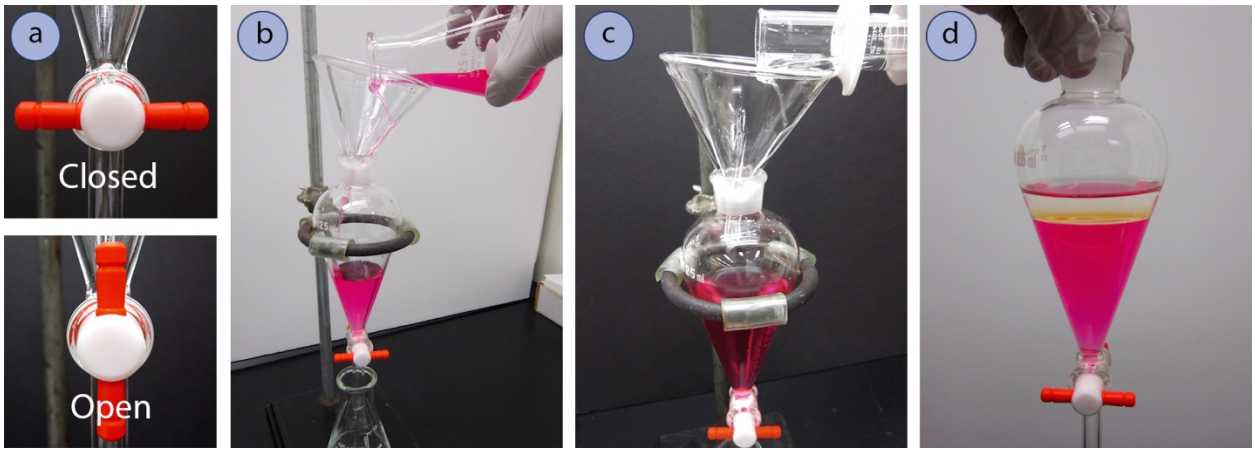

Figure 4.24: a) Closed and open stopcocks, b) Pouring in liquid with a funnel: notice the Erlenmeyer flask positioned below as a fail-safe, c) Pouring in the organic solvent, d) Separatory funnel before mixing.

Add the Solutions

3. Before pouring anything into a separatory funnel, be sure that the stopcock is in the "closed" position, where the stopcock is horizontal (Figure 4.24a).

   As a fail-safe, always position an Erlenmeyer flask beneath the separatory funnel before pouring (Figure 4.24b). This can catch liquid in case the stopcock is accidentally left open, or if the stopcock is loose and liquid leaks through unintentionally.

4. Using a funnel, pour the liquid to be extracted into the separatory funnel (Figures 4.24b + 4.25). A separatory funnel should never be used with a hot or warm liquid.

   The ground glass joint atop a separatory funnel is more prone to stick to the stopper if there was liquid in the joint at some point. Pouring liquid into the separatory funnel using a short-stemmed funnel avoids getting the joint wet, so that it will be less likely to freeze during mixing.

Figure 4.25: Student adds liquid to a separatory funnel.

5. Pour a quantity of the extractive solvent into the separatory funnel, as indicated by the procedure (Figure 4.24c).

   It is unnecessary to use precise quantities of solvent for extractions, and the volumes can be measured in a graduated cylinder. If a procedure calls for 20 mL of solvent, it is acceptable if between 20-25 mL of solvent is used each time.

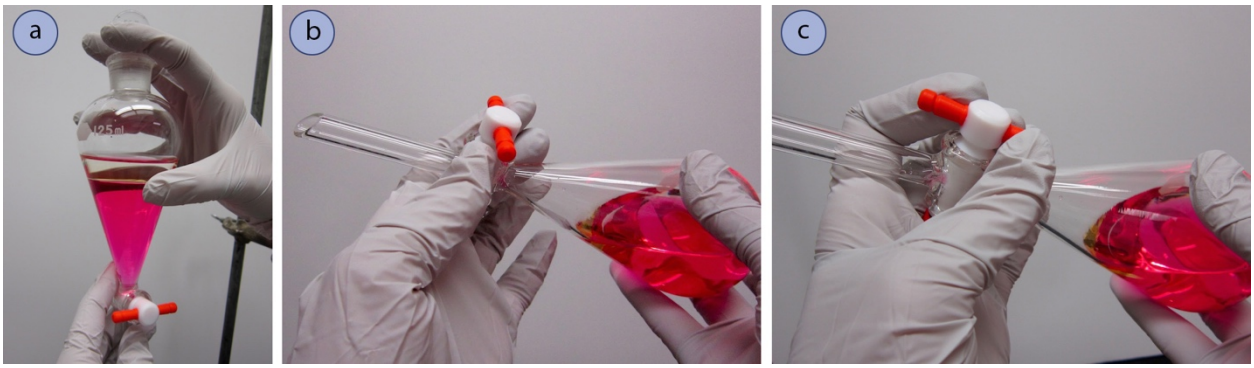

Figure 4.26: a) Holding the separatory funnel before shaking, b) Inverting the funnel to mix the components, c) Venting to release pressure.

Mix the Solutions

6. Place the stopper on the funnel, and hold the funnel such that the fingers of one hand securely cover the stopper, while the other hand grips the bottom of the funnel (Figure 4.26a).

7. Gently invert the funnel (Figure 4.26b), and swirl the mixture a little.

   Although it is not uncommon for *some* liquid to creep into the ground glass joint when inverted, it should be minimal. If liquid drips onto your fingers or gloves when you invert the funnel, the stopper is probably the wrong size.

8. Pressure may build up inside the separatory funnel when solutions are mixed, so immediately after swirling, and with the funnel still inverted, "**vent**" the funnel by briefly opening the stopcock to allow for a release of pressure (Figure 4.26c).

   Pressure builds in the funnel as solvent evaporates into the headspace and contributes additional vapor to the initial ~1 atmosphere of air pressure in the funnel. With highly volatile solvents (like diethyl ether), a definite "swoosh" can be heard upon venting, and small amounts of liquid may even sputter out the stopcock. If liquid spits out the stopcock, try to allow it to drain back into the funnel. The noise associated with venting normally ceases after the second or third inversions, as the headspace becomes saturated with solvent vapors and the pressures inside and outside the funnel are equalized.

   Safety note: Never point the stopcock toward someone as you vent, as it's possible some liquid may splatter onto him or her.

9. Close the stopcock and mix the solutions a bit more vigorously, periodically stopping to vent the system.

   There are differences of opinion on how vigorously solutions should be mixed in separatory funnels, and for how long. As a general guide, a mild mixing for **10-20 seconds** should be enough. With some solutions (*e.g.* dichloromethane), care should be taken to not shake too vigorously, as these solutions often form emulsions (where the interface between the solutions doesn't clarify). With solutions prone to emulsions, a funnel should be gently *rocked* for one minute.

10. Place the separatory funnel upright in the ring clamp to allow the layers to fully separate. The interface between the layers should settle rather quickly, often within 10 seconds or so. If the interface is clouded or not well defined (an emulsion has formed), see the troubleshooting section for tips.

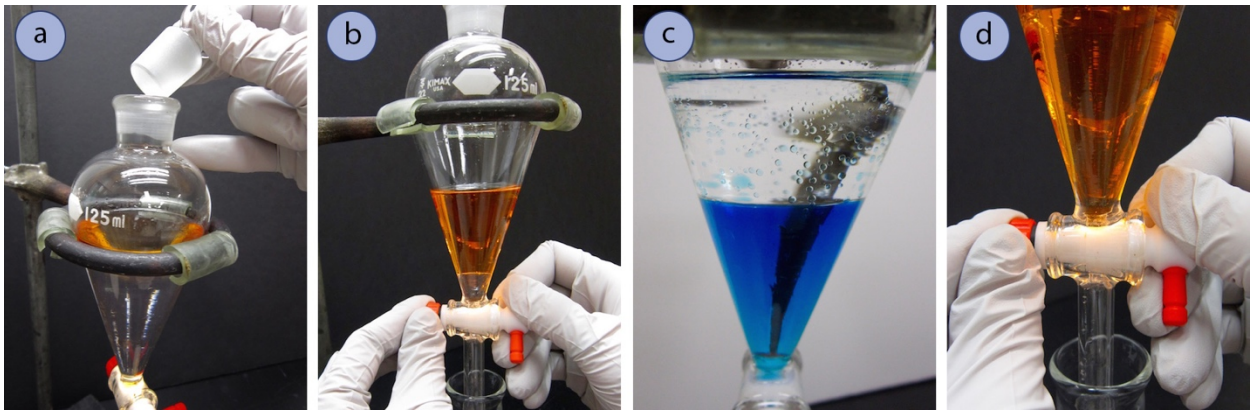

Figure 4.27: a) Taking the stopper off before draining the funnel, b) Draining to the interface, c) Clinging droplets (using a different system), d) Stopping when the interface is in the stopcock.

Separate the Layers

11. Liquid will not drain well from a separatory funnel if the stopper remains on, as air cannot enter the funnel to replace the displaced liquid. If liquid did drain from the funnel without replacement by an equal volume of air, a negative pressure would form in the funnel. Thus, before draining liquid from a separatory funnel, remove the stopper (Figure 4.27a).

12. Drain the majority of the bottom layer into a clean Erlenmeyer flask, positioning the ring clamp so that the tip of the separatory funnel is nestled in the Erlenmeyer flask to prevent splashing (Figure 4.27b). Stop draining when the interface is within 1 cm of the bottom of the stopcock.

13. Gently swirl the funnel to dislodge any droplets clinging to the glass (Figure 4.27c). A glass stirring rod can be used to knock down stubborn clinging droplets.

14. Further drain the bottom layer, stopping when the interface just enters the stopcock chamber (Figure 4.27d). Label the Erlenmeyer flask (*e.g.* "bottom layer").

    When labeling flasks, it's often best to use terminology that is without question correct, such as "top layer" or "bottom layer." If the layers are labeled with statements like "organic layer" or "aqueous layer," it's possible that the layers have been incorrectly identified. It may be best to use combined statements like "top organic layer," as it's quite useful to track the aqueous and organic layers. If the layers have been incorrectly identified, at least the "top" part of the label will always be correct.

    Flasks can be labeled using labeling tape or by writing directly on the glass with a permanent marker (*e.g.* Sharpie). Marker ink can be removed from glass by rubbing it with a KimWipe moistened with a bit of acetone.

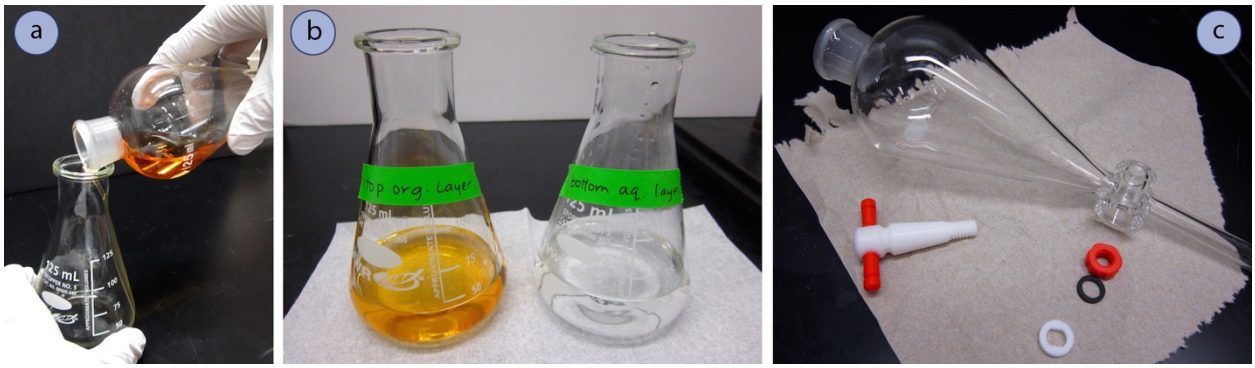

Figure 4.28: a) Pouring out the top layer, b) Labeled layers, c) Drying the separatory funnel with a disassembled stopcock.

15. Pour out the top layer from the top of the separatory funnel into another clean Erlenmeyer flask (Figure 4.28a), making sure to again label this flask (Figure 4.28b).

    It is proper technique to drain the bottom layer through the stopcock, and to pour out the top layer from the top of the funnel. This method minimizes re-mixing the solutions, as only the lower layer touches the stem of the funnel.

16. **Never throw away any liquids from an extraction** until you are *absolutely* sure that you have the desired compound. Undesired layers can be properly disposed of when the desired compound is *in your hands* (*e.g.* after the rotary evaporator has removed the solvent).

    Mistakes made during extractions (*e.g.* carrying on with the wrong layer), can be solved as long as the solutions have not been placed in the waste container! The layers should also be saved until after evaporation because the desired compound may not be very soluble in the solvent used. If the compound failed to extract in one solvent, a different solvent could be tried later, again only if the layers had not yet been thrown away.

