Lecture 4: Charge Calculations, Environment & pK$_a$, Titration curves & Monoprotic Buffers.

Goals:

- Calculate charges on molecules, given pK$_a$s
- Predict environmental effect on pKa.
- Obtain pKa from a titration curve.
- Understand the molecular nature of buffers.
- Buffer construction, monoprotic.

A. Charge Calculations:

The overall charge on a molecule as a function of pH can be calculated by summing the contribution from each ionizable group, as indicated in the equation on the right.

$$q_{total} = \sum (f_{HA} \cdot q_{HA} + f_{A-} \cdot q_{A-})$$

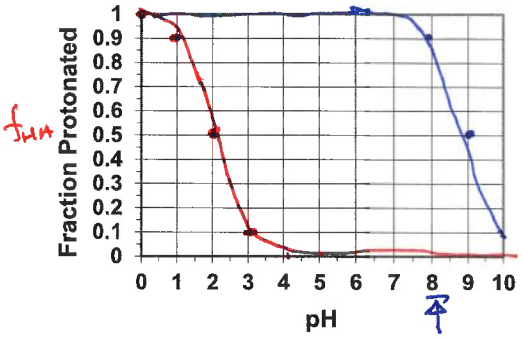
AVE, all groups

Approach:

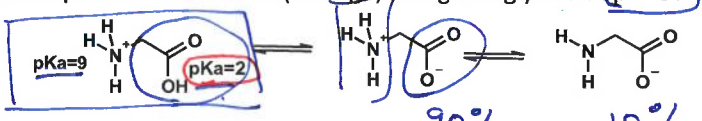
i) Identify all ionizable groups on the molecule & their charge when protonated and deprotonated.

ii) Use the known pK$_a$ of each group to determine the fraction protonated (f$_{HA}$) and deprotonated (f$_{A-}$) at the required pH.

iii) Calculate the overall charge by summing the contribution of each group.



Example: What is the net (average) charge on glycine at pH=8?



pH = 8

$q_{COOH} = f_{HA} q_{HA} + f_A q_A$

$= (0)(0) + 1(-1) = -1$

$q_{NH} = f_{HA} q_{HA} + f_A q_A = +0.9$

$= (0.9)(+1) + (0.1)(0)$

$\rightarrow -0.1$

Short cuts:

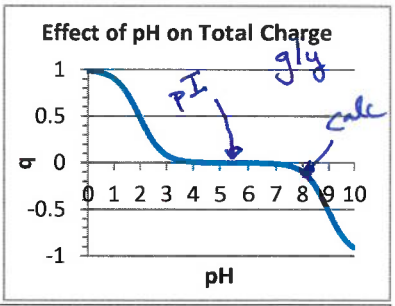
i) if pH << pKa, fully protonated, f$_{HA}$ =1

ii) if pH >> pKa, fully deprotonated, f$_A$=1

iii) ignore neutral species.

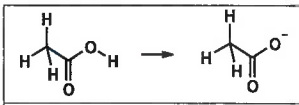
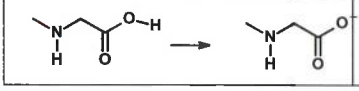
Zwitterion: a compound that is ionized but has no *net* charge. (Middle compound)

Isoelectric pH (pI) pH where the net charge is zero.



B. Effects on pKa Values:

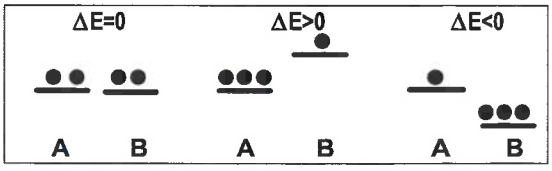
1. Chemical

	Acetic Acid pK$_a$ ~ 4.0 (Glu,Asp sidechain)	*Negative charge delocalized over C=O, lower in energy, therefore a carboxylate is a stronger acid.*
	Carboxy pK$_a$ ~ 2.0 group on an amino acid.	*Electronegative N withdraws charge from the neg. carboxylate, giving a stronger acid.*

2. Environmental Effects due to near-by Charges.

Analysis by Energy: The relative populations (n_a, n_b) of two states depends on the energy difference between them, ΔE (Boltzmann)

$$\frac{n_b}{n_a} = e^{-\Delta E / kT} = K_{eq}$$



The electrostatic environment of an ionizable group can change the pKa of that group, by affecting the energy of either the protonated or deprotonated states - it is the **relative** energy difference between the HA and A- states that determines the equilibrium constant: $K_{EQ}=n_b/n_a$.

