
بسم الله الرحمن الرحيم

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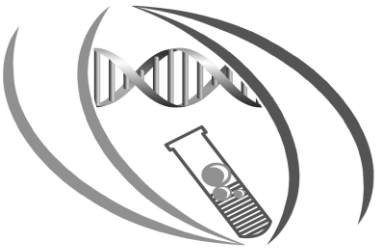

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Practical Note

BIOCHEMICAL CALCULATIONS

(BCH 312)

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Experiment 1

Identification of the common laboratory glassware, pipettes and Equipment .

1.1. Identification of the common laboratory glassware:

A volumetric flask is used for preparing large amounts of standard solutions and reagents that require highly accurate concentrations. This flask is a vessel with a tall, slender neck and a pear-shaped body with a flat bottom and is designed for a single volume, as indicated by the etched line on the neck of the bottle. The most common volumes are 250, 500 ,1000 , and 2000 ml. However smaller volumes are available such as 100, 50 , 25 , and even 1,2, and 5 ml. Figure 1. shows a volumetric flask, Ehrlenmeyer flask graduated cylinder, and beaker of similar sizes.

Ehrlenmeyer flasks are also used in solution preparation but are less accurate than volumetric flasks. This type of flask is a conical container with multiple volume markings that sever as estimate of volume and is available in a variety of sizes, such as 250, 500 and 1000 ml. The purpose of an Ehrlenmeyer flask is to help dissolve a solid solute into solution before transfer to a volumetric flask for final volume adjustment. The flask provides a larger surface area with its straight conical sides, so when a solution is swirled, it has maximum contact with the sides of the flask to help with dissolution.

Beakers are the simple flasks that are shaped like a drinking glass with a small pour spout. They also have volume marking and are available in a variety of total volume sizes. Beakers have the least accuracy of the three types of flasks. They would not be accurate in determining the total volume of an unknown fluid because the markings are not calibrated to a level of accuracy for that purpose. Beakers are used to hold stock solutions or diluents for short-term storage or during laboratory procedures.

Graduated cylinder is a tall flask with multiple volume gradation. The purpose of this flask is to aliquot volumes of a fluid in making up a reagent dilution or to determine the volume of an unknown fluid.

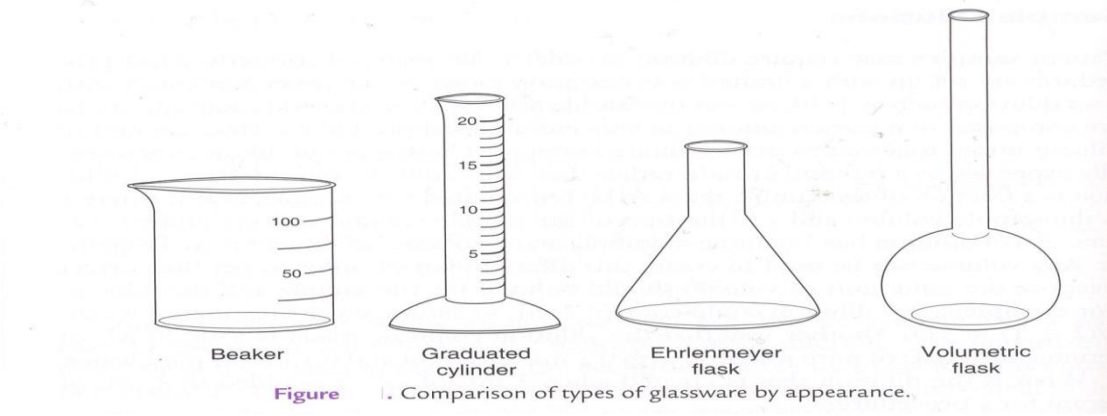


Figure . Comparison of types of glassware by appearance.

1.2. Pipetting techniques

Objective:

(1) For students to become familiar with the use of pipetting techniques.

1.2.1 Introduction:

There are two main type of pipettes are used in biochemical laboratory:

(a)Volumetric or transfer pipettes

(b)Graduated or measuring pipettes

(a)Volumetric pipettes (Transfer):

are designed to deliver a fixed volume of liquid and consists of a cyclindrical bulb joined at both ends to narrowed glass tubing.

Steps of the Use of the pipettes:

-The pipette is first washed with water ,then rinsed several times with a little of the solution to be used and finally filled to just above the mark , the liquid is allowed to fall to the mark .

The solution is allowed to drain into the appropriate vessel with the jet of the pipette touching the wall of the vessel .

After the flow of the liquid has stopped, the jet is held against the wall for some times and then removed . A certain amount of liquid will remain at the tip and this must not be "blown out".

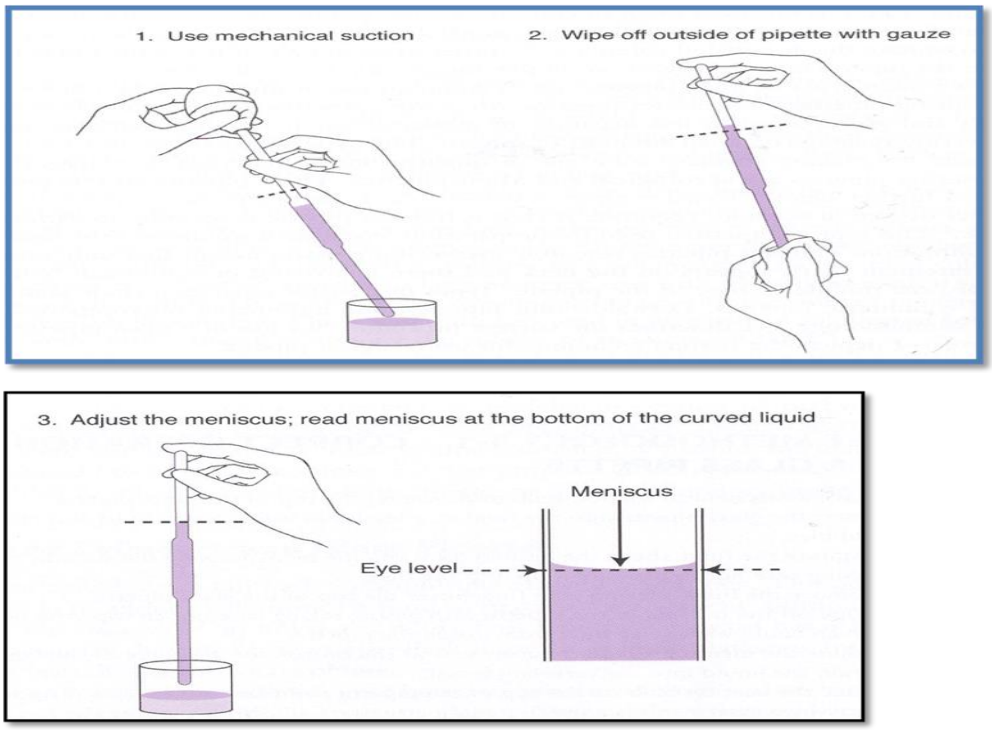


Figure 2. Correct operation of a glass pipette.

