BT203-Mid Sem Question Paper Date: 22.09.2022
Marks: 30

Part A (Marks: 14)

A separate sheet has been provided for Part A questions. The answers have to be entered on the reverse side of that sheet only.

Part B (Marks: 8 x 2 = 16)

Answer the following questions in the answer booklet provided to you. For numerical problems, it is neccessary to show all the steps neatly. Part marking applies only when supported with proper steps. If applicable, the numerical answers should be accompanied by units.

1. What is the pH of a 10^{-8} M solution of hydrochloric acid?

2. How will you prepare 5 litres of 0.3 molar acetate buffer of pH 4.47, if you are given 2 molar solution of acetic acid and 2.5 molar solution of KOH?

3. Under the condition of very low substrate concentration, find out the first order rate constant for an enzyme catalyzed reaction which has a maximum velocity of 4.6 micromoles per liter per minute and Km of 2 micromolar.

4. A peptide was digested with trypsin, and the individual fragments were subjected to Edman degradation. This yielded the following sequence data: PQTSGM, YSRHGQKPTM, STSYP. The same peptide was then fragmented with CNBr and resultant peptides were sequenced by Edman degradation yielding the following results: PQTSGMYSR, HGQK, PTMSTSYP. Deduce the sequence of the original peptide.

5. A cell maintained at 37°C has ATP, ADP and Pi at concentrations of 10^{-3}, 10^{-4}, and 10^{-2} molar, respectively. What is the **actual** free energy change for **ATP synthesis** in this cell?
[The standard free energy of hydrolysis of ATP is -30.5 kJ/mole]

6. An enzyme has a Km of 67 milimolar and exhibits a maximum velocity of 300 nanomoles per liter per minute. This enzyme catalyzed reaction carried out in the presence of 20 micromolar concentration of substrate and 10 micromolar concentration of a competitive inhibitor yielded a reacion velocity of 1.5 nanomoles per liter per minute. Find out the dissociation constant of the enzyme-inhibitor complex.

7. A researcher collects enzyme kinetics data by determining initial velocity for increasing substrate concentrations, in the presence or absence of a non-competitive inhibitor. On the same graph, show the Lineweaver-Burk plots for data generated in the presence and absence of this inhibitor.

8. Consider an amino acid with a basic R group. The pK_{COOH}, p_{amino}, and pK_R of this amino acid is 2.18, 8.95, and 10.53, respectively. What will be the pI of this amino acid? If a solution of this amino acid maintained at pH of 6 is electrophoresed, then towards which electrode will this amino acid move, negative electrode or positive electrode?

Q1 pH = 8 is **not** the answer. Although 10^{-8} M HCl is a very dilute solution of HCl, the solution will never become alkaline.

Secondly, one must not forget that H^+ ions will be contributed by H_2O. In fact, it will contribute more H^+ than HCl.

1st: Approximate Solution

You may neglect H^+ contributed by HCl.

∴ pH of the solution will be **7** [∵ $[H^+]$ due to H_2O will be 10^{-7} M]

2nd Approximate Solution

Total $[H^+]$ in solution = 10^{-7} (from H_2O) + 10^{-8} (from HCl)

$= (1 \times 10^{-7}) + (0.1 \times 10^{-7})$

$\therefore pH = -\log[(1 \times 10^{-7}) + (0.1 \times 10^{-7})] = \log \frac{1}{(1 \times 10^{-7}) + (0.1 \times 10^{-7})}$

$= \log \frac{1}{1.1 \times 10^{-7}} = \log(0.909 \times 10^{7})$

$= \log(9.09 \times 10^{6}) = \log 9.09 + \log 10^{6}$

$= 0.959 + 6 = \boxed{6.959}$

Most Accurate answer: $H_2O \rightleftharpoons H^+ + OH^-$

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

Let X be the $[H^+]$ contributed by H_2O [i.e. $[H^+]_{H_2O} = X$]

$\Rightarrow [OH^-] = X$

$[H^+]$ from HCl = 10^{-8} M

$\therefore$ Total $[H^+] = X + 10^{-8}$ and $[OH^-] = X$

We know

$[H^+][OH^-] = 10^{-14} \Rightarrow (X + 10^{-8})(X) = 10^{-14}$

$\Rightarrow X^2 + 10^{-8}X = 10^{-14} \Rightarrow X^2 + 10^{-8} - 10^{-14} = 0.$

