Column chromatography:

Column chromatography is suitable for the physical separation of gram quantities of material. A solvent acts as the mobile phase while a finely divided solid surface acts as the stationary phase. The stationary phase will adsorb the components of the mixture to varying degrees. As the solution containing the mixture passes over the adsorbent, the components are distributed between the solvent and adsorbent surface. This process may be described by a three-way equilibrium between the sample, the solvent and the adsorbent.

The solvent and sample compete for positions on the solid adsorbent, the solvent displacing the sample reversibly and continuously in the direction of the solvent flow. Consequently, a weakly adsorbed compound will spend more time in the solvent, and will therefore be eluted first.

Adsorbents:
The most common solid adsorbents are alumina (aluminum oxide) and silica gel (silicon dioxide). A variety of grades and activities is available depending upon the specific application. Silica gel is slightly acidic while alumina may be acidic, neutral or basic. For greatest effectiveness the particles of solid adsorbent should be of uniform size and large surface area (for instance, 150 mesh alumina has a surface area of 155 m^2/g). The weight of adsorbent used is usually 20 - 50 times the sample weight, the greater ratio used for more difficult separations.

The strength of adsorption depends upon the compounds involved. Since the adsorbents are polar, the more polar compounds are adsorbed more strongly. Thus, non-polar compounds are eluted first. The order of elution from a column usually follows the series: alkyl halides < saturated hydrocarbons < unsaturated hydrocarbons < ethers < esters < ketones < amines < alcohols < phenols < acids. Polymeric compounds and salts will often not elute.

Solvents:
The solvent will compete with the sample for adsorbent sites. The choice of solvent is therefore an important consideration in column chromatographic separations. Consider two situations. If the solvent is much more polar than the compounds, the three-way equilibrium will be shifted in favour of the solvent - adsorbent interactions. The compounds will remain in the mobile phase, and separation will not occur. Alternatively, if the compounds are much more polar than the solvent, the three-way equilibrium will shift in favour of the sample - adsorbent interactions. No compounds will elute since the solvent is unable to move compounds from the adsorbent sites.

Selection of solvents requires a balancing act between solvent and compound polarities. For most separations, the solvent should be less polar than the compounds. The compounds must also be soluble in the solvent so they are not permanently adsorbed. The elutropic series (order of polarity) for silica gel and alumina is as follows: hexane ≈ petroleum ether (Petroleum ether is a generic name given to a mixture of pentanes, hexanes and heptanes. It does not contain any ethers of the R-O-R' structure.) < carbon tetrachloride < toluene < dichloromethane < chloroform < diethyl ether < ethyl acetate < acetone < propanol < ethanol < methanol < acetic acid < water.

In simple separations a single solvent (or solvent mixture) is used throughout the process. In complex separations, a series of increasingly polar solvents is used. By starting with a non-polar

solvent, most of the compounds should remain adsorbed at the top of the column. Small systematic increases in solvent polarity will ideally elute these components one at a time.

A large increase in polarity might cause all of the components to elute at once, as well as cause other problems with the column packing. Consequently, small polarity changes are accomplished by careful use of mixed solvents. For example, pure hexane may be used as the first solvent. This may be followed by a mixture of 90% hexane - 10% dichloromethane. Successive mixtures containing 20%, 50%, 80%, and 100% dichloromethane would complete the transition in polarity.

Columns:
The columns available in the department are simple glass tubes, varying in length and diameter. They usually have a stopcock attached to control the solvent flow, and may have a fritted plate to support the adsorbent. Some columns may have a flask attached to the top of the column to act as a solvent reservoir.

The variation in size allows one to select the best column for the separation. A large diameter column will handle larger amounts of compound. The resolution of the column depends both upon diameter and length of the adsorbent in the column. Resolution increases with increasing length, and decreases with increasing diameter. Thus, 25 g of adsorbent will provide a better separation in a 1 cm diameter column than in a 2 cm diameter column.

Experimental aspects of column chromatography:
The best conditions for running the separation may be determined by experimental tlc (see above section). This will indicate the best adsorbent and the best solvent for the separation. The desired material should have an R_f of about 0.35 if the separation is to be run as a single solvent. It is faster to run many experimental tlc plates than to set up a column that does not work.

