Chromatography and electrophoresis are powerful analytical techniques that both separate a sample into its components and provide a means for determining each component’s concentration. Chromatographic separations utilize the selective partitioning of the sample’s components between a stationary phase that is immobilized within a column and a mobile phase that passes through the column.

The effectiveness of a separation is described by the resolution between two chromatographic bands and is a function of each component’s retention factor, the column’s efficiency, and the column’s selectivity. A solute’s retention factor is a measure of its partitioning into the stationary phase, with larger retention factors corresponding to more strongly retained solutes. The column’s selectivity for two solutes is the ratio of the their retention factors, providing a relative measure of the column’s ability to retain the two solutes. Column efficiency accounts for those factors that cause a solute’s chromatographic band to increase in width during the separation. Column efficiency is defined in terms of the number of theoretical plates and the height of a theoretical plate, the latter of which is a function of a number of parameters, most notably the mobile phases’ flow rate. Chromatographic separations are optimized by either increasing the number of theoretical plates, by increasing the column’s selectivity, or by increasing the solute retention factor.

In gas chromatography the mobile phase is an inert gas and the stationary phase is a nonpolar or polar organic liquid that is either coated on a particulate material and packed into a wide-bore column, or coated on the walls of a narrow-bore capillary column. Gas chromatography is useful for the analysis of volatile components.

In high-performance liquid chromatography the mobile phase is either a nonpolar solvent (normal phase) or a polar solvent (reversed-phase). A stationary phase of opposite polarity, which is bonded to a particulate material, is packed into a wide-bore column. HPLC can be applied to a wider range of samples than GC; however, the separation efficiency for HPLC is not as good as that for GC.

Together, GC and HPLC account for the largest number of chromatographic separations. Other separation techniques, however, find specialized applications. Of particular importance are: ion-exchange chromatography for separating anions and cations; size-exclusion chromatography for separating large molecules; and supercritical fluid chromatography for the analysis of samples that are not easily analyzed by GC or HPLC.

In capillary zone electrophoresis a sample’s components are separated based on their ability to move through a conductive medium under the influence of an applied electric field. Positively charged solutes elute first, with smaller, more highly charged cationic solutes eluting before larger cations of lower charge. Neutral species elute without undergoing further separation. Finally, anions elute last, with smaller, more negatively charged anions being the last to elute. By adding a surfactant, neutral species can be separated by micellar electrokinetic capillary chromatography. Electrophoretic separations also can take advantage of the ability of polymeric gels to separate solutes by size (capillary gel electrophoresis), and the ability of solutes to partition into a stationary phase (capillary electrochromatography). In comparison to GC and HPLC, capillary electrophoresis provides faster and more efficient separations.

### 12.8.1 Key Terms

- adjusted retention time
- adsorption chromatography
- band broadening
- gas–solid chromatography
- general elution problem
- guard column
- packed columns
- partition chromatography
- peak capacity
baseline width
bleed
bonded stationary phase
capillary column
capillary electrochromatography
capillary electrophoresis
capillary gel electrophoresis
capillary zone electrophoresis
chromatogram
chromatography
column chromatography
counter-current extraction
cryogenic focusing
electrokinetic injection
electroosmotic flow
electroosmotic flow velocity
electron capture detector
electropherogram
electrophoresis
electrophoretic mobility
electrophoretic velocity
exclusion limit
flame ionization detector
fronting
gas chromatography
gas–liquid chromatography
gradient elution
headspace sampling
high-performance liquid chromatography
hydrodynamic injection
inclusion limit
ion-exchange chromatography
ion suppressor column
isocratic elution
isothermal
Joule heating
Kovat's retention index
liquid–solid adsorption chromatography
longitudinal diffusion
loop injector
mass spectrometer
mass spectrum
mass transfer
micelle
micellar electrokinetic capillary chromatography
mobile phase
monolithic column
multiple paths
nonretained solutes
normal-phase chromatography
on-column injection
open tubular column
planar chromatography
polarity index
porous-layer open tubular column
purge-and-trap
resolution
retention factor
retention time
reversed-phase chromatography
selectivity factor
single-column ion chromatography
solid-phase microextraction
split injection
splitless injection
stacking
stationary phase
supercritical fluid chromatography
support-coated open tubular column
tailing
temperature programming
theoretical plate
thermal conductivity detector
van Deemter equation
void time
wall-coated open-tubular column
zeta potential

References


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