CONCEPT: HPLC

●_____ (High Performance Liquid Chromatography): separates molecules under *high pressure* & *resolution* columns.

□ Uses automated computerized instrumentation for extremely _____ separation of molecules.

□ _____ resolution columns create more interaction sites & *greater* resolving/separation power.

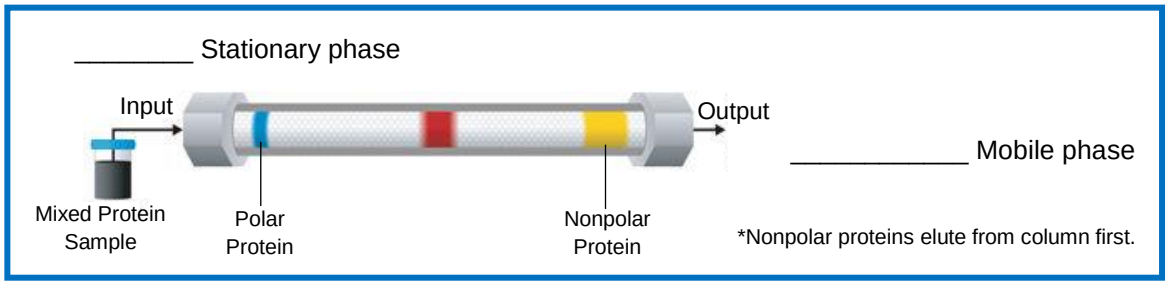
□ High _____ increases the *speed* of the separation through the high-resolution column matrix.

Normal-Phase HPLC Purifies Polar Molecules

●_____ *Phase HPLC*: Stationary phase is _____ and liquid mobile phase is *nonpolar*.

□ *Polar* molecules stay in the column *longer* while _____ molecules elute _____ & earlier.

EXAMPLE: Normal-phase HPLC.



PRACTICE: What is the order of elution (first → last) of the following amino acids in normal-phase HPLC: Phe, Gly, Glu.

a) Phe → Gly → Glu.
b) Phe → Glu → Gly.
c) Glu → Phe → Gly.
d) Glu → Gly → Phe.
e) Gly → Phe → Glu.
f) Gly → Glu → Phe.

Reverse Phase HPLC Purifies Nonpolar Molecules

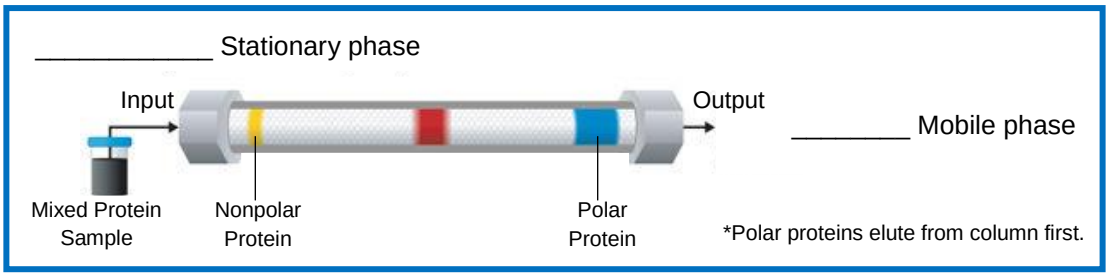
●*Reverse Phase HPLC* is the _____ of normal phase HPLC.

□ A *nonpolar* stationary phase _____ nonpolar molecules in the column via the hydrophobic effect.

□ A *polar* liquid _____ phase flows over the stationary phase.

□ Nonpolar molecules remain in the column longer while more _____ & soluble molecules elute faster & earlier.

EXAMPLE: Reverse-phase HPLC.



PRACTICE: What is the order of elution (first → last) of the following amino acids in reverse-phase HPLC: Ala, Arg, Leu.

a) Leu → Ala → Arg.
b) Leu → Arg → Ala.
c) Arg → Leu → Ala.
d) Arg → Ala → Leu.
e) Ala → Leu → Arg.
f) Ala → Arg → Leu.