(b)Graduated pipettes:

These consists of a plain narrow tube drawn out to a tip and graduated uniformly along its length. There are two types of graduated pipettes are available:

1-Mohr, graduated between two marks

2-Serological, pipettes with graduation marks down to the tip.

Some are of the blown out type(stated on the stem); the last drop being blown out against the vessel wall .

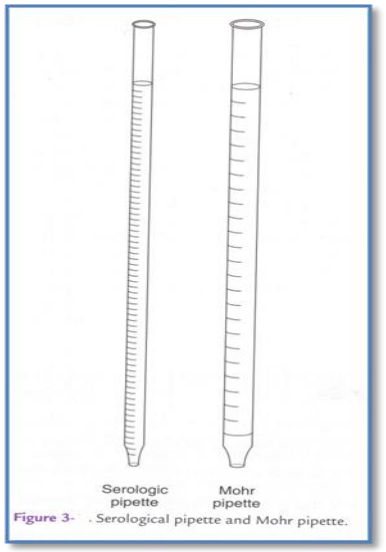


Figure 3- Serological pipette and Mohr pipette.

1.2.2 Material and Apparatus:

pipettes , Beaker , Distilled water , balance , Measuring cylinder

1.2.3 Method

Part 1

Examine the 3 pipette placed on your laboratory bench . Record their types and the volume of their smallest division .

1.2.4A. Result:

Pipette	***Type***	***Smallest division***
A		
B		
C		

Part 2

By using distilled water , pipette into weighted beaker

1-with 5ml graduate pipette(Mohr) =5ml water

2-with 5ml measuring cylinders =5ml water

1.2.4B. Result:

	Weight of the beaker	**Weight of beaker +water**	**Weight of water**
1- graduate pipette			
2-measuring cylinder			

1.2.5. Discussion

1.2.6.Questions:

1-Measuring cylinder cannot be a substitute for the pipette or a burette, why?

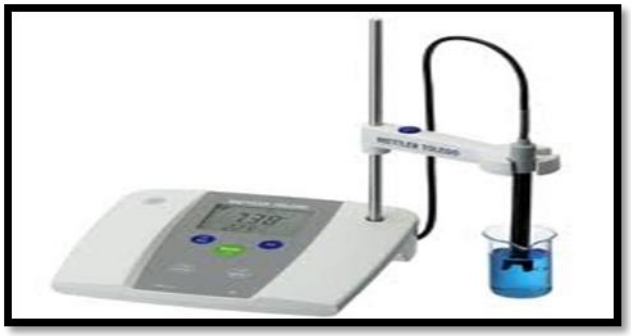
1.3 Identification of the common laboratory Equipment:

(A)Balance

(B) pH meter

(C) Spectrophotometer

1.3.A. pH Meter:



1.3.a1.Objective:

To learn how to handle the pH meter and to measure pH values

1.3.a2.Introduction:

As the hydrogen ion concentration of many solution is low and difficult to measure accurately, Sorenson (in 1909) introduce the term pH as a convenient way of expressing hydrogen ion concentration .There are many ways in biochemical laboratory to measure PH value such as ; litmus paper, a field kit and pH meter .

The most accurate and reliable method is PH meter.

PH define as the negative logarithm of the hydrogen ion concentration .

PH = - log10 [H+]

The measurement of pH is one of the most important and useful analytic procedure in biochemical lab. Since the PH determines many important aspects of the structure and activity of biological macromolecules and thus of the behavior of the cell and organisms .

Note: PH range value (0 - 14) ,, the higher PH number , the lower the hydrogen ion concentration and vice versa..

-There are two type of PH meter hydrogen electrode and glass electrode ; the glass electrode is use in biochemical lab.

Glass electrode:

the glass electrode consist of a very thin bulb ,blown onto a hard glass tube. The bulb is made of high conductivity glass which is sensitive to pH. The bulb contains a solution of hydrochloric acid (0.1N) and is connected to a platinum lead via silver - silver chloride electrode which is reversible with respect to hydrogen ions . The glass electrode is very sensitive and readily responds to changes in hydrogen ion concentration .

1.3.a3.Material and apparatues:

PH meter , buffer solution of known pH value , distilled water , Acetic acid 0.2M(A) , Beaker , Solid sodium hydroxide 0.1M(B) , Measuring cylinders .

1.3.a4.Method:

1-standardize the PH meter by placing the electrode in a solution of known PH(PH 4 , 7 , 9) .

2-Wash the electrode with distilled water and dry by tissue then put it into sample solution A & B , read PH .

Note: After use the electrode you should storage it in distilled water and never be allowed to dry out .IF the electrode get dry it will required reactivation.

Solution	***PH Value***
Standard PH 4	
Standard PH 7	
Standard PH 9	
A	
B	

1.3.a5.Result

1.3.a6.Discussion:

1.3.a7.Questions:

1-Why it is important to measure pH accurately?

2-A pH meter must be standardized, why ?

3-Three different solution have pH Values 3 , 7 and 10

solution 1 is basic (T or F)

solution 2 is neutral (T or F)

solution 3 is acidic (T or F)

1.3.B. Spectrophotometer

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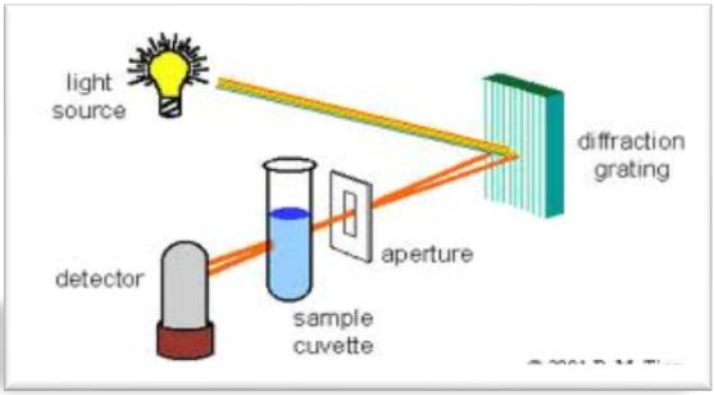


1.3.B .1.Objective:

To learn how to handle the spectrophotometer and to identify its application.

1.3.B .2.Introduction:

spectrophotometer is instrument used to measure the intensity of light at a given wavelength that is transmitted or absorbed by a sample.

It consist of two parts:

- Spectrometer is designed to emit the light at different wavelength
- Photometer contains photoelectric cell and the potentials are recorded on a scale which read out as absorbance or transmittance.

***Wavelength** in this instrument divided into:

-Invisible range(ultraviolet) from 100 to 360 nm [Quartz cuvette are used]

-Visible range (400 -700 nm) [Glass or plastic cuvette are used]

Blank : contain everything except the compound to be measure.

