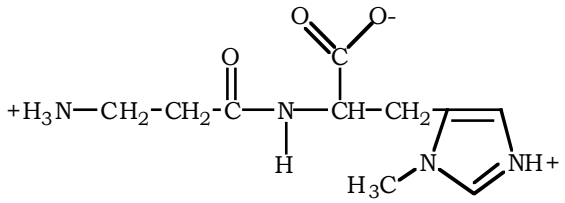
12. How to prepare a buffer solution: an anserine buffer

Draw the titration curve for anserine (Figure 2.16). The isoelectric point of anserine is the pH where the net charge on the molecule is zero; what is the isoelectric point for anserine? Given a 0.1 M solution of anserine at its isoelectric point and ready access to 0.1 M HCl, 0.1 M NaOH and distilled water, describe the preparation of 1 L of 0.04 M anserine buffered solution, pH 7.8?

Answer:



The structure of anserine is shown above. It has three ionizable groups: a carboxyl group pK_{a1} = 2.64, imidazole nitrogen pK_{a2} = 7.04 and amino group pK_{a3} = 9.49. Starting at acidic pH, all three groups will be protonated and thus the molecule will have a +2 charge. As base is added, the carboxyl group will be the first to deprotonate with a midpoint at 2.64. When fully deprotonated at about 4.64, anserine will have a +1 charge. As the pH approaches 7.0, the imidazole group will deprotonate leaving anserine uncharged. Finally, as the pH passes 9.49, the amino group will deprotonate and by about pH 11.5 anserine will have a –1 charge.

The titration curve is shown below with the pK_a's labeled. The isoelectric point, pI, is the pH at which the molecule is uncharged. Clearly, this will happen at a pH at which the carboxyl group's –1 charge is balanced by positive charges from both the imidazole group and the amino group. The isoelectric point must be between the pK_a's of the imidazole and amino groups. Thus, the sum of the protonated imidazole group and the protonated amino group must equal to one equivalent of charge.

Let I and IH^+ be the unprotonated and protonated imidazole groups respectively.
Let A and AH^+ be the unprotonated and protonated amino groups.

$$[IH^+] + [AH^+] = \text{one equivalent}$$

That is, the sum of the positively charged species for the imidazole and the amino groups must sum to the one equivalent of negative charge from the carboxylate. (Remember, there is one of each group.) The concentrations of the imidazole species, protonated and unprotonated, must sum to one equivalent. The same is true for the amino species. Thus,

$$[I] + [IH^+] = [A] + [AH^+] \text{ and so}$$

$$[I] + \cancel{[IH^+]} = \cancel{[IH^+]} + [AH^+] \quad (1)$$

$$[A] + \cancel{[AH^+]} = [IH^+] + \cancel{[AH^+]} \quad (2)$$

From equations (1) and (2) we can see that:

$$[AH^+] = [I] \text{ or } [IH^+] = [A] \quad (3)$$

The Henderson-Hasselbalch equations for each are:

$$pH = pK_2 + \log\frac{[I]}{[IH^+]} = pK_3 + \log\frac{[A]}{[AH^+]}$$

Substituting (3) into these we have:

$$pH = pK_2 + \log\frac{[AH^+]}{[IH^+]} \text{ and}$$

$$pH = pK_3 + \log\frac{[IH^+]}{[AH^+]}$$

Solving these two equations for the log terms, which are inversely related, and setting them equal we have:

$$\text{pH-pK}_2 = \log\frac{[\text{AH}^+]}{[\text{IH}^+]}$$

$$\text{-pH+pK}_3 = -\log\frac{[\text{IH}^+]}{[\text{AH}^+]} = \log\frac{[\text{AH}^+]}{[\text{IH}^+]}$$

Thus,

$$\text{pH-pK}_2 = \text{-pH+pK}_3$$

$$\text{pH} = \frac{\text{pK}_2 + \text{pK}_3}{2} = \text{pI} = \frac{7.04 + 9.49}{2}$$

$$\text{pI} = 8.27$$

In order to prepare 1 L of 0.04 M anserine we need to use 400 ml (0.4 L) of 0.1 M anserine stock. (1 L×0.04 M = 0.1 M×0.4 L). Since the pH = 8.27 we will have to titrate to pH = 7.2 using 0.1 M HCl. At pH = 8.27 the amino group is nearly fully protonated whereas the imidazole group is nearly unprotonated. Using the Henderson-Hasselbalch equation for the imidazole group, we can determine what the ratio of unprotonated to protonated form at pH 8.27 and 7.20.

$$\text{pH} = 7.04 + \log\frac{[\text{I}]}{[\text{IH}^+]}$$

$$\text{At 7.2, } \frac{[\text{I}]}{[\text{IH}^+]} = 10^{(7.2-7.04)} = 1.445$$

And since $[\text{I}] + [\text{IH}^+] = 0.04$

$$1.445 \times [\text{IH}^+] + [\text{IH}^+] = 0.04$$

$$[\text{IH}^+]_{7.2} = \frac{0.04}{2.445} = 0.0164$$

$$\text{At 8.27, } \frac{[\text{I}]}{[\text{IH}^+]} = 10^{(8.27-7.04)} = 16.98$$

And since $[\text{I}] + [\text{IH}^+] = 0.04$

$$16.98 \times [\text{IH}^+] + [\text{IH}^+] = 0.04$$

$$[\text{IH}^+]_{8.27} = \frac{0.04}{17.98} = 0.0022$$

$$[\text{IH}^+]_{7.2} - [\text{IH}^+]_{8.27} = 0.0164 - 0.0022 = 0.0142$$

Thus, we will need to add 142 mL of 0.1 M HCl to adjust the imidazole group. We will need additional HCl to titrate the amino group from pH 8.27 to 7.20.