Clean Up

17. To clean a separatory funnel, first rinse it with acetone into a waste container. Then wash the funnel with soap and water at your benchtop. Disassemble the Teflon stopcock (if used). After rinsing with distilled water, allow the parts to dry separated in your locker (Figure 4.28c).

4.5.B SINGLE EXTRACTION SUMMARY

			
Use slit tubing to cushion the separatory funnel in the ring clamp. Close the stopcock on the separatory funnel and position an Erlenmeyer flask beneath the setup, in case it drips. Into the separatory funnel pour the liquid to be extracted using a funnel: this prevents liquid from getting on the ground glass joint which can cause it to stick. Pour the extractive solvent into the funnel.	Hold the separatory funnel so that your fingers firmly cover the stopper. Invert the funnel and shake gently for **10-20 seconds.** Periodically "vent" the funnel (open the stopcock while inverted to release pressure). Never point the tip at someone while venting.	Return the separatory funnel to the ring clamp, and allow the layers to separate. Remove the stopper (it won't drain otherwise). Drain the majority of the bottom layer into an Erlenmeyer flask. Stop when roughly 1 cm of the bottom layer is in the funnel, and swirl to dislodge clinging droplets. Drain the rest of the bottom layer, stopping when the interface is inside the stopcock.	Label the flask (*e.g.* "bottom aqueous layer"). Pour out the top layer into another Erlenmeyer flask (and label it). **Don't throw away either layer** until you are sure you've accomplished the goal of the extraction.

Table 4.3: Procedural summary for single extraction.

4.5.C MULTIPLE EXTRACTIONS

4.5.C.1 ORGANIC LAYER IS ON THE TOP

In this section are stepwise instructions on how to extract an aqueous solution with an organic solvent that is less dense than water (the organic layer will be on the top). As an example, the instructions are written to extract an aqueous solution three times using 25 mL diethyl ether each time (**3 × 25 mL diethyl ether**). A procedural summary of the first two extractions is in Figure 4.29.

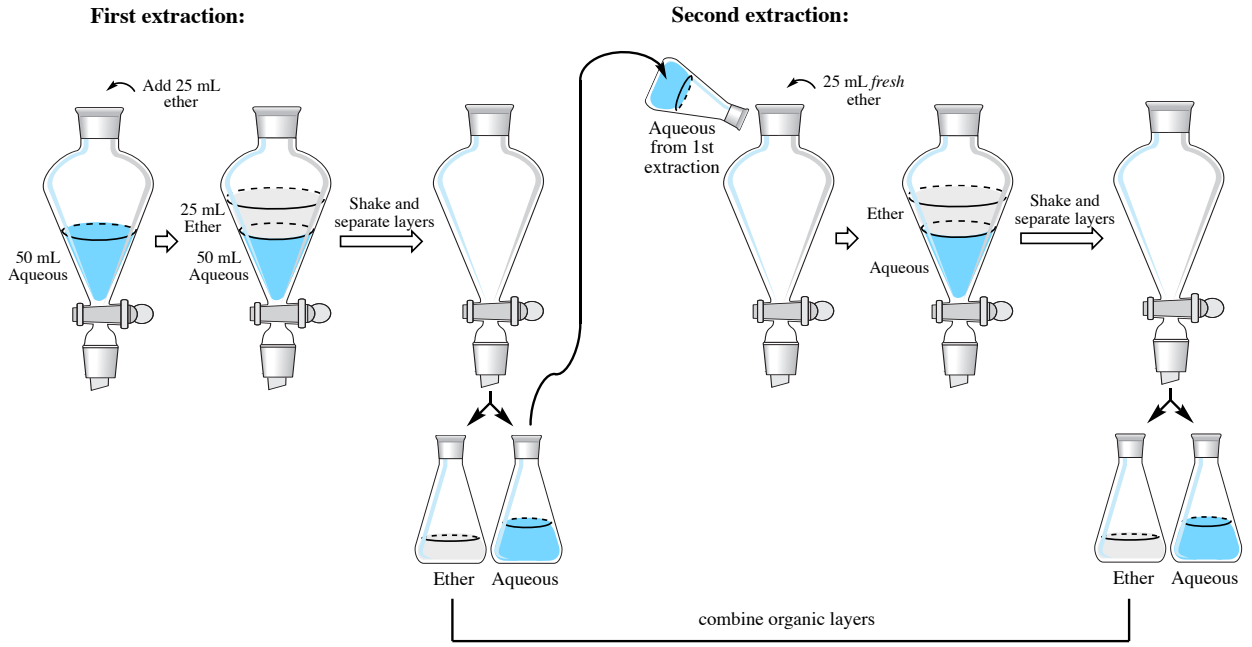

Figure 4.29: Two extractions when the organic layer is on the top.

Extraction #1

1. Perform a single extraction using approximately 25 mL of diethyl ether (an exact amount is not necessary), as described previously, making sure to appropriately label each layer (*e.g.* "top organic layer" and "bottom aqueous layer").

Extraction #2

2. Return the **aqueous layer** to the separatory funnel. There is no need to wash the funnel in between extractions.

3. Add a ***fresh*** 25 mL portion of diethyl ether to the separatory funnel. Stopper the funnel, invert and shake with venting, then allow the layers to separate.

   At this step, there should be two layers in the separatory funnel. If two layers aren't present, it's likely that the wrong layer was added to the funnel in step 2 (a common mistake). One way to test if this was the mistake is to add a bit of water from a squirt bottle. If the layer returned to the separatory funnel is the organic layer (incorrect), the squirt bottle water will not mix with the solution, and will instead fall as droplets to the bottom.

If the organic layer (incorrect) was accidentally returned to the separatory funnel, there is no harm done, as the organic layer was simply diluted. Pour the liquid back into the flask designated for the organic layer, and instead add the aqueous solution to the funnel.

4. Drain the bottom aqueous layer into an Erlenmeyer flask: it is acceptable to use the same flask that was used for the aqueous layer in the first extraction (that may have been labeled "bottom aqueous layer").

5. Since it is most common to combine the organic layers in multiple extractions, the top organic layer can be poured out of the separatory funnel into the same flask that was used for the organic layer in the first extraction (that may have been labeled "top organic layer"). In this flask, there should be roughly 50 mL of diethyl ether from the two extractions.

Extraction #3

6. Repeat the extraction a third time by adding the aqueous layer from the second extraction into the separatory funnel, followed by another ***fresh*** 25 mL portion of diethyl ether. Stopper the funnel, invert and shake with venting, then allow the layers to separate.

7. Drain the aqueous layer into the appropriate flask, and again pour the top layer into the organic layer flask, where there should be roughly 75 mL of diethyl ether from the three extractions.

4.5.C.2 ORGANIC LAYER IS ON THE BOTTOM

In this section are stepwise instructions on how to extract an aqueous solution with an organic solvent that is denser than water (the organic layer will be on the bottom). As an example, the instructions are written to extract an aqueous solution three times using 25 mL CH_2Cl_2 each time (**3 × 25 mL CH_2Cl_2**, Figure 4.28).

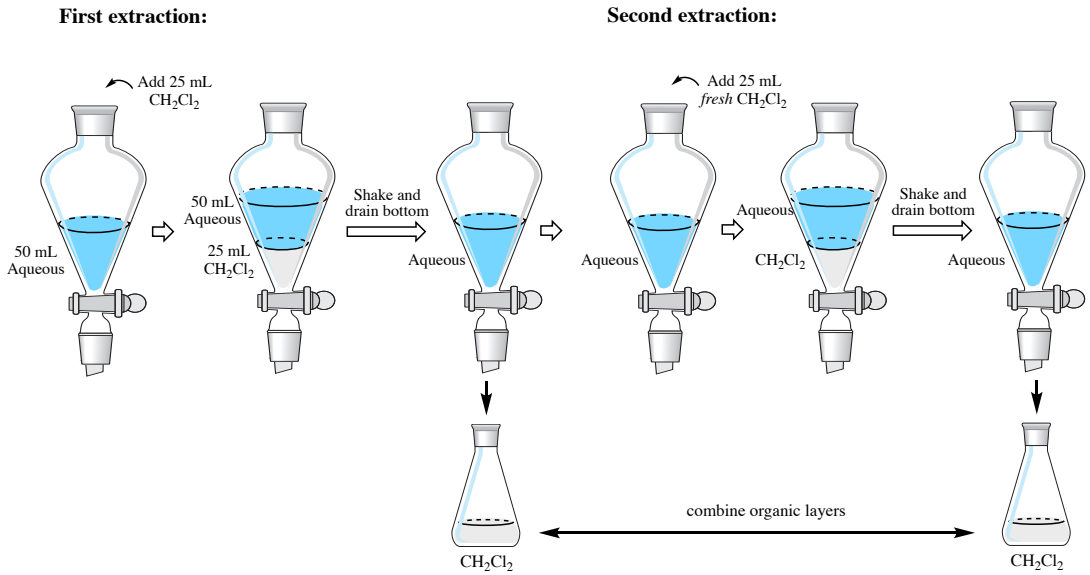

Figure 4.30: Two extractions when the organic layer is on the bottom.

Extraction #1

1. Perform a single extraction using approximately 25 mL of dichloromethane (CH_2Cl_2, an exact amount is not necessary), as described previously, with the following differences:
   a. As CH_2Cl_2 is prone to emulsions, invert the funnel and shake *gently* for one minute with venting.
   b. After allowing the layers to separate in the funnel, drain the bottom organic layer into a clean Erlenmeyer flask (and label the flask, *e.g.* "bottom organic layer"). Do not drain the top aqueous layer from the funnel.

Extraction #2

2. To the aqueous layer remaining in the funnel, add a ***fresh*** 25 mL portion of dichloromethane. Stopper the funnel, invert and shake *gently* for 1 minute with venting, then allow the layers to separate.

3. Since it is most common to combine the organic layers in multiple extractions, the bottom organic layer can be drained from the separatory funnel into the same flask that was used for the organic layer in the first extraction (that may have been labeled "bottom organic layer"). In this flask, there should be roughly 50 mL of dichloromethane from the two extractions.

Extraction #3

4. Repeat the extraction by adding another ***fresh*** 25 mL portion of dichloromethane to the aqueous layer in the separatory funnel. Stopper the funnel, invert and shake gently with venting, then allow the layers to separate.

5. Drain the bottom organic layer into flask used previously, where there should be roughly 75 mL of dichloromethane from the three extractions.

4.5.D TROUBLESHOOTING

This section describes common problems and solutions in extractions.

4.5.D.1 THERE IS ONLY ONE LAYER

The most common reason for having only one layer in a separatory funnel when there should be two (as in when the procedure tells you to "separate the layers"), is to have made a mistake. What likely happened is that the wrong layer was added to the separatory funnel- for example the organic layer was unknowingly added instead of the aqueous layer. When organic solvent is added to an organic layer in the separatory funnel, the result is only one layer. The mistake can be remedied as long as the layers have not yet been thrown away! If the correct layer is added to the funnel, everything will work out as planned.

To prevent making this mistake in the future, be sure to label the Erlenmeyer flasks. Also, be sure to never throw away a layer until you are absolutely sure that you've done everything correctly.

An occasional reason that only one layer forms in a separatory funnel is if there are large quantities of compounds present that dissolve in both solvents, for example if large amounts of ethanol are present, which dissolve well in both aqueous and organic solvents. In this situation, the best approach is to remove the troublesome compound (*i.e.* the ethanol) on a rotary evaporator before extraction.

4.5.D.2 THERE ARE THREE LAYERS

The most common reason for three layers in a separatory funnel is inadequate mixing (Figure 4.31a). If the funnel is shaken with more vigor it will likely settle into two layers (Figure 4.31b).

It is also possible that a middle third layer is an emulsion, where the two layers are not fully separated.

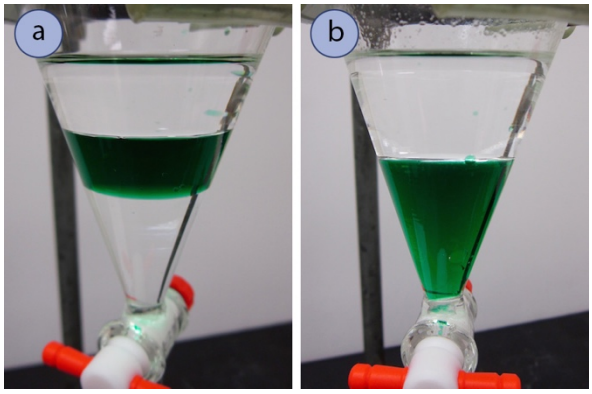

Figure 4.31: a) Three initial layers from inadequate mixing, b) Two layers resulting from more vigorous mixing.

4.5.D.3 THERE IS INSOLUBLE MATERIAL AT THE INTERFACE

A small amount of insoluble film between two layers is not uncommon during an extraction. Polymeric materials tend to rest between layers as solvent interactions are minimized at the interface. A minor film is not something to worry about because if a small amount does make it into the organic layer, a subsequent drying and filtration step will often remove it.

