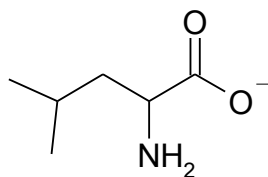


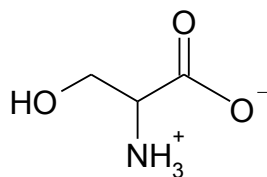
- (1) Give brief definitions or unique descriptions of the following terms:
- (a) oligopeptide
 - (b) stationary phase
 - (c) neutral drift
 - (d) quaternary structure
 - (e) zwitterion
 - (f) salting out
- (2) Consider the 20 amino acids that commonly occur in proteins. Draw a stick diagram of each of the following:
- (a) a non-polar residue at pH 7.0
 - (b) a polar, uncharged residue at pH 4.0
 - (c) a polar, charged residue at pH 10.0
- (3) What are the names and 3-letter abbreviations of the three aromatic amino acids? Draw their structures.
- (4) Which amino acid is not chiral? Draw the structure.
- (5) Show the difference between the two negatively charged amino acids by drawing their structures.
- (6) What is the difference between glutamate and glutamine? Show by drawing the structure.
- (7) Which amino acid has a thiol group? Provide the full name, the 3-letter abbreviation and the structure.
- (8) Describe in words and with structures the difference between Leucine and Isoleucine.
- (9) What is special about proline? Explain and draw the structure.
- (10) Which amino acid has a side chain which is able to accept or donate a proton close to the physiological pH? Provide the name, 3-letter abbreviation, structure and pKa of the side chain.
- (11) Which amino acid has an amine group in its side chain? Provide the name, 3-letter abbreviation, structure at physiological pH and pKa of the side chain.
- (12) Draw the structures of the following amino acids at physiological pH and classify them as polar, aromatic, nonpolar, positively or negatively charged.
- (a) Arginine
 - (b) Tyrosine
 - (c) Valine
 - (d) Asparagine

(13) For the following forms of amino acids, provide the name, the 3-letter abbreviation and indicate in which pH range this would be the predominant form and justify your choice.

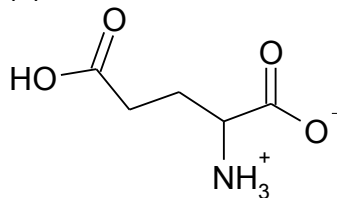
(a)



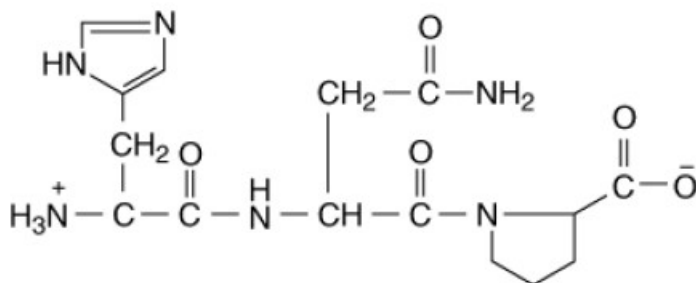
(b)



(c)



(14) Give the full name and the 3-letter abbreviation of the following tripeptide. Indicate the N- and the C-terminus of the structure.



(15) Draw the structure of alanylseryltryptophan at physiological pH.

(16) The Lys-Asp-Gly sequence is a characteristic feature of SH3 domains involved in protein-protein interactions. Calculate the charge associated with a synthetic Lys-Asp-Gly tripeptide sequence under the following conditions: (Note: show your work)

(a) pH 2.2

(b) pH 6.0

(c) pH 11.0

(17) You have titrated the free amino acid Glu with strong acid:

(a) Draw a reasonable titration curve, label the axis and significant transitions

(b) Calculate the isoelectric point

(18) Draw the Lys-Asp-Gly tripeptide at pH 7.0 and draw a box around the peptide bonds.

(19) What is the net charge of the following amino acid? Draw the corresponding structure.

(a) Glutamate at pH 10

(b) Lysine at pH 1.5

(c) Serine at pH 5.8

(d) Isoleucine at pH 1.0

(20) Show the complete reaction equation for formation of Ala-Arg from the individual amino acids at physiological pH (use complete structures).

(21) Draw the structures of the two amino acids containing sulfur and name these with both the full name and the 3-letter abbreviation.

(22) Consider all possible tripeptides made of the amino acids tyrosine, histidine and proline. Answer the following questions.

