

# BL/CH 401 Lecture #3 - Acids, Bases, and Buffers

## 1. Principle of Ionization of Weak Acids:

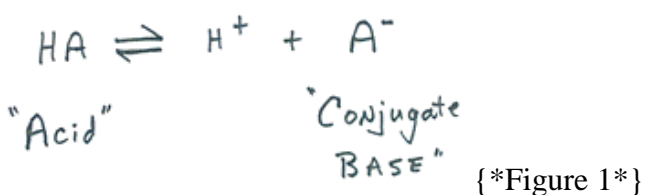
The Fundamental Concept of Buffers is: **A Buffer Resists Change**

pH buffers resist change in pH when either acid (H<sup>+</sup>) or base (OH<sup>-</sup>) is added to it.

Chemicals which are pH buffers are **weak acids or bases**

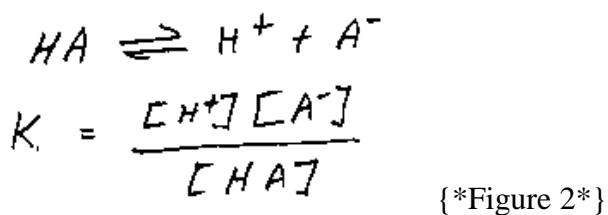
Acids = Proton (H<sup>+</sup>) donors

Bases = Proton Acceptors



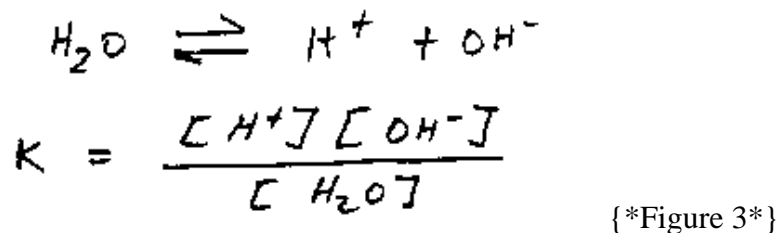
Acids and their conjugate bases are in equilibrium. Since equilibria are related to the properties of the reactants and products, so for weak acids, the tendency to give up its proton determines its buffering property.

This **tendency** to ionize can be put in terms of an equation for the equilibrium:



Where [ ] = Molar concentration; K = Ionization constant

Simplest example is water (H<sub>2</sub>O):



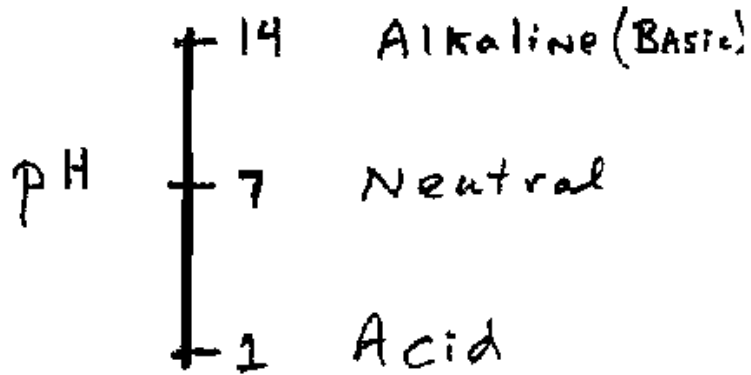
But since [H<sub>2</sub>O] (water concentration) = Constant (55.5 M), K<sub>w</sub> = [H<sup>+</sup>][OH<sup>-</sup>] = 10<sup>-14</sup> M

In pure water, [H<sup>+</sup>] = [OH<sup>-</sup>] = 10<sup>-7</sup> M

To make this easier to use, the pH scale was invented.

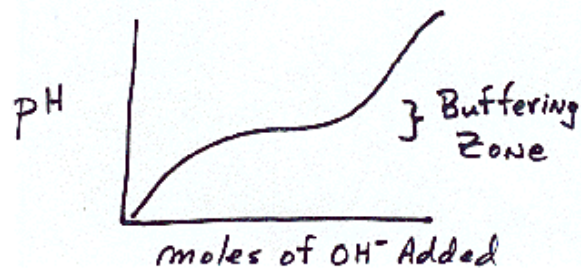
$\text{pH} = -\log [\text{H}^+]$ ; thus when  $[\text{H}^+] = 10^{-7} \text{ M}$ ,  $\text{pH} = 7$

This is called **Neutral pH** because it is in the middle of the pH scale. At pH greater than neutral, the solution is alkaline; while at pH less than neutral, the solution is acid.



