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Laboratory Techniques of Purification and Isolation

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Abstract

Purification is the main basis for synthesis of new molecules and drug discovery and sometimes we need to use proper techniques for isolation and purification so that we can get pure drug. This article describes about solution of problems which we have to face during laboratory experiments like selection of proper solvent for reaction, isolation procedures and purification techniques.

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Introduction:

In order to obtain satisfactory results in synthesis, it may be necessary to use proper solvents, to create proper condition for reaction, to develop a suitable mobile-phase for checking purity of synthesized compound, to do isolation properly and recrystallize it with using suitable solvent. For all this purpose it is necessary to remind polarity and solubility chart of different polar and non-polar solvents. It is as described as below.

Selection of Proper Solvent[4-6]

The choice of solvent is perhaps the most critical step in the process of recrystallization since the correct solvent must be selected to form a product of high purity and in good recovery or yield. Consequently a solvent should satisfy certain criteria for use in recrystallization.

The desired compound should be reasonably soluble in the hot solvent, about 5 g/100 mL (5 mg/100 µL) being satisfactory and insoluble or nearly insoluble in the cold solvent. Note that the reference temperature for determination of the solubility in "cold" solvent is often taken to be room temperature. This

combination of solute and solvent will allow dissolution to occur in an amount of solvent that is not unduly large and will also permit recovery of the purified product in high yield. A solvent having this type of solubility properties as a function of temperature would be said to have a favorable temperature coefficient for the desired solute.

Conversely, the impurities should either be insoluble in the solvent at all temperatures or must remain at least moderately soluble in the cold solvent. In other words, if the impurities are soluble, the temperature coefficient for them must be unfavorable; otherwise the desired product and the impurities would both crystallize simultaneously from solution.

Sr. No.	Solvent	Formula	Boiling point (°C)	Melting point (°C)	Density (g/mL)	Solubility in H_2O (g/100g)	Relative polarity
1	Cyclohexane	C_6H_{12}	80.7	6.6	0.779	0.005	0.006
2	Pentane	C_5H_{12}	36.1	-129.7	0.626	0.0039	0.009
3	Hexane	C_6H_{14}	69	-95	0.655	0.0014	0.009
4	Heptanes	C_7H_{16}	98	-90.6	0.684	0.0003	0.012
5	Carbon tetrachloride	CCl_4	76.7	-22.4	1.594	0.08	0.052
6	Carbon disulfide	CS_2	46.3	-111.6	1.263	0.2	0.065
7	p-Xylene	C_8H_{10}	138.3	13.3	0.861	0.02	0.074
8	Toluene	C_7H_8	110.6	-93	0.867	0.05	0.099
9	Benzene	C_6H_6	80.1	5.5	0.879	0.18	0.111
10	Ether	$C_4H_{10}O$	34.6	-116.3	0.713	7.5	0.117
11	Methyl t-butyl ether	$C_5H_{12}O$	55.2	-109	0.741	4.8	0.124
12	Diethylamine	$C_4H_{11}N$	56.3	-48	0.706	M	0.145
13	Dioxane	$C_4H_8O_2$	101.1	11.8	1.033	M	0.164
14	N,N-Dimethylaniline	$C_8H_{11}N$	194.2	2.4	0.956	0.14	0.179
15	Chlorobenzene	C_6H_5Cl	132	-45.6	1.106	0.05	0.188
16	Anisole	C_7H_8O	153.7	-37.5	0.996	0.10	0.198
17	Tetrahydrofuran	C_4H_8O	66	-108.4	0.886	30	0.207
18	Ethyl acetate	$C_4H_8O_2$	77	-83.6	0.894	8.7	0.228
19	Ethyl benzoate	$C_9H_{10}O_2$	213	-34.6	1.047	0.07	0.228
20	Dimethoxyethane	$C_4H_{10}O_2$	85	-58	0.868	M	0.231
21	Diglyme	$C_6H_{14}O_3$	162	-64	0.945	M	0.244
22	Methyl acetate	$C_3H_6O_2$	56.9	-98.1	0.933	24.4	0.253
23	Chloroform	$CHCl_3$	61.2	-63.5	1.498	0.8	0.259
24	1,1-dichloroethane	$C_2H_4Cl_2$	57.3	-97.0	1.176	0.5	0.269
25	di-n-Butyl phthalate	$C_{16}H_{22}O_4$	340	-35	1.049	0.0011	0.272
26	Cyclohexanone	$C_6H_{10}O$	155.6	-16.4	0.948	2.3	0.281
27	Pyridine	C_5H_5N	115.5	-42	0.982	M	0.302
28	Dimethylphthalate	$C_{10}H_{10}O_4$	283.8	1	1.190	0.43	0.309
29	Methylene chloride	CH_2Cl_2	39.8	-96.7	1.326	1.32	0.309
30	2-Pentanone	$C_5H_{10}O$	102.3	-76.9	0.809	4.3	0.321
31	2-Butanone	C_4H_8O	79.6	-86.3	0.805	25.6	0.327
32	1,2-Dichloroethane	$C_2H_4Cl_2$	83.5	-35.4	1.235	0.87	0.327
33	Benzonitrile	C_7H_5N	205	-13	0.996	0.2	0.333
34	Acetone	C_3H_6O	56.2	-94.3	0.786	M	0.355
35	Dimethylformamide	C_3H_7NO	153	-61	0.944	M	0.386
36	t-Butyl alcohol	$C_4H_{10}O$	82.2	25.5	0.786	M	0.389
37	Aniline	C_6H_7N	184.4	-6.0	1.022	3.4	0.420
38	Dimethylsulfoxide	C_2H_6OS	189	18.4	1.092	M	0.444
39	Acetonitrile	C_2H_3N	81.6	-46	0.786	M	0.460
40	3-Pentanol	$C_5H_{12}O$	115.3	-8	0.821	5.1	0.463
41	2-Pentanol	$C_5H_{12}O$	119.0	-50	0.810	4.5	0.488
42	2-Butanol	$C_4H_{10}O$	99.5	-114.7	0.808	18.1	0.506
43	Cyclohexanol	$C_6H_{12}O$	161.1	25.2	0.962	4.2	0.509
44	1-Octanol	$C_8H_{18}O$	194.4	-15	0.827	0.096	0.537
45	2-Propanol	C_3H_8O	82.4	-88.5	0.785	M	0.546
46	1-Heptanol	$C_7H_{16}O$	176.4	-35	0.819	0.17	0.549
47	1-Hexanol	$C_6H_{14}O$	158	-46.7	0.814	0.59	0.559
48	1-Pentanol	$C_5H_{12}O$	138.0	-78.2	0.814	2.2	0.568
49	Acetyl acetone	$C_5H_8O_2$	140.4	-23	0.975	16	0.571
50	Ethyl acetoacetate	$C_6H_{10}O_3$	180.4	-80	1.028	2.9	0.577
51	1-Butanol	$C_4H_{10}O$	117.6	-89.5	0.81	7.7	0.586
52	Benzyl alcohol	C_7H_8O	205.4	-15.3	1.042	3.5	0.608
53	1-Propanol	C_3H_8O	97	-126	0.803	M	0.617
54	Acetic acid	$C_2H_4O_2$	118	16.6	1.049	M	0.648
55	2-Aminoethanol	C_2H_7NO	170.9	10.5	1.018	M	0.651
56	Ethanol	C_2H_6O	78.5	-114.1	0.789	M	0.654
57	Diethylene glycol	$C_4H_{10}O_3$	245	-10	1.118	M	0.713
58	Methanol	CH_4O	64.6	-98	0.791	M	0.762
59	Ethylene glycol	$C_2H_6O_2$	197	-13	1.115	M	0.790
60	Glycerin	$C_3H_8O_3$	290	17.8	1.261	M	0.812
61	Water, heavy	D_2O	101.3	4	1.107	M	0.991
62	Water	H_2O	100.00	0.00	0.998	M	1.000