$$\text{pH} = 9.49 + \log\frac{[\text{N}]}{[\text{NH}^+]}$$

$$\text{At 7.2, } \frac{[\text{N}]}{[\text{NH}^+]} = 10^{(7.2-9.49)} = 0.00513$$

And since $[\text{N}] + [\text{NH}^+] = 0.04$

$$0.00513 \times [\text{NH}^+] + [\text{NH}^+] = 0.04$$

$$[\text{NH}^+]_{7.2} = \frac{0.04}{1.00513} = 0.0398$$

$$\text{At 8.27, } \frac{[\text{N}]}{[\text{NH}^+]} = 10^{(8.27-9.49)} = 0.06026$$

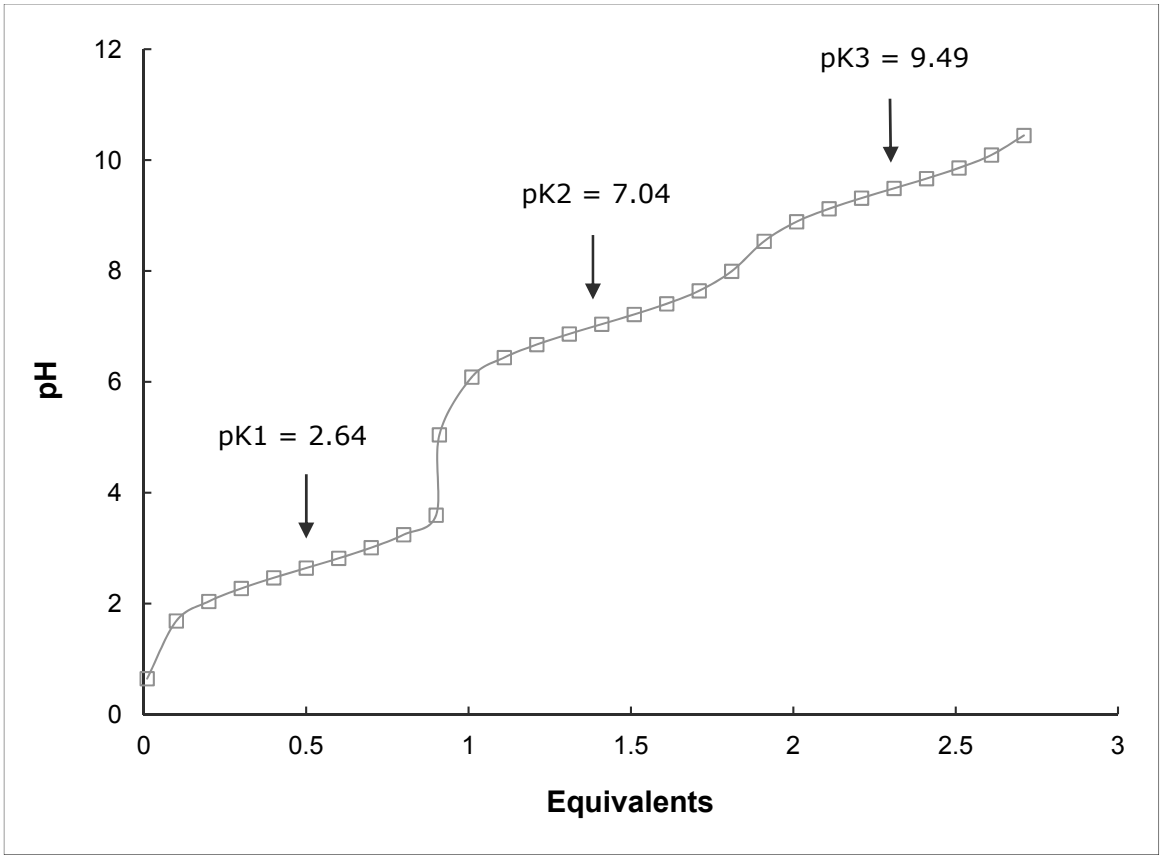
And since $[\text{N}] + [\text{NH}^+] = 0.04$

$$0.06026 \times [\text{NH}^+] + [\text{NH}^+] = 0.04$$

$$[\text{IH}^+]_{8.27} = \frac{0.04}{1.06026} = 0.03773$$

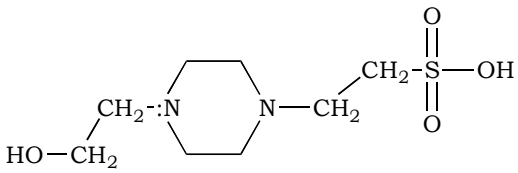
$$[\text{IH}^+]_{7.2} - [\text{IH}^+]_{8.27} = 0.0398 - 0.03773 = 0.00207$$

Thus, we will need to add 21 mL of 0.1 M HCl to titrate the amino group. The total HCl need will be 163 ml.



13. How to prepare a buffer solution: a HEPES buffer
Given a solution of 0.1 M HEPES in its fully protonated form, and ready access to 0.1 M HCl, 0.1 M NaOH and distilled water, describe the preparation of 1 L of 0.025 M HEPES buffer solution at pH 7.8?

Answer: The structure of HEPES is shown below.