4.5.D.4 THE INTERFACE CANNOT BE SEEN

On occasion the compounds in a separatory funnel are so dark that they obscure the interface between the two layers. If this happens, there are several methods that might help you see the interface. One is to hold the separatory funnel up to the light, or to shine a flashlight onto the glass (Figure 4.32b). Additional light sometimes allows you to see the interface. A second method is to carefully observe the layers while tilting the funnel back and forth to the side (Figure 4.32c). Your eye can sometimes pick up on subtle differences in the way the liquids flow. A third method is to add a bit more solvent to the funnel to somewhat dilute one of the layers, or to add a different solvent to alter the index of refraction.

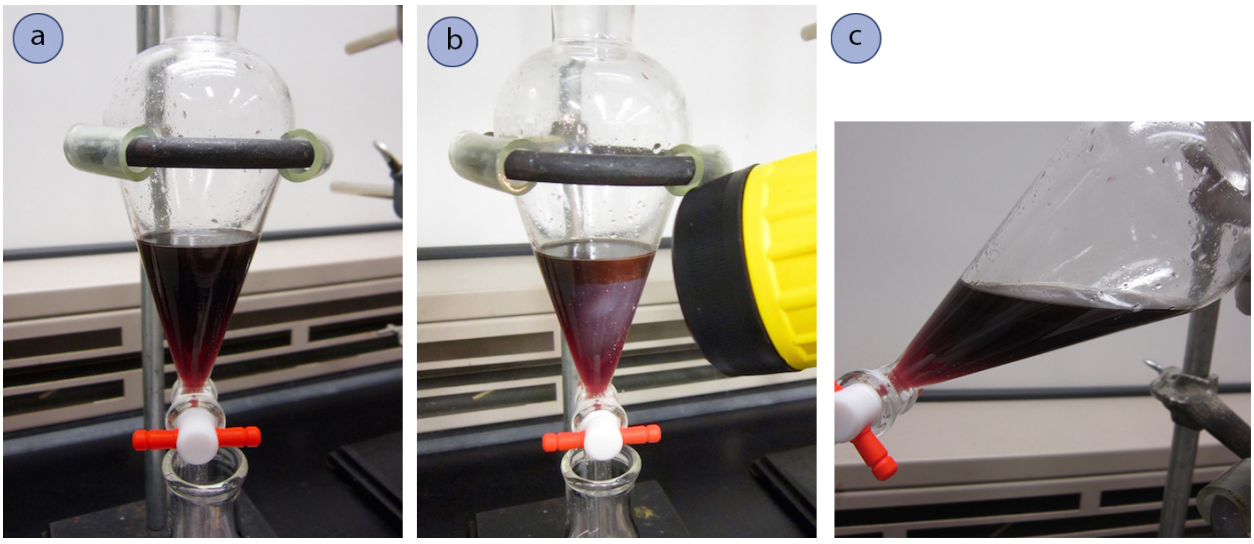

Figure 4.32: a) Interface is too dark to easily see, b) Flashlight visualizes the interface, c) Tilting also subtly visualizes the interface, although it is much less dramatic.

4.5.D.5 THE LAYERS DON'T SEPARATE WELL (AN EMULSION FORMED)

Emulsions are when tiny droplets of one layer are suspended in the other layer, resulting in no distinct interface between the two layers (Figure 4.33). Often an emulsion looks like a bubbly mess near the interface, and can even appear to be an odd-looking third layer.

Emulsions can happen for several reasons:

1) The density of each layer may be so similar that there is weak motivation for the liquids to separate.

2) There may be soap-like compounds or other emulsifying agents present that dissolve some of the components in one another.

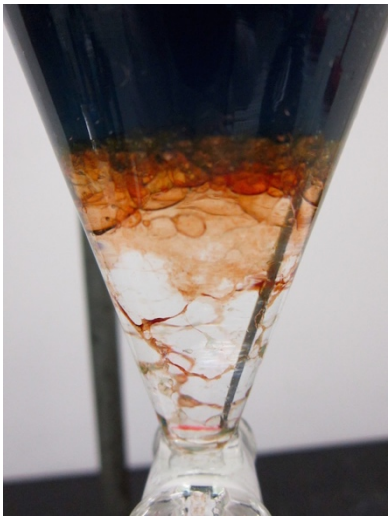

Figure 4.33: Emulsion formed between dichloromethane and brine (with food coloring).

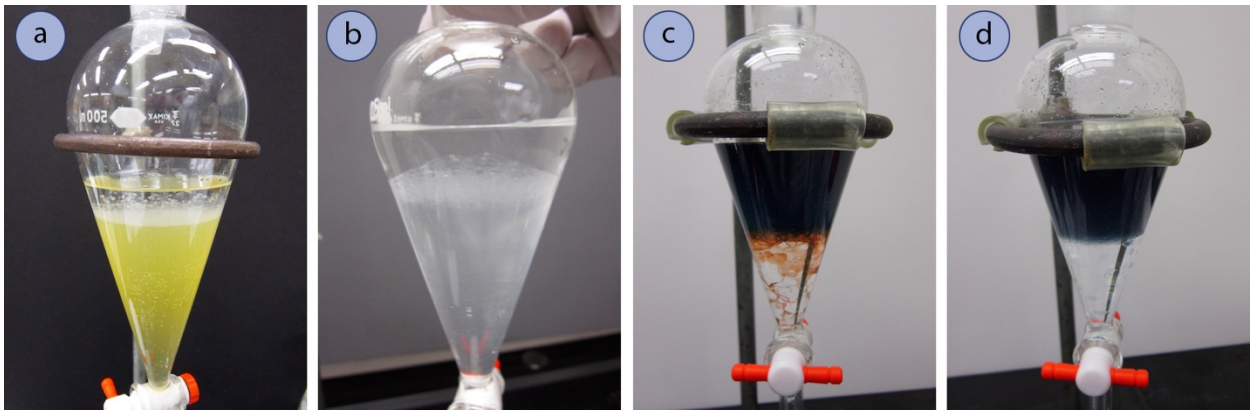

Figure 4.34: a) An emulsion with biodiesel and methanol, b) An emulsion with brine and ethyl acetate, c) An emulsion with dichloromethane and brine (as well as food coloring, d) The emulsion is resolved after addition of water that decreases the density of the top brine layer.

Emulsions can be very difficult to rectify, and it's best if they are avoided in the first place by shaking solutions that are prone to emulsions (*e.g.* dichloromethane with highly basic or dense solutions) *gently* in the separatory funnel. Nonetheless, if an emulsion does form, there are some ways to attempt to clarify them:

a) For mild emulsions, gently swirl the layers and try to knock down suspended droplets with a glass stirring rod.

b) Allow the solution to sit for a period of time (even until the next lab period) if possible. With enough time, some solutions do settle out on their own. This of course may not be practical.

c) For small volumes, use a centrifuge if one is available. A centrifuge hastens the process of letting an emulsion settle on its own. Remember that a centrifuge needs to be balanced or it may wobble off the benchtop. Divide the solutions equally, putting tubes of equal volume opposite one another inside the centrifuge.

d) If an emulsion is formed because the two layers having similar densities, try to alter the density of each layer to make them more different. To help clarify an emulsion, try to decrease the density of the top layer or increase the density of the bottom layer.

   For example, if an emulsion occurs with ethyl acetate (top layer) and an aqueous solution (bottom layer), add some NaCl. NaCl will dissolve in the aqueous layer and increase the density of the aqueous solution. Alternatively add additional ethyl acetate, which will dilute the organic layer and lower its density. As a last resort add some pentane, which will mix with the top organic layer and decrease its density (pentane is one of the least dense organic solvents). The addition of pentane is used as a final effort as it will negatively affect the ability of the organic layer to extract somewhat polar compounds.

   If an emulsion occurs with an aqueous solution (top layer) and dichloromethane (bottom layer), add some water from a squirt bottle to dilute the top layer and decrease its density. This method worked well to clarify the emulsion in Figure 4.32c, as evidenced by Figure 4.32d.

e) Try decreasing the solubility of one component in the other. One method is to add NaCl or NH_4Cl to the separatory funnel, which dissolves in the aqueous layer and decreases the ability of organic compounds to dissolve in water ("salting out").

4.5.E MICROSCALE EXTRACTIONS

Microscale work involves the manipulation of less than 300 mg of compound, and usually involves solvent volumes of 5 mL or less. A separatory funnel would be impractical when working with such small quantities, and conical vials (Figure 4.35) or centrifuge tubes are typically used instead.

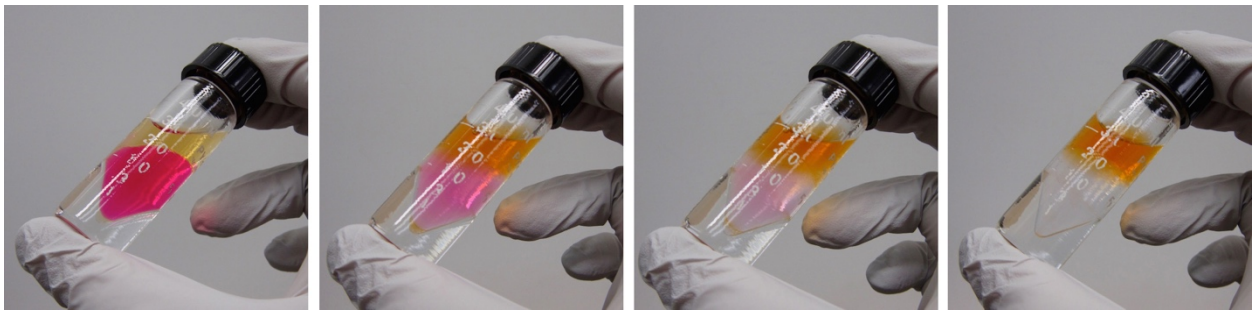

Figure 4.35: Progress of the extraction of methyl red from the acidic aqueous layer (bottom) into the organic layer (top). The inversions were done very slowly in order to see the extraction stepwise. With even gentle mixing, the methyl red (and thus color) extracts rapidly.

The pictures in this section show the extraction of 2 mL of a mildly acidic aqueous solution containing a single drop of methyl red solution into 2 mL of ethyl acetate. The color (the methyl red), is extracted from the aqueous layer (bottom) into the ethyl acetate layer (top).

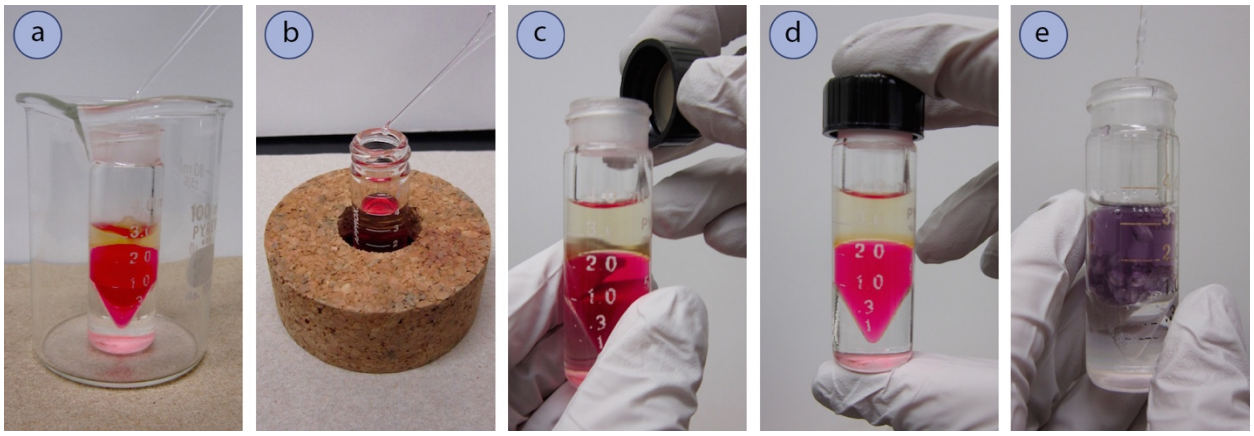

Figure 4.36: a) Adding solvent to the conical vial by pipette, and using a beaker to support the vial, b) Supporting the vial with a cork ring, c+d) Attaching the Teflon-lined cap to the vial, e) Manual mixing of layers (using a different system).

Mix the solutions

1. Pour the contents to be extracted into a conical vial, or a glass tube with a tapered end (*e.g.* centrifuge tube). As these containers are prone to tip, use a beaker (Figure 4.36a) or inverted cork ring (Figure 4.36b) for support.

2. Add the extractive solvent by pipette (Figure 4.36a). If using a conical vial, the volume markings on the glass maybe be helpful.

3. Gently mix the two solutions using one of the following methods:

    a. Secure a cap firmly on the vial (Figures 4.36 c+d) then invert and shake the tube for 10-20 seconds (Figure 4.35). Conical vials and centrifuge tubes tend to be less airtight than separatory funnels, so there should be no need to vent the system during shaking unless $NaHCO_3$ or Na_2CO_3 solutions are used.

    b. Alternatively, manually mix the layers using a pipette. Withdraw a pipette-full of the bottom layer from the vial, and then vigorously expunge the solution through the top layer (Figure 4.36e). Do this repeatedly for at least one minute. Manual mixing is not recommended when using low-boiling solvents (*e.g.* diethyl ether), as the volume often decreases dramatically after mixing. Instead use the first mixing method described.