Table 1: Solvents Polarity Index[1-3] (M=Miscible)

Selection of Proper Solvent[4-6]

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Conversely, the impurities should either be insoluble in the solvent at all temperatures or must remain at least moderately soluble in the cold solvent. In other words, if the impurities are soluble, the temperature coefficient for them must be unfavorable; otherwise the desired product and the impurities would both crystallize simultaneously from solution.

The boiling point of the solvent should be low enough so that it can readily be removed from the crystals.

The boiling point of the solvent should generally be lower than the melting point of the solid is being purified. The solvent should not react chemically with the substance being purified. The chemical literature is a valuable source of information about solvents suitable for recrystallizing known compounds. If the compound has not been prepared before, it is necessary to resort to trial-and-error techniques to find an appropriate solvent for recrystallization. The process of selection can be aided by consideration of some generalizations about solubility characteristics for classes of solutes. Polar compounds are normally soluble in polar solvents and insoluble in non-polar solvents, for example, whereas non-polar compounds are more soluble in non-polar solvents. Such characteristics are summarized by the adage, "like dissolves like." Of course, although a highly polar compound is unlikely to be soluble in a hot, non-polar solvent, it may be very soluble in a cold, very polar solvent. In this case, a solvent of intermediate polarity may be the choice for a satisfactory recrystallization.

Occasionally a mixture of solvents is required for satisfactory recrystallization of a solute. The mixture is usually comprised of only two solvents; one of these dissolves the solute even when cold and the other one does not.

Solvent Pairs: Sometimes no single satisfactory solvent can be found, so mixed solvents, or solvent pairs are used. To use a solvent pair, one dissolves the crystals in the better solvent and adds the poorer solvent to the hot solution until it becomes cloudy, which means that the solution is saturated with the solute. The two solvents must be miscible with each other.

First requirement for the reaction is to choose a solvent, which is not reacting with any starting materials and catalyst or which is act as a catalyst itself or which is act as a reactant. Sometimes there is no need of solvents and reactants are act as a solvent. Like reaction of chlorination with thionyl chloride is used as a solvent as well as reactant for reaction. It also depends on the type of reaction like S_N1 or S_N2, in both types of reactions different solvents are being used. Like in S_N1 reaction ethanol, methanol is used and in reaction of S_N2 acetone, dimethyl formamide, etc are being used. Some of the reactions contain property of hydrolysis means they are hydrolyses by using water or moisture, like in reaction of chlorination, moisture free atmosphere is necessary. In order to obtain satisfactory results in many syntheses involving air moisture sensitive reactions,

it may be necessary to purify solvents to remove reactive impurities such as water.

Protic/acidic materials, or atmospheric contaminants such as oxygen. A commonly employed purification method is the solvent still, which involves the reflux/distillation of an organic solvent in the presence of a dehydrating/deoxygenating reagent.

Isolation of Oil[7-8]

Extract the distillate (collected in receiving flask) with dichloromethane (3 × 10 mL portions). Dry the organic layer over Na_2SO_4, filter and divide into two portions: one portion in a shortly vial, capped (used for GC and GC/MS analysis) and one portion put in a 20-mL vial. The 20-mL vial portion should be concentrated down (with a light stream of nitrogen gas) then dissolve in acetone. This portion will be used for the biological assay.

Oils may form from the hot solution and then solidify to an amorphous mass at lower temperatures; in the meantime, crystals of the solute may precipitate from the mother liquor. Because the oil is not a pure liquid, the solid mass produced from it will be impure, as noted earlier. In a case such as this, the usual remedy is to reheat the entire mixture to affect dissolution, add a few milliliters of additional pure solvent and allow the resulting solution to cool.

Sublimation[9-10]

Technique for purifying organic solid compounds with sublimation. Sublimation is a purification technique, in which a solid is directly converted to vapor phase without passing through liquid phase. However, the compound must have a relatively high vapor pressure and the impurities must have significantly lower vapor pressures. By heating, the solid will be vaporized and become solid again when the vapor contacts with the cold surface. Some solid compounds, such as iodine, camphor, naphthalene, acetanilide, benzoic acid, can be purified by sublimation at normal pressure. Several compounds will sublime when heating under reduced pressure.

