V.V.VANNIAPERUMAL COLLEGE FOR WOMEN
VIRUDHUNAGAR

LAB MANUAL ON

GRAVIMETRIC ANALYSIS AND ORGANIC PREPATATION

(UNDER DBT STAR COLLEGE SCHEME)
Department of Biotechnology, Ministry of Science and Technology,
MHRD, New Delhi

M.Amutha
R.Nagasathya
Department of Chemistry

V.V.VANNIAPERUMAL COLLEGE FOR WOMEN
(Belonging to Virudhunagar Hindu Nadars)
An Autonomous Institution Affiliated to Madurai Kamaraj University
Re-accredited with 'A' Grade (3rd cycle) by NAAC
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DBT STAR COLLEGE SCHEME
Department of Biotechnology, Ministry of Science and Technology
Government of India, New Delhi

Chairman & Principal : Dr.(Tmty.) S.M. MEENA RANI, M.Sc.,M.Phil.,P.G.D.C.A.,Ph.D.,
Contact number: 9498088703
e-mail:smmeenarani@gmail.com
Coordinator & Member Secretary : Dr.(Tmty.) M.TAMILSELVI, M.Sc.,M.Phil.,Ph.D.,
Contact Number:9894883106
e- mail:tamilasc.selvi08@gmail.com

FOREWORD

The Lab Manual for **"GRAVIMETRIC ANALYSIS AND ORGANIC PREPARATION"** is designed to acquaint the student with essential skills and techniques in accordancewith the updated syllabus under DBT Star College Scheme sponsored by the Department of Biotechnology, Ministry of Science and Technology, MHRD, New Delhi. The skill of analysis and performing experiments will reinforce the theoretical knowledge of learnt concepts.

We thank the **Department of Biotechnology, The Ministry of Science and Technology, MHRD, New Delhi** for providing a good opportunity under Star College Scheme (No HRD11011/163/2020-HRD-DBT Dt. 24.08.2020). Under this scheme, we have purchased Analytical balance, Distillation Unit, Chemicals and Glassware. This provision enables the students for better understanding of basic concepts in Chemistry and to develop curiosity for further progress.

We hope this manual surely fulfil the student's need to enhance their attitude towards research and empower them as a better chemist.

S. M. Meena Rani

MEMBERSECRETARY/COORDINATOR **CHAIRMAN/PRINCIPAL**

LIST OF PRACTICALS

GRAVIMETRIC ANALYSIS

PRE LABORATORY WORK AND BASIC CONCEPTS OF GRAVIMETRIC ANALYSIS

Gravimetric analysis is a method of quantitative analysis, in which the analyte (the substance to be precipitated) is treated with the precipitant (reagent used to precipitate the analyte) in the proper ratio and produce the precipitate. Analytes such as Ba, Pb, Ni, Ca and Cu are taken as their soluble salts and weighed accurately as shown in table 1 then the stock solution is prepared in 1000ml standard measuring flask. From the stock solution known volumes of solution are distributed in given bottles. The given solution is treated with specific precipitants which precipitate desired metals (analytes) alone and leave other ions in the solution. The precipitate of the particular metal is separated as mentioned in the procedure and weighed accurately to find out the amount of desired metal present in the whole of the given solution. Obtaining 100% pure form of precipitate with chemical formula is difficult, so that all gravimetric analysis is subjected to some degree of error. To minimize the error, the precipitating processes such as digestion, washing and drying of the precipitate should be done carefully. Wash the crucible in chromic acid and keep it in the hot oven then cool in the desiccator and the weight of empty crucible should be noted.

LIST OF THE EXPERIMENTS

1	**Stock solution**		**Given solution**	**Experiment**			**Weight (g)**		**Calculation**	
	Salt	**Weight of the salt(g) in 1000mL**	**1.Stock solution is distributed with different volumes in given bottles.**	**Analyte**	**Precipitant (Reagent) in acidic/ basic medium**	**Formula of the precipitate**	**analyte (g)/mol**	**precipitate (g)mol**	**Wt of the ppt in given solu.**	**Weight of the analyte present in the given solution 100mL**
1	Barium chloride $BaCl_2$	208.23		Ba	**K_2CrO_4 / Acetic acid**	**$BaCrO_4$**	137.3	253.3	W3	137.3/253.3 x w_3x 10
2	Lead nitrate $Pb(NO_3)_2$	331.2		Pb	**K_2CrO_4 / NH_4OH**	**$PbCrO_4$**	207.2	323.2	W3	207.2/323.2 x w_3 x 10
3.	Nickel sulphate$NiSO_4$	154.69	**2)Given solution is made up to 100 ml Standard measuring flask**	Ni	**DMG/NH_4OH**	**Ni-DMG**	**58.69**	288.69	W3	58.69/288.68 x w_3 x 10
4	Calcium chloride$CaCl_2.2H_2O$	147.02		Ca	**$(NH_4)_2C_2O_4$**	**CaC_2O_4**	40.08	146.1	W3	40.08/146.1 x w_3 x 10
5	Copper sulphate$CuSO_4.5H_2O$	249.50		Cu	**NH_4SCN**	**Cu-SCN**	137.3	253.3	W3	137.3/253.3 x w_3 x 10