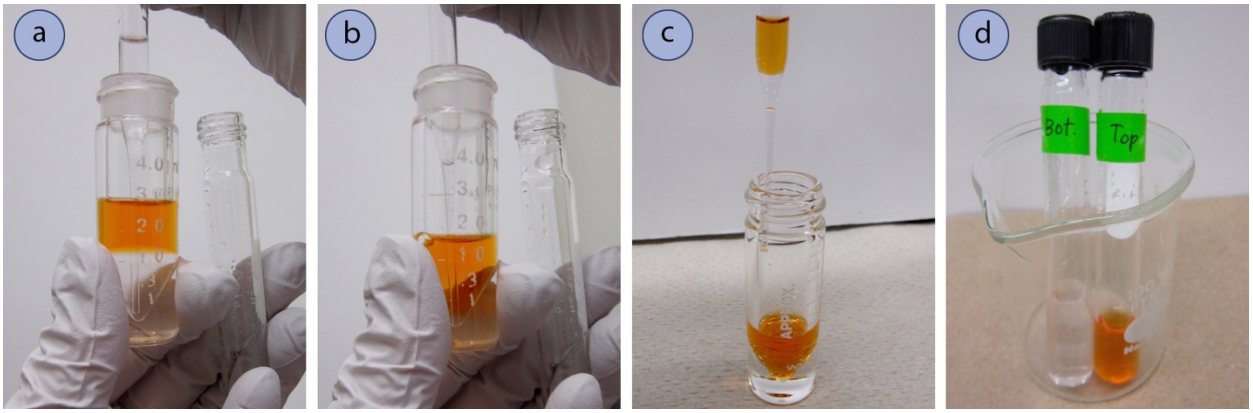

Figure 4.37: a+b) Withdrawal of the bottom layer, with pipette into the tip of the vial, c) Removal of a residual drop of the bottom layer by allowing the layers to separate inside a Pasteur pipette, d) Separated layers.

Separate the layers

4. Separate the layers with a Pasteur pipette. The design of conical vials and centrifuge tubes allows for efficient separation of the layers through withdrawal of the *bottom* layer by pipette. This means that even if the top layer is to be reserved, the bottom layer still needs to be removed *first*.

    a. Hold the conical vial or tapered tube in the same hand as a container for the bottom layer (label it). Withdraw the majority of the bottom layer by Pasteur pipette, and dispense into the container (Figure 4.37a).
    b. When withdrawing, always place the pipette tip to the point of the conical vial or tapered tube (Figure 4.37b).
    c. It may be difficult to remove the very last drop of bottom layer from the point of the vial. To do so, withdraw the entirety of the bottom layer and a small amount of top layer into the pipette. Allow the layers to separate inside the pipette (Figure 4.37c), then delicately expel the bottom layer from the pipette into the container. Return the rest of the top layer to the conical vial.

5. If the bottom layer is the desired layer, and another extraction is to be done, add fresh organic solvent to the top layer still in the conical vial and repeat the extraction and separation.

6. If the top layer is the desired layer, remove it from the conical vial using a fresh pipette into a clean container. If another extraction is to be done, return the bottom layer to the conical vial, add fresh solvent and repeat the extraction and separation.

4.6 REACTION WORK-UPS

4.6.A PURPOSE OF A WORK-UP

When the goal of an experiment is to conduct a reaction and isolate the product, the general sequence of events is shown in Table 4.4.

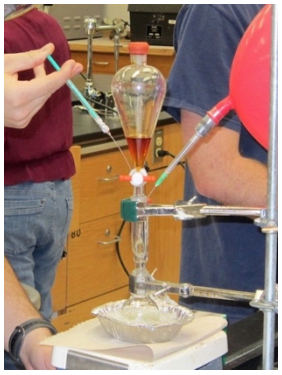			
a) Conduct the reaction.	b) Perform multiple extractions and/or washes to partially purify the desired product.	c) Remove trace water with a drying agent. d) Filter or decant the drying agent.	e) Remove the solvent with a rotary evaporator. f) Further purify if necessary.

Table 4.4: Typical reaction sequence of events.

A key step in this sequence comes immediately after the reaction is complete, and is called the reaction "**work-up**" (step *b* in Table 4.4). The work-up refers to methods aimed at purifying the material, and most commonly occur in a separatory funnel. Solutions are added to the funnel to either extract or wash the mixture, with the goal of isolating the product from excess reagents, catalysts, side products, solvents or compounds formed from side reactions.

4.6.B COMMON WASHES

4.6.B.1 WATER

The most common wash in separatory funnels is probably water. Water is cheap, non-hazardous, and works well to remove many impurities found alongside a desired product.

Water can potentially remove water-soluble impurities from an organic layer, as long as they are present in quantities that do not exceed their water solubility. The following are common materials that can be removed with a water wash: unconsumed acid or base, many ionic salts, and compounds that can hydrogen bond with water (have an oxygen or nitrogen atom) and are relatively small (*e.g.* CH_3CH_2OH or CH_3COCH_3).

To demonstrate the effectiveness of a water wash, a Fischer esterification reaction was conducted to produce isoamyl acetate (Figure 4.38). In this reaction, an excess of acetic acid is used to drive the reaction through Le Châtelier's principle, and the acetic acid had to be removed from the product during the purification process.

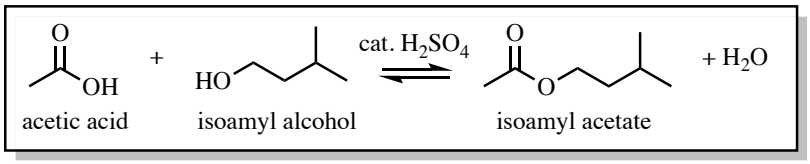

Figure 4.38: Reaction scheme for the synthesis of isoamyl acetate.

The ^{1}H NMR spectrum in Figure 4.39a was taken of the reaction mixture immediately after ceasing heating and *before* the work-up. As expected, a significant signal for acetic acid is seen at 2.097 ppm.

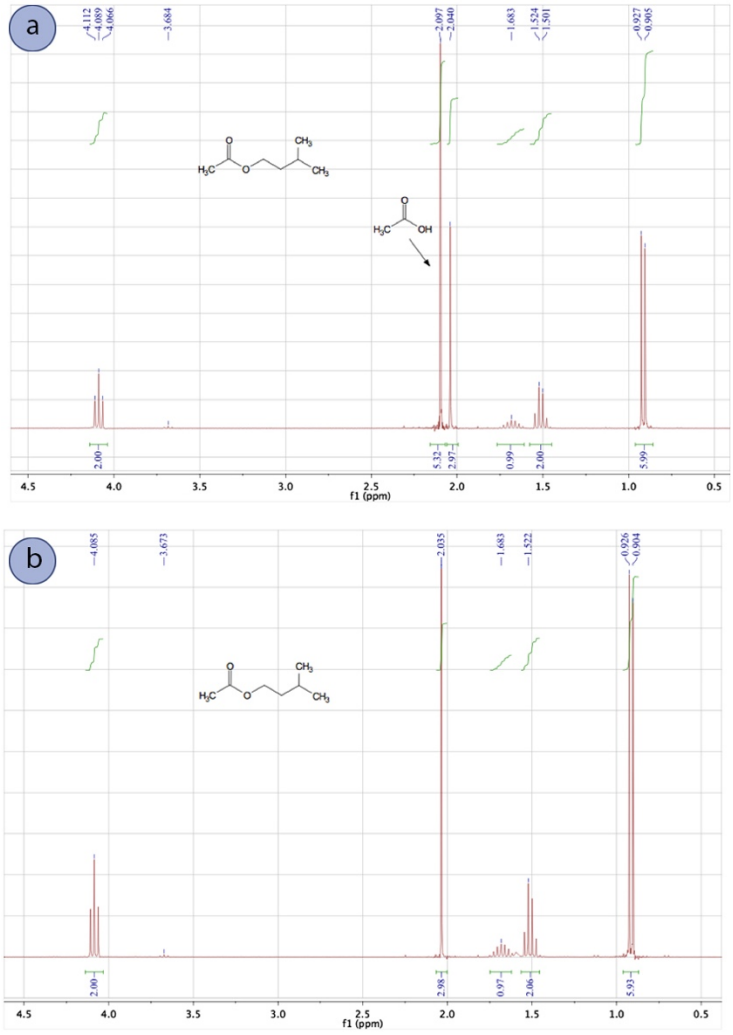

Figure 4.39: 300 MHz ^{1}H NMR spectra of the Fischer esterification mixture, a) Before the work-up, b) After the complete work-up (water, sodium bicarbonate, and brine washes).

The reaction was then "worked up" by pouring the reaction mixture into a separatory funnel and washing the organic layer with water, sodium bicarbonate, and brine in succession. The main purpose of the water wash was to remove the majority of the catalytic sulfuric acid and the excess acetic acid, while the sodium bicarbonate wash neutralized the rest. The ^{1}H NMR spectrum of the final product (Figure 4.39b) showed the washes were effective as the acetic acid signal at 2.097 ppm is absent.

The sodium bicarbonate wash in this example was necessary (and discussed in the next section) because a water wash alone may not fully remove the acetic acid. It's important to know that when a compound is "water soluble" it does not necessarily mean it is "organic insoluble," a common misconception that arises from the "like dissolves like" principle. For example, acetic acid has a K of 0.5 when partitioning between diethyl ether and water, meaning acetic acid favors the aqueous layer *only twice* as much as the organic layer.[4] The ability of acetic acid and other polar compounds to dissolve in the organic layer of a separatory funnel should not be ignored.

[4] A. Seidell, *Solubilities of Inorganic and Organic Substances*, D. Van Nostrand Company, **1907**.

4.6.B.2 SODIUM BICARBONATE AND SODIUM CARBONATE

How They Work

A normal part of many work-ups includes neutralization. It is important to neutralize any organic solvent that was exposed to an acidic or basic solution as trace acid or base may cause undesired reactions to occur when the solutions are concentrated. Also, samples intended for GC analysis must be neutral as acidic solutions degrade the polymeric coating of the GC column. In addition, it is preferable to manipulate neutral materials rather than acidic or basic ones, as spills are then less hazardous.

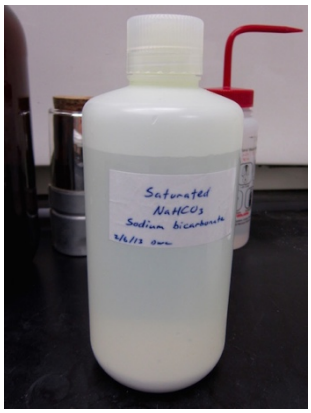

Figure 4.40: Bottle of saturated sodium bicarbonate.

Aqueous solutions of saturated sodium bicarbonate ($NaHCO_3$) and sodium carbonate (Na_2CO_3) are basic, and the purpose of these washes is to **neutralize an organic layer that may contain trace acidic components**. Even if an organic layer should not in theory dissolve very polar components such as acid, acid sometimes "hitches a ride" on polar components that may dissolve in an organic layer, such as small amounts of alcohols or water.

The following reactions occur between bicarbonate ion (1), carbonate ion (2) and acid (H^+) during a wash:

(1) $HCO_3^- (aq) + H^+ (aq) \rightarrow H_2CO_3 (aq) \rightleftharpoons H_2O (l) + CO_2 (g)$

(2) $CO_3^{2-} (aq) + H^+ (aq) \rightarrow HCO_3^- (aq)$

The initial product of reaction (1) is carbonic acid (H_2CO_3), which is in equilibrium with water and carbon dioxide gas. This means that solutions of bicarbonate ion often bubble during a neutralization wash in a separatory funnel. The product of reaction (2) is the bicarbonate ion, which can subsequently undergo reaction (1). This means that solutions of carbonate ion also often bubble during neutralizations.

Safety note: To prevent excess pressure from being generated by the release of carbon dioxide gas into a separatory funnel during neutralization, the layers should be gently swirled together before placement of the stopper. **They should be vented directly after inversion, and more frequently than usual.** Figure 4.41 shows a strongly acidic organic layer (top) in contact with an aqueous solution of 10% sodium bicarbonate (bottom). A vigorous stream of bubbles is seen originating from a small portion of organic layer trapped on the bottom of the funnel. The bubbling was even more vigorous when the layers were mixed together.