For Example- Sublimation of impure acetanilide, Iodine, Camphor

Place 50 mg of impure acetanilide (mixed acetanilide with a minute amount of carbon black or other substance) in a suction flask.

Assemble the cold finger with water hoses connected to a miniature water pump and place the flask in a well with a window for observation in a heat dissipation block as shown below.

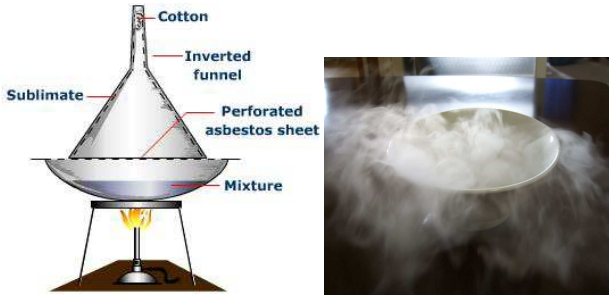


Figure 1: Sublimation

Ice can be added in a water container to obtain much cooler water for circulating in the cold finger. Turn on the heat and keep temperature stable at 135-140°C. Crystals will form on the cold finger. Continue heating until sublimation is complete and no more crystals form on the cold finger. Turn off the heat. Remove the apparatus from the heat and allow it to cool at room temperature. Remove the cold finger from the suction flask gently. Scrape the crystals onto a tare piece of weighing paper and reweigh. Record the mass of pure acetanilide. Determine its melting point.

Isolation Procedures[11]

- Pour reaction mass to water, stir it and extract out product using organic solvent like Ethyl acetate, Dichloromethane.
- It is not useful in case of pyridine, thionyl chloride, DMF, methanol.

- In case of Pyridine, Pyridine cause impotence so distilled it in wood or removed by dilute HCl and 10% citric acid.
- DMF removed from Reaction mixture by cold water wash.
- In case of $SnCl_2$+EtOH,
- Distilled the EtOH first
- Add 10% NaOH then add organic solvent
- Filter it through high flow bed of celite
- Collect filtrate and separate the organic layer by separating funnel and distilled it.
- In case of **Pd or Zn**, first filter RM through vacuum filter
- Pour funnel in dilute HCl or Water before inorganic metal become dry.
- Pd is flammable in dry condition.
- In case of excess of thionyl **chloride**, use formic acid to remove it.
- There are so many techniques also used in work up like filtration, extraction, distillation etc.

Filtration[12]

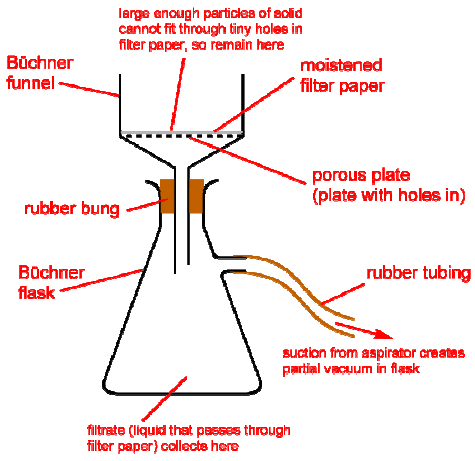
- It contains two types like gravity filtration and suction filtration.

Gravity filtration

Figure 2: Gravity Filtration

- In chemistry, even filtration is more sophisticated than it might seem. Take gravity filtration; all you do is stick a piece of filter paper in a funnel and let it go.

Suction filtration



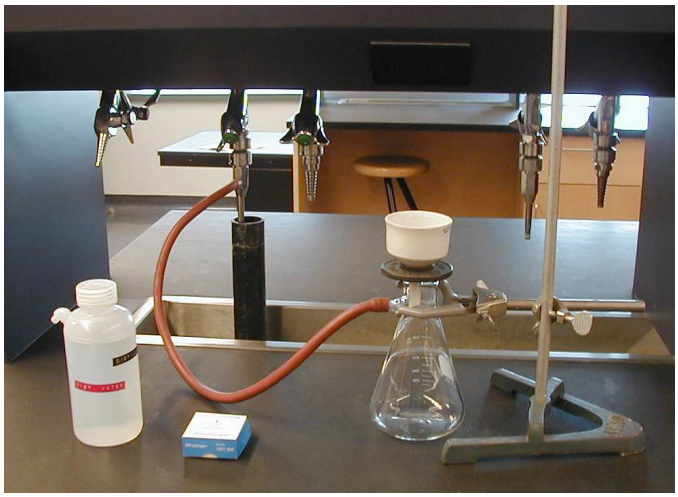


Figure 3: Suction filtration

Extraction[13-16]

- Solvent extraction is often used for acidic or basic compounds. Here the impure compound is dissolved in an organic solvent. The mixture can be filtered if necessary.
- For acidic compounds, aqueous sodium hydroxide is added. Two layers form which are then well-mixed. The acid compound reacts with the base to form a salt which is then dissolved in the aqueous solution. The organic layer – which contains the impurities - is

discarded. You then acidify and extract into a new layer of organic solvent.

- For basic compounds you use an acid first then base. The procedure can be repeated several times although you lose quite a lot of product.

The Separating Funnel used to separate the aqueous and organic layers is shown below.