The sulfonic acid group has a pK_a of around 3 and the ring nitrogen has a pK_a of 7.55. HEPES in its fully protonated form would have its tertiary nitrogen protonated and hence positively charged and a protonated, uncharged sulfonic acid group. To achieve this state a strong acid, like HCl must be added making the chloride salt of HEPES. To bring the pH to 7.8 would require a strong base like NaOH. One equivalent of NaOH would have to be added to deprotonate the sulfonic acid group. Additional NaOH would be required to deprotonate the tertiary nitrogen. To make 1 L of 0.025 M HEPES using 0.1 M stock we need:

$$0.025\frac{\text{mole}}{\text{L}} \times 1\ \text{L} = 0.1\frac{\text{mole}}{\text{L}} \times \text{x}$$

Solving for x we find that x = 0.25 L or 250 mL

The amount of base needed to adjust the pH to 7.8 can be calculated using the Henderson-Hasselbalch equation. Let [$Hepes^{1-}$] = the concentration of the unprotonated tertiary amine form (whose charge would be 1- due to the sulfonate group) and let [Hepes-H] be the protonated tertiary amine form (which is uncharged because the protonated tertiary nitrogen's positive charge balances the sulfonate's negative charge).

$$pH = pK_a + \log \frac{[Hepes^{1-}]}{[Hepes\text{-}H]}$$

$$7.8 = 7.55 + \log \frac{[Hepes^{1-}]}{[Hepes\text{-}H]}$$

$$\frac{[Hepes^{1-}]}{[Hepes\text{-}H]} = 10^{0.25} = 1.78 \quad (1)$$

Since $[Hepes^{1-}] + [Hepes\text{-}H] = 0.025$ M, we can use this equation and (1) to calculate $[Hepes^{1-}]$ at pH = 7.8.

$$[Hepes^{1-}] + \frac{[Hepes^{1-}]}{1.78} = 0.025 \text{ M}$$

$$[Hepes^{1-}] = 0.025 \times \frac{1.78}{2.78} = 0.016 \text{ M}$$

Thus, in addition to the 0.025 mol NaOH we must add to deprotonate the sulfonic acid group, we must add an additional 0.016 mol NaOH to titrate the tertiary amine. The total amount of NaOH we must add is 0.041 moles or 410 ml of 0.1 M NaOH. To complete the solution we must add 340 ml of water.

14. Determination of the molecular weight of a solution by freezing point depression
A 100-g amount of a solute was dissolved in 1000 g of water. The freezing point of this solution was measured accurately and determined to be –1.12°C. What is the molecular weight of the solute?

Answer: In the section dealing with colligative properties we learn that 1 mol of an ideal solute dissolved in 1000 g of water (a 1.0 molal solution) depresses the freezing point by 1.86°C. We can set up a proportionality to calculate how many mol was added in this problem.

$$\frac{1.0 \text{ molal}}{-1.86^\circ\text{C}} = \frac{x}{-1.12^\circ\text{C}}$$

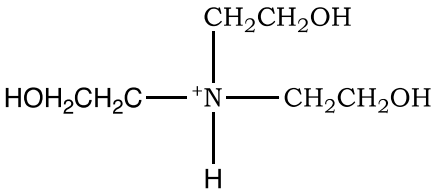
$$x = \frac{1.12}{1.86} \times 1.0 \text{ molal}$$

$$x = 0.60 \text{ molal}$$

The 0.6 molal solution was made by adding 100 g solute, which represents 0.6 mol. Therefore, the solute's molecular weight is:

$$\frac{100 \text{ g}}{0.6 \text{ mol}} = 167 \text{ Da}$$

15. How to prepare a buffer solution: a triethanolamine buffer
Shown here is the structure of triethanolamine in its fully protonated form:



Its pK_a is 7.8. You have available at your lab bench 0.1 M solutions of HCl, NaOH, and the uncharged (free base) form of triethanolamine, as well as ample distilled water. Describe the preparation of a 1 L solution of 0.05 M triethanolamine buffer, pH 7.6.

Answer: The free base form of triethanolamine is the molecule shown above but with its tertiary nitrogen unprotonated. The solution we are asked to make must be 0.05 M triethanolamine. So, the first step is to calculate the amount of triethanolamine that is needed to make 1 L of 0.05 M solution using a 0.1 M solution.

$$0.05 \text{ M} \times 1 \text{ L} = 0.1 \text{ M} \times x$$

$$x = 0.5 \text{ L} = 500 \text{ ml}$$

The pH of the free base solution is basic because when added to water triethanolamine protonates and

depletes the solution of free protons. To adjust the pH to 7.6 we will have to add HCl. The amount of HCl needed is determined by application of the Henderson-Hasselbalch equation using 7.8 for pK_a and 7.6 for pH.

$$pH = pK_a + \log\frac{[\text{Triethanolamine}]}{[\text{Triethanolamine}\cdot H^+]}$$

$$\frac{[\text{Triethanolamine}]}{[\text{Triethanolamine}\cdot H^+]} = 10^{(pH-pK_a)}$$

$$\frac{[\text{Triethanolamine}]}{[\text{Triethanolamine}\cdot H^+]} = 10^{(7.6-7.8)} = 10^{(-0.2)} = 0.6310$$

At pH 7.6 the ratio of free base to protonated triethanolamine is 0.631 and we know that the sum of the concentrations of these species is 0.05. Or,

$$\frac{[\text{Triethanolamine}]}{[\text{Triethanolamine}\cdot H^+]} = 0.6310$$

And,

$$[\text{Triethanolamine}] + [\text{Triethanolamine}\cdot H^+] = 0.05\ \text{M}$$

There are two equations with two unknowns. Solving for one unknown and substituting gives:

$$[\text{Triethanolamine}] = 0.6310 \times [\text{Triethanolamine}\cdot H^+]$$

And,

$$[\text{Triethanolamine}] + [\text{Triethanolamine}\cdot H^+] = 0.05\ \text{M}$$

