1. a. For the strong acid HCl, $pH = -\log[H^+]$

$[HCl] = 40\ mM$ ∴ $pH = -\log[4\times10^{-2}M]$

$= 1.4$

b. $[H^+] = \text{antilog}\ -pH = 10^{-pH}$

$pH = 4.0$ ∴ $[H^+] = 10^{-4}M \equiv 0.1\ mM$

c. If 90% of $[H^+]$ in (b) is titrated with the strong base LiOH, $[H^+]$ remaining $= 10^{-4}M - (0.9\times10^{-4}M) = 10^{-5}M$

$pH = -\log[H^+] = -\log 10^{-5} = 5$

2. a. For the weak acid acetic acid ($K_a = 4.76$),

$H_3C\text{-}CO_2H \overset{K_a}{\rightleftharpoons} H^+ + H_3C\text{-}CO_2^{\ominus}$

$K_a = \text{antilog}\ pK_a = 10^{-4.76} = 1.74\times10^{-5}M$

$K_a = [H^+]^2 / [H_3C\text{-}CO_2H]_0 - [H^+] = 1.74\times10^{-5}M$

Assuming $[H_3CCO_2H]_0 \gg [H^+]$, the $[H^+]$ can be eliminated from the denominator.

∴ $[H^+]^2 \simeq 1.74\times10^{-5}M\ [H_3CCO_2H]_0$

with a $[H_3CCO_2H]_0 = 40\ mM \equiv 4\times10^{-2}M$,

$[H^+] \simeq (1.74\times10^{-5}M \times 4\times10^{-2}M)^{1/2}$

$\simeq (6.96\times10^{-7}M^2)^{1/2} = 8.3\times10^{-4}M$

To see if means the < 5% of acetic acid is dissociated,

$[H^+] / [H_3CCO_2H]_0 = \frac{8.3\times10^{-4}M}{4\times10^{-2}} \simeq 2.1\%$

this is < 5%, thus answer is accurate enough.

2.2. cont. $pH = -\log[H^+] = -\log(8.3 \times 10^{-4} M)$

$= 3.1$

b. $pH = 4$, what is $[H_3CCO_2H]_0$?

$K_a = [H^+]^2 / [H_3CCO_2H]_0 - [H^+]$

$1.74 \times 10^{-5} M = (10^{-4} M)^2 / [H_3CCO_2H]_0 - 10^{-4} M$

$[H_3CCO_2H]_0 = (10^{-8} M^2 + 1.74 \times 10^{-9} M^2) / 1.74 \times 10^{-5} M$

$= 6.7 \times 10^{-4} M \equiv 0.67 mM$

c. If 2× amt. of LiOH used in (1c) is added to the acetic acid in (2b), what is pH change?

$[LiOH]$ added $= 2 \times (0.9 \times 10^{-4} M) = 1.8 \times 10^{-4} M$

To calculate pH, use H-H eq. and assume a stoichiometric titration of acetic acid by LiOH.

$pH = pKa + \log \frac{[CH_3CO_2^-]}{[CH_3CO_2H]}$

$= 4.76 + \log [LiOH] / [H_3CCO_2H]_0 - [LiOH]$

$= 4.76 + \log 1.8 \times 10^{-4} M / (6.7 \times 10^{-4} M - 1.8 \times 10^{-4} M)$

$= 4.76 + \log(3.67 \times 10^{-1})$

$= 4.3$

d. Although 2×[LiOH] added in 2c, pH changed only $(4.3 - 4.0) = 0.3$ units vs. $(5.0 - 4.0) = 1.0$ unit in (1c).

2. d. cont. Reasons for difference: (need to state 2)

1. More acetic acid present (0.67 mM vs 0.1 mM)
   ∴ not as high a fraction titrated.
2. Titration is within the buffer range of acetic acid
3. Assumption of stoichiometric titration of acetic acid by LiOH not valid at start of titration curve for acetic acid due to significant dissociation of untitrated acetic acid at this pH.

3. a. EGTA has 4 pKa values. When dissolved in water, the lowest pKa group will dissociate first, and if the pH that is established is pKa_2, only the pKa_1 will significantly affect pH. ∴ check pH due to pKa_1.

Use Ka eq.

