II B.Sc., Biochemistry **2018-2019**

TOOLS AND TECHNIQUES IN BIOCHEMISTRY PRACTICAL

Semester III **Subject Code: 18BCU314A**

Course objectives

- To train the students on the preparation of different molar, normal, percentage and ppm solution.

Course outcomes (CO's)

- Students acquire the skills to prepare various strength solutions, buffers and to estimate proteins and nucleic acids.

1. Preparation of a molar solutions
2. Preparation of normal solutions
3. Preparation of percentage and ppm solutions.
4. Preparation of dilute acids from concentrated acids
5. Preparation of various dilute solutions
6. Conversion of milli equivalent solution to milli molar equivalent solution (eg-KCl, $MgSO_4$)
7. Preparation of a buffer of given pH and molarity.
8. Determination of the absorption maxima and molar extinction coefficient (of a relevant organic molecule).
9. Measurement of UV spectrum of compounds .
10. Determination of concentration of a protein solution by Lowry/BCA method.
11. Determination of nucleic acid concentration and purity

SUGGESTED READING

1. Sheehan, D., (2010). Physical Biochemistry: Principles and Applications 2nd ed., Wiley Blackwell (West Sussex), ISBN:978-0-470-85602-4 / ISBN:978-0-470-85603-1.

2. Freifelder, D., (1982). Physical Biochemistry: Applications to Biochemistry and Molecular Biology 2nd ed., W.H. Freeman and Company (New York), ISBN:0-7167-1315-2 / ISBN:0-7167-1444-2.

3. Plummer D. T., (1998). An Introduction to Practical Biochemistry 3rd ed., Tata McGraw Hill Education Pvt. Ltd. (New Delhi), ISBN:13: 978-0-07-099487-4 / ISBN:10: 0-07-099487-0.

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University)
(Established Under Section 3 of UGC Act 1956)
Coimbatore - 641021.
(For the candidates admitted from 2016 onwards)

DEPARTMENT OF BIOCHEMISTRTY

SUBJECT: TOOLS AND TECHNIQUES IN BIOCHEMISTRY PRACTICAL -A

SEMESTER: III

SUBJECT CODE: 18BCU314A **CLASS: II B. Sc. BC**

EXPERIMENTAL DETAILS
DEPARTMENT OF BIOCHEMISTRTY

S.No	Lecture Duration Hour	Topics to be Covered	Support Material/Page Nos
1	3	Preparation of a molar solutions	Plummer D. T., (1998). An Introduction to Practical Biochemistry 3rd ed., Tata McGraw Hill Education Pvt. Ltd. (New Delhi), ISBN:13: 978-0-07-099487-4 / ISBN:10: 0-07-099487-0.
2	3	Preparation of normal solutions	
3	3	Preparation of percentage and ppm solutions.	
4	3	Preparation of dilute acids from concentrated acids	
5	3	Preparation of various dilute solutions	
6	3	Conversion of milli equivalent solution to milli molar equivalent solution (eg-KCl, $MgSO_4$)	
7	3	Preparation of a buffer of given pH and molarity.	
8	3	Determination of the absorption maxima and molar extinction coefficient (of a relevant organic molecule).	
9	3	Measurement of UV spectrum of compounds	
10	3	Determination of concentration of a protein solution by Lowry/BCA method.	
11	3	Determination of nucleic acid concentration and purity	
Total No Of Hours Planned For Practical= 33			

REFERENCES

Sheehan, D., (2010). Physical Biochemistry: Principles and Applications 2nd ed., Wiley Blackwell (West Sussex), ISBN:978-0-470-85602-4 / ISBN:978-0-470-85603-1.

Freifelder, D., (1982). Physical Biochemistry: Applications to Biochemistry and Molecular Biology 2nd ed., W.H. Freeman and Company (New York), ISBN:0-7167-1315-2 / ISBN:0-7167-1444-2.

Plummer D. T., (1998). An Introduction to Practical Biochemistry 3rd ed., Tata McGraw Hill Education Pvt. Ltd. (New Delhi), ISBN:13: 978-0-07-099487-4 / ISBN:10: 0-07-099487-0.

PRACTICAL SYLLABUS

1. Preparation of a molar solutions
2. Preparation of normal solutions
3. Preparation of percentage and ppm solutions.
4. Preparation of dilute acids from concentrated acids
5. Preparation of various dilute solutions
6. Conversion of milli equivalent solution to milli molar equivalent solution (eg-KCl, $MgSO_4$)
7. Preparation of a buffer of given pH and molarity.
8. Determination of the absorption maxima and molar extinction coefficient (of a relevant organic molecule).
9. Measurement of UV spectrum of compounds .
10. Determination of concentration of a protein solution by Lowry/BCA method.
11. Determination of nucleic acid concentration and purity

EXPERIMENT NO: 1 **DATE:**

PREPARATION OF MOLAR SOLUTIONS

AIM: To prepare 100 ml of 2 M Sodium chloride solution

PRINCIPLE: Molarity is the number of moles of solute dissolved in 1Liter of solution.

