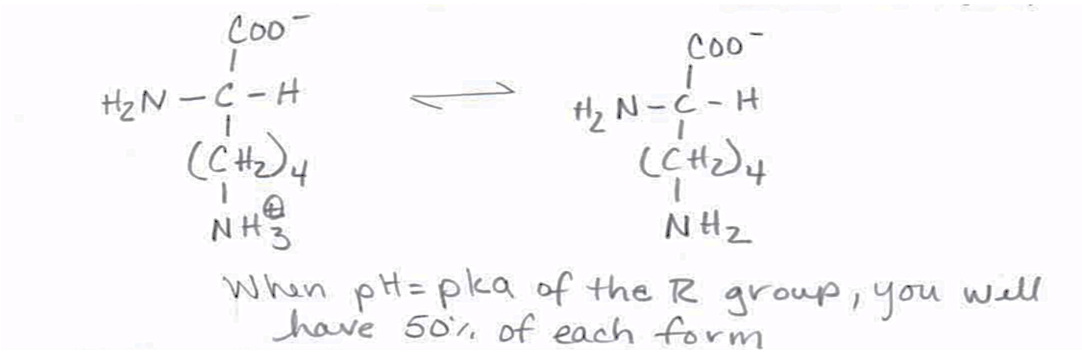
Q1: Draw the form(s) of the amino acid lysine that would exist at pH 10.8.

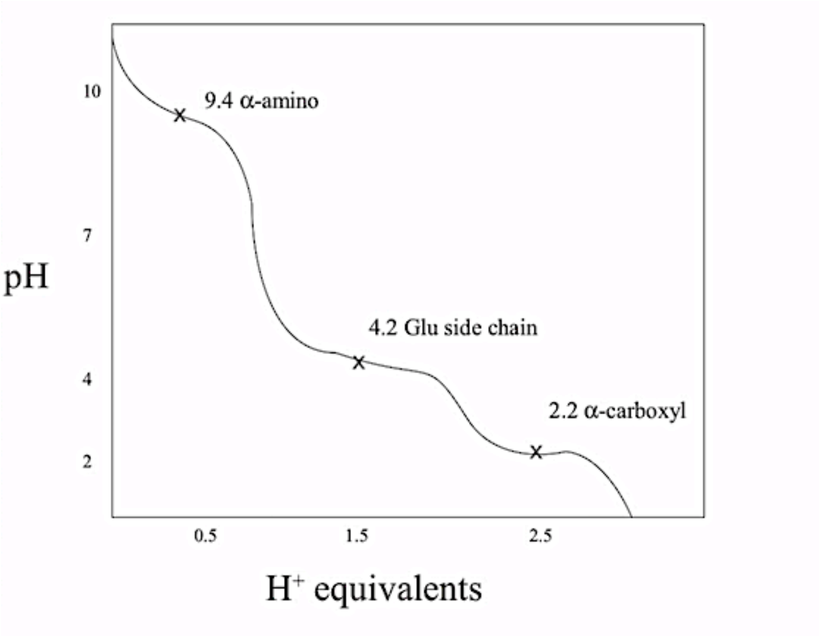
In what relative percentage will these forms exist? Why?

pKa_1= 2.18 , pKa_2 = 8.95 , and pKa_3=10.53



Q2: You have titrated the a.a. -glutamic acid- with strong acid:

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



b) Calculate the isoelectric point ***$pKa_{\alpha\text{-COOH}}$= 2.19 , $pKa_{\alpha\text{-amino}}$ = 9.67 , and $pKa_{R\text{-COOH}}$=4.25***