$[EGTA]_o = 38\ g\ l^{-1} / 380\ g\ mole^{-1} = 0.1\ M$

$pKa_1 = 2.00$; $pKa_2 = 2.65$

$Ka_1 = 1.00 \times 10^{-2}\ M$; $Ka_2 = 2.24 \times 10^{-3}\ M$

$$Ka_1 = \frac{[H^+]^2}{[EGTA]_o - [H^+]} \cong \frac{[H^+]^2}{[EGTA]_o}$$

$$[H^+] \cong (1.00 \times 10^{-2}\ M \times 0.10\ M)^{1/2} = 3.16 \times 10^{-2}\ M$$

$$\%\ EGTA\ dissociated = \frac{3.16 \times 10^{-2}\ M}{0.1\ M} \times 100 = 32\%$$

32% >> 5% limit for elimination of $[H^+]$ in denominator. Must solve quadratic eq.

3.a. cont. $K_{a_1} = \dfrac{[H^+]^2}{[EGTA]_o - [H^+]}$

$$[H^+]^2 + 1.00\times10^{-2}\,M[H^+] - (1.00\times10^{-2}\,M)(10^{-1}\,M) = 0$$

$$[H^+] = \frac{-10^{-2}\,M + \sqrt{(10^{-2}\,M)^2 - 4(-10^{-3}\,M^2)(1)}}{2(1)}$$

$$= 2.70\times10^{-2}\,M$$

$$pH = -\log(2.70\times10^{-2}\,M) = 1.57$$

This pH is >1 pH unit from pK_{a_2} (2.65) so H^+ dissociation from pK_{a_2} group can be discounted.

b. ml of 1 M KOH needed to titrate 1 l of 0.1 M EGTA to pH 7.8?

$pK_{a_1} = 2.00$; $pK_{a_2} = 2.65$; $pK_{a_3} = 8.85$; $pK_{a_4} = 9.46$

Since pH 7.8 lies between well beyond pK_2 and close to pK_{a_3}, 2 equivalents of KOH and a fraction of a 3rd will be needed to titrate through pK_{a_1} & $_2$ and part way through the pK_{a_3} area.

Use H-H eq. to determine this fraction. Since pH 7.8 is closest to pK_{a_3}, set up H-H eq. with pK_{a_3} for best accuracy.

$$pH = pK_{a_3} + \log [EGTA^{3-}]/[EGTA^{2-}]$$

$$7.8 = 8.85 + \log [EGTA^{3-}]/[EGTA^{2-}]$$

$$\frac{[EGTA^{3-}]}{[EGTA^{2-}]} = 10^{-1.05} = \frac{1}{10^{1.05}} = \frac{1}{11.2}$$

$$\text{Fraction } [EGTA^{3-}] = \frac{1}{1+11.2} = 8.2\times10^{-2}$$

3. b. cont. equivalents of KOH needed = 2.08

From [EGTA], 1 equivalent = 1.0 l. × 0.1 M = 0.1 mole

∴ 2.08 × 0.1 mol = 2.08×10^{-1} moles KOH needed

[KOH] = 1 M ≡ 10^{-3} mol/ml

ml needed = 2.08×10^{-1} mol / 10^{-3} mol·ml^{-1} = 208 ml

For 2 liters of final solution,

water added = 2000 ml − 1000 ml − 208 ml

= 792 ml

c. If equal volumes of 3b solution and 5.0 mM K_4EGTA are mixed, what is pH?

The 3b solution is 0.05 M EGTA and is comprised of $8.2 \times 10^{-2} \times 0.05\,M = 4.1 \times 10^{-3}\,M$ $EGTA^{3-}$

and $0.05\,M - 4.1 \times 10^{-3}\,M = 45.9 \times 10^{-3}\,M$ $EGTA^{2-}$

If $EGTA^{4-}$ is added H^+ will move from the $EGTA^{2-}$ to the $EGTA^{4-}$, converting both to $EGTA^{3-}$.

