Many substances are polyprotic in nature, that is they contain more than 1 acid or base function. This is especially true in biological materials. All of your amino acids are polyprotic, as well as other goodies like ADP, ATP, ETC. The acid-base behavior of these compounds is a bit more complicated so let’s look at them in a little more depth.

### 11-1 Amino acids are polyprotic

Proteins are made from amino acids. Since these are common polyprotic species found in biology the book focuses on them to help teach the concepts of polyprotic ions. Amino acids are not the only polyprotic species found in biology. The nucleic acids are polyprotic, phosphate is polyprotic, and many drug compounds are polyprotic. Thus don’t get the idea that begin polyprotic is unique for amino acids.

Amino acids have a structure like this:

\[
\begin{align*}
R & \\
\text{NH}_2 - & \text{C-COOH} \\
& H
\end{align*}
\]

The amino group acts as a ? (base)  
The carboxylic group as an? (Acid)  
And the R can be neutral, acid or basic

At neutral pH the carboxylic acid is in the \( \text{COO}^- \) form, and the amino group is in the \( \text{NH}_3^+ \) form, so the amino acid has a + and a - charge, called the zwitterion (for it + and - charge character.)

Typically the \( \text{COOH} \) in an amino acid has a \( pK_a \) is about 2 ( about 2 unit lower than the \( pK_a \) alone) and the \( pK_a \) of the \( \text{NH}_2 \) is about 9 ( about 1 unit lower than a typical amine)

In the fully protonated for an amino acid would be \( H_2A^+ \)  
The acid is then deprotonated to form \( HA^{+/−} \)  
And then the base deprotonated to give you \( A^- \)

Typically we number \( K_a \)'s and \( K_b \) in the following way:

\[
\begin{align*}
K_{a_1}^- & \quad K_{a_2}^- \\
H_2A^+: & \quad HA^{+/−} \quad A^- \\
- K_{b_2} & \quad - K_{b_1}
\end{align*}
\]

Note that our conjugate acid/base pairs then make the following equations true:  
\( K_{a_1}K_{b_2} = K_w \);  \( K_{a_2}K_{b_1} = K_w \)
We will usually refer to $K_{A_1}$ as simply $K_1$ and $K_{A_2}$ as $K_2$

11-2 Finding the pH in Diprotic systems

Your book uses leucine as an example amino acids. Just to give you some variety, I will use a different amino acid, serine. It has two $p_a$'s 2.187 and 9.209 Can you see the groups responsible for this chemistry?

As written, this is the fully protonated form. At what pH would you expect this to appear? (Low) We will call it $H_2\text{Ser}^+$. Out 1st pK_a is associated with the deprotonation of the COOH group. I will call this form $H\text{Ser}^-$. Notice $H\text{Ser}^-$ has a negative charge on the COO$^-$ and a positive charge on the NH$_3^+$. A molecule with both positive and negative charges is called zwitterionic. Our second pK_a is associated with the loss of the proton on the secondary amine. I will call this $\text{Ser}^+$, and this form appears at high pH values.

Let's diagram this simply

```
\begin{align*}
OH & \\
\text{CH}_2 & \\
\text{NH}_3^+ & \text{\textrightarrow COOH} & \text{H} \\
\text{H} & \\
\end{align*}
```

$pK_{a1} = 2.187$; $2.187 = -\log(K_{a1}); -2.187 = \log(K_{a1}); K_{a1} = 6.50 \times 10^{-3}$

$pK_{a2} = 9.209$; $9.209 = -\log(K_{a2}); -9.209 = \log(K_{a2}); K_{a2} = 6.18 \times 10^{-10}$

We therefore use the weak acid equation with $K_{a1}$ and get

$$K_{a1} = 6.50 \times 10^{-3} = X^2/(0.1-X)$$

$$6.50 \times 10^{-4} - 6.50 \times 10^{-3}X = X^2, 0 = X^2 + 6.50 \times 10^{-3}X - 6.50 \times 10^{-4}$$

and using the quadratic

$$[H^+] = [H\text{Ser}^-] = 2.245 \times 10^{-2}; \text{pH}=1.65; H_2\text{Ser}^+ = .1-.0225 = .00775$$

(Q&D sqrt(.1\times6.50 \times 10^{-3}) = 2.55 \times 10^{-2}; .1-.0255 is 20% error, can't do Q&D)
To solve this solution we assumed that not much $\text{Ser}^-$ was formed. Was this a good assumption?

$$K_a = \frac{[\text{H}^+][\text{Ser}^-]}{[\text{HSer}^+]}$$

And using the $[\text{H}^+]$ and $[\text{HSer}^+]$ we just calculated

$$6.22 \times 10^{-10} = 2.245 \times 10^{-2} [\text{AA}] / 2.245 \times 10^{-2}$$

$[\text{AA}] = 6.22 \times 10^{-10}$; Yes this is very, very tiny!