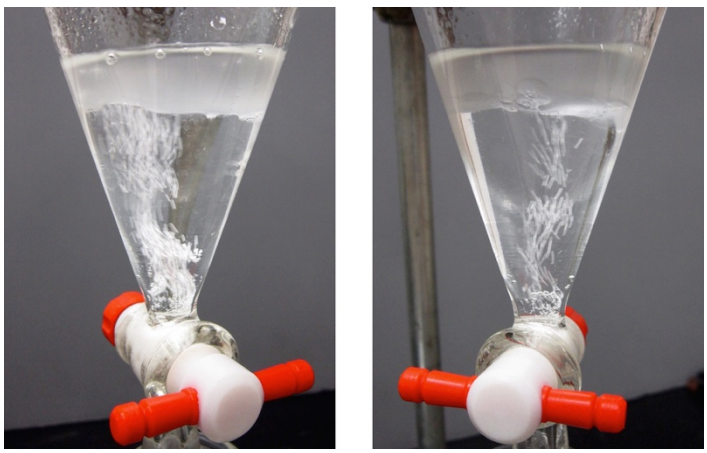

Figure 4.41: Dilute $NaHCO_3$ solution (bottom layer) bubbling during the wash of an acidic organic (top) layer.

Testing the pH After a Wash

To test whether a base wash with $NaHCO_3$ or Na_2CO_3 was effective at removing all the acid from an organic layer, it is helpful to test the pH. It is not possible to test the pH of an organic solution directly, however it is possible to test the pH of an aqueous solution that the organic solution has been in contact with. If the aqueous layer is on the top of a separatory funnel, insert a glass stirring rod into the top layer and touch the wet rod to blue litmus paper. An acidic solution turns blue litmus paper pink (or red), while a neutral or basic solution gives blue litmus paper only a darkened "wet" appearance (Figure 4.42d). If the litmus paper turns pink at all[5], the base wash has not fully neutralized the organic layer, and subsequent base washes are needed.

If the aqueous layer is on the bottom of the separatory funnel, test an "**aliquot**" of the aqueous layer (or tiny sample) on litmus paper through the following method:

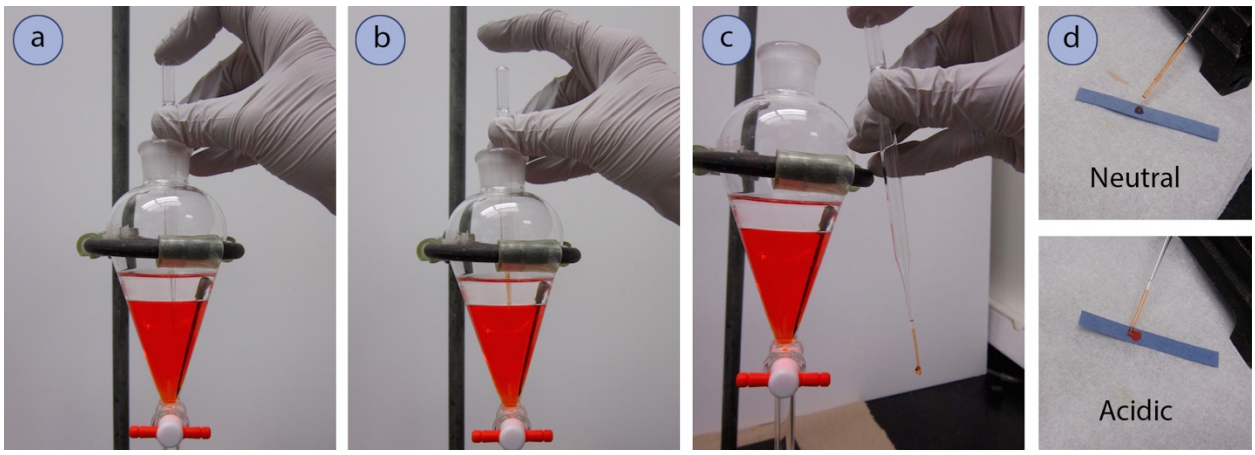

Figure 4.42: a) Positioning a finger on the pipette, b) Lifting the finger off, c) Positioning the finger on again and removal of an aliquot, d) Testing the aliquot on blue litmus paper.

1. With a finger placed atop a glass pipette, insert the pipette into the separatory funnel so the tip is positioned in the bottom aqueous layer (Figure 4.42a).

2. Remove the finger on the pipette to allow a sample of the aqueous layer to enter the pipette through capillary action (Figure 4.42b).

3. With a finger placed atop the glass pipette again, remove the pipette from the separatory funnel. A bit of liquid should remain in the pipette tip, an aliquot of the bottom layer (Figure 4.42c).

4. Touch the aliquot to blue litmus paper and observe the color (Figure 4.42d).

5. If the litmus paper turns pink at all, the base wash has not fully neutralized the organic layer, and subsequent base washes are needed.

[5] When assessing the result of a litmus paper test, look at the center of the drop. The center is the most concentrated spot, and it's possible a color change may not be seen on the outside where the solution has spread and diluted. Any pink seen on blue litmus paper means the solution is acidic.

4.6.B.3 BRINE (SATURATED NaCl)

Removing Water

In some experiments, an organic layer may be washed with brine, which is a saturated solution of NaCl *(aq)*. The purpose of this wash is to **remove large amounts of water** that may be dissolved in the organic layer. Although the organic layer should always be later exposed to a drying agent (*e.g.* anhydrous sodium sulfate, magnesium sulfate, or calcium chloride), these reagents do best to remove only small amounts of water.

The organic solvents that require a brine wash before exposure to a solid drying agent are diethyl ether and ethyl acetate. These solvents dissolve large quantities of water in comparison to other solvents (Table 4.5).

Solvent	Grams water dissolved in 100 mL solvent
Diethyl ether	1.24 g
Ethyl acetate	2.92 g
Dichloromethane (CH_2Cl_2)	0.32 g
Hexanes	0.007 g

Table 4.5: Quantity of water dissolved in various solvents.[6]

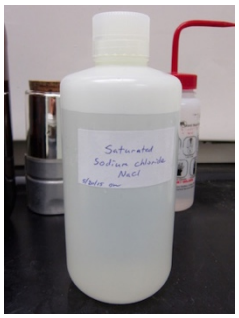

Figure 4.43: Bottle of brine.

Brine works to remove water from an organic layer because it is highly concentrated (since NaCl is so highly water soluble). A saturated NaCl *(aq)* solution is highly ordered, causing a large motivation for water to draw into the solution from the organic layer to increase the entropy of the salt solution (to dilute the solution).

Figure 4.44 shows a qualitative difference in the amount of water present in an organic layer with and without the use of a brine wash. Ethyl acetate was shaken with water (Figure 4.42a), then dried with a portion of anhydrous $MgSO_4$. The large clumps of drying agent in Figure 4.44b indicate that this ethyl acetate layer is still noticeably wet. Ethyl acetate was then shaken with brine (Figure 4.44c), and dried with the same quantity of anhydrous $MgSO_4$. There is little clumping of the drying agent in this ethyl acetate layer, and fine particles are seen (Figure 4.44d), signifying this layer contained very little water.

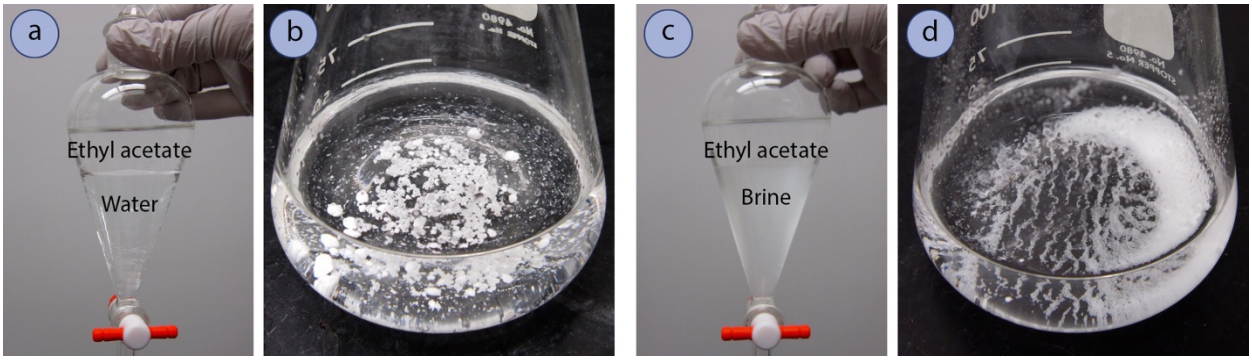

Figure 4.44: a) Ethyl acetate and water layers shaken together, b) Appearance of the ethyl acetate layer upon addition of anhydrous $MgSO_4$, c) Ethyl acetate and brine layers shaken together, d) Appearance of the ethyl acetate layer with anhydrous $MgSO_4$.

[6] From: Fessenden, Fessenden, Feist, *Organic Laboratory Techniques*, 3rd ed., Brooks-Cole, **2001**.

If drying agents are used to remove water, you might wonder "Why bother with brine; why not use lots of drying agent when the time comes?" The main reason to limit the amount of water present in an organic solution *before* the drying agent step is that the drying agent will often adsorb compound along with water. Using as little as possible will maximize the yield.

To demonstrate, Figure 4.45 shows an ethyl acetate solution that has a faint pink tint because it contains some dissolved red food dye. The solution was swirled with white anhydrous $MgSO_4$, and the drying agent turned pink as it adsorbed the red food dye compound (Figure 4.45a). Addition of more anhydrous $MgSO_4$ made the drying agent pinker (Figure 4.45b), as more dye was removed from solution. In this example, even after filtering and rinsing the drying agent with additional solvent, the drying agent remained pink (Figure 4.45c). Thus, the more drying agent that is used, the more compound that may be irrecoverably lost.

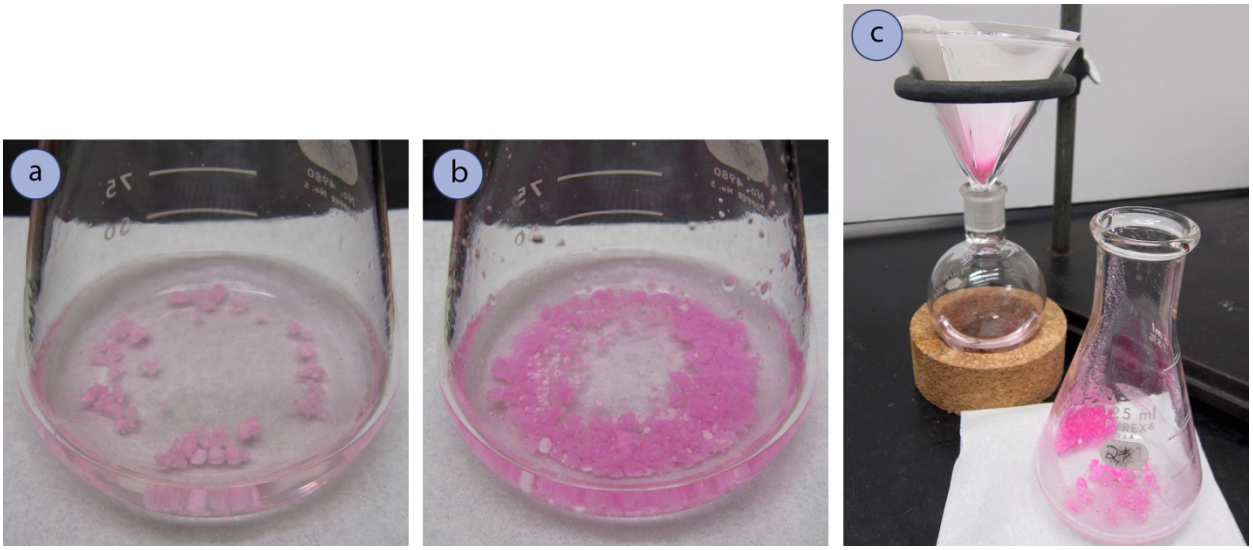

Figure 4.45: a) Ethyl acetate layer with a small amount of dissolved red food dye. With addition of white anhydrous $MgSO_4$, the drying agent turned pink, showing that the drying agent has adsorbed the dye, b) With more $MgSO_4$, the drying agent became pinker, c) Even after a rinsing step, the drying agent retained its pink color.

Decreasing Water Solubility of Organic Compounds ("Salting Out")

Saturated ionic solutions may be used to decrease the solubility of organic compounds in the aqueous layer, allowing more of a compound to dissolve in the organic layer. If a desired product can hydrogen bond with water and is relatively small, it may be difficult to keep it in the organic layer when partitioning with an aqueous phase (*K* will be < 1). However, the equilibrium can favor the organic layer if all aqueous washes contain high concentrations of ions (*e.g.* saturated $NaHCO_3$, NaCl, or NH_4Cl). With water being so tightly "occupied" in dissolving the ions in these solutions, they are less capable of dissolving organic compounds. Additionally, ionic solutions have high dielectric constants, making them less compatible with organic compounds.

Figure 4.47 shows how brine affects the partitioning of red food dye in ethyl acetate and aqueous solutions. Figure 4.47a shows addition of one drop of red food dye to a layer of water in a separatory funnel, and the dye dissolves easily even without swirling. Figure 4.47b shows the water layer containing the dye after shaking with a portion of ethyl acetate. The organic layer has only a very faint pink color, signifying that little dye has dissolved. The dye has obviously partitioned toward the aqueous layer, which is consistent with its very polar structure (Figure 4.46).