1.3.B .3.Material

1. Spectrophotometer
2. Quartz cuvette
3. Blank (Water)
4. Standard solution

5. Solution of unknown concentration

1.3.B .4.Method

- Adjust the spectrophotometer to zero using blank solution in the cuvette and read the absorbance of standard solution and the solution of unknown concentration at 280 nm.
- Read your result in the table below:

NO.	Solution	Absorbance
1	Standard solution (0.5 gm/100 ml of BSA)	
2	Solution of Unknown concentraton	

- Calculate the concentration of unknown solution from the following formula:

$$\frac{Au}{As} \times Cs$$

Where

Au= Absorbance of the solution of unknown concentration

As= Absorbance of the solution of standard solution

Cs= concentration of standard solution

- Concentration of unknown solution is =

1.3.B .5.References:

1. Freifelder , D.M. Physical Biochemistry .(1976). Freeman, USA.

2. Arneson,W and Brickell ,J.(2007).Clinical chemistry .F.A.Davis company .

Expermint-2

2. Preparation of Biological Solutions and Serial Dilutions

2.1 Objectives:

1) To learn how to prepare solutions.
2) To get familiar with solution dilutions.

2.2 Introduction:

Understanding how to prepare solutions and make dilutions is an essential skill for biochemists which is necessary knowledge needed for example in preparing various solutions diluting antibodies etc. In chemistry a solution is composed of one or more substance (the solute) dissolved in another substance (the solvent) forming a homogenous mixture .

A. Preparation of biological solutions:

1) Molar solutions:

A 1 Molar solution is a solution in which 1 mole (mole = wt_g /MW) of solute is dissolved in a total volume of 1 liter, for example:

The MW of NaCl is 58.44 so 1 mole of Nacl has a weight of 58.44g. To prepare a 1M solution of NaCl 58.44g of NaCl should be dissolved in a final volume of 1 liter.

2) w/v %:

The number of grams of solute dissolved in 100 mL of solution is indicated w/v% ,
for example:

a 1% solution has one gram of solute dissolved in 100 ml of solution .

To prepare the solution properly 1.0 gram of solid should be weighed and dissolved in slightly less than 100 ml once the solid is dissolved the volume is brought up to a final volume of 100 ml.

3) w/w/%:

The number of grams of solute dissolved in 100 gram of solution is indicated w/w%.

The concentrations of many commercial acids are giving in terms of w/w%. In order to calculate the volume of the stock solution required for a given preparation the density (specific gravity) of stock solution should be provided.

B .Dilution of Solution

1) **Volume to volume dilutions** :

This type of dilutions describes the ratio of the solute to the final volume of the dilute solution, for example to make 1:10 dilution of a 1.0 M NaCl solution, one part of the 1.0 M NaCl solution, should be mixed with nine parts of water, for a total of ten parts, therefore 1 :10 dilution means 1 part + 9 parts of water . Thus if 10 ml of the 1:10 dilution was needed then 1ml of 1.0 M NaCl should be mixed with 9 ml of water. If 100 ml of 1:10 dilution was needed then 10 ml of the 1.0 M NaCl should be mixed with 90 ml of water. The final concentration of NaCl in both cases will be 0.1 M.

1. Preparing dilutions by using the $\mathbf{V_{1X}C_1=V_{2X}C_2}$ formula:

Sometimes it is necessary to use one solution to make a specific amount of a more dilute solution .To do this the following formula can be used: $\mathbf{V_{1X}C_1=V_{2X}C_2}$**.**

Where:

V_1= Volume of starting solution needed to make the new solution.

C_1= Concentration of starting solution.

V_2= Final volume of new solution.

C_2= Final concentration of new solution.

For example:

Make 5ml of 0.25M solution from a 1.0M solution.

Since: $\mathbf{V_{1x}C_1=V_2xC_2}$**.**

(V_1) (1M) = (5ml) (0.25M).

So 1.25ml of the 1M solution is needed since the diluted solution should have a final volume of 5ml thus,

(V_1-$V_{2)}$ = 5ml – 1.25ml = 3.75ml).

3.75ml of diluent (generally water) should be added to the 1.25ml of starting solution.

2.3 Materials and Equipments:

- Solid NaOH.
- Solid NaCl.
- Concentrated HCl, 37 w/w%, S.G =
- Volumetric flasks (50ml), (100ml).
- Pipettes.
- Beakers.
- Glass rod.
- Filter paper.
- Balance.
- Measuring cylinders.

2.4 Method and Calculations:

A. Preparation of solutions:

You are provided with solid NaOH solution.

1) Prepare 50ml of a 0.08M NaOH solution.

Calculations:

To prepare the 0.08M NaOH solution ……..g of solid NaOH should be dissolved in a little volume of water then the volume made up to ……ml ,by the addition of water.

2) Prepare 50ml of a 1.5 w/v% solution of NaCl**.**

Calculations:

To prepare the 1.5 w/v% solution ……g of NaCl should be dissolved in little water and the volume made up to …….ml by the addition of water.

3) Prepare 100ml of 0.4 M HCl solutions starting with the concentrated HCl solution you are provided with. (w/w% , S.Gr =).

Calculations:

To prepare the 100ml of 0.4M HCl solution

…….ml of stock (i.e. concentrated HCl) solution is needed and the volume made up to ……..ml by the addition of water.

B. Solution dilutions:

1) Prepare 50ml of a 1:20 dilution of the 0.08M solution you previously prepared.

Calculations:

To prepare the 1:20 dilution ……..ml of the starting solution (0.08M NaOH) is needed and volume made up to a final volume of ……..ml.

2) Prepare 50ml of a 1:60 dilution of the 0.4M HCl solution you previously prepared.

Calculations:

To prepare the 1:60 dilution ……..ml of the starting solution (0.4M HCl) is needed and volume made up to a total volume of ……..ml by adding water.

Concentration of final solution ……M.

3) Prepare 10ml of a $2.5x10^{-3}$M of the previously prepared 0.4M HCl.

Calculations:

To prepare the $2.5x10^{-3}$M HCl solution ……..ml of the starting solution is taken and final volume made up to ……ml by the addition of water.

2.5 Questions:

Q_1- A student needed to prepare 1L of a 1M NaCl solution, which of the following methods is more accurate in preparing the solution? Why?

a)Weighing 58.5g of solid NaCl carefully ,dissolving it in 300ml of water, then adding 700ml of water.

b)Weighing 58.5g of solid NaCl carefully ,dissolving it in a small volume of water then making the final volume up to 1L by adding water.

Q_2-List the most important points to be considered when preparing solutions.

Q_3-A solution was prepared by taking 6ml of a 0.22M solution and then the volume was made up to a final volume of 30ml .What is the concentration of the final solution.?

Q_4-How would you prepare 80ml of a 1:25 dilution of a 2.1M KCl solution?

2.6 References:

Segel,Irwin H. Biochemical Calculations. 2nd Edition .

Farrell ,Shawn O. and Ranallo , Ryan T. Experiments in Biochemistry .2000.Harcourt Brace &company.

Experiment 3

3. Preparation of Different Buffer Solutions

3.1 Objectives:

1) To understand the nature of buffers solutions.

2) To learn how to prepare buffers.

3.2 Introduction:

All biochemical reactions occur under strict conditions of the concentration of hydrogen ion. Biological life cannot withstand large changes in hydrogen ion concentrations which we measure as the pH. Those solutions that have the ability to resist changes in pH are called buffers. A buffer is a solution that resists changes in pH upon the addition of limited amounts of acid or base. A buffer is made up of a weak acid and its conjugate base. It resists pH changes when it's two components are present in specific proportions. A buffer is best used close to its pK_a ($pK_a = -\log K_a$)

The Henderson-Hasselbalch equation is an equation that is often used to perform the calculations required in preparation of buffers for use in the laboratory. It relates the K_a of a weak acid , HA and the pH of a solution of the weak acid .