If you apply $X = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$ formula $X = 9.5125 \times 10^{-8}$

$\therefore [H^+] = X + 10^{-8} = 9.5125 \times 10^{-8} + 10^{-8} = 10.5125 \times 10^{-8}$

$pH = -\log(10.5125 \times 10^{-8}) = \boxed{6.978}$

[Note: Any one of the answers is fine]

Q2. $pH = pK_a + \log \frac{[CH_3COO^-]}{[CH_3COOH]} \Rightarrow 4.17 = 4.76 + \log \frac{[CH_3COO^-]}{[CH_3COOH]}$

(pK_a of acetic acid.)

$\Rightarrow -0.3 = \log \frac{[CH_3COO^-]}{[CH_3COOH]} = \frac{1}{2}$

$\Rightarrow$ The ratio $[CH_3COOH] : [CH_3COO^-] = 2 : 1$

$\therefore$ The buffer is 0.3 M acetate buffer, the final solution should contain

$\frac{2}{3} \times 0.3M = 0.2M$ CH_3COOH

$\Rightarrow$ 1 mole in 5 litres of ~~sol~~ buffer

and $\frac{1}{3} \times 0.3M = 0.1M$ CH_3COO^-

$\Rightarrow$ 0.5 moles in 5 litres.

$\therefore$ The total of $1 + 0.5 = 1.5$ moles of acetate should be there in 5 lit of the buffer.

So, for 1.5 moles of acetate we need $\frac{1.5}{2}$ litres of 2M Acetic acid solution

$= 0.75$ lit

Also, $\frac{1}{3}$ of 1.5 moles have to be deprotonated by KOH. For this we need 0.5 moles of KOH. This can be contributed by $\frac{0.5}{2.5}$ litres of 2.5 M KOH solution.

$= 0.2$ litres of KOH solution (2.5 M).

[Ans: We have to mix 750 ml of 2M Acetic acid, and 200 ml of 2.5 M KOH and make up the volume to 5 litres.]

Q3 $v_0 = \frac{V_{max}[S]}{K_M + [S]}$

$v_0 = \frac{V_{max}}{K_M}[S]$ when [S] is very low.

here $\frac{V_{max}}{K_M}$ is the first order rate constant

$= \frac{4.6 \times 10^{-6}\ M/min}{2 \times 10^{-6}\ M} = \boxed{2.3\ min^{-1}}$

or $\boxed{0.0383\ sec^{-1}}$

Q4

CNBR fragments	Trypsin fragments
PQTSGM	PQTSGMYSR
YSRHGQKPTM	HGQK
STSYP	PTMSTSYP

The peptide sequence is as follows, with indicated sites for CNBR and Trypsin cleavage

CNBr ↓ CNBr ↓

P Q T S G M Y S R H G Q K P T M S T S Y P

↑ Trypsin ↑ Trypsin

Q5 $\Delta G = \Delta G^{10} + RT \ln Q = \Delta G^{10} + RT \ln \frac{[ATP]}{[ADP][P_i]}$

Reaction for ATP synthesis

$ADP + P_i = ATP$

ΔG^{10} for ATP synthesis = 30.5 KJ/mole

Actual free energy change

$\Delta G = 30.5\ \frac{KJ}{mole} + 8.315\ \frac{J}{mol.K} \cdot 310K \cdot \ln \frac{10^{-3}\ M}{10^{-4}_M \times 10^{-2}_M}$

$= 30.5 \times 10^3\ \frac{J}{mole} + 8.315\ \frac{J}{mole.K} \cdot 310K \cdot \ln \frac{10^{-3}}{10^{-4} \times 10^{-2}}$

$$\Delta G = 30.5 \times 10^3 \frac{J}{mole} + 8.315 \frac{J}{mol.K} \cdot 310\,K \cdot \ln 10^3$$

$$= 30500 \frac{J}{mole} + 17805.77 \frac{J}{mole}$$

$$= 48305.77 \frac{J}{mole}$$

$$= 48.3\ kJ/mole.$$

Q6 $K_M = 67 \times 10^{-3}\ M$ $\quad v_0 = 1.5 \times 10^{-9}\ M/min$

$V_{max} = 300 \times 10^{-9}\ M/min$

$[S] = 20 \times 10^{-6}\ M$

$[I] = 10 \times 10^{-6}\ M$

$$v_0 = \frac{V_{max}[S]}{K_M^* + [S]}$$ (in the presence of a competitive inhibitor).

