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Techniques Course: Practical Chromatography

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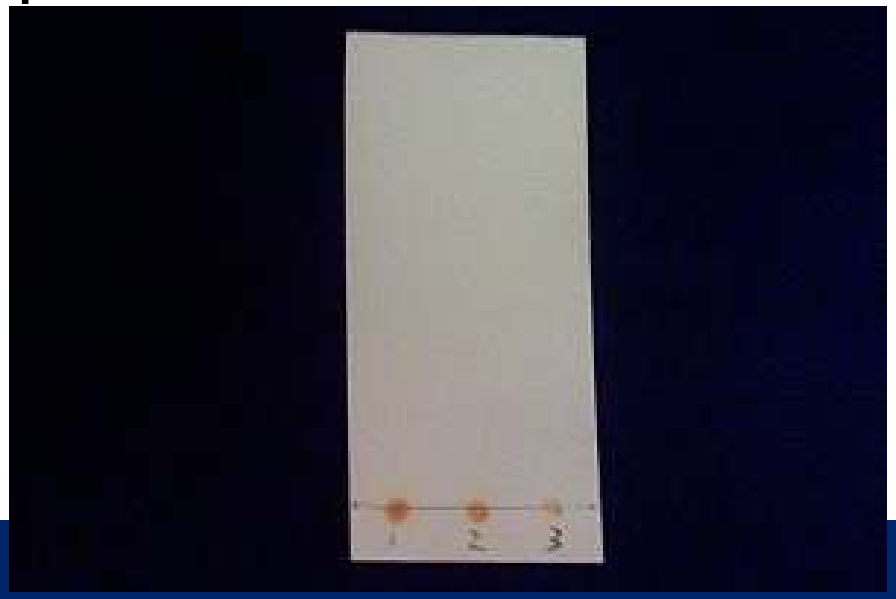
Thin Layer Chromatography (TLC)

- Thin layer chromatography is a quick and simple procedure that allows you to determine the number of components in a mixture, and is especially useful for monitoring the progression of reaction.
- This type of chromatography is usually run on a sheet of glass, metal or plastic coated in a thin layer of silica.



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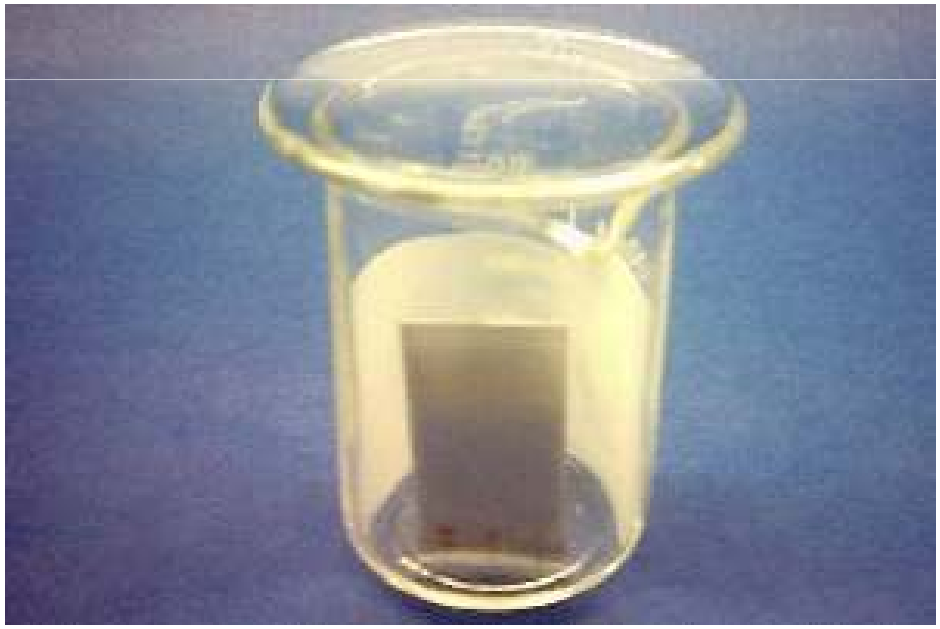
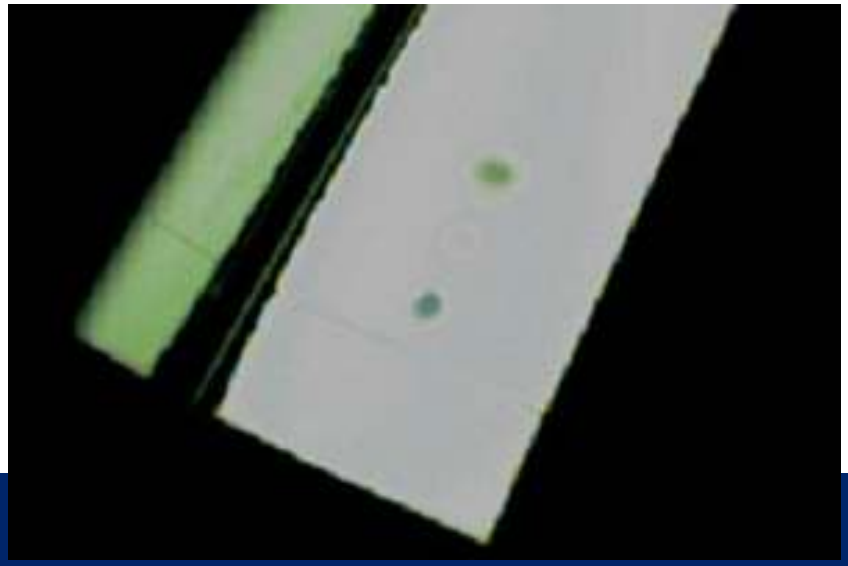
Thin Layer Chromatography (TLC)

