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General and Organic Chemistry Laboratory Experiments for Science , Pre-medical, and Pharmacy students
Chapter IV: Identification and Purification of Organic Compounds

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Abstract

In order to identify an organic compound, a chemist needs to apply the suitable technique, depending on the compound state. Accordingly, melting point identification is applied for solids, boiling point for liquids, and sublimation for some compounds which have the tendency to sublime. On the other hand, When an organic compound is isolated from its natural source such as plants, it can be contaminated with some other undesirable compounds, called impurities. Therefore, a special technique, called re-crystallization is used to obtain the original compound in a crystalline form. This technique belongs to the purification techniques. This chapter consists of two main parts, the identification and the purification techniques of some important organic compounds. Another techniques is used depending on the solubility of the organic in a special solvent compared to another in which the organic compound is sparingly soluble, depending on compound's polarity. This technique is called the solvent extraction. The same situation is found with the qualitative technique, the chromatography, which determines the compound's purity. In this chapter, highlights will be thrown on most of these techniques used to identify and purify some organic compounds, based on the compound's polarity, solubility, in addition to its physical state and capability to change from a physical state to another.

Keywords: Melting; Boiling; Purification; identification, Centrifugation.

Part I: Introduction to Organic Compound Identification

While qualitative in nature, identification methods determine compound's identity by measuring specific physical characteristics. These properties are often determined by the physical state of the substance—whether it exists as a solid, liquid, or gas.

Common identification techniquess include:

- **Melting Point Determination:** For solid materials.
- **Boiling Point Analysis (Distillation):** Used for liquid substances.
- **Chromatography:** Identification via specific R_f values.
- **Solubility Profiles:** Based on the principle of polarity.
- **Physical Constants:** Such as density and refractive index.

Part II: Purification Techniques

Purification is a critical, sometimes complicated, stage following the synthesis or natural extraction of organic molecules. The goal is to eliminate unwanted contaminants from the target substance. The chemical makeup of the compound and its contaminants determines the procedure to be used.

Common methods include: Sublimation, crystallization, distillation types (fractional, vacuum, or steam), differential extraction, chromatography, and centrifugation.

Purity Assessment: A compound's purity can be verified by observing a sharp melting or boiling point. Additionally, Thin Layer Chromatography (TLC) can detect impurities when the sample is viewed under a UV lamp.[1]

UV lamp

Experiment 1: Melting Point and Sublimation

Melting Point Concept The melting point is the specific temperature range where a solid transitions into its liquid phase. This physical constant is vital for substance identification.

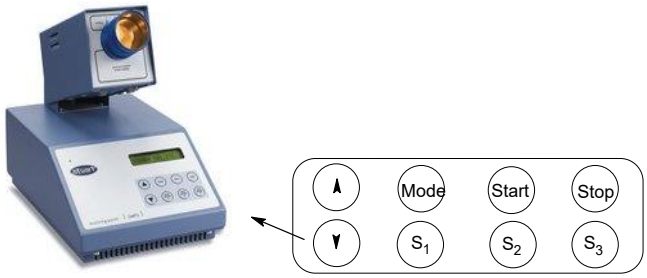
Measurement: A small quantity of the solid is heated gradually while being monitored, often through a capillary tube. The "melting range" refers to the temperature interval from the initial formation of liquid to the point at which the solid becomes completely liquefied.

Purity Indicators: Pure organic compounds typically exhibit sharp melting points with a narrow range (0.5–1.0 °C). Conversely, the presence of miscible impurities will cause the melting point to drop and the range to widen.[2]

Theoretical Background Contaminants almost always lower the melting point of a sample (a phenomenon known as melting point depression) and broaden its melting range. Organic substances generally melt at lower temperatures (below 350 °C) than inorganic ones (above 750 °C) because they are held by covalent bonds rather than strong ionic (electrostatic) bonds. Factors influencing these values include molecular weight, polarity, and crystalline structure.[3]

Experimental Procedure (Melting Point)

1. Pack 1–2 mm of finely ground solid into a capillary tube by tapping it on a hard surface.
2. Utilize a Stuart Scientific 3 apparatus.

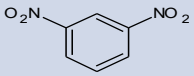
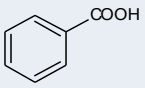
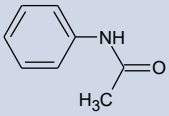
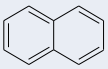
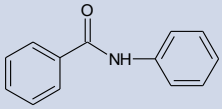
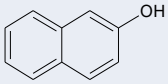
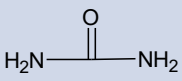


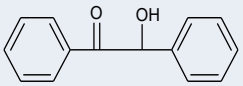
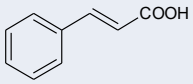
The Stuart 3 has a button named 'Mode' which can have either of three functions, 'Cooling to ambient', 'Plateau', and 'Slope'. The first function means that the device is set on cooling to lab temperature. The 'Plateau' function allows you to set a plateau temperature several degrees below the expected melting point; called the pre-adjusted temperature. The 'Slope' function means the rate of heating in ^{o}C / minute.

3. Set the **Plateau** temperature to roughly 20 °C below the expected melting point.
4. Begin with a rapid heating **Slope** (10 °C/min), then switch to a slower rate (2.5 °C/min) as the melting point approaches for increased accuracy.
5. Record the start and end of the melting process and compare these results against literature values to identify the compound.[4]

The melting points of some important organic compounds are shown in Table 1.