The Henderson–Hasselbalch equation is derived from the acid dissociation constant equation by the following steps:

$$K_{\mathrm{a}} = \frac{[\mathrm{H}^+][\mathrm{A}^-]}{[\mathrm{HA}]}$$

Rearranging the equation to solve for [H^+];

$$\frac{1}{[H^+]} = \frac{1}{[K_a]}\frac{[A^-]}{[HA]}$$

By definition, log 1/ [H^+] = pH , and log 1/K_a = pK_a , so that by taking the log of the equation above, we get the Henderson–Hasselbalch equation;

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

Buffers resist pH changes because they use up excess hydrogen ion or hydroxide ion. If we have a solution with both weak acid and its salt, and we add some H^+ then the following reaction occurs:

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

Conversely, if we added OH^- the following occurs:

$HA + OH^- \leftrightarrow A^- + H_2O$

Thus a buffer can protect against pH changes from added H^+ or OH^- ion as long as there is sufficient basic and acidic forms respectively. As soon as you run out of one of the forms you no longer have a buffer. To act as a good buffer the pH of the solution must be within one pH unit of the pK_a. The proper choice and preparation of a buffer is paramount to your success in a biochemistry lab.

3.3 Materials and Equipments:

- Acetic acid.
- Sodium acetate.
- Na_2HpO_4.
- NaH_2PO_4 .
- KCl.
- Standard buffer pH(7,4,9)
- 2M HCl solution.
- 0.2M acetic acid, 0.2M sodium acetate.
- 2M HCl solution.
- 0.2M acetic acid, 0.2M sodium acetate.
- pH meters.
- Volumetric flask.
- Glass rod.

- Beakers.
- Balance.

3.5 Method Results and Calculations:

1) Nature of buffers:

You are provided with :

0.2M solution of CH_3COOH/0.2M solution of CH_3COONa.

0.2M solution of NaH_2PO_4/ Na_2HPO_4.

a) Determine for your acid-base pair which is the acid component and which is the base component.

b) Prepare mixtures from previously mentioned solutions ,(i.e. acid –base pairs) 20 ml final volume for each .

i) 100 % HA.

ii) 75 % HA , 25% A^- .

iii) 50% HA , 50 % A^-.

iv) 25% HA , 75% A^-.

Mix solutions properly and measure the pH of final solution.

Calculate the pH of each solution mixture and record results in following table,

(pK_a acetic acid = 4.76 , pK_a phosphate = 7.2)

Table 1: For acetic acid/sodium acetate.

Solution.	**ml HA**	**ml A^-**	**Calculated pH**	**Measured pH**
100% HA.				
75%HA,25%A^-.				
50%HA,50%A^-.				
25%HA,75%A^-.				

Table 2.

For NaH_2PO_4/Na_2HPO_4.

Solution	ml HA	ml A^-	Calculated pH	Measured pH
100% HA.				
75%HA, 25%A^-				
50%HA, 50%A^-				
25%HA ,75%A^-				

2) Preparation of buffer:

You are provided with 0.2M solution of acetic acid and solid sodium acetate , pK_a =4.76).Prepare 45ml of a 0.2M acetate buffer pH =4.86.

Calculations:-

3) Testing for buffering behavior:

Follow instructions.

Table 3. For the acetic acid /sodium acetate mixture.

Solution(10ml of each)	Add 2M HCl	Measured pH
100%HA.	0.1ml	
75%HA,25% A	0.1ml	
50%HA,50%A	0.1ml	
25%HA,75%A	0.1ml	

Table 4. For the Na_2HPO_4/NaH_2PO_4 mixture.

Solution (10ml of each)	Add 2M HCl	Measured pH
100%HA	0.1ml	
75%HA,25%A^-	0.1ml	
50%HA,50%A^-	0.1ml	
25%HA,75%A^-	0.1ml	

Table 5. For the 0.2M acetate buffer prepared**:**

Solution(10 of each)	Measured pH	Add 2M HCl(0.1ml)	pH after HCl
0.2M acetate buffer.			
o.2M KCl.			

3.6 Discussion:

- Compare the calculated pH values with the measured pH values in table 1 and 2.
- From the results in table 3, 4 and 5 comment on how the solutions were affected by the (pH of solution) addition of 0.1ml of 2M HCl.
- Show the chemical reaction by which the acetate buffer resisted the change in pH upon addition of HCl?

3.7 Questions:

Q_1 -State which solutions showed buffering behavior and why?

Q_2-How did the measured pH of the 0.2M acetate buffer you prepared in section 2 compare with the requested pH ? Comment.

Q_3-If the pH of the acetate buffer you prepared was 5.8 instead of the requested 4.86 what do you think you did wrong that caused this difference in pH?.

3.8 References :

Segel,Irwin.H. Biochemical Calculations. 2nd Edition .

-Farrell ,Shawn O. and Ranallo , Ryan T. Experiments in Biochemistry .2000.Harcourt Brace &company.

Experiment 4

4 Buffer Capacity

4.1 Objectives:

- To understand the concept of buffer capacity.
- To determine the maximum buffer capacity of a number of buffer solutions.
- To establish the relationship between buffer capacity and buffer concentration.

4.2 Introduction:

Buffer solutions are solutions that can resist changes in pH upon addition of small amounts of acid/base. Common buffer mixtures contain two substances, a conjugate acid and a conjugate base .Together the two species (conjugate acid and conjugate base) resist large changes in pH by absorbing the H^+ ions or OH^- ions added to the system. When H^+ ions are added to the system they will react with the conjugate base in the buffer as follows,

$$\mathbf{H^+ + A^- \longleftrightarrow HA}$$

When OH^- ions are added they will react with the conjugate acid in the buffer as follows,

$$\mathbf{OH^- + HA \longleftrightarrow A^- + H_2O}$$

Thus the buffer is effective as long as it does not run out of one of its components.

Quantitative measure of this resistance to pH changes is called buffer capacity. Buffer capacity can be defined in many ways, it can be defined as the number of moles of H^+/OH^- ions that must be added to one liter of the buffer in order to decrease /increase the pH by one unit respectively. The buffer capacity is expressed as β and can be derived from Henderson-Hasselbalch equation;

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

$$\mathbf{= pKa + \log[A^-] - \log([C] - [A^-])}$$

$$\mathbf{= pKa + \frac{\ln[A^-]}{2.3} - \frac{\ln([C] - [A^-])}{2.3}}$$

Where C = the total concentration of buffer components = $[A^-]$ +[HA]

Differentiating with respect to $[A^-]$:

$$\frac{dpH}{d[A^-]} = \frac{1}{2.3[A^-]} + \frac{1}{2.3([C] - [A^-])} = \frac{[C]}{2.3[A^-]([C] - [A^-])}$$

$d[A^-]$ is the same as $d[H^+]$ or $d[OH^-]$ because for every mole of H^+ added a mole of A^- is utilized : for every mole of OH^- added a mole of A^- is produced . Substituting and inverting:

$$\frac{d[H^+]}{dpH} = \frac{d[OH^-]}{dpH} = \frac{2.3[A^-]([C] - [A^-])}{[C]} = \beta$$

$$\beta = \frac{2.3[A^-][HA]}{[A^-] + [HA]}$$

Further substitution from the expression for Ka yields :

$$\beta = \frac{2.3\,K_a[H^+][C]}{(K_a + [H^+])^2}$$

Where $[H^+]$ = the hydrogen ion concentration of the buffer ,β is the buffer capacity.

