Chromatography and Electrophoresis

Type	Paper Chromatography	Thin Layer Chromatography (TLC)	Gas/Liquid Chromatography (GLC)	High Performance Liquid Chromatography (HPLC)	
Stationary Phase	**Polar** liquid. Layer of permanently bonded water molecules to the –OH groups of cellulose.	**Polar** solid. Thin layer of polar solid adsorbents support in an inert material (e.g. glass). Adsorbents can be silica, alumina or cellulose. Silica and alumina are slightly acidic and basic and good adsorbents for basic and acidic solutes respectively.	**Non-polar** or **polar** liquid. Stationary phase has low volatility, are chemically inert, thermally stable, and can be polar or non-polar. Non-polar liquids are used for non-polar solutes and polar liquids are used for polar solutes. Coated on solid support column.	**Polar** solid. Silica particles with-OH groups. Particles may be coated with fragments of a polar solvent joined by covalent bonds, ensuring they do not dissolve in the mobile phase.	**Non-polar** solid. Usually modified silica with long hydrocarbon chains attached to its surface, which act like a solvent.
Mobile Phase	Usually **less polar** than stationary phase. (Mixture of) solvents.	**Non-polar** or **polar**. (Mixture of) solvents. Non-polar solvents are used for non-polar solutes and polar solvents are used for polar solutes.	**Inert** gas. Must be dry by passing through anhydrous $CuSO_4$ to avoid interference from water molecules. Organic solvent vapors can be removed by passing through activated charcoal.	**Non-polar**. (Mixture of) high purity solvents.	Highly **polar**. Solvent must be filtered and free of particulates that may clog or contaminate the column and free of dissolved gases that may form bubbles under high pressure.
Principle	Compounds **partition** themselves between the water stuck to the cellulose surface and the less polar moving solvent.	Solutes have different **adsorption** affinities to the polar stationary phase and solubility in the mobile phase.	Solutes are **partitioned** between gaseous mobile phase and a non-volatile liquid stationary phase based on differences in boiling points and relative solubility in the liquid stationary phase.	Solutes have different **adsorption** affinities to the stationary phase and solubility in the mobile phase.	Solutes have different **partition** affinities to the stationary phase and solubility in the mobile phase.
Elution Order	Increasing polarity.	Increasing polarity/acidity/basicity.	Increasing boiling points.	Increasing polarity.	Decreasing polarity.
Solute	-	Non-volatile solid organic and inorganic compounds. Volatile liquid compounds should be avoided due to evaporation from the TLC plate.	Gas or have appreciable vapor pressure at the temperature GLC is conducted at (may not be rtp). Liquid/solids can be easily vaporized at the injection chamber.	Thermally unstable and not volatile (not suitable for GC).	
Detection	Conjugated compounds absorb UV light. Less polar solutes preferentially absorb gaseous I_2, appearing brown. Amino acids and small peptides appear purple in ninhydrin. Sugars appear red or purple in Molisch's reagent. Reducing sugars give a silver mirror in Tollen's reagent.		The eluate is burned in a mixture of H_2 and air. Carbon atoms produce CH● radicals that are oxidized to CHO^+ ions and electrons in the flame, producing a current which is converted to a digital signal. Thermal conductivity detectors measure the ability of a substance to transport heat from a hot to cold region. When the eluate flows over the hot filament, the conductivity of the gas decreases and the filament gets hotter, changing its resistance and producing a change in voltage which is measured. Mass spectrometers can be used.	Spectrometers (mass, IR, UV/Vis).	
		The stationary phase can be impregnated with a fluorescent insoluble compound that absorbs UV light and emits it as visible light. When placed under a UV lamp, UV absorbing compounds appear as dark spots.			
Analysis	R_f = distance moved by solute/distance moved by solvent. Each solute has a characteristic R_f value for a given solvent. However, more than one solute can have the same R_f value.		Identification of compounds is done by comparing the retention time between sample injection and the peak at the detector with known solutes under identical conditions. Spiking can be used, where a known compound is added to the unknown mixture. If the added compound is identical with a component in the mixture, the relative area of the peak will increase. Area = width at half-height x height is proportional to the quantity of the component. This can be compared to a known quantity of an internal standard. Percentage of compound = its area/sum of all areas.		
Advantages	-	<u>Over paper chromatography:</u> Results are more reproducible since TLC plates can be made according to specifications. Separation is more efficient due to smaller particle size of the stationary phase. <u>Over GLC and HPLC:</u> Ability to run many samples simultaneously for immediate and direct comparison with standards. Cheaper, more versatile and quicker. All solutes, including those that do not migrate from the origin are detectable.	-	Faster with superior separation and sensitivity since high pressures are used to force the mobile phase through closed columns. High efficiency since the size of the stationary phase particles are small and the solute can equilibrate more rapidly between the stationary and mobile phases. This results in sharper peaks and shorter retention times. -	
Remarks	Solvent is buffered to achieve reproducible results. Two way paper chromatography can be used, with two different solvents, resulting in more efficient separation of solutes with similar R_f in one solvent.	Solute is dissolved in highly volatile organic solvent (e.g. acetone) which evaporates almost immediately and does not interfere with analysis. Done in a closed vessel such that the atmosphere is saturated with solvent vapor to minimize evaporation.	Temperature of the GLC oven can be increased gradually over a required range, giving a better separation if the boiling points of the components are close. it also gives a faster separation if some components are relatively non-volatile.	Buffers may be used to ionize acidic/basic groups to minimize interactions with the stationary phase to prevent tailing in the peaks.	

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Separation occurs by two processes: participation between the liquid stationary phase and mobile phase, or adsorption onto the solid stationary phase.

Adsorption: solutes adsorb onto and desorb from a solid stationary phase as the mobole phase passes through the stationary phase. Adsorption when molecules in mobile phase interact with the molecules on the surface of a solid by dipole-dipole interactions, hydrogen bonding or van der Waals' interactions. Separation of the solutes is dependent on the differences in their polarity. The tighter they adsorb to the stationary phase, the slower they travel through the chromatography column. Highly polar solutes are best separated by partition since they bind to the solid polar stationary phase too astrongly for desproption and hence will not separate efficiently.

Partition: the compounds partition themselves between a stationary liquid phase and a mobile liquid or gas phase. The separation of a mixture is due to their relative solubilities in the liquid stationary and mobile phase. The greater the attraction a compound has for the stationary phase, the slower it travels.