(a) Give the names of each possible tripeptide using 3-letter code.

(b) Of the tripeptide in question (a), draw the structure of the tripeptide which has a C-terminal proline and a N-terminal tyrosine at pH 1.0

(c) What charge will this tripeptide have at pH 12? Explain.

(23) A mixture of the following dipeptides was applied to a cation exchange column.

Ala-Cys His-Asp Tyr-Glu Leu-Arg Val-Lys

(a) Which dipeptide(s) would bind at pH 8.0

(b) Would the result differ at pH 4.5? Explain your reasoning.

(24) What is the role of 2-mercaptoethanol (β-mercaptoethanol) in SDS-PAGE?

(25) *Vibrio cholera* (causative agent of “cholera”) secretes a protein toxin with an AB₅ quaternary structure. The single “A” subunit has a molecular mass near 22 kDa and each “B” subunit has a mass of near 12 kDa.

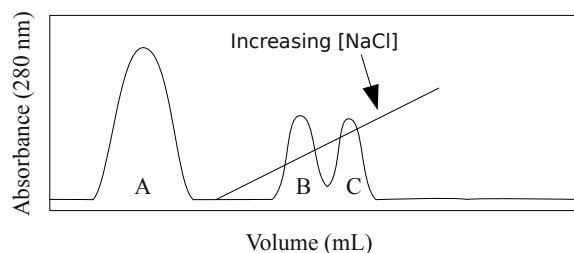
(a) Sketch and label a figure of the result of an SDS-PAGE experiment.

(b) Sketch and label a figure of the result of size exclusion chromatography experiment.

(26) What is the retarding force in size exclusion chromatography?

(27) You have isolated a novel, globular protein. Describe an experiment or set of experiments to prove (or disprove) the protein is composed of more than one subunit.

(28) Consider the following cation exchange chromatogram run at pH 6.0. A mixture of proteins were applied to the column in a low [NaCl] buffer. Proteins retained by the cation exchange column were eluted by gradually increasing the NaCl concentration within the buffer. What can be concluded regarding the pI and relative charge of the protein(s) in each chromatogram peak?



Answers:

(1a) – a short polymer of up to ~20 residues

(1b) – inert, porous material that exerts retarding force

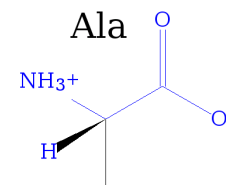
(1c) – random mutations in proteins that do not affect function

(1d) – spatial arrangement of subunits in a multisubunit protein

(1e) – molecule containing both a positive and a negative charged functional group

(1f) – decrease in protein solubility at high salt concentrations (due to competition for solvent)

(2a) – Any of Ala, Val, Leu, Ile, Met, Pro, Phe or Trp (Gly, Cys, Tyr also acceptable) as the L-stereoisomer. At pH 7.0, the α -amino and the α -carboxyl will be fully ionized.
eg. Ala



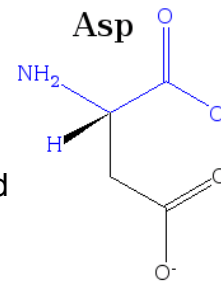
(2b) – Any of Ser, Thr, Asn or Gln (Gly, Cys, Tyr, His also acceptable) as the L-stereoisomer. At pH 4.0, the α -amino and the α -carboxyl will be fully ionized.
eg. Asn



(2c) - Any of Asp, Glu, Arg or Lys (His also acceptable) as the L-stereoisomer. At pH 10.0, the α -amino is largely deprotonated and the α -carboxyl fully ionized.

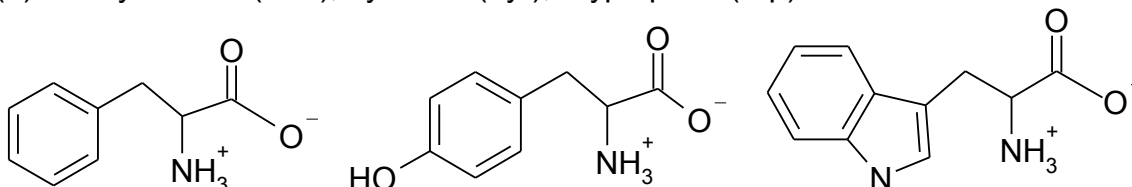
Asp and Glu side chain functional groups are fully deprotonated at pH 10.0. The Lys side chain is largely protonated and Arg is fully protonated at pH 10.0.

