Basic Principle:

It is TLC by partition method

Chemicals:

2% solution of amino acid, ninhydrin, developing solvent (mobile phase)

Preparation of Ninhydrin:

0.3 g ninhydrin + 97 ml ethanol + 3ml acetic acid

Developing Solvent:

Butanol, acetic acid, water

Observations and Calculations:

S. No	Distance covered by solvent	Solvent front	Rf Value
1	Glycine = 8 cm	10 cm	0.8
2	Lycine = 6cm	"	0.6
3	Mixture	"	
i.	Glycine = 8 cm	"	0.8
ii.	Lycine = 6 cm.	"	0.6

Result:

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The mixture of amino acids has been separated, visualized and their Rf values are 0.8 (Glycine) and 0.6 (Lycine)

Practical No. 1

Separation and Identification of Amino Acids by TLC

Theory:

Chromatography is a physical method of separation by which mixture of compounds are separated between the two phases. one of them is stationary phase and other is mobile phase which passes through the stationary phase and brings along separation.

The term chromatgraphy has been derived from two words (Greek) chroma = colour and graphine = writing.

There are various types of chromatography which are used for the separation, identification and quantification of various components in a mixture Some of them are following.

1. Thin Layer Chromatography (TLC)
2. Paper Chromatography
3. High Performace Liquid Chromatography (HPLC)
4. Ion Exchange Chromatography
5. Ion Pair Chromatography

6. Counter Current Chromatography
7. Soap Chromatography
8. Gel Chromatography (Size Exclusion)
9. Chiral Chromatography
10. Affinity Chromatography and many more.

Thin Layer Chromatography:

TLC is a planner form of chromatography that is largely used for qualitative analysis of broad ranges of compounds and may also be used for quantitative analysis.

In TLC stationary phase is a thin layer of finely powdered solid which is supported on glass, aluminium or plastic slaip. It is adhered with the help of specific binder such as gypsum, plaster of paris, H_2O and polyvinyl alcohol. The mobile phase comprises of a single liquid or a mixture of liquids. As mobile phase pases along the thin layer, separation of components takes place.

Procedure:

1. Select TLC plate of dimension 6x20cm
2. Draw a base line by led pencil at about 1.5 cm.
3. Place spot of sample and standard on the base line.
4. Allow it to dry.
5. Placed it in chromatographic tank containing mobile phase.
6. Allow it to run for 45 minutes. (about 3/4 path).
7. Remove TLC plate and mark solvent front by led pencil.
8. Spray ninhydrin on plate
9. Placed it in oven at 110°C for about 5-10 minutes.
10. Calculate R_f values from spots.

Basic Poinciple:

It is TLC by partition method.

Chemicals:

0.1 M $Cu(NO_3)_2$, 0.1 M $Ni(NO_3)_2$, 0.1 M $Fe(NO_3)_3$ and sample of them.

Eluting Solvent:

45 mg Acelone + 5 mL of 6 M HCl

Locating Reagent:

Ageous ammonia and 1% DMG in ethanol

Observations and Calculations:

S. No.	Metal ion	Distance covered	Solvent front	R_f value
1.	Cu^{+2} (Blue)	8 cm	15 cm	0.53
2.	Ni^{+2} (Red)	10.5 cm	"	0.7
3.	Fe^{+3} (Rust)	6.5 cm	"	0.43
4.	Cu^{+2} (Blue)	8 cm	"	0.53
5.	Ni (Red)	10.5 cm	"	0.7

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Results:

Given mixture of ions contain Cu^{+2}, Ni^{+2} and Fe^{+3} ions which were identified by their colous. Their R_f values are 0.53, 0.7 and 0.43 respectively.

Practical No. 2

Separation and Identification of Metal Ions using Paper / TLC.

Theory:

Chromatography is a physical method of separation by which mixture of compounds are separated between the two phases, one of them is the stationary phase and other is mobile phase which passes through or along the stationary phase and brings about separation.

The term chromatography has been derived for two greek words chroma = colour and graphine = writing or pattern.

Types of Chromatography:

There are many types of chromatography, some of which are followings.