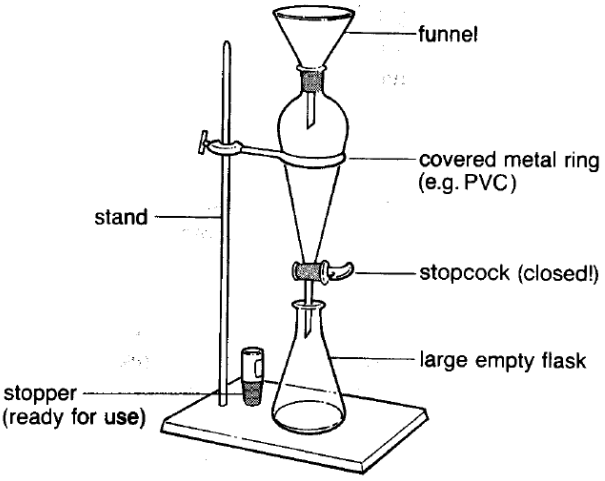
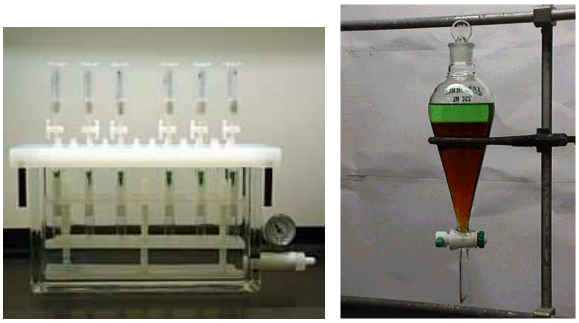


Figure 4: Extraction

Solid-phase extraction method

Solid-phase extraction (SPE) is an extraction method that uses a solid phase and a liquid phase to isolate the impurity of interest from a solution. It is usually used to clean up a sample before using a chromatographic or other analytical method to quantitate the amount of analyte(s) in the sample. SPE uses the affinity of solutes dissolved or suspended in a liquid which act as a mobile phase for a solid through which the sample is passed which act as the stationary phase to separate a mixture into desired and undesired components. The result is that either the desired analytes of interest or undesired impurities in the sample are retained on the stationary phase. When the sample passes through the stationary phase, the analytes in the sample will interact and retain on the sorbent but the solvent, salts and other impurities pass through the cartridge. After the sample is loaded, the cartridge is washed with buffer or solvent to remove further impurities. Then, the analyte is eluted with a non-polar solvent or a buffer of the appropriate pH.



(a) **(b)**

Figure 5: (a) Solid Phase Extraction (b) Liquid-Liquid Extraction

Liquid-liquid extraction method

In this type of extraction, two immiscible liquids are selected. Usually, one phase is aqueous (hydrophilic) and the other is a (hydrophobic) organic solvent. In that the solute is distributed between two immiscible solvents. The extraction is based on Distribution Co-efficient or Partition Co-efficient (K_d), which is the ratio of concentration of solute in two different solvents.

Figure 6: Accelerated solvent extraction

Accelerated solvent extraction method

- Accelerated solvent extraction (ASE) is a fully automated technique that uses common solvents to rapidly extract solid and semisolid samples. ASE operates at temperatures above the normal boiling point of most solvents, using pressure to keep the solvents in liquid form during the extraction process.

Typically, ASE methods are completed in 15–25 min, while consuming only 15–50 mL of solvent.

Purification techniques

- Recrystallization (if impurities are less than 5 %)
- Trituration (used for less than 500mgs)
- Chromatography

Recrystallization[17-18]

- In the laboratory, most compounds are not soluble in water. This means that a solvent has to be found in which the compound or the impurity is soluble under either hot or cold conditions.
- In other words, if compound A is contaminated with an impurity, the solvent must be one in which the impurity is either completely insoluble or more freely soluble than compound A. In either case, a saturated solution of compound A is prepared. Compound A then will recrystallize (or precipitate) from the solution. The impurity will stay in solution when the impurity is more soluble than compound A or will not dissolve when less soluble than A.
- The best solvents for recrystallization are those in which compound A is poorly soluble at room temperature but soluble at higher temperatures. In this case, the solid to be purified is placed in the solvent, the solvent is heated and the compound will dissolve. If necessary, the solution can be filtered while hot to remove an insoluble impurity. Then the solution is cooled. The compound will form crystals and precipitate from the solution if a saturated solution has been prepared. The crystals will be rather pure. It is essential that the solvent and the compound do not react with each other. Frequently, a compound has to be purified and a solvent has to be chosen for the recrystallization for purification).

The rule of thumb for choosing a solvent is like dissolves like. This means that compounds with similar functional groups are usually mutually soluble in each other. Sucrose that is a polyhydroxy compound is soluble in water. Alcohols and carboxylic acids, which also contain the -OH group, are soluble in water, which contains the -OH group. Benzene is not water-soluble but will dissolve other hydrocarbons like hexane. Grease is soluble in gasoline because both are hydrocarbons. It is desirable to have a compound dissolve upon heating and then precipitate when cool. If precipitation does not occur after a compound has been dissolved, there are two techniques that may be used to attempt precipitation:

Figure 7: Hot Filtration (Recrystallization)

- Cool the solution in an ice bath and scratch the inside of the container with a glass rod.
- Cool the solution in an ice bath and add a small crystal of the same compound dissolved in the solution. This method is called "seeding".
- Some common recrystallization solvents are water, ethyl alcohol, benzene, acetone, acetic acid, chloroform, carbon disulfide and ethyl acetate.
- In the case in which the impure compound is soluble in the recrystallization solvent, but the impurities are not, the hot solution must be filtered. The filtration has to be done with the hot solution and has to be rapid. If the hot solution filters too slowly, precipitation will occur in the stem of the funnel and plug it so badly that the solution will not flow from the funnel. This type of precipitation can be avoided by placing the funnel under hot water jacket during its use. The inconvenience of this

method is the use of another piece of hot equipment. A short-stemmed funnel is definitely better than a long-stemmed one for filtration.

- When a recrystallization has been completed, the crystals are collected on a Buchner (or Hurch) funnel.