Molarity= Number of moles / Volume in liters

First, we must calculate number of moles so we can calculate the weight in grams.

Number of moles=Molarity x volume

=2 x 0.1 = 0.2 moles
Now, we need to calculate grams of NaCl:

Weight= mole x molecular weight

=0.2 x (23 + 35.5)=11.7 grams

We dissolve 11.7 grams of NaCl and make up the volume to 100 ml

PROCEDURE:

1. Place a beaker in a balance and zero the balance.
2. Weight 11.7 grams of NaCl, in the beaker and dissolve in a very small volume of water, once the solid is dissolved, the volume is transferred to 100 ml volumetric flask.
3. Wash the beaker at least 2 times with small amount of distilled water and transfer it to the volumetric flask, to make sure all the solute is dissolved and there is no left overs.
4. Bring up to a final volume 100 ml.

RESULT:

The molarity of the solution prepared was found to be _____

EXPERIMENT NO: 2 **DATE:**

PREPARATION OF NORMAL SOLUTIONS

AIM: To prepare 1N normal solution of Sodium hydroxide

PRINCIPLE: Normal solution is one, which contains one equivalent weight of the reagent in one litre of the solution. Normality is expressed as N.

PROCEDURE:

1. Place a beaker in a balance and zero the balance.
2. Weight 11.7 grams of NaCl , in the beaker and dissolve in a very small volume of water ,once the solid is dissolved, the volume is transferred to 100 ml volumetric flask.
3. Wash the beaker at least 2 times with small amount of distilled water and transfer it to the volumetric flask, to make sure all the solute is dissolved and there is no left overs.

4. Bring up to a final volume 100 ml.

RESULT:

EXPERIMENT NO: 3 **DATE:**

PREPARATION OF PERCENTAGE AND PPM SOLUTIONS

AIM: 1) How would you prepare 500 mL of a 5 % (w/v) solution of NaCl?

2) How would you prepare 1L of a 5 ppm solution of Chlorine?

PRINCIPLE:

Percent composition is the ratio of one part of solute to one hundred parts of solution and is expressed as a percent. Determine the mass of solute and solution and then divide the mass of the solute by the total mass of the solution. This number is then multiplied by 100 and expressed as a percent. In dilute water solutions, we can assume that 1 mL of water-based solution has a mass of 1 gram, so 1 liter of solution has a mass of 1000 grams.

Parts per million (ppm) is a ratio of parts of solute to one million parts of solution, and is usually applied to very dilute solutions. It is often found in reports of concentration of water contaminants. To calculate parts per million, divide the mass of the solute by the total mass of the solution. This number is then multiplied by 10^6 and expressed as parts per million (ppm). In dilute water solutions, we can assume that 1 mL of water-based solution has a mass of 1 gram, so 1 liter of solution has a mass of 1000 grams.

PROCEDURE:

1. **Percentage solution**:

1. Place a beaker in a balance and zero the balance.

2. Weight 5 grams of NaCl , in the beaker and dissolve in a very small volume of water, once the solid is dissolved, the volume is transferred to 100 ml volumetric flask.

3. For preparing for 500 mL it needs five times concentration of the solute of previous scenario.

4. Bring up to a final volume 500 ml of distlled water.

By definition: 5 % = 5 g / 100 mL

5 g / 100 mL = X / 500 mL

X = 25 g which is the amount of solute to be dissolved in 500 mL of distilled water

2. Parts per million solution:

1. Place a beaker in a balance and zero the balance.

2. Weight 5 mg of chlorine, in the beaker and dissolve in a very small volume of water, once the solid is dissolved, the volume is transferred to 1L volumetric flask.

3. For preparing for 1L it needs 10^6 dilutions of the solute.

5 ppm chlorine = 5 g chlorine / 10^6 mL water

= 5 mg / 1 L water

RESULT:

EXPERIMENT NO: 4 **DATE:**

PREPARATION OF DILUTE ACIDS FROM CONCENTRATED ACIDS

AIM: What volume of 10 M acetic acid is required to prepare 1.0 L of 0.50 M acetic acid?

PRINCIPLE: When preparing a dilution, decide the volume and molar concentration of the resulting solution you require. Use the following equation to determine how much of the concentrated reagent is needed to prepare the diluted solution,

$$M_{reagent} \times V_{reagent} = M_{dilution} \times V_{dilution}$$

where M is molarity and V is volume.