Table 1. Melting points of some organic compounds

Compound name	Compound structure	M. Point°C
m-Dinitrobenzene		90-89
Benzoic acid		118.5-117
Acetanilide		114-113
Naphthalene		80-79
Benzanilide		161-160
2-Naphthol		121-120
Urea		133-132

Benzoin		133-132
Cinnamic acid		133-134

Sublimation

The process by which a solid transforms straight into a gas without going through the liquid phase is known as sublimation. This technique is effective for separating mixtures where only one component, such as ammonium chloride, sublimes. Symmetrical molecules such as iodine and naphthalene can sublime.[5]

Method: Heat the impure mixture; the sublimed vapors will condense into a pure solid on a cool surface, such as the walls of an inverted funnel.

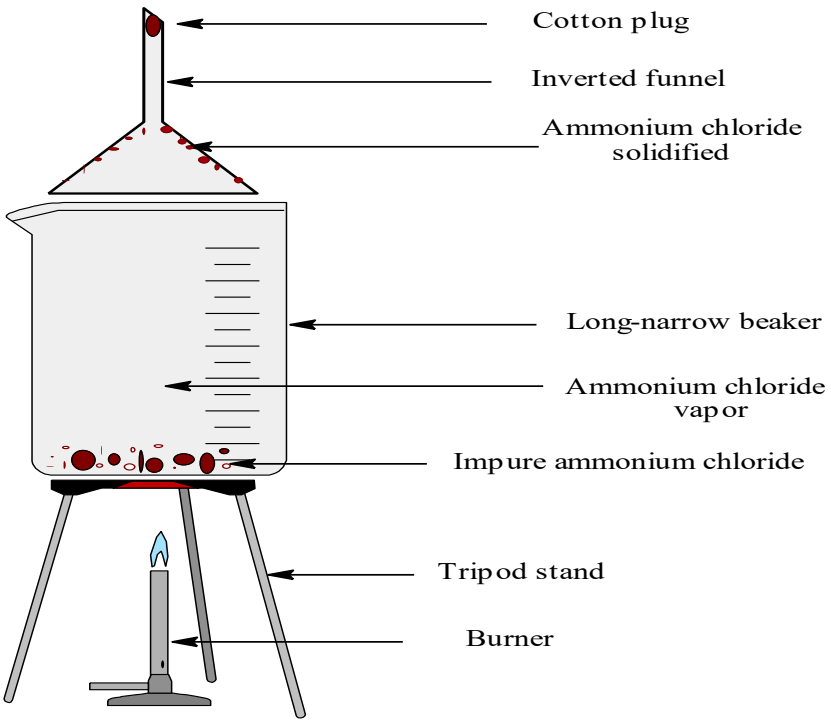


Fig. Sublimation of ammonium chloride

As a result, this can be used as a purification technique, for instance when a mixture of sodium chloride and ammonium chloride is heated, the sodium chloride, unlike the ammonium chloride, will not sublime. [6]

Procedure:

1. Weigh the impure sample. Heat it in a long-narrow beaker either on a hot plate, a Bunsen burner, or a sand bath.
2. Watch the white clouds evaporating from the sample and condensing on the inner side of the funnel.
3. Once the evaporation is finished, turn off the heat and allow the sample to cool.
4. Weigh the solid after scraping it off the funnel. Determine the recovery percentage.
5. Weigh the solid after scraping it off the beaker's bottom. Determine the percentage of contaminants.[7]

Experiment 2: Simple Distillation

Distillation is the primary technique for the purification and separation of liquid mixtures.

Principle: A liquid is heated until its vapor pressure matches the surrounding atmospheric pressure, reaching its boiling point. The generated vapors are subsequently condensed into liquid form and collected.