- Small amount of mixture to be analysed is placed on the silica plate near the bottom and the plate placed in a shallow pool of solvent, which is drawn up the plate by capillary action.



Thin Layer Chromatography (TLC)

- As the solvent moves past the spot an equilibrium is established for each component of the mixture between the molecules of that component which are adsorbed on the solid and the molecules which are in solution. These will differ in solubility and in the strength of their adsorption to the silica and some components will be carried farther up the plate than others.



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Running a TLC

- Usually a beaker with watch glass will suffice. Put in ~0.5cm depth of solvent. Lining with beaker with some filter paper will aid saturation of the chamber with solvent vapours.
- Draw a line ~0.5cm from bottom of the plate. Spot your samples on this line. This is the origin.

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Running a TLC

- For a TLC of a reaction place 3 spots. i) starting material (SM), ii) Co-spot of SM and reaction mixture (RM), iii) RM.
- If not already in solution you will need to dissolve your sample in a volatile solvent (~1mg in 1ml) and apply this to the plate with a capillary tube.
- Place plate in the TLC tank and let it run until the solvent front is just below the top of the plate. Remove the plate and draw a line at the solvent front.
- If you can see any spots circle them with a pencil.

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Running a TLC

- Look at your TLC plate under UV light and circle any spots which appear. Develop your plate using a visualisation dip.
- The retention factor (Rf) of a spot is the distance travelled by the spot from the origin divided by the distance travelled by the solvent

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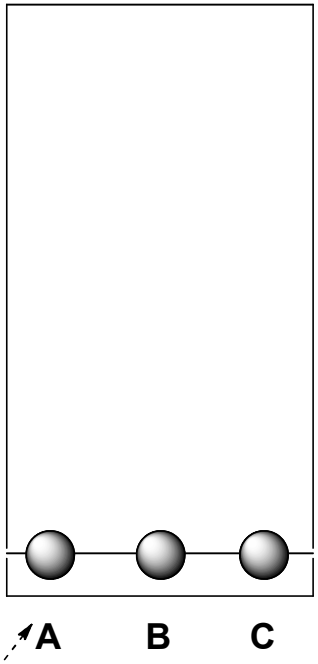
Running a TLC

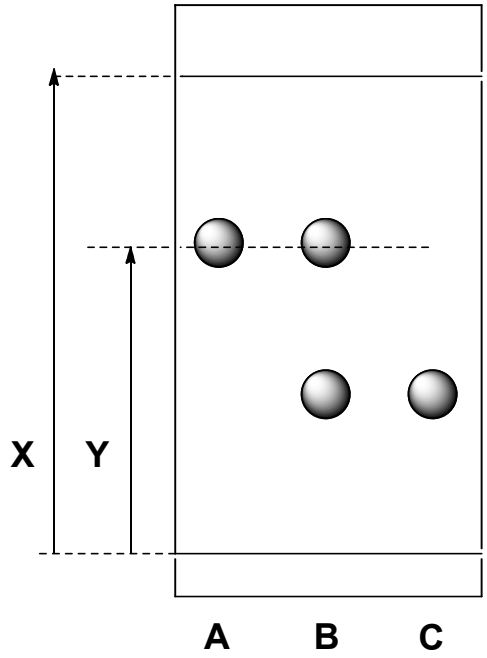
A = SM
B = mixed spot (SM & RM)
C = RM

Before elution



Mark the position of the sample spots. Make sure you label them.

After elution



Mark this position where the solvent reaches. This is the solvent front.

X = distance moved by the solvent front
Y = distance moved by the sample

$R_F = Y/X$

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TLC Troubleshooting

- Runs as a streak: TLC was overloaded, re-run it but dilute the sample. Or, there are just too many components in the sample. Or sample may be decomposing on silica (see 2D TLC).
- Runs as a smear or upward crescent: Compound possess strongly acidic or basic groups. Add a few drops of AcOH (acidic compound) or NH_4OH (basic compound) to the mobile phase.
- Runs as a downward crescent: Likely the silica was disturbed while spotting the sample.