Slowly add the calculated volume of concentrated reagent to the proper-size volumetric flask half filled with distilled or deionized water and swirl the flask to mix. Once the solution is at room temperature, dilute to the mark with water and invert the flask several times to mix.

PROCEDURE:

1. Place a beaker in a balance and zero the balance.
2. About 10 M of acetic acid is prepared in first step.
3. A volume of 50 mL of 10 M acetic acid is required to prepare 1.0 L of 0.50 M acetic acid.

$$10\ M \times V_{reagent} = 0.50\ M \times 1.0\ L$$

$$V_{reagent} = 0.050\ L = 50\ mL$$

RESULT:

EXPERIMENT NO: 5 **DATE:**

PREPARATION OF VARIOUS DILUTE SOLUTIONS

AIM: 1. How would you prepare 1000 mL of a 1 M solution of Tris buffer from a 3 M stock of Tris buffer?

2. A can of frozen orange juice is labeled 4X. How would you dilute it to make 1L of drinkable drinkable juice?

PRINCIPLE: When preparing a dilution, decide the volume and molar concentration of the resulting solution you require. Use the following equation to determine how much of the concentrated reagent is needed to prepare the diluted solution,

$$M_{reagent} \times V_{reagent} = M_{dilution} \times V_{dilution}$$

where M is molarity and V is volume.

Slowly add the calculated volume of concentrated reagent to the proper-size volumetric flask half filled with distilled or deionized water and swirl the flask to mix. Once the solution is at room temperature, dilute to the mark with water and invert the flask several times to mix.

PROCEDURE:

A. Tris buffer dilutions

1. The concentrated solution is 3 M, and is C_1.
2. The volume of stock needed is unknown, X, and is V_1.
3. The final concentration required is 1 M, and is C_2.
4. The final volume required is 1000 mL and is V_2.

$$C_1 \times V_1 = C_2 \times V_2$$

$$3\ M \times X = 1\ M \times 1000\ mL$$

$$\mathbf{X = 333.33\ mL}$$

So, take 333.33 mL of the concentrated stock solution and diluted with 1 L of distilled water.

B. Orange juice dilutions

1. Using the $C_1 \times V_1 = C_2 \times V_2$ equation

$$4X \times K = 1X \times 1L$$

$$K = 0.25\ L$$

Use 0.25 L of orange juice is taken from the stock solution and diluted with 1L of distilled water.

RESULT:

EXPERIMENT NO: 6 **DATE:**

CONVERSION OF MILLI EQUIVALENT SOLUTION TO MILLI MOLAR EQUIVALENT SOLUTION (EX-KCL, $MgSO_4$)

AIM: What is the concentration of a solution containing 4 mEq/L of KCl?

PRINCIPLE: mEq represents the amount in milligrams, of a solute equal to 1/1000 of its gram equivalent weight taking into account the valence of the ions.

Equivalent weight = formula weight divided by the total valence

mEq = mg x valence /atomic, molecular or formula weight

PROCEDURE:

Step 1: Calculate the molecular weight of KCl

MW of potassium (K) = 39

MW of chloride (Cl) = 35.5

MW KCl = MW K + MW CL = 39 + 35.5 = 74.5 g

Step 2: Calculate equivalent weight

Equiv weight = molecular weight KCl divided by valence

Since valence of KCl = 1, Equiv weight = 74.5/ 1

Step 3: 1 mEq KCl = 1/1000 x 74.5 g = 0.0745 g = 74.5 mg

Step 4: 4 mEq KCl = 74.5mg x 4 = 298 mg/ml

OR using the equation listed before:

mg/ml = mEq/ml * atomic, molecular or formula weight /valence

= (4x74.5 / 1) = 298*mg /ml*

RESULT:

EXPERIMENT NO: 7 **DATE:**

PREPARATION OF BUFFER

AIM: To prepare the buffer at required pH.

PRINCIPLE: The pH meter measures at electrical potential developed by pair of electrode pins in a solution. For measurement of pH, an electrode system sensitive to change in H+ ion concentration of solution is taken. The electrode system consists of sequence of electrode whose potential raise with pH (H+ concentration of the solution).

PROCEDURE:

1. ACETIC ACID- SODIUM ACETATE BUFFER:

REAGENTS REQUIRED:

Acetic Acid 0.2M: 1.5 ml of glacial acetic acid is made upto 100ml with distilled water.

Sodium Acetate Solution**:** 0.64 gm of sodium acetate or 2.72gm of sodium acetate trihydrate is dissolved in 100ml Distilled water.