Or,

$$0.6310 \times [\text{Triethanolamine}\cdot H^+] + [\text{Triethanolamine}\cdot H^+] = 0.05\ \text{M}$$

And,

$$1.6310 \times [\text{Triethanolamine}\cdot H^+] = 0.05\ \text{M}$$

$$[\text{Triethanolamine}\cdot H^+] = \frac{0.05\ \text{M}}{1.6310} = 0.0307\ \text{M}$$

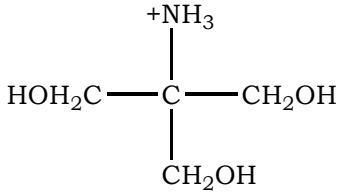
To adjust the pH to 7.6, which we should recognize is below the pK_a, we will have to add 0.0307 moles of HCl. (We are making up 1L.) Using 0.1 M HCl, we will need

$$\frac{0.0307\ \text{mole}}{0.1\ \text{M}} = 0.307\ \text{L} = 307\ \text{ml}$$

So, the solution is made by mixing 500 ml of 0.1 M triethanolamine (free base) with 0.1 M HCl using 307 ml to drop the pH to 7.6. Then adjust the final volume to 1 L.

16. How to prepare a buffer solution: a Tris buffer solution

Tris-*hydroxymethyl aminomethane (TRIS) is widely used for the preparation of buffers in biochemical research. Shown here is the structure TRIS in its protonated form:*



Its acid dissociation constant, K_a, is 8.32 x 10^{-9}. You have available at your lab bench a 0.1 M solution of TRIS in its protonated form, 0.1 M solutions of HCl and NaOH, and ample distilled water. Describe the preparation of a 1 L solution of 0.02 M TRIS buffer, pH 7.8.

Answer: Using the 0.1 M TRIS solution the amount needed to make 1 L of 0.02 M is determined as follows:

$$\frac{x \times 0.1\ \text{M}}{1\ \text{L}} = 0.02\ \text{M}$$

Where x is the volume of 0.1 M to be used.

Solving for x we find:

$$x = \frac{0.02\ \text{M} \times 1\ \text{L}}{0.1\ \text{M}} = 0.2\ \text{L} = 0.2\ \text{L} \times \frac{1000\ \text{ml}}{\text{L}} = 200\ \text{ml}$$

Next let's use the Henderson-Hasselbalch equation to calculate the ratio of TRIS base to protonated TRIS at pH = 7.6. Note: We are given the value for K_a, 8.32 x 10^{-9}. The pK_a is calculated as follows:

$$pK_a = -\log\left(8.32 \times 10^{-9}\right) = 8.0799$$

Use the Henderson-Hasselbalch equation as follows:

$$pH = pK_a + \log\frac{[\text{Tris base}]}{[\text{Tris} \cdot H^+]}$$

$$7.6 = 8.0799 + \log\frac{[\text{Tris base}]}{[\text{Tris} \cdot H^+]}$$

$$\frac{[\text{Tris base}]}{[\text{Tris} \cdot H^+]} = 10^{(7.6-8.0799)} = 10^{-0.4799} = 0.331$$

But,

$$[\text{Tris base}] + [\text{Tris} \cdot H^+] = 0.02\ \text{M}$$

Use the last two equations to solve for the concentration of each species.

$$[\text{Tris base}] = 0.331 \times [\text{Tris} \cdot H^+]$$

But,

$$0.331 \times [\text{Tris} \cdot H^+] + [\text{Tris} \cdot H^+] = 0.02\ \text{M}$$

$$1.331 \times [\text{Tris} \cdot H^+] = 0.02\ \text{M}$$

$$[\text{Tris} \cdot H^+] = \frac{0.02\ \text{M}}{1.331} = 0.0150$$

And,

$$[\text{Tris base}] = 0.02\ \text{M} - [\text{Tris} \cdot H^+] = 0.02\ \text{M} - 0.0150 = 0.005\ \text{M}$$

The protonated Tris solution was likely made using Tris·HCl, which is the chloride salt of Tris base. Thus, it contains equal amounts of chloride and Tris distributed between its protonated and unprotonated forms but mainly as the protonated form. The 200 ml represents 0.02 mole of protonated Tris. To adjust the pH to 7.6 we will need to lower the protonated Tris from 0.02 moles to 0.015 mole and so we will need to add NaOH in the amount of 0.005 mole. This corresponds to the following volume of 0.1 M NaOH:

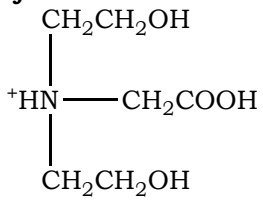
$$x \times 0.1\ \text{M} = 0.005\ \text{mol}$$