From the equation it is obvious that the buffer capacity is directly proportional to the buffer concentration.

4.3 Materials and Equipments:

-Standard buffers(pH 4,7,9).

-0.05M CH_3COOH solution.

-0.05M CH_3COONa solution.

-0.1M CH_3COONa solution.

-0.3M CH_3COOH solution.

-0.3M CH_3COONa solution.

-0.5M NaOH solution.

-pH meter.

-Conical flasks 250ml.

-Beakers 150ml.

-Burettes.

4.4 Method:

a) To determine the buffer capacity three measuring series with mixtures prepared from acetic acid and sodium acetate solutions of different concentrations are to be prepared.

b) First prepare the following three mixtures from 0.05M acetic acid and 0.05M sodium acetate as indicated in table 1, filling the two solutions (0.05M acetic acid, 0.05M sodium acetate) into separate burettes and transferring the amounts for each of the three mixtures into a separate labeled 250ml conical flask.

Table 1.

solution	Acetic acid	Sodium acetate
A	40ml	10ml
B	25ml	25ml
C	10ml	40ml

a) Now prepare another three mixtures of composition as given in table 1 but from 0.1M solutions of acetic acid and a further three mixtures from the 0.3M solutions of acetic acid and sodium acetate.

b) Calibrate your pH meter using the standard buffers provided .

c) Fill a burette with 0.5M NaOH solution .

d) Take 20ml of the first buffer mixture (solution A) ,prepared from the 0.05M solutions of acetic acid and sodium acetate, transfer to a 250ml conical flask, then measure and record the pH value of the solution.

e) Add successive 0.5ml portions of 0.5M NaOH solution to the buffer mixture with continuous mixing ,measure and record pH value after each addition ,until you reach pH=10.

f) Carry out the same procedure with the other two remaining solution mixtures prepared from the 0.05M solution of acetic acid/sodium acetate (the B and C solutions). -Also subsequently carry out the same procedure with the 0.1M solution mixtures but here with the difference that 1.0ml portions of NaOH is added ,the same is repeated for the 0.3M solution mixtures but here 2.0ml portions of NaOH is added.

g) The results should be recorded in the following result section.

4.5 Results and Calculations:

Table 2. For the 0.05M mixture solutions of acetic acid/sodium acetate.

Solution A		**Solution B**		**Solution C**	
ml NaOH	**pH value**	**ml NaOH**	**pH value**	**ml NaOH**	**pH value**

Table 3. For the 0.1M acetic acid / sodium acetate solutions

Solution A		**Solution B**		**Solution C**	

Table 4. For the 0.3M acetic acid / sodium acetate solution.

Solution A		**Solution B**		**Solution C**	
ml NaOH	**pH value**	**ml NaOH**	**pH value**	**ml NaOH**	**pH value**

To determine the buffer capacity of each buffer solution prepared the buffer capacity β should be calculated for each point according to the following formula;

β =ΔC/ΔpH,

where $\Delta C_{base} = C_{NaOH} \times V_{NaOH} / V_{total}$.

and, C_{NaOH} = concentration of NaOH added.

V_{NaOH} = volume of NaOH solution added.

V_{total} = volume of the buffer mixture plus the volume of added NaOH solution.

ΔpH is the difference in the pH values before and after the addition of NaOH.

Table 5. β (buffer capacity) for the 0.05M mixtures of acetic acid /sodium acetate.

Solution A		**Solution B**		**Solution C**	
β	**pH value**	**β**	**pH value**	**β**	**pH value**

Table 6. For the 0.1M acetic acid / sodium acetate solutions.

Solution A		**Solution B**		**Solution C**	
β	**pH value**	**β**	**pH value**	**β**	**pH value**

Table 7. For 0.3M acetic acid / sodium acetate solutions.

Solution A		**Solution B**		**Solution C**	
β	**pH value**	**β**	**pH value**	**β**	**pH value**

-From results in tables 5,6 and 7, plot curves of β on y-axis and pH on x-axis to determine maximum buffer capacity.

Results of 0.05M solution mixtures should be plotted in graph 1, results of 0.1M solution mixtures should be represented in graph2, and finally those of the 0.3M solutions should be represented in graph3.

4.6 Discussion:

From graph 1:

a) What was the maximum buffer capacity of buffer solutions A,B,and C?

b) Which solution A, B, or C showed the lowest buffer capacity and why?

c) Explain why the buffer capacity of solution A was low at first then it reached a maximum buffer capacity ?

d) Why did buffer solutions A and C show a decline in buffer capacity after the maximum capacity peak?

4.7 Questions:

$Q_{1;}$ By comparing the maximum buffer capacity values of solutions A, and B in graphs 1 , 2 and 3 ,which graph recorded the highest buffer capacity and why?

$Q_{2:}$What was the corresponding pH value at the maximum buffer capacity for solutions A and B in graphs 1 , 2 and 3?

$Q_{3:}$What do you conclude finally about the relationship between;

a) Buffer concentration and buffer capacity .

b) pH at maximum buffer capacity and pK_a of the buffer.

c) What are the proportions of the buffer components at maximum buffer capacity?

4.8 References:

Segel, Irwin.H. Biochemical Calculations. 2nd Edition .

Experiment 5

5 Preparation of Buffer Solutions by Different ways :

5.1 Objectives:

1) To learn how to prepare the potassium phosphate buffer by different ways.

5.2 Introduction:

For example : prepare 0.1 liters of 0.045 M potassium phosphate buffer, PH7.5 Solution

The PH of this buffer is a little above the pka2 of H_3PO_4 , consequently , the two major ionic species present are $H_2 Po_2^-$ (conjugate acid) and HPO^{2-}_4 (conjugate base) with the HPO^{2-}_4 predominating.

The buffer can be prepared in any one of several ways :

(1) by mixing kH_2PO_4 and k_2HPO_4 in the proper proportions ,

(2) by starting with H_3PO_4 and converting it to kH_2PO_4 plus k_2HPO_4 by adding the proper amount of KOH ,

(3) by starting with kH_2PO_4 and converting a portion of it to k_2HPO_4 by adding KOH ,

(4)by starting with k_2HPO_4 and converting a portion of it to kH_2PO_4 by adding a strong acid such as HCL ,

(5) by starting with k_3PO_4 and converting it to kH_2PO_4 plus k_2HPO_4 by adding HCL ,and

(6) by mixing k_3PO_4 and kH_2PO_4 in the proper proportions .