PROCEDURE:

Pipette out exactly 36.2ml of sodium acetate solution into 100ml of standard flask and add 14.8ml of glacial acetic acid, make the volume 100ml using distilled water using distilled water. This gives 0.2 M of acetic acid and sodium acetate buffer. The pH is measured with pH meter.

The pH meter is first standararised with pH buffer. Wash electrode with distilled water and introduced into 0.2M acetic acid-sodium acetate buffer prepared, the pH of solution is 4.6.

RESULT:

36.2ml Sodium acetate and 14.8 ml glacial acetic acid were mixed and buffer was prepared. pH was measured initial reading observed was 4 which made upto 4.6 with 5N NaOH.

2. BARBITONE BUFFER:

REAGENTS REQUIRED:

- Diethyl barbituric acid.
- Sodium diethyl barbititrate

PROCEDURE:

Dissolve 2.85gm of diethyl barbituric acid and 14.2gm of sodium diethyl barbititrate in distilled water and upto 1 liter. This gives the barbitone buffer.

The pH meter is first standararised with pH buffer. Wash electrode with distilled water and introduced into barbitone buffer prepared, the pH of solution is 6.8.

3. CITRATE BUFFER:

REAGENT S REQUIRED:

- Citric acid: Dissolve 2.101 gm of citric acid in 100ml distilled water.
- Sodium citrate solution 0.1 M: Dissolved 2.941gm of sodium citrate in 100ml distilled water.

PROCEDURE:

46.5ml of citric acid with 3.5ml of sodium citrate solution and upto 100ml with distilled water. It corresponds to 0.1 M citrate buffer and standardised with pH meter and measures the pH of the prepared solution. This gives citrate buffer at pH 2.5.

RESULT:

Citrate buffer was prepared and the pH observed was 4.8 which was adjusted to 2.5 using 1N Hcl and 5N NaoH.

4. CARBONATE- BICARBONATE BUFFER:

REAGENTS REQUIRED:

- Sodium carbonate solution 0.2M: Dissolve 2.12gm of anhydrous sodium carbonate in 100ml Distilled water.
- Sodium bicarbonate solution: Dissolve 1.68gm of sodium bicarbonate in 100ml of distilled water.

PROCEDURE:

Pipette out exactly 27.5ml of sodium carbonate (Na_2Co_3) solution. To this add 22.5ml of sodium bicarbonate solution and made upto 100ml with distilled water which corresponds to 0.2 M sodium carbonate and bicarbonate buffer.

Standardise pH meter and measure the pH of required buffer. This gives the Carbonate- bicarbonate buffer pH 10.2.

RESULT:

Carbonate bicarbonate buffer was prepared and pH observed was 7.5 which was adjusted to 10.2 using 1N Hcl and 5N NaoH.

5. PHOSPHATE BUFFER:

REAGENTS REQUIRED:

- Monobasic: Dissolve 2.78gm of sodium dihydrogen phosphate in 100ml of distilled water.
- Dibasic sodium phosphate (0.2M): Dissolve 5.3gm of disodium hydrogen phosphate or 7.17 gm sodium hydrogen phosphate in 100ml distilled water.

PROCEDURE:

39 ml of dihydrogen sodium phosphate is mixed with 61 ml of disodium hydrogen phosphate This made up to 200ml with distilled water .This gives phosphate $(Po_4)_2$ buffer of 0.2M.

Standardized pH meter with standard buffer. Washed electrode with distilled water and introduced it into phosphate buffer prepared. The pH of the solution is 6.8.

RESULT:

Phosphate buffer was prepared and pH was observed 8.5 which was made upto 6.8 using 1N Hcl and 5N NaoH.

6. POTASSIUM PHOSPHATE BUFFER:

REAGENTS REQUIRED

- Dipotassium hydrogen phosphate
- Potassium dihydrogen phosphate

PROCEDURE:

174.18 g/mol dipotassium hydrogen phosphate and 136.09 g/mol potassium dihydrogen phosphate was taken and made up to 200ml using distilled water. This gives the potassium buffer.

Standardised pH meter with standard buffer. Washed electrode with distilled water and introduced it into potassium buffer prepared. The pH of the solution is 6.5.

RESULT:

Dipotassium hydrogen phosphate (K2HPO4) and potassium dihydrogen phosphate (KH2PO4) solution will be prepared and the pH was measured to be 9.87 and 4.23 respectively, the solution were made using 1N Hcl and 5N NaoH respectively and the pH was found to be 6.5.

EXPERIMENT NO: 8 **DATE:**

DETERMINATION OF THE ABSORPTION MAXIMA AND MOLAR EXTINCTION COEFFICIENT (OF A RELEVANT ORGANIC MOLECULE).

Aim: To determine the absorption maxima and molar extinction coefficient.