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TLC Troubleshooting

- Solvent front runs crookedly: Either the silica has flaked off the side of the plate or the plate was touching the side of the container. Rf values measured will be incorrect.
- Random spots on the plate: Plate contaminated. Compounds have been splashed on the plate accidently.
- Blur of blue spots on the plate: You used an ink pen to mark the origin.

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TLC Troubleshooting

- There are no spots: Did you spot the plate? If you did then your sample may be too dilute.
- Is the solvent level in the container above the origin line of your plate?
- If none of these then try different methods for visualising the plate.

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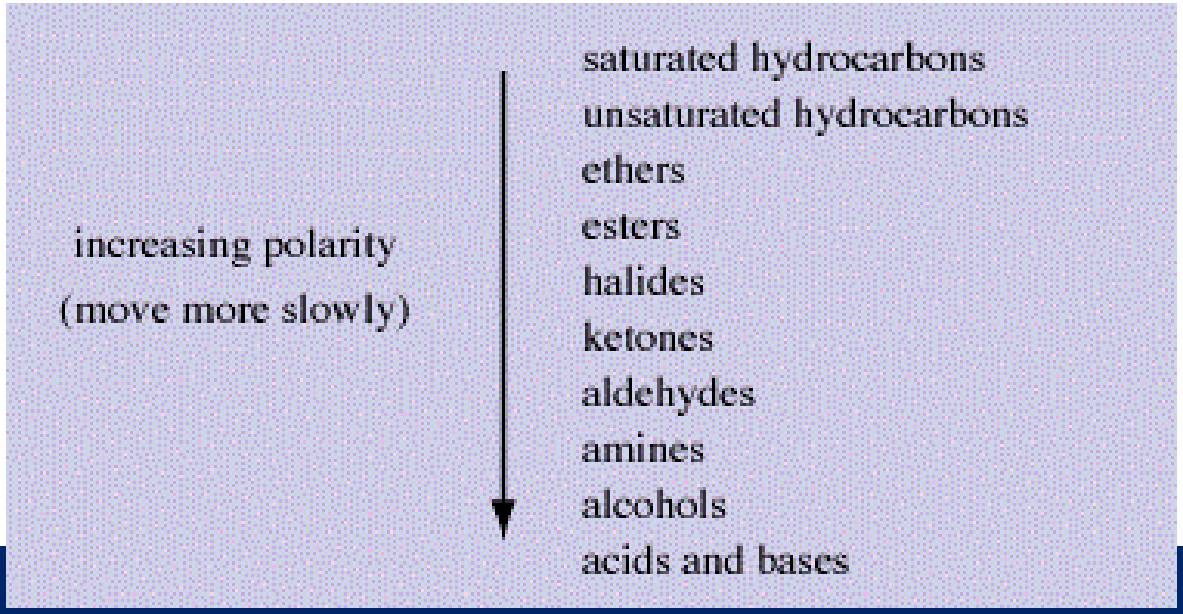
TLC Solvent Choice

- More polar solvents will cause the compound to move faster (higher) up the plate.
- More polar compounds will move more slowly (lower) up the plate.
- Ideally an Rf value of 0.3-0.4 is optimal.

The expected elution order of organic classes.



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TLC Solvent Choice

- Good general solvent choices are:
- EtOAc/Hexane mixtures and CH_2Cl_2/MeOH mixtures.

Eluting power of organic solvents.

increasing polarity
(more powerful eluters)

alkanes (hexanes, petroleum ether)
toluene
halogenated hydrocarbons (methylene chloride)
diethyl ether
ethyl acetate
acetone
alcohols
acetic acid

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Visualising a TLC

- UV light: Aromatic and highly unsaturated compounds.
- p-Anisaldehyde: General purpose, good with groups with nucleophilic properties.
- Ninhydrin: For amino acids and other amines.
- $KMnO_4$: Olefins and other readily oxidized groups.
- Cerium Sulfate: General stain, good for alkaloids.
- Cerium Ammonium Molybdate: General purpose good for substrates which can be oxidised. Requires heating.

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Visualising a TLC

- 2,4-DNP: Aldehydes and ketones.
- Bromocresol Green: Acidic (pKa <5) groups.
- Phosphomolybdic Acid: General purpose.

- The additional handout provides recipes for the preparation of these dips.