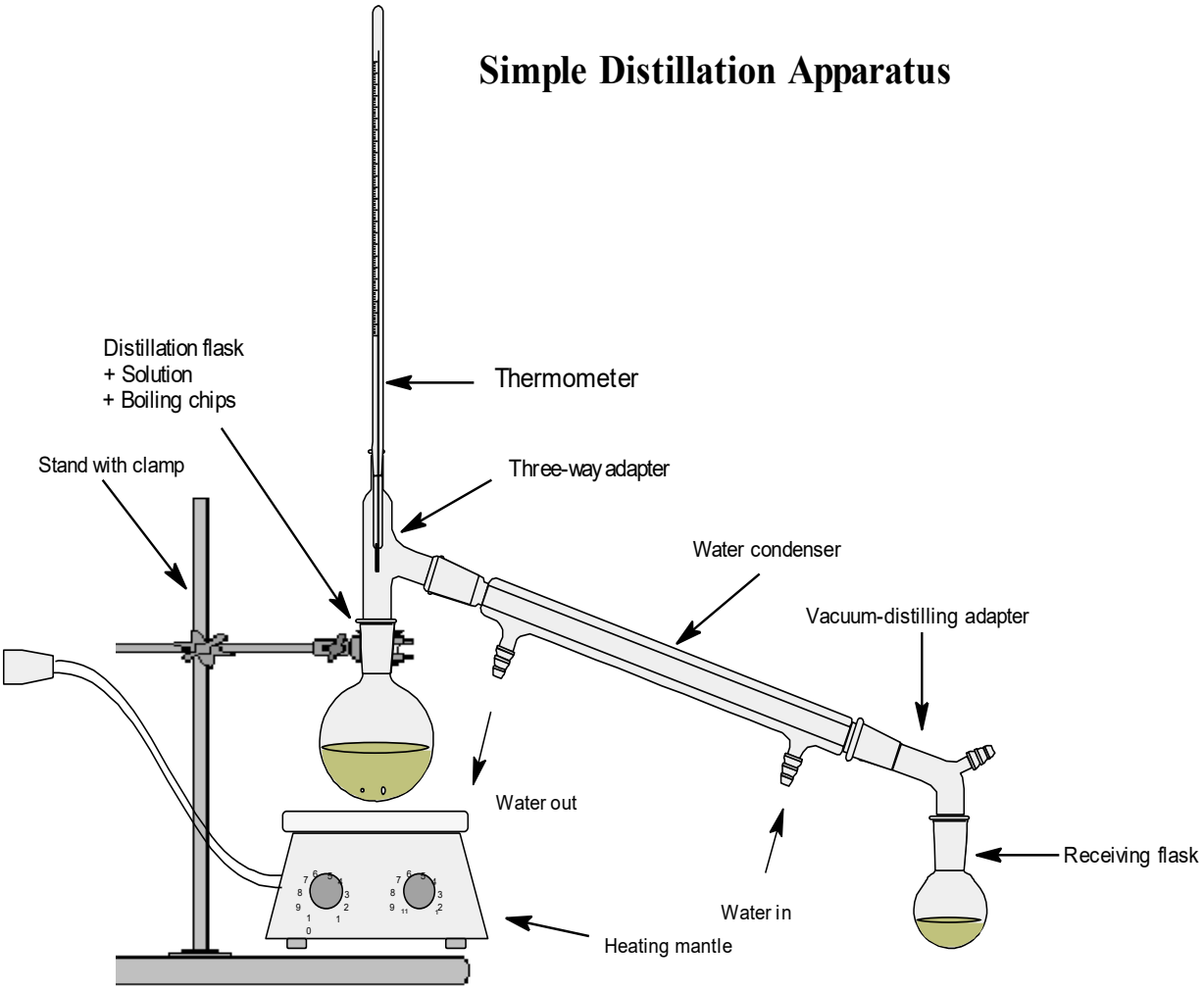


Fig 1: Fractional distillation: A fractionating column is used to separate liquids having similar boiling points.

Its high surface area allows for repeated evaporation-condensation cycles, resulting in a significantly purer distillate shown in fig 1.

Azeotropes: Some mixtures, like 95.6% ethanol and water, form constant-boiling solutions that cannot be further separated by simple distillation.[8]

Experiment 3: Chromatography

All chromatography involves a **mobile phase** (moving) and a **stationary phase** (fixed). Separation occurs because different compounds have varying affinities for these two phases.

Fig 2 shows the Compounds are identified via TLC (Thin Layer Chromatography) based on their Rf value, which is the ratio of the compound's travel distance to the solvent front's travel distance. Because the solubility and

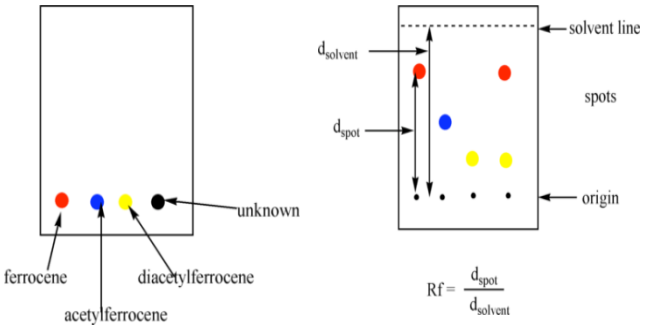
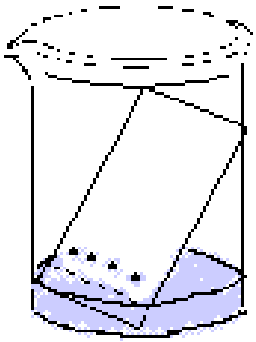


Fig 2: Compounds are identified via TLC (Thin Layer Chromatography

affinity of every compound for every stationary and cell segment might be exceptional, separation happens as every compound travels a specific distance alongside the column (or the slide) in a hard and fast time. The distance traveled by a compound during separation is expressed as the Rf

value in TLC and as retention time or retention volume in column chromatography. The retention factor, or Rf, in TLC is used to identify compounds during separation and analysis. It is defined as the ratio of the compound's travel distance to the solvent front's travel distance during plate development. Retention time in GC and HPLC is the amount of time needed for a compound to elute from the column. Retention volume in column chromatography is the amount of mobile phase required to elute a molecule. The volume of mobile phase that a packed column can hold is



represented by one column volume, which is how this value is commonly described.[9]

Column Chromatography: A preparative separation technique in which a sample is introduced onto a packed column, commonly containing silica or alumina, and eluted using a suitable solvent. The components are separated based on their differing migration rates and collected in individual fractions as they elute from the column shown in fig 3.

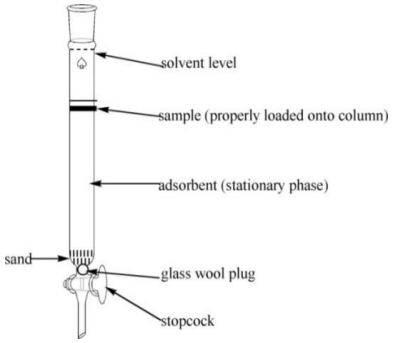
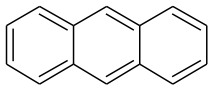


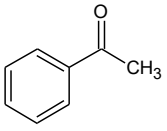
Fig 3: Column Chromatography

Separation of Colorless Compounds by Thin Layer Chromatography

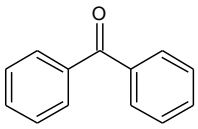
In this experiment, a set of compounds with sufficiently different Rf values to enable component identification are separated by TLC using a suitable solvent or solvent mixture. There are 1% solutions of anthracene, acetophenone, and benzophenone in dichloromethane. For use in solvent selection, a mixed solution comprising all three chemicals in dichloromethane is also provided.