1. Thin Layer Chromatography
2. Paper Chromatography
3. HPLC / UPLC

4. Ion exchange chromatography
5. Ion pair chromatography
6. Counter current chromatography
7. Soap chromatography
8. Chiral chromatography
9. Affinity chromatography

Paper Chromatography:

Paper is made up of cellulose fiber having hydroxyl group upon which water is bounded by hydrogen banding. which act as stationary phase and some organic solvent as mobile phase.

Procedure:

1. Select a TLC or paper plate.
2. Draw a base line by led pencil at about 1.5 cm.
3. Place spot of sample and all standard on the base line.
4. Allow it to dry.
5. Placed it in chromatographic tank containing mobile phase.

6. Allow it to run for 45 minutes.
7. Remove the plate and mark the solvent foont.
8. Spray ninhydrin (locating reagent) on the plate.
9. Placed it in oven at 110°C for about 5-10 minutes.
10. Mark the spots and calculate the R_f values.

Basic Poinciple:

It is Ultra Violet (UV) visible absoption spectrophotometry.

Chemicals Required:

0.1 M Co^{++} solution, 0.01% DPC solution and 0.2N H_2SO_4.

Obseovations and Calculations:

No.	Wavelength	Absoebance	No	Wavelength	Absoebara
1.	450 nm	0.4	9.	530 nm	1.71
2.	460 nm	0.56	10.	540 nm	1.83
3.	470 nm	0.78	11.	550 nm	1.80
4.	480 nm	0.89	12.	560 nm	1.76
5.	490 nm	1.09	13.	570 nm	1.68
6.	500 nm	1.22	14.	580 nm	1.57
7.	510 nm	1.43	15.	590 nm	1.49
8.	520 nm	1.62	16.	600 nm	1.31

Result:

The λ_{max} Value of Co^{++} DPC complex at 540 nm.

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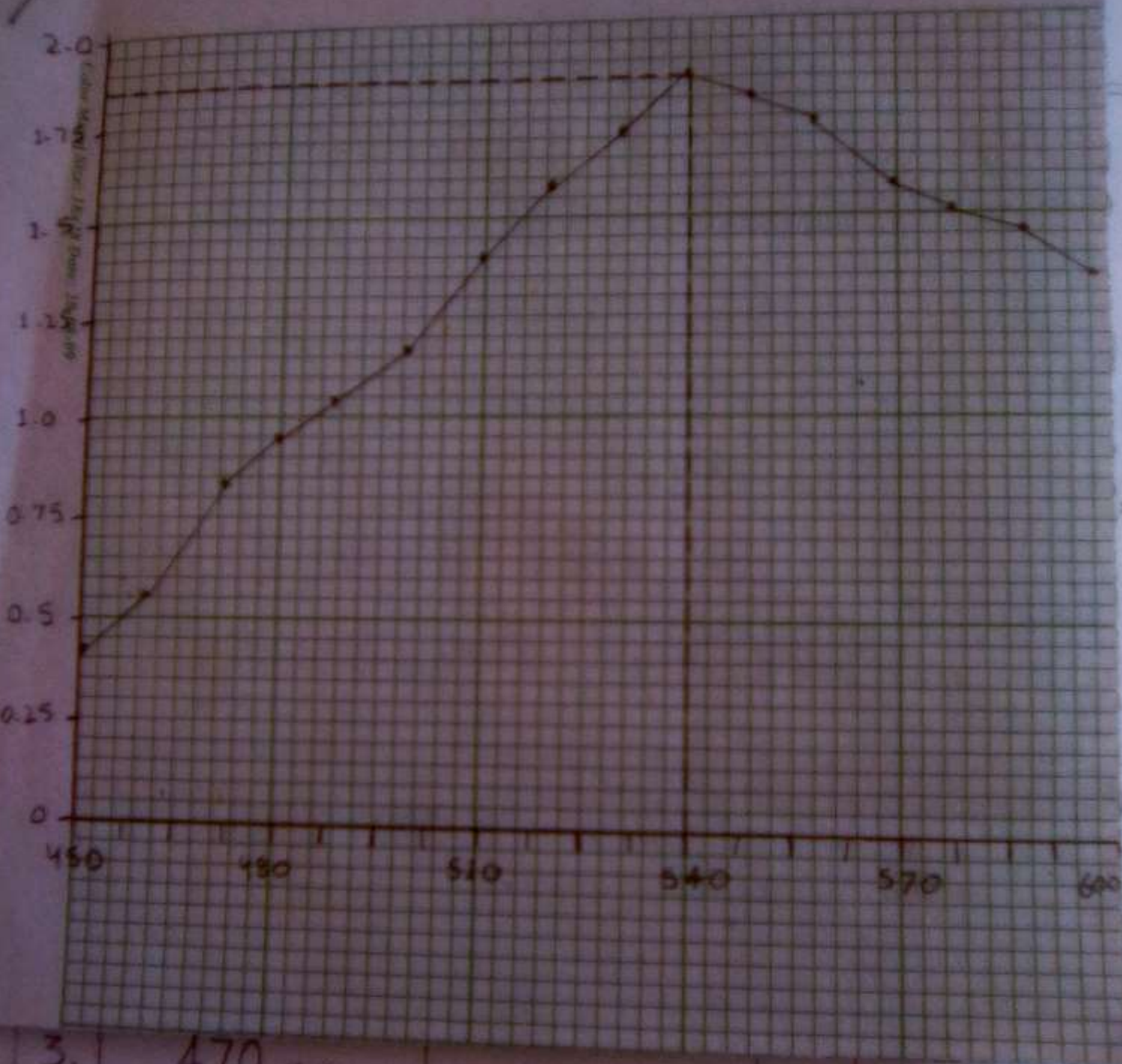