{\*Figure 4\*}

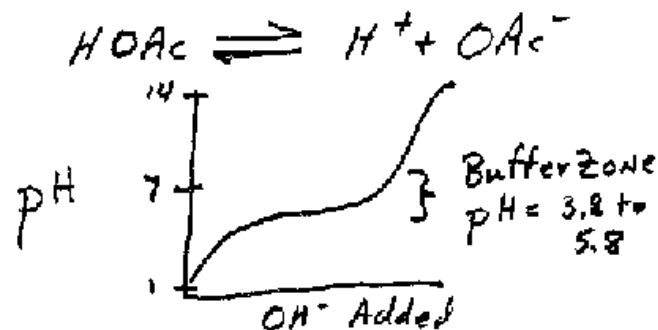
## 2. Titration of a Weak Acid illustrating its Ionization and Buffering Property:



{\*Figure 5\*}

All weak acids have titration curves like this one. Bases (like ammonium,  $\text{NH}_4^+$ ) are also **weak acids** and have similar titration curves. The position where the **Buffering zone** is on the pH scale is related to the chemical nature of the **weak acid**:

Acetic acid ionizes in the **Acidic** portion of the pH scale



{\*Figure 6\*}

Titration curves can also be put in the form of an equation:

$$pH = pK + \log \frac{[A^-]}{[HA]}$$

for  $HA \rightleftharpoons H^+ + A^-$



{\*Figure 7\*}

The pK of a weak acid is the pH where  $[A^-] = [HA]$

At pH below the pK,  $[HA] > [A^-]$

At pH above the pK,  $[HA] < [A^-]$

Therefore the pK determines the buffering zone for a weak acid.

For example, Acetic Acid has a pK = 4.8 and a buffering zone from pH 3.8 to 5.8.

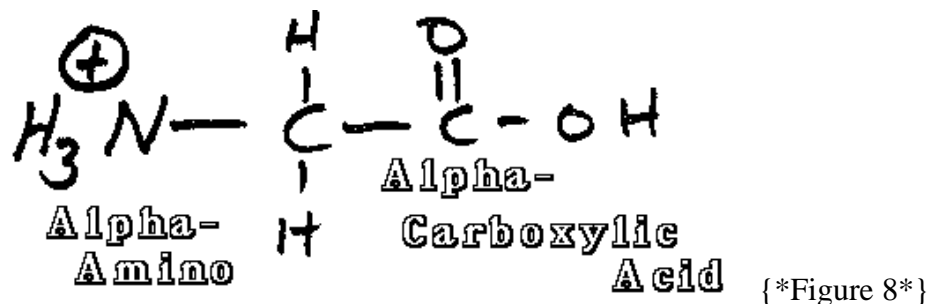
So a weak acid will be an effective buffer at pH = pK +/- 1 pH unit.

To buffer at a specific pH, use a weak acid with a pK near the pH you want to maintain.

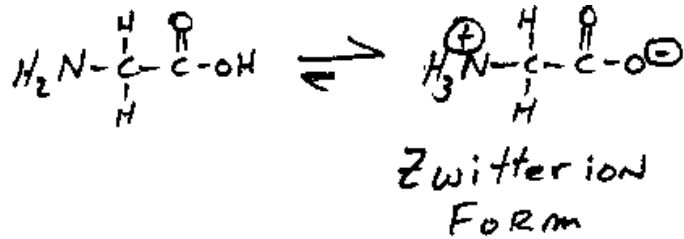
### 3. Ionization of Simple Amino Acids:

**Amino acids are more complicated than simple weak acids since amino acids have at least 2 ionizing groups.**

Glycine (abbreviation is Gly), for example, has both a carboxylic acid and an amino group that can ionize:



If we dissolve the free base of Gly in pure water (ie neutral pH), it will ionize by protonating itself.

