CHROMATOGRAPHY

All of the purification/isolation techniques described thus far (crystallization, distillation, extractions) are suitable for the mixtures that contain compounds with quite different chemical and physical properties. Neither of them would be efficient in separating compounds that possess similar physical properties, such as the mixture of *para*- and *ortho*-bromonitrobenzenes obtained in the previous lab.

One of the best ways to separate structurally and functionally similar compounds is chromatography. There is a great deal of variety of chromatographic methods, yet all of them rely on a common principle: components of the mixture have to distribute differently between a mobile phase and a stationary phase. The mobile phase, usually a liquid or gas, is flowing over the mobile phase, which could be either a solid or liquid. The compound(s) are dissolved in the mobile phase and are placed onto the stationary phase. Continuous partition of the compounds between the mobile and stationary phases, while the mobile phase moves along the stationary phase, is responsible for the separation of the compounds. Therefore, the interactions between the compound(s) and the mobile phase as well as the compound(s) and the station phase will determine whether the mixture of compounds could be separated. The exact nature of the stationary and mobile phases will be indicative of the type of the chromatographic technique.

Column and thin-layer chromatography

Both column chromatography and TLC are examples of solid-liquid adsorption chromatography. The differences between TLC and column chromatography are primarily in the amounts of the material that can be effectively separated. For TLC micro- or milligram-quantities are suitable, whereas gram-quantities should be separated using column chromatography. Hence, TLC is more of an analytical technique, whereas column chromatography is primarily used for preparative separations. Another distinction is that in TLC the solvent is usually moves up the stationary phase, *i.e.*, against the gravity force, and in column chromatography the solvent is moving downwards, along with the gravity force. TLC is normally used to find a solvent system, often called eluant that assures the appropriate separation of the compounds in a given mixture. This particular solvent system will be used to perform a column chromatography.

The strength of adsorption of a compound to the stationary phase will depend on the polarity of the stationary phase. The stronger a compound is interacting with the stationary phase, the slower will it move upon the influence of the mobile phase.

Adsorbent for both TLC and column chromatography is usually silica gel (SiO_2 x H_2O) or alumina (Al_2O_3). Both adsorbents are polar materials that have almost uniform particle size and very high surface area. The higher the surface area of the adsorbent, the better the separation, since the equilibrium between the compound in the

Silica gel is usually available in one form, which is somewhat acidic. Hence, the separation of acid-sensitive compounds should not be done on silica gel. Alumina is commercially available in neutral, acidic or basic forms. Neutral compounds are best separated on neutral alumina, while basic and acidic alumina are more suited for basic and acidic compounds, respectively. Therefore, when choosing which adsorbent to use, the nature of the functional groups should always be considered. In general, the trend shown in Figure 1 will hold true.

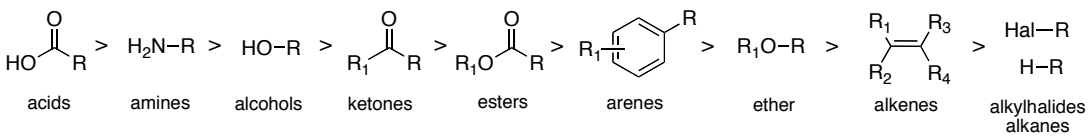


Figure 1. Compound binding strength to polar stationary phase

Another important factor that determines the efficiency of the separation is the mobile phase, *i.e.*, eluant. Although some criteria do exist to select the specific mobile phase, in general, a trial and error method is used to select the appropriate eluant solvent system. Eluant can consists of one, two or more solvents. Usually, two-solvents systems are used. The ratio of those two components could be adjusted for better separations. The compound would not move from the baseline if it is not soluble in the eluant. Thus, the compound should be completely soluble in at least one component of the mobile phase. The solvent should not be too polar, since it might bind to the stationary phase in preference of the compounds in the mixture. In other words, if only the eluant interacts with the stationary phase, the mixture will be moving vary rapidly, providing no separation of the compounds. On the other hand, if the solvent is very apolar, it would not be capable of modulating compound-stationary phase interactions, and the compounds would not be moving at all. An ability of different solvents to move a compound up a TLC place is shown in Figure 2.