Solvent Selection for recrystallization

- Although different criteria are used for defining solubility, plan to use the following definitions in this experiment: (a) soluble-20 mg of solute will dissolve in 0.5 mL of solvent; (b) slightly soluble-some but not all of the 20 mg of solute will dissolve in 0.5 mL of solvent; (c) insoluble-none of the solute appears to dissolve in 0.5 mL of solvent.
- For known compounds, place about 20 mg (a spatula-tip full) of the finely crushed solid in a test tube and add about 0.5 mL of water using a calibrated Pasteur pipette. Stir the mixture with a glass rod or spatula to determine whether the solid is soluble in water at room temperature. If the solid is not completely soluble at room temperature, warm the test tube in the hot-water or steam bath and stir or swirl its contents to determine whether the solid is soluble in hot water.
- Repeat the solubility test for the solutes using 95% ethanol and then petroleum ether (b.p. 60-80℃, 760 Torr). After completing these additional tests, record which of the three solvents you consider best suited for recrystallization of each of the solutes.
- For unknown compounds, a systematic approach is important for determining their solubility, and the following protocol accomplishes this. The following solvents may be tried: water, ethanol, diethyl ether and hexanes.
- After selecting the solvents, obtain enough clean, dry test tubes so that there is one for each solvent to be tested. Place about 20 mg (a spatula-tip full) of the finely crushed unknown in each test tube and add about 0.5 mL of a solvent to a tube containing the solid. Stir each mixture and determine the solubility of the unknown in each solvent at room temperature. Use the definitions of soluble, slightly soluble, or insoluble given earlier.
- If the unknown sample is insoluble in a particular solvent, warm the test tube in the hot-water. Stir or swirl the contents of the tube and note whether the unknown is soluble in hot solvent. If the solid is soluble in the hot solvent but only slightly soluble or insoluble at room temperature, allow the hot solution to cool to room temperature slowly. If crystals form in the cool solution, compare their quantity, size, color, and form with the original solid material and with those obtained from other solvents.
- It is a good idea to test the solubility of a solute in a variety of solvents. Even though nice crystals may form in the first solvent you try, another one might prove better if it provides either better recovery or higher-quality crystals. To assist in determining the best solvent to use in recrystallizing an unknown, you should construct a table containing the solubility data you gather by the systematic approach described above.
- If these solubility tests produce no clear choice for the solvent, mixed solvents might be considered. Before trying any combinations of solvent pairs take about 0.2 mL of each pure solvent being considered and mix them to ensure that they are miscible in one another. If they are not, that particular combination cannot be used.

Figure 8: Trituration

Trituration[19]

- Some compounds contain stickiness and non-polar impurities.
- To remove stickiness from the compound trituration technique is being used.
- Generally non-polar solvents like n-hexane, n-pentane, diethyl ether are used as a trituration solvent to remove non-polar impurities.
- Oils may persist on cooling with no evidence of crystallization. These may often be induced to crystallize by scratching the oil against the side of the flask with a glass rod at the interface of the oil and the solution. If this fails, several small seed crystals of the original solid may be added to the oil, and the mixture allowed to stand for a period of time. Failure of these alternatives may necessitate separation of the oil from the solution and crystallization of it from another solvent.

Chromatography

- Chromatography is an effective and very useful method for separation and purification of organic compounds. Chromatography separates components of a mixture based upon the principle that how well they are adsorbed on the stationary phase, versus how well they dissolve in the mobile phase. The components with greater affinity for the mobile phase will move faster than those components with greater affinity for the stationary phase, causing the components to separate. There are many chromatographic methods characterized by the nature of the stationary and mobile phases. Among these methods, column chromatography, thin-layer chromatography and paper chromatography are more common ones.

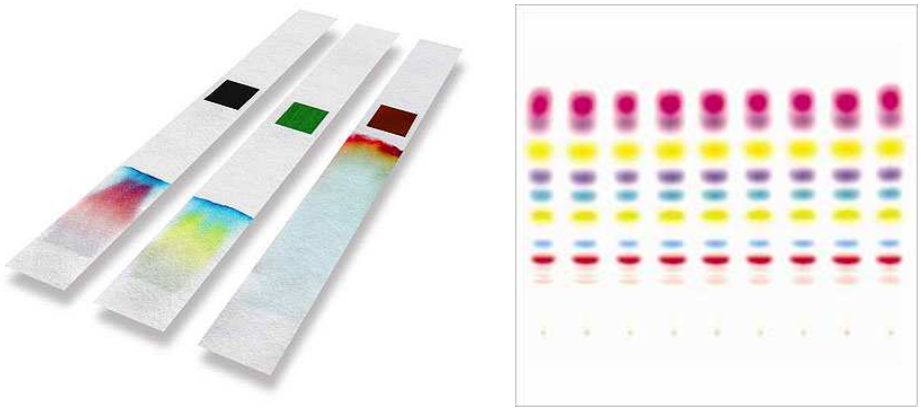


Figure 9: Chromatography

Thin-layer chromatography[20-23]

- Generally first of all confirm the purity of compound by taking TLC.
- Prepare 1 TLC plate (4 × 7 cm dimension).
- Handle it only on the edges, as fingerprints contain UV-active materials. Using a pencil draw a very light line across the sheet (short dimension) about 1 cm from one end. Then make 4 small light marks at even intervals along the line for spotting the samples. Draw another light line about 1 cm from another end of the plate for the solvent front.
- Obtain a TLC chamber and place solvent, a 5% ethyl acetate in dichloromethane to 0.5 cm height. Place a piece of filter paper around the inside surface of the container and extend into the solvent.
- A glass jar with a lid or a beaker with a watch glass or a cover of a Petri dish can be used as a TLC chamber, but it should be large enough so that the TLC plate can lean against one side.
- Using clean capillary tubes carefully spot four samples at four pencil marks.