$$1.5 \times 10^{-9}\ M/min = \frac{300 \times 10^{-9}\ M/min \times 20 \times 10^{-6}\ M}{K_M^* + 20 \times 10^{-6}\ M}$$

This will yield

$$K_M^* = 3980 \times 10^{-6}\ M$$

$$= K_M\left(1 + \frac{[I]}{K_i^o}\right)$$

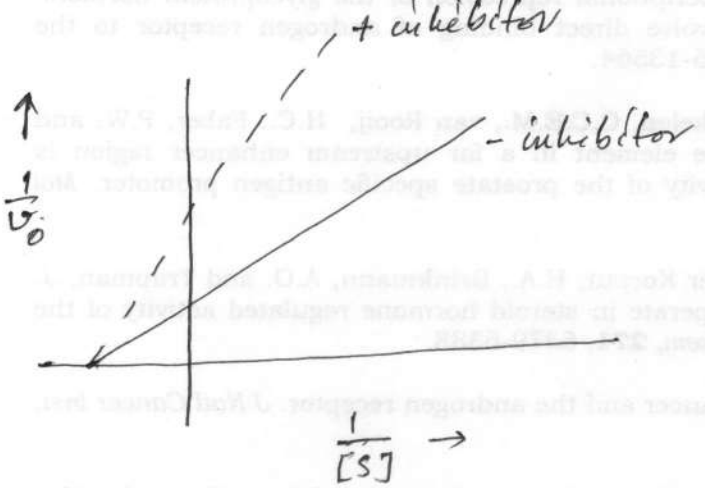
$$\Rightarrow 3980 \times 10^{-6}\ M = 67 \times 10^{-3}\ M \left(1 + \frac{[I]}{K_i^o}\right)$$

$$59.40 \times 10^{-3} = 1 + \frac{[I]}{K_i^o}$$

Here $\frac{[I]}{K_i^o}$ will have ~~not~~ to be negative

$\therefore$ There obviously a problem with the values provided in the question.

Q7 In non-competitive inhibition V_{max} decreases V_{max}/α K_M is not effected.

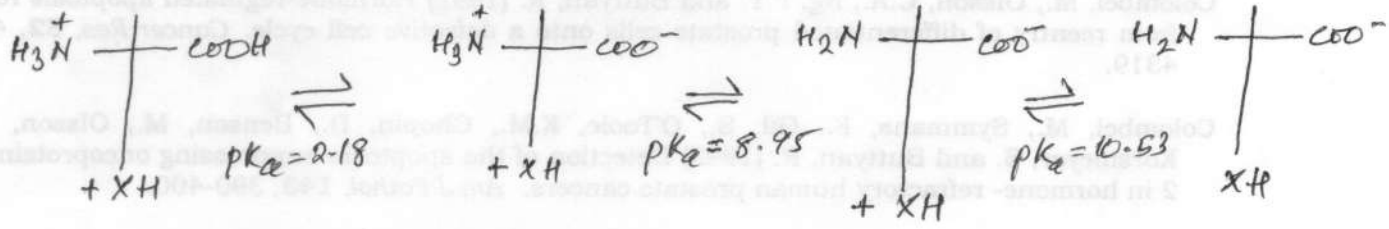


$$v_0 = \frac{V_{max}[S]}{K_M + [S]}$$

Lineweaver Burk Equation

$$\frac{1}{v_0} = \frac{K_M}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

Q8 Schematic representation of all species of the aminoacid is as follows



charge: +2 +1 0 −1

pH at neutral charge = $pI = \frac{10.53 + 8.95}{2} = \boxed{9.74}$

At pH of 5.56 (i.e $\frac{8.95 + 2.18}{2}$) the aminoacid will exist in 1:1 ratio of +2 form and +1 form. At pH=6, concentration of +1 form will be slightly higher than the +2 form. Nonetheless, all molecules will have + charge.

i.e. The aminoacid will move to the negative electrode