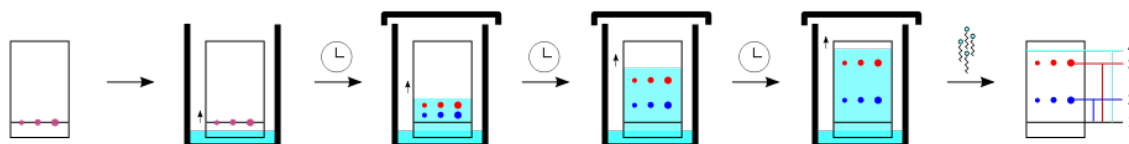


Chromatography is the term used by chemists to describe a family of separation techniques. A modern chemical laboratory might use gas chromatography (GC), column chromatography, high performance liquid chromatography (HPLC), paper chromatography, thin-layer chromatography (TLC), and others. What all of these techniques have in common is that separation of a mixture takes place by having the sample in contact with two phases, one stationary and the other moving. Because of the different solubility of adsorptions of each component of the mixture, the components are partitioned and separation is achieved.

In thin-layer chromatography (TLC) the stationary phase that we will use is a thin coating of silica gel on a sheet of flexible plastic. The moving (or mobile) phase is a solvent consisting of 4 parts ethyl acetate to 1 part toluene. As the solvent carries the mixture up through the stationary phase we say that the elution is taking place. When elution is complete the original mixture should be separated into its component parts and should be ready for identification.

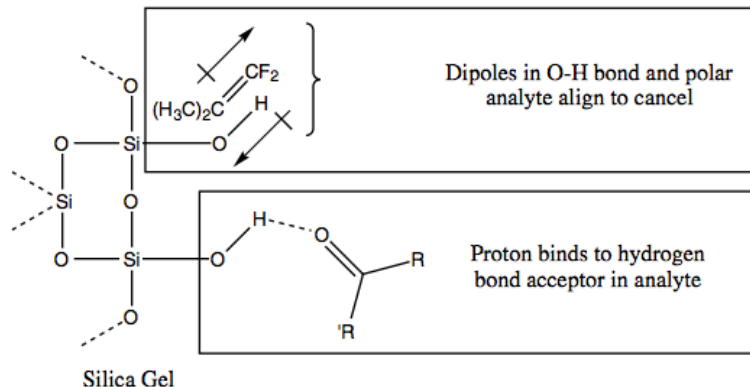


TLC has been used in police laboratories to quickly an initial identification of suspected illegal drugs. Other, more accurate, methods are then employed when needed. Pharmaceutical laboratories also use TLC to separate and identify unknown compounds.

Stationary Phase

Silica gel, the most commonly used stationary phase, has the empirical formula SiO_2 . However, at the surface of the silica gel particles, the dangling oxygen atoms are bound to protons. The presence of these hydroxyl groups renders the surface of silica gel highly polar. Thus, polar functionality in the organic analyte interacts strongly with the surface of the gel particle and nonpolar functionality interact only weakly. Polar functionality in the analyte molecules can bind to the silica gel in two ways: through hydrogen bonds and through dipole-dipole interactions. The total strength of the interaction is a sum of these two components. It should be noted that the shape of the organic analyte is also a factor in predicting the strength of its interaction with silica gel. Thus, an analyte that displays multiple polar groups in position to interact with the surface of the stationary phase with interact more strongly than an analyte that displays the same polar functionality in a way that does not permit multi-dentate binding.

Modes of Interaction of Analyte with Silica Gel

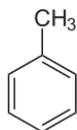


Mobile Phase

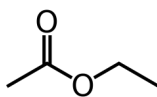
For silica gel chromatography, the mobile phase is an organic solvent or mixture of organic solvents. As the mobile phase moves past the surface of the silica gel it transports the analyte past the particles of the stationary phase. However, the analyte molecules are only free to move with the solvent if they are not bound to the surface of the silica gel. Thus, the fraction of the time that the analyte is bound to the surface of the silica gel relative to the time it spends in solution determines the retention factor of the analyte. The ability of an analyte to bind to the surface of the silica gel in the presence of a particular solvent or mixture of solvents can be viewed as a the sum of two competitive interactions. First, polar groups in the solvent can compete with the analyte for binding sites on the surface of the silica gel. Therefore, if a highly polar solvent is used, it will interact strongly with the surface of the silica gel and will leave few sites on the stationary phase free to bind with the analyte. The analyte will, therefore, move quickly past the stationary phase. Similarly, polar groups in the solvent can interact strongly with polar functionality in the analyte and prevent interaction of the analyte with the surface of the silica gel. This effect also leads to rapid movement of the analyte past the stationary phase. The polarity of a solvent to be used for chromatography can be evaluated by examining the dielectric constant (ϵ) and dipole moment (δ) of the solvent. The larger these two numbers, the more polar is the solvent. In addition, the hydrogen bonding ability of the solvent must also be considered. For example methanol is a strong hydrogen bond donor and will severely inhibit the ability of all but the most polar analytes to bind the surface of the silica gel.