Approach:

i) Set the pH = pKa of the free acid, at this pH the energy of HA and A are equal (making comparisons easier).

ii) Determine which state will be affected by electrostatics in the new environment, i.e. is the protonated (HA) charged, or is the deprotonated (A) charged. For example, in the case of a carboyxylate (COOH), HA has no charge while A has a negative charge.

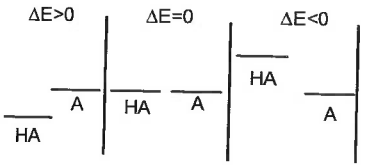
iii) The energy level of the uncharged state remains the same as the free acid in solution.

iv) The energy of the charged state is:

- Lowered if the environment is opposite to its charge (favorable electrostatics)
- Raised if the environment has the same charge (electrostatic repulsion)

v) Evaluate ΔE:

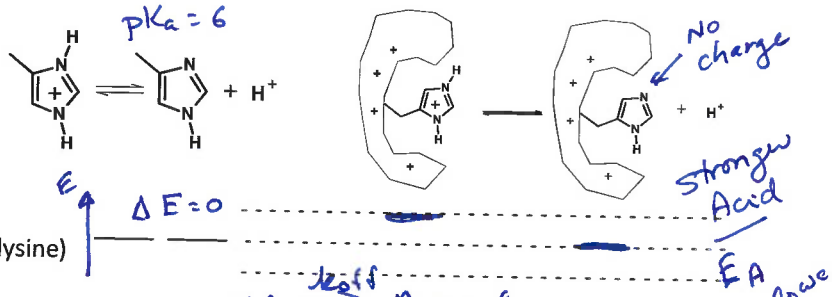
- If the energy of A is now higher than HA, then the deprotonated state is less favorable, therefore the acid is weaker (left system).
- If the energy of A is now lower than HA, then the deprotonated state is more favorable, therefore the acid is stronger (right system).



Example: How will a positively charged environment affect the pKa of histidine (pKa of free His =6.0)

The left shows the relative energy of the protonated and deprotonated group for the free weak acid (made equal by setting the pH=pKa)

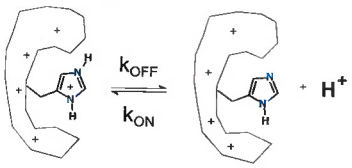
The right shows the relative energy of the same group, but within the context of a local positive charge from other groups (e.g. lysine) on the protein, *at the same pH.*



Alternative approach - Analysis by Chemical Kinetics: $K_A = k_{OFF}/k_{ON}$

If the environment is positive (e.g. lysine) proton collisions will decrease,

∴ k_{ON} decrease, ∴ K_A larger, ∴ -logK_A (pKa) lower.



C. Titration Curves:

K_a values, or acidity constants, must be measured by direct experiment, usually with a pH titration. Known amounts of a strong base (NaOH) are added to a solution of **weak acid.** The effect of the added base can be measured by two ways:

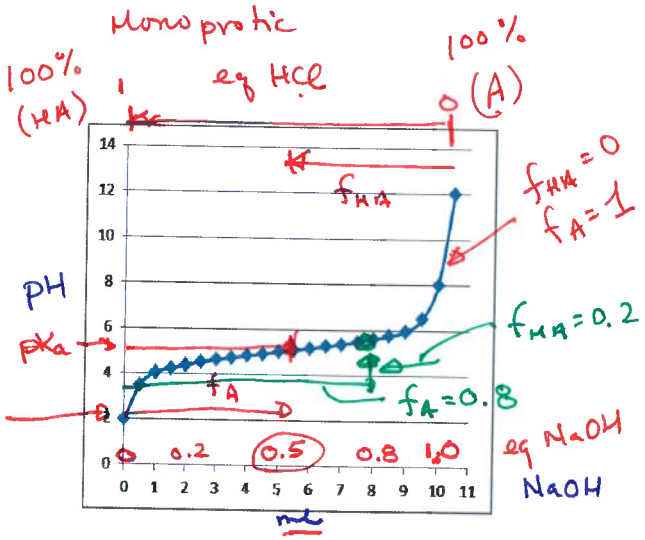
i) Changes in the pH of the solution, this is appropriate for a solution with only one weak acid. As the base is added it removes the proton from the acid.