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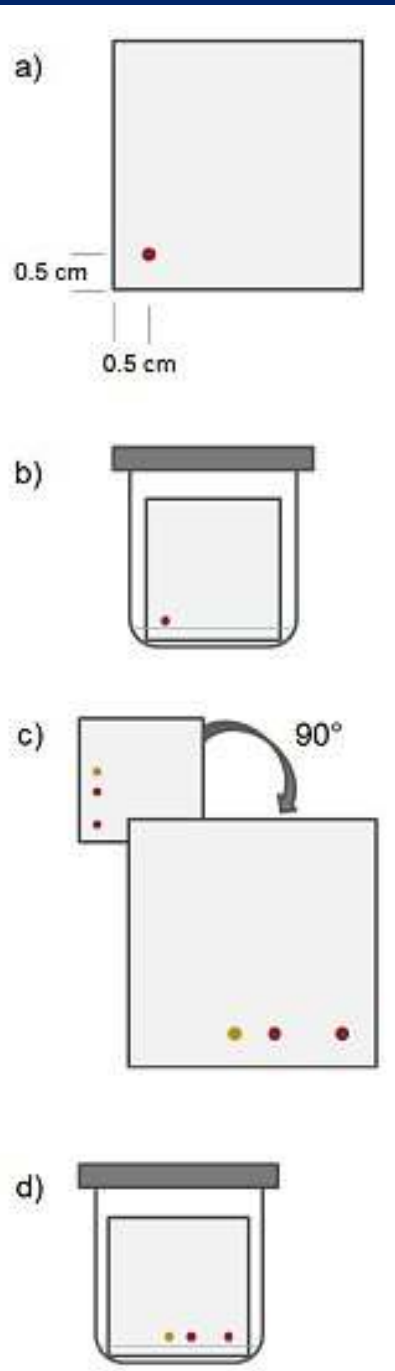
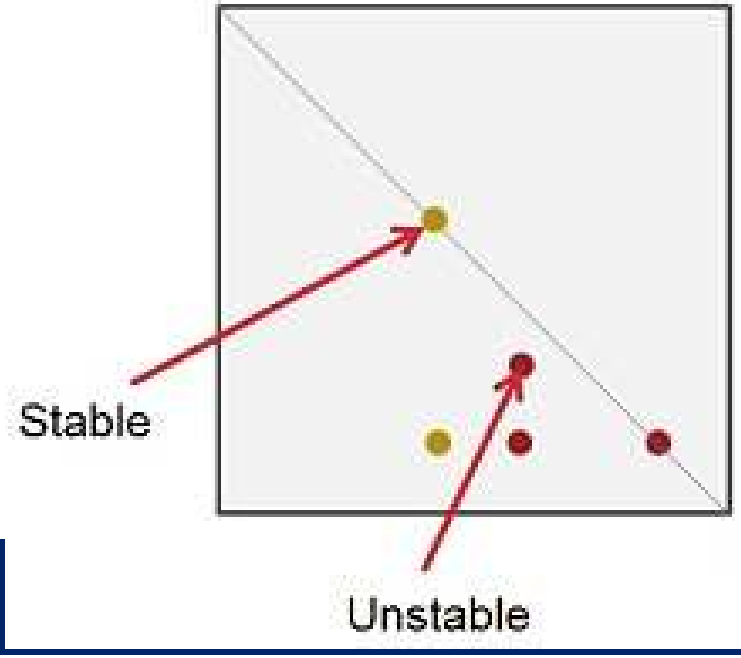
2D-TLC

- Sometimes a TLC will have multiple spots that you are convinced are not in the sample. It is possible that your sample is decomposing on the silica.
- You will need to use a larger plate ~7cm x 7cm.
- Spot the sample in the left hand corner ~0.5cm from each side. Run the TLC as normal. When complete rotate the TLC by 90 O so the original sample spot is in the right hand corner and re-run it.



2D-TLC

- When this elution is complete, remove the plate and develop it. Draw a diagonal line from the corner where the original spot was spotted through the sample spot to the opposite corner. Mark the position of all spots.
- Compounds that are stable to silica will appear on the diagonal.