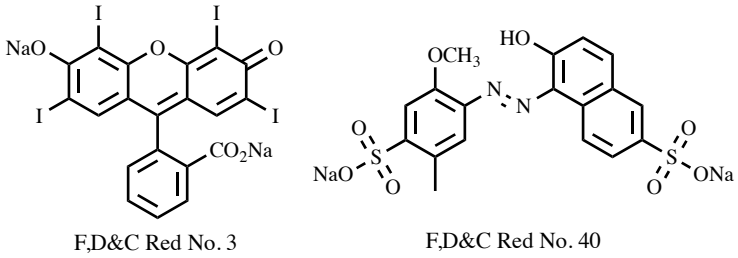

Figure 4.46: Structure of the two components in red food dye.

Figure 4.47c shows addition of one drop of red food dye to a brine solution, and the dye does not appear to mix with the brine at all. Figure 4.47d shows the brine layer containing the dye after shaking with a portion of ethyl acetate. The organic layer is pinker, signifying that more dye has now partitioned toward the organic layer. The polar dye molecules are much less soluble in the brine solution than in pure water (they have been "**salted out**"). In fact, some of the dye precipitated in the funnel (Figure 4.47d) as it had such low solubility in both brine and ethyl acetate.

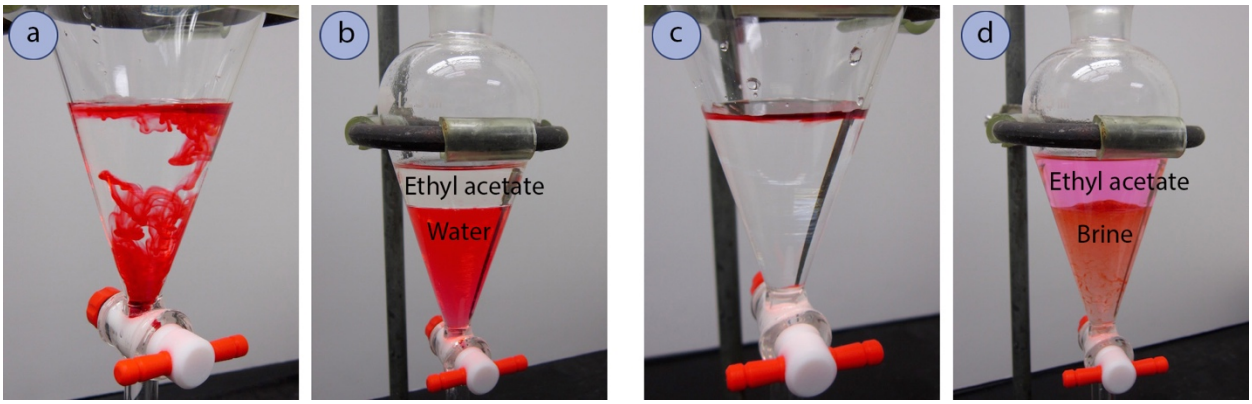

Figure 4.47: a) Water with the addition of one drop of red food dye, before swirling, b) Ethyl acetate, water and dye at equilibrium, c) Brine with the addition of one drop of red food dye, before swirling, d) Ethyl acetate, brine and dye at equilibrium.

4.6.C DRYING AGENTS

4.6.C.1 WHY THEY ARE USED

The general sequence of events for most reactions is shown in Table 4.6.

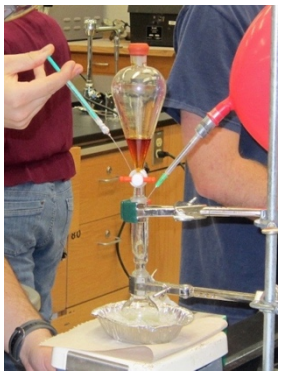			
a) Conduct the reaction.	b) Perform multiple extractions and/or washes to partially purify the desired product.	c) Remove trace water with a drying agent. d) Filter or decant the drying agent.	e) Remove the solvent with a rotary evaporator. f) Further purify if necessary.

Table 4.6: Typical reaction sequence of events.

An organic layer is always treated with a drying agent after having been exposed to water in a separatory funnel (step *c* in Table 4.6). Drying agents are anhydrous inorganic materials that favorably form "hydrates," which incorporate water molecules into their solid lattice structure (for example $Na_2SO_4 \cdot 7\ H_2O$). A drying agent is swirled with an organic solution to **remove trace amounts of water**.

Many organic solvents dissolve a significant portion of water (Table 4.7[7] that must be removed before rotary evaporation, or else water will be found in the concentrated product. After solvent removal using a rotary evaporator, it occasionally happens that so much water is present that droplets or a second layer is seen amongst the oily liquid in a round-bottomed flask. The presence of water with the product makes the yield inaccurate, and water also must be removed before GC-MS analysis, as water is incompatible with mass-spectrometer detectors.

Solvent	Grams water dissolved in 100 mL solvent
Diethyl ether	1.24 g
Ethyl acetate	2.92 g
Dichloromethane (CH_2Cl_2)	0.32 g
Hexanes	0.007 g

Table 4.7: Quantity of water dissolved in various solvents.

Drying agents must be used with even relatively nonpolar organic solvents that do not theoretically dissolve much water, as water may cling to the sides of the separatory funnel and inadvertently travel with the organic layer while draining. Additionally, solutes dissolved in an organic layer with polar functional groups (*e.g.* alcohols, carboxylic acids) can hydrogen-bond with water and increase the likelihood of water dissolving in the organic layer.

[7] From: Fessenden, Fessenden, Feist, *Organic Laboratory Techniques*, 3rd ed., Brooks-Cole, **2001**.

Figure 4.48: Various drying agents.

Drying agents (Figure 4.48) remove trace amounts of water from organic solutions by forming hydrates. The most useful drying agents indicate when they have completely absorbed all of the water from the solution. Anhydrous magnesium sulfate ($MgSO_4$) is a fine, loose powder (Figure 4.49a), but its hydrate is clumpy and often clings to the glass (Figure 4.49b). A typical drying procedure is to add anhydrous $MgSO_4$ to an organic solution until it *stops* clumping and fine particles are seen, which indicate that there is no longer water available to form the clumpy hydrates.

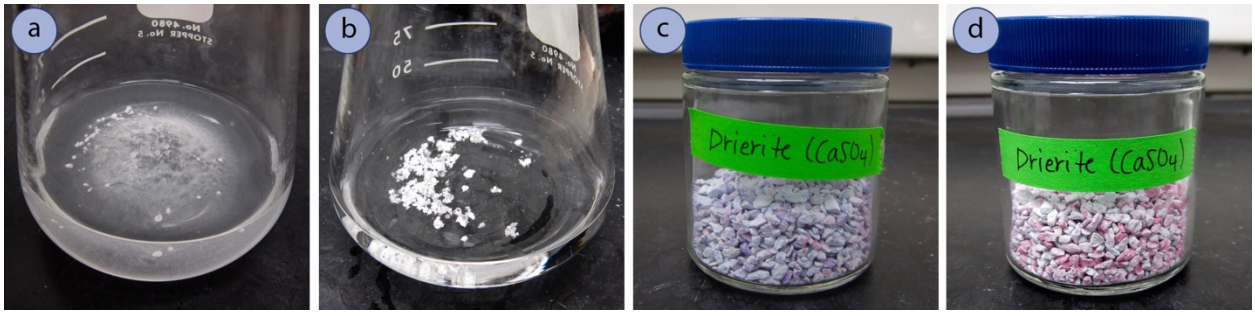

Figure 4.49: a) Ethyl acetate solution containing fine particles of anhydrous $MgSO_4$, b) Ethyl acetate with clumpy $MgSO_4$ hydrate, c) Dry Drierite (blue), d) Wet Drierite (pink).

Anhydrous calcium sulfate ($CaSO_4$), can be purchased containing a cobalt compound that is blue when dry and pink when wet (this is then sold under the name Drierite, Figures 4.49 c+d). In this way, blue Drierite can be used as a visual indicator for the presence of water.[8]

The most common drying agents used to remove water from organic solutions are anhydrous sodium sulfate (Na_2SO_4) and anhydrous magnesium sulfate ($MgSO_4$). Many chemists consider $MgSO_4$ the "go-to" drying agent as it works quickly, holds a lot of water for its mass, and the hydrates are noticeably chunkier compared to the anhydrous form, making it easy to see when you've added enough. A drawback to using $MgSO_4$ is that it is a fine powder, and so the solutions must be subsequently filtered to remove the drying agent. Another drawback to $MgSO_4$ is that all fine powders heavily adsorb product on their surface (which is why they must be rinsed with solvent after filtration), and sometimes more granular drying agents are used to minimize the loss of product by adsorption.

[8] Blue Drierite is expensive, so is commonly used by mixing it together with white Drierite ($CaSO_4$ without the cobalt indicator). Pink (wet) Drierite can be dried by spreading it on a watch glass and drying in a 110 ˚C oven overnight.

In some procedures Na_2SO_4 or $CaCl_2$ are used if they seem to work just as well as $MgSO_4$, or if the solution is incompatible with $MgSO_4$ (see Table 4.8). A procedural advantage to these drying agents is that their granules are not easily dispersed, allowing for the solutions to be easily decanted (poured). In many situations drying agents are interchangeable (see Table 4.5 for a survey of drying agents). However, it is most common for desiccators and drying tubes to use $CaSO_4$ or $CaCl_2$ (Figure 4.50), as they can be easily manipulated in their pellet or rock forms.

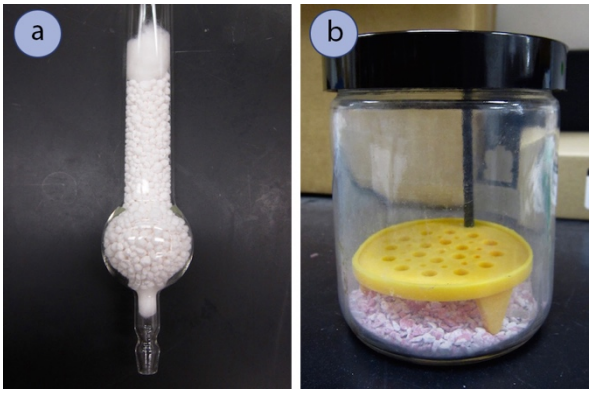

Figure 4.50: a) Drying tube filled with $CaCl_2$, b) Desiccator using $CaSO_4$.

Drying Agent	Hydrate formula(s)	Practical Comments	Other Comments
Magnesium sulfate	$MgSO_4 \cdot 7\ H_2O$	Quickly removes most water, and can hold a lot for its mass (0.15-0.75 g water per g desiccant).[9] Is a fine powder, so must be gravity filtered. Its high surface area means it will somewhat adsorb compound: be sure to rinse after filtering.	$Mg(H_2O)_4^{2+}$ is somewhat acidic, so is incompatible with highly acid-sensitive groups.
Sodium sulfate	$Na_2SO_4 \cdot 7\ H_2O$ $Na_2SO_4 \cdot 10\ H_2O$	Removes water at a moderate rate, so the solution should be allowed to sit with the drying agent for some time. Can hold a lot of water for its mass (1.25 g water per g desiccant), but may leave small amounts of water remaining. Solutions with Na_2SO_4 can usually be decanted.	Cannot dry diethyl ether well unless a brine wash was used.
Calcium chloride	$CaCl_2 \cdot 2\ H_2O$ $CaCl_2 \cdot 6\ H_2O$	Quickly removes water well, although larger quantities are needed than other drying agents (holds 0.30 g water per g desiccant). If using a fine powder, the solution must be gravity filtered and drying agent rinsed. If using pellets, the solution should be allowed to sit for a few minutes, then decanted.	Absorbs water as well as methanol and ethanol.
Calcium sulfate (Drierite)	$CaSO_4 \cdot ½\ H_2O$ $CaSO_4 \cdot 2\ H_2O$	Quickly removes water, but needs large quantities as it holds little water per gram. Are most often used in desiccators and drying tubes, not with solutions.	

Table 4.8: Survey of drying agents.

[9] Grams water per gram of desiccant values are from: J.A. Dean, *Lange's Handbook of Chemistry*, 15th ed., McGraw-Hill, **1999**, Sect. 11.2. $CaCl_2$ value is quoted for the formation of $CaCl_2 \cdot 2\ H_2O$.