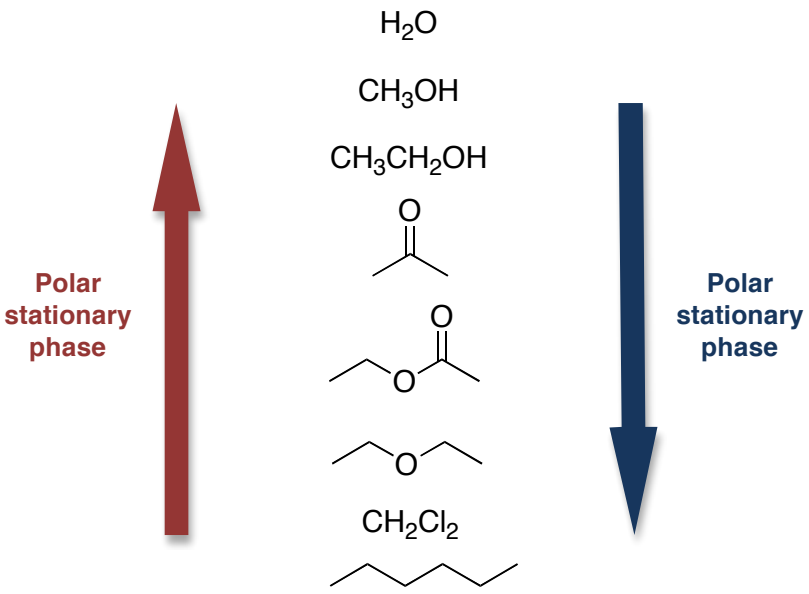


Figure 2. Eluting power of some common solvents

The main steps for the preparing the column for the chromatographic separation of a mixture of the compounds using silica gel are outlined as follows:

The column should be securely clamped vertically. Next, a small piece of cotton or glass wool should be placed at the bottom of the column, followed by a small amount of sand (usually about 1 cm layer of sand is enough). Sand is not used for the separation process, but rather to provide support for the adsorbent, and prevent the small particles of the adsorbent from being washed from the column. About a quarter to a half of the column is then filled with a solvent. If the eluant system consists of more than one component, the column should be filled with the least polar solvent. To the appropriate amount of silica gel eluant (or the least polar component of the eluant) is added, and the mixture is suspended by swirling, and the mixture should be poured gently and steadily into the column. The pitch-clamp should be open during the addition, such that the solvent is dripping out of the column. While the silica gel settles, tapping on the side of the column has to be done to assure that uniform packing and remove air that might be trapped within the solid. Allow the solvent to go through (about a quarter of the length of the solvent above the silica gel. The solvent should be drained to only slightly above the level of silica gel. At this stage, no taping on the column should be done. The mixture of compounds is dissolved in the minimum amount of the appropriate solvent;

often the most polar component of the eluant system. This concentrated solution is placed on top of the silica gel, and the level of the solvent is brought right at the level of the silica gel. Next, small amount of sand is added (usually about 1-2 cm layer of sand is enough). The sand on top of the column will prevent the disruption of silica gel layer when the solvent is added. The eluant is slowly added to fill the column. The pitch-clamp then is open and the solvent is collected in test tubes. The solvent should be added periodically, by pouring along the wall of the column. The level of the solvent should never be allowed to go below the top sand layer (this introduced air into the column, and the efficiency of the separation process will decrease; when this happens, one can observe the formation of "cracks" in the column as the solvent drains). Solvent should flow steadily and slowly enough. A set of the test tubes is collected and their content is analyzed using TLC. Tubes containing same composition (preferably a single spot) should be combined, and the solvent is removed to yield the desired compound(s).

There are two approaches for solvent flow: gravity controlled column chromatography, when the solvent goes downwards due to the gravity force, and a flash chromatography, when the solvent is forced by a stream of air or an inert gas, such as nitrogen for example. Gravity chromatography leads to better separation, but it is slower; flash chromatography is faster, yet the separation efficiency decreases. Flash chromatography is often used to separate compounds that have very large ΔR_f values.

High pressure (or high performance) liquid chromatography

The greater surface area on TLC adsorbent than the surface area of the silica gel used for column chromatography, is often responsible for superior separation achieved on TCL. However, the finer the particles, the slower the rate at which the solvent can pass through the stationary phase. In order to increase the rate of the separation process a pressure has to be applied to force the solvent through the column. This type of chromatography is often refereed to as a high pressure liquid chromatography, HPLC (Figure 3). Due to superior separation achieved by this process, this technique also known as high performance liquids chromatography (conveniently abbreviated as HPLC). The equipment consists of a pump, which draws the solvent from the solvent reservoir to a chromatography column, which is packed with very fine adsorbent. The sample is introduced into a continuous flow of the solvent (which is equivalent to the eluant in column chromatography). Once the compound goes through the column it enters into a detector. Usually a UV/vis detector is mostly used to monitor the separation process, however other types of detectors could be used.