$$x = 0.05\ \text{L} = 50\ \text{ml}$$

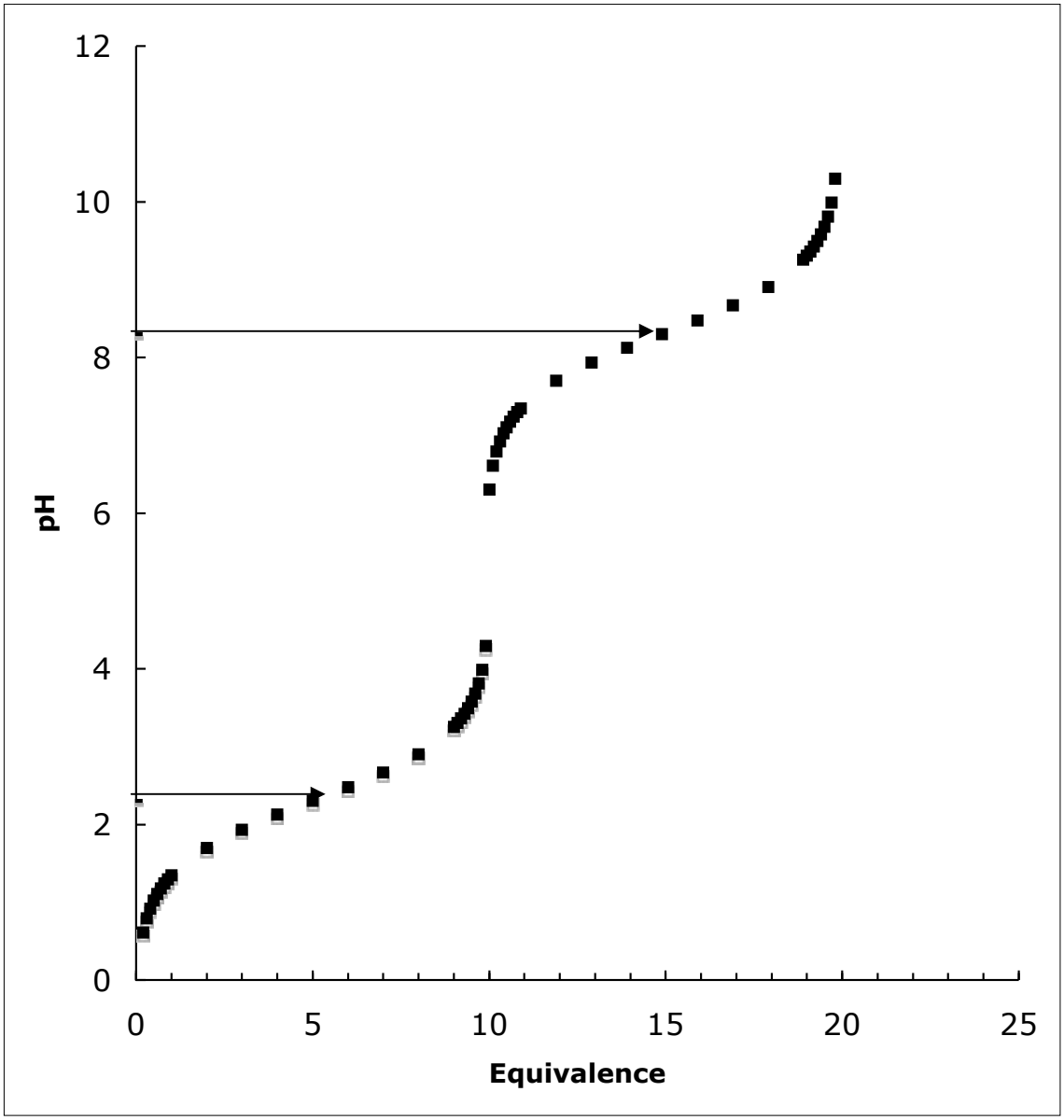
The final recipe is to use 200 ml of 0.1 M protonated Tris solution and add 50 ml of 0.1 M NaOH.

The final solution will actually be 0.02 M Tris buffer at pH = 7.6 but it will contain 5 mM NaCl produced when Tris HCl was adjusted with NaOH. A better way of preparing this solution is to use Tris base and then titrate with HCl to pH = 7.6.

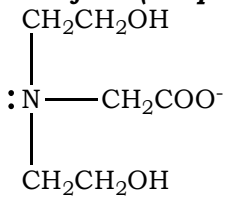
17. Plot the titration curve for Bicine and calculate how to prepare a pH 7.5 Bicine buffer solution
Bicine (N, N–bis (2-hydroxyethyl) glycine) is another commonly used buffer in biochemistry labs. The structure of bicine in its fully protonated form is shown below:



a. Draw the titration curve for Bicine, assuming the pK_a for its free COOH group is 2.3 and the pK_a for its tertiary amino group is 8.3.



b. Draw the structure of the fully deprotonated form (completely dissociated form) of bicine.



c. You have available a 0.1 M solution of Bicine at its isoelectric point (pH_I), 0.1 M solutions of HCl and NaOH, and ample distilled H_2O. Describe the preparation of 1 L of 0.04 M Bicine buffer, pH 7.5.

Answer: The volume of 0.1 M bicine needed is:

$$\frac{x \times 0.1\ M}{1\ L} = 0.04\ M$$

Solving for x :

$$x = \frac{1\ L \times 0.04\ M}{0.1\ M} = 0.4\ L = 400\ ml$$

The isoelectric point of Bicine is simply the average of the two pK_as.

$$pI = \frac{pK_{a1} + pK_{a2}}{2}$$

$$pI = \frac{2.3 + 8.3}{2} = 5.3$$

At this pH the carboxyl group is mainly unprotonated whereas the tertiary nitrogen is nearly fully protonated and thus the average charge is zero. Using the Henderson-Hasselbalch equation we can calculate the ratio of protonated to unprotonated species for each group. For the carboxyl group:

$$pH = pK_{COOH} + \log\frac{[COO^-]}{[COOH]}$$

$$\frac{[COO^-]}{[COOH]} = 10^{(pH - pK_{COOH})} = 10^{(5.3-2.3)}$$

$$\frac{[COO^-]}{[COOH]} = 10^{(3)} = 1000$$

This calculation shows that at pH 7.5 only approximately 0.1% is protonated. The calculation for the tertiary nitrogen of bicine is as follows:

$$pH = pK_N + \log\frac{[N]}{[NH^+]}$$

$$\frac{[N]}{[NH^+]} = 10^{(pH - pK_N)} = 10^{(5.3-8.3)}$$

$$\frac{[N]}{[NH^+]} = 10^{(-3)} = 0.001$$

We should have anticipated this result, namely the ratios are inverse, because we are starting at a pH that is equidistant from each pK_a.

