AVIT
AARUPADAI VEEDU INSTITUTE OF TECHNOLOGY

VINAYAKA MISSION'S RESEARCH FOUNDATION
(Deemed to be University under section 3 of the UGC Act 1956)

Accredited by NAAC

Approved by AICTE

DEPARTMENT OF BIOTECHNOLOGY

NAME OF THE LAB

17BTCC87- BIOINSTRUMENTATION LAB

HOD

AVIT
AARUPADAI VEEDU INSTITUTE OF TECHNOLOGY
VINAYAKA MISSION'S RESEARCH FOUNDATION
(Deemed to be University under section 3 of the UGC Act 1956)
Accredited by NAAC
Approved by AICTE

DEPARTMENT OF BIOTECHNOLOGY

17BTCC87-BIOINSTRUMENTATION LAB

LIST OF EXPERIMENTS

1. Validating Lambert – Beer's law using $KMnO_4$.
2. Determination of complementary color and complementary wavelength
3. Precision and Validity in an experiment using Absorption spectroscopy.
4. Finding the Stoichiometry of the Fe (1,10Phenanthroline Complex) using Absorption spectroscopy.
5. UV spectra of Nucleic Acid.
6. Estimation of Alizarin Aluminium complex
7. Estimation of Al^{3+} concentration using Alizarin in the spectrometer.
8. Estimation of Sulphate by Nephelometry.
9. Experiments on

a. Conductivity meter

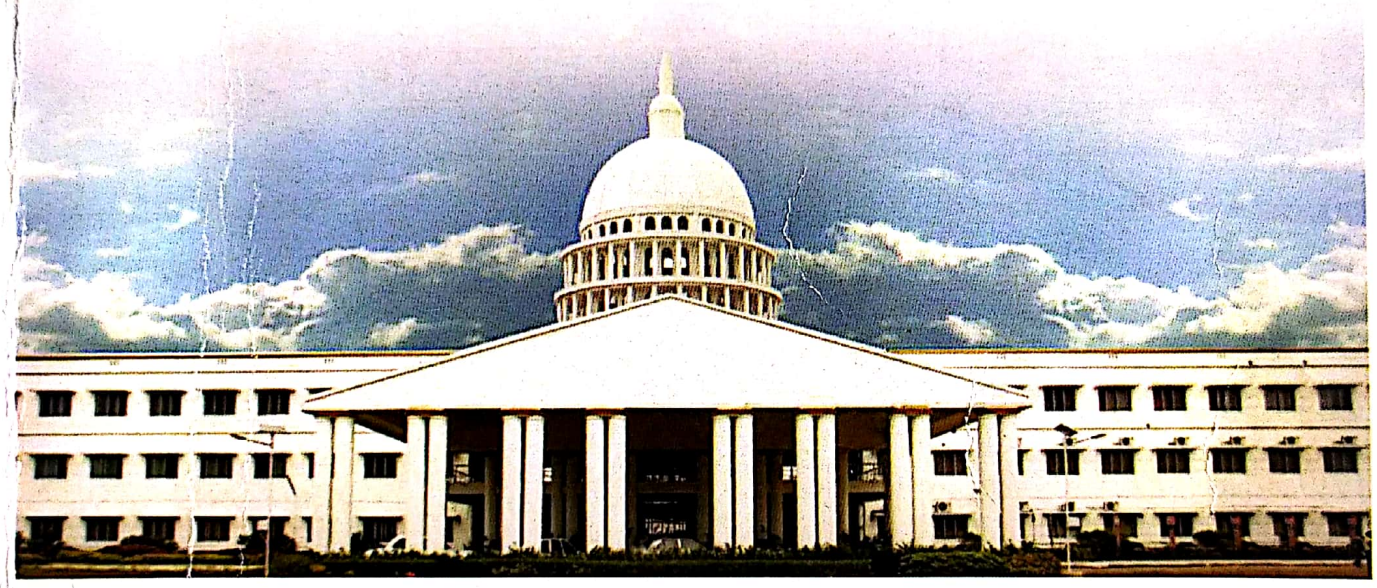
b. Turbidity meter.

10. Estimation of Dissolved oxygen.
11. Determination of Fe^{2+} content in fruit juices

HOD

AARUPADAI VEEDU
INSTITUTE OF TECHNOLOGY
OMR, PAIYANOOR, CHENNAI - 603104

VINAYAKA MISSION'S
RESEARCH FOUNDATION
(Deemed to be University under section 3 of the UGC Act 1956)



B.Tech - BIOTECHNOLOGY

ADVANCED BIOCHEMISTRY - LABORATORY MANUAL

17BTCC84

DEPARTMENT OF BIOTECHNOLOGY

AARUPADAI VEEDU INSTITUTE OF TECHNOLOGY

VINAYAKA MISSION'S RESEARCH FOUNDATION

(Deemed to be University)

Paiyanoor, Chennai

TABLE OF CONTENTS

Expt. No.	NAME OF THE EXPERIMENT	PAGE NO.
1	Validating Lambert – Beer's law using KMnO4.	
2	Determination of complementary color and complementary wavelength	
3	Precision and Validity in an experiment using Absorption spectroscopy	
4	Finding the Stoichiometry of the Fe (1,10 Phenanthroline Complex) using Absorption spectroscopy	
5	UV spectra of Nucleic Acid	
6	Estimation of Alizarin Aluminium complex	
7	Estimation of Al 3+ concentration using Alizarin in the spectrometer	
8	Estimation of Sulphate by Nephelometry	
9	Experiments on a. Conductivity meter b. Turbidity meter	
10	Estimation of Dissolved oxygen.	
11	Determination of Fe2+ content in fruit juices	

EXPT. NO. :

DATE :

VALIDATING LAMBERT – BEER'S LAW USING POTASSIUM PERMANGANATE

AIM

To validate Lambert – Beer's law using Potassium permanganate.

PRINCIPLE

Beer – Lambert's law states that when a beam of monochromatic light passes through a solution, the rate of decrease in the intensity of the radiation with the thickness of the solution is proportional to the intensity of incident radiation as well as the concentration of the solution.

