must know that the two forms of the side chain are zero and minus (not plus!) charged. The answer to this example is:

\[ pI = (6.0 + 9.9) / 2 = 7.95 \]

**BUFFER PROBLEM**

To one mole of alanine at its pI are added a) 0.1 moles of HCl or b) 0.1 moles of NaOH or c) 0.5 moles of HCl or d) 1.5 moles of HCl. What pH results in each of these examples?

The Henderson Hasselbalch equation is \( pH = pK_a + \log(A/HA) \). The numerator, A, is a form with one less proton than HA.

a) \[ pH = pK_a + \log(A/HA) \]. NEVER use the pI where the pK_a is called for! You must remember that pI and pK_a have opposite meanings. Always insert the pK_a value toward which you are moving. Here you are starting at the isoelectric point and adding acid so (see your charge form drawings on the previous page) you will be moving left from the zero charge form, or toward 6.0. To determine A and HA: you will be adding a proton to 10% of the dipeptide, and leaving the other 90% unchanged, so A/HA should equal 9/1 or 0.9/0.1 (and NOT 10/1!!). Remember that A + HA = original concentration of amino acid or peptide.

\[ pH = 6.0 + \log(0.9/0.1) = 6.0 + \log 9 = 6.95 \]

b) Here you are moving to the right, deprotonating 10% of the available dipeptide, and leaving 90% unchanged (as HA).

\[ pH = 9.9 + \log(0.1/0.9) = 9.9 - \log 9 = 8.95 \]

c) Here you are moving further to the left – protonating half of the peptide, and leaving the other half unchanged.

\[ pH = 6.0 + \log(0.5/0.5) = 6.0 + \log 1 = 6.0 \]

d) Here you move beyond 6.0. The first mole completely protonates the first ionizable group, taking the compound from the zero to the (+1) charge form, and then the second 1/2 mole of acid moves it toward 1.8.

\[ pH = 1.8 + \log(0.5/0.5) = 1.8 + \log 1 = 1.8 \]