Anthracene



Acetophenone



Benzophenone

In this experiment, the unknown sample could be a single drug or a combination of compounds. Hexane, toluene, cyclohexane, dichloromethane, ethyl acetate, and methanol are the solvents used in this test.[10]

Procedure

Preparing TLC plates spotted with each compound to be separated and

testing them with each possible solvent are steps in the solvent selection process. This can be done by developing six TLC plates, each containing the three-component mixture and a different solvent, or by using a faster method such as micro-TLC development. You'll select a solvent by means of the micromethod. On a precut TLC plate, place the three-element combination at three locations that are roughly equal apart. To allow the solvent to drain down and through the TLC plate, draw the solvent into a capillary spotter and contact the problem area. For every place, use a different solvent. To complete the experiment using all six solvents, you'll need TLC plates. To see the results, use the UV lamp. As the plate grows, you are looking for a hoop pattern that results from the compounds' separation.

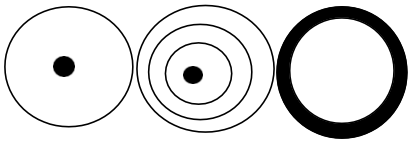


Figure A: An illustration of what happens when no compound moves in a solvent system and all materials stay at the baseline (origin). The deeper hue is caused by the solvent, either by partial drying or by small contaminants in the solvent that quench the fluorescent dye. A successful separation is depicted in Figure B. Each component of the combination is shown as rings and is isolated from the opposing components. The outcome of every compound changing with the solvent is displayed in Figure C. At the solvent front, every component is arranged in a ring. Because they are ineffective at separating the compounds, the solvents utilized in A and C are not suitable for separations. The preferred solvent is the one utilized in B.

Obtain an unknown from the lab instructors. Pick up two precut TLC plates and spot compounds and your unknown per plate. In the event that the plates do not develop precisely the same, this will enable you to identify all three compounds and the unknown across two plates. Use the solvent used for the micro TLC test to develop the plates as previously mentioned. Examine the formed plates under the UV lamp once they have dried. Prior to drying, don't forget to indicate the solvent front. Outline the spots with a pencil. Find the compounds in your unknown by calculating the Rf's for each area and using the Rf records in addition to examination.

Separation of Analgesics

Obtain an unidentified amount from the lab instructors. To identify your plates and raise your TLCs as specified for the colorless chemicals experiment, you will follow the same well-known protocol. A specific solvent will be placed on the solvent bench; you won't have to pick one

anymore. Identify your unknown and the four requirements, then develop the plate or plates in the specified solvent. You could use two plates. If all five cannot fit on one plate, place your unknown and two standards on each plate. To determine which compounds are present in your unknown sample, use the Rf values and eye inspection. Select the analgesic product that contains these substances using the chart on the bulletin board.

Column Chromatography

Acquire a stationary phase, glass wool, sand, and a column (25 mL buret). Your TA may supply the quantity of stationary phase. As mentioned earlier, dry-pack the column. Make sure to faucet the column to release as much air as possible. Carefully pour petroleum ether into the column to fill it. ether along the column's margins. Empty the column until the silica gel level is just above the petroleum ether level. Obtain a sample of ferrocene derivatives that has already been blended. As previously mentioned, load the column after dissolving the sample in a little amount of methylene chloride. Upload a pet. Add ether slowly dropwise using a pipette along the walls of the column until the solvent level reaches about 1–2 cm above the surface. Allow it to drain down to the top of the silica gel, then repeat this process 5–10 times. Take care not to disturb the column bed, as this can affect the separation efficiency. Once the sample has been properly loaded, gently fill the column with petroleum ether.

This procedure will be carried out as flash chromatography, meaning the solvent will be driven through the column using air pressure. Ensure the pressure is not too high, and avoid forcing the stopper tightly into the burette—simply keep it positioned at the top. Further clarification will be provided during the demonstration.

Begin the elution and collect the fractions, typically in volumes of 20–40 mL, using beakers or Erlenmeyer flasks. You will be able to observe the movement of the bands as they travel down the column, which is relatively uncommon since most organic compounds are colorless and not easily visible during separation. Always ensure that the solvent level remains above the silica gel and never allow the column to run dry.

After the first band has completely eluted, switch the mobile phase to an 80:20 mixture of petroleum ether and ethyl acetate. Allow the petroleum ether to drain until it is about 1 cm above the silica gel, then carefully introduce the new solvent mixture. Continue the elution process, adding

more of the 80:20 mixture as the solvent level decreases.

Once the second band has eluted, again reduce the solvent level to approximately 1 cm above the silica gel, and then gently add a 50:50 petroleum ether and ethyl acetate mixture to proceed further.

Continue the elution, adding the 50:50 petroleum ether–ethyl acetate mixture as the solvent level drops to keep it near the top of the column. Stop the process once the third band has fully eluted.

Collect and combine all fractions corresponding to each band into separate beakers—one for band 1, one for band 2, and one for band 3. If needed, use TLC to help identify which fractions belong to each compound. Remove the solvent from each combined fraction using a steam bath under a fume hood. This can be aided by a gentle stream of air or nitrogen, or preferably by using a rotary evaporator if available. By the end, you should obtain three separate samples (more if the separation was not efficient).