The most important experimental consideration is the preparation of the packed column. The packing should be homogeneous and should not contain trapped air or vapour bubbles. The trapped gasses lead to channelling through the adsorbent, and a loss of resolution. The best method of packing is different between the two adsorbents.

Silica gel:
The column is clamped vertically, and half-filled with the solvent to be used at the start of the separation. Remember to close the stopcock.

A small piece of glass or cotton wool is used as a plug to support the adsorbent. Use a long glass rod to place the wool at the bottom of the column. The wool should be compressed enough to support the column packing yet loose enough that the solvent flow will not be hindered. There should be no air bubbles in the wool.

The silica gel is weighed out in an Erlenmeyer flask, and enough solvent is added with stirring to form a slurry. All of the air bubbles should be removed from the slurry before filling the column. With the aid of a powder funnel, the slurry is added to the column. As soon as the column begins to build, the stopcock is opened. This allows the excess solvent to drain, and helps to settle the silica gel. The adsorbent is evenly packed by tapping the column with a piece of vacuum tubing. Tap

gently, and don't take out your life's frustrations on the column.

Once the silica has packed, a final layer of sand (≈ 5 mm) is added to the column. The sand protects the top of the packed silica gel. Pack the sand in the same manner as the silica gel, and allow the solvent level to drop until it is just above the level of the sand. The solvent must not be allowed to drop below the sand level, as this may introduce bubbles of air that would disrupt the continuity of the packing and lead to a loss in resolution.

The column is now ready for use. If some cracking of the column occurs before your mixture is placed on the column, this does not mean starting over. You may expel some of the air by placing a pipette bulb on top of the column, and increasing the pressure over the column by compressing the bulb with the stopcock open. This will cause the solvent to flow at a faster rate, often taking the air with it. Obviously, the solvent must not drop below the level of the sand, as this would rapidly introduce air into the column.

Alumina:
The column is clamped vertically. Remember to close the stopcock.

A small piece of glass or cotton wool is used as a plug to support the adsorbent. Use a long glass rod to place the wool at the bottom of the column. The wool should be compressed enough to support the column packing yet loose enough that the solvent flow will not be hindered. There should be no air bubbles in the wool.

Columns made with alumina perform better when packed dry. Weigh out the desired amount of alumina into a flask (or do a 'dummy' fill to estimate the amount needed). Half-fill the column with the non-polar solvent, and with the aid of a powder funnel, add the alumina to the column. Tap the column gently to pack the alumina. When the alumina is well packed, add a layer of sand (≈ 5 mm) to the top of the column. Open the stopcock and allow the solvent to drain to the level of the sand.

If some cracking of the column occurs before your mixture is placed on the column, this does not mean starting over. You may expel some of the air by placing a pipette bulb on top of the column, and increasing the pressure over the column by compressing the bulb with the stopcock open. This will cause the solvent to flow at a faster rate, often taking the air with it. Obviously, the solvent must not drop below the level of the sand, as this would rapidly introduce air into the column. The column is now ready for use.

Running the separation:
Add the sample to the top of the column, either as a neat liquid, or dissolved in the absolute minimum amount of good solvent. The sample should be added directly to the sand layer (if possible) or carefully down the side of the column so as not to disrupt the sand surface.

Solvent is drawn from the bottom of the column until the level of the liquid is just above the level of the sand. Small quantities of eluting solvent are carefully added to the column, with the stopcock opened so that the solvent is continuously flowing through the column. Only when the sample is well adsorbed on to the column can the head space above the column be filled with eluant. It is very

important that this liquid above the column is not a solution of your sample but is only the eluting solvent(s). Fractions of a standard volume are collected. The volume of solvent collected for each fraction should correspond to the amount of material being separated *ie* larger fractions for larger quantities.

The identity of the fractions may be determined by one of several methods. If the compounds are coloured, they can be seen to separate and visible spectroscopy can confirm the degree of separation. For colourless compounds, either tlc or gc may be used to identify the compounds present in the different fractions. The preferred method is usually tlc. Once the desired fractions are identified, the solvent may be removed by rotary evaporation and the compound isolated.