Consider a hexapeptide with the following composition of amino acids:
The N-terminal residue has the shortest side chain (R group) containing 2 methyl groups.
The next residue's side chain contains an aromatic ring that can be phosphorylated.
The next residue has a nonpolar aliphatic side chain containing only methylene groups.
The next residue's side chain is readily oxidized to form covalent bonds.
The next residue has the shortest side chain that is negatively charged at neutral pH.
Lastly, the C-terminal residue has the longest side chain with an amido group.

Answer the following questions. Use the pKa values for R groups from Table 3-1 in Lehninger or from the lecture slides. You can assume the "common" values for the terminal carboxyl and amino groups: pK_1=2.3 and pK_2=9.7, respectively.

(A) Write the amino-acid sequence of this peptide in 3-letter and 1-letter abbreviations.

Val-Tyr-Pro-Cys-Asp-Gln or VYPCDQ

(B) What is the total charge on the peptide at pH = 12?

The pH is above all the relevant pKa values for this peptide, so all ionizable groups are deprotonated. In the deprotonated state only the carboxy-terminus and the side chains of Cys, Tyr, and Asp are charged, with the total charge = - 4 (see also the pI calculation table below)

(C) Estimate the isoelectric point of this peptide. Show the equation you used and briefly explain your reasoning.

pI = $(pK_1+pK_{Asp})/2$ = 2.975 (see the pI calculation schematics below)

(D) What fraction of the peptide molecules is in the zwitterionic state at the isoelectric point? Show your calculation and briefly explain your reasoning.

$$fraction = \frac{[zw]}{[total]} \approx \frac{1}{1+1/10^{pI-pK_1}+1\times10^{pI-pK_{Asp}}} = \frac{1}{1+2\times10^{pI-pK_{Asp}}} \approx 0.703$$

(see the calculations below the pI schematics)

(E) You want to use ion-exchange chromatography to purify this peptide by retaining it on the column. Do you need cation or anion column for this if the peptide is in TRIS buffer at pH=8.2? Explain your reasoning.

Since this pH is above the isoelectric point, the peptide will have a negative charge (actually, -1.5 on average, because the pH = pK_{Cys}), and therefore will stick to positively charged beads. Thus, an anion exchange column should be used in order to retain the peptide on the column.

The charge states of the hexapeptide:

NH_3^+-Val-Tyr-Pro-Cys-Asp-Gln-COO^-

pK_2=9.7 pK_{Tyr}=10.07 pK_{Cys}=8.18 pK_{Asp}=3.65 pK_1=2.3

pK_a	pH	NH_3^+	Tyr	Cys	Asp	COO^-	Total Charge	
	1	+	0	0	0	0	+1	
2.3						pK_1		
	3	+	0	0	0	-	0	← zw
3.65					pK_{Asp}			
	6	+	0	0	-	-	-1	
8.18								
	9	+	0	-	-	-	-2	
9.7								
	9.9	0	0	-	-	-	-3	
10.07								
	12	0	-	-	-	-	-4	

$$pI = (pK_1 + pK_{Asp})/2 = 2.975$$

Calculation of the peptide fraction in zwitterionic state.

Let's first account for all possible charge states of the peptide:

[+1] + [zw] + [-1] + [-2] + [-3] + [-4] + [0] = [total] (Eq.1)

Here [+1], [zw] etc are molar concentrations of the corresponding charge states/species of the peptide (see the pI calculation schematics). [0] corresponds to the nonionic state; as we discussed in class, the concentration of this species is practically negligible, and I will ignore it later. Now let's use the Henderson-Hasselbalch equation to relate the concentrations of the two species that are the conjugate acid and base and make a quick assessment if some of them can be neglected:

$\frac{[zw]}{[+1]} = 10^{pI-pK_1}$, and from here I get $[+1] = [zw]/10^{pI-pK_1}$. Note that $10^{pI-pK_1} \approx 4.7$, so [+1] is smaller than [zw] but not negligibly smaller.

$\frac{[-1]}{[zw]} = 10^{pI-pK_{Asp}}$ ➔ $[-1] = [zw] \times 10^{pI-pK_{Asp}}$; Likewise, because $10^{pI-pK_{Asp}} \approx 0.21$, [-1] < [zw] but is not negligible.

$\frac{[-2]}{[-1]} = 10^{pI-pK_{Cys}}$ ➔ $[-2] = [-1]\times 10^{pI-pK_{Cys}} = [-1]\times 10^{-5.2} \ll [-1]$, so [-2] is practically negligible compared to [-1] and, by extension, compared to [zw].

$\frac{[-3]}{[-2]} = 10^{pI-pK_{NH_3}}$ ➔ $[-3] = [-2]\times 10^{pI-pK_{NH_3}} = [-2]\times 10^{-6.7} \ll [-2]$, so [-3] is also negligible

$\frac{[-4]}{[-3]} = 10^{pI-pK_{Tyr}}$ ➔ $[-4] = [-3]\times 10^{pI-pK_{Tyr}} = [-3]\times 10^{-7.1} \ll [-3]$, so [-4] is also negligible

Therefore, if I remove all the negligible terms from Eq.1, I get

[+1] + [zw] + [-1] ≈ [total]

And if I now use the above equations that relate [+1] and [-1] to [zw], I get

$$[zw](1+1/10^{pI-pK_1}+1\times 10^{pI-pK_{Asp}}) = [total]$$

Solving it for [zw] gives

$$fraction = \frac{[zw]}{[total]} = \frac{1}{1+1/10^{pI-pK_1}+1\times 10^{pI-pK_{Asp}}} = \frac{1}{1+2\times 10^{pI-pK_{Asp}}} \approx 0.703$$

(note that $1/10^{pI-pK_1} = 10^{pI-pK_{Asp}}$ because $pI = \left(pK_1 + pK_{Asp}\right)/2$).

Above I discarded the negligible terms – and this simplified the calculation. Of course, you can keep those terms in Eq.1. For this, you can use the above equations relating [-2] to [-1] and [-1] to [zw] to relate [-2] to [zw], and similarly relate [-3] and [-4] to [zw], and put all the terms into Eg.1. That will result in 3 more terms containing products of 10^{pI-pK_a} in the denominator of the final equation, but because these terms have negligible values they will not affect the answer.

***For those who are curious, here is how you can do this, step by step:

$$[-2] = [-1]\times 10^{pI-pK_{Cys}} = [zw]\times 10^{pI-pK_{Asp}}\times 10^{pI-pK_{Cys}}$$

$$[-3] = [-2]\times 10^{pI-pK_{NH_3}} = [zw]\times 10^{pI-pK_{Asp}}\times 10^{pI-pK_{Cys}}\times 10^{pI-pK_{NH_3}}$$

$$[-4] = [-3]\times 10^{pI-pK_{Tyr}} = [zw]\times 10^{pI-pK_{Asp}}\times 10^{pI-pK_{Cys}}\times 10^{pI-pK_{NH_3}}\times 10^{pI-pK_{Tyr}}$$

and substituting these results together with the above equations for [+1] and [-1] into Eq.1, gives the complete equation that you can then solve for [zw]:

$$[zw](1+1/10^{pI-pK_1}+1\times10^{pI-pK_{Asp}}+10^{pI-pK_{Asp}}\times10^{pI-pK_{Cys}}+10^{pI-pK_{Asp}}\times10^{pI-pK_{Cys}}\times10^{pI-pK_{NH_3}}+$$
$$+10^{pI-pK_{Asp}}\times10^{pI-pK_{Cys}}\times10^{pI-pK_{NH_3}}\times10^{pI-pK_{Tyr}})=[total]$$