ii) In complex mixtures of acids, a spectroscopic signal (e.g. NMR) can be used to determine the pKa of individual groups.

Key features of titration curves:

Equivalents: the ratio of the moles of the strong base to the weak acid:

$$eq = \frac{\text{moles NaOH}}{\text{moles Weak acid}}$$



varies from 0 to 1 for an acid that releases one proton (monoprotic), from 0 to 2 for a diprotic acid, etc.. This scale is useful since it removes the concentration of the weak acid.

It is also possible to define equivalents in terms of an HCl if you started the titration with the salt (e.g. NaA), and add HCl. In this case the scale is reversed.

- ***The number of NaOH equivalents added = f_A***
- ***The number of HCl equivalents added = f_{HA}.***

pKa Determination (Inflection point): There is an infection point at the point where the weak acid is ½ deprotonated. Since the two forms of the acid (HA, A) are equal, the pH=pKa at this point. For multi-protic buffers there would be multiple infection points, one at each pKa.

***Equivalence Point*:** Complete deprotonation of the weak acid occurs when the amount of added base is equal to, or *equivalent*, to the total number of ionizable protons that were originally on the weak acid. This point in the titration is referred to as the *equivalence* point. The equivalence point can be used to determine the concentration of the acid. Multi-protic buffers have multiple equivalence points.

D. Buffers: A pH buffer is an acid that resists changes in the solution pH by absorbing or releasing protons. Buffers play an important role in cellular processes because they maintain the pH at an optimal level for biological processes. They are also widely used to control pH in laboratory processes.