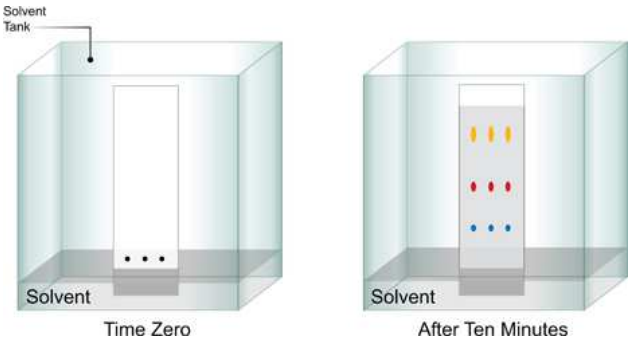


Figure 10: Thin-layer chromatography

- The spots should be as small as possible in order to minimize tailing and overlapping when the TLC plate is developed. If a more intense spot is desired, let the spot dry and re-spot in the same location.
- When the spots are dry, place the TLC plate in the developing chamber. Then gently close the chamber.
- Be sure that the bottom edge of the TLC plate is in the solvent but the spots are above the solvent and the filter paper does not touch the chromatographic sheet. Place a TLC plates at a time in a TLC chamber.
- When the solvent has moved to the front line, remove the plate. Lay it on a clean surface in a fume hood or well ventilated area and allow the solvent to evaporate until the plate appears dry.
- Visualize the plate under UV light and immediately draw a light pencil line around each spot.
- UV radiation is harmful to eyes. Do not look directly at the UV lamp.
- Alternatively, the spots can be visualized in an I_2 chamber (small bottle containing a few I_2 crystals).
- Measure all the distances traveled by the compounds and solvent. Calculate the retention factor (R_f) for each compound.
- Mobile phase: minimum polarity and desired resolution (R_f between 0.3 to 0.7)
- Identification of spots:
- Ninhydrin for primary amines
- Dragendorff for amides
- Molybdic acid for alcohols
- $KMnO_4$ for oxidizing substances
- 2,4-DNPH for Ketone and Aldehydes

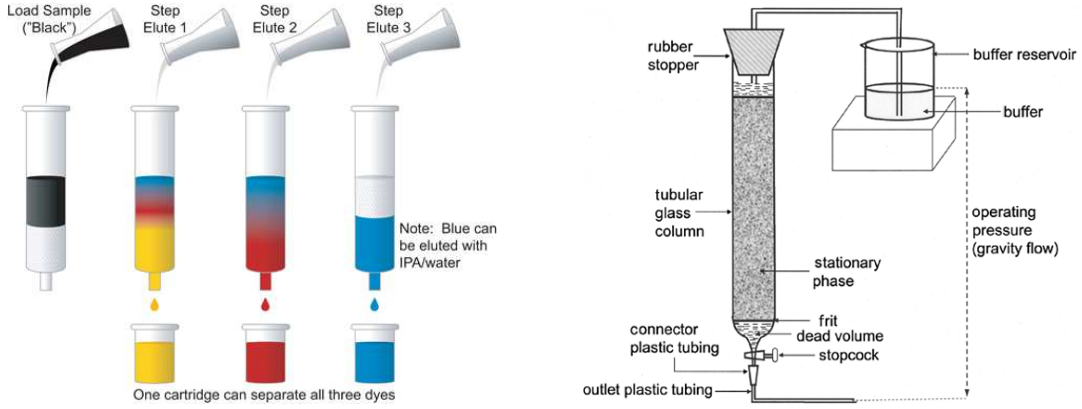


Figure 11: Column Chromatography

Column Chromatography[20-23]

- Clamp a Pasteur pipette in a vertical position to a lab stand. Push a small piece of cotton wool with a copper wire to loosely pack at the neck of a Pasteur pipette. Add a small amount of fine sand to make a small layer before adding the adsorbent.
- Weigh alumina 1 g in a 50-mL beaker or a small vial, add 4 mL of ethanol. Swirl or stir gently with a glass rod to obtain the slurry of alumina.
- Transfer the slurry of alumina drop wise using another Pasteur pipette into the prepared column containing 4 mL of ethanol (at the beginning, push gently at the tip of the pipette column with a finger

until the alumina column 1 cm high is obtained). Tap the side of the column gently to produce even packing of the adsorbent in the column.

- Allow the solvent to drain to the level of alumina. Add 1 drop of the mixture of impure compound to the top of alumina. Allow the mixture to adsorb into the top of the alumina. Add a few drops of ethanol and allow ethanol to drain to the top of adsorbent.
- Fill up the column with ethanol as a polar solvent.
- When the first band comes down to the neck of the pipette column, collect it in a container and stop adding ethanol.
- Allow the solvent to drain to the level of alumina. Switch to the second eluting solvent, water and fill up the column with water. Collect the second band into another container.

Figure 12: Flash chromatography

Flash chromatography[20-23]

Flash Chromatography is a rapid form of preparative column chromatography based on an optimized pre-packed column through which is pumped solvent at a high flow rate. It is a simple and economical approach to Preparative LC. It is "an air pressure driven hybrid of medium and short column chromatography optimized for rapid separation." This approach was pioneered by W.C. Still at Columbia University. Flash chromatography utilizes a plastic column filled with some form of solid support, usually silica gel, with the sample to be separated placed on top of this support. The rest of the column is filled with an isocratic or gradient solvent which, with the help of pressure, enables the sample to run through the column and become separated. Flash chromatography used air pressure initially, but today pumps are used to speed up the separation. This technique is considered a low to medium pressure technique and may be scaled up for separations from a few mg to many tens or hundreds of gams. grams.