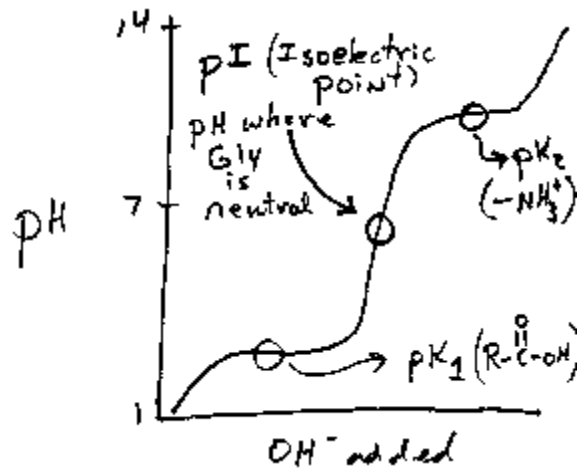


{\*Figure 9\*}

The equilibrium is far to the right so most of the Gly is in the charged form called the Zwitterion and

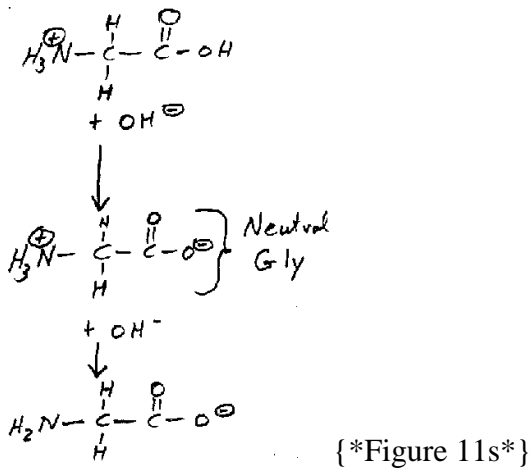
**Gly is still neutral because the + charge is neutralized by the - charge.** Gly is always in the Zwitterion form at neutral pH.

Now if we put Gly at an acid pH where it is fully protonated (ie. it has all the protons bound to it which it bind), we can titrate it to reveal its 2 pK values for the alpha-carboxylic acid group and the alpha-amino group.

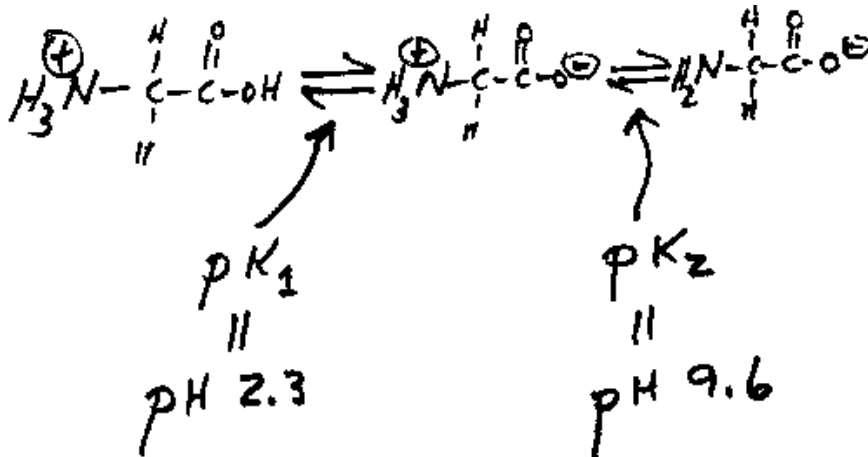


{\*Figure 10\*}

Here is what happens to glycine when titrated:



Another way to write this:



{\*Figure 12\*}

From the pK values, the pI (called the isoelectric point or the place where Gly has no net charge) can be calculated

$$\text{pI} = \frac{2.3 + 9.6}{2} \approx 6$$

{\*Figure 13\*}

Glycine is neutral at pH 6; it has no net charge here.

#### 4. Ionization Rules 1:

**How do you tell which group (carboxylic acid or amino) on Gly ionizes first?**

It depends on the chemical nature of the group.