Introduction:

Estimation of protein concentration in a given protein preparation is one of the most commonly performed tasks in a biochemistry lab. There are several ways of estimating the protein concentration such as amino acid analysis following acid hydrolysis of the protein; analyzing the changes in the spectral properties of certain dyes in the presence of proteins; and spectrophotometric estimation of the proteins in near or far UV region. Although dye-binding

assays and amino acid analysis following acid hydrolysis of the protein can be used for estimating the protein concentration for both pure as well as an unknown mixture of proteins; UV spectroscopic quantitation holds good for the pure proteins. If a protein is pure, UV spectroscopic quantitation is the method of choice because it is easy and less time- consuming to perform; furthermore, the protein sample can be recovered back.

Absorption of ultraviolet radiation is a general method used for estimating a large number of bioanalytes. The region of the electromagnetic radiation ranging from ~10-400 nm is identified as the ultraviolet region. For the sake of convenience in referring to the different energies of UV region, it can be divided into three regions:

- Near UV region (UV region nearest to the visible region; $\lambda \sim 250 - 400$ nm)
- Far UV region (UV region farther to the visible region; $\lambda \sim 190 - 250$ nm)
- Vacuum UV region ($\lambda < 190$ nm)

This division is not strict and you may find slightly different wavelength ranges for these regions. We shall, in this course, stick to the above-mentioned definitions. Absorption of UV light is associated with the electronic transitions in the molecules from lower to higher energy states (Figure 4.1).

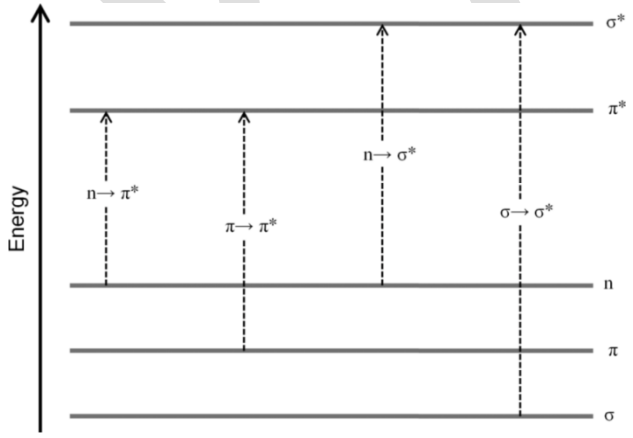
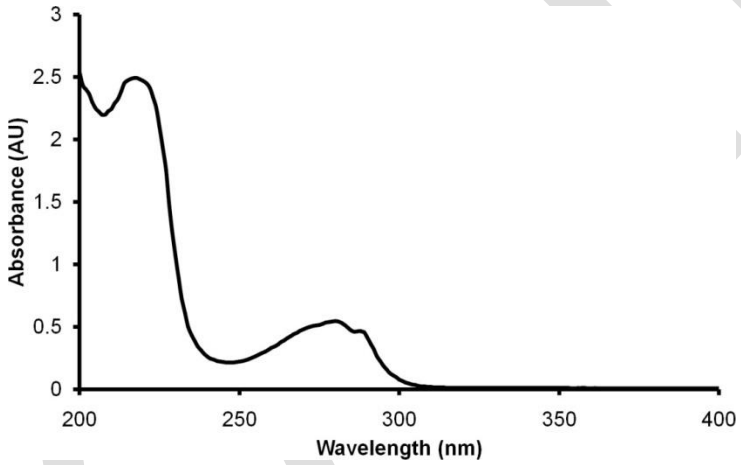


Figure 4.1 A diagrammatic representation of the energy levels of molecular orbitals; the vertical arrows represent electronic transitions.

As is clear from figure 4.1, $\sigma \rightarrow \sigma^*$ transition involves very high energy and usually lies in the vacuum UV region. Saturated hydrocarbons, that can undergo only $\sigma \rightarrow \sigma^*$transition, therefore show absorption bands at ~150 nm wavelength. Compounds that have unsaturation and/or lone pair of electrons *i.e.* the ones that can undergo $\pi \rightarrow \pi^*$ or $n \rightarrow \pi^*$ transitions, absorb at higher wavelengths that may lie in far or near UV regions, the regions of UV radiation the biochemical spectroscopists are usually interested in. The group of atoms in a molecule that comprise the orbitals involved in the transition is said to constitute a chromophore.Figure 4.2 shows an absorption spectrum of a peptide. The spectrum immediately suggests that the proteins can absorb both in near UV and far UV regions.



Absorption of UV radiation is usually represented in terms of absorbance and %transmittance:

$$Absorbance\ (A) = -log \frac{I}{I_0} \quad \text{---------- (4.1)}$$

$$\%Transmittance\ (\%T) = \frac{I}{I_0} \times 100 \quad \text{---------- (4.2)}$$

where, I_0 and I represent the intensities of light entering and exiting the sample, respectively.