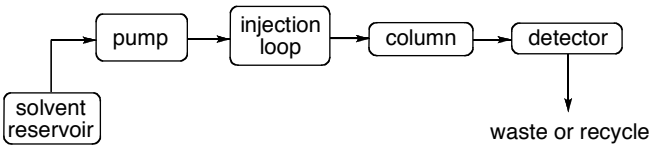


Figure 3. Schematic presentation of a HPLC system

Depending on the size of the column, HPLC can be used either for analytical purposes, *i.e.*, to monitor the progress of the reaction, determine the composition of the mixture, etc, or it could be used for preparative purposes. The latter case, also known as preparative HPLC is reminiscent to column chromatography. However, preparative HPLC is much more expensive, in terms of equipment (pump and the column should be able to handle larger quantities of materials), than column chromatography, yet the ability to separated mixtures that could not be separated by a column chromatography, makes preparative HPLC a very valuable tool.

Gas chromatography

Gas chromatography (GC), also known as gas-liquid chromatography, is based on the partitioning of the compounds between the mobile gaseous phase (usually an inert gas such as nitrogen or helium is used, but other gasses or mixtures of gases could be used as well) and a thermostable stationary phase, which is usually a clay, wax, rubber or silicone. Specific examples of stationary phases include various polymeric species, such as polyesters, polyamides, silicone polymers as well as hydrocarbons.

Instrumental set-up of GC is somewhat similar to HPLC, yet there are a few important distinctions (Figure 4). The carrier gas constantly flows through the whole system. The sample, which is prepared in a suitable solvent, is introduced at the injection point through a septum, and upon entering the injection point, the sample vaporizes immediately, and it is carried by the gas into the column, which has an appropriate stationary phase. The temperature of the oven can be adjusted to control the separation process. The oven temperature can be held constant through out the analysis (known as isothermal mode) or changed throughout the analysis (for example the initial temperature could be set at 100^{o}C and then increased with a rate of 5^{o}C/min). Upon leaving the column, the compound(s) enters the detector. A flame ionization detector is one of the most common detectors used, and it detects the number of ions produced by passing the mobile phase through a hydrogen flame.

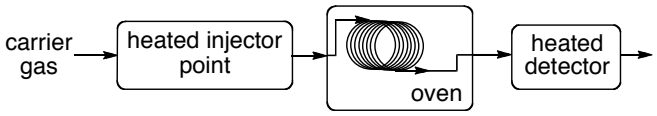


Figure 4. Schematic presentation of a GC system

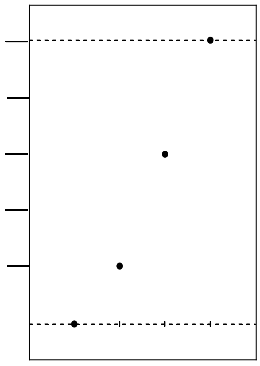
The nature of the stationary phase will be very important in achieving suitable separation of the compounds in a given mixture. Also, the efficiency of the resolution (or separation) generally increases with increasing length of the decreasing diameter of the column. Temperature and the flow rate of the carrier gas are the other two important factors that control the separation in GC.

Considering how the compound is introduce into GC instrument, it is evident that non-volatile compounds or thermally unstable compounds are not suitable for GC analysis, and they should be analyzed using other chromatographic techniques, such as HPLC, for example.

PROBLEMS

Some questions are based on the material cover in the lecture

1. Calculate the R_f values for spots on the following TLC plate:



2. Which of the following solvent combinations are suitable as mobile phases for a column chromatography:
   a) dichloromethane-ethanol; b) hexane-water;
   c) acetonitrile-hexane; d) acetone-water;
   e) toluene-cyclohexane; f) ethyl acetate-methanol.

3. What is the purpose for putting sand at the bottom and at the top of the column?

4. Arrange the following compounds in their ability to bind to silica gel:
   alkanes, amines, alcohols, aromatic hydrocarbons, salts

5. Arrange the following solvents according to increasing eluting power from silica gel packed column:
   hexane, chloroform, acetic acid, petroleum ether, acetone, ethanol.

6. True or false:
   a) one spot on TLC means that the compound is pure;
   b) the sample for TLC should be as concentrated as possible so that the spots could be easily seen;
   c) the eluent system that works best for a TLC should be used for the column chromatography;
   d) the diameter of the spots on TLC should be as small as possible;
   e) the spots should be immersed in the solvents in the developing chamber.

7. Why the solvent should be evaporated from the surface of the TLC plate before it is placed under a UV lamp?