#### GENERAL RULES:

1. Alpha Carboxylic acids ionize at acidic pH and have pKs less than 6; So in titrating a fully

protonated amino acid, carboxylic acids lose the proton first

2. Alpha Amino groups ionize at basic pH and have pKs greater than 8; So after acids lose their protons, amino groups lose their proton second.

### 5. Ionization of Complex Amino Acids:

Most of the 20 amino acids are like Gly in their ionization properties because their side chains do not ionize at biological pHs.

However, 5 amino acids have side chains that ionize (really 2 others do also but we ignore them).

---

The 5 Complex Amino Acids are:

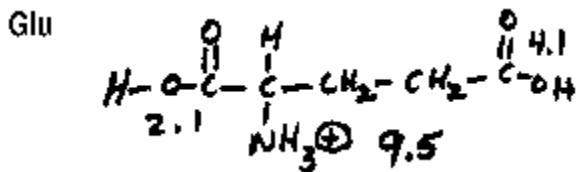
glutamic acid (Glu), aspartic acid (Asp), lysine (Lys), arginine (Arg) and histidine (His).

Each of these 5 amino acids has 3 ionizable groups and therefore, 3 pKs.

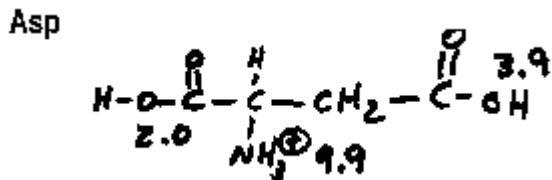
---

The pKs for these 5 amino acids are shown below in the order they ionize:

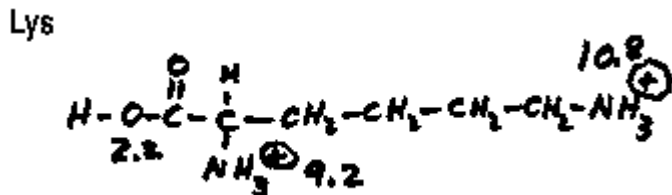
Glu - pKs = 2.1 (alpha-carboxylic acid), 4.1 (side chain-carboxylic acid), 9.5 (amino)



Asp - pKs = 2.0 (alpha-carboxylic acid), 3.9 (side chain-carboxylic acid), 9.9 (amino)



Lys - pKs = 2.2 (alpha-carboxylic acid), 9.2 (amino), 10.8 (side chain-amino group)



Arg - pKs = 1.8 (alpha-carboxylic acid), 9.0 (amino), 12.5 (side chain-amino group)



calculating the pH of 1 M HCl and 1 M NaOH. Use the equation  $K_w = 10^{-14} = [H^+] \times [OH^-]$ , to find the pH of 1 M NaOH.

3. pH of a buffered solution can be found by using the Henderson-Hasselbach equation:  $pH = pK + \log([A^-]/[HA])$

All acids and bases have a conjugate acid (HA) and a conjugate base (A<sup>-</sup>). At  $[HA] = [A^-]$ ,  $pH = pK$  (prove this with the above equation). At the pK, a buffer maximally resists change in pH and the buffering zone is considered to be 1 pH unit above and below the pK.

### Useful Concepts:

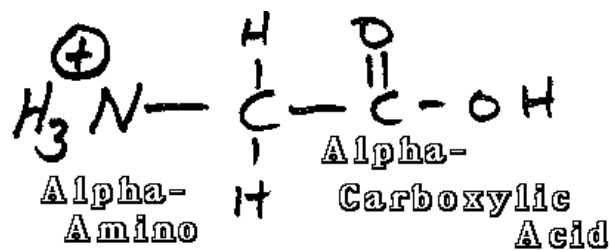
**Buffering zone = pK +/- 1 pH unit**

**At pH = pK,  $[HA] = [A^-]$**

**At pH below pK,  $[HA] > [A^-]$**

**At pH above pK,  $[HA] < [A^-]$**

4. Amino acids (AAs) are more complex than simple buffers and have at least two pK values because AAs have at least two ionizing groups:



### Glycine structure

For example, Glycine has an alpha-amino group and alpha-carboxylic acid group. Each ionizable group has a pK (called pK-1 and pK-2). Glycine pK-1 = 2.3 (alpha-carboxylic acid); pK-2 = 9.6 (alpha amine). Draw a titration curve for Glycine for practice.