Reaction occuring in region A & C: large conc. change in H^+

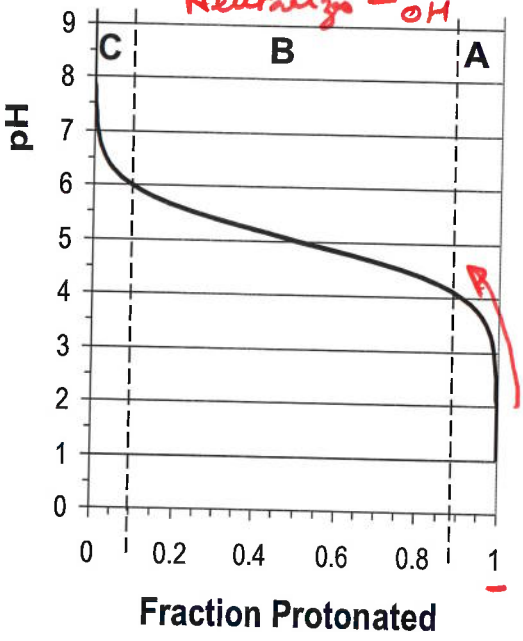
$$H_3O^+ + OH^- \rightarrow H_2O$$

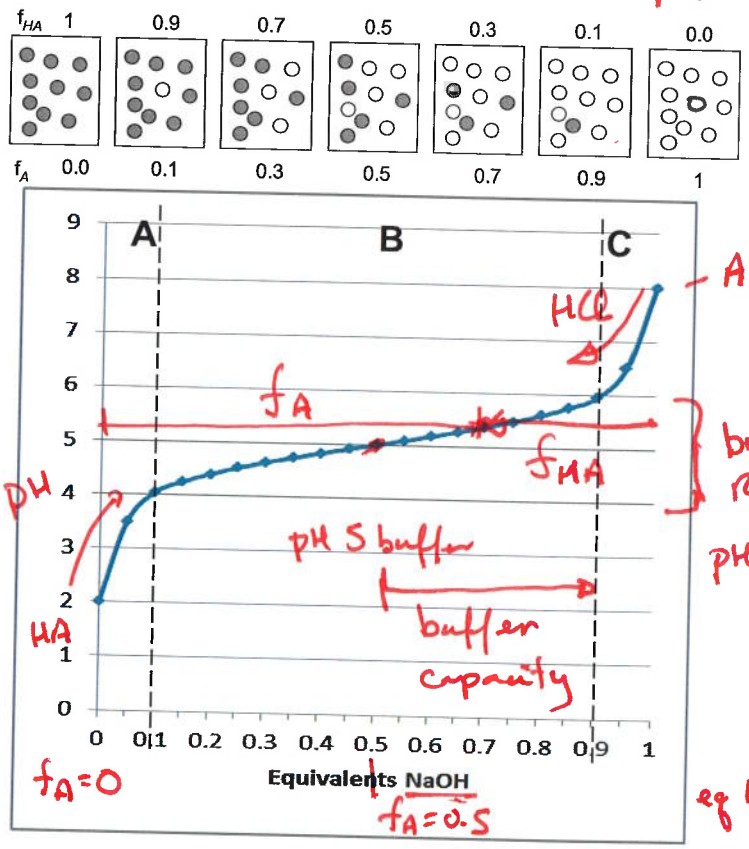
Reactions occuring in region B: monoprotic

$$H_3O^+ + OH^- \rightarrow H_2O$$

$$HA \rightarrow A + H^+$$

Neutralize OH^-





pH = ± 1 from pKa

eq NaOH = moles NaOH / moles W.A.

Buffering range/region:

$$pH = pK_a \pm 1$$

Buffering capacity: Total moles of a strong acid or base that can be absorbed by a buffer solution and keep the pH within the buffer region. It depends on the concentration of the weak acid, and where the pH is relative to the edges of the buffer region. The higher concentration of weak acid, the higher the capacity.

E. Buffers Construction: Need to determine the ratio of [A-] to [HA] (=R) to give desired pH of the solution.

Typical Problems - Monoprotic Buffer:

- concentration [A_T] , [A_T] = [HA] + [A]
- volume V, pH
- List of weak acids and their pKa values.

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

← calculate

pH: desired; pK_A: selected

Method:

1. Select a weak acid whose pK_a is within one pH unit of the desired pH.
2. Determine the fraction protonated and deprotonated at the desired pH, f_{HA} & f_{A^-}.
3. Obtain this ratio of [HA] to [A^-] in solution by one of the following methods:
   i) Mix the indicated concentration of the weak acid (HA) and its conjugate base (NaA) to give the desired pH:
   moles (HA)= $f_{HA} \times [A_T] \times V$ moles (A-) = $f_{A^-} \times [A_T] \times V$
   ii) Use [A_T] amount of the *acid form* of the weak acid and add sufficient *strong base* (e.g. NaOH) to make the required concentration of [A^-] to attain the desired pH. You are titrating starting from the left side and converting enough of the fully protonated acid to give the correct amount of the deprotonated acid. The added base converts HA to A^-.
   The amount of strong base to add is f_{A^-} equivalents. *moles NaOH = $f_{A^-} \times [A_T] \times V$*
   iii) Use [A_T] amount of the *conjugate base* form of the weak acid and add sufficient *strong acid* (e.g. HCl) to make the required concentration of [HA] to attain the desired pH. You are protonated the fully deprotonated acid by just the right amount to give the correct amount of the protonated acid. The added acid converts A^- to HA.
   The amount of strong acid to add is f_{HA} equivalents. *moles HCl = $f_{HA} \times [A_T] \times V$*

Example: Make 1L of 1 M buffer solution at pH 5.0 using either imidazole (pKa~6), or pyruvate (pKa~2.5). You have both the protonated and deprotonated species (e.g. Na salt) in hand.