3.	470 nm	0.78	11.	550 nm
4.	480 nm	0.89	12.	560 nm
5.	490 nm	1.09	13.	570 nm
6.	500 nm	1.22	14.	580 nm
7.	510			

Practical No. 3

Determination of λ_{max} of Cr^{+6} complex with Diphenylcarbazine (DPC)

Theory:

Spectroscopy is the term that is used in science to describe the interaction of electromagnetic radiation with water. But Now a days, spectroscopy has been broadened to include the interaction of matter with other forms of energy such as cathode rays, neutrons, alpha, acoustic waves (sound waves)

Classification of Spectroscopic Methods:

Spectroscopic methods are classified into many types on the basis of followings.

1. On the basis of species involved:
   - Atomic Spectroscopy
   - Molecular Spectroscopy
2. On the basis of phenomenon Involved:
   - Emission
   - Absorption
   - Scattering
   - Flouresecence
3. One the basis of Radiation Involved
   - X Ray
   - Gamma Ray
   - UV Visible Spectroscopy
   - IR
   - Microwave
   - Radiowave Spectroscopy

Procedure:

1. Wash the apparatus.
2. Turn on spectrophotometer 10 minutes before work.
3. Calibrate it by using blank.
4. Take 5 ml Cr^{6+} solution in a beaker and mix it with 5 ml DPC solution.
5. Now place the sample in the spectrophotometer.
6. Takes values on spectrophotometer.

Basic Principle:

It is UV visible absorption spectroscopy

Chemicals:

0.1 M stock solution of Cr^{6+} ions, 1% DPC solution, Acetone, Acetic acid and distilled water.

Preparation of Stock Solution:

$$M = \frac{\text{mass in grams}}{\text{molar weight}} \times \frac{1}{\text{volume of solution in } dm^3}$$

$$0.1 = \frac{x}{294} \times \frac{1}{0.275} \qquad x = 0.275 \times 294 \times 0.1 = 8.085 \text{ gm}$$