This assumption, That we can work with $K_a$ independently of $K_{a2}$ will work pretty well until $K_{a2}$ is $> 0.1 K_a$

The basic form: pH of Ser$^-$

What about the pH of serine in the fully deprotonated, basic form, $\text{Ser}^-$?

Same story, but just use $K_b$’s. Remember how to get $K_b$’s from $K_a$’s?

$$K_{b2} = K_w / K_{a1}$$
$$K_{b1} = K_w / K_{a2}$$
$$K_{b1} = K_w / K_{a2} = 1 \times 10^{-14} / 6.22 \times 10^{-10} = 1.61 \times 10^{-5}$$

Let’s say we start with a solution that is .05F [Ser$^-$] this time.

Again we can ignore the second ionization and use the regular equation for a monoprotic base and we have:

$$X = [\text{OH}^-] = [\text{HSer}^+] ; [\text{Ser}^-] = 0.05 - X$$

$$K_{b1} = [\text{OH}^-][\text{HSer}^+] / [\text{Ser}^-]$$

$$1.61 \times 10^{-5} = X^2 / (0.05 - X)$$

Here, since $K_{b1}$ is so small, you can probably get away with the Q&D, lets try it:

$$1.61 \times 10^{-5} \sim X^2 / (0.05)$$

$$X \sim \sqrt{0.05 \times 1.6 \times 10^{-5}}$$

$$X \sim 8.97 \times 10^{-4}; \text{pOH} = 3.05, \text{pH} = 10.95$$

$X$ is $.000897 / .05 = 1 \text{ or } 2 \% \text{ of total, so we are probably good, Let's try it the long way just to be sure:}$

$$8.05 \times 10^{-7} - 1.61 \times 10^{-5} X = X^2$$

$$0 = X^2 + 1.61 \times 10^{-5} X - 8.05 \times 10^{-7}$$

Using the quadratic we have

$$[\text{OH}^-] = [\text{HSer}^+] = 8.89 \times 10^{-4} ; [\text{OH}^-] = 3.051, \text{pH} = 10.949$$

The same answer!

Here, since $K_{b1}$ is so small, you can probably get away with the Q&D, lets try it

The intermediate form: pH of HSer$^{+\cdot}$

Now for the 20$^\$$ question, What is the pH in the middle? For the HSer$^{+\cdot}$ form?

This is quite a bit trickier because we have two potential reactions, acid and base, because our HSer$^{+\cdot}$species is amphiprotic

$$\text{HSer}^{+\cdot} \leftrightarrow \text{H}^+ + \text{Ser}^-; \quad K_a = K_{a2} = 1.0 \times 10^{-10}$$
HSer^- + H_2O ⇌ H_2Ser^+ + OH^-;  \[ K_b = \frac{K_w}{K_{a1}} = 1 \times 10^{-14}/6.50 \times 10^{-3} = 1.53 \times 10^{-12} \]

And you can see that both pK’s are similar in magnitude, so both reaction will be going on. (even if pK’s weren’t too close the H^+ from one reacts with the OH^- of the other so one reaction will always drag the other one along by Le C’s principle)

With this complicated system with several unknows. To be able to solve it we need to have a few more equations. Here is where I would like to introduce the **Charge Balance equation** (The book does this is chapter 12)

**Charge Balance**
The Charge Balance equation can be a use equation in solving equilibria problems. The idea of the charge balance equation is simple - Since solutions, as a whole, are electrically neutral, the sum of the + charges in a solution = sum of negative charges.

Thus in a strong acid (HCl), strong base (NaOH) titration, what where this ions in solution? H^+, OH^-, Cl^-, and Na^+

and our charge balance equation is

\[ [\text{Na}^+] + [H^+] = [\text{OH}^-] + [\text{Cl}^-] \]

How about a weak acid (HA) and NaOH titration

\[ [\text{Na}^+] + [H^+] = [A^-] + [\text{OH}^-] \]

Now how about something a little more interesting

H_3PO_4 and NaOH?