Dissolve each isolated sample in a minimal volume of methylene chloride and spot them individually on a TLC plate. Also prepare a plate with standard samples of the three ferrocene derivatives. Develop the plates using an 80:20 petroleum ether–ethyl acetate solvent system. Finally, visualize the spots under a UV lamp and identify the compounds present in each band.

Experiment 4

Compound Purification through Re-Crystallization

Purifying compounds, whether synthesized in the laboratory or isolated from natural sources, is an essential aspect of organic chemistry. A variety of techniques can be employed for this purpose, including distillation, sublimation, extraction, various chromatographic methods, and recrystallization. The basic procedure of re-crystallization involves dissolving the substance in a hot solvent to dispose of insoluble impurities then letting the preferred compound crystallize. Products received from a natural response are seldom natural whilst isolated immediately from the reaction mixture. The product purification depends on types of impurities therein. If the product is strong, it is able to be purified with the aid of re-crystallization from a appropriate solvent in which a few impurities are insoluble and subsequently they may be separated with the aid of filtration of hot solution of the product. Alternatively, the soluble

impurities within the filtrate are filtered after the product had shaped crystals. In most cases, filtration is carried out in two stages—first while the solution is hot and then again after it has cooled. Therefore, an ideal recrystallization solvent should dissolve a reasonable amount of the target compound at elevated temperatures but only a minimal amount at lower temperatures. Additionally, impurities should either remain insoluble or be highly soluble in the solvent so they can be easily removed. Ultimately, these properties ensure efficient purification and high-quality crystal formation. the solvent must be eliminated with no trouble from the purified product. This commonly procedure must have a fantastically low boiling point. Anyways, the selected solvent must have low boiling point and should neither be highly priced nor toxic. Similarly, the chosen solvent ought to neither react with the product nor with the impurities. Once in a while, a single solvent will not be sufficient, relying on form of the natural compound and a aggregate of or 3 solvents is required.
A chemist can consult the literature for information concerning solvents for re-crystallizing a specific substance, or if that data isn't always available, take a look at numerous solvents. Solubility is then referred to each at cold and high temperatures. The first-class and amount of crystals obtained while the solution is cooled also are mentioned.

Procedure

Solubility tests

Add approximately 10 mg of anthracene to each of four reaction tubes. Initially weigh the 10 mg portions until you become comfortable estimating this amount by observation. Then add different solvents to each tube: water (tube 2), toluene (tube 3), and ligroin (tube 4). A substance is considered dissolved when the solution appears clear, without any visible cloudiness or solid particles. Even if the solution shows color, it is still regarded as dissolved as long as no solid remains.

Take particular care with the ligroin samples, as ligroin has a low boiling point and can easily evaporate completely. If this occurs, simply add 0.25 mL of solvent again and continue the procedure.

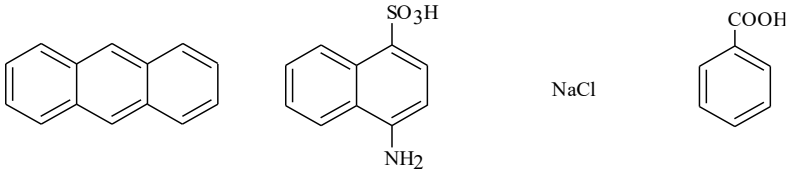
Repeat the same experiment using 4-amino-1-naphthalenesulfonic acid (sodium salt), and then again with benzoic acid. Record all observations carefully in your laboratory notebook.

Did the sample dissolve in a solvent at room temperature?

Did the compound dissolve in warm solvent?

Did the compound precipitate (crystallize) upon cooling of the solvent?

Recording these data in a table form works better.



Re-crystallization of Acetylsalicylic Acid (Aspirin) from Water:

Determine the minimum volume of warm water needed to dissolve 60 mg of acetylsalicylic acid. Its solubility is 1.0 g in 300 mL at 25 °C and 1.0 g in 100 mL at 37 °C. For the calculation, use the solubility at 37 °C, then reduce the calculated solvent volume by about one-third to one-half to account for increased solubility at higher temperatures .Keep in mind that aspirin can hydrolyze in boiling water, so avoid prolonged heating. Warm the solution only until slight convection currents (wavy lines) or the first signs of bubbling are observed Place 60 mg of acetylsalicylic acid in a test tube, add the calculated minimum amount of water, introduce a boiling stick, and heat gently using a sand bath.

Because the solvent starts off evolved to boil, add water drop-wise till the sample just dissolves. Add 1 more drop of water. Record the full extent of water had to dissolve the sample (21 drops = 1 mL). Put off the solution from the heat and put in the tube holder to reach the room temperature undisturbed. If crystals have no longer appeared upon cooling, scratch the wall of the reaction tube with a tumbler stir rod. If crystals still fail to form, consult your instructor for further guidance. Once crystallization begins, place the test tube in an ice bath and allow the process to complete. When the tube is fully cooled and no additional crystals are forming, proceed to the isolation step.Carefully insert a Pasteur pipette to the bottom of the tube—firm enough to reach the liquid, but not so forceful that the pipette may break. Gently withdraw the solvent, leaving the crystals behind as much as possible. After drying, weigh the collected crystals. Finally, determine the melting point of both the purified crystals and the original crude sample for comparison.

Re-crystallization of Naphthalene from 80% Methanol/Water:

Recrystallize 40 mg of naphthalene using an 80% methanol–water solvent mixture. Since the minimum solvent volume cannot be calculated in

advance, determine it experimentally as you proceed. If crystals do not initially form, follow the standard recrystallization techniques to induce crystallization.