1. ESTIMATION OF BARIUM AS BARIUM CHROMATE

CALCULATION

Mass of sintered glass crucible with Barium Chromate (W_1) = -----g

Mass of sintered glass crucible (W_2) = --- g

Mass of bariumchromate (W_1) - (W_2) = (W_3)= ----- g(W_3)

253.3g of barium chromatecontain 137.3 g of Barium

Mass of barium in -----g (w_3) of $BaCrO_4$ = 137.3 x ----- (C) /253.3

Mass of the barium in the whole of the given solution = -------x 5 g

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1. ESTIMATION OF BARIUM AS BARIUM CHROMATE

AIM

To estimate gravimetrically the amount of barium present in the whole of the given $BaCl_2$ solution by precipitating it as $BaCrO_4$.

REQUIREMENTS

EQUIPMENTS

Hot oven, digital balance, suction pump

CHEMICALS

Barium chloride, potassium chromate, 1N acetic acid, ammonium chloride

GLASSWARES

G4 sintered crucible, desiccator, water bath, tripod stand, gravimetric beaker, police man rod, watch glass

PRINCIPLE

Barium is precipitated as barium chromate in acetic acid medium by the addition of K_2CrO_4. The $BaCrO_4$ precipitate is filtered in a sintered crucible, washed, dried at 120^{0}C and weighed as $BaCrO_4$.

$$BaCl_2 + K_2CrO \longrightarrow BaCrO_4 + 2KCl$$

PROCEDURE

- The given $BaCl_2$ solution is made up to 200 ml in a standard measuring flask.
- 20 ml of it is pipetted out into a beaker provided with a watch glass and glass rod, diluted to 100 ml added 10 ml of 1N acetic acid and 5 g of NH_4Cl and stirred well till the solid dissolved.
- Heated to boiling and added 10 ml of 10% K_2CrO_4 solution slowly with constant stirring and boiled gently for 5 minutes and allowed the precipitate to settle.
- The supernatant liquid must be coloured yellow. The $BaCrO_4$precipitate is filtered through a G-4 sintered crucible, washed with cold water, dried at 120^{0}C for one hour and is allowed to cool then weighed.
- The precipitate is reheated, cooled and weighed to get constant weight. A duplicate is also carried out.
- From the weight of $BaCrO_4$,the weight of Ba is calculated knowing that 253.3 g of $BaCrO_4$contains 137.3g of Ba.

RESULT

Mass of the Barium in the whole of the give solution = ----- g.

INFERENCE

As the specific precipitant potassium chromate precipitate barium ion alone. Hence yellow colourbarium chromate is obtained.

NO. OF BENEFICIARIES: 43

OUTCOME:

Students are able to

- Know the basic gravimetric precipitation process
- Determine the weight of barium as barium chromate by gravimetric method.

2. ESTIMATION OF LEAD AS LEAD CHROMATE

CALCULATION

Mass of sintered glass crucible with lead chromate (w_1) = -----g

Mass of sintered glass crucible (w_2) = --- g

Mass of lead chromate (w_1) - (w_2)(w_3) = ----- g (C)

323.2g of lead chromate contain 207.2 g of lead

Mass of lead in -----g of $PbCrO_4$ = 207.2 x ----- (C)/323.2

Mass of lead in the whole of the given solution = -------x 5 g

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2. ESTIMATION OF LEAD AS LEAD CHROMATE

AIM

To estimate gravimetrically the amount of Lead present in the whole of the given Lead nitrate solution by precipitating it as $PbCrO_4$.

REQUIREMENTS

EQUIPMENTS

Hot oven, digital balance, suction pump

CHEMICALS

Lead nitrate, potassium chromate, ammonium hydroxide

GLASSWARES

G4 sintered crucible, desiccator, water bath, tripod stand, gravimetric beaker, police man rod, watch glass

PRINCIPLE

Lead is precipitated as lead chromate in acetic acid medium by the addition of K_2CrO_4 solution. The precipitated $PbCrO_4$ is filtered in a sintered crucible, washed, dried at 120^{0}C and weighed as $PbCrO_4$.

$$Pb(NO)_3 + K_2CrO_4 \longrightarrow PbCrO_4 + 2KNO_3$$

PROCEDURE

- The given lead nitrate solution is made up to 200 ml in a standard measuringflask.
- 20 ml of the made up solution is pipetted out into a 250 ml beaker provided with a watch glass and glass rod.
- Then 10 ml NH_4OH is added drop by drop till the precipitate is formed. Dilute acetic acid is added to make the solution slightly acidic.
- It is then diluted to125 ml and heated to boiling. To the hot solution, 20 ml of 4% K_2CrO_4solution is added slowly with constant stirring and boiled gently for 5 -10 minutes to make the precipitate granular and allowed to settle.
- The supernatant liquid is tested for complete precipitation and must be colored in yellow. This indicates that potassium chromate is sufficient.
- The $PbCrO_4$ precipitate is filtered with previously weighed sintered glass crucible G-4 and washed with cold water, dried at 120^{0}C for one hour and weighed.
- It is reheated to constant weight and duplicateexperiment is also carried out.