8.085 gm $K_2Cr_2O_7$ + Water upto 275 ml = 0.1 M $K_2Cr_2O_7$

Preparation of Standard:

$$M_1V_1 = M_2V_2$$

$$0.1 \times V_1 = 0.005 \times 100$$

$$V_1 = \frac{0.005 \times 100}{0.1} = 5 \text{ ml}$$

5 ml stock solution + water upto 100 ml = 0.005 M

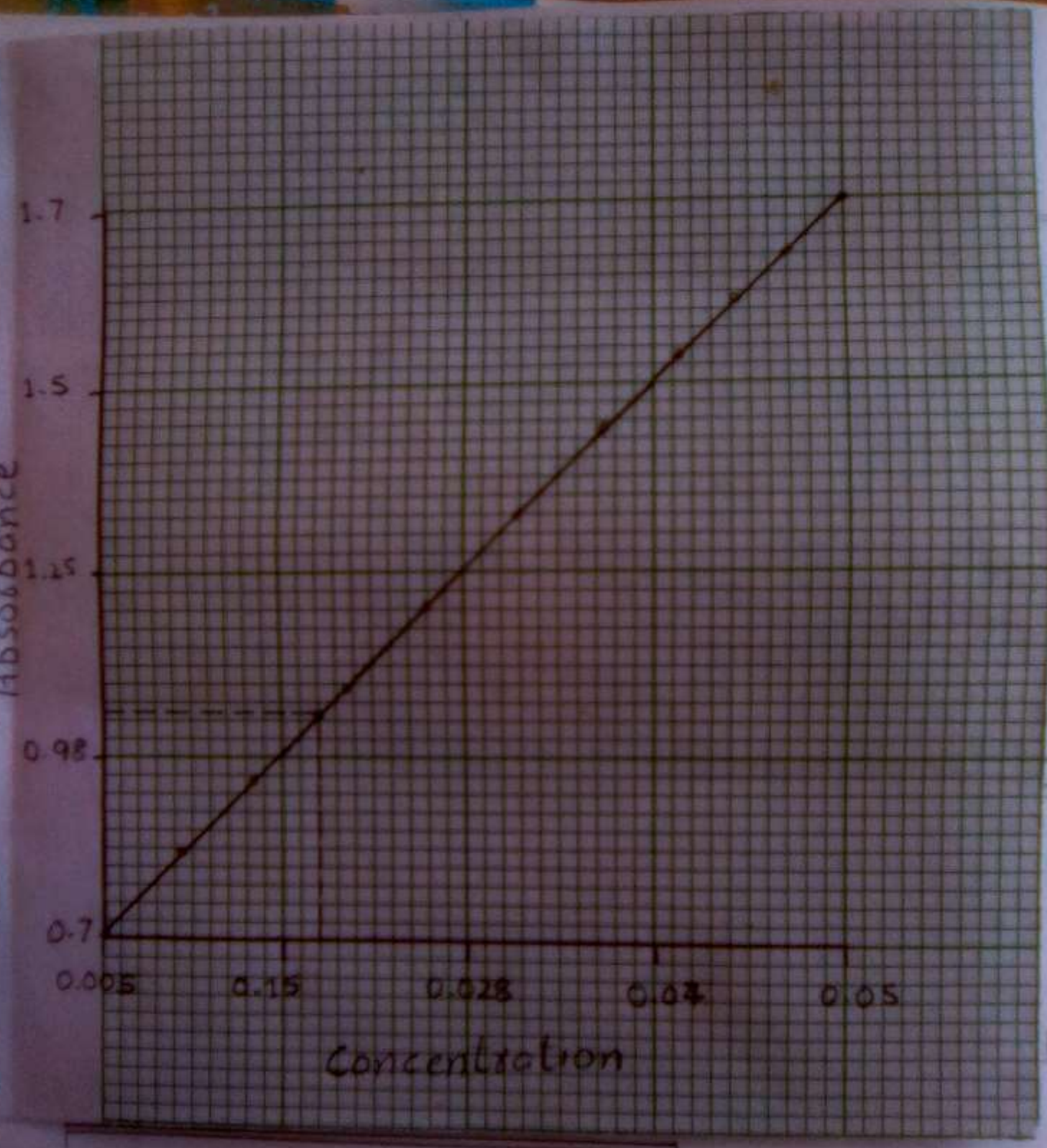
10 ml " " + " " " = 0.01 M

15 ml " " + " " " = 0.015 M

20 ml " " + " " " = 0.02 M

25 ml " " + " " " = 0.025 M

30 ml " " + " " " = 0.03 M



$$M_1 V_1 = M_2 V_2$$

$$0.1 \times V_1 = 0.005 \times 100$$

$$V_1 = \frac{0.005 \times 100}{0.1} = 5 \text{ ml}$$

Practical No. 4

Spectroscopic Determination of Cr^{+6} Ions by using diphenylcarbazide in Water Sample

