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Practical Note
BIOCHEMICAL CALCULATIONS
(BCH 312)

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Experiment 1

1. Preparation of Biological Solutions and Serial Dilutions

1.1 Objectives:

1) To learn how to prepare solutions.
2) To get familiar with solution dilutions.
3)

1.2 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.

1.3 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.

1.4 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.

1.5 Method and Calculations:

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, 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.

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.

1.6 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?

1.7 References:

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

Experiments in Biochemistry , by Shawn O. Farrell . Ryan T. Ranallo.

Experiment 2

2. Preparation of Different Buffer Solutions

2.1 Objectives:

1) To understand the nature of buffers solutions.

2) To learn how to prepare buffers.

2.2 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:

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

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.

2.3 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.

2.4 Method Results and Calculations:

1) Nature of buffers:

You are provided with :

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

0.2Msolution 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.			

2.5 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?

2.6 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?.

2.7 References :

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

Experiments in Biochemistry by.Shawn O. Farrell , Ryan T. Ranallo.

Experiment 3

3. Buffer Capacity

3.1 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.

3.2 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;

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

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

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

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.

3.3 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.

3.4 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.

3.5 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.

3.6 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?

3.7 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?

3.8 References:

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

Experiment 4

4. Titration of a weak acid with strong base

4.1 Objectives:

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

4.2 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.

4.3 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.

4.4 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.

4.5 Results:

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

4.6 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.

4.7 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?

4.8 References:

Experiments in Biochemistry Shawn O. Farrell ,Ryan T. Ranallo.

Biochemical Calculations 2nd Edition by .Irwin.H.S

Experiment 5

5. Beer's- Lambert Law and Standard Curves

5.1 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.

5.2 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*:*

$$A = a_m \, x \, c \, x \, l$$

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;

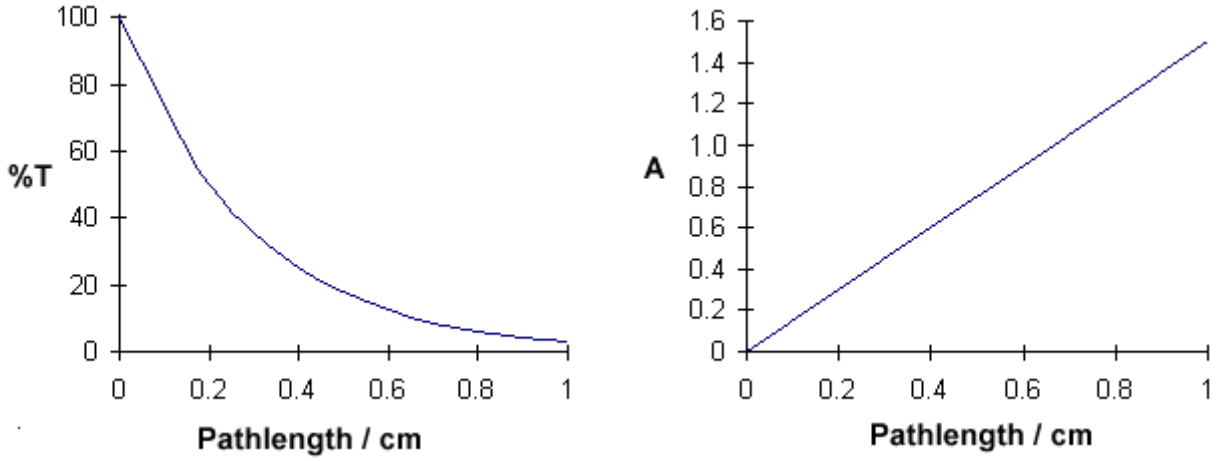
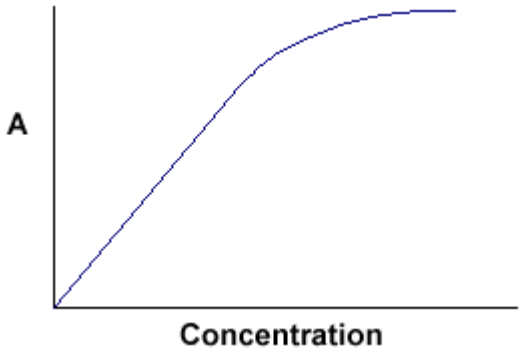


Figure 1.

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.
5- 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

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

5.3 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.

5.4 Materials and Equipments:

- 0.1 M Copper Sulfate stock solution.
- Unknown Copper Sulfate solution.
- Test tubes.
- pipettes.
- Spectrophotometer.

5.5 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.

5.6 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.

5.7 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?

5.8 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.

5.9 References:

Experiments in Biochemistry Shawn O.Farrell, Ryan T. Ranallo.

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

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