Absorbance of an analyte depends on the concentration of the analyte and the path length of the solution (Beer-Lambert Law):

$$A = \varepsilon cl \text{ ----------(4.3)}$$

where, ε is the molar absorption coefficient, c is the molar concentration of the analyte and l is the path length of the cell containing the analyte solution. If molar absorption coefficient of the analyte and the path length of sample cell are known, concentration can directly be determined using Beer-Lambert law.

Let us see how protein concentration is estimated using near and far UV radiation.

Near-UV radiation

Aromatic amino acids, tryptophan, tyrosine, and phenylalanine and the disulfide linkage constitute the chromophores that absorb in the near UV region. Absorption of near UV radiation by proteins is usually monitored at 280 nm due to very high absorption by Trp and Tyr at this wavelength. Table 4.1 shows the molar absorption coefficient of the protein chromophores that absorb the light of 280 nm.

Table 4.1 Molar absorption coefficientsof protein chromophores at 280 nm

	$\varepsilon_{280}(M^{-1}cm^{-1})$		
	Trp	Tyr	S-S
Average value in folded proteins	5500	1490	125
Value in unfolded proteins	5690	1280	120

where, ε_{280} is the molar absorption coefficient at 280 nm.

It is therefore straightforward to calculate the molar absorption coefficientof a folded protein if its amino acid sequence or composition is known:

$$\varepsilon_{280} = (5500 \times n_{Trp}) + (1490 \times n_{Tyr}) + (125 \times n_{S-S}) \quad \text{---------- (4.4)}$$

For short peptides that are usually unfolded in water, the molar absorption coefficients can be calculated using the following equation:

$$\varepsilon_{280} = (5690 \times n_{Trp}) + (1280 \times n_{Tyr}) + (120 \times n_{S-S}) \quad \text{---------- (4.5)}$$

Far-UV radiation

The proteins and peptides that lack aromatic residues and disulfide linkage do not absorb the near UV radiation. The concentration of such proteins and peptides can be estimated using far UV radiation. Peptide bond is the major chromophore in the far UV region with a strong absorption band around 190 nm ($\pi \rightarrow \pi^*$ transition) and a weak band around 220 nm ($n \rightarrow \pi^*$ transition). As oxygen strongly absorbs 190 nm radiation, it is convenient to measure absorptionat 205 nm where molar absorption coefficient of peptide bond is roughly half of that at 190 nm. A 1 *mg/ml* solution of most proteins would have an extinction coefficient of ~30 – 35 at 205 nm. This means that the result obtained can have more than 15% error. An empirical formula, proposed by Scopes [1] provides the $A^{1\ mg/ml}_{205}$ within ± 2%:

$$A^{1\ mg/ml}_{205} = 27 + 120\left(\frac{A_{280}}{A_{205}}\right) \quad \text{---------- (4.6)}$$

Alternatively, the concentration can be estimated using Wadell's method[2] that relies on

the absorbance at 215 and 225 nm:

$$Protein\ concentration\ \left(\frac{\mu g}{ml}\right) = 144(A_{215} - A_{225})\text{----------}\ (4.7)$$

Materials:

1. A UV/Visible spectrophotometer
2. Pipettes
3. Pipette tips
4. Disposable microfuge tubes
5. Quartz cuvettes (suitable for wavelengths smaller than 205 nm)
6. Pure protein solution in a buffer (or in water)
7. The buffer the protein is dissolved in (will act as the blank).

Procedure:

1. Switch 'ON' the UV/visible spectrophotometer and allow it 30 minutes warm up.
2. Determine the number of tryptophans, tyrosines, and disulfide linkages present in the protein.
3. Determine the molar absorption coefficient of the protein at 280 nm using equation 4.4.
4. Take the bufferused for protein dissolution in the quartz cuvettes.
    a. The volume of buffer has to be sufficient enough to cover the entire aperture the light beampasses through and depends on the capacity of the quartz cuvette; typically cuvettes with 1 *ml* capacity are used.
5. Place the cuvettes in the reference cell and sample cell slots in the spectrophotometer.
6. 'ZERO' the baseline for the 250 – 350 nm range.
7. Remove the quartz cuvette placed in the sample cell slot and discard all the

contents.

8. Add the same volume of the given protein solution into the cuvette and place it back in the sample cell slot.
9. Record the absorbance at 280 nm A_{280}^{Sample} and 330 nm A_{330}^{Sample}
    a. Proteins do not absorb at wavelengths higher than 320 nm; any absorbance obtained at 330 nm therefore arises due to scattering.
    b. If the absorbance at 280 nm does not lie between 0.05 – 1.0, dilute the protein solution in the same buffer so as to obtain an absorbance in this range.
10. Switch off the spectrophotometer.
11. Take out the quartz cell and clean them using detergent solution and deionized water.