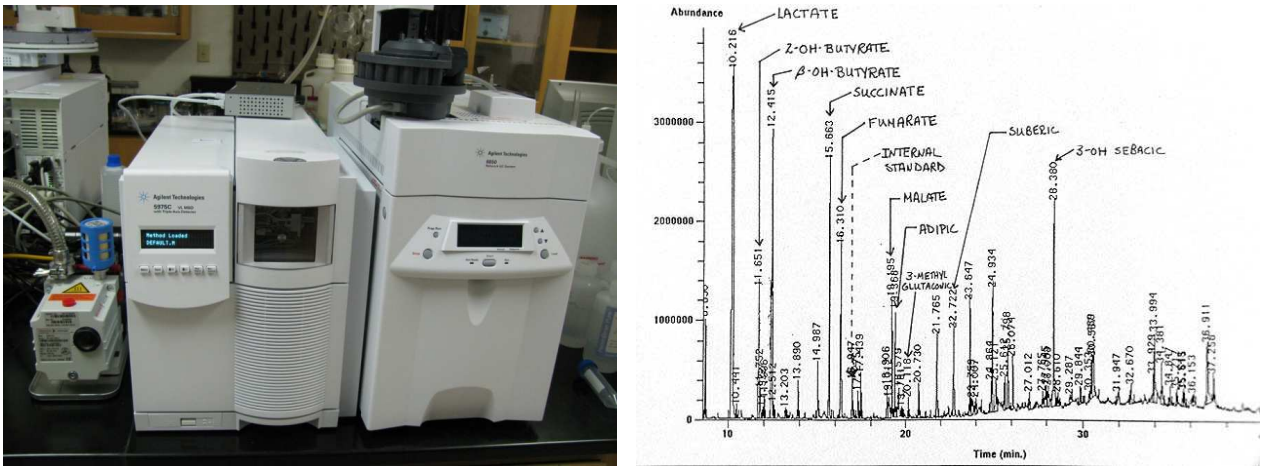


Figure 13: Gas chromatography (GC)

Gas chromatography (GC)[20-23]

Gas chromatography is an analytical technique for separating compounds based primarily on their volatilities. Gas chromatography provides both qualitative and quantitative information for individual compounds present in a sample. Compounds move through a GC column as gases with their linear velocity and flow rates, because the compounds are normally gases or they can be heated and vaporized into a gaseous state. The compounds partition between a stationary phase, which can be either solid or liquid and a mobile phase (gas). The differential partitioning into the stationary phase allows the compounds to be separated in time and space.

High performance Liquid chromatography (HPLC)[20-23]

Normal-phase HPLC separates analytes based on adsorption to a stationary surface chemistry and by polarity. NP-HPLC uses a polar stationary phase and a non-polar, non-aqueous mobile phase, which effectively separates the analytes that are readily soluble in non-polar solvents. The analyte associates with and is retained by the polar stationary phase. Adsorption strengths increase with increased analyte polarity and the interaction between the polar analyte and the polar stationary phase (relative to the mobile phase) increases the elution time. The interaction strength depends not only on the functional groups in the analyte molecule, but also on steric factors. Reversed phase HPLC (RP-HPLC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is silica which has been treated with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}. With these stationary phases, retention time is longer for molecules which are less polar, while polar molecules elute more readily. The retention time can be increased by adding more water to the mobile phase; thereby making the affinity of the hydrophobic analyte for the hydrophobic stationary phase stronger. Similarly, the decreasing of retention time by adding more organic solvent to the eluent can be done.

Figure 14: HPLC

Figure 15: HPTLC

High performance thin layer chromatography (HPTLC)[20-23]

Similar to other chromatographic methods HPTLC is also based on the principle of separation. The separation depends on the relative affinity of compounds towards stationary and mobile phase. The compounds under the influence of mobile phase (driven by capillary action) travel over the surface of

stationary phase. During this movement the compounds with higher affinity to stationary phase travel slowly while the others travel faster. Thus separation of components in the mixture is achieved. Once separation occurs individual components are visualized as spots at respective level of travel on the plate. Their nature or characters are identified by means of suitable detection techniques.

Capillary Electrophoresis (CE)[20-23]

Capillary Electrophoresis (CE) is a separation technique based on the differential transportation velocities of charged species in an electric field through a conductive medium. Primary candidate for CE separation is ions (+/-). The basic instrumental set-up consists of a high voltage power supply (0 to 30 kV), a fused silica (SiO_2) capillary, two buffer reservoirs ,two electrodes and an column detector.

Figure 16: CE

Figure 17: SCF

Supercritical fluid chromatography(SCF)[20-23]

A pure supercritical fluid (SCF) is any compound at a temperature and pressure above the critical values (above critical point). Above the critical temperature of a compound the pure, gaseous component cannot be liquefied regardless of the pressure applied. The critical pressure is the vapor pressure of the gas at the critical temperature. In the supercritical environment only one phase exists. The fluid, as it is termed, is neither a gas nor a liquid and is best described as intermediate to the two extremes. This phase retains solvent power approximating liquids as well as the transport properties common to gases.

Ultra Performance Liquid Chromatography (UPLC)[24-25]

High performance liquid chromatography (HPLC) is a proven technique that has been used in laboratories worldwide over the past 30-plus years. One of the primary drivers for the growth of this technique has been the evolution of packing materials used to effect the separation. The underlying principles of this evolution are governed by the van Deemter equation, which is an empirical formula that describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency). Since particle size is one of the variables, a van Deemter curve can be used to investigate chromatographic performance. According to the van Deemter equation, as the particle size decreases to less than 2.5 µm, not only is there a significant gain in efficiency, but the efficiency does not diminish at increased flow rates or linear velocities. By using smaller particles, speed and peak capacity (number of peaks resolved per unit time in gradient separations) can be extended to new limits, termed Ultra Performance Liquid Chromatography, or UPLC. The technology takes full advantage of chromatographic principles to run separations using columns packed with smaller particles and/or higher flow rates for increased speed, with superior resolution and sensitivity. They shows a stability

indicating assay of five related substances accomplished in under one minute, proving that the resolving power of UPLC is not compromised even at high speed. The current USP lists multiple HPLC methods for the analysis of these same compounds with run times approaching 20 min, with broad, tailed peaks.

