CONCEPT: ISOELECTRIC FOCUSING

●*Isoelectric Focusing* (*IEF*): electrophoresis technique separating proteins only based on _____ points (pI).

□ Recall: pI is the pH where the net charge of the protein is _____.

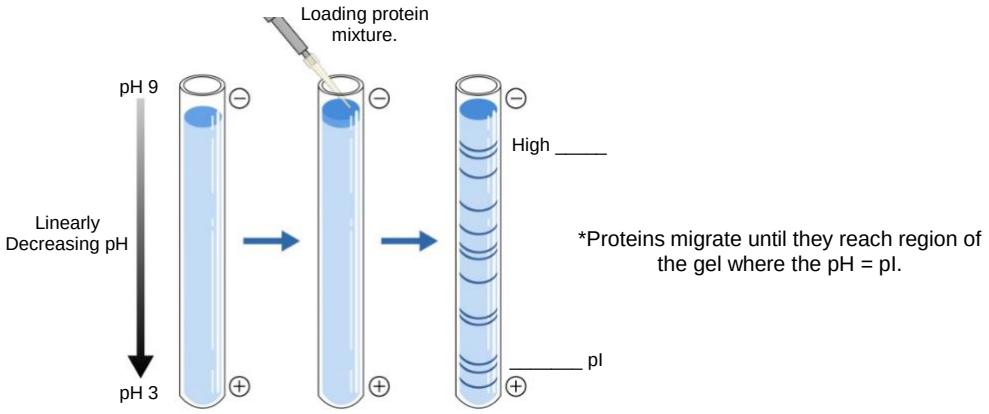
●A stable/immobile _____ gradient is established into the gel.

□ Proteins alter their _____ as they migrate through different regions of the gel with different pH.

□ Proteins continue to migrate until they reach the portion of the gel that has a pH _____ to its pI.

□ When the pH = pI of a protein, it has a *neutral* net charge of *zero* & does _____ migrate in an electric field.

EXAMPLE: Isoelectric focusing (IEF).



PRACTICE: At some point during isoelectric focusing, proteins stop moving through the gel because:

a) The proteins do not have ionized groups at that pH.

b) The proteins have a net charge of zero at that pH.

c) The proteins have a net positive or net negative charge at that pH.

d) Their mass is too large to be moved at that position in the gel.

PRACTICE: Electrophoretic separation at pH 6 of a sample mixture with Peptide #1 (MW 100) Peptide #2 (MW 200) and Peptide #3 (MW 400) would result in which of the following? (Note: the pI of each peptide occurs at pH 6).

a) Peptide #1 would move the farthest.

b) Peptide #2 would move the farthest.

c) Peptide #3 would move the farthest.

d) None of the peptides would move.

PRACTICE: Mark the approximate final position of the following tripeptide on the isoelectric focusing gel: Glu-Met-Asp.

Hint: calculate the isoelectric point of the peptide.

Ionizable Group		pK_a
C-terminus	—COOH	3.5
Asp	—CH(CH$_2$)—COOH	3.9
Glu	—CH(CH$_2$)—CH$_2$—COOH	4.1
N-terminus	—$\overset{\oplus}{N}$H$_3$	8.0