Mathematically, this law is stated as

$$I_t = I_0 \, e^{-kcl}$$

$$\log I_0 / I_t = \varepsilon cl = A$$

Where A - Absorbance of the sample.

ε - Constant called molar absorptivity.

l - Distance that the incident radiation travels through the sample.

C - Concentration of the absorbing species in the sample.

Absorbance has no units. It 'l' is expressed in cm and 'C' in mol dm^{-3} then 'ε' has units of $mol^{-1} dm^3 cm^{-1}$.

MATERIALS AND REAGENTS REQUIRED

(i) Spectrophotometer

(ii) Cuvette

(iii) Test tube

(iv) Beaker

(v) Potassium permanganate (0.1%)

(vi) Distilled water

(vii) Volumetric flask.

PROCEDURE

(i) Prepare 0.1% solution of potassium permanganate in distilled water. Switch ON the spectrophotometer and warm up for 15 to 20 minutes. Take clean dry test tubes, pipette out 1mL to 10mL of the test solution with a gradual increase of 1mL to different test tubes. Add distilled water to make the final volume in each tube to 10mL and mix well.

(ii) Using the complementary coloured filter or complementary wavelength, adjust the blank to 100% transmittance, then read each tube, and record the absorbance.

(iii) Plot absorbance against concentration and find out up to what concentration of the solution obeys the Beer's law.

(iv) The concentration of unknown from the mixture can be determined by two methods.

- From the calibration graph
- Formulae method

(v) If the absorbance value of unknown lies between or near the range of the curve, generally the first method is followed.

(vi) If the absorbance lies outside, the second method has to be followed to determine the concentration of unknown.

OBSERVATION

From the graph, it is found that the absorbance at 525 nm increased with an increase in concentration of the solution.

RESULT

Thus, the Beer – Lambert's law is validated

The concentration of potassium permanganate in the given unknown sample is found to be ----------

Stock Standard $KMnO_4$ (0.01%)

0.01g of $KMnO_4$ is dissolved in 100mL of distilled water

Tabular Column

S. No.	Concentration of Standard $KMnO_4$ (%)	Volume of Standard $KMnO_4$ (mL)	Volume of Distilled Water (mL)	Concentration of Working Standard $KMnO_4$ (%)	Absorbance (O. D.)
1.	-	Blank	10		
2.	0.1	1	9		
3.	0.1	2	8		
4.	0.1	3	7		
5.	0.1	4	6		
6.	0.1	5	5		
7.	0.1	6	4		
8.	0.1	7	3		
9.	0.1	8	2		
10.	0.1	9	1		
11.	0.1	10	0		
12.	0.1	Unknown	-		

CALCULATION

Formulae method

To determine the concentration of the unknown potassium permanganate

$$\text{Concentration of unknown solution} = \frac{\text{Absorbance of unknown x Concentration of standard}}{\text{Absorbance of standard}}$$

EXPT. NO. :

DATE :

DETERMINATION OF COMPLEMENTARY COLOUR AND COMPLEMENTARY WAVELENGTH

AIM

To determine the complementary wavelength and complementary colour for the given solution.

PRINCIPLE

When a coloured solution absorbs the light from a particular colour of the filter to a maximum extent then that colour is said to be complementary to the colour of the solution.

When a coloured solution absorbs light maximally at a particular wavelength then their wavelength is said to be complementary wavelength of the solution. Complementary colour or wavelength is used to estimate the concentration of the substance because at the colour or wavelength, the given substance absorbs maximally and contribute to high sensitivity to the estimation and also the less inference from other substance.

MATERIALS REQUIRED

(i) Spectrophotometer

(ii) Cuvette

(iii) Test tubes

(iv) Beaker

(v) Potassium dichromate (0.1%)

(vi) Potassium permanganate (0.01%)

(vii) Methyl orange (0.1%)

(viii) Methyl red (0.1%)

(ix) Distilled water

PROCEDURE

(i) Prepare 0.1% solution of potassium dichromate, (0.1%) methyl orange, (0.1%) methyl red and (0.01%) potassium permanganate.

(ii) Warm up the spectrophotometer for 15 – 30 minutes with one of the filter in position.

(iii) Place the Cuvette containing the blank in the cuvette holder and adjust the reading top 100% transmittance.

(iv) Now take the test solution in the cuvette and place it in the cuvette holder and note the readings.

(v) Repeat the steps iii and iv, for each filter and for every 5 or 10 wavelengths in nm.

(vi) Find out the colour of the filter, which absorbs maximally. This colour is the complementary colour to the colour of the solution.

(vii) For finding the complementary wavelength, plot absorbance against wavelength, the peak gives the wavelength of maximum absorption of the solution.

RESULT

The complementary colour of the given sample is

(i) Potassium permanganate -

(ii) Potassium dichromate -

(iii) Methyl orange -

(iv) Methyl red -

The complementary wavelength for the given sample is

(i) Potassium permanganate -

(ii) Potassium dichromate -

(iii) Methyl orange -

(iv) Methyl red -

Complementary Colours

S. No.	Wavelength (nm)	Hue Transmitted	Complementary
1.	400 – 435	Violet	Yellowish Green
2.	435 – 480	Blue	Yellow
3.	480 – 490	Greenish Blue	Orange
4.	490 – 500	Bluish Green	Red
5.	500 – 560	Green	Purple
6.	560 – 580	Yellowish Green	Violet
7.	580 – 595	Yellow	Blue
8.	595 – 610	Orange	Greenish Blue
9.	610 – 750	Red	Bluish Green

Determination of Complementary Wavelength for $KMnO_4$

S. No.	Wavelength (nm)	Absorbance (O. D.)
1.	400	
2.	450	
3.	500	
4.	550	
5.	600	
6.	650	
7.	700	

Determination of Complementary Wavelength for $KMnO_4$

S. No.	Wavelength (nm)	Absorbance (O. D.)
1.	500	
2.	505	
3.	510	
4.	515	
5.	520	
6.	525	
7.	530	
8.	535	
9.	540	
10.	545	
11.	550	

Determination of Complementary Wavelength for $K_2Cr_2O_7$

S. No.	Wavelength (nm)	Absorbance (O. D.)
1.	400	
2.	450	
3.	500	
4.	550	
5.	600	
6.	650	
7.	700	

Determination of Complementary Wavelength for $K_2Cr_2O_7$

S. No.	Wavelength (nm)	Absorbance (O. D.)
1.	350	
2.	355	
3.	360	
4.	365	
5.	370	
6.	375	
7.	380	
8.	385	
9.	390	
10.	395	
11.	400	

Determination of Complementary Wavelength for Methyl Orange

S. No.	Wavelength (nm)	Absorbance (O. D.)
1.	400	
2.	450	
3.	500	
4.	550	
5.	600	
6.	650	
7.	700	

Determination of Complementary Wavelength for Methyl Orange

S. No.	Wavelength (nm)	Absorbance (O. D.)
1.	430	
2.	435	
3.	440	
4.	445	
5.	450	
6.	455	
7.	460	
8.	465	
9.	470	
10.	475	
11.	480	

Determination of Complementary Wavelength for Methyl Red

S. No.	Wavelength (nm)	Absorbance (O. D.)
1.	400	
2.	450	
3.	500	
4.	550	
5.	600	
6.	650	
7.	700	

Determination of Complementary Wavelength for Methyl Red

S. No.	Wavelength (nm)	Absorbance (O. D.)
1.	430	
2.	435	
3.	440	
4.	445	
5.	450	
6.	455	
7.	460	
8.	465	
9.	470	
10.	475	
11.	480	
12.	485	
13.	490	

EXPT. NO. :

DATE :

PRECISION AND VALIDITY OF AN EXPERIMENT USING ABSORPTION SPECTROSCOPY

AIM

To study and verify the Precision and Validity of an experiment using spectrophotometer.

THEORY

Precision may be defined as the concordance of a series of measurements of the same quantity. Accuracy (validity) may be defined as the concordance between the experimental value and the most probable value.

Accuracy expresses the correctness of measurement, the precision express the reproducibility of the measurements, and the precision always accompanies accuracy.

If a solution of 'X' concentration is taken in duplicate, it should give some change in absorbance for the same concentration due to error in dilution. Similarly, for a given concentration of solution there should not be any change in absorption when it is read repeatedly. Any difference in this reading reflects the precision of the instrument.

MATERIALS REQUIRED

(i) UV – Visible spectrophotometer

(ii) Beaker

(iii) Pipette

(iv) Cuvette

(v) Measuring cylinder

(vi) Potassium permanganate solution (0.01%)

(vii) Distilled water.

PROCEDURE

Using the stock solution of potassium permanganate (0.01%) the following dilutions were made in duplicates, which is shown in the column. The concentration of 0.35 mg/mL was prepared for validation. The above dilutions was prepared and mixed well for homogeneity and their absorbances were analyzed. One of the duplicates was reanalyzed for their concordance using spectrophotometer, the readings were tabulated.

A standard graph was plotted using average of absorbance of the duplicates and their respective concentrations. From the graph drawn, the average values of the duplicates and the absorbance of the repeated sets were plotted as bar diagrams.

OBSERVATION

The sample has shown an insignificant variation in its absorption. The variation could be due to error in dilution. The difference in absorption for the repeated set was found to be insignificant, there by the precision of the instrument was ensured. The concentration of the sample was checked from the standard graph and validated.

RESULT

(i) Percentage precision of the Instrument -

(ii) Percentage precision of the Experiment -

(iii) Percentage validity of the Experiment -

Thus the precision and validity of an experiment are verified.

Tabular Column – 1

Stock solution - 0.01% $KMnO_4$

Dissolve 10 mg of $KMnO_4$ in 100mL distilled water.

S. No.	Particulars	B	S_1	S_2	S_3	S_4	S_5	A
1.	Volume of Standard $KMnO_4$ (mL)							
2.	Concentration (mg)							
3.	Volume of distilled water (mL)							

Tabular Column – 2

S. No.	Concen-tration (mg)	Duplicate (1) Absorbance at 520nm A	Duplicate (2) Absorbance at 520nm B	Recheck Duplicate (2) {B}	Average of Duplicate 1 & 2	Difference Between B & A	Difference Between 1& 2	% Error of the Instrument = B&A x 100 / Average (A & B)	% Error of the Dilution = Dup 1& 2 x 100 / Average (1& 2)

CALCULATION

- % Precision of the Instrument = 100 - % error of the Instrument
- % Precision of the Experiment = 100 - % error of the dilution
- % Accuracy (validity) = $\dfrac{\text{Observed value - Theoretical Value}}{\text{Theoretical value}}$

EXPT. NO. :

DATE :

DETERMINATION OF STOICHIOMETRY OF Fe (II) - ORTHO 1, 10 PHENANTHROLINE COMPLEX

AIM

To determine the stoichiometry of Fe (II) – 1, 10 o - Phenanthroline complex by using Absorption spectroscopy.

PRINCIPLE

Heterocyclic compounds like 1, 10 Phenanthroline form coloured complexes with ferrous salts. When equimolar amounts of the two solutions are mixed in varying proportions, the maximum amount of complex is formed in equilibrium. When the proportions of the reactants corresponding to the empirical formula of the Complex, absorption is maximum.

The optimum pH range for the stability of Fe (II) – 1, 10 o- Phenanthroline complex is 2-9. This is obtained by adding sodium acetate buffer and hydroxylamine hydrochloride is added to reduce any Fe^{3+} to Fe^{2+} and to maintain it in that state.

REACTIONS

REAGENTS REQUIRED

- **Standard Iron (II) solution (10ppm)**

  Dissolve 0.0702g of Ferrous ammonium sulphate hexa hydrate [$Fe(NH_4)_2(SO_4)_2.6H_2O$] in 1000mL water. Add 2.5mL of concentrated sulphuric acid to the solution.

- **1, 10 Phenanthroline solution : (2×10^{-4} M)**

  Dissolve 0.0396g of 1, 10, o - Phenanthroline monohydrate in 1000mL water. Store it in a plastic bottle.

- **Hydroxyl amine hydrochloride solution (10%)**

  Dissolve 10g of Hydroxyl amine hydrochloride in 100mL water.

- **Sodium acetate solution**

  Dissolve 10g of sodium acetate in 100mL water.

MATERIALS REQUIRED

(i) Spectrophotometer
(ii) Cuvette
(iii) Beaker
(iv) Conical flask
(v) Standard flask
(vi) Test tubes
(vii) Pipettes

PROCEDURE

(i) Firstly, equimolar solutions of Ferrous Ammonium Sulphate and 1, 10 Phenanthroline are prepared.

(ii) Into a series of 100mL volumetric flask, 2mL of standard iron solution is taken.

(iii) To each of these flasks 10mL of 10% Hydroxylamine hydrochloride solution is added.

(iv) Followed by the addition of 4mL of 0.2M sodium acetate buffer to adjust the pH to a value at which the orange red colour complex forms.

(v) The Iron solution is then treated with varying amounts of 1, 10 Phenanthroline and the solution is then diluted to 100mL using distilled water.

(vi) Thus, the various standard solution of the complex is prepared and the absorbance is measured at maximum of 515nm.

(vii) A graph is plotted by taking L / M ratio of x – axis and absorbance on y – axis.

(viii) From the plot, the stoichiometry of the complex is determined. The mole ratio corresponding to the maximum absorbance value gives the stoichiometry of the complex.

Tabular Column

S. No	Volume of FAS [M] (mL)	Volume of 1, 10 Phenanthroline [L] (mL)	Volume of Hydroxyl Amine Hydrochloride (mL)	Volume of Sodium Acetate Buffer (mL)	L / M Ratio	Absorbance (O. D.)
1.	Blank	2	10	4		
2.	2	2	10	4		
3.	2	3	10	4		
4.	2	4	10	4		
5.	2	5	10	4		
6.	2	6	10	4		
7.	2	7	10	4		
8.	2	8	10	4		
9.	2	9	10	4		
10.	2	10	10	4		

EXPT. NO. :

DATE :

UV – SPEPCTRA OF NUCLEIC ACIDS

A. ABSORPTION MAXIMUM AND ABSORPTION SPECTRUM OF DNA

AIM

To determine the Absorption spectrum of DNA and to find out the wavelength of Maximum absorption (λ_{max}).

PRINCIPLE

The light absorbing molecules absorb light maximally at a particular wavelength. This wavelength is called λ_{max} of that compound. Since 'λ_{max}' is characteristic of that compound. Compounds are usually estimated at same λ_{max}. Therefore for estimation of any compound it 'λ_{max}' should be found out.

MATERIALS REQUIRED

(i) UV – Visible spectrophotometer

(ii) DNA sample (0.001 %): 1mg of DNA in 100mL of 0.9% saline

(iii) 0.9% saline

(iv) Cuvette

(v) Test tubes

(vi) Distilled water

PROCEDURE

(i) Prepare the solution of DNA sample at a concentration of 1mg in 100mL of 0.9% saline buffer. Adjust the pH to 8.

(ii) Switch 'ON' the spectrophotometer and allow it to warm for about 10 minutes. select the wavelength 200nm.

(iii) Set the instrument at zero absorbance with buffer solution.

(iv) Replace the cuvette containing the blank by the one with DNA solution and record the absorbance.

(v) Change the wavelength to 210nm and repeat the above step.

(vi) Record the reading for other wavelengths up to 320nm.

(vii) Plot the absorbance against wavelength on a graph and find the wavelength of maximum absorption for DNA.

RESULT

The optical densities at various wavelengths were studied using a spectrophotometer and the λ_{max} for DNA is found to be ----------nm.

Tabular Column

Absorption Spectrum of DNA

S. No	Wavelength (nm)	Absorbance (O. D.)
1.	200	
2.	210	
3.	220	
4.	230	
5.	240	
6.	250	
7.	260	
8.	270	
9.	280	
10.	290	
11.	300	
12.	310	
13.	320	

EXPT. NO. :

DATE :

UV – SPEPCTRA OF NUCLEIC ACIDS

B. ABSORPTION MAXIMUM AND ABSORPTION SPECTRUM OF RNA

AIM

To determine the Absorption spectrum of RNA and to find out the wavelength of Maximum absorption (λ_{max})

PRINCIPLE

The light absorbing molecules absorb light maximally at a particular wavelength. This wavelength is called λ_{max} of that compound. Since 'λ_{max}' is characteristic of that compound. Compounds are usually estimated at same λ_{max}. Therefore, for the estimation of any compound it 'λ_{max}' should be found out.

MATERIALS REQUIRED

(i) UV – Visible spectrophotometer
(ii) RNA Sample (0.001 %): 1mg of RNA in 100mL of 0.9% saline
(iii) 0.9% saline
(iv) Cuvette
(v) Test tubes
(vi) Distilled water

PROCEDURE:

(i) Prepare the solution of RNA sample at a concentration of 1mg in 100mL of 0.9% saline buffer. Adjust the pH to 8.
(ii) Switch 'ON' the spectrophotometer and allow it to warm for about 10 minutes. Select the wavelength 200nm.

(iii) Set the instrument at zero absorbance with buffer solution.

(iv) Replace the cuvette containing the blank by the one with RNA solution and record the absorbance.

(v) Change the wavelength to 210nm and repeat the above step.

(vi) Record the reading for other wavelengths up to 320nm.

(vii) Plot the absorbance against wavelength on a graph and find the wavelength of maximum absorption for RNA.

RESULT

The optical densities at various wavelengths were studied using a spectrophotometer and the λ_{max} for RNA is found to be ----------nm.

Tabular Column

Absorption Spectrum of RNA

S. No	Wavelength (nm)	Absorbance (O. D..)
1.	200	
2.	210	
3.	220	
4.	230	
5.	240	
6.	250	
7.	260	
8.	270	
9.	280	
10.	290	
11.	300	
12.	310	
13.	320	

EXPT. NO. :

DATE :

ESTIMATION OF ALUMINIUM ION IN ALUM – ALIZARIN COMPLEX

AIM

To estimate the amount of Aluminium ion in Alum – Alizarin complex by using Absorption spectroscopy.

PRINCIPLE

Alizarin is an Anthraquininoid compound that reacts with aluminium ion to form a deeply red colour complex and the absorbance is measured with a spectrophotometer. The spectrum is plotted to determine that absorption maximum of Alum – alizarin complex.

MATERIALS REQUIRED

(i) Alizarin : Dissolve 7.3mg in 1L of 0.1N NaOH

(ii) 0.01M Alum

(iii) Sodium Hydroxide (0.1N): Dissolve 4g in 1L of distilled water.

(iv) Spectrophotometer'

(v) Pipette

(vi) Cuvette

(vii) Test tubes

(viii) Distilled water

PROCEDURE

(i) Into a series of test tubes 0.01M Alum solution is taken

(ii) To each of these 1mL of alizarin is added and the solution is made up to 10mL using 0.01M sodium hydroxide solution.

(iii) The sodium hydroxide solution is taken as blank.

(iv) The given unknown solution is made up to 10mL by suing 0.01M NaOH solution.

(v) The absorption spectrum for Alum – alizarin complex is obtained by measuring the absorbance form about 400nm to 700nm and the graph is plotted between wavelength and absorbance.

(vi) By suing maximum wavelength the absorbance for Alum – alizarin complex is measured and the concentration of unknown alum complex is determined from calibration graph.

RESULT

The absorption spectrum of Alum complex is found to be -------nm.

The concentration of Al^{3+} ion in the given unknown alum complex is found to be --

---------g.

Tabular Column

S. No.	Volume of Alum (mL)	Volume of Alizarin (mL)	Volume of NaOH (mL)	Concentration of Al^{3+} ions (g)	Absorbance (O. D.)
1.	Blank	-	10	-	
2.	1	1	8	0.0003	
3.	2	1	7	0.0006	
4.	3	1	6	0.00039	
5.	4	1	5	0.0012	
6.	5	1	4	0.0015	
7.	Unknown	1	5	To be determined	

CALCULATION

630.39g of Alum contains 26.98g of Aluminium ions

0.63g of Alum contains $= \dfrac{0.63 \times 26.98}{630.39}$

$= 0.0269$g of Al^{3+} ions

$= \sim 0.03$g of Al^{3+} ions

Therefore, 100mL of Alum contains 0.03g of Al^{3+} ions

To determine the concentration of unknown

From the Calibration graph,

xmL of unknown Alum complex contains --------g of Al^{3+} ion

Therefore, 100mL of unknown Alum complex contains ----------g

EXPT. NO. :

DATE :

DETERMINATION OF STOICHIOMETRY OF ALUM – ALIZARIN COMPLEX

AIM

To determine the Stoichiometry of Alum – alizarin complex by absorption spectroscopy.

PRINCIPLE

Anthraquininoid compounds like alizarin forms coloured complex with aluminium sulphate. When equimolar amounts of the two solutions are mixed in varying proportions, the maximum amount of complex is formed in equilibrium. When the proportions of the reactants correspond to the empirical formula of the complex, absorption is maximum.

MATERIALS REQUIRED

(i) Alizarin : Dissolve 7.3mg in 1L of 0.1N NaOH

(ii) 0.01M Alum

(iii) Sodium Hydroxide (0.1N): Dissolve 4g in 1L of distilled water.

(iv) Spectrophotometer'

(v) Pipette

(vi) Cuvette

(vii) Test tubes

(viii) Distilled water

PROCEDURE

(i) Firstly, equimolar solutions of Alum and Alizarin are prepared.

(ii) Into a series of test tubes, 2mL of Alum is taken.

(iii) The Alum solution is then treated with varying amounts of alizarin and the solution is then diluted to 10mL by using 0.01M sodium hydroxide.

(iv) Thus, the various Alum complexes are prepared and the absorbance is read at maximum of 570nm.

(v) A graph is plotted by taking L / M ratio of x – axis and absorbance on y – axis.

(vi) From the plot, the stoichiometry of the complex is determined. The mole ratio corresponding to the maximum absorbance value gives the stoichiometry of the complex.

RESULT

The stoichiometry of Alum complex is found to be --------

Tabular Column

S. No.	Volume of Alum (mL)	Volume of Alizarin (mL)	Volume of NaOH (mL)	L / M ratio	Absorbance (O. D.)
1.	-	-	10	-	
2.	2	1	7	0.5	
3.	2	2	6	1.0	
4.	2	3	5	1.5	
5.	2	4	4	2.0	
6.	2	5	3	2.5	
7.	2	6	2	3.0	
8.	2	7	1	3.5	
9.	2	8	-	4.0	

EXPT. NO. :

DATE :

DETERMINATION OF SULPHATE BY NEPHELOMETRY

AIM

To determine the amount of Sulphate present in the given unknown solution.

PRINCIPLE

The turbidity of the dilute barium sulphate suspension in difficulty to reproduce, it is therefore essential to adhere rigidity to the experimental procedure detailed below. The velocity of precipitation, as well as the concentration of the reactants, must be controlled by adding pure solid barium chloride of definite grams size. The rate of solution of barium chloride controls the velocity of the reaction. Sodium chloride and hydrochloric acid are added before the precipitation in order to inhibit the growth of micro crystals of barium sulphate. The optimum pH is maintained and minimizes the effect of the variable amounts of other electrolytes present in the sample upon the size of suspended barium sulphate particles.

A glycerol - ethanol solution helps to stabilize the turbidity. The reaction vessel is shaken gently in order to obtain the uniform particle size. Each vessel should be shaken at the same rate; and the same number of times. The unknown must be treated exactly like the standard solution. The interval between the time of precipitation and measurement must be kept constant.

REAGENTS REQUIRED

(i) **Standard Sulphate Solution**

Dissolve 0.018141g of Potassium Sulphate in 100mL distilled water

(ii) **Sodium Chloride - Hydrochloride Reagent**

Dissolve 60g sodium chloride in 200mL distilled water and 5mL pure concentrated hydrochloric acid.

(iii) **Barium Chloride**

0.08 g packets

(iv) **Glycerol - Ethanol Mixture**

Dissolve 1 volume of glycerol and 2 volumes of ethanol.

MATERIALS REQUIRED

(i) Nephelometer
(ii) Volumetric flask
(iii) Beaker
(iv) Pipette
(v) Conical flask
(vi) Measuring cylinder

PROCEDURE

(i) 2mL to 10mL of standard potassium sulphate solution is taken in a 100mL of volumetric flask separately.

(ii) 5mL of glycerol - ethanol mixture is added to the above solution followed by 2.5mL of NaCl - HCl solution.

(iii) The solution in each flask is diluted to 100mL by using distilled water.

(iv) Finally 0.08g of barium chloride is added to form a nice precipitate.

(v) Each solution is allowed to stand for 2 - 3 minutes to get clear precipitate.

(vi) The blank and unknown solution is prepared by repeating the steps (ii) to (iv).

(vii) The blank solution is placed in the Nephelometer and adjusted to zero reading of the galvanometer suspension.

(viii) The intensity of scattered radiation for the standard and the unknown solution is measured by using nephelometer.

(ix) The graph is plotted by taking concentration of sulphate on x - axis of scattered radiation on y - axis.

(x) From the calibration curve, the concentration of unknown is determined.

RESULT

The amount of sulphate present in the unknown sample is found to be-------

Tabular Column

S. No.	Particulars	B	S_1	S_2	S_3	S_4	S_5	U_1
(i)	Volume of standard Potassium Sulphate solution (mL)	0.0	2.0	4.0	6.0	8.0	10.0	x
(ii)	Concentration of Sulphate ion (mg)	-	0.2	0.4	0.6	0.8	1.0	-
(iii)	Volume of Glycerol -ethanol mixture (mL)	5.0	5.0	5.0	5.0	5.0	5.0	5.0
(iv)	Volume of NaCl - HCl solution (mL)	2.5	2.5	2.5	2.5	2.5	2.5	2.5
(v)	Weight of Barium chloride (g)	0.08	0.08	0.08	0.08	0.08	0.08	0.08
(vi)	Intensity of Scattered radiation (NTU)							
(vii)	Difference in the Intensity of scattered radiation (NTU)							

EXPT. NO. :

DATE :

CONDUCTOMETRIC TITRATIONS

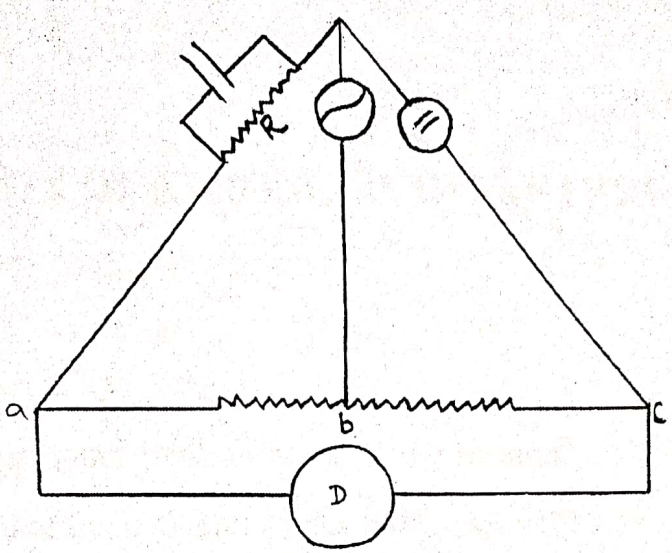
BASIS OF CONDUCTOMETRIC TITRATIONS

The addition of an electrolyte to a solution of another electrolyte under conditions producing no appreciable change in volume will affect the conductance of the solution according to whether or not ionic reactions occur. If no ionic reaction takes place, such as in the addition of one simple salt to another (eg : KCl, $NaNO_3$) the conductance will simply rise. If ionic reaction occurs, the conductance may either increase or decrease owing to the replacement of hydrogen ion of high conductivity by another cation of low conductivity. This is the principle underlying conductometric titrations.

INSTRUMENTATION

The underlying principle of use of an alternating current is that as absorbance result reversal of the direction of the current about absorbance thousand times per second, the polarization produced by each pulse of the current is completely neutralized by the next, provided the alternations or symmetry. There is also exact compensation of any concentration changes, which may occur.

The electrolyte was placed in a cell and its resistance was measured by absorbance Wheat stone bridge arrangement as shown in figure.



The cell "A" is in the one arm and resistance box "R" constitutes the other arm, the source of alternating current is represented as "S" and "ad" is calibrated slide wire of uniform resistance and "D" is current detectors.

The variable resistance and the two arms "ab" and "bd" of the wheat stone bridge are adjusted until "D" can detect no current. Then the resistance is related by absorbance simple proportion.

$$\mathbf{A / r = bd / ab}$$

EXPT. NO. :

DATE :

TITRATION OF STRONG ACID Vs STRONG BASE

AIM

To determine the neutralization point of the titration of strong acid against strong base and also to determine the concentration of the unknown acid.

PRINCIPLE

When a strong base like NaOH is added to a strong acid like HCl, the reaction occurs as follows.

$$H^+Cl^- + Na^+OH^- \longrightarrow Na^+ + Cl^- + H_2O$$

Conductance is directly proportional to mobility and the number of ions. Hydrogen ions has higher mobility and as NaOH is added H^+ ions are replaced by Na^+ which has lower conductance. Thus, there is a decrease in conductance as we continue adding NaOH. After the end point, the addition of NaOH increases the conductance, as the OH^- ions are no longer used in chemical reaction. Due to increase in the number of OH^- ions conductance of the system will have minimum value.

MATERIALS REQUIRED

(i) Hydrochloric acid (0.1N)
(ii) Sodium hydroxide (1M)
(iii) Distilled water
(iv) Beaker
(v) Conductivity meter
(vi) Pipette
(vii) Conical flask

PROCEDURE

About 50mL of unknown HCl is taken in a beaker and titrated against 1M NaOH taken in the burette. After each addition, conductance is measured and the end point is determined by plotting the volume of titrant on x - axis and conductance on y - axis.

RESULT

The concentration of the given unknown Hydrochloric acid is found to be -------

Tabular Column

S. No	Volume of NaOH added (mL)	Conductance measured (mS)
(i)	0.0	
(ii)	0.5	
(iii)	1.0	
(iv)	1.5	
(v)	2.0	
(vi)	2.5	
(vii)	3.0	
(viii)	3.5	
(ix)	4.0	
(x)	4.5	
(xi)	5.0	
(xii)	5.5	
(xiii)	6.0	
(xiv)	6.5	
(xv)	7.0	
(xvi)	7.5	
(xvii)	8.0	
(xviii)	8.5	
(xix)	9..0	
(xx)	9.5	
(xxi)	10.0	
(xxii)	10.5	
(xxiii)	11.0	
(xxiv)	11.5	
(xxv)	12.0	

CALCULATION

Volume of NaOH (V_1) = mL

Strength of NaOH (N_1) = N

Volume of HCl (V_2) = mL

Strength of HCl (N_2) = ?

$$\text{Therefore } (N_2) = \frac{V_1 N_1}{V_2}$$

Strength of HCl = ---------- N

PROCEDURE

About 50mL of unknown acetic acid is taken in a beaker and titrated against 1M NaOH taken in the burette. After each addition, conductance is measured and the end point is determined by plotting the volume of titrant on x - axis and Conductance on y - axis.

RESULT

The concentration of the given unknown acetic acid is found to be --------

Tabular Column

S. No.	Volume of NaOH Added (mL)	Conductance Measured (mS)
(i)	0.0	
(ii)	0.5	
(iii)	1.0	
(iv)	1.5	
(v)	2.0	
(vi)	2.5	
(vii)	3.0	
(viii)	3.5	
(ix)	4.0	
(x)	4.5	
(xi)	5.0	
(xii)	5.5	
(xiii)	6.0	
(xiv)	6.5	
(xv)	7.0	
(xvi)	7.5	
(xvii)	8.0	
(xviii)	8.5	
(xix)	9..0	
(xx)	9.5	
(xxi)	10.0	
(xxii)	10.5	
(xxiii)	11.0	
(xxiv)	11.5	
(xxv)	12.0	

CALCULATION

Volume of NaOH (V_1) = mL

Strength of NaOH (N_1) = N

Volume of CH_3COOH (V_2) = mL

Strength of CH_3COOH (N_2) = ?

Therefore (N_2) = $\frac{V_1 N_1}{V_2}$

Strength of CH_3COOH = ----------N

EXPT. NO. :
DATE :

ESTIMATION OF DISSOLVED OXYGEN IN WATER SAMPLE BY WINKLER'S METHOD

AIM

To determine amount of dissolved oxygen in the given water sample by Winkler's method.

PRINCIPLE

The dissolved oxygen content in the water sample should be 8mg/L at 25^0C, But this amount may decrease due to the aerobic oxidation of organic impurities .Thus dissolved oxygen content in water sample gives an idea about the level of organic impurities. The dissolved oxygen concentration is determined by Winkler's method. When $MnSO_4$ solution is added in the presence of an alkaline medium to the water sample the following reaction occurs

$$Mn^{2+} + 2OH^- \rightarrow Mn(OH)_2 \quad \textbf{(White precipitate)}$$

$$2Mn(OH)_2(s) + O_2(aq) \rightarrow 2\,MnO(OH)_2(s) \quad \textbf{(Yellow precipitate)}$$

When concentrated sulphuric acid is added the following reaction occurs

$$MnO(OH)_2 + 2I^- + 4H^+ \rightarrow I_2 + Mn^{2+} + 3H_2O$$

When the solution is titrated with sodium thiosulphate the following reaction occurs

$$2S_2O_3^{2-} + I_2 \rightarrow S_4O_6^{2-} + 2I^-$$

One equivalent weight of I_2 = One equivalent weight of O_2 =

One molecular weight of $Na_2S_2O_3$

1 mole of O_2 → 2 moles of $MnO(OH)_2$ → 2 mole of I_2 → 4 mole of $S_2O_3^{2-}$

REAGENTS REQUIRED

(i) Manganous Sulphate Solution

Dissolve 480g of $MnSO_4 . 4H_2O$ or 400g of $MnSO_4 .2H_2O$ or 360g of $MnSO_4.H_2O$ in 100mL of distilled water

(ii) Alkaline – Iodide - Sodium - Azide solution

(a) Dissolve 700g of KOH and 150g of KI in 600mL to 700 mL distilled water. After the solution is cooled, dilute to 1Litre.

(b) Dissolve 10g of sodium azide in 40mL of distilled water

(c) Add the sodium azide solution with constant stirring to the cooled alkaline iodide

(iii) Concentrated Sulphuric Acid

(iv) Starch Indicator

Dissolve 2g of Starch powder in 1000mL distilled water

(v) Sodium Thiosulphate Solution (0.025N)

Dissolve 6.25g of Sodium thiosulphate pentahydrate, 1.5mL of 6N Sodium hydroxide and diluted to 1Litre.

MATERIALS REQUIRED

(i) Burette
(ii) Burette stand
(iii) 300mL of glass stoppered BOD bottle
(iv) 500mL of wide mouthed bottle
(v) Pipette
(vi) 250mL Erlen Meyer flask

PROCEDURE

Estimation of Dissolved Oxygen in the Given Water Sample

(i) The sample to be tested is collected in a 300mL BOD bottle taking special care to avoid adding air to the liquid being collected. The bottle is filled completely and stoppered

(ii) The bottle stopper is removed and 1mL of the Manganous sulphate solution is added to the surface of the liquid.

(iii) 1mL of alkaline - potassium iodide - sodium azide solution is added at the surface of the liquid.

(iv) The stopper is replaced carefully to avoid trapping air bubbles and shaked well by inverting the bottle several times. The shaking is repeated after the floc is settled halfway and the floc is again allowed to settle a second time.

(v) 1mL of concentrated sulphuric acid is added by allowing the acid to run down the neck of the bottle above the surface of the liquid.

(vi) The bottle is stoppered and rinsed to remove any acid and shaked well until the precipitate is dissolved.

(vii) A volume of treated sample which corresponds to 200mL of the original sample is titrated. This corrects for the sample during the addition of reagents.

This volume is calculated using the formula

mL of sample to titrate **= 200*[300 / 300 - 2]**

= 201

(viii) 100mL of sample from the BOD bottle is poured into an Erlenmeyer flask.

(ix) If the solution is reddish brown colour a drop of starch indicator is added and it is titrated with sodium thiosulphate solution until the blue colour disappears .The titration is repeated for the amount of titrant used and is recorded.

RESULT

The Dissolved Oxygen content present in the given sample is found to be ---------

Titration

Estimation of Dissolved Oxygen in the Water sample

Burette Solution : Standard sodium thiosulphate solution

Pipette Solution : 100mL given water sample + 1mL alkaline iodide – Sodium Azide solution + 1mL Manganous sulphate solution + 1mL of concentrated sulphuric acid

Indicator : Starch

Endpoint : Disappearance of blue colour

Tabular Column

S. No.	Volume of Water Sample (mL)	Burette Reading		Volume of Sodium Thiosulphate Consumed (mL)	Concordant Value (mL)
		Initial (mL)	Final (mL)		
(i)	100	0	x	x	x
(ii)	100	0	x	x	
(iii)	100	0	x	x	

CALCULATION

Volume of Standard sodium thiosulphate (V_1) = xmL

Strength of Standard sodium thiosulphate (N_1) = 0.025N

Volume of Water sample (V_2) = 100mL

Normality of Water sample (N_2) = ?

$$\text{Therefore } (N_2) = \frac{V_1 N_1}{V_2}$$

$$= \frac{\text{x mL x } 0.025}{100}$$

$$= \text{----------N}$$

Normality of Water sample = ----------N

The Concentration of dissolved oxygen in Water sample = Normality of water sample x Equivalent weight of oxygen x 1000

= ----------mg / L

Therefore, the Concentration of dissolved oxygen in Water sample = --------- mg / L

EXPT. NO. :

DATE :

DETERMINATION OF IRON CONTENT IN FRUIT JUICES

AIM

To determine the iron content in the given fruit juice

PRINCIPLE

Iron present in biological sample is released by acid treatment, which undergoes reduction to ferrous state in the presence of thioglycolic acid. This iron complex with bathophenanthroline (1, 10 - Phenanthroline) reagent to yield a pink colour, whose intensity is measured at 535 nm.

REAGENTS REQUIRED

(i) Protein Precipitant Reagent

Dissolve 100g of trichloro acetic acid (TCA) in 250 – 300mL of deionized or double distilled water. Separately mix 30mL of thioglycolic acid and 2mL of conc. HCl, add it to TCA solution and make up the volume to 1L in a volumetric flask with deionized or double distilled water.

(ii) Chromogen Reagent

Weigh 25mg Phenanthroline, dissolve and make up the volume to 100mL with 2M sodium acetate solution in a volumetric flask.

(iii) Sample

Apple juice and grape juice (use any commercially available product, which may be procured from market).

(iv) **Standard Iron Solution** : Stock solution (100µg of iron / mL):

Dissolve 70.22mg of ammonium ferrous sulphate [$Fe(NH_4)_2(SO_4)_2 . 6H_2O$] in distilled water and make up the volume to 100mL. Dilute the stock solution 1 : 10 in distilled water to give a final concentration of 10µg of iron/mL.

PROCEDURE

(i) Take 1mL of samples in an iron - free test tube, add 1mL of protein precipitant reagent and vortex for 1 minute. Allow the sample to stand at room temperature for 5 minutes and centrifuge at 2500 x g for 15 minutes.

(ii) Run a set of iron standards separately in the range of 1 – 10µg mL^{-1} and a reagent blank, along with sample. Add 1mL of protein precipitant reagent to all tubes.

(iii) Carefully collect and transfer the supernatant of the test sample into a separate test tube. Add 1mL of chromogen solution to test tubes containing blank, standards and test solutions. Mix and allow the test tubes to stand for 10 – 12 minutes at room temperature before recording the absorbance at 535 nm in a photometer. Construct a calibration curve and compute the concentration of iron in the fruit juices. Express the value per 100mL of the juices.

RESULT

(i) Amount of iron content present in the commercially available Apple juice-------

(ii) Amount of iron content present in the Fresh Apple juice--------

(iii) Amount of iron content present in the Fresh Grape juice----------

Tabular Column

S. No	Vol. of Standard Solution (mL)	Concentration of Iron (µg)	Vol. of Distilled Water (mL)	Precipitant Reagent (mL)	Chromogen Reagent (mL)	Absorbance at 535 nm
i.	Blank	--	2.0	1.0	1.0	
ii.	0.2	2.0	1.8	1.0	1.0	
iii.	0.4	4.0	1.6	1.0	1.0	
iv.	0.6	6.0	1.4	1.0	1.0	
v.	0.8	8.0	1.2	1.0	1.0	
vi.	1	10.0	1.0	1.0	1.0	
vii.	Sample – Commercial apple juice	To be determined	1.0	1.0	1.0	
viii.	Sample – Fresh apple juice	To be determined	1.0	1.0	1.0	
ix.	Sample – Fresh grape juice	To be determined	1.0	1.0	1.0	