Adjusting for this change, the H-H eq. can be used to solve for pH.

$$pH = pKa_3 + \log \frac{[EGTA^{3-}]}{[EGTA^{2-}]}$$

$$= 8.85 + \log \frac{(4.1 \times 10^{-3}\,M + 2 \cdot 5.0 \times 10^{-3}\,M)/2}{(45.9 \times 10^{-3}\,M - 5.0 \times 10^{-3}\,M)/2}$$

$$= 8.39$$

4. a. $pH_{\text{for blood}} = pK_{\text{overall}} + \log \frac{[HCO_3^-]}{[CO_2(d)]}$

If pH ↑ to 7.6 and $[HCO_3^-]$ ↓ 2mM/0.3mM ↓ in $CO_2(d)$, what fraction of normal 1.2mM $CO_2(d)$ is lost?

$$7.6 = 6.1 + \log \frac{[HCO_3^-]_0 - x\,(2mM/0.3mM)}{[CO_2(d)]_0 - x}$$

where $x = [CO_2(d)]_{lost}$

$$1.5 = \log \frac{24mM - 6.7x}{1.2mM - x}$$

$$24mM - 6.7x = 10^{1.5}\,(1.2mM - x)$$

$$31.6x - 6.7x = 37.9mM - 24mM$$

$$x = 0.56mM$$

Fraction $CO_2(d)$ lost = 0.56mM / 1.2mM = 0.46

b. Level of $[HCO_3^-]$ need to return pH to normal 7.4

$$7.4 = 6.1 + \log \frac{[HCO_3^-]_{new}}{CO_2(d)\ remaining}$$

$$= 6.1 + \log \frac{[HCO_3^-]_{new}}{1.2mM - 0.56mM}$$

$$10^{1.3} = [HCO_3^-]_{new} / 0.64mM$$

$$[HCO_3^-]_{new} = 13mM$$

5. For the Rx, $dCMP + H^+ + H_2O \rightleftharpoons NH_4^+ + dUMP$

40mM dCMP converted to dUMP in reaction containing 100mM Tris·HCl (pH 7.1). pH at end of RX?
Determine starting $Tris^\circ$ & $Tris^+$ concentrations, and after adjusting them for the 40mM of H^+ consumed calculate pH at the new $Tris^\circ/Tris^+$ ratio.

Tris pKa = 8.1

$$pH_{init.} = 7.1 = pKa + \log \frac{[Tris^\circ]}{[Tris^+]}$$

$$= 8.1 + \log \frac{[Tris^\circ]}{[Tris^+]}$$

$$[Tris^\circ]/[Tris^+] = 10^{-1} = \frac{1}{10} \text{ of 11 parts total}$$

$$[Tris]_0 = 100mM \times \frac{1}{11} = 9.1\ mM$$

$$[Tris^+]_0 = 100mM - 9.1\ mM = 90.9\ mM$$

At end of reaction,

$$pH = 8.1 + \log \frac{[Tris^\circ]_0 + [H^+]_{consumed}}{[Tris^+]_0 - [H^+]_{consumed}}$$

$$= 8.1 + \log \frac{9.1\ mM + 40mM}{90.9mM - 40mM}$$

$$= 8.1 + \log 0.96$$

$$= 8.1$$

This pH is 1.14 units below the pKa of 9.24 for $NH_4^\oplus$ so its contribution to the pH can be considered insignificant.
The 50mM NaCl is just to provide a good environment for the enzyme.

6. $ATP \rightleftharpoons ADP + P_i$ ($\Delta G^{\circ\prime} = -32.5\ kJ/mol$, $\Delta S^{\circ\prime} = 5.37\ J/^\circ mol$)

a. $\Delta H^{\circ\prime} = \Delta G^{\circ\prime} + T\Delta S^{\circ\prime}$

$= -32.5\ kJ/mol + (273^\circ + 25^\circ)\left(5.37\ J/^\circ mol\right)\left(\frac{10^{-3}\ kJ}{J}\right)$

$= -32.5\ kJ/mol + 1.6\ kJ/mol$

$= -30.9\ kJ/mol$

b. Both the enthalpy and entropy favor this reaction, but $\Delta H^{\circ\prime}$ at $-30.9\ kJ/mol$ far outdoes the $-T\Delta S^{\circ\prime}$ at $-1.6\ kJ/mol$. Thus mainly enthalpy driven.

c. $\Delta G^{\circ\prime} = \Delta H^{\circ\prime} - T\Delta S^{\circ\prime}$

$= -30.9\ kJ/mol - (273^\circ + 37^\circ)\left(5.37\ J/^\circ mol\right)\left(\frac{10^{-3}\ kJ}{J}\right)$