Once crystals begin to appear, place the reaction tube in an ice bath to allow the process to complete. After crystallization is finished, remove the solvent using a pipette as described earlier. Transfer the crystals onto filter paper and let them air dry for about 10–15 minutes.

Avoid applying heat, as naphthalene can readily sublime. For the same reason, do not use a drying oven. Also, do not leave the crystals exposed to air for long periods, as this may lead to product loss through sublimation. If storage is required, keep the naphthalene in a tightly sealed screw-cap vial.

Re-crystallization of an Unknown

Dissolving the impure substance

Evaluate the solubility of the unknown compound by testing ~10 mg samples in water, ethanol, and methanol. Note whether the compound dissolves at room temperature, upon heating, and whether crystals reform upon cooling. Use these observations to determine the most appropriate solvent for recrystallization. Remember that your unknown may contain insoluble impurities, so not all of the substance will dissolve. Check whether any colored material remains undissolved; this is an insoluble impurity and does not affect your choice of a suitable recrystallization solvent. Accurately weigh approximately Weigh 1.0 g of the impure unknown and transfer it to a 50–125 mL Erlenmeyer flask. Add the chosen solvent dropwise until the solid is just covered. Heat the mixture gently on a hot plate until it begins to boil lightly, continuing to add solvent dropwise until all the crystals dissolve. Any insoluble impurities will remain undissolved and may appear different in shape or form compared to the main compound.

Once dissolution is complete, add an additional ~5% of solvent. Record the total volume used (note: 21 drops ≈ 1 mL). Remove the insoluble impurities by performing hot gravity filtration using fluted filter paper and the appropriate setup. Ensure the plastic funnel is preheated on a steam bath just before use, and keep hot solvent ready if needed during filtrationThe receiving Erlenmeyer flask should be preheated, either on a steam bath or a hot plate (if using a hot plate, add a small amount of solvent to the flask).

Place fluted filter paper into a clean plastic funnel and position it over the warmed receiving flask, which should be set on a ceramic disk just before use.

Quickly pour the hot recrystallization mixture into the filter paper. Use a clamp (such as a burette clamp), a paper towel ring, or another protective method to handle the hot vessel safely and prevent burns. Allow the solution to pass through the filter. If crystals begin to form in the funnel or on the filter paper, rinse them with a small amount (about 1–2 mL) of hot solvent to ensure complete transfer.

Cooling the solution

Allow the solution to cool slowly to room temperature, then place the flask in an ice bath. Avoid speeding up this step, as rapid cooling can lead to the formation of very fine crystals that are difficult to isolate, or may cause the compound to separate as an oil instead of forming crystals. If crystals do not appear upon cooling, follow the standard procedures to induce crystallization.

Gathering the Crystals

If no additional crystals form, allow the solution to stand for about five more minutes before proceeding with collection using a vacuum filtration setup. Gently swirl the flask to keep the crystals suspended, then transfer the mixture into the funnel while maintaining the vacuum. Rinse the collected crystals with a small amount of ice-cold solvent to remove impurities. The liquid collected in the vacuum flask is known as the mother liquor. Keep the vacuum applied for several minutes to ensure thorough drying of the crystals.

During this stage, additional crystals may sometimes form in the mother liquor. These are referred to as the second crop of crystals and typically appear as the solvent cools and partially evaporates. Depending on the compound and solvent system, further crops may also form, although they will not be collected in this procedure. However, their approximate quantity should be noted, as it may be useful when evaluating potential material loss. Finally, dry the crystals in an oven as directed by the laboratory staff, and once completely dry, measure and record the final mass.

Determining the m.p.

Measure the melting point of the unknown sample, then compare the

observed value with the reference charts provided on the bulletin board to identify the compound.

Pre-Lab Questions

1. **Experiment 5**

Solvent Extraction

Objectives

The solvent extraction is a method used to separate natural compounds from mixtures or flowers. Sometimes, the solvent will extract the natural compound with impurities; in this case it would be possible to extract it in natural way, using any other solvent. For example, at the end of oganic synthesis, the product is contaminated with unwanted inorganic catalysts; in this case, ether is used for dissolving natural compound whereas water is introduced to dissolve the inorganic compound, considering those are immiscible, thereafter the ether layer is evaporated to achieve the pure organic compound.

Definition of Extraction

It is a procedure of isolating an organic compound found with impurities in a certain solvent with the use of some other solvent wherein the natural compound is pretty soluble and the impurities are sparingly soluble or insoluble. The procedure of extracting a substance from a mixture or from its natural source is made via shifting its solution in a separating funnel and adding an organic solvent which is immiscible with the compound's solution and wherein the compound's solubility is excessive. Shake the funnel several times and open the stopcock once in a while to release excessive stress. Leave the funnel untouched on the iron ring with the stopper left open to permit separation of the two layers, then draw the lower layer in conical flask A. If the aggregate is composed of a non-polar compound and an inorganic compound, we are able to use water and ether for keeping apart the compounds. The isolation is done in one extraction, because the organic compound is insoluble in water whilst the inorganic compound is insoluble in ether. But if the aggregate is composed of an inorganic compound and a polar compound (with hetero-atoms inclusive of oxygen or nitrogen) like aldehydes, ketones, esters and amines in which these compounds make hydrogen bonds and are miscible with water, then a single extraction is not enough due to the fact that part of the organic compound is soluble within the inorganic solvent (water). In this example, extraction of the organic compound from the inorganic solvent is repeated with the aid of including an organic solvent to the aqueous layer isolated from the primary extraction with repeating shaking

and separation several times in step with the K_{dist} within the two stated solvents and in on every occasion a in addition portion of the natural compound migrates to the organic solvent. Subsequently, those quantities are gathered in a beaker where the solvent is distilled or evaporated leaving the natural organic compound.