Calculation:

The absorbance at 280 nm is corrected for light scattering:

$$A_{280(corrected)}^{Sample} = A_{280}^{Sample} - 1.929 \times \left(A_{330}^{Sample}\right)$$

The amount of the given protein is determined using Beer-Lambert law (equation 4.3):

$$A_{280(corrected)}^{Sample} = \varepsilon c l$$

$$c(M) = \frac{A_{280(corrected)}^{Sample}}{\varepsilon(M^{-1}cm^{-1})\, l(cm)}$$

Notes:

1. If the given protein lacks Trp, Tyr, and disulfide linkages, the concentration can be estimated using A_{205} or A_{215} and A_{225} using equations 4.6 and 4.7.
2. If the protein solution is turbid, it will scatter light leading to inflated absorbance

values. The solution should therefore be cleared either by filtering it through a 0.2 *μm* filter or through centrifugation.

EXPERIMENT NO: 9 **DATE:**

MEASUREMENT OF UV SPECTRUM OF COMPOUNDS

Aim: To determine the UV absorption spectrum of compounds

Principle:

UV- spectrophotometer is used for enumeration of compounds like DNA/RNA. The DNA/RNA has maximum and minimum absorption at 260 and 234 nm, respectively. The λ_{max} is used to assess purity of the DNA/RNA and concentration present in the sample. DNA/RNA absorbs UV more or less strongly depending upon the wavelength. By using a UV-Visble spectrophotometer, which takes measurements at wavelengths of 260 and 280 nm, and in addition determines an absorption spectrum from 220 – 350 nm.

Materials Required:

1. DNA and RNA sample
2. UV spectrophotometer
3. Quartz cuvette
4. Distilled water
5. Wash bottle

Procedure:

1. Make the dilution of the given DNA sample in the ratio 1:20.

2. Take the absorbance from 220 to 350 nm.

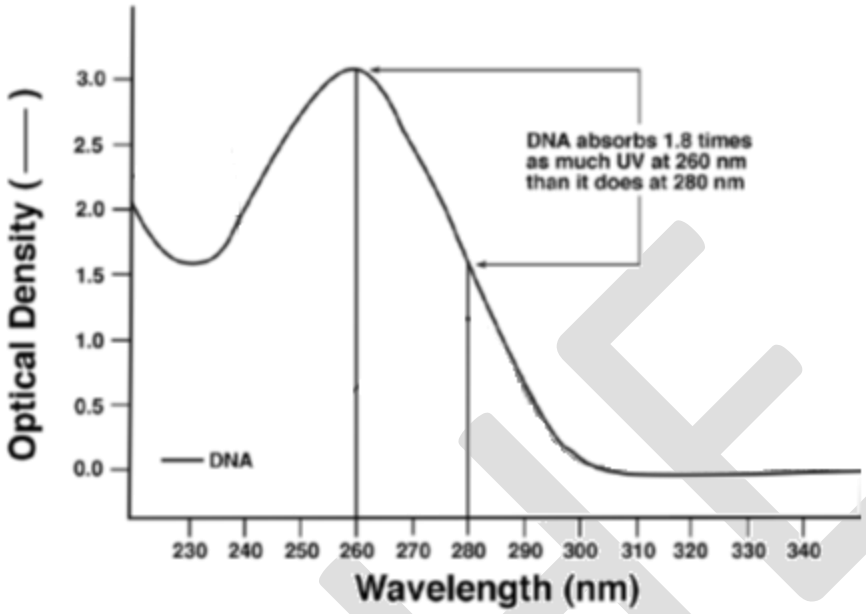
3. Note the readings and plot the graph of absorbance against the wavelength and make the conclusions.

Result:

Nucleic acids DNA/RNA absorbs maximally at 260 nm. Hence, maximum absorbance at 260 nm indicates presence of nucleic acids.

Table: UV absorption spectrum of DNA and RNA

Si.No.	Absorption Spectrum (nm)	O.D values
1	220	2.0
2	230	1.5
3	240	1.8
4	250	2.0
5	260	2.5
6	270	2.0
7	280	1.5
8	290	0.5
9	300	0.1
10	310	0.0
11	320	0.0
12	330	0.0
13	340	0.0
14	350	0.0



EXPERIMENT NO: 10 **DATE:**

DETERMINATION OF CONCENTRATION OF A PROTEIN SOLUTION BY LOWRY/BCA METHOD.

AIM:

To estimate the amount of Protein present in given unknown solution by Lowry's method.