$pI = (pKa_{\alpha\text{-COOH}} + pKa_{R\text{-COOH}})/2$

$pI = (2.19 + 4.25)/2 = 3.2$

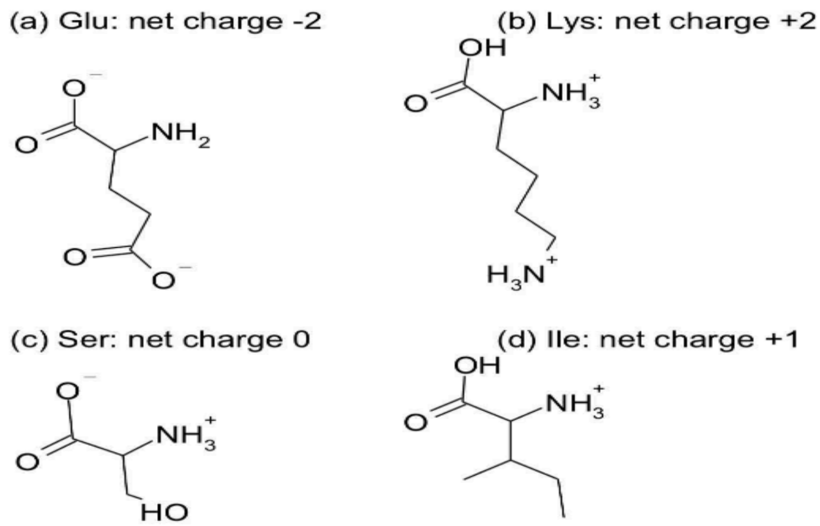
Q3: What is the net charge of the following amino acids:

a) Glutamate at pH 10 ***pKa_1= 2.19 , pKa_2 = 4.25 , and pKa_3=9.67***

b) Lysine at pH 1.5 ***pKa_1= 2.18 , pKa_2 = 8.95 , and pKa_3=10.53***

c) Serine at pH 5.8 ***pKa_1= 2.21 , pKa_2 = 9.15***

d) Isoleucine at pH 1.0 ***pKa_1= 2.36 , pKa_2 = 9.68***



Q4: Choose the correct answer:

<u>**A)**</u> The amino acid arginine contains a guanidino R-group and has pKa values of 2.2, 9.0, and 12.5. A sample of arginine is titrated from pH=1.0 to pH=14.0 with NaOH.

1._____ At pH=2.2,

a) all of the amino acid molecules will be in the fully protonated form.

<u>**b) half of the amino acid molecules will be in the fully protonated form.**</u>

c) all of the amino acid molecules will be in the zwitterion form.

d) half of the amino acid molecules will be in the zwitterion form.

2._____ At pH=12.5,

a) half the amino acid molecules have a -2 charge.

b) all the amino acid molecules have a -2 charge.

<u>**c) half the amino acid molecules have a -1 charge.**</u>

d) all the amino acid molecules have a -1 charge.

3._____ For arginine molecules at pH=14,

a) all the ionizable groups will be charged.

b) all the guanidino groups will be charged.

c) all the amino groups will be charged.

d) all the carboxyl groups will be charged.

4_____ What is the isoelectric point of arginine?

a) 5.60

b) 7.00

c) 7.90

d) 10.75

5._____ A solution with a pH of 2.2 contains 6.0 mmol of arginine. If 12.0 mmol of NaOH is added to the solution, what will be the pH after the NaOH has completely reacted with the arginine?

a) 14.00

b) 12.50

c) 10.75

d) 5.60

Explanation:

* pH 2.2 = pKa 1 → half **α-COOH** is ionized.

* 6.0 mmol of arginine **+ 6 mmol of NaOH** →all **α-COOH** and half **α-NH_3 are ionized (pH =pKa2)**

* **+ 6 mmol of NaOH** → all **α-COOH**, all **α-NH_3 are ionized and half R-NH_3 are ionized (pH = pKa3 = 12.5)**

B) The amino acid tyrosine contains a phenolic R-group and has pKa values of 2.2, 9.0, and 10.2. A sample of tyrosine is titrated from pH = 1.0 to pH = 14.0 with NaOH.

1._____ At which pH will all the amino acid molecules be in their fully protonated form?

a) 1.0

b) 2.2

c) 5.6

d) 9.0

2._____ At which pH will half the amino acid molecules be in their zwitterion form?

a) 5.6

b) 9.0

c) 9.6

d) 10.2

3._____ At which pH will all the amino acid molecules have a -1 charge?

a) 12.0

b) 10.2

c) 9.6

d) 5.6

4._____ For a solution of tyrosine molecules at pH = 10.2

a) all the α-carboxyl groups will be uncharged.

b) all the α-amino groups will be uncharged.

c) all the phenolic R-groups will be uncharged.

d) all the ionizable groups will be uncharged.

Q4: Below is a table prepared by a biochemistry student to construct a standard curve for protein analysis. The Bradford assay was used with bovine serum albumin (BSA, 0.1mg/ml), as standard protein . Complete the table by filling in the approximate A_{595} that will be obtained for each tube. Assume the procedure was conducted correctly.