eg. Asp

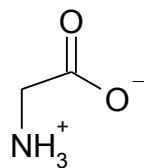
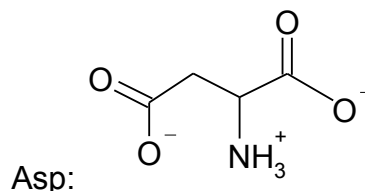
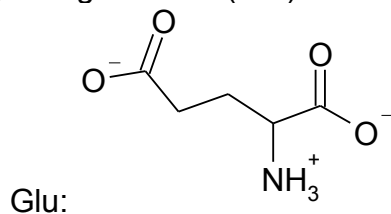


Note: If you are asked to draw an L-amino acid, L-residue or L-peptide, you must show the correct stereoisomer. In all other cases, you may omit the α -hydrogen.

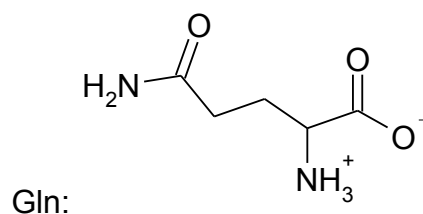
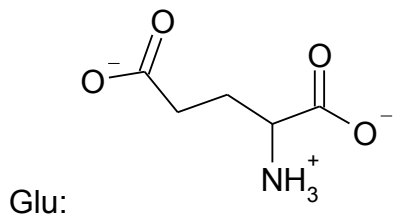
(3) Phenylalanine (Phe), Tyrosine (Tyr), Tryptophan (Trp)



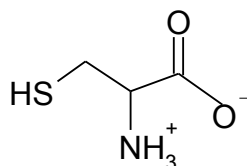
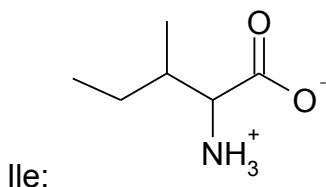
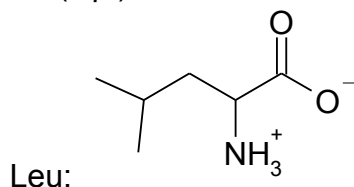
(4) Glycine (Gly)

(5) The glutamate (Glu) side-chain is one $-\text{CH}_2-$ group longer than Aspartate (Asp):

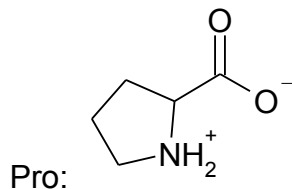
(6) The side chain of glutamate ends with a carboxylate group whereas the side chain of glutamine ends with an amide group:



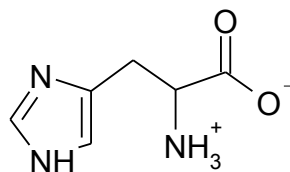
(7) Cysteine (Cys)

(8) Leucine and isoleucine are constitutional isomers. Leucine has a methyl group attached to the second C (C_γ) of its side chain whereas isoleucine has the methyl group attached to the first C (C_β) of its side chain.

(9) In Proline (Pro) the side chain is linked to the backbone amino group to form a cyclic structure. Thus, the backbone amino group is a secondary amine (instead of a primary amine as in the other amino acids) in free proline.



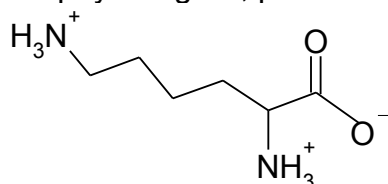
(10) The side chain of Histidine (His) has a pKa of 6.0 and can thus act as an acid or base at physiological pH:



Three acceptable structures of His when no pH is identified – the one above (H on Nε2), its tautomer (H on Nδ1) and the charged form where both N are protonated.

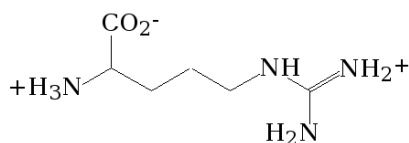
Note: If you are unsure how to answer a question on an exam, ASK the invigilator.

(11) The side chain of Lysine (Lys) contains a primary amine. The side-chain amine is drawn in the physiological, protonated form as its pKa is ~10.5.