Idea and summary

This method includes the separation of an organic substance from a combination of solvents, particularly aqueous natural combination (e.g. water-ether) or steam distillate. The solvent used for extraction ought to have the following properties:

1. It easily dissolves the substance to be extracted.
2. It must have low b. p. so that it is able to be removed.
3. It must not react with the solute or with the alternative solvent.
4. It must not be flammable or poisonous.
5. It should not be expensive.
6. It must not be miscible with water.
7. It must not dissolve the impurities.

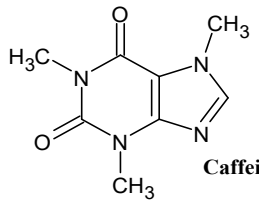
Glaringly, no solvent meets these kinds of standards however numerous come close. The maximum commonplace solvent used for extraction is diethyl ether, although it is extremely flammable. It is used for setting apart many natural compounds present in animals and flora. Every so often, opportunity water-immiscible solvents consisting of hexane, petroleum ether, benzene, chloroform, methylene chloride, and carbon tetrachloride are used. Extraction approach relies upon at the unequal distribution of the natural compound among the 2 immiscible solvents, primarily based on its solubility in these solvents. The ratio of a compound's concentration in each solvent is defined as the distribution coefficient, $K_{dist.}$

.

$$K_{dist} = \frac{\text{Compound's concentration in ether}}{\text{Compound's concentration in water}} = \frac{\text{Solubility of solute in ether (g/100 ml)}}{\text{Solubility of solute in water (g/100 ml)}}$$

The higher the K_{dist} , the more efficient is the separation.

Extraction of Caffeine from Tea Leaves



Caffeine Structure

With a view to extract caffeine from tea leaves, the following steps should be executed:

1. Weigh about 150 g of dry red tea leaves obtained from the market.
2. In a 2-liter beaker boil the dry tea leaves in about 2 L of water for 1 h.
3. Filter out the dark solution combination and cool the filtrate.
4. Transfere 50 mL of the tea filtrate to a 250 mL beaker.
5. Add 12 mL of lead acetate solution (10%) to the solution and hold stirring for 10 min to precipitate tannin, a competitor of caffeine, as a white precipitate of lead tannate.
6. Filter out the solution in a tumbler funnel and bring the filtrate to a 250-mL isolating funnel.
7. Add 5 mL of chloroform to the separating funnel, agitate the solution and open the funnel vent occasionally to launch immoderate strain. Restorate the funnel on a stand permitting the two layers to split. Pour the lower layer in a 50-mL beaker.
8. Repeat step 7 three times.
9. Accumulate the three quantities of the lower layer (approximately 15 mL) and heat the solution on a heating mantle to evaporate the chloroform, leaving white precipitate of caffeine (about 0.1-0.3 g).

Calculation of caffeine % yield

Every I Kg of the tea leaves contains at the least 50 g of caffeine. Assuming the concentration of the prepared boiled solution of tea extract is 150 g of tea leaves in 1000 ml water, i.e. 15 %; consequently, 1000 g of tea leaves comprise 50 g of caffeine and therefore 150 g of tea leaves incorporate X g of caffeine.

$$X = \frac{50 \times 150}{} = 7.50 \text{ g caffeine}$$

1000

This means 1000 ml of the organized conc. tea solution include 7.50 g of caffeine.
Therefore, the 50 ml of the organized conc. tea solution comprise Y g of caffeine, in which Y is the theoretical yield.

$$Y = \frac{7.50 \times 50}{1000} = 0.375 \text{ g caffeine}$$

The % yield of caffeine is calculated as follows:

$$X = \frac{\text{Experimental yield}}{\text{Theoretical yield}} \times 100 = \ldots\ldots\ldots \%$$

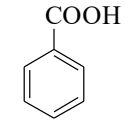
Q.1 Calculate the caffeine % yield if the weight of caffeine received is 0.15 g.

Q.2 Draw the structural method of caffeine.

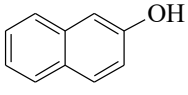
Separation of a 3 element mixture using extraction strategies

Extraction is a method often used by organic chemists for the rapid crude separation of mixtures containing acidic and/or basic compounds, frequently in the presence of neutral substances. The separation utilizes acid–base chemistry and two mutually insoluble solvent layers (water and tert-butyl methyl ether).

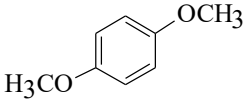
You'll be isolating three compounds: benzoic acid, 2-naphthol, and *p*-dimethoxybenzene.



Benzoic acid



2-Naphthol



p-Dimethoxybenzene

Dedication of a Partition Coefficient

On this experiment, you may extract a dichloromethane solution of benzoic acid with water.

This requires cautious pipette capabilities. Upload anhydrous $CaCl_2$ to the CH_2Cl_2 layer until you do not observe any similarly clumping of the pellets.

Experiment 6

Separating natural Compounds by Centrifugation

The precept of Centrifuge

A centrifuge operates based on the principle of sedimentation: when subjected to a strong centrifugal force (g-force), components of a mixture separate according to their density, with denser particles moving outward more rapidly than less dense ones. Particles become concentrated as a pellet at the bottom of the centrifuge tube and are separated from the solution, called the supernatant.