To adjust the pH from 5.3 to 7.5 will require addition of NaOH. The exact amount is determined by application of the Henderson-Hasselbalch equation to both groups to determine the ratio of protonated to unprotonated forms of both species. For the carboxyl group:

$$pH = pK_{COOH} + \log\frac{[COO^-]}{[COOH]}$$

$$\frac{[COO^-]}{[COOH]} = 10^{(pH - pK_{COOH})} = 10^{(7.5-2.3)}$$

$$\frac{[COO^-]}{[COOH]} = 10^{(5.2)} = 1.58 \times 10^5$$

For the amino group:

$$pH = pK_N + \log\frac{[N]}{[NH^+]}$$

$$\frac{[N]}{[NH^+]} = 10^{(pH-pK_N)} = 10^{(7.5-8.3)}$$

$$\frac{[N]}{[NH^+]} = 10^{(-0.8)} = 0.158$$

Using these equations and remembering that the total sum of COO- and COOH is equal to 0.04 mol (0.04 M times 1 L) and that the same is true for the protonated and unprotonated nitrogen we can calculate the moles of each species at the starting pH (i.e., the pI) and at pH = 7.5. The results are shown to five places. The column labeled "delta" is the change in the species from pI to pH = 7.5.

	pI = 5.3	pH = 7.5	delta
COO-	0.03996	0.04000	-0.00004
COOH	0.00004	0.00000	0.00004
N	0.00004	0.00546	-0.00542
NH+	0.03996	0.03454	0.00542
		Sum =	0.00546

Using 0.1 M NaOH we need 54.6 ml. The final solution is then adjusted to a final volume of 1L with 545.4 ml of water.

d. What is the concentration of fully protonated form of Bicine in your final buffer solution?

Answer: At any pH there will be four possible forms of bicine shown in the chart below. The fraction of each species is simply the fraction of the carboxyl species times the fraction of the nitrogen species.

For example, COO-/NH refers to Bicine with unprotonated carboxyl group and protonated nitrogen. The value 0.863 under the fraction column was calculated using data in the chart in part c. The fraction of carboxyl group that is unprotonated is calculated by dividing the value for COO- in the above chart by the sum of COOH and COO-. The same is done for the nitrogen. The molar amount is the value under fraction times 0.04. The sum shows that all species are accounted for. There are only two species at significant levels, both have the carboxyl group unprotonated. Fully protonated Bicine is only at 0.2 μM.

Species	Fraction	Molar Amount
COO-/N	0.1364413	0.0054577
COO-/NH	0.8635524	0.0345421
COOH/N	0.0000009	0.0000000
COOH/NH	0.0000055	0.0000002
		Sum = 0.04

18. Calculate the concentration of Cl^- in gastric juice
Hydrochloric acid is a significant component of gastric juice. If chloride is the only anion in gastric juice, what is its concentration if pH =1.2?

Answer: Strong acids like HCl fully dissociate in solution and so the pH, which is –log[H^+], is closely approximated by –log[HCl]

$$HCl \rightarrow H^+ + Cl^-$$

Thus, pH = –log[HCl] = 1.2 giving

[HCl] = $10^{-1.2}$= 0.0631 or 63.1 mM
So the chloride concentration is 63.1 mM.

19. Calculate the concentration of lactate in blood plasma at pH 7.4 if [lactic acid] = 15 μM. From the pK_a for lactic acid given in Table 2.4, calculate the concentration of lactate in blood plasma (pH = 7.4) if the concentration of lactic acid is 1.5 μM.

Answer: From Table 2.4 the pK_a of lactic acid is 3.86. To determine the concentration of lactate at pH = 7.4 we need to use the Henderson-Hasselbalch equation.

$$pH = pK_a + \log\frac{[\text{lactate}]}{[\text{lactic acid}]}$$

$$pH = 7.4,$$
$$pK_a = 3.86,$$
$$[\text{lactic acid}] = 1.5\ \mu M = 1.5\times10^{-6} M$$
$$[\text{lactate}] = [\text{lactic acid}]\times10^{(7.4-3.86)} = 1.5\times10^{-6} M\times10^{3.54}$$
$$[\text{lactate}] = 0.0052\ M = 5.2\ mM$$

Note: The statement that the concentration of lactic acid is 1.5 μM was taken literally. It is possible that the concentration refers to the sum of lactate and its protonated, lactic acid form. In this case the solution is as follows:

$$pH = pK_a + \log\frac{[\text{lactate}]}{[\text{lactic acid}]}$$

$$pH = 7.4,$$
$$pK_a = 3.86,$$
$$[\text{lactic acid}] = 1.5\ \mu M = 1.5\times10^{-6} M = [\text{lactate}] + [\text{lactic acid}]$$
$$[\text{lactate}] = [\text{lactic acid}]\times10^{(7.4-3.86)} = [\text{lactic acid}]\times10^{3.54} = 3{,}467\times[\text{lactic acid}]$$
$$3{,}467\times[\text{lactic acid}] + [\text{lactic acid}] = 1.5\times10^{-6} M$$
$$[\text{lactic acid}] = \frac{1.5\times10^{-6} M}{3{,}468} = 4.32\times10^{-10} M, \text{ and}$$
$$[\text{lactate}] = 3{,}467\times[\text{lactic acid}] \approx 1.5\times10^{-6} M$$

That is, the pH is so far from the pKa that essentially all the lactic acid is in the unprotonated lactate form.