1. Which buffer would you use, why?

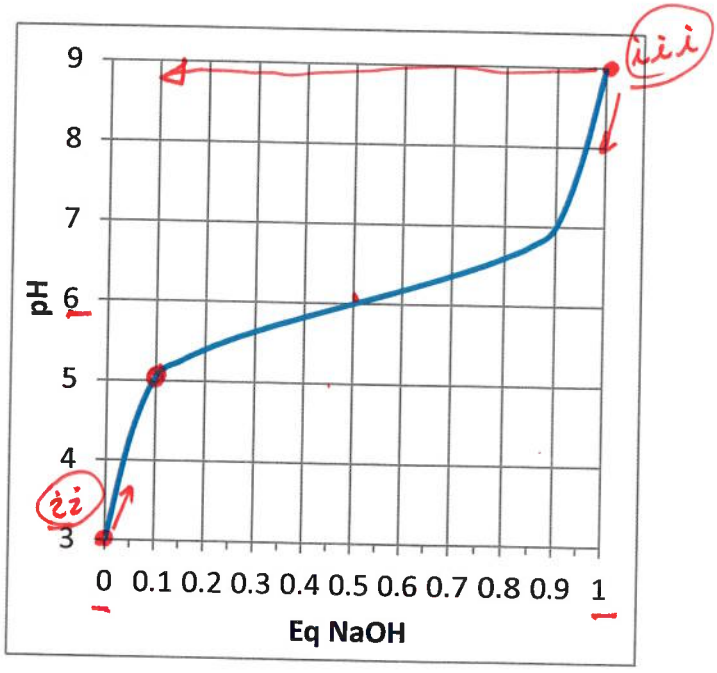
pKa is 1 unit from pH

2. Determine fraction protonated and deprotonated at the desired pH:

$R = 10^{(pH-pKa)}$ $= 10^{5-6} = 10^{-1} = 0.1$

$f_{HA} = \frac{1}{1+R} \approx 0.9$ $f_{A^-} = \frac{R}{1+R} \approx 0.1$

3. Since we have both forms (HA), (A) we can use any of the three methods to make the buffer:



i) mole HA = $A_T \cdot f_{HA}$
mole A = $A_T \cdot f_A$
= A_T

ii) Start with HA (100%)
mole HA = $[A_T] \cdot V$
add NaOH.
eq NaOH = 0.1
moles NaOH = (eq.) A_T

iii) Start NaA
mole A = $[A_T] \cdot V$
(eq): $\frac{\text{moles NaOH}}{\text{mol } A_T} \times$ ~~mol A_T~~
add HCl. 0.9 eq
mole HCl = 0.9 eq · A_T

Questions on buffer Capacity:

1. *What is the capacity of this buffer to the addition of acid? How many equivalents of acid could be added and the pH would still be within the buffer region?*

No capacity (edge)

2. *What is the capacity of this buffer to the addition of base? How many equivalents of base could be added and the pH would still be within the buffer region?*

absorb 0.8 eq of Base still be in buffer region.

Polyprotic Buffers:

Any of the pKas in a poly-protic acid can be used to buffer the solution. For example, phosphate has three pKas and therefore three buffer regions. The overall approach in generating buffers using polyprotic acids is to do all of the calculations using one of the pKas and then modify how you make the buffer. As before, we are going to work in equivalents for all of the calculations, and then convert the equivalents to moles at the end.

Approach:

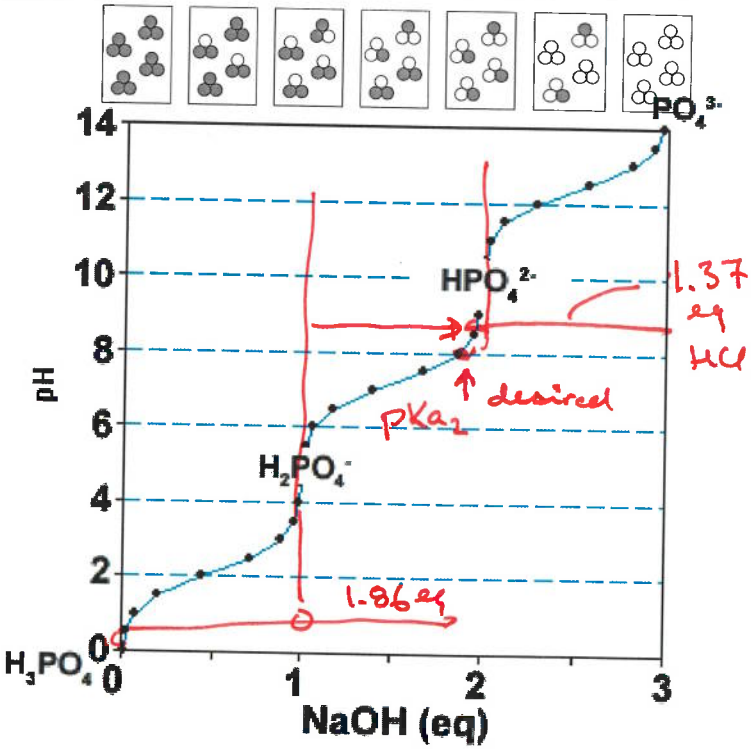
1. Select pKa that is closest to the desired pH, use that one for your calculations.
2. Calculate f_{HA} and f_A using that pKa, and the desired pH
3. Adjust the pH using either of the three methods:
   - i) mix the acid form and the base form of the compound in the ratio of f_{HA} to f_A. The actual chemicals will depend on the buffer region that was used
   - ii) Start with the fully protonated form and add sufficient base to reach desired pH. The number of equivalents is f_A plus additional equivalents to reach the buffer region you used.
   - ii) Start with the fully deprotonated form and add sufficient strong acid to reach desired pH. The number of equivalents is f_{AH} plus additional equivalents to reach the buffer region you used.

Example: Make 1L of a 0.2M phosphate buffer with a pH = 8.0. pKa values are 2.1, 7.2, and 12.7 for phosphoric acid.

1. Use pKa closest to desired pH.
   pK₂ should be used.

2. Calculate f_{HA} and f_{A^-} *(Note: "HA" = H_2PO_4, "A"=HPO_4)*
   $R=10^{pH-pKa} = 10^{(8.0-7.2)} = 10^{0.8} = 6.31$
   $f_{HA} = 1/(1+6.31) = 1/7.31 = 0.137$
   $f_A = 0.863$

3. Select one of the following three methods:
   i) Use chemical forms of "(HA)" and "(A-)" that represent the species present at the pKa you used, in this case. NaH_2PO_4 = "HA" , Na_2HPO_4="A".
   moles $NaH_2PO_4 = f_{HA} \times [A_T] \times V = 0.137 \times 0.2$ mole/L x 1 L
   moles $Na_2HPO_4 = f_A \times [A_T] \times V = 0.863 \times 0.2$ mole/L x 1 L



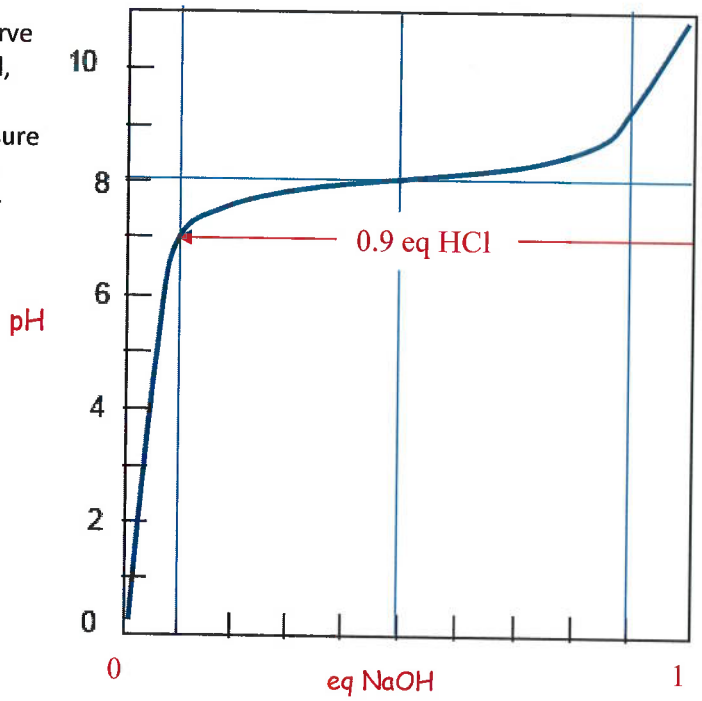
   ii) Starting from completely protonated form (H_3PO_4). Add sufficient whole equivalents (n) to reach the buffer region you are using, plus and additional f_{A^-} to reach the pH within that buffer region.
   eq NaOH = 1 + f_A, moles NaOH = eq x V x A_T