Solvent	formula	boiling point (°C)	melting point (°C)	density (g/mL)	relative polarity
cyclohexane	C6H12	80.7	6.6	0.779	0.006
toluene	C7H8	110.6	-93	0.867	0.099
ethyl acetate	C4H8O2	77	-83.6	0.894	0.228
acetone	C3H6O	56.2	-94.3	0.786	0.355
methanol	CH4O	64.6	-98	0.791	0.762
water	H2O	100	0	0.998	1

Toluene



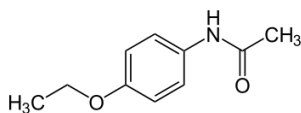
Ethyl Acetate



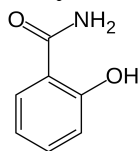
The Experiment

In our laboratory exercise we will work with three compounds: a) phenacetin, an analgesic (pain-killer) and antipyretic (fever reducer); b) salicylamide, a compound similar to aspirin; and c) caffeine, a stimulant found in coffee, tea, and cola drinks. Each of them is already in solution and may be found in labeled test tubes in the front of the lab.

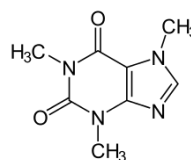
Phenacetin



Salicylamide



Caffeine



After you have experimented with these 3 known solutions, your instructor will give you an unknown sample. It may contain any one of the three known chemicals or any combination thereof. Your job is to determine the composition of your unknown solution.

PROCEDURE

Cut, with a pair of scissors, two chromatographic plates so that they will fit into the glass developing chamber with its cover on. Do not touch the dull side of the sheet with your fingers. With a sharp pencil gently draw a line across the entire plate about 1 cm from the bottom. On one plate lightly mark four dots, evenly spaced across the line, so that the line is now divided into 5 equal segments.

Take four open-ended capillary tubes and draw them into micropipettes. To do this, hold the ends of the tubes and roll them very slowly in a Bunsen burner flame so that the center of the capillary is heated. When the glass glows bright orange and begins to sag,

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remove it from the flame and quickly stretch the tube. Gently break the center of the stretched portion with your fingers.

Dip one of the micropipettes into the known solution and then touch its tip to the first dot on the chromatographic plate. Do the same with the other two knowns and the reference sample and write with pencil under each dot, respectively: sal; caf; phen; ref. The liquid drop that you place on each dot should not be much larger than the letter “o” shown here. The same capillary tube should not be used for more than one solution. Discard the used capillaries in a “waste glass” container.

Pour solvent into the developing chamber to a depth of about ½ cm. Place the chromatographic plate into the chamber so that the horizontal line and sample dots are above the solvent level. Cover the chamber and don't move it.

Watch the solvent elute upward as it carries the samples by capillary action.

When the solvent reaches about 1 cm or less from the top of the plate, remove the plate, place it on your bench-top and draw a pencil line across the plate to indicate where the solvent stopped rising.

Take the developed plate to the ultra-violet (UV) light box and mark, with pencil, the outlines of the six (6) spots on your chromatogram. Mark a dot in the center of each spot outline.

Next, show the developed plate to your instructor and ask for an unknown. Record the number of your unknown. Dissolve your unknown in a solvent mixture containing 50% methylene chloride and 50% ethanol. Use as little solvent as possible to dissolve all of the unknown sample.

On the second chromatography sheet that you have cut, also draw a light horizontal pencil line across the plate about 1 cm from the bottom and lightly mark 2 dots on that line so that it is divided into 3 segments. Using two new capillary tubes, place small amounts of your unknown solution and the reference solution on each dot, respectively. As before, allow this plate to develop until the solvent reaches about 1 cm from the top of the plate. Then remove the plate from the chamber, draw a light line to indicate the solvent front and outline all of the spots while under the UV light.

Measurement must now be taken of each spot on both plates relative to the solvent front to ascertain the relative flow (Rf) value for each substance.

$$Rf = \frac{\text{distance traveled by substance}}{\text{distance traveled by solvent}}$$

Remember, distances are measured from the lower line of the chromatogram, not the lower edge. All distances are to be measured in centimeters (CM) and tenths of centimeters (millimeters) so that each measurement is recorded with 2 significant figures.

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(Example – 3.7 cm) When calculating the Rf values only 2 significant figures should be recorded (example – Rf = 0.63). Please note that the substance distance and the solvent distance are measured in centimeters, the Rf value has no units because they cancel each other.

Finally, in writing the conclusion section of your report, you should compare the Rf values of the reference to the Rf value(s) of your unknown. Colors under the UV light may also be compared. (Don't expect the Rf values of the known compounds and unknowns to be exactly the same, but they should be close.)