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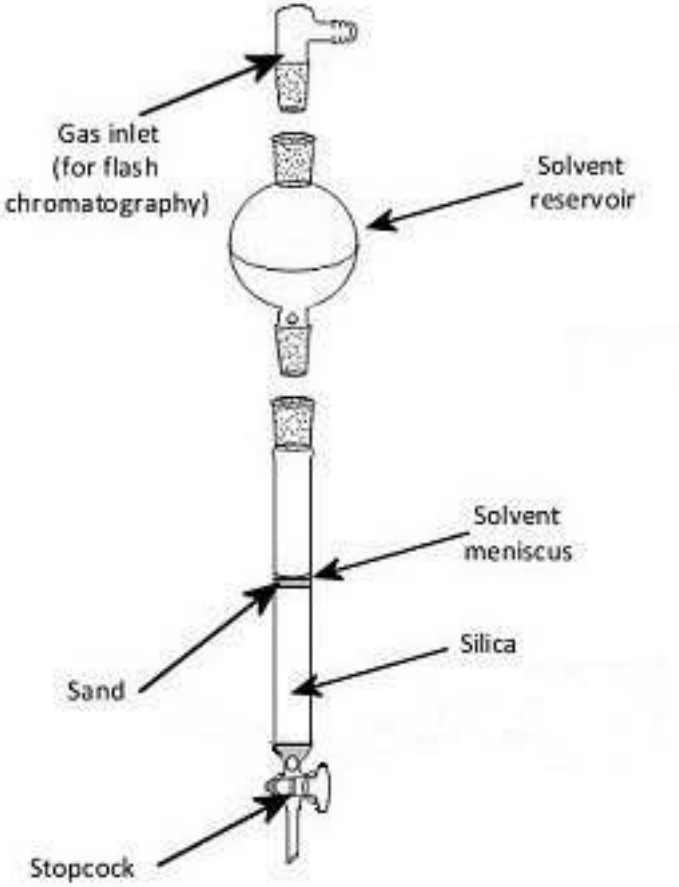
Flash (Forced Flow) Chromatography

- A commonly used preparative purification technique.
- Usually carried out using silica or alumina as a stationary phase in a vertical glass column. The mobile phase is added to the top of the column and flows down under external pressure (air or N_2). The mobile phase containing the sample is collected in vials and analysed by TLC.



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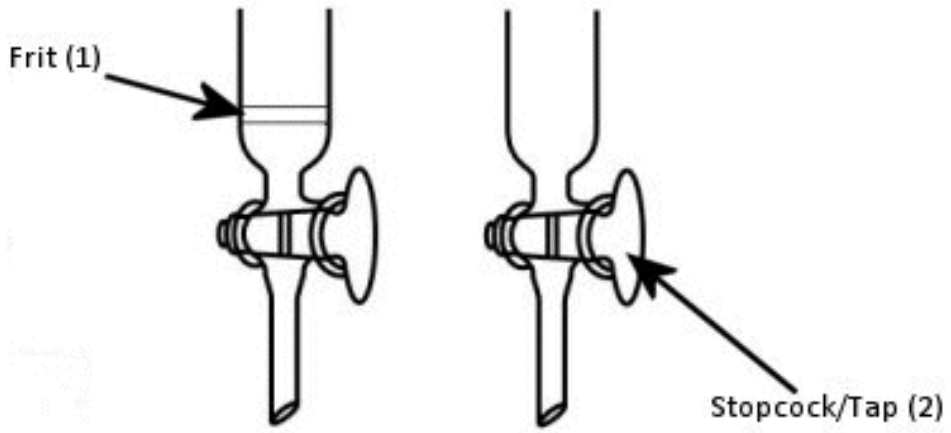
Setting up a Flash Column

- Some columns have glass frits and others do not. If possible avoid the use of glass frit columns as these usually have larger “mixing areas” after the frit and the frits can also be a source of impurities.



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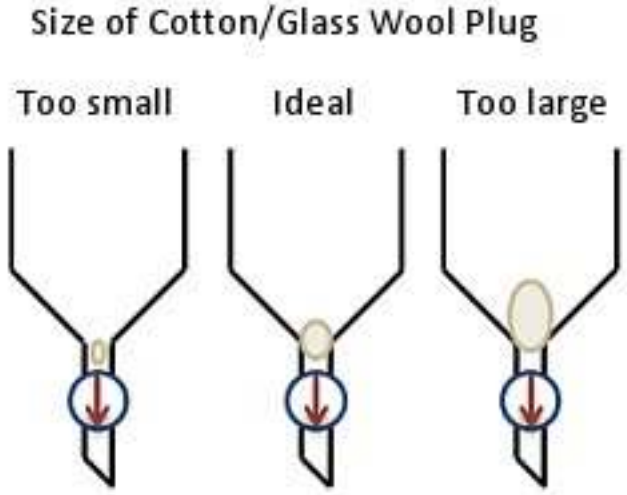
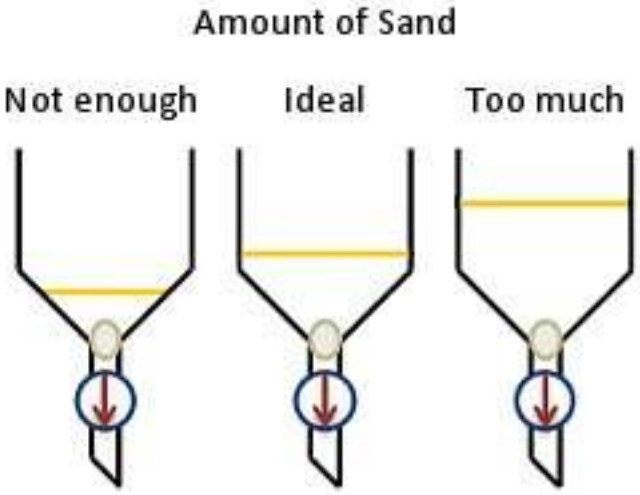
Setting up a Flash Column

- Non-fritted columns will require a cotton wool plug and a layer of sand.