In this class, we treat all ionizing groups within a molecule as acting independently of other ionizing groups. The pH on the titration curve where an AA has no net charge is called the pI or isoelectric point. The pI is calculated by averaging the two pK values on either side of the neutral form (ie form with no net charge). All AAs must have at least 3 ionic forms: "AA+1", "AA0", & "AA-1". Where "AA+1" = AA form with net charge of +1, "AA0" = neutral form with no net charge, & "AA-1" = AA form with net charge of -1. While an AA may have other ionic forms, only these 3 forms count when finding pI: AA+1 goes to AA0 via pK-below; AA0 goes to AA-1 via pK-above.

### USEFUL CONCEPTS:

**pI = average of (pK-below) + (pK-above)**

**where pK-below is the pK between AA+1 and AA0**

**and pK-above is the pK between AA0 and AA-1.**

5. For more complex AAs, like Asp, Glu, Lys, Arg & His - with 3 ionizing groups, other forms exist with charges other than +1, zero, & -1. For these AAs, the best approach is to start with the fully protonated form (ie. One with all the protons it can take on its ionizable groups). Calculate the net charge on this group (it must be positively charged), then titrate the AA to remove the first proton and find net charge again. Continue doing this until all the protons have been removed. Keep track of this by making a simple model (see below for His). Then, find the form with no net charge (ie. AA0) and use the pK values, which govern the transition from AA+1 to AA0 and AA0 to AA-1, as the ones to calculate the pI.

- **His has 3 ionizing groups, alpha-carboxylic acid (pK 1.8),**
- **side-chain amino (pK 6.0) and alpha-amino (pK 9.2):**
- **His+2 goes to His+1 via pK 1.8;**
- **His+1 goes to His0 via pK 6.0;**
- **His0 goes to His-1 via pK 9.2.**
- **Therefore, pI = (6.0 + 9.2)/2 = 7.6.**

**6. Some of you have trouble figuring out which group ionizes first.**

Here are some rules to apply to help you:

A. Carboxylic acids ionize at acidic pH; ie. Carboxylic acids give up their protons at acid pHs.

B. Amino groups ionize at basic pH; ie. Amines give up their protons at basic or alkaline pHs.

C. When groups with a similar chemical nature are present:

i. Carboxylic acids near an amino group have a more acidic pK than isolated carboxylic acids.

ii. Amino groups near a carboxylic acid have a more acidic pK than isolated amines.

iii. Aromatic amines (like in His side chain) have a pK near neutral.

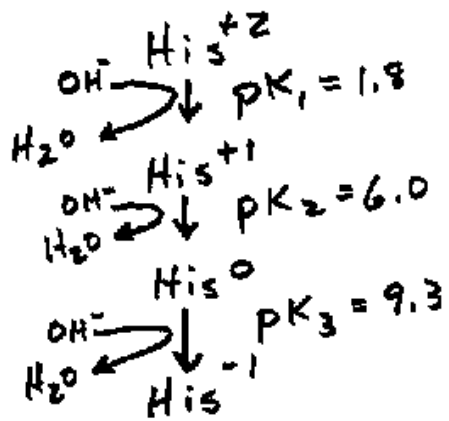
Apply the above rules and concepts to calculate the pI for all the other complex AAs:

Asp, Glu, Lys, and Arg.

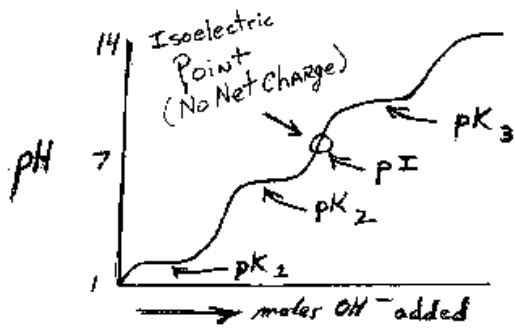
## **6. Ionization of His:**

**Complex amino acids can be handled just like the simpler amino acids:**

For example- titration of Histidine (His) goes like this:

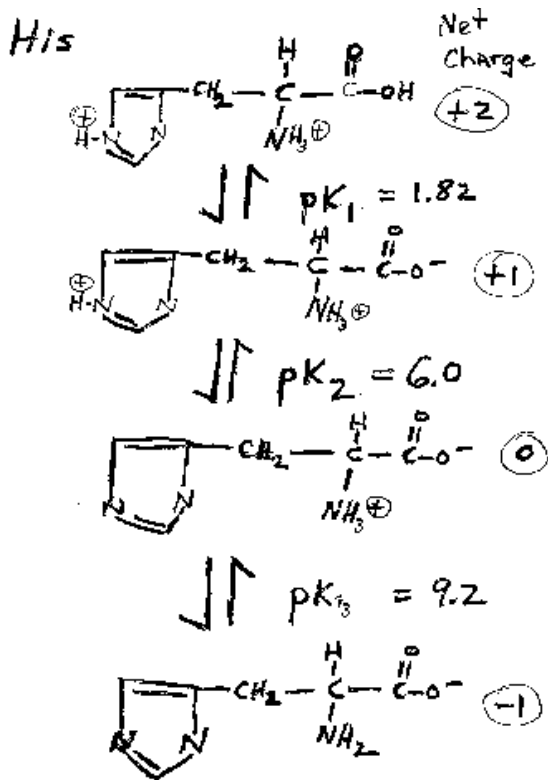


Here is the titration curve:



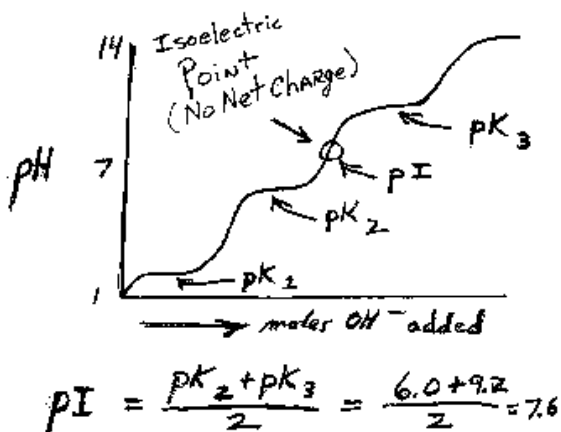
$$\text{pI} = \frac{\text{pK}_2 + \text{pK}_3}{2} = \frac{6.0 + 9.2}{2} = 7.6$$

Here is how to find the charges on the different protonated forms of His:



## 7. Calculating pI (Isoelectric Point or pH) of His:

Here is how to calculate the pI (for His in this example and for all other complex amino acids too): After you find the His form with no net charge, then take the pK going toward the His+1 form as the lower pK, Next find the pK going toward the His-1 form as the upper pK, and finally average these two pKs to get pI. The calculation for the pI of His is shown below:



So for any amino acid, finding the AA 0 form (ie. form with no net charge), then average pK going to AA+1 with the pK going to AA-1, to get pI.

**Try to calculate the pI of another of the 5 complex amino acids or even all!**

## **8. Ionization Rules 2:**

**Again- which group ionizes first?**

It still depends on the chemical nature of the group and the general rules apply

(acids ionize first, amino groups second).

But some amino acids have two groups of similar chemical nature and some additional information about the whole molecule must be used to know which ionizes first. Use the rules below to help you figure out which group ionizes 1st, 2nd, 3rd etc. in complex amino acids.

---

### **Additional Rules:**

3. Carboxylic groups near an amino group in a molecule have more acidic pK than isolated carboxylic acid groups.

Therefore, alpha carboxyl groups ionize before side chain carboxylic acids.

4. Amino groups near a carboxylic acid group also have more acidic pK than isolated amines.

Therefore, alpha amino groups ionize before side chain amino groups.

5. Aromatic amines like in His have pK about pH 6.

## **SUMMARY:**

When titrating an amino acid that is fully protonated (ie starting at pH = 1), the alpha carboxylic acids lose their proton first (all free amino acids have this group), then side chain carboxylic acids lose their proton next ( for example, Asp and Glu), then aromatic-like amino side chains lose their proton next (His is the only example), then alpha amino groups lose their proton next (all free amino acids have this group), then side chain amino groups lose their proton last (for example, Lys and Arg).