$= -32.56\ kJ/mol$

$K_{eq}' = 10^{-\Delta G^{\circ\prime}/2.303RT}$

$= 10^{32.56\ kJ/mol \,/\, 2.303 \cdot 8.314\ J/^\circ mol \cdot 10^{-3}\ kJ/J \cdot (273^\circ + 37^\circ)}$

$= 10^{32.56\ kJ/mol \,/\, 5.94\ kJ/mol} = 10^{5.48}$

$= 3.02 \times 10^5$

d. $\Delta G' = \Delta G^{\circ\prime} + 2.303RT \log \frac{[ADP][P_i]}{[ATP]}$

$= -32.56\ kJ/mol + 5.94\ kJ/mol \log \frac{[ADP][3 \times 10^{-3}\ M]}{10[ADP]}$

$= -32.56\ kJ/mol - 20.93\ kJ/mol$

$= -53.49\ kJ/mol$

7. $tRNA^{Gly}$ + glycine + ATP ⇆ glycyl-tRNA + AMP + $2P_i$

a. This reaction is a coupled reaction, which is the sum of the following reactions:

$ATP \leftrightarrows AMP + PP_i$ $\Delta G^{o\prime}$ = -31 kJ/mol (from Table 4.7, Chap. 3)

$PP_i \leftrightarrows 2P_i$ $\Delta G^{o\prime}$ = -33 kJ/mol (from Table 4.7, Chap. 3)

$tRNA^{Gly}$ + glycine ⇆ glycyl-tRNA $\Delta G^{o\prime}$ = +33 kJ/mol (given in problem)

Summing the $\Delta G^{o\prime}$ values = $\Delta G^{o\prime}$ for the overall charging reaction = -31 kJ/mol

H_2O and H^+ are not included in the chemical equation since they are constant and subsumed in the $\Delta G^{o\prime}$ values.

b. $\Delta G^{o\prime} = -2.303RT\log Keq'$

$Keq = 10^{\exp -\Delta G^{o\prime}/5.71 kJ/mol} = 10^{\exp -(-31 \text{ kJ mol}^{-1}/5.71 \text{ kJ mol}^{-1})}$

$= 10^{5.43} = 2.69 \times 10^5$

c. If glycine, ATP, AMP and P_i are present at typical intracellular values of 0.15 mM, 1 mM, 0.1 mM, and 3 mM, what will be the equilibrium ratio of charged to uncharged $tRNA^{Gly}$?

When $\Delta G' = 0$ the reaction is at equilibrium

$Keq = [\text{glycyl-tRNA}][AMP][P_i]^2/[tRNA^{Gly}][Gly][ATP] = 2.69 \times 10^5$

$[\text{glycyl-tRNA}]/[tRNA^{Gly}] = 2.69 \times 10^5 [Gly][ATP]/[AMP][P_i]^2$

$= 2.69 \times 10^5 (1.5 \times 10^{-4}M)(1 \times 10^{-3}M)/(1 \times 10^{-4}M)(3 \times 10^{-3}M)^2$

$= 4.48 \times 10^7$

d. If $[\text{glycyl-tRNA}]/[tRNA^{Gly}]$ reduced 10x

$\Delta G' = \Delta G^{o\prime} + 2.303RT \log (1/10)([\text{glycyl-tRNA}][AMP][P_i]^2/[tRNA^{Gly}][Gly][ATP])$

$= -31 \text{ kJ/mol} + 5.71 \text{ kJ mol}^{-1} \log (Keq/10)$

$= -31 \text{ kJ/mol} + 5.71 \text{ kJ mol}^{-1} \log 10^{5.43} + 5.71 \text{ kJ mol}^{-1} \log 10^{-1}$

$= -31 \text{ kJ/mol} + 31 \text{ kJ/mol} - 5.71 \text{ kJ mol}^{-1}$

$= -5.71 \text{ kJ mol}^{-1}$

If $[\text{glycyl-tRNA}]/[tRNA^{Gly}]$ reduced 100x

$\Delta G' = \Delta G^{o\prime} + 2.303RT \log (1/10)([\text{glycyl-tRNA}][AMP][P_i]^2/[tRNA^{Gly}][Gly][ATP])$