CONCEPT: HPLC

PRACTICE: What is the basis for the separation of proteins by the following techniques?

a) Gel-filtration chromatography: _____

b) Affinity chromatography: _____

c) Ion-exchange chromatography: _____

d) Reverse phase HPLC: _____

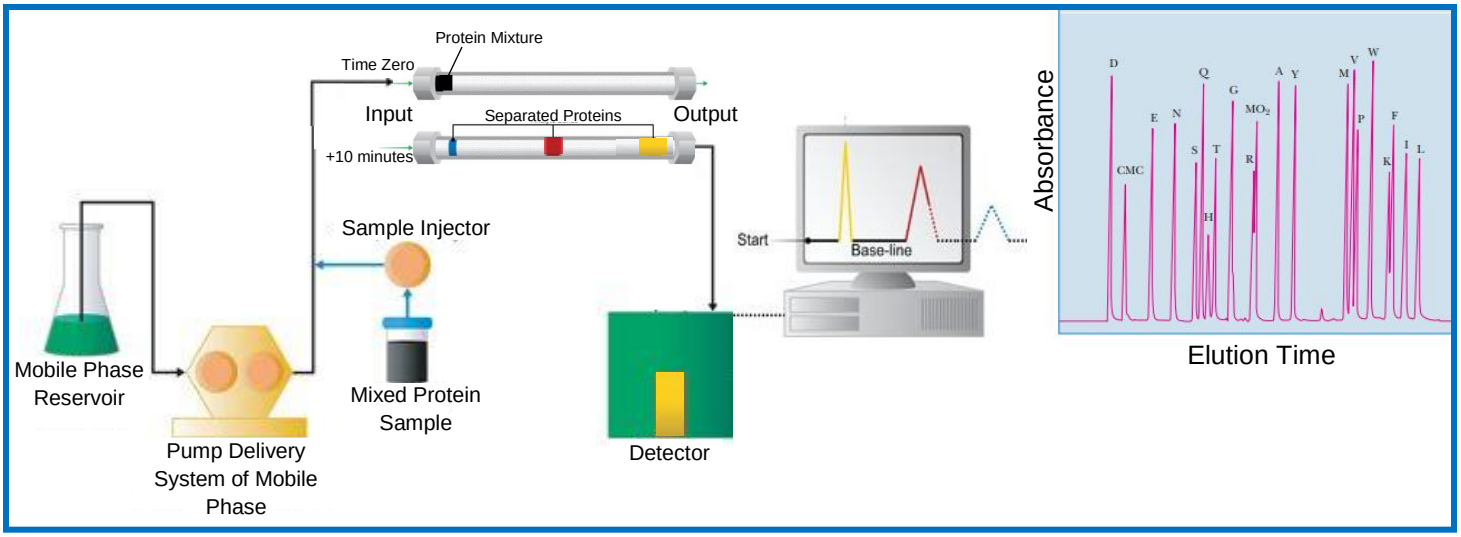
HPLC Chromatogram

●HPLC separation results show up in a data plot called a _____.

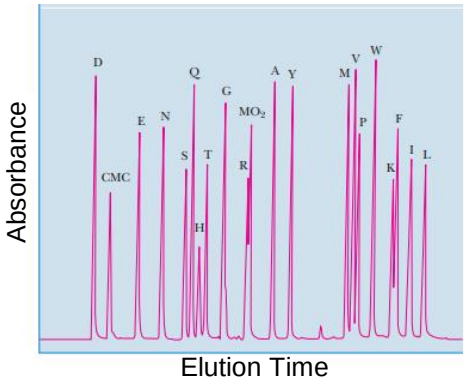
□ Plots elution _____ (X-axis) vs. the light _____ (y-axis) for each separated molecule.

□ Absorbance indicates the _____ of separated molecule.

EXAMPLE: HPLC process & chromatogram.



PRACTICE: In the following HPLC chromatogram, which amino acid was the third substance eluted from the column?



a) Aspartic acid.

b) Leucine.

c) Phenylalanine.

d) Glutamic Acid.