CONCEPT: ANION EXCHANGE CHROMATOGRAPHY

2) Anion Exchange Chromatography

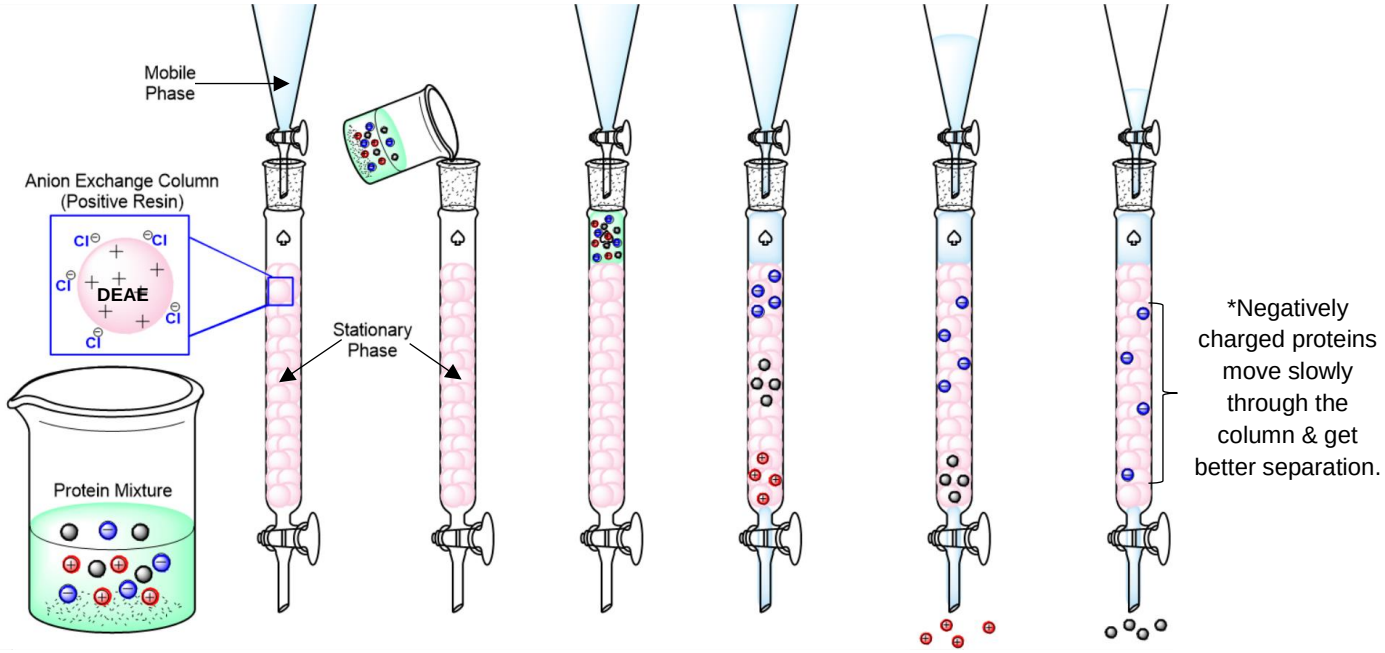
●Anion exchange chromatography is the _____ of cation exchange chromatography:

□ Uses (+) stationary resin like diethylaminoethyl (_____) in the column to collect & purify (-) proteins.

□ Neutral or (+) proteins do not bind the resin & pass quickly through the column.

□ The greater the net positive charge, the faster & _____ the unwanted proteins come out.

EXAMPLE: Anion Exchange Chromatography.



*_____ charged proteins elute from column *first*.

●Negatively charged target protein is later _____ with addition of salt.

EXAMPLE: Circle the peptide below that elutes last during anion-exchange chromatography if the pH is ~7?

Peptide #1: Gly-Ala-Asp-Lys-Glu-Ser or Peptide #2: Leu-Thr-Ile-His-Gly-Arg

PRACTICE: Which amino acid elutes last from an anion-exchange column at physiological pH?

a) Lysine. b) Alanine. c) Glutamate. d) Asparagine. e) Glycine.

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PRACTICE: Use the chart to determine which tripeptide would elute last from an anion-exchange column at pH = 9.3.

a) Tyr-Lys-Met. b) Gly-Pro-Arg. c) Asp-Trp-Tyr. d) Asp-His-Glu.

Ionizable Group	pKR/pKa
Arg	12.5
Asp	3.9
Glu	4.1
His	6
Lys	10.53
Tyr	10.07
α-amino group	8.5
α-carboxyl group	3.5

PRACTICE: Which type of ion exchange chromatography would be best to separate a mixture of histidine and arginine?

His: $pK_1 = 1.8$, $pK_2 = 9.3$, $pK_R = 6.0$ Arg: $pK_1 = 1.8$, $pK_2 = 9.0$, $pK_R = 12.5$

a) Anion-exchange chromatography at pH = 2.

b) Anion-exchange chromatography at pH = 4.

c) Cation-exchange chromatography at pH = 2.

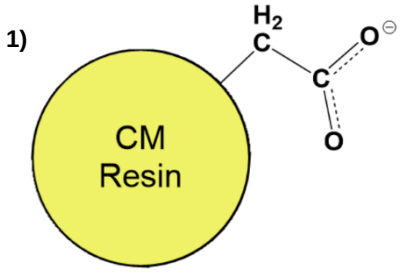
d) Cation-exchange chromatography at pH = 4.

e) Cation-exchange chromatography at pH = 9.

PRACTICE: Stationary resin compounds with carboxymethyl (CM) and diethylaminoethyl (DEAE) groups are shown below. Indicate which one is likely used in a cation exchange column and which one is likely used in an anion exchange column. Considering the following peptide at pH 7, should DEAE or CM groups be used as the stationary resin to purify the peptide?

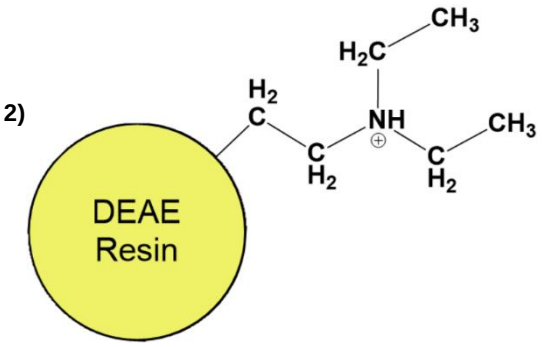
Peptide: G-R-W-K-R-H

1)



Used in _____ exchange columns.

2)



Used in _____ exchange columns.