

Chromatography is the most effective technique for isolating and purifying all types of biomolecules. In addition, it is widely used as an analytical tool to measure quantitative properties.

The mobile phase (gas or liquid) moves the sample through a stationary phase that has ability to “bind” sample.

Thin-layer chromatography (TLC) is a powerful, mainly analytic, technique for rapidly separating lipids and other small molecules such as amino acids, nucleotides, vitamins, and drugs. The *sample* being analyzed is applied to a *plate* made of glass, aluminum, or plastic, which is covered with a thin layer of silica gel or other material. The plate is then placed in a chromatography chamber that contains some *solvent*. Drawn by capillary forces, the solvent moves up the plate. The substances in the sample move with the solvent. The speed at which they move is determined by their distribution between the *stationary phase* (the hydrophilic silica), and the *mobile phase* (the hydrophobic solvent). When the solvent reaches the top edge of the plate, the chromatography is stopped. After evaporation of the solvent, the separated substances can be made visible using appropriate staining methods or with physical processes (e. g., ultraviolet light).

Gel permeation chromatography (“gel filtration”) separates proteins according to their size and shape. This is done using a *chromatography column*, which is filled with spherical *gel particles* (diameter 10–500 μm) of polymeric material. The insides of the particles are traversed by channels that have defined diameters. A protein mixture is then introduced at the upper end of the column and *elution* is carried out by passing a buffer solution through the column. Large protein molecules are unable to penetrate the particles, and therefore pass through the column quickly. Medium-sized and small particles are delayed for longer or shorter periods. The proteins can be collected separately from the *eluate*.