20. Draw the titration curve for a weak acid and determine its pK_a from the titration curve When a 0.1 M solution of a weak acid was titrated with base, the following results were obtained:

Equivalence of base added	*pH Observed*
0.05	*3.4*
0.15	*3.9*
0.25	*4.2*
0.40	*4.5*
0.60	*4.9*
0.75	*5.2*
0.85	*5.4*
0.95	*6.0*

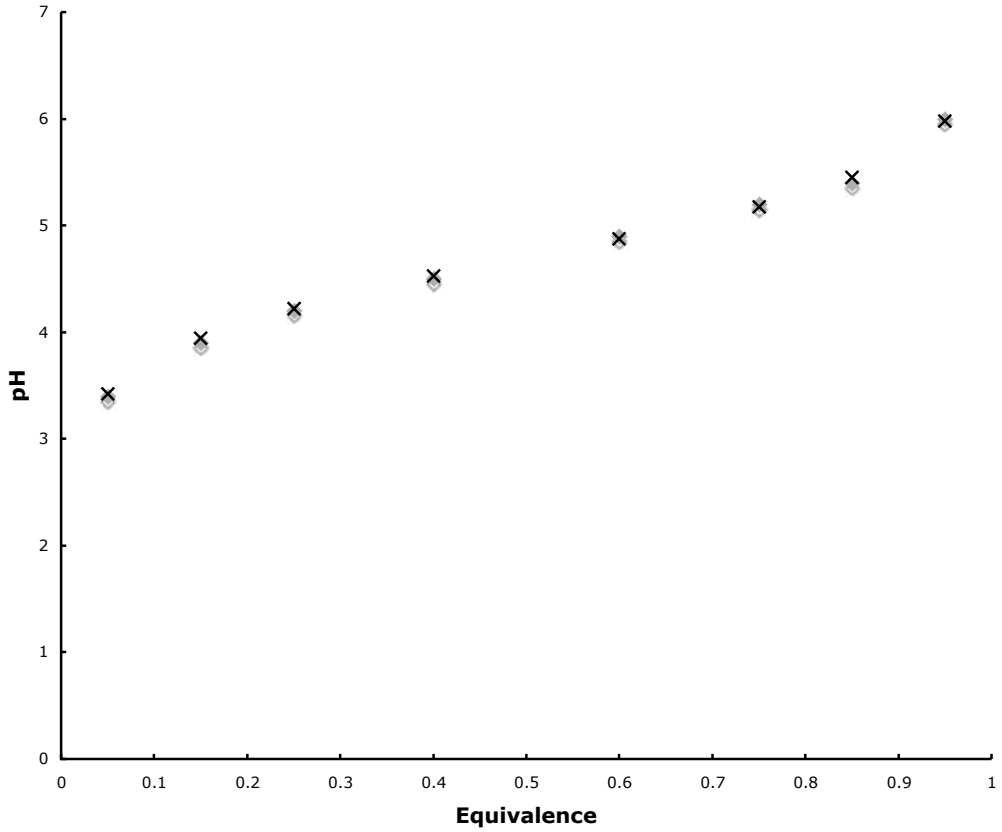
Plot the results of this titration and determine the pK_a of the weak acid from your graph.

Answer: In the chart shown below the values for $pH_{calculated}$ were determined using the Henderson-Hasselbalch equation with a guess for pK_a shown. Values of $[A^-]$ were assumed to be equal to the amount of

base added while the values of [HA] were 1- [A^-].

equivalence	pH	$pH_{calculated}$	pK_a	A^-	HA
0.05	3.4	3.42	4.7	0.05	0.95
0.15	3.9	3.95		0.15	0.85
0.25	4.2	4.22		0.25	0.75
0.4	4.5	4.52		0.4	0.6
0.6	4.9	4.88		0.6	0.4
0.75	5.2	5.18		0.75	0.25
0.85	5.4	5.45		0.85	0.15
0.95	6	5.98		0.95	0.05

In the graph shown below pH is plotted against equivalence for the observed data (closed circles) and for the $pH_{calculated}$ (×). There is very close agreement between the two data sets indicating that the guess for 4.7 is close to the true value. We know we are dealing with an acid (something with pK_a lower that 7.0) because the initial pH is 3.42.



Preparing for the MCAT® Exam

21. The enzyme alcohol dehydrogenase catalyzes the oxidation of ethyl alcohol by NAD^+ to give acetaldehyde plus NADH and a proton:

$$CH_3CH_2OH + NAD^+ \rightarrow CH_3CHO + NADH + H^+$$

The rate of this reaction can be measured by following the change in pH. The reaction is run in 1 ml 10 mM TRIS buffer at pH 8.6. If the pH of the reaction solution falls to 8.4 after ten minutes, what is the rate of alcohol oxidation, expressed as nanomoles of ethanol oxidized per sec per ml of reaction mixture?

Answer: The number of protons produced after 10 minutes can be determined using the Henderson-Hasselbalch equation. The reaction is being conducted using Tris buffer at an initial pH of 8.6. In problem 16 we were given the following information about Tris:

$$pK_a = -\log\left(8.32 \times 10^{-9}\right) = 8.0799$$

We will use the Henderson-Hasselbalch equation to determine the ratio of protonated and unprotonated Tris at pH 8.6 and at pH 8.4.