Regardless of which method is used , the first step involves calculating the proportion and amounts of the two ionic species in the buffer .

-The buffer contains a total of 0.1 liters ×0.045 M = 0.0045 mole of phosphate .

PH =PK_{a2} + log [HPO^{2-}_4] / $H_2PO_4^-$]

7.5 = 7.2 + log [HPO^{2-}_4] / [$H_2PO_4^-$] 0.3 = log [HPO^{2-}_4] / [$H_2PO_4^-$]

[HPO^{2-}_4] / [$H_2PO_4^-$] = antilog of 0.3 = 2=$\frac{2}{1}$ ratio

$\frac{2}{3}$ × 0.0045 mole = 0.003 mole of HPO^{2-}_4 is needed and $\frac{1}{3}$ × 0.0045 mole = 0.0015 mole of $H_2PO_4^-$ is needed.

5.3 Materials and Equipments:

- Solid kH_2PO_4 , k_2HPO_4 . k_3PO_4
- 15 MH_3PO_4
- 1.5 M KOH
- solid KOH
- 2 M solution of HCL
- 2 Volumetric flasks (100ml) .
- Pipettes.
- Beakers.
- PH meter

5.4 Method and Calculations:

Preparation of solutions:

1-You are provided with solid kH_2PO_4 and k_2HPO_4 .

Calculations:

Weigh out 0.0030 mole of k_2HPO_4 (…….g) and 0.0015 mole of kH_2PO_4 (……g) and dissolve in sufficient water to make 0. 1 liters final volume . Or , if stock solutions of the two phosphates are available, measure out the appropriate volumes of each and dilute to 0.1 liters.

OR-In practice , we might prepare 0.045 M k_2HPO_4 and 0.045 M kH_2PO_4 and simple mix the two until the PH (as measured with a PH meter) is 7.5. since both stock solutions are 0.045 M , the total phosphate concentration will remain 0.045 M regardless of what volumes of each are added .

2-You are provided with concentrated (15M)H_3PO_4 and solution of 1.5 M KOH .

Calculations:

— start with 0.0045 mole of H_3PO_4 and add sufficient KOH to titrate completely 1 hydrogen and $\frac{2}{3}$ of the second hydrogen .

$$H_3PO_4 \xrightarrow{OH^-} H_2PO_4^- \xrightarrow{OH^-} HPO^{2-}_4$$

YOU need 0.0045 mole of H_3PO_4 .

Liters × M = number of moles

Liters ×15 = 0.0045 mole

Liters = $\frac{0.0045}{15}$ = 0.0003 liter

∴ take 0.3 ml of H_3PO_4 . Add 0.0045 mole of KOH to convert all the H_3PO_4 to $H_2PO_4^-$; then add another $\frac{2}{3}\times 0.0045 = 0.0030$ mole of KOH to convert 0.003 mole of $H_2PO_4^-$ to HPO^{2-}_4 . In other words, a total of 0.0075 mole of KOH is required .

-YOU can calculate how much of 1.5 M KOH solution to add.

Liters × M = number of moles

Liters = $\frac{\text{number of moles}}{M} = \frac{0.0075}{1.5} = 0.005$

∴ Add 5ml of KOH to the 0. 3 ml of concentrate H_3PO_4; then add sufficient water to bring the final volume to 0.1 liters.

3- You are provided with solid kH_2PO_4 and KOH

Calculations:

You can start with kH_2PO_4 and add sufficient KOH to convert $\frac{2}{3}$ of the $H_2PO_4^-$ to HPO^{2-}_4.

$$H_2PO_4^- \xrightarrow{OH^-} HPO^{2-}_4$$

You need 0.0045 mole of kH_2PO_4 .

Wt g / MW = number of moles

Wt g = (0.0045)(136) = ……. g

Dissolve the kH_2PO_4 in some water; then add 0.0030 mole of KOH (solid or dissolved in some water).

Wt g = (0.0030)(56) = …….. g of KOH

Next , add sufficient water to bring the volume to 0. 1 liters.

4- You are provided with solid k_2HPO_4 and 2 M solution of HCL

Calculations:

-The HPO^{2-}_4 may be converted to $H_2PO_4^-$ by adding HCL .

$$HPO^{2-}_4 \xrightarrow{H^+} H_2PO_4^-$$

Because we want to end up with an HPO^{2-}_4 / $H_2PO_4^-$ of $\frac{2}{1}$, we want to convert only $\frac{1}{3}$ of the HPO^{2-}_4 to $H_2PO_4^-$.

- weigh out 0.0045 moles of k_2HPO_4 .

Wt g = number of moles × MW

Wt g = (0.0045)(174) = ……..g

Dissolve the kH_2PO_4 in some water ; then add $\frac{1}{3}$×0.0045 mole = 0.0015 mole of HCL

Liters × M = number of moles

$$\text{Liters} = \frac{\text{number of moles}}{M} = \frac{0.0015}{2} = \text{……..liter}$$

∴ Add …….ml of 2M HCL . Then add sufficient water to bring the volume to 0. 1 liters .

5- You are provided with solid k_3PO_4 and 2 M solution of HCL

Calculations:

-Start with 0.0045 mole of k_3PO_4 and add sufficient HCL to convert all the PO^{3-}_4 to HPO^{2-}_4 .

Then add additional HCL to convert $\frac{1}{3}$ of the HPO^{2-}_4 to $H_2PO_4^-$

$$PO_4^{3-} \xrightarrow{H^+} HPO^{2-}_4 \xrightarrow{H^+} H_2PO_4^-$$

-You need 0.0045 mole of k_3PO_4 .

$$\text{Wt g} = \text{number of moles} \times MW$$

$$\text{Wt g} = (0.0045)(212) = \text{……. g}$$

Dissolve the k_3PO_4 in water . Add 0.0045 mole of HCL to convert all the $PO43^-$ to HPO^{2-}_4

Liters × M = number of moles

$$\text{Liters} = \frac{\text{number of moles}}{M} = \frac{0.0045}{2} = \text{……..liter}$$

- Then add another $\frac{1}{3}$×0.0045 = 0.0015 mole of HCL to convert 0.0015 mole of HPO^{2-}_4 to $H_2PO_4^-$.

Liters × M = number of moles

$$\text{Liters} = \frac{\text{number of moles}}{M} = \frac{0.0015}{2} = \text{……..liter}$$

The final solution then contains 0.0015 mole of $H_2PO_4^-$ and 0.0030 mole of HPO^{2-}_4 . Now add sufficient water to make 0.1 liters .

6- You are provided with solid kH_2PO_4 and k_2PO_4

Calculations:

- The kH_2PO_4 and k_3PO_4 react to from k_2HPO_4 .

The $H_2PO_4^-$ acts as an acid and the PO_4^{3-} acts as a base .

$$H_2PO_4^- + PO_4^{3-} \leftrightarrows 2\ HPO^{2-}_4$$

The reaction is the reverse of the disproportionation reaction .

Note that each mole of $H_2PO_4^-$ and $PO^{3}_{4}^-$ yields 2 moles of $HPO^{2}_{4}^-$.Thus to produce 0.0030 mole of $HPO^{2}_{4}^-$, 0.0015 mole of $H_2PO_4^-$ and 0.0015 mole of $PO^{3}_{4}^-$ are required.