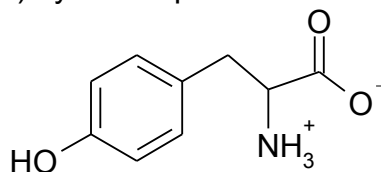


(12)

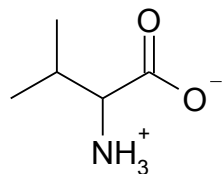
(a) Arg: positively charged



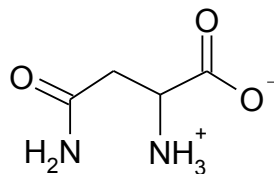
(b) Tyrosine: polar & aromatic



(c) Val: nonpolar (aliphatic)



(d) Asn: polar

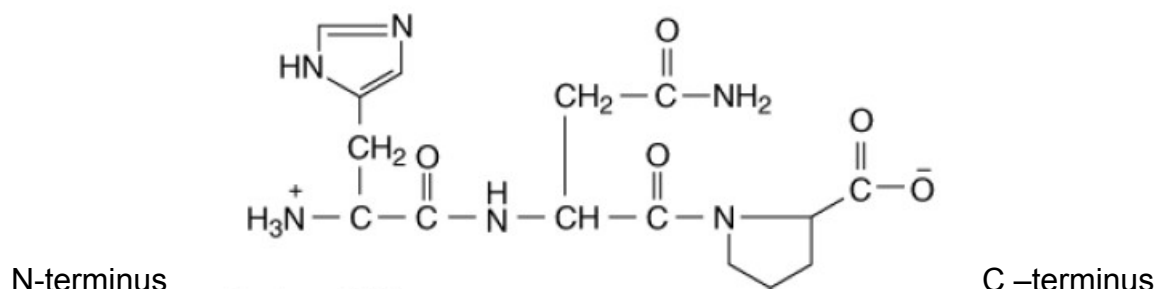


(13) (a) Leucine (Leu): the negatively charged form where the amino group is deprotonated exists at pH higher than 9.4 which is the pKa for the α-amino group.

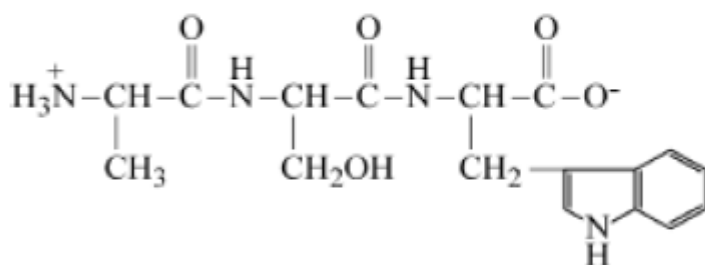
(b) Serine (Ser): the zwitterion with a net charge of zero exist between the pKas for the carboxylate (2.2) and the amino (9.4) group i.e. when the carboxylate group is deprotonated and the amino group is protonated. More precisely, the uncharged zwitterion exists at the isoelectric point pI which is the average of the two pKas, i.e. at pH 5.8.

(c) Glutamate (Glu): this is the primary form of glutamate between pH 2.2 and 4.1. The protonated side chain exists at pHs below its pKa of 4.1 and the deprotonated main chain carboxylate exists at pH higher than its pKa of 2.2.

(14) Histidylasparagylproline = His-Asn-Pro



(15)



(16a) – Using pK_a 's of 2.2 (α -carboxyl), 9.4 (α -amino), 3.9 (Asp side chain) and 10.5 (Lys side chain) the charge as a function of pH is as follows (this is a highly detailed example that is more than required to answer the question):

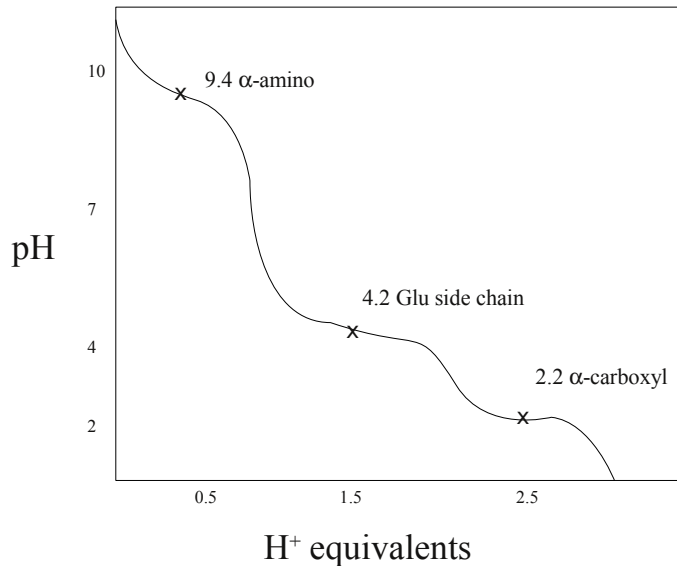
	<2.2	2.2	2.2-3.9	3.9	3.9-9.4	9.4	9.4-10.5	10.5	>10.5
α -carboxyl	0	-0.5	-1	-1	-1	-1	-1	-1	-1
α -amino	+1	+1	+1	+1	+1	+0.5	0	0	0
Asp	0	0	0	-0.5	-1	-1	-1	-1	-1
Lys	+1	+1	+1	+1	+1	+1	+1	+0.5	0
Net Charge	+2	+1.5	+1	+0.5	0	-0.5	-1	-1.5	-2

