HOW TO PERFORM BUFFER AND ISOELECTRIC POINT CALCULATIONS

by Frank Deis

First, visualize what will happen at low and high pH. At low pH there are a lot of protons around (H^+), and things tend to be protonated. At high pH, there are very few protons around, and functional groups are stripped of ionizeable protons. What happens between the extremes of pH is determined by the pK_a's of the groups involved. You are usually told the relevant pK_a's in a problem.

It is best to think "on paper" about all of this. Try drawing the low pH, high proton form on the far left of the page, and the high pH, low proton form on the far right. Then arrange the pK_a numbers in increasing sequence from left to right between the two forms. Now start from the left hand, fully protonated form; as you increase the pH to the first pK, the group whose pK it is has become 50% protonated. Draw the protonated form minus *that* proton. Continue with this process until you have passed all of the pK_a's and you will have drawn all possible ionization states for that compound. Now figure the charge on each ionization form. The zero charge form is the iso-electric form, and the isoelectric point is the average of the two pK_a values immediately on both sides. All other pK_a values are irrelevant for this calculation.

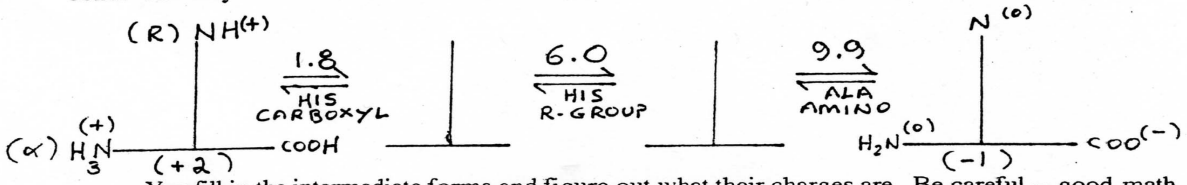
On past exams, dipeptides or tripeptides have been used in problems of this sort. In this case, you have to remember that amides are not ionizeable in aqueous solution, and that you have to identify and ignore the pK_a's of carboxyl and α-amino groups bound in peptide bonds. Another useful thing to remember is that it is not really necessary to draw the entire compound, just the ionizeable groups.

EXAMPLE: What is the isoelectric point of Alanyl Histidine? pK_a values: Ala COOH = 2.3, α-NH_2 = 9.9, His COOH = 1.8, α-NH_2 = 9.2, R = 6.0 This problem is solved below, but please try to solve it by yourself.

This sort of visualization of chemical change in response to shifting pH can also help in solving buffer problems with the **Henderson Hasselbalch Equation.** It is useful to remember that at the pK_a, a compound tends to be a good buffer – that is, you can add acid or base without significantly changing the pH. The iso-electric point, or pI, has the *opposite* property. Adding small amounts of acid or base at the pI or any of the midpoints between two adjacent pK_a values (where 100% of some specific charge form is present) produces a *large* change in pH. The 100% charge forms could be thought of as "anti-buffers." If you bought a bottle of alanyl-histidine, it would come in the zero charge "iso-electric" form. That is one reason why buffer problems start at the pI and then add acid or base.

Adding a strong acid or base to an amino acid has a very predictable effect. A strong acid completely protonates anything which can be protonated, whereas a strong base completely strips away protons – the amino acid reacts quantitatively with however much base or acid is added. You don't have to worry about it "fighting back." There is an example of a problem using the Henderson Hasselbalch equation below.

The name of the compound, "alanyl histidine" tells us that alanine's α-NH_2 is free, and the histidine carboxyl is also free. Alanine's carboxyl and histidine's α-NH_2 group are bound in a peptide bond. The only ionizeable side chain is histidine's.



You fill in the intermediate forms and figure out what their charges are. Be careful – good math can be frustrated by bad chemistry in problems like this. For example, if cys or tyr are involved, you