$= -31 \text{ kJ/mol} + 5.71 \text{ kJ mol}^{-1} \log (Keq/100)$

$= -31 \text{ kJ/mol} + 5.71 \text{ kJ mol}^{-1} \log 10^{5.43} + 5.71 \text{ kJ mol}^{-1} \log 10^{-2}$

$= -31 \text{ kJ/mol} + 31 \text{ kJ/mol} - (2 \times 5.71 \text{ kJ mol}^{-1})$

$= -11.4 \text{ kJ mol}^{-1}$

e. $\Delta \Delta G'$ value for each successive 10-fold decrease in $[\text{glycyl-tRNA}]/[tRNA^{Gly}]$ is -5.7 kJ mol^{-1}. This will be the case for all reactions when the denominator of the log term increases by a factor of 10..

8. $H_3\overset{\oplus}{N}$-Lys - Ala - Gln - Met - Arg - Thr - Asp - $CO_2^{\ominus}$

pKa 9.5 10.0 - - - 12.0 4.7 4.5

pKas on the α-amino & side chain of Lys may be switched. Same is true for α- and side chain carboxyls of Asp.

a. CNBr cleavage will occur after Met, yielding

$H_3\overset{+}{N}$-Lys-Ala-Gln-Met + $H_3\overset{+}{N}$-Arg-Thr-Asp-$CO_2^{\ominus}$

the Met will be modified by the cleavage to be a lactone, eliminating the C-ter $-CO_2^{\ominus}$.

b. At ~pH2 where ESI MS is run the 2 peptides will have the following charges:

$\overset{\oplus}{\oplus}$K A Q M $\overset{+}{\oplus}$R T D

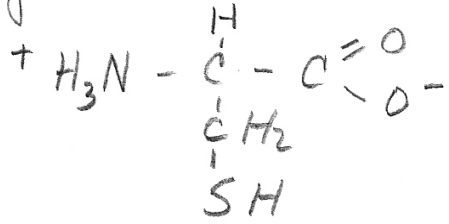
The tripeptide RTD will reach the detector 1st since it has the higher charge/mass (z/m) ratio. ESI TOF MS separates on the basis of z/m with the highest z/m gaining the greater momentum and thus striking the detector first.

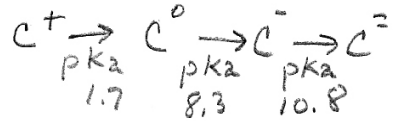
c. At pH 7, the peptides would have the following charges:

$\overset{+}{+}$K A Q M + R T D −

8. c. cont. CM-chromatography has $^{\ominus}O_2C-$ groups linked to the matrix. It binds positive molecules such as $KAQM^{2+}$. The neutral RTD° would likely flow through during loading.