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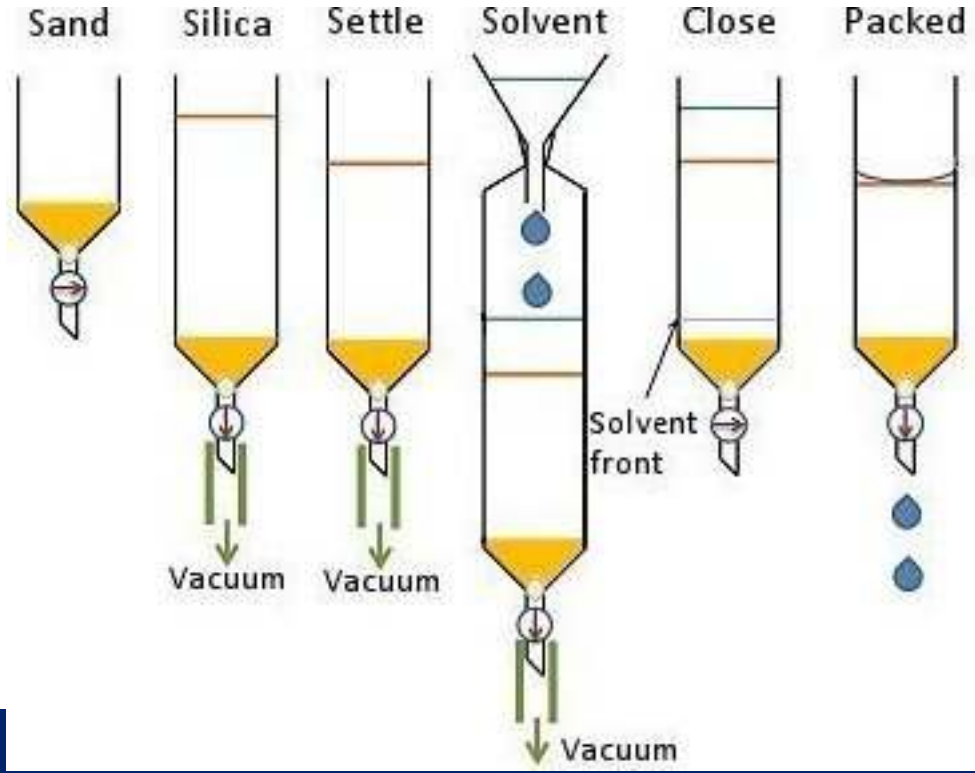
Setting up a Flash Column

- Dry-packing: Add about 6” of silica to the column. Pour in the solvent. Allow solvent to run until it is just above the silica. Level the silica by light tapping. Carefully run the solvent until it is at the level of the silica.



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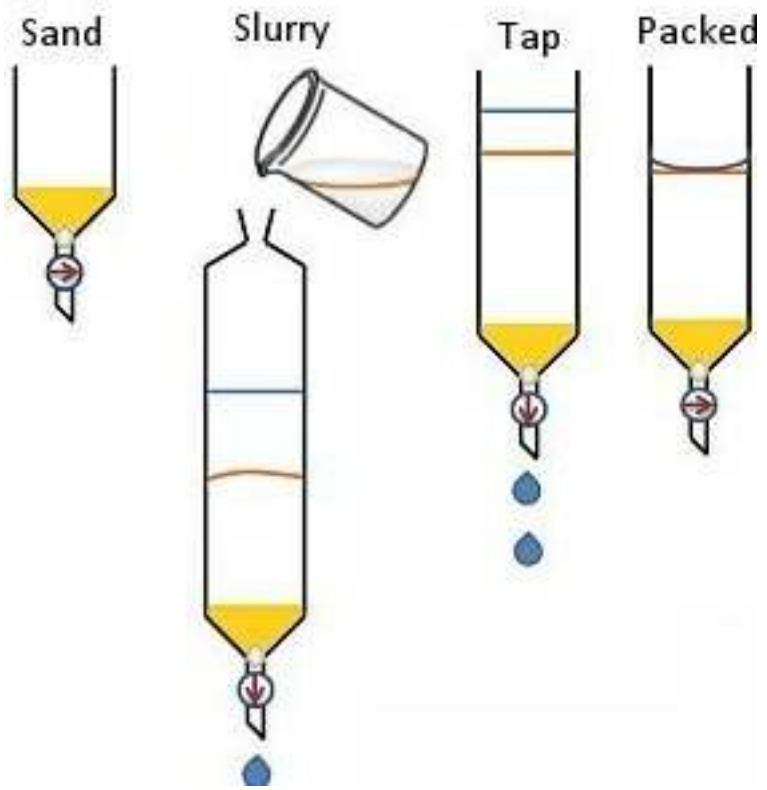
Setting up a Flash Column