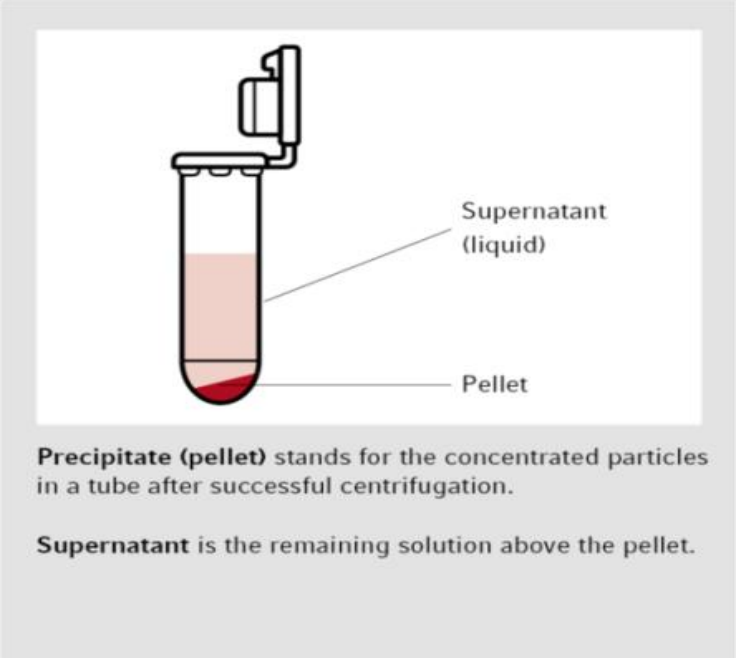


Fig 4: Separating natural Compounds by Centrifugation

- Centrifugation is a method of isolating substances which entails the use of centrifugal force.

The components of a mixture are separated based on differences in size, shape, density, the viscosity of the medium, and the speed of the rotor shown in fig 4.

Precept of Centrifugation

- In a solution, particles that are denser than the solvent tend to settle down (sediment), while those with lower density rise and remain near the top.
- The greater the difference in density between particles, the faster they separate; if their densities are equal (isopycnic conditions), no separation occurs and the particles remain suspended.
- To exploit even very small differences in density, normal gravitational force can be replaced by the much stronger centrifugal force generated by a centrifuge.
- A centrifuge is a device that rotates samples around a fixed axis, producing a strong outward force perpendicular to the axis of rotation, which facilitates separation.

- A centrifuge operates on the principle of sedimentation, where centripetal acceleration causes denser particles to move outward in the radial direction.
- At the same time, less dense particles are displaced inward, toward the center.
- In a laboratory centrifuge using test tubes, this radial acceleration drives denser particles to settle at the bottom of the tube, while lower-density materials remain toward the top.

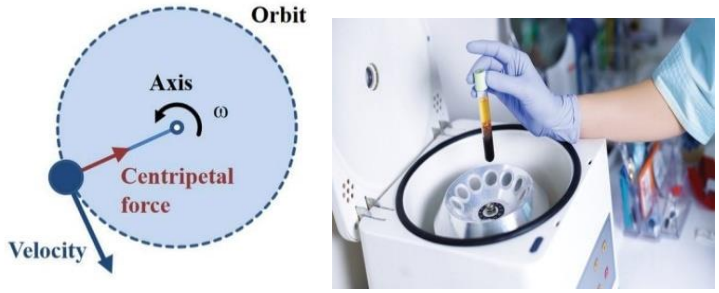


Fig 5: Sludge Suspended Solid Separation

Centrifuge applications

Centrifuges can be used for multiple applications, because there are many different sorts available in today's market. Some applications for centrifuges are listed underneath:

- Separation of mixtures with near densities
- Separating immiscible liquids

- Sedimentation of suspended solids
- Blood separation
- Separating insoluble particles (e.g. insoluble proteins in a protein solution)
- Isotope Separation
- Gravity simulation environments for astronauts
- Separation of creams
- Spin function of washing machines
- Separation of wastewater sludge
- Compound synthesis in a high gravity environment

Sludge Suspended Solid Separation

Concentration of suspended solids (SS) is an essential parameter in waste water analysis that is used to recognize dealing with characteristics and to assess treatment options and efficiencies. Samples from anaerobic lagoon sludge frequently have high stages of SS which can not be easily measured using standard filtration techniques. This method employs centrifugation to separate solid particles from liquid samples and measures the dissolved solids (DS) present in the supernatant. The suspended solids (SS) are then determined as the difference between total solids (TS) and dissolved solids (DS) shown in fig 5.

The technique is based on the assumption that dissolved substances are not significantly influenced by centrifugal forces up to about 10,000 × g, and therefore remain uniformly distributed between the liquid phase and the residual water associated with the sedimented pellet.

This approach has been evaluated against the conventional filtration method, as well as another centrifuge-based technique, using lagoon sludge samples with total solids (TS) concentrations ranging from 61,000 to 100,000 mg L^{-1}.

Conclusion

The identification and purification of natural compounds are important procedures in organic chemistry that make sure the accuracy, reliability, and the use of synthesized or naturally derived substances. Thru diverse analytical and separation strategies, chemists can verify the structure, composition, and purity of compounds before they are applied in research, material science, pharmaceuticals, or industrial applications.

Techniques including re-crystallization, distillation, extraction, and chromatography, play an essential position in getting rid of impurities and separating favored compounds with excessive purity. Further, modern-day analytical tools such as spectroscopy and chromatographic techniques provide specific identity by by way of revealing structural and molecular properties. Those combined tactics no only improve the performance of chemical research but also enhance reproducibility and quality control in laboratories and industries. Ordinary, the systematic identification and purification of organic compounds continue to be essential to the advancement of chemical technologies, permitting the improvement of new substances, medicines, and sustainable chemical methods.

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