Reagents	1	2	3	4	5	6
H_2O (ml)	1.0	0.9	0.8	0.6	0.2	------
BSA volume (ml)	-----	0.1	0.2	0.4	0.8	1.0
Bradford reagent (ml)	5.0	5.0	5.0	5.0	5.0	5.0
A_{595}		0.08	? **(0.16)**	? **(0.32)**	? **(0.64)**	? **(0.8)**

Known BSA volume → Known Absorbance

0.1 → **0.08**

0.2 → ?

Absorbance of tube 3 = (0.2 x 0.08) /0.1 = 0.16

Q5: Calculate the absorbance and the transmission at 260 nm and 340 nm of the following solutions in a 1 cm cuvette.

a) 2.2×10^{-5} M NADH

b) 7×10^{-6} M NADH plus 4.2×10^{-5} M ATP.

	a_m	
	260nm	**340nm**
NADH	15000	6220
ATP	15400	0.0

a) This solution contains one absorbing substance (NADH)

$A_{260} = a_m \times C \times l$

$A_{260} = 15000 \times (2.2 \times 10^{-5}) \times 1 = 0.33$

$A = \text{Log } I_\circ / I$

$0.33 = \log 1.0 - \log I$

$0.33 = -\log I$

$I = \text{antilog} - 0.33 = 0.464$

Absorbance and transmission at 340nm

$A = 6220 \times 2.2 \times 10^{-5} \times 1 = 0.1368$

$A = \text{Log } I_\circ / I$

$0.1368 = \log 1.0 - \log I$

$0.1368 = -\log I$

$I = \text{antilog } -0.1368$

$I = 0.729$

b) The solution contains two absorbing substances

At 260nm → $A = A_{NADH} + A_{ATP}$

$A_{NADH} = 15000 \times (7 \times 10^{-6}) \times 1 = 0.105$

$A_{ATP} = 15400 \times (4.2 \times 10^{-5}) \times 1 = 0.646$

$A_{Total} = 0.105 + 0.646 = 0.751$

$A = \text{Log } I_\circ / I = 0.751 = \log 1.0 - \log I$

$0.751 = -\log I$, $I = \text{antilog } - 0.751$, $I = 0.177$.

At 340 nm only NADH absorbs

$A = 6220 \times (7 \times 10^{-6}) \times 1 = 0.043$

$A = \text{Log } I_\circ / I = 0.043 = \log 1.0 - \log I$, so $I = \text{antilog } - 0.043$, $I = 0.905$

Q6: Calculate the concentration of ATP and NADPH in solutions with absorbance's:

a) 0.15 at 340 nm and 0.9 at 260 nm.

b) Zero at 340 nm and 0.750 at 260 nm.

c) 0.22 at 340 nm and 0.531 at 260 nm.

	a_m	
	260nm	**340nm**
NADPH	15000	6220
ATP	15400	0.0

a) Since this solution contains two absorbing substances , thus we will start with absorbance at 340nm since only NADPH absorbs

$A_{340nm} = A_{NADPH}$ only

$A = a_m \times C \times l = 6220 \times C \times 1$

$C = 0.15 / 6220 = 2.4 \times 10^{-5}$ M

$A_{260nm} = A_{ATP} + A_{NADPH}$

$A_{NADPH} = a_m \times C \times l = 15000 \times 2.4 \times ^{10-5} \times 1 = 0.36$

$A_{ATP} = A_{Total} - A_{NADPH} = 0.9 - 0.36 = 0.54$

$A_{ATP} = a_m \times C \times l = 0.54 = 15400 \times C \times 1$

$C = 0.54 / 15400 = 3.5 \times 10^{-5}$ M

b) Since Absorbance at 340 nm is zero , and NADPH is the only absorbing substance at that wavelength thus the concentration of NADPH is zero

Accordingly, the absorbance 0.75 at 260 nm is the absorbance of ATP only.

$A_{ATP} = a_m \times C \times l \rightarrow C_{ATP} = 0.751 / 15400 = 4.8 \times 10^{-5}$ M

c) At 340nm only NADPH absorbs

$0.22 = 6220 \times C \times 1$

$C_{NADPH} = 0.22 / 6220 = 3.5 \times 10^{-5}$ M

At 260nm both ATP and NADPH absorb

Thus $A = A_{ATP} + A_{NADPH}$

$A_{NADPH} = 15000 \times (3.5 \times 10^{-5}) \times 1 = 0.53$

Since $A_{NADPH} = A_{total} \rightarrow$ Thus ATP concentration must be Zero