- Wet-packing: Weigh the silica into a beaker. Measure solvent ~1.5 x the volume of silica into another beaker. Slowly pour the solvent into the silica beaker. Stir the mixture until a uniform slurry develops. Pour the slurry into the column. Level the silica and run the solvent to the top of the silica.



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Loading a Flash Column

- Wet-Loading: Dissolve the sample in a minimum (5-10 drops) of solvent. If you need to use a more polar solvent for this then consider CH_2Cl_2 rather than a higher EtOAc/hexane ratio.
- With a pipette, drip the solution of sample slowly onto the silica. Try not to disturb the silica level.
- Once the sample has been added, let the column drain until the solvent level is at the silica level.
- Add a layer of sand (~0.5cm) to the top of the column and carefully fill the column with solvent. Take care not to disturb the silica level.

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Loading a Flash Column

- Dry-Loading: If the sample can't be wet-loaded.
- Dissolve the sample in a rb flask, add silica ~20 x weight of sample and swirl until all the silica is suspended in solution.
- Gently rota-vap away the solvent until the silica runs free.
- Carefully add 2-3cm of solvent to the top of your column. Do not disturb the silica level.
- Add the sample silica to the column, and tap to make sure it is level.
- Continue as for wet-loading.

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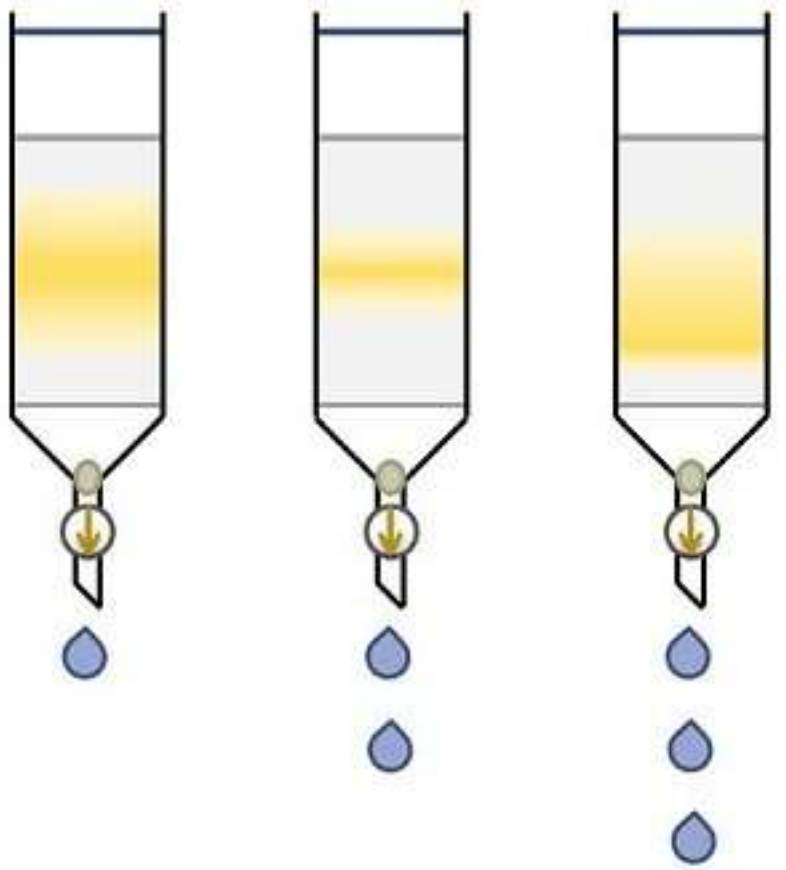
Fraction Collection

- Collect fractions in to test tubes. If the flow rate is too slow, diffusion will lead to band widening. If it is too fast the compound will be forced down the column and leave a long tail. Optimal flow rate is dependant upon column diameter.
- Never let the solvent level go below the silica level.



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Fraction Collection

- If you can see a line corresponding to the polar solvent used to dissolve the sample, this is a good guide for when you should start collecting fractions.
- If you can't see such a line you can estimate the dead volume as 2/3 the height of the silica.

