CONCEPT: ION EXCHANGE CHROMATOGRAPHY

●*Ion Exchange Chromatography*: purifies a protein based on the magnitude of its net _____.

●There are _____ main types of *ion exchange chromatography*:

1) _____ *Exchange Chromatography:* used to purify *positively* charged proteins.

2) _____ *Exchange Chromatography:* used to purify *negatively* charged proteins.

1) Cation Exchange Chromatography

●Uses *negatively* charged stationary resin in the column to collect & purify _____ charged proteins.

□ Example of negative resin: Carboxymethyl (_____) groups.

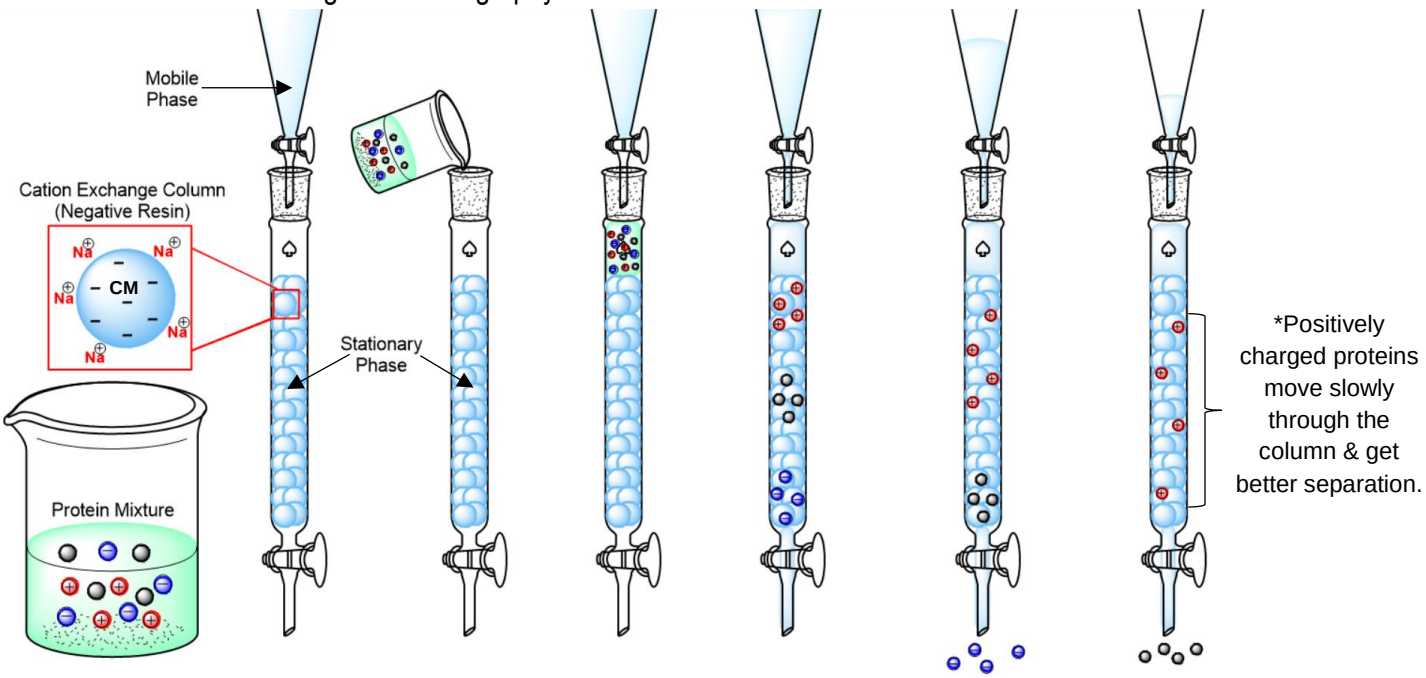
□ _____ (Na^+) are loosely bound to the resin but are _____ with the target protein.

●Positively charged proteins bind to the negatively charged stationary resin & do _____ move through the column.

□ Neutral or _____ charged proteins do not bind the resin & pass through the column.

□ The greater the net negative charge, the _____ & earlier the unwanted proteins come out.

EXAMPLE: Cation Exchange Chromatography.



*_____ charged proteins elute from column *first*.

●Positively charged target protein is later _____ from the column with addition of salt.

EXAMPLE: Which protein elutes first during cation-exchange chromatography?

a) Protein A, net charge = -4.

b) Protein B, net charge = +2.

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PRACTICE: What is the order of elution of the following proteins from a cation-exchange chromatography column?

Net charges of Proteins: Protein A = +1 Protein B = -2 Protein C = -5 Protein D = +3.

a) A → B → C → D. b) D → A → B → C. c) C → B → A → D. d) B → C → D → A.

PRACTICE: In a cation-exchange column at neutral pH, which peptide would elute last?

a) A peptide that contains mostly Asp and Glu residues.
b) A peptide that contains mostly Tyr and Trp residues.
c) A peptide that contains mostly Ala and Gly residues.
d) A peptide that contains mostly Lys and Arg residues.

PRACTICE: Mixtures of amino acids can be analyzed by first separating the mixture into its components through ion exchange chromatography. Certain amino acids placed on a cation-exchange resin containing sulfonate groups (—SO_3^-) flow down the column slowly because of two factors that influence their movement: (1) ionic attraction between the sulfonate residues on the column and positively charged functional groups on the amino acids, and (2) hydrophobic interactions between amino acid R-groups and the strongly hydrophobic backbone of the polystyrene resin. For each pair of amino acids listed below, circle the amino acid that is eluted first from the cation-exchange column by a buffer at pH 7.

a) Asp and Lys. b) Arg and Met. c) Glu and Val. d) Gly and Leu. e) Ser and Ala.

PRACTICE: Give the order of elution of the following peptides when using cation-exchange chromatography at pH 7.2.

Peptide #1: A-D-G-H-E. Peptide #2: K-L-M-R-A. Peptide #3: M-D-L-I-V. Peptide #4: I-L-R-P-M.

Order of Elution: _____, _____, _____, _____

(1st to elute) (Last to elute)