- From the weight of $PbCrO_4$,the weight of Pb in the whole of the given solution is calculated. Knowing that 323.2.3 g of $PbCrO_4$ contains 207.2g of Pb.

RESULT

Mass of lead present in the whole of the give solution = ----- g.

INFERENCE

As the specific precipitant Potassium chromate precipitate lead ion alone, yellow colour lead chromate is obtained.

NO. OF BENEFICIARIES:43

OUTCOME:

Students can able to

- Determine the weight lead as lead chromate by gravimetric method

3. ESTIMATION OF NICKEL AS NICKEL DIMETHYL GLYOXIME

CALCULATION

Mass of sintered glass crucible with nickel dimethyl glyoxime complex (w_1) = -----g

Mass of sintered glass crucible (w_2) = --- g

Mass of nickel dimethyl glyoxime complex $(w_2) - (w_1) = w_3$ = ----- g

288.69 g ofnickel dimethyl glyoxime complex contain 58.69 g of Ni

Mass of Ni in -----gof nickel dimethyl glyoxime complex = 58.69x(C)/288.69

Mass of the Ni in the whole of the given solution = -------x 5 g

3. ESTIMATION OF NICKEL AS NICKEL DIMETHYL GLYOXIME

AIM

To estimate gravimetrically the amount of nickel present in the whole of the given nickel ammonium sulphate solution by precipitating it as nickel dimethyl glyoxime complex.

REQUIREMENTS

EQUIPMENTS

Hot oven, digital balance, suction pump

CHEMICALS

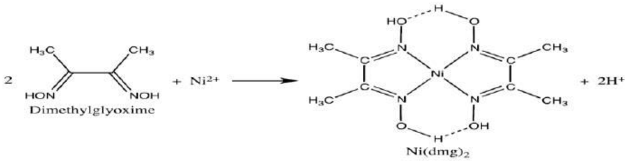
Nickel ammonium sulphate, dimethyl glyoxime, ammonium hydroxide

GLASSWARES

G4 sintered crucible, desiccator, water bath, tripod stand, gravimetric beaker, police man rod, watch glass

PRINCIPLE

Nickel in a solution is precipitating as dimethyl glyoxime complex. The alcoholic solution of dimethyl glyoxime and NH_4OH to a hot acidic solution of the nickel salt is added in slight excess. The precipitated complex is filtered, washed and dried at 110^0C.



PROCEDURE

- The given nickel sulphate salt solution is made up to 100 ml in a standard measuring flask.
- 20 ml of it is pipetted out in a clean beaker provided with watch glass and glass rod.
- Add about 5 ml of 1:1 HCl and diluted to 100 ml. The solution is then heated to about 80^0 C, added about 20 ml of 1% alcoholic solution of dimethyl glyoxime in drops with stirring.
- Dilute NH_4OH is added at once in drops, directly to the solution and not along the sides of the beaker till the solution is slightly alkaline (indicates the smell of ammonia).

- The solution containing the rosy red precipitate is heated on a steam bath for about an hour and it is allowed to stand for an hour preferably within a basin of cold water. Test for completion of precipitation by adding a few drops of dimethyl glyoxime into it.
- The supernatant liquid is filtered through a weighed sintered crucible G-3.
- The precipitate is washed with cold water till it is free from chloride. The crucible is placed in a dry 100 ml beaker and heated in an air oven at 110-120^{0}C for an hour.
- It is allowed to cool in a desiccator and weighed. Heating, cooling and weighing are repeated to get constant weight.
- A duplicate is also carried out. From the weight of dimethylglyoxime complex, the weight of nickel is calculated knowing that 288.69g of nickel complex contains 58.69 g of nickel.

RESULT

Mass of the nickel in whole of the given solution = ----- g.

INFERENCE

As the specific precipitant DMG precipitate Ni ion is alone, rosy red precipitate of Ni-DMG complex is obtained

NO. OF BENEFICIARIES: 43

OUTCOME:

Students can able to

- Determine the weight Ni as Ni-DMG by gravimetric method.
- Understand coordination complex formation reactions

4. ESTIMATION OF CALCIUM AS CALCIUM OXALATE

CALCULATION