The net charge at pH 2.2 is +1.5.

(16b) – The net charge at pH 6.0 is 0.

(16c) – The net charge at pH 11.0 is -2.

(17a) -



(17b) -

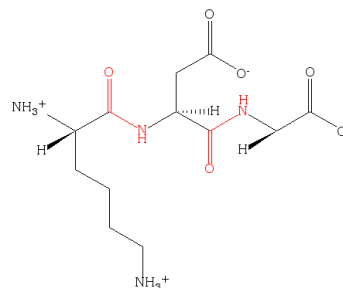
	< 2.2	2.2-4.1	4.1-9.4	> 9.4
α-carboxyl	0	-1	-1	-1
Glu	0	0	-1	-1
α-amino	+1	+1	+1	0
Net Charge	+1	0	-1	-2

The isoelectric point is the pH of the average of the pK_as at either extreme of the zero charge state.

$$(2.2 + 4.1) / 2 = 3.2$$

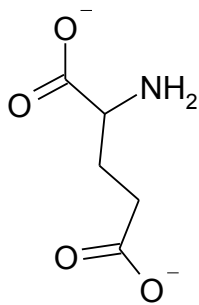
Please Note: In the above table the net charge has been reported as the nearest integer. To be completely accurate, the net charge at pH 2.2 is +0.5, then net charge at pH 4.1 is -0.5 and the net charge at 9.4 is -1.5.

(18) The peptide bonds are shown in red.

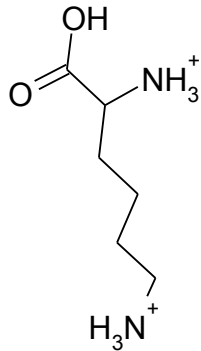


(19)

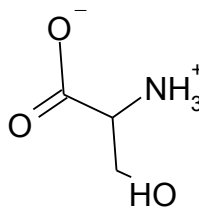
(a) Glu: net charge -2



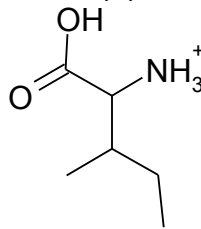
(b) Lys: net charge +2



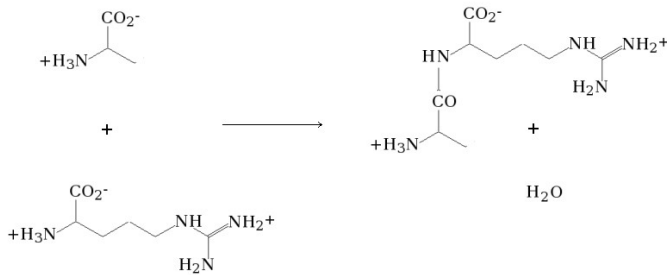
(c) Ser: net charge 0



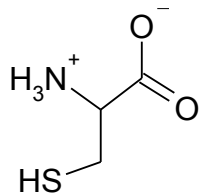
(d) Ile: net charge +1



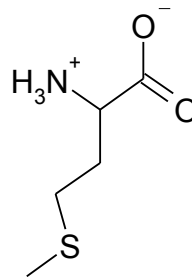
(20) Ala + Arg → Ala-Arg + H₂O



(21) Cysteine (Cys)



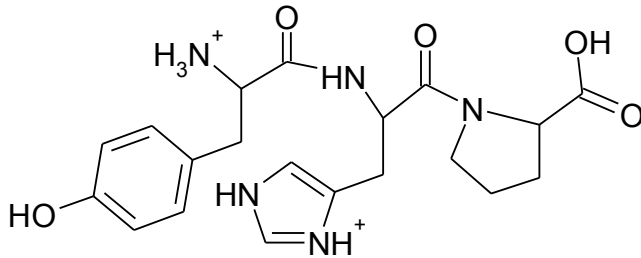
Methionine (Met)