Theory:

It is the term that is used in science to describe the interaction of electromagnetic radiation with water

Now a days, It also include other forms of energy such as the cathode rays, neutrons, alpha rays and acoustic waves

Electromagnetic Radiations:

Cosmic rays, Gamma rays, X-Rays, UV Visible Rays, IR Rays, Micro waves and Radio waves

Material Rays:

Cathode rays, Neutrons, Positrons, Acoustic waves.

Characteristics:

Energy, Wavelength, Velocity, Amplitude, Frequency, Wave Number, Absorption, Emission, Dispersion, Refraction, Diffraction, Polarization, Scattering, Transmission etc

35 ml stock solution + Water upto 100 ml = 0.035 M
40 ml " " + " " " " = 0.04 M
45 ml " " + " " " " = 0.045 M
50 ml " " + " " " " = 0.05 M

Preparation of DPC Solution:

1 gm DPC + 100 ml acetone + CH_3COOH

Observations:

S. No	Cr^{+6} Concentration	Absorbance
1.	0.005 M	0.78
2.	0.01 M	0.88
3.	0.015 M	0.98
4.	0.02 M	1.08
5.	0.025 M	1.18
6.	0.03 M	1.28
7.	0.035 M	1.39
8.	0.04 M	1.47
9.	0.045 M	1.58
10.	0.05 M	1.7
11.	Sample	1.06

Result:

The concentration of Cr^{+6} ions in the given solution (sample) is 0.02 M.

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Procedure:

1. Took 11 volumetric flasks of 100 ml
2. Pipette out 5 ml, 10 ml, 15 ml etc. from stock solution and dilute upto 100 ml.
3. Take 5 ml from each standard in a test tube and add 5 ml of DPC solution in it.
4. Allow the mixture to develop colour.
5. Set spectrophotometer at zero by using water.
6. Set spectrophotometer at λmax of 540 nm for measurements of standards and sample.
7. Plot a graph between concentration on X-axis and absorbance on Y-axis
8. Determine the concentration of sample from graph.

Basic Principle:

It is adsorption Chromatography.

Apparatus:

Burette, pipettes, stand, funnel.

Chemicals:

Mixture of $KMnO_4$ and $K_2Cr_2O_7$.

Eluting Solvent:

Distilled water

Result:

The given mixture was separated by the column chromatography and the components were $KMnO_4$ (Pink) and $K_2Cr_2O_7$ (Orange).

Practical No. 5

Separation of Mixture of $KMnO_4$ and $K_2Cr_2O_7$ by Column Chromatography.

Theory:

Chromatography is a physical method of separation by which mixture of the compounds are separated between two phases, one of them is stationary phase and other is mobile phase which pass through stationary phase and brings the separation

History:

The term chromatography has been derived from two greek words, chroma = colour and graphine = writing / pattern

Chromatographic Methods:

There are many types of chromatographic methods, some of which are followings.

1. TLC
2. Paper Chromatography

3. HPLC / UPLC
4. Ion Exchange Chromatography
5. Ion Pair "
6. Counter Current "
7. Soap Chromatography
8. Chiral "
9. Affinity Chromatography

Column Chromatography:

In column chromatography stationary phase is solid which is adsorbant, while mobile phase is a gas or liquid Based on difference of absorbance and desorbance of compounds.

Procedure:

1. Wash column with distilled water.
2. Fix the column in stand
3. Place about 40 gm of silica gel in it and add water till above silica gel.
4. Place cotton at the bottom.
5. Pour about 1-2 ml of mixture of the

solution ($K_2Cr_2O_7 + KMnO_4$).