But, in addition to the 0.0030 mole of HPO^{2}_{4}-, the final solution contains 0.0015 mole of $H_2PO_4^-$.

Therefore , dissolve 0.0030 mole of kH_2PO_4 and 0.0015 mole of k_3PO_4 in water .

- Of the original 0.0030 mole of kH_2PO_4 , 0.0015 mole reacts with the $PO^{3}_{4}^-$ to produce 0.0030 mole of $HPO^{2}_{4}^-$, leaving 0.0015 mole as $H_2PO_4^-$.

-You need 0.0015 mole of k_3PO_4 .

Wt g = number of moles × MW

Wt g = (0.0015)(212) = ……. g

You need 0.0030 mole of kH_2PO_4 .

Wt g / MW = number of moles

Wt g = (0.0030)(136) = ……. g

Dissolve the kH_3PO_4 and k_3PO_4 in some water, then add sufficient water to make 0.1 liters.

5.5 Discussion:

5.6 Reference:

Segel ,Irwin.H. Biochemical Calculations. 2nd Edition .

Experiment 6

6. Titration of a weak acid with strong base

6.1 Objectives:

To study titration curves, determine the pKa value of a weak acid, and reinforce the understanding of buffers.

6.2 Introduction:

Determining the pH of solutions of weak acids or bases is not as easy as is the case when dealing with strong acids or bases, since weak acids or bases do not dissociate completely, an equilibrium expression with Ka must be used. The K_a is a measure of the strength of the acid, since it's value is always very low (i.e 10^{-8}, $4.3x10^{-6}$ and so on) pK_a value have been introduced to simplify the matter.

pKa = -log Ka

pK_a values of weak acids can be determined mathematically or practically by the use of titration curves. Titration Curves are produced by monitoring the pH of a given volume of a sample solution after successive addition of acid or alkali. The curves are usually plots of pH against the volume of titrant added.

6.3 Materials and Equipments:

- 0.1M Weak acid (Acetic acid)
- 0.1M NaOH.
- Standard buffers (pH 4,7,9)
- pH meter.
- Conical flask.
- Burettes.
- Measuring cylinder.

6.4 Method:

You are provided with 10 ml of a 0.1M CH_3COOH weak acid solution, titrate it with 0.1m NaOH adding the base drop wise mixing, and recording the pH after each 0.5 ml NaOH added until you reach a pH=10.

6.5 Results:

Volume of NaOH (0.1M) added (ml)	**pH**	**Volume of NaOH (0.1M) added (ml)**	**pH**

6.6 Discussion :

a) Plot a Curve of pH versus ml of NaOH added.

b) Calculate the pH of the weak acid HA solution after the addition of 3ml, 5ml, and 10ml of NaOH.

c) Compare your calculated pH values with those obtained from Curve.

6.7 Questions:

Q_1-Determine the pK_a value of the weak acid, how does it compare to the theoretical value?

Q_2 -At what pH-range did the acid show buffering behavior? What are the chemical species at that region, what are their proportions?

6.8 References:

-Segel, Irwin .H. Biochemical Calculations 2nd Edition .

-Farrell ,Shawn O. and Ranallo , Ryan T. Experiments in Biochemistry .2000.Harc0urt Brace &company.

Experiment 7

7. Titration curves of amino acids

7.1 Introduction

Titration curves are produced by monitoring the pH of a given volume of a sample solution after successive addition of acid or alkali . The curves are usually plots of PH against the volume of titrant added or more correctly against the number of equivalents added per mole of the sample.

7.2 Materials and apparatus

1. Hydrochloric acid (0.1 M)

2. Sodium hydroxide (0.1 M)
3. Amino acids (0.1 M alanine, 0.1 M lysine and 0.1 M glutamic acid)
4. pH meter
5. Burette (10 ml)

7.2 METHOD :

1.Titration of alanine

Pipette 10 ml of alanine solution (0.1 M) into a 100 ml bearker. Standardise the pH meter and determine the pH of the solution. The solution should be constantly shaken. Add 0.5 ml of (0.1 M) HCL from the burette and determine the pH of the solution after each addition. Continue adding acid in until pH falls to about 1.3 .

Wash the electrode in distilled water, restandardize the pH meter and titrate a further 10 ml of alanine solution with 0.1 M NaOH to pH 12.5.

Plot a titration curve for alanine (pH verses titrant in ml).

Do the titration of the other amino acids in a similar way and plot their titration curves.

Determine the pka values from your curves and compare them with the standard values.

Determination of pka values

pka values can be obtained from titration data by the following methods :

1. The pH at the point of inflection is the pka value and this may be read directly.
2. By definition the pka value is equal to the pH at which the acid is half titrated . The pka can therefore be obtained from a knowledge of the end point of the titration.

7.3 RESULTS :

Name of the amino acid titrated

Volume of NaOH added	pH	Volume of HCl added	pH

pka value of the amino acid =

7.4 Discussion:

7.5 Reference:

Segel, Irwin. H. Biochemical Calculations. 2nd Edition .

Experiment 8

8. Beer's- Lambert Law and Standard Curves

8.1 Objectives:

1) To understand the concept of Beer-Lambert law and its application.
2) To get introduced to standard curves, their applications, and to learn how to design protocols for the creation of a standard curve.

8.2 Introduction:

Spectrophotometry (the measure of light absorption or transmission), is one of the most valuable analytical techniques available to biochemists. Unknown compounds may be identified by their characteristic absorption spectra in the ultraviolet(100-400nm), visible(400-800nm), or infrared(800nm-100µm) parts of the spectrum, thus being a very useful tool in colorimetric tests. Colorimetric tests are commonly used in both biology and chemistry. The idea behind using a colorimetric test is that the amount of compound X can be measured if compound X absorbs light. The higher the concentration of compound X in a solution, the higher the absorbance. In many cases, compound X itself is not a chromophore, but it can be reacted with some other compound and the product is a chromophore. Colorimetric tests are based on the proportional relationship between concentration of a chromophore and the amount of light absorbed by that chromophore.

Concentrations of unknown compounds in solutions may be determined by measuring the light absorption at one or more wavelengths. The fraction of the incident light that is absorbed by a solution depends on the thickness of the sample ,and the concentration of the absorbing compound .The relationship between concentration, length of the light path, and the light absorbed by a particular substance are expressed mathematically as*:*

$$A = a_m \times c \times l$$

Where:

A is the absorbance of the solution

a_m is the molar extinction coefficient

C is the concentration of the absorbing substance.

l is the length of the light path.