H_3PO_4 is polyprotic so it can undergoes 3 different equilibria

\[ H_3PO_4 = H_2PO_4^- + H^+ = HPO_4^{2-} + H^+ = PO_4^{3-} + H^+ \]

Your first attempt at writing a charge balance equation for this system might be

\[ [H^+] + [\text{Na}^+] = [\text{OH}^-] + [H_2PO_4^-] + [\text{HPO}_4^{2-}] + [\text{PO}_4^{3-}] \]

But you would be WRONG Why?

Say you had 1L of 1M [HPO_4^{2-}] How many moles of -charges would you have?

1L of 1M means 1mole of HPO_4^{2-} but, because HPO_4^{2-} has a -2 charge your total negative charge is -2 mole! [HPO_4^{2-}] x 2 mole charge/1 mole
HPO$_4^{2-}$

The PO$_4^{-3}$ is even worse because it has a -3 charge.

Bottom line the equation should be

\[
[H^+] + [Na^+] = [OH] + [H_2PO_4^-] + 2[HPO_4^{2-}] + 3[PO_4^{3-}]
\]

The charge balance equation applies to any solution, so we will be seeing it again when we need to solve complicated equilibrium calculations.

**Returning to our problem**

What is the charge balance equation for this system

\[
[H^+] + [H_2Ser^+] = [Ser^-] + [OH^-]
\]

(Note: HSer$^+$ has no net charge so doesn’t fit here)

Will now solve the hairy system exactly. In 2006 I skipped this and went directly to answer:

\[
[H^+] + [H_2Ser^+] - [Ser^-] - [OH^-] = 0
\]

Since we are starting with [HSer$^+$] let’s express [H$_2$Ser$^+$] and [Ser$^-$] in terms of [HSer$^+$]

\[
K_{a2} = [H^+][Ser^-]/[H_2Ser^+] ; \quad [Ser^-] = K_{a2}[H_2Ser^+]/[H^+]
\]

\[
K_b = [OH^-][H_2Ser^+]/[H_2Ser^+]= [H_2Ser^+] = K_b[HSer^+]/[OH^-]
\]

\[
[H^+] + [H_2Ser^+] - [Ser^-] - [OH^-] = 0
\]

\[
[H^+] + K_{a2}[HSer^+]/[OH^-] - K_{a2}[H_2Ser^+]/[H^+] - [OH^-] = 0
\]

\[
K_b = K_w/K_{a1} ; \quad [OH^-] = K_w[H^+]
\]

\[
[H^+] + K_w/K_{a1}[HSer^+]/[H^+] - K_{a2}[H_2Ser^+]/[H^+]/K_w[H^+] = 0
\]

\[
[H^+] + K_w[HSer^+][H^+] - K_{a2}[H_2Ser^+][H^+] - K_w[H^+]K_{a1}K_w = 0
\]

Removing $K_w/K_w$ and Multiplying by [H$^+$] to get it out of the numerator

\[
[H^+]^2 + [HSer^+][H^+]^2/K_{a1} - K_{a2}[HSer^+]K_w = 0
\]
\[
[\text{H}^+]^2 \left(1 + [\text{HSer}^+] / K_{a_1}\right) = K_w + K_{a_2}[\text{HSer}^+]
\]

\[
\left[\text{H}^+\right] = \frac{\left(K_w + K_{a_2} [\text{HSer}^+]\right)}{1 + [\text{HSer}^+] / K_{a_1}} = \frac{\left(K_w + K_{a_2} [\text{HSer}^+]\right)}{\left(K_{a_1} + [\text{HSer}^+] \right) / K_{a_1}} = \frac{K_{a_1} K_w + K_{a_1} K_{a_2} [\text{HSer}^+]}{K_{a_1} + [\text{HSer}^+]}
\]

Returning to final solution

\[
[\text{H}^+] = \sqrt{\frac{K_{a_1} K_{a_2} [\text{HSer}^+] + K_{a_1} K_w}{K_{a_1} + [\text{HSer}^+]}}
\]

IN the more general form:

\[
[\text{H}^+] = \sqrt{\frac{K_1 K_2 F + K_1 K_w}{K_1 + F}}
\]

Where \(F\) = Formal concentration of the intermediate species

Up to this point we have made no assumptions and we have \([\text{H}^+]\) in terms of \([\text{HSer}^+]\). But what is \([\text{HSer}^+]\)? Well we recognize that neither \(K_b\) nor \(K_{a_2}\) were very big so \([\text{HSer}^+]\) is essentially our initial concentration.