PRINCIPLE:

Alkaline $CuSo_4$ catalyses the oxidation of aromatic amino acids with subsequent reduction of sodium potassium molybdate tungstate of Folin's reagent giving a purple colour complex the intensity of the colour is directly proposition to the concentration of the aromatic amino acid in the given sample solution.

REAGENTS REQUIRED:

1. Stock Solution:

Bovine Serum albumin of 100 mg is weighed accurately and dissolved in 100ml of distilled

water in a standard flask (concentration 1 mg /ml).

2. Working Standard:

The Stock Solution of 10 ml is distilled to 100ml with distilled water in a standard flask (concentration 100 µg/ml).

3. Folin's Phenol Reagent:

Folin's Phenol Reagent is mixed with distilled water in the ratio 1:2.

4. Alkaline copper reagent:

Solution A: 2% sodium carbonate in 0.1 N sodium hydroxide.

Solution B: 0.5% copper sulphate in 1% sodium potassium tartarate.

Solution A, B, C is mixed in the proportion of 50:1:0.5.

PROCEDURE:

Working standard of 0.2 -1ml is pipette out into clean test tube and labeled as S1-S5. Test solution of 0.2ml is taken into test tube and labeled as T1. The volume is made up to 1ml of distilled water. Distill water of 1ml serve as blank. To all the test tube 4.5 ml of alkaline Copper sulphate reagent is added and incubated at room temperature for 10 minutes. All the test tube 0.5ml of folin's phenol reagent is added. The contents are mixed well and the blue colour developed is read at 640 nm after 15 minutes. From the standard graph the amount of protein in the given unknown solution is calculated.

RESULT:

The amount of protein present in the given unknown solution is _____ (mg of protein).

EXPERIMENT NO: 11 **DATE:**

DETERMINATION OF NUCLEIC ACID CONCENTRATION AND PURITY

Aim: To determine the absorption spectrum of nucleic acid and its purity.

Principle:

Once the contamination has been removed from DNA preparation, the concentration of the DNA in the solution can be determined. The method most commonly used to determine DNA

concentration involves the use of UV absorption spectroscopy. Just as all organic compounds have characteristic absorption spectra, the nitrogenous bases of dsDNA exhibit strong absorption maxima at a wavelength of 260 nm. At this wavelength, the extinction coefficient of DNA, E_{260} = 20, indicates that DNA at a concentration of 1 mg/mL will have an absorption (A_{260}) = 20.

As the relationship between DNA concentration and A_{260} is linear to an A_{260} = 2, the concentration of DNA in a solution can be determined. For example, A_{260} = 0.5 corresponds to 25 µg/mL, A_{260} = 0.1 corresponds to 5 µg/mL, and so on. Use of the conversion factor, 50 µg/mL = 1 A_{260} unit, enables the concentration of most DNA solutions to be determining easily. However, this relationship only applies to purify dsDNA with a G + C content of 50%. The presence of RNA, proteins, detergents, and organic solvents will also contribute to absorbance at this wavelength. Since the absorption maxima for DNA and protein are 260 nm and 280 nm, respectively, an approximate measure of the purity of the isolated DNA can be obtained by determining the A_{260}/A_{280} ratio. Pure *E. coli* DNA has A_{260}/A_{280} = 1.95. This ratio, however, is dependent on the overall base composition of the DNA and will vary with different organisms. UV-spectrophotometer is used for enumeration of DNA. The DNA concentration can be calculated by the formula DNA conc. (µg/mL) = $A_{260}/\in_{260}$.

Where, A_{260} is absorbance at 260 nm and ε_{260} is the DNA extinction coefficient. For dsDNA, the ε_{260} is 0.02 $(\mu g/mL)^{-1}$ cm^{-1}. Thus, an absorbance of 1 at 260 nm gives a DNA concentration of 50 µg mL^{-1} (1/0.2 = 50), the ε_{260} is 0.027 $(\mu g/mL)^{-1}$ cm^{-1} for ssDNA at absorbance of 1 (1/0.027 = 37).

The purity of DNA can be determined by the formula = the ratio of A 260 nm/A 280 nm.

The concentration can be determined by DNA conc. (g/mL) = OD 260 nm × 50 × dilution factor (i.e., 20).

Materials Required:

1. DNA sample
2. UV spectrophotometer
3. Quartz cuvette

4. Distilled water
5. Wash bottle

Procedure:

1. Make the dilution of the given DNA sample in the ratio 1:20.

2. Take the optical density (OD) at 260 nm and 280 nm.

3. Note the readings and make the conclusions.

Result:

The purity of DNA was found to be = 1.5/0.85 = 1.8

The concentration of DNA conc. (g/mL) was found to be = 1,500