For pH = 8.6

$$pH = pK_a + \log\frac{[\text{Tris base}]}{[\text{Tris} \cdot H^+]}$$

$$8.6 = 8.0799 + \log\frac{[\text{Tris base}]}{[\text{Tris} \cdot H^+]}$$

$$\frac{[\text{Tris base}]}{[\text{Tris} \cdot H^+]} = 10^{(8.6-8.0799)} = 10^{0.5201} = 3.312$$

For pH =8.4

$$pH = pK_a + \log\frac{[\text{Tris base}]}{[\text{Tris} \cdot H^+]}$$

$$8.4 = 8.0799 + \log\frac{[\text{Tris base}]}{[\text{Tris} \cdot H^+]}$$

$$\frac{[\text{Tris base}]}{[\text{Tris} \cdot H^+]} = 10^{(8.4-8.0799)} = 10^{0.3201} = 2.090$$

The total concentration of Tris is 10 mM and we are dealing with a volume of 1.0 ml. The total number of moles of Tris is calculated as follows:

$$10 \text{ mM} \times 1 \text{ ml} = \frac{10 \times 10^{-3} \text{ mol}}{\text{L}} \times 1 \text{ ml} \times \frac{1 \text{ L}}{1000 \text{ ml}} = 1.0 \times 10^{-5} \text{mol} \times \frac{1 \times 10^{9} \text{ nmol}}{\text{mol}} = 10{,}000 \text{ nmol}$$

At pH 8.6, the ratio of unprotonated Tris to protonated Tris is 3.312. Using this information and the fact that both forms must sum to 10,000 nmol we can calculate the moles of each form at both pH values.

$$\text{Tris base} + \text{Tris} \cdot H^+ = 10{,}000 \text{ nmol}$$

$$\frac{\text{Tris base}}{\text{Tris} \cdot H^+} = 3.312$$

$$\text{Tris base} = 3.312 \times \text{Tris} \cdot H^+$$

Substituting into the top equation

$$3.312 \times \text{Tris} \cdot H^+ + \text{Tris} \cdot H^+ = 10{,}000 \text{ nmol}$$

Or,

$$4.312 \times \text{Tris} \cdot H^+ = 10{,}000 \text{ nmol}$$

$$\text{Tris} \cdot H^+ = \frac{10{,}000}{4.312} \text{ nmole} = 2319 \text{ nmol}$$

And, substituting this back into the top equation

$$\text{Tris base} + 2319 \text{ nmol} = 10{,}000 \text{ nmol}$$

$$\text{Tris base} = 7681 \text{ nmol}$$

The moles of each species at the two pH values are shown below

	pH		
	8.6	8.4	Delta
Tris base	7681	6764	917
$TrisH^+$	2319	3236	917
Total	10000	10000	

The column headed "Delta" is the amount of acid in nmoles that must have been produced to change the ratio of Tris species. To be correct we should calculate the amount of acid needed to lower the pH from 8.6 to 8.4 without regard to buffering. This value, however, is very small. It is calculated as follows:

$$pH = -\log[H^+]$$

$$[H^+] = 10^{-pH}$$

At pH 8.6

$$[H^+] = 10^{-8.6} = 2.51 \times 10^{-9}\text{M}$$

At pH 8.4

$$[H^+] = 10^{-8.4} = 3.98 \times 10^{-9}\text{M}$$

The change in protons is the difference inconcentrations times the volume

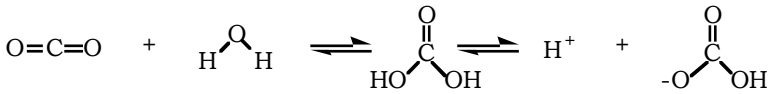
$$\text{moles produced} = \left(3.98 \times 10^{-9}\text{M} - 2.51 \times 10^{-9}\text{M}\right) \times 1 \times 10^{-3}\text{L} \times \frac{1 \times 10^{9}\text{nmol}}{\text{mol}} = 0.0015 \text{ nmol}$$

So, we have determined that 917 nmol of proton were produced in 10 minutes. The rate is calculated as follows:

$$\text{rate} = \frac{917 \text{ nmole}}{10 \text{ min}} \times \frac{1 \text{ min}}{60 \text{ sec}} = 1.53 \ \frac{\text{nmole}}{\text{sec}}$$

22. In light of the Human Biochemistry box, what would be the effect on blood pH if cellular metabolism produced a sudden burst of carbon dioxide?

Answer: The enzyme carbonic anhydrase rapidly hydrates carbon dioxide to form carbonic acid, which dissociates into a proton and bicarbonate as shown below.



Thus, production of carbon dioxide should lead to additional protons and hence a decrease in pH (acidosis). The body can compensate by increasing exchange of carbon dioxide in the lungs. In the lungs, since carbon dioxide is being eliminated the above equilibrium shifts to the left causing a decrease in proton concentration and an increase in pH.

23. On the basis of Figure 2.12, what will the pH of acetate-acetic acid solution when the ratio of [acetate]/[acetic acid] is 10?

a. 3.76
b. 4.76
c. 5.76
d. 11.24

Answer: The pK_a of acetic acid is 4.76. To realize a ratio of acetate to acetic acid of 10 nearly an equivalent of base (NaOH) must be added and from Figure 2.12 we see that this corresponds to a pH of around 6. So, the correct answer must be "c", 5.76. One can easily calculate this value using the Henderson-Hasselbalch equation, $pH = pK_a + \log[A^-]/[HA]$. The term $[A^-]/[HA]$ is the ratio of acetate to acetic acid, which is given as 10. The log 10 = 1.0, thus the pH = 4.76 +1 = 5.76.