Figure 18: Ultra Performance Liquid Chromatography

Figure 19: Fast Performance Liquid Chromatography

Fast Performance Liquid Chromatography[26-27]

Liquid chromatography is a term which refers to all chromatographic methods in which the mobile phase is liquid. The stationary phase may be a liquid or a solid. Fast performance liquid chromatography (FPLC) is a type of liquid chromatography where the solvent velocity is controlled by pumps. The pumps control the constant flow rate of the solvents. The solvents are accessed through tubing from an outside reservoir. The flow rate of the solvent is set through computer input and controlled by pumps. There are various columns used in liquid chromatography depending on the type of separation preferred. Each column contains a small diameter packing material. The column is a large (mm id) tube containing small (u) particles (gel beads) known as stationary phase. The chromatographic bed is composed by the gel beads alone when they are inside the column. The sample is introduced into the injector and then carried into the column by the flowing solvent. Once in the column, the sample mixture separates as a result of different components adhering to or diffusing into the gel. As the solvents is forced into the chromatographic bed by the flow rate, the sample separates into various zones of sample components. These zones are referred to as bands.

Conclusion:

Nowadays it is necessary to have compounds with maximum purity to get accurate result for analytical datas (UV, IR, NMR, Mass spectras) & biological activity both *in-vivo* and *in-vitro*. This review provides depth of knowledge on importance of Purification of New Drug Substance and New Drug Product with various techniques of isolation and purification of intermediate and final compounds either obtained from natural, synthetic, semi-synthetic or mineral sources.

References:

1) Christian Reichardt, *Solvents and Solvent Effects in Organic Chemistry*, (3rd ed.). Wiley-VCH Publishers, 2003. ISBN: 978-3-527-32473-6

2) Freed BK, Biesecker J, Middleton WJ, Spectral polarity index: a new method for determining the relative polarity of solvents. *Journal of Fluorine Chemistry* 48(1), 63–75, 1990.

3) Rafiqul G and David JC. Method for selection of solvents for promotion of organic reactions. *Computers & Chemical Engineering* 29(7), 1661–1676, 2005.

4) Lowery, T.H. and Richardson, K.S. *Mechanism and Theory in Organic Chemistry*. (3rd ed.) Harper Collins Publishers. 181-183, 1987. ISBN 0063640449

5) Lide DR. *CRC Handbook of Chemistry and Physics* (86th ed.). Boca Raton (FL): CRC Press. 486, 2005. ISBN 0-8493-0486-5.

6) Petr K, Jun WS, Akio T, Takeshi I. Solvent selection for pharmaceuticals, *Fluid Phase Equilibria* 194–197, 771–782, 2002.

7) Nabil B, Maryline AV, Mohamed AF, Emmanuel P, Brahim YM. Comparison of two isolation methods for essential oil from rosemary leaves: Hydrodistillation and microwave hydro diffusion and gravity. *Food Chemistry* 114(1), 355–362, 2009.

8) Harwood LM. and Moody CJ. *Experimental Organic Chemistry: Principles and practice*. Oxford: Blackwell Science, 778, 1989. ISBN 0632020172

9) Carberry JJ. Designing Laboratory Catalytic Reactants. *Industrial Engineering Chemistry*. 56(39), 39–46, 1964.

10) Souza Mendes PR. The naphthalene sublimation technique. *Experimental Thermal and Fluid Science* 4(5), 510–523, 1991.

11) Nnane IP, Hutt AJ, Damani LA. Essential Guides for Isolation/Purification of Drug Metabolites. *Encyclopedia of Separation Science* 1, 4539–4547, 2003.

12) Wilfred LF. *Chapter 1 – Common Physical Techniques Used in Purification*. Purification of Laboratory Chemicals. (6th ed.) 1–60, 2009. ISBN 978-1-85617-567-8

13) Berset JD, Ejem M, Ruth H, Peter L. Comparison of different drying, extraction and detection techniques for the determination of priority polycyclic aromatic hydrocarbons in background contaminated soil samples. *Analytica Chimica Acta* 383(3), 263–275, 1999.

14) Richardson JA. and Rase HF. Continuous stirred microreactors as laboratory tool. *Industrial Engineering Chemical products research and development* 17(4), 287-296, 1978.

15) Weekman VW. Laboratory reactors and their limitations, *American institute of chemical engineering Journal* 20(5), 833-839, 1974.

16) Yip MT. and Dalton DR. *Organic chemistry in the laboratory*. New York: D Van Nostrand Co. 362, 1979.

17) Zwir FA and Biziuk M. Solid Phase Extraction Techniques-Trends, opportunities and applications. *Polish Journal of Environmental studies* 15(5), 677-690, 2006.

18) Anderson NG. *Chapter 11 – Workup*. (2nd ed.). Practical Process Research and Development, 289–327, 2012.

19) Riddick JA and Bunger WB, *Organic Solvents*, Wiley-Interscience, NewYork, 145-147, 638-69, 1970.

20) Beckett AH and Stenlake JB. *Practical Pharmaceutical Chemistry*. (4th ed.) CBS Publishers and Distributors, New Delhi, 1997.

21) Willard HH, Meritt LL, Dean JA and Settle FA. *Instrumental methods of analysis*, (7th ed.) CBS Publishers and Distributors, New Delhi, 1986.

22) Sharma BK. *Instrumental methods of chemical analysis*, (18th ed.), Krishna Prakashan Media Pvt Ltd, Meerut, 1999.

23) Chatwal G and Anand S. *Instrumental methods of chemical analysis*. (5th ed.) Himalaya publishing house, New Delhi, 2002.

24) Mutton IM. Ultra Performance Liquid Chromatography (UPLC): An Introduction. *Chromatographia J* 47, 291, 1998.

25) Ayrton J, Dear GJ, Leavens WJ, Mallett DN and Plumb RS, Use of generic fast gradient liquid chromatography–tandem mass spectroscopy in quantitative bioanalysis. *Journal of Chromatography B: Biomedical Sciences and Applications* 709(2), 243–254, 1998.

26) Tangvarasittichai S, Tangvarasittichai O, Jermnim N. Comparison of fast protein liquid chromatography (FPLC) with HPLC, electrophoresis & microcolumn chromatography techniques for the diagnosis of β-thalassaemia, *Indian J Med Res* 129, 242-248, 2009.

27) Goke B. and Keim V. Recent progress in the use of automated chromatography systems for resolution of pancreatic secretory proteins. *International Journal of Gastrointestinal Cancer* 11(2), 109–116, 1992.