9. a. Cysteine structure & charge at pI



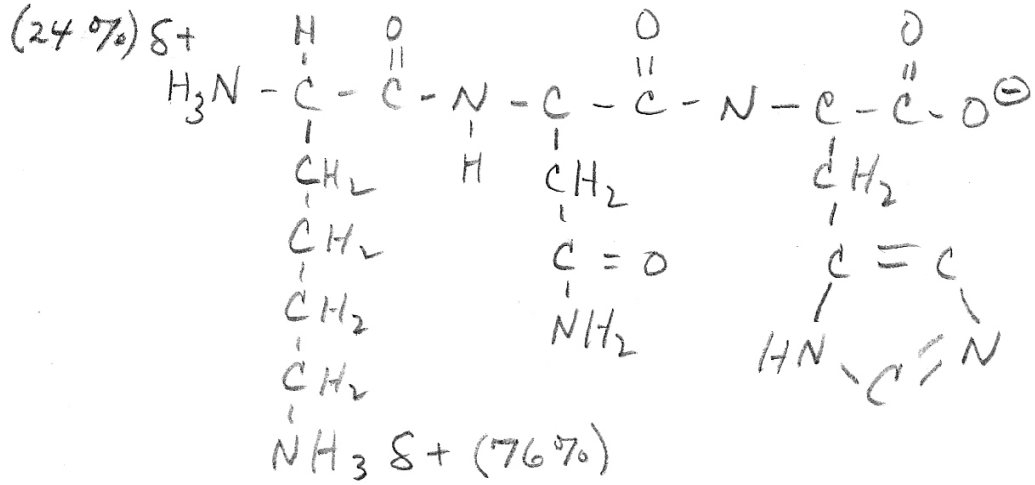


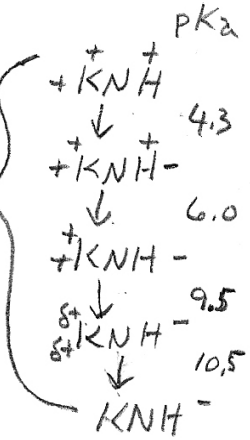
pKa 10.8 8.3 1.7

The pI will lie half way between the deprotonation of the α-carboxyl and the thiol groups

$$\therefore \quad pI = \frac{1.7 + 8.3}{2} = 5.0$$

b. K - N - H at pI





pKa 9.5 10.5 6.0 4.3

9. b. cont. $pI = \frac{pK_a \text{ N-ter} + pK_a \text{ Lys side chain}}{2}$

$= \frac{9.5 + 10.5}{2} = 10$

Use H-H eq. to calculate fractional charge at this pH.

For lys $pH = pK_a + \log \frac{Lys^0}{Lys^+}$

$10 = 10.5 + \log \frac{Lys^0}{Lys^+}$

$Lys^0/Lys^+ = 10^{-0.5} = \frac{1}{3.2}$

$\therefore \frac{3.2}{4.2} = 0.76 \equiv 76\%$ + charged on ϵ-NH_2

For N-ter

$10 = 9.5 + \log \frac{[-NH_2]}{[-NH_3^+]}$

$-NH_2/-NH_3^{\oplus} = 10^{0.5} = \frac{3.2}{1}$

$\therefore \frac{1}{4.2} = 0.24 \equiv 24\%$ + charged on N-ter

10. Given proteins A (M_r 18,245; pI 9.82), B (M_r 36,556; pI 7.44), and C (M_r 74, 172; pI 6.54)

a. In SDS PAGE, proteins are denatured by binding to SDS, which converts them all into a similar shape (prolate ellipsoid) coated by the negatively charged SDS. Because of this, distance migrated by a protein is a function only of log M_r. The lower the M_r, the further the protein migrates. The exception to this would be proteins that are rich in positively charged residues, which could neutralize enough of the negative SDS charge to make the protein migrate slower than expected for their size.
Protein A is half the size of protein B, but both migrate together. This indicates that protein A is rich in Lys and/or Arg residues and thus has a larger positive charge than most proteins.

b. In isoelectric focusing, proteins band at their pI in a pH gradient. The cathode at the basic end of the gradient will be negative and attract cations. In the part of the pH gradient below their pI values, proteins will carry a positive charge and migrate toward the cathode. As they enter higher pH regions, they lose protons and finally come to rest at their pI. Thus the higher the pI, the closer they band to the cathode. As a result, protein A, with the highest pI (9.82), will band closest to the cathode.

c. CM (carboxymethyl)-chromatography is cation exchange chromatography, thus the more positive the protein, the stronger the binding to the negative CM matrix. To carry a negative charge the CM must be in a buffer above its pKa. Conversely, to carry a postive charge, the proteins must be in a buffer below their pI. The lower the pH is below the pI, the greater the positive charge on the protein; and, in turn, the stronger the binding to the CM matrix. Similarly, the higher the pI above the pH, the stronger the binding will be.
   As a result, the buffer should be between the pI of the protein and the pKa of the CM, and preferably < pI -1 and < pka + 1. The proteins will elute from lowest to highest pI.
   Thus, the order of elution will be protein C, then B, then A.

d. In gel filtration, proteins are separated on the basis of M_r and shape, with the largest, most compact proteins emerging first and the others in order of decreasing size and compactness.
   Assuming similar shape, the order or emergence should be protein C, B, then A.

e. The lower the charge on a protein, the less soluble it is and the more easily it is outcompeted by salt for water of hydration. Furthermore, the closer the pI of a protein is to the pH of the buffer, the less charge it will carry and ,thus, the easier it can aggregate for precipitation. As a result, proteins will be salted out in order of increasing difference between their pI and the pH of the buffer.
   Because of this, the order of salting out at pH 6, should be protein C, then B, and finally A.