4.6.C.3 DRYING AGENTS PROCEDURE

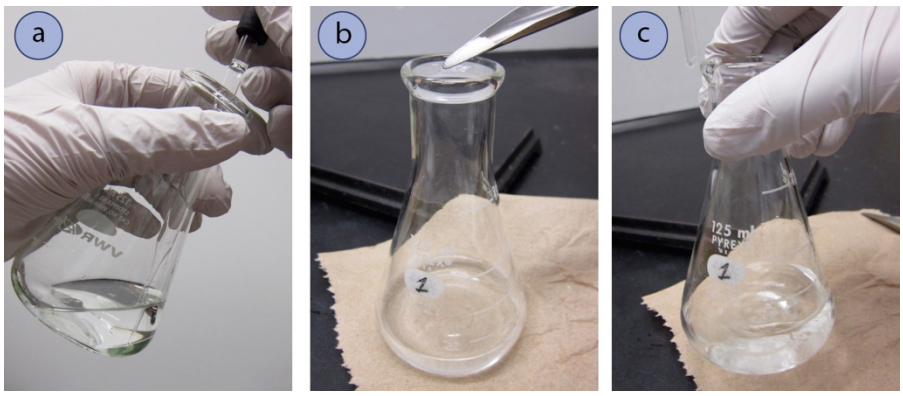

Figure 4.51: a) Pipetting out a droplet of water at the bottom of a flask, b) Addition of an initial portion of drying agent, c) Swirling.

1. The organic solution to be dried must be in an **Erlenmeyer flask**, as solutions can easily splash out of beakers when swirled.

2. First inspect the solution to see if it's homogeneous, or if there is a second layer of liquid (typically a puddle on the bottom). If a second layer is noticed, this is probably water and the majority of it should be pipetted out before continuing on (Figure 4.51a). It can be difficult to completely remove a water layer by pipette, so leaving a tiny bit is acceptable.

3. Add a small portion of drying agent to the flask, the size of one pea for macroscale work (Figure 4.51b), and swirl the solution (Figure 4.51c). Be sure to close the jar of drying agent when not in use, as the reagents are hygroscopic. After a short period of time, inspect the mixture closely.

    a. If the entire drying agent clumps into pieces that are much larger than the original size (Figures 4.52 b+c), there is still water remaining in the flask. Add another portion of drying agent and swirl.

    b. A solution is nearing dryness when fine particles are noticed that don't cling to other particles (Figures 4.52 a+c) or to the glass when swirled (Figure 4.53a). A wet organic solution can be cloudy, and a dry one is always clear.

    c. If using anhydrous Na_2SO_4, allow the solution to sit for at least 5 minutes before declaring the solution dry, as this reagent takes time to work.

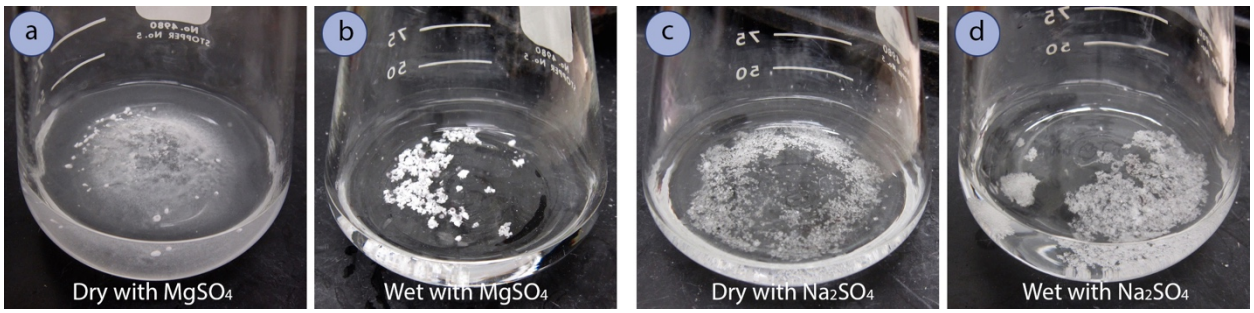

Figure 4.52: Drying ethyl acetate with anhydrous $MgSO_4$ and Na_2SO_4: a) Dry $MgSO_4$ (very fine particles), b) Wet $MgSO_4$ (hydrate clumps), c) Dry Na_2SO_4 (small particles), d) Wet Na_2SO_4 (hydrate clumps).

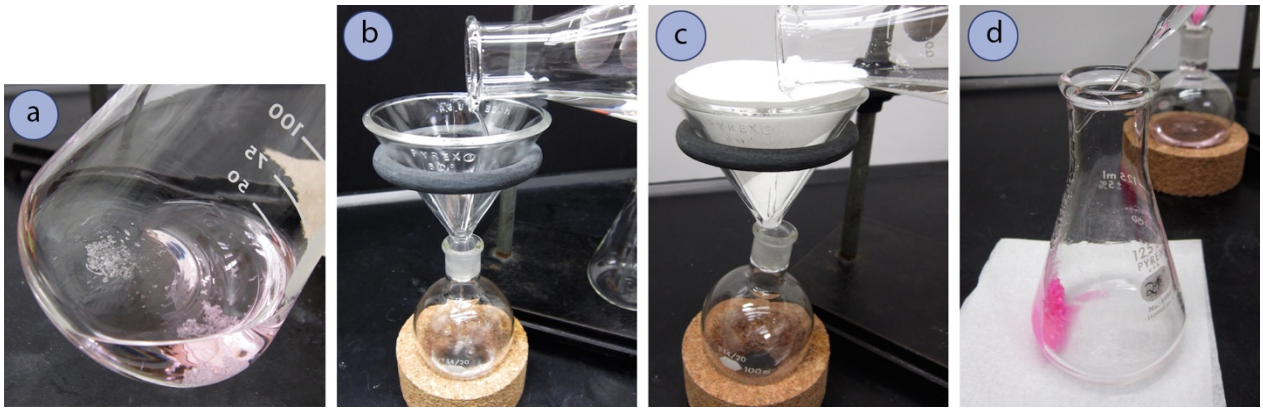

Figure 4.53: a) Wet hydrate of Na_2SO_4 can stick to the glass, b) Decanting a solution, c) Gravity filtering a solution, d) Rinsing the residual drying agent in the flask.

4. When the solution is dry, separate the drying agent from the solution:

   a. If using Na_2SO_4, $CaCl_2$ pellets, or $CaSO_4$ rocks, carefully decant the solution into an appropriately sized round-bottomed flask (Figure 4.53b), being sure to fill the flask no more than half way. Reminder: a mass of the *empty* flask should be obtained if the solvent will be evaporated on the rotary evaporator.

   b. If using $MgSO_4$, gravity filter the solution into an appropriately sized round bottomed flask (Figure 4.53c). When pouring, leave the solid behind as long as possible (essentially decant the solution, but into a funnel lined with filter paper). Solid can slow drainage in the filter paper.

   c. With all drying agents, rinse the drying agent (in the flask and in the filter funnel) with a few mL of fresh organic solvent, and add the rinsing to the round-bottomed flask (Figure 4.53d). Remove the solvent using a rotary evaporator.

4.7 ACID-BASE EXTRACTION

4.7.A HOW THEY WORK

A modification of the extractions previously discussed in this chapter is to perform a *chemical reaction* in the separatory funnel in order to change the polarity and therefore partitioning of a compound in the aqueous and organic layers. A common method is to perform an acid-base reaction, which can convert some compounds from neutral to ionic forms (or vice versa).

For example, imagine that a mixture of benzoic acid and cyclohexane is dissolved in an organic solvent like ethyl acetate in a separatory funnel. To separate the components, a water wash may be attempted to remove benzoic acid, but benzoic acid is not particularly water-soluble due to its nonpolar aromatic ring, and only small amounts would be extracted into the aqueous layer (Figure 4.54a).

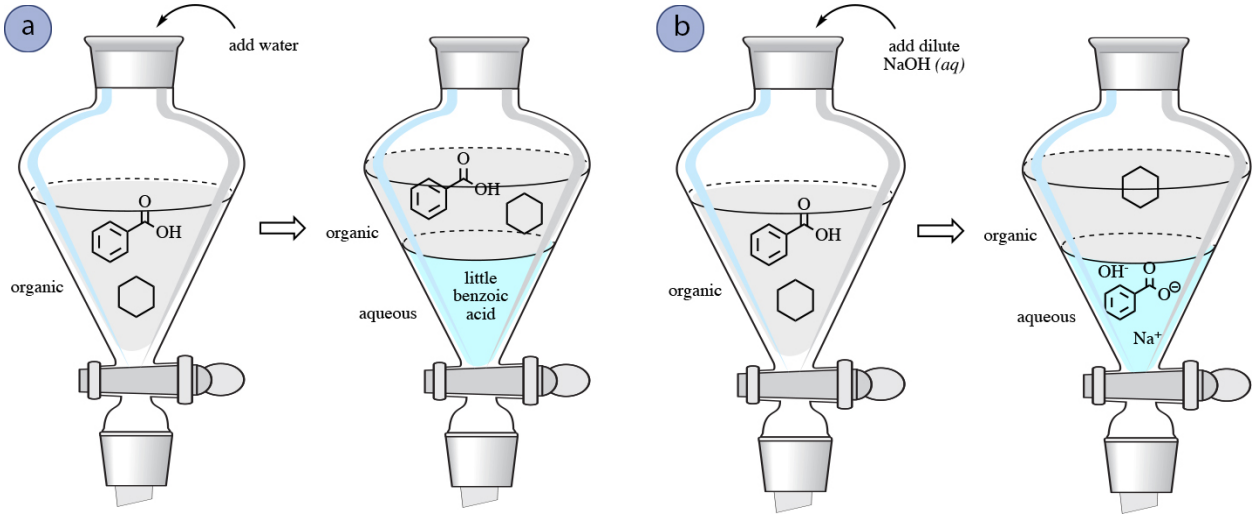

Figure 4.54: Washing a mixture of benzoic acid and cyclohexane with: a) water, b) aqueous NaOH.

Separation of a mixture of benzoic acid and cyclohexane is however possible using a wash with a base such as NaOH. Due to its acidic nature, benzoic acid can undergo a reaction with NaOH as follows, resulting in the carboxylate salt sodium benzoate.

$$PhCO_2H\ (aq) + NaOH\ (aq) \rightarrow H_2O\ (l) + PhCO_2Na\ (aq) \quad (\text{or } PhCO_2^-\ Na^+)$$

Benzoic acid — Sodium benzoate

The solubility properties of carboxylic acids are substantially different than their corresponding carboxylate salts. Sodium salicylate is roughly 350 times more soluble in water than salicylic acid due to its ionic character (Figure 4.55), and it is rather insoluble in organic solvents such as diethyl ether.

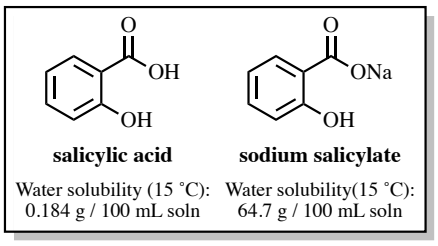

Figure 4.55: Aqueous solubility data for salicylic acid and sodium salicylate (Ref 4).

Therefore, a wash with NaOH would convert benzoic acid into its ionic carboxylate form, which would then be more soluble in the aqueous layer, allowing for the sodium benzoate to be extracted into the aqueous layer. Cyclohexane would remain in the organic layer as it has no affinity for the aqueous phase, nor can react with NaOH in any way. In this manner, a mixture of benzoic acid and cyclohexane can be separated (Figure 4.54b). The aqueous layer may be later acidified with HCl *(aq)* if desired to convert the benzoic acid back to its neutral form.

4.7.B SODIUM BICARBONATE WASHES

An acid-base extraction can be used to extract carboxylic acids from the organic layer into the aqueous layer. As was discussed in the previous section, NaOH can be used to convert a carboxylic acid into its more water-soluble ionic carboxylate form. However, if the mixture contains a desired compound that can *react* with NaOH, a milder base such as sodium bicarbonate should be used. A similar reaction occurs:

$$\underset{\text{Benzoic acid}}{PhCO_2H\ (aq)} + NaHCO_3\ (aq) \rightarrow \underset{\text{Sodium benzoate}}{PhCO_2Na\ (aq)} + H_2CO_3\ (aq) \rightleftharpoons H_2O\ (l) + CO_2\ (g)$$

One difference in using the base $NaHCO_3$ instead of NaOH is that the byproduct carbonic acid (H_2CO_3) can decompose to water and carbon dioxide gas. When shaking an acidic solution with sodium bicarbonate in a separatory funnel, care should be taken to swirl gently and **vent more frequently** to release pressure from the gas.

An example of a reaction that often uses a sodium bicarbonate wash in the work-up is a Fischer Esterification reaction. To demonstrate, benzoic acid was refluxed in ethanol along with concentrated sulfuric acid in order to form ethyl benzoate (Figures 4.56 a+b). A TLC plate of the reaction mixture at 1 hour of reflux showed residual unreacted carboxylic acid (Figure 4.56c), which is not uncommon due to the energetics of the reaction.