Silica (g)	$R_f = 0.15$	$R_f = 0.3$
2	5 ml	1–2 ml
10	10 ml	5 ml
30	15 ml	10 ml
50	30 ml	15 ml
100	60 ml	30 ml

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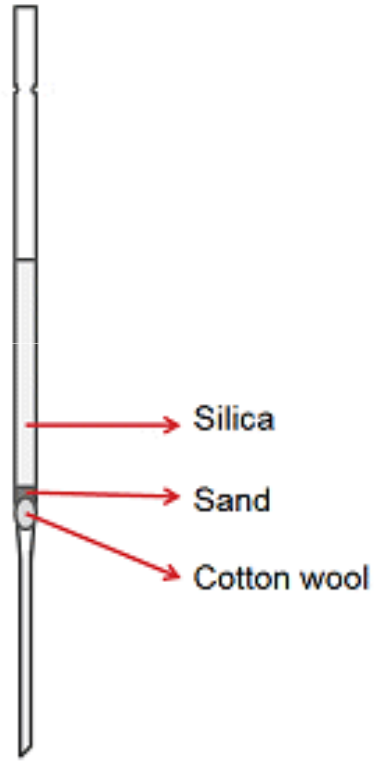
Micro-Columns

- If you only have a few mg of sample then you can run a micro-column.
- Use a Pasteur pipette, set up and run it as a normal flash column.
- Reduce the polarity of the mobile phase so the compound has an Rf = 0.2 (rather than the usual 0.3).
- Force the solvent through using a pipette bulb. Take care you do not break the pipette while attaching & removing the bulb.



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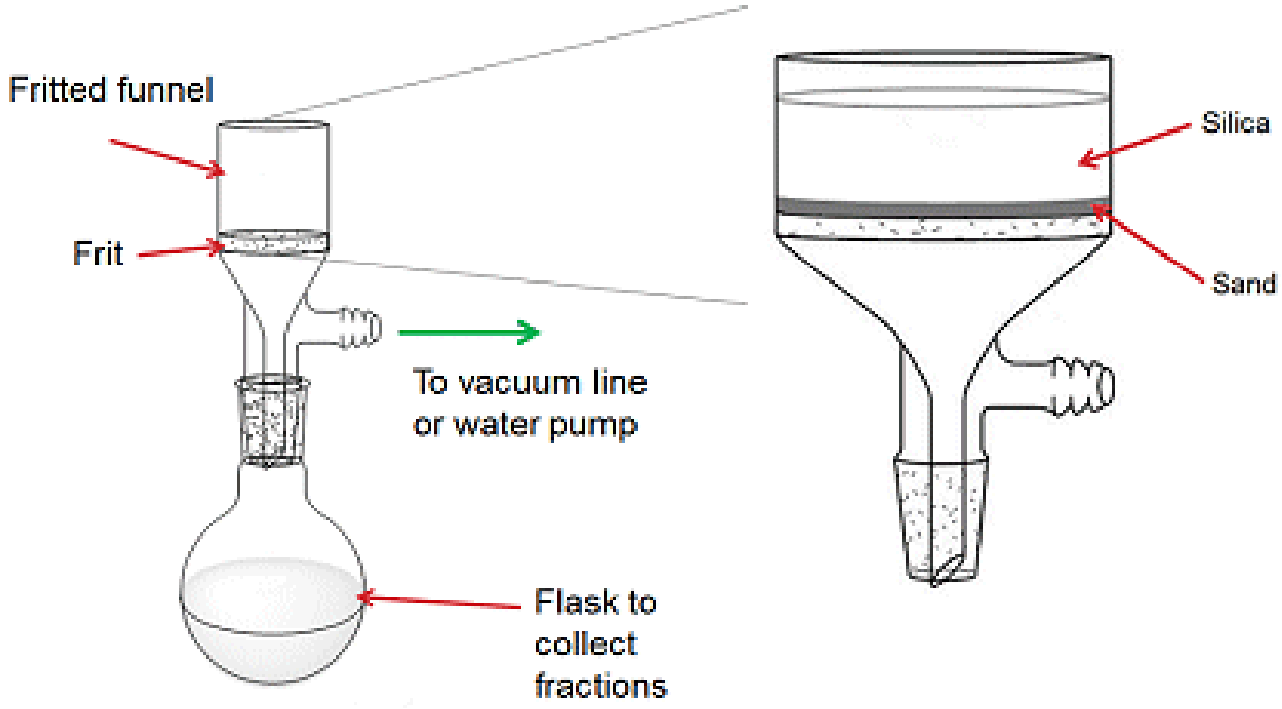
Plug Filtration

- An alternative to flash column. Suitable only for compounds of high Rf with a few very polar impurities.



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Plug Filtration

- Dry pack the plug “column”.
- Load the sample as usual.
- Add enough solvent so the plug does not run dry in the next step. Do not disturb the silica level.
- Apply vacuum and collect in rb flask. Before plug runs dry disconnect the vacuum and change flask.
- Add more solvent and repeat as needed.