Lecture II

PROBLEM 1: PREPARATION OF A BUFFER SOLUTION AND TEST

1) **Prepare a Buffer Solution**

Calculate the pH of a buffer solution prepared by dissolving 0.1 mole of cyanic acid, HCNO, and 0.5 mole of sodium cyanate, NaCNO, in enough water to make 0.5 L of solution. For HCNO, Ka=$2.0\text{x}10^{-4}$

Solution:

n_{HCNO} = 0.1 mol (cyanic acid)
n_{NaCNO} = 0.5 mol (sodium cyanate)
V_f = 0.5 L
$K_a = 2\times10^{-4}$
pH = ?

First approach

We first write the equation for the equilibrium reaction and the expression for the K_a:

$$HA \leftrightarrow A^- + H^+$$

$$K_a = \frac{[A^-][H^+]}{[AH]} = 2\times10^{-4}$$

Let's convert now the moles in molarity amounts:

$$Molarity = \frac{nr.mole}{Volume}$$

[HCNO] = $[AH] = {}^{0.1}/_{0.5} = 0.2\ M$
[NaCNO] = $[A^-] = {}^{0.5}/_{0.5} = 1\ M$
NaCNO dissolves completely in the buffer solution: $NaCNO \rightarrow CNO^- + Na^+$
$[H^+] = \frac{[AH]\,K_a}{[A^-]} = \frac{0.2\times2\times10^{-4}}{1} = 4\times10^{-5}$
pH = - $log_{10}([H^+]) = 4.39$

Second approach

We converts mole to molarity quantities as the previous approach;
[HCNO] = $[AH] = {}^{0.1}/_{0.5} = 0.2\ M$
[NaCNO] = $[A^-] = {}^{0.5}/_{0.5} = 1\ M$
In the buffer solution, NaCNO complete dissolves: $NaCNO \rightarrow CNO^- + Na^+$
We can summarize the information in a table based on the chemical equation:

	$HCNO$ $\leftrightarrow$	CNO^- +	H^+
Initial Concentration (M)	0.2	1	-
Concentration change	-x	+x	+x
Concentration @ equilibrium	0.2-x	1+x	x

$$K_a = \frac{[CNO^-][H^+]}{[HCNO]} = \frac{(1+x)x}{0.2-x} = 2\times10^{-4}$$

Since x << 0.2M, we can approximate like:

$$K_a = \frac{[CNO^-][H^+]}{[HCNO]} = \frac{x}{0.2} = 2{\times}10^{-4}$$

Then:

$$x = [H^+] = 0.2{\times}2{\times}10^{-4} = 4{\times}10^{-5}$$

$$\text{pH} = -\,log_{10}([H^+]) = 4.39$$

2) Add a strong base to a Buffer Solution. Will the pH change?

Calculate the pH of the buffer solution after 0.02 mole of NaOH (sodium hydroxide) has been added to it.

n_{NaOH} = 0.02 mol (sodium hydroxide)

[NaOH] = $^{0.02}/_{0.5} = 0.04\ M$

it completely dissociates in buffer solution: $NaOH \rightarrow\ OH^- +\ Na^+$

the product reacts with cyanic acid: $OH^- + HCNO \rightarrow\ CNO^- +\ H_2O$

So 0.04 M of OH^- consumes 0.04 M of HCNO.

Therefore the table changes as follow:

	$HCNO$ $\leftrightarrow$	CNO^- $+$	H^+
Initial Concentration (M)	0.2	1	-
Buffer action	-0.04	+0.04	-
Concentration change	-x	+x	+x
Concentration @ equilibrium	0.16-x	1.04+x	x

By substituting it in the K_a formula:

$$K_a = \frac{[CNO^-][H^+]}{[HCNO]} = \frac{(1.04 + x)x}{0.16 - x} = 2{\times}10^{-4}$$

we can approximate the equation, since x << 0.16 M:

$$K_a = \frac{[CNO^-][H^+]}{[HCNO]} = \frac{x{\times}1.04}{0.16} = 2{\times}10^{-4}$$

Then:

$$x = [H^+] = \frac{0.16{\times}2{\times}10^{-4}}{1.04} = 3.1{\times}10^{-5}$$

$$\text{pH} = -\,log_{10}([H^+]) = 4.51$$

We can also arrive to the same conclusion by considering only the K_a equation and the changes on these quantities. Indeed, while $[CNO^-]$ increases with the addition of NaOH, [HCNO] decreases of the same quantity since it is reacting with the NaOH. Since we have added 0.04 M of NaOH, we can modify the equation as follow:

$$K_a = \frac{([A^-] + 0.04)[H^+]}{[AH] - 0.04} = \frac{([CNO^-] + 0.04)[H^+]}{[HCNO] - 0.04} = 2{\times}10^{-4}$$

Then:

$[H^+] = \frac{([HCNO]-0.04)\ K_a}{[CNO^-]+0.04} = \frac{0.16{\times}2{\times}10^{-4}}{1.04} = 3.1{\times}10^{-5}$ which means again pH = 4.51

PROBLEM 2 (BUFFER SOLUTION FOR DNA/RNA)
How much Tris-Acetate-EDTA (TE buffer) stock buffer (3.25 M) is required to make 400 ml of solution that has a concentration of 250 mM? How much water?

Solution:

We need to use the formula for dilutions:

$$V_i C_i = V_f C_f$$

Where $V_i C_i$ are Volume and Concentration of the stock solution and $V_f C_f$ are Volume and Concentration of the final solution we want to obtain.
Therefore:
$C_i = 3.25\ M\ TEbuffer$
$V_f = 400\ mL$
$C_f = 250\ mM$
$V_i = ?\,;V_{H2O} = ?$

$$V_i = {V_f C_f}/{C_i} = {400mL \times 250mM}/{3250\ mM} = 30.7\ mL$$

$$V_{H2O} = V_f - V_i = 400\ mL - 30.7\ mL = 369.3\ mL$$

PROBLEM 3 (BUFFER SOLUTION FOR PROTEINS)
You wish to make 3 reactions with the specified amounts of protein. The remainder of each reaction consists entirely of buffer. The source of your protein is a stock solution that has a concentration of 0.5 mg/ml. What is the volume of stock solution required for each reaction (column 3). What is the volume of buffer required (column 4)?

Reaction number	Amount of protein (µg)	Volume of protein solution (µl)	Required Volume of buffer solution (µl)	Required Final Volume (ml)
1	2			1
2	25			2.5
3	100			4
Column 1	**Column 2**	**Column 3**	**Column 4**	**Column 5**

Solution:
We need to use the formula for dilutions: $V_i C_i = V_f C_f$
Where:
Stock Solution: $C_i = 0.5\ mg/ml \quad V_i\ is\ Column\ 3$
Final Solution: $V_f\ is\ Column\ 5 \quad C_f = Column\ 2/Column\ 5$

$$V_i = Column\ 3 = \frac{V_f C_f}{C_i} = \frac{Column\ 5 \times (Column\ 2/Column\ 5)}{0.5\ mg/ml}$$

Column 4 = $V_f - V_i = Column\ 5 - Column\ 3$

Reaction number	Amount of protein (µg)	Volume of protein solution (µl)	Required Volume of buffer solution (µl)	Required Final Volume (ml)
1	2	4	996	1
2	25	50	2450	2.5
3	100	200	3800	4
Column 1	**Column 2**	**Column 3**	**Column 4**	**Column 5**