6. Open the knob of pipette with the continously adding water till components emerge into the recieving beaker.

Basic Principle:

This practical aims to convert soluble calcium caesin into insoluble caisen by centrifugation.

$Ca\text{-}Caisen + CH_3COOH \longrightarrow Caisen\downarrow + (CH_3COO)_2Ca$

Materials:

Centrifuge, Falcon tube (50 ml), Centrifuge tube (15 ml), micropipetts, pipette filter, blue tips, 50% CH_3COOH, Fresh milk (5 ml).

Result:

The coagulated milk was separated using centrifugation. It was found that separation increases with increase in speed for the centrifugation.

Practical No. 6

Separation of Coagulated Milk from Fresh Milk and Acetic Acid by Centrifuge

Theory:

Centrifugation is a separation process which uses the action of the centrifugal force to separate the components in a mixture on basis of density.

Procedure:

1. 10 ml CH_3COOH in falcon tube + 5 ml milk
2. Invest falcon tube to mix
3. Centrifuge falcon tube at 1300 rpm, for 10 minutes.
4. As a result coaggulated milk pelleted to solid
5. Pour 1 ml supernatant in centrifuge tube and centrifuge it at 13.000 rpm for 15 minutes. As a result 2 layers are formed. upper layers contain caesin

microparticles and lower layer contain acetic acid.

Basic Principle:

It is UV visible absorptions Spectroscopy.

Chemicals:

- 0.1% O-phenanthroline in ageous solution.
- 10% hydroxylamine hydrochloride
- 10 gm CH_3COONa in 100 ml distilled water

Preparation of Stock Solution of Fe^{+2}:

1gm $Fe^{2+}SO_4 . (NH_4)_2 SO_4 . 6H_2O$ (Mohr's salt) + H_2O upto 1 liter

Preparation of Standards:

- Bottle No. 1:

1 ml stock solution + 1 ml hydroxylamine + 5ml of O-phenanthroline + 5 ml acetate buffer = 10ppm

- Bottle No. 2 = 20 ppm
- Bottle No. 3 = 30 ppm
- Bottle No. 4 = 40 ppm
- Bottle No. 5 = 50 ppm
- Bottle No. 6 = 60 ppm

Geometry of Complex:

$$Fe^{+2} + O\text{-phe} \xrightarrow{PH=4.5} [Fe(O\text{-phen})_3]^{2+}$$

Practical No. 7

Determination of Fe^{+2} ions in Ageous sample using the 1,10 -phenanthroline spectrophotometrically

Theory:

It is the term used in the science to describe the interaction of electromagnetic radiation with water. Now a days, it also include the other forms of energy such as the cathode rays, neutrons, alpha rays, and acoustic waves.

Classification of Spectroscopic Methods:

Spectroscopic methods are classified on the basis of.