(22a) There are 6 different tripeptides with this composition:

Tyr-His-Pro, Tyr-Pro-His, His-Pro-Tyr, His-Tyr-Pro, Pro-Tyr-His, Pro-His-Tyr

(22b)



(22c) At pH 12, this peptide will have a net charge of -2. pH 12 is higher than all pK_as of side chains (His, Tyr) and main chain carboxylate and amino groups. Thus, all groups will be deprotonated. The C-terminal carboxylate group and Tyr will have a negative charge and the other groups will be uncharged.

(23a) Cation exchange columns will typically bind dipeptides with a net positive charge. At pH 8.0, only the Leu-Arg and Val-Lys dipeptides have a net positive charge.

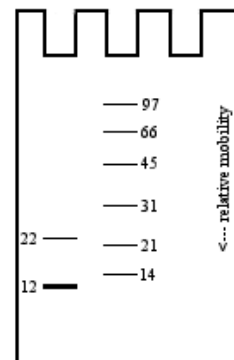
(23b) At pH 4.5, only the Leu-Arg and Val-Lys dipeptides will have a positive net charge. There will be no change in the results.

(24) 2-mercaptoethanol is a reducing agent that breaks disulfide bonds (cystines) into free thiols (cysteines). This breaks covalent attachments between subunits in multisubunit proteins.

(25a)

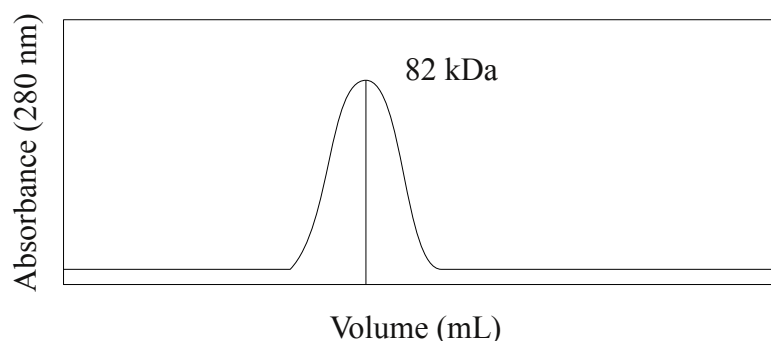
The size information and relative intensity are required while the molecular weight markers in lane 2 are not.

SDS-PAGE denatures the protein structure and 2-mercaptoethanol breaks any disulfide bonds. As a result, each polypeptide chain migrates according to its size.



(25b) The label and determined size of the peak are all required.

SEC does not disrupt the quaternary structure of the protein.



(26) Each bead of the stationary phase contains pores with a defined minimum and maximum size. Solute that diffuses into a bead flows more slowly through the column as the pores restrict flow down the column.

(27) The unknown protein must be exposed to conditions that (i) disrupt quaternary structure and (ii) maintain quaternary structure.

(i) SDS-PAGE in the presence of reducing agent (2-mercaptoethanol) will denature the protein and break any disulfide bonds. There are two possible results:

case 1: two or more bands are present and indicate the protein has multiple subunits

case 2: one band is present. This is an inconclusive result as the sample may have one subunit or be composed of multiple identical subunits

(ii) To resolve case 2, use SEC in the absence of denaturant:

If the mass determined by SEC is significantly larger (an \sim integer multiple) than that determined by SDS-PAGE the sample has multiple subunits. Otherwise the sample contains a single polypeptide chain.

(28) Peak A – The protein(s) do not bind to the cation exchange column indicating their pI is likely 6.0 or less and their net charge is neutral or negative at pH 6.0.

(eg. A protein has a net charge of 0 at pH 5.0 ($pI = 5.0$); as the pH is raised (less H^+) its charge will be negative)

Peak B – The protein(s) bind to the cation exchange column indicating the pI is likely greater than 6.0 and has a net positive charge at pH 6.0.

(eg. A protein has a net charge of 0 at pH 7.0 ($pI = 7.0$); as the pH is lowered (more H^+) its charge will be positive)

Peak C – The protein binds to the cation exchange column indicating the pI is likely greater than 6.0 and has a net positive charge at pH 6.0. The net positive charge of Peak C is likely greater than that of Peak B as it requires higher salt concentration to elute.

Note – It does not follow that Peak C has a higher pI than Peak B.