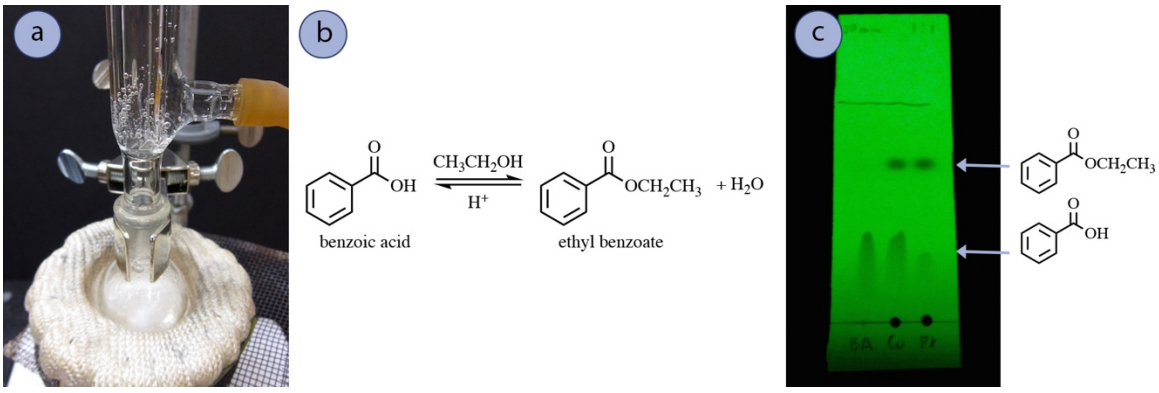

Figure 4.56: a) Refluxing reagents, b) Reaction scheme, c) TLC after 1 hour reflux, where the first lane (BA) is benzoic acid, the second lane (Co) is the co-spot and the third lane (Pr) is the reaction mixture (ran with 1:1 hexanes:ethyl acetate and visualized with UV light).

The residual carboxylic acid can be removed from the desired ester product using an acid-base extraction in a separatory funnel. A wash with sodium bicarbonate converts benzoic acid into its more water-soluble sodium benzoate form, extracting it into the aqueous layer (Figure 4.57). Additionally, the sodium bicarbonate neutralizes the catalytic acid in this reaction.

Sodium bicarbonate is preferable to NaOH in this process, as it is a much weaker base; washing with NaOH could cause hydrolysis of the ester product.

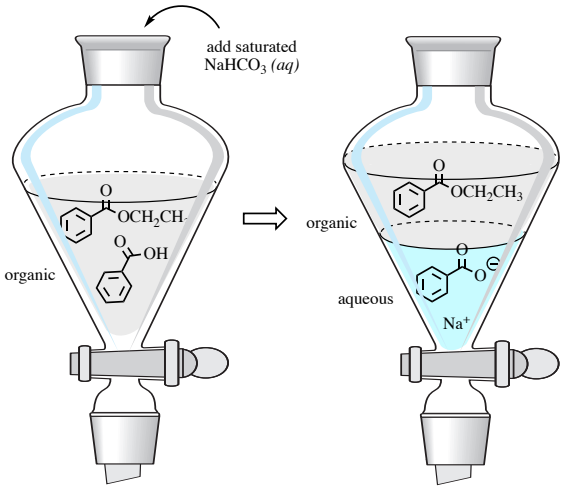

Figure 4.57: Saturated sodium bicarbonate wash to remove residual carboxylic acid from the reaction mixture of a Fischer esterification. The ester is then isolated in the organic layer.

4.7.C MIXTURES OF ACIDS AND BASES

As has been discussed previously, the acid-base properties of compounds can be utilized to selectively extract certain compounds from mixtures. This strategy can be extended to other examples.

4.7.C.1 EXTRACTING BASES

Basic compounds such as amines can be extracted from organic solutions by shaking them with acidic solutions to convert them into more water-soluble salts. In this way, they can be extracted from an organic layer into an aqueous layer.

$$PhNH_2\ (aq) + HCl\ (aq) \rightarrow PhNH_3Cl\ (aq)\ (\text{or } PhNH_3^+\ Cl^-)$$

Basic amine — Ammonium salt

4.7.C.2 EXTRACTING CARBOXYLIC ACIDS VS. PHENOLS

As previously discussed, carboxylic acids can be extracted from an organic layer into an aqueous layer by shaking them with basic solutions, which converts them into their more water-soluble salts.

$$PhCO_2H\ (aq) + NaOH\ (aq) \rightarrow H_2O\ (l) + PhCO_2Na\ (aq) \quad (\text{or } PhCO_2^-\ Na^+)$$

Carboxylic acid — Carboxylate salt

A similar reaction occurs with phenols (PhOH), and they too can be extracted into an aqueous NaOH layer (Figure 4.58a).

However, phenols are considerably less acidic than carboxylic acids, and are not acidic enough to react completely with $NaHCO_3$, a weaker base. Therefore, a solution of bicarbonate can be used to separate mixtures of phenols and carboxylic acids (Figure 4.58b).

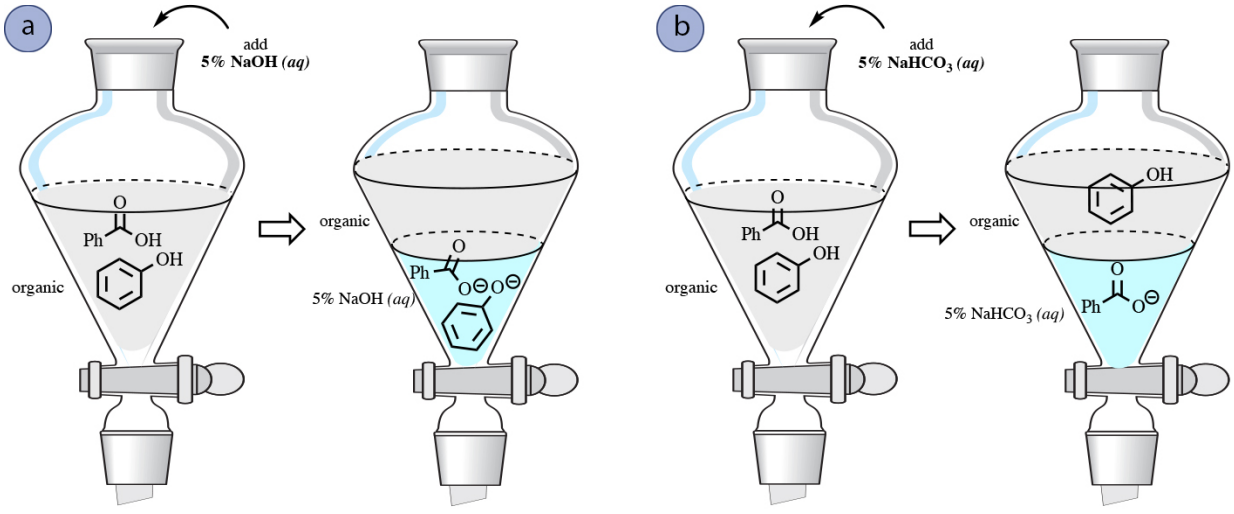

Figure 4.58: a) Extraction of both carboxylic acids and phenols into 5% NaOH *(aq)*, b) Extraction of only carboxylic acids into 5% $NaHCO_3$ *(aq)*.

4.7.C.3 EXTRACTING ACID, BASE, AND NEUTRAL COMPONENTS

The acid-base properties previously discussed allow for a mixture containing acidic (*e.g.* RCO_2H), basic (*e.g.* RNH_2), and neutral components to be purified through a series of extractions, as summarized in Figure 4.59 (which uses an organic solvent less dense than water).

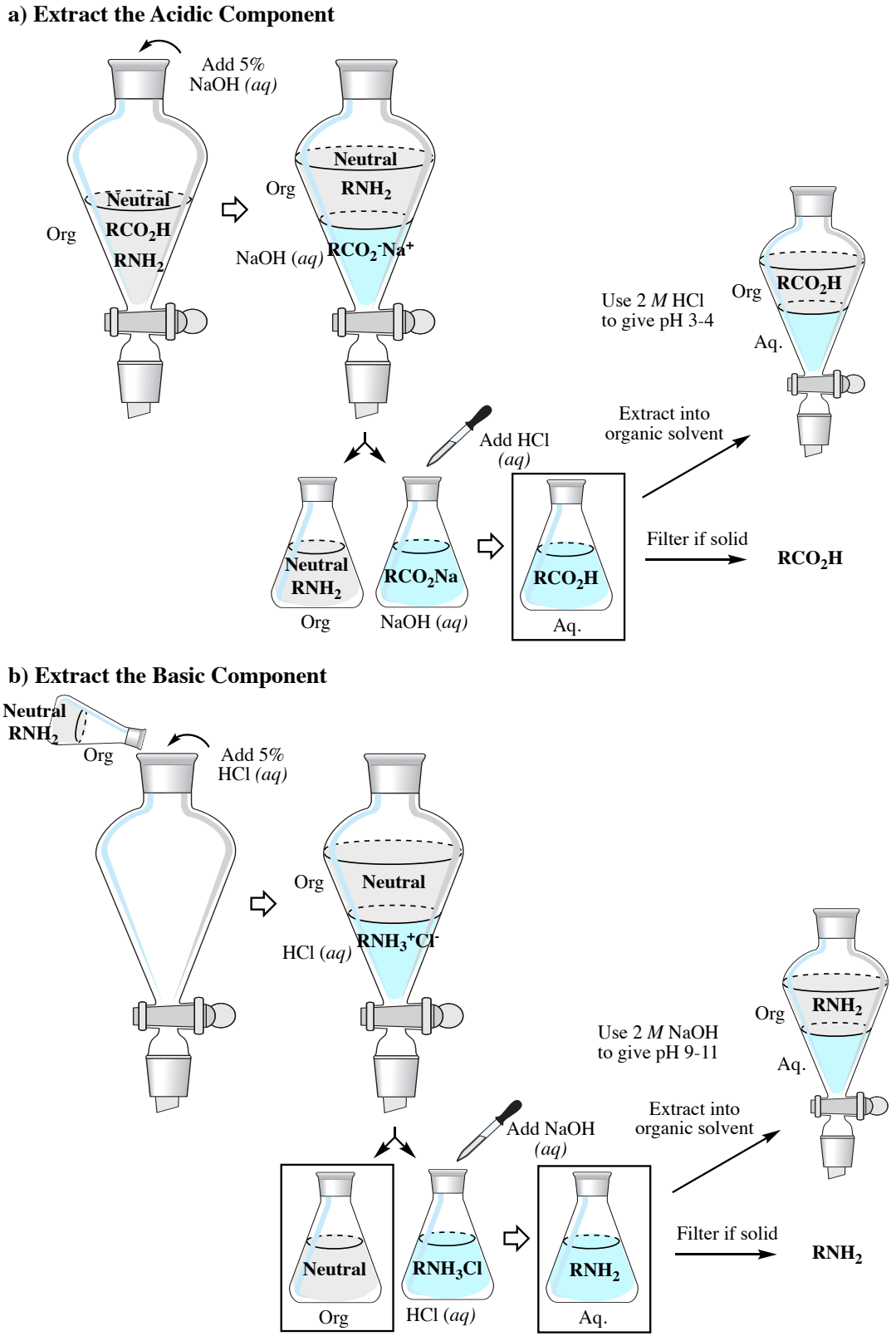

Figure 4.59: Flowchart for separating a mixture containing acidic, basic and neutral components.

It is assumed that readers conducting this type of experiment are familiar with performing single and multiple extractions. In this section are described differences between general extraction procedures and the process as summarized in Figure 4.59.

1. Isolating the Acidic component:

    a. When the acidic component is in the aqueous layer in an Erlenmeyer flask, it can be converted back to the neutral component through addition of 2 *M* HCl *(aq)* until the solution gives a pH of 3-4 (as determined by pH paper). If large quantities of acid are present such that acidification would require too great a volume of 2 *M* HCl *(aq)*, concentrated HCl *(aq)* may be instead added dropwise. Lower concentrations of HCl *(aq)* are less hazardous, but increasing the volume of the aqueous layer by a large amount would affect the efficiency of subsequent extractions and filtering steps.

    b. After acidification, two routes may be taken, depending on if the acidic component is solid or liquid.

        - If a solid forms upon acidification of the ionic salt, it can be collected through suction filtration. This method should only be used if large quantities of large-sized crystals are seen. If fine crystals form (which are quite common), they will clog the filter paper and interfere with adequate drainage. If only a small amount of solid is seen compared to the theoretical quantity, it is likely the compound is quite water-soluble, and filtration would lead to low recovery.

        - If no solid forms upon acidification (or if fine crystals or low quantity of solid forms), extract the acidic component back into an organic solvent (×3). As a general rule of thumb, use one-third as much solvent for the extractions as the original layer (*e.g.* if using 100 mL aqueous solution, extract with 33 mL organic solvent each time). Be sure to first cool the aqueous solution in an ice bath before extraction if the acidification created noticeable heat. Follow up with a brine wash (×1) if using diethyl ether or ethyl acetate, dry with a drying agent, and remove the solvent via rotary evaporator to leave the pure acidic component.

2. Isolating the Basic component:

    Use a similar process as the isolation of the acidic component, except basify the solution using 2 *M* NaOH *(aq)* until it gives a pH of 9-10 as determined by pH paper.

3. Isolating the Neutral component:

    The neutral component will be the "leftover" compound in the organic layer. To isolate, wash with brine (×1) if using diethyl ether or ethyl acetate, dry with a drying agent, and remove the solvent via rotary evaporator to leave the pure neutral component.