If \([\text{HSer}^+]\) is .05, let us plug in and see what we get.

\[
[\text{H}^+] = \sqrt{\frac{6.50 \times 10^{-3} \times (6.22 \times 10^{-10}) \times 0.05 + 6.50 \times 10^{-3} \times (1 \times 10^{-14})}{6.50 \times 10^{-3} + 0.05}}
\]

\[= 1.89 \times 10^6, \text{ pH} = 5.72\]

You can check to see if our assumption (\(\text{HSer}^+ \rightarrow \text{H}_2\text{Ser}^+\) or \(\text{Ser}\)) was a good one if you want
The above equation is pretty hairy to remember. If we can make the following two assumptions we can get to something much easier to use.

1. \( K_{HSer} \gg K_a \) so \( K_{HSer} \ll K_1 K_2 F \)
2. \( K_1 \ll HSer^- \) so \( K_1 + F \approx K_1 \)

What happens if these two statements are true?
If (1) is true then we can ignore the second term in the numerator
If (2) is true then the denominator simplifies to \( HSer^- \)
and we get the equation:

\[
[H^+] = \sqrt{\frac{K_{d1} K_{d2} [HSer^+]}{[HSer^-]}}
\]

And the \([HSer^-]\) term cancels out and we get a far nicer equation:

\[
[H^+] = \sqrt{K_1 K_2}
\]

or, taking the negative logs:

\[
pH = (pK_1 + pK_2) / 2
\]

Which works out to 5.70 in this example and is usually a pretty good first guess.

11-3 Principal species

Another property that is frequently useful to analyze for is called the principal species. What this means is that given any pH quickly look at the situation and figure out what the major ionic form of the compound that occurs in the solution at that pH.

Let’s us a more complicated amino acid for our example here, Aspartic acid

\( R=CH_2 COOH \), pKa’s = 1.99 (main chain COOH), 3.90 (side chain COOH), and 10.02 (main chain NH) (2, 4 and 10 for my purposes)

First, let’s identify the various species and label them, going from most protonated to least protonated

\( H_3A \)  Actually \( H_3A^- \) (2 cooh no charge 1 NH3+ charge)
\( H_2A \)  Actually \( H_2A^{+/-} \) (1 COOH, 1 COO−, 1 NH3+)
\( HA \)  Actually \( HA^{+/-} \) (2 COO−, 1NH3+)
\( A \) \( A^{2-} \) (2 COO−, 1 NH3)
Now let’s think of what the pK/a tell us about what species dominate at what pH. Let’s start by laying out a pH scale that we are going to make a diagram from.

### Principle Species

<table>
<thead>
<tr>
<th>pH</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
</tr>
</thead>
</table>

First, use the Henderson Hasselbalch equation to find a few pH’s where you have a 50:50 mix of acid and conjugate base forms. Where would these be?

\[
pH = pK_a + \log \left( \frac{[A^\text{-}]}{[HA]} \right)
\]

Principle Species

<table>
<thead>
<tr>
<th>pH</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
</tr>
</thead>
</table>

50:50 mixes

at the lowest pH we will be in the fully protonated form, \( H_3A \)
at pH=\( pK_1 \) we have equal amounts of \( H_3A \) and \( H_2A \)
at pH=\( pK_2 \) we have equal amounts of \( H_2A \) and HA
at pH=\( pK_3 \) we have equal amounts of HA and A
and at high pH we have \( A^\text{-} \)

Let's put this information on a diagram

\[
\begin{array}{cccccccccccc}
\text{pH} & 1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 & 10 & 11 & 12 & 13 \\
H_3A & H_2A & | & HA & | & A & & & & & & & \\
pK_1 & pK_2 & & pK_3
\end{array}
\]

A simple diagram like this helps you to keep straight what is going on, and can also help you to decide what species you should be using in buffer problems or titration curves (next section)

For instance, If I said the pH of the solution were 8, what \( pK_a \) and what concentration would you use in the H-H equation?

\[
8 = pK_{a3} + \log \left( \frac{[A^\text{-}]}{[HA]} \right)
\]

pH 10?

\[
10 = pK_{a3} + \log \left( \frac{[A^\text{-}]}{[HA]} \right)
\]
11-4 Titrations in Polyprotic systems

Our final task is to compare the titration curve of a simple monoprotic amino acid to a more complicated polyprotic.

We will start with one simplifying assumption, that the $K_a$'s of various groups are >100 times different, or that the $pK_a$'s differ by at least 2. This is not a good assumption for many polyprotics, so you can’t always use this approach. However it makes the math much easier, and gives you enough information for this class.