The relationship is expressed in following curves;

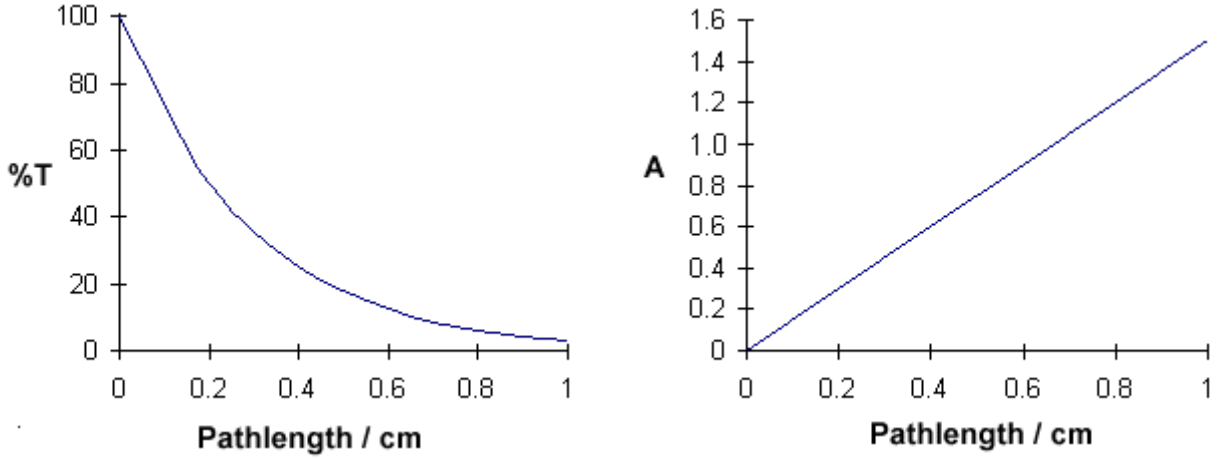
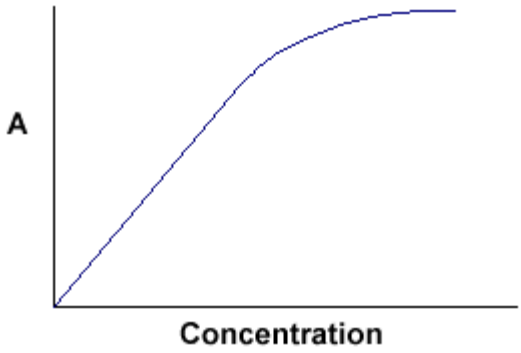


Figure 1.

A = a_mc l : tells us that absorbance depends on the total quantity of the absorbing compound in the light path through the cuvette. If we plot absorbance against concentration, we get a straight line passing through the origin (0,0).

The linear relationship between concentration and absorbance is both simple and straightforward, which is why we prefer to express the Beer-Lambert law using absorbance as a measure of the absorption rather than %T (% transmittance).



Note that the law is not obeyed at high concentrations.

Figure 2.

Using this relationship, the concentration of a compound can be determined directly from the absorbance provided molar absorptivity and path length are known.

This linear relationship between absorbance and concentration is true for the useful absorbance range; this is generally considered from 0.2 to 1.2. At absorbance greater than 1.2, the linear relationship shows a negative deviation and thus any absorbance value above 1.2 will underestimate the concentration. Absorbance below 0.2 is difficult to read with great accuracy because of the faint color. The higher level of error produces values that are less useful. Molar absorptivity is a constant under a given set of conditions; if pH, temperature or other conditions differ, this value will change. It is also possible to relate the absorbance of a compound to its concentration by use of the standard curve.

Some points to consider:

1- Absorbance has no units, it is read off of the spectrophotometer, the wavelength is often specified along with the absorbance ,such as $A_{540} = 0.3$.
2- The extinction coefficient has units of reciprocal concentration and path length.
3- The path length is usually in cm and if not specified is assumed to be 1cm.
4- Lots of things can interfere with the spectrophotometer reading .If the cuvette is smudged or scratched, light will be scattered rather than absorbed by the solution .If there is insufficient volume the light may pass over the solution instead of going through it.

A proper blank should be used in every spectrophotometry study.
In order to effectively use a spectrophotometer we must first zero the machine, we do this using "the blank." The blank contains everything except the compound of interest which

5- absorbs light. Thus, by zeroing the machine using "the blank," any measured absorbance is due to the solute of interest only.

- Standard Curve:

The idea of a standard curve is that the relationship between absorbance and concentration is linear provided that the absorbance value is in the useful range. The amount of X in an unknown sample can be measured by comparing the absorbance of the unknown with a series of standards (a series of tubes with varying amounts of solute in them).

The absorbance of standards is measured and Abs is plotted against amount of solute. Once you have the standard curve you can determine the concentration of the unknown. The standard is constructed by plotting the absorbance values vs amount of X and solving for the best straight line which is given in terms of :

$$y = mx + b$$

where; y is absorbance, x amount of X, b is the y intercept and m is the slope.
The best straight line will give values for the slope and y intercept. It is possible to mathematically solve for amount of X in an unknown sample.

Since the standard curve is so important for figuring out the unknown concentration, it is imperative that it be accurate. One way of increasing the accuracy is to use a large number of standards; in this course typically six standards, including a blank, will be used to construct the standard curve.

Once the standard curve is established the unknown concentration can be determined.. The absorbance of the unknown must fall within the line of the standard curve, preferably within the linear region, you should not extrapolate your line beyond the highest concentration standard you have.

8.3 Materials and Equipments:

- 0.1 M Copper Sulfate stock solution.

- Unknown Copper Sulfate solution.
- Test tubes.
- pipettes.
- Spectrophotometer.

8.4 Method:

1) Set up the spectrophotometer at 600 nm.
2) Set up 8 test tubes ,clean and label ,test tubes A,B,C,D,E,F for standard solution ,tube G the blank and tube H the unknown solution.
3) Prepare a series of known standard solutions by diluting the stock solution following the protocol in table 1.

Table 1.

Tube	0.1 M Copper Sulfate Standard Solution.	Unknown	H_2O
A	2ml	-	8ml
B	4ml	-	6ml
C	6ml	-	4ml
D	8ml	-	2ml
E	10ml	-	0ml
G	-	-	10ml
H	-	10 ml	-

Mix contents, measure the absorbance of each tube at 600 nm against the blank ,and record results in table 2.

8.5 Results:

Calculate the concentration in each standard tube, and record with absorbance in table 2.

Table 2.

Tube	Absorbance at 600nm	Concentration M
A		
B		
C		
D		
E		
G		
H		

Plot the standard curve (Absorbance vs. Concentration), determine the concentration of unknown from graph.

8.6 Discussion:

What was the shape of the standard curve you obtained? What relationship does it reflect between the Absorbance (of absorbing substance) and the concentration (of absorbing substance)? Explain?

8.7 Questions:

Q_1-If your unknown sample had an absorbance higher the highest absorbance recorded by standard, how will you determine its concentration correctly ?

Q_2-What is the purpose of the blank tube (tube G)?

Q_3- If you repeated the same experiment but used cuvettes of 2cm path length instead of the 1cm cuvettes used in the original experiment, how do you think the absorbance will be affected?

Q_4- Calculate the extinction coefficient of your Copper sulfate solution.

8.8 **References:**

-Segel,Irwin .H. Biochemical Calculations. 2nd Edition .

-Farrell ,Shawn O. and Ranallo , Ryan T. Experiments in Biochemistry .2000.Harcourt Brace &company.

- (http://www.fgsc.net/teaching/keenan.pdf)