**NOTE WELL: The rules apply to small peptides and you need to practice using these rules with peptides...Go to [pK Problems](#)**

### **Extra Problems -- pK of Amino Acid Side Chains and Net Charge**

1. For the dipeptide, Glu-Ala, write out the structure and estimate the pK of all ionizable groups.

Using your assigned pK values, determine the net charge at pH 1, 3, 5, 7, 10, 11.

Calculate the pI. (Remember pI = pH where peptide has no net charge.)

2. For the tetrapeptide, Glu-Ala-Lys-Tyr, write out structure and estimate pK of all ionizable groups.

With your assigned pK values, determine the net charge at pH 1, 3, 5, 7, 10, 11.

Calculate the pI of this tetrapeptide.

The pK values of the amino acids used in this problem set are:

Ala- 2.4, 9.9

Glu - 2.1, 4.1, 9.5

Lys - 2.2, 9.2, 10.8

Tyr - 2.2, 9.7

**Points to Remember:**

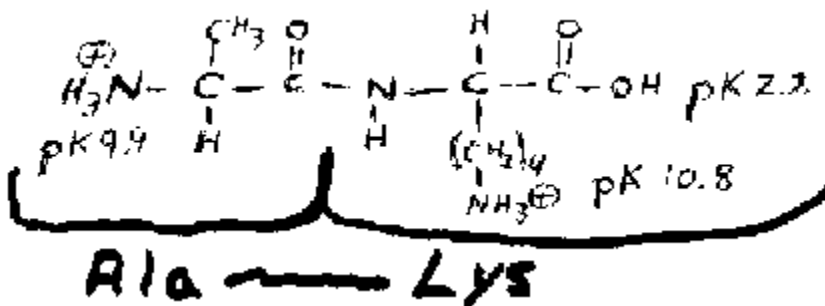
**A.** In assigning pK values to ionizable groups in a peptide, assume that the ionizable groups will have same pK values as they have in the free amino acid.

**B.** To assign pK values, you must know which groups in the amino acid are associated with which pK value shown after the name of the amino acid in the above list.

**C.** After you assign pK values, then use them to decide what the charge on the group is at the pH of interest.

**D.** After you have given a charge to all groups at the pH of interest, then sum them to arrive at the net charge at that pH.

**EXAMPLE**



For Ala-Lys, there are 3 ionizable groups:

- 1) alpha-amino group contributed by Ala - assign pK 9.9.
- 2) alpha-carboxylate group from Lys - assign pK 2.2.
- 3) side chain amino group from Lys - assign pK 10.8.

**Table of Charges at the pH values of interest:**

Group	pH 1	pH 3 & 5	pH 7	pH 10	pH 11
Alpha-Amino	+1	+1	+1	0	0
Alpha-Carboxylate	0	-1	-1	-1	-1
Side-Chain Amino	+1	+1	+1	+1	0
<b>Net Charge</b>	<b>+2</b>	<b>+1</b>	<b>+1</b>	<b>0</b>	<b>-1</b>
Peptide Form	"+2 Form"	"+1 Form"	"+1 Form"	"Zero Form"	"-1 Form"

- **pI = average of pK values for ionizable groups on either side of Peptide Form with Zero Net Charge:**
- **For peptide Ala-Lys example**,  $pI = ((9.9) + (10.8))/2 = 10.35$  or about  $\sim 10.4$ .
- **For peptide to go from +1 Form to Zero Form**, Alpha Amino Group must give up its proton which can be stated as the peptide passes through the pK of Alpha-Amino Group being at pH 9.9.
- **For peptide to go from Zero Form to -1 Form**, the Side Chain Amino Group must give up its proton, which means that the peptide passes through the pK of the Side Chain Amino Group being at 10.8.

**General Rule:**

Average the pK values for transition **from +1 form to zero form** and **zero form to -1 form**

All peptides have a +1, a Zero and a -1 form; so it does not matter what types of ionizable groups are present in the peptide since it always has the 3 forms you need to use to find the pK values to average for finding the pI.