If we make the above assumption, then we can treat the titration of each chemical moiety as a separate titration and put the titrations together sequentially. For instance our Aspartic acid, with $pK_a$'s of 2, 4, and 10 would titrate into a curve with 3 buffer regions (2, 4, and 10) and 3 equivalence points, on for each group bring titrated.

As usual, let’s look at the curve and look for places where we can use familiar equations

(10 ml 0.2M ASP titrated with .25M NaOH)
1 As usual, decide where the equivalence point is to you can get an idea of what the graph should look like

\[(10(.2) = X(.25) \]
\[X = 10(.2)/.25 = 8 \text{ ml} \]
3 ionizable groups so will have 3 equivalence points, 8, 16, and 24 ml

1. Initial point - Same as always $K_i = X^2/(F-X)$

\[1.02 \times 10^{-2} = X^2/(.2-X) \]
1st approx: $X = 0.101$
2nd approx: $X = 0.032$
3rd approx: $X = 0.041$
4th

Exact answer $0.0404 \times 10^{-2}$, pH = 1.395

2, 3, 4 Buffer regions

Use H-H Equation $pH = pK_a + \log A^-/HA$

The only problem is deciding the appropriate $K$ and $A^-$ and $HA$

Buffer 1 $K_1$, $HA = H_2A$, $A^- = H_2A$
Buffer 2 $K_2$, $HA = H_2A$, $A^- = H_3A$
Buffer 3 $K_3$, $HA = H_3A$, $A^- = A$

In an open ended problem you will probably need to use multiple reaction table to keep track of each species. For instance, say I had added 23 ml of base then
Moles of base = 23(.25) = 5.75 mmole
Moles of $H_A = 10(.2) = 2$ mmole

<table>
<thead>
<tr>
<th>Initial</th>
<th>$H_A + OH^- \rightarrow H_2A$</th>
<th>$H_2A + OH^- \rightarrow HA$</th>
<th>$HA + OH^- \rightarrow A$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>5.75</td>
<td>0</td>
</tr>
<tr>
<td>L.R.</td>
<td>-2</td>
<td>-2</td>
<td>+2</td>
</tr>
<tr>
<td>Rxn1</td>
<td>0</td>
<td>3.75</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Initial 2</th>
<th>2</th>
<th>3.75</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.R.</td>
<td>-2</td>
<td>-2</td>
<td>+2</td>
</tr>
<tr>
<td>Rxn2</td>
<td>0</td>
<td>1.75</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Initial 2</th>
<th>2</th>
<th>1.75</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.R.</td>
<td>-1.75</td>
<td>-1.75</td>
<td>+1.75</td>
</tr>
<tr>
<td>Rxn3</td>
<td>.25</td>
<td>0</td>
<td>1.75</td>
</tr>
</tbody>
</table>

\[ \text{pH} = 10.02 + \log \left( \frac{1.75}{.25} \right) = 10.865 \]

Final equivalence point just like the usual equivalence points
\[ K_b = \frac{K_w}{K_a} = \frac{X^2}{(F-X)} \]
\[ K_b = 1 \times 10^{-14} / 1 \times 10^{-10} = 1 \times 10^{-4} \]
\[ F = .2(10)/(10+24) = .059 \]
\[ 1 \times 10^{-4} = \frac{X^2}{(.059-X)} \]

1st approx $X = .01$
2nd approx $X = .002$
3rd approx $X = .0024$

exact .00238, $pOH = 1.62$, $pH = 12.38$

After the equivalence point just like any excess base

\[ [OH^-] \times \text{dilution} = \text{ml excess base/total volume} \]

That only leaves 2 points that you don't know, the intermediate equivalence points. For these you have $H_2A$ and $HA$ in solution, and these are intermediate, amphiprotic forms that can act as acids or bases. Do you remember how to calculate these $pH$'s
Long and short equations

\[ [H^+] = \sqrt{\frac{K_{a1}K_{a2}[HSer^*] + K_{a1}K_{a2}}{K_{a1} + [HSer^*]}} \]

Or the simpler \( pH = (pK_1 + pK_2)/2 \)

The last is easier to remember, and it also makes sense when you look at the graph. Notice how these equivalence points are sandwiched between the buffers that are defined by the pKₐ’s.

For completeness, what happens when the \( K_a \) are <100 apart? No distinct equivalence points, things merge into each other. This is the kind of behavior you see in proteins that might have 40-100 different charged amino acids all merging together.