   iii) Starting from completely ionized form (Na_3PO_4). Add sufficient whole equivalents (n) to reach the buffer region you are using, plus and additional f_{AH} equivalents *of* HCl to get to the desired pH in that buffer region.
   eq HCl = 1 + f_{AH} and moles HCl = eq HCl x V x A_T

You want to make a 500 ml solution of a 0.1 M weak acid to use as a buffer at pH 7.0. This is a monoprotic acid with pKa value of 8.0.

$$R = \frac{[A^-]}{[HA]} = 10^{(pH-pKa)}$$
$$f_{HA} = \frac{1}{1+R} = \frac{[HA]}{[A_T]} \quad f_{A^-} = \frac{R}{1+R}$$

a) Sketch the titration curve for this acid, using the graph. Be sure to label the axis or your sketch.



b) Calculate the total number of moles of the weak acid you will need to make this buffer solution.

*This is **always** $[A_T] \times V = 0.1$ moles/L x 0.5 L = 0.05 moles. It is completely **independent** of the pH. It is how many moles of the buffering acid that you would have to add, depending on the method of making the buffer: i) $A_T = AH + A$ (mixing acid and conj base), ii) $A_T = AH$ (starting with acid form), iii) $A_T = NaA$ (starting with deprotonated salt,*

c) Calculate the fraction deprotonated and protonated at the desired pH (7.0). Is this what you would have expected, based on the titration curve for this acid?

Calculate R first: $R = 10^{(pH-pKa)} = 10^{(7-8)} = 10^{-1} = 0.1$

$f_{HA} = 1/(1.1) \sim 0.9$, $f_A = 1-0.9 = 0.1$

At pH 7, 0.1 eq of NaOH was added, this would have converted 1/10 (0.1) of the fully protonated acid to the deprotonated form, so:

$f_{HA} = 1.0 \rightarrow f_{HA} = 0.9$

$f_A = 0.0 \rightarrow f_A = 0.1$

This is consistent with the calculated values.

d) Assume that you started with fully deprotonated acid (e.g. sodium salt, A), how many *equivalents* of HCl will you have to add to bring the pH of the solution to 7.0?

You are starting with f_A=1.0 and f_{HA}=0.0 (fully deprotonated). You need to add HCl to make f_A=0.1 and f_{HA}=0.9. Therefore, you need to add 0.9 equivalents of HCl.

e) How many *moles* of HCl would you need to add?

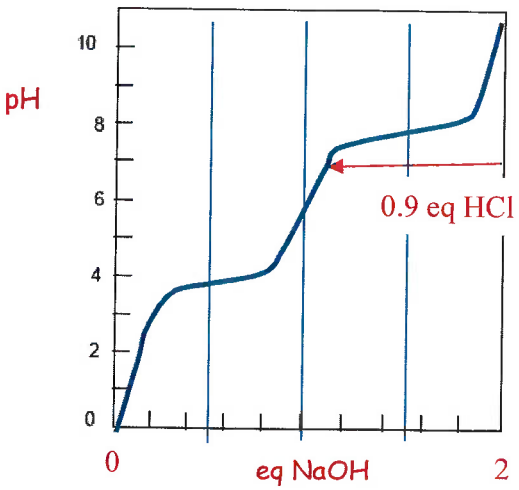
The definition of equivalent is:

moles of titrant (NaOH or HCl)/moles of weak acid.

eq HCl = moles HCl /moles A_T

moles HCl = eq HCl x moles A_T = 0.9 x 0.05 = 0.045 moles

(HCl would be added to bring the total solution volume to 500 ml)



f) How would your answer to question d) change is this were a diprotic acid whose pKa values were 4.0 and 8.0?

The answer would be the same, since you are using the 2nd buffer region and you are starting from the right side of the titration curve.