1. On the basis of species
   - Atomic
   - Molecular
2. On the basis of phenomenon
   - Absorption
   - Emission

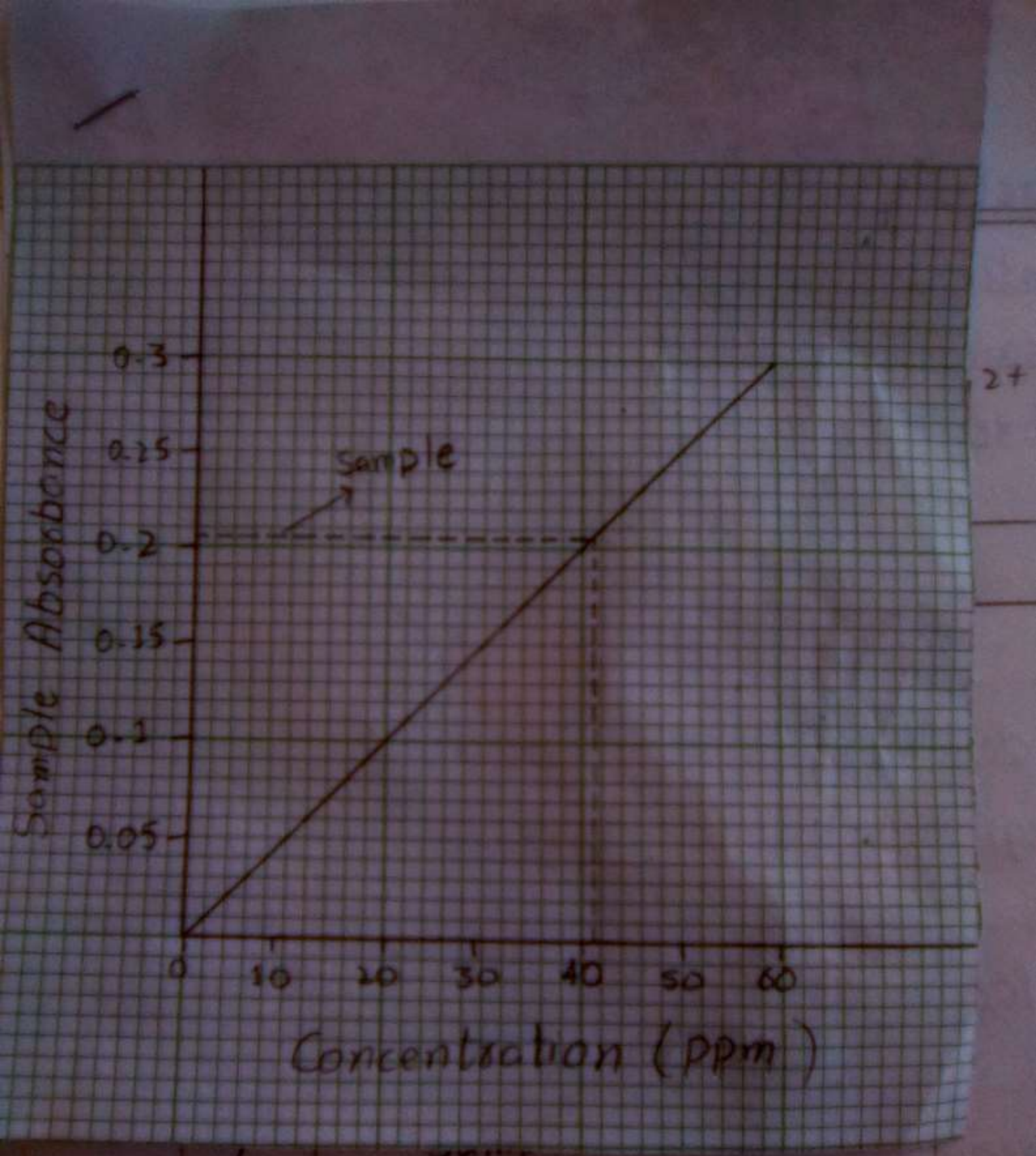
Observations:

λ_{max} of $[Fe(O\text{-}phen)_3]^{2+}$ = 508 nm

S. No	Concentration (ppm)	Absorbance
1	10	0.05
2	20	0.1
3	30	0.15
4	40	0.2
5	50	0.25
6	60	0.3
7	Sample	0.21

Result:

The concentration of Fe^{+2} ions in the given (aqeous) sample is 40 ppm.


0.3
0.25
0.2
0.15
0.1
0.05
Sample Absorbance
Sample
0
10
20
30
40
50
60
Concentration (ppm)

Result:

- Flourescence
- Phosphorescence
- Scattering

3. On the basis of radiations:
   - γ - Rays
   - X - Rays
   - UV Visible
   - IR
   - Microwaves
   - Radiowaves

Procedure:

1. Took 7 volumetric flask of 100 ml
2. Pipette out 10 ppm, 20 ppm and so on in volumetric flask
3. Take 5 ml from each standard in a test tube and add 5ml of O-phenanthroline solution in it.
4. Allowed the mixture to develop colour.
5. Set spectrophotometer at zero by using water

6. Set spectrophotometer at λ max of 508 nm for measurement of sample and standards.
7. Plot a graph between concentration on X-axis and absorbance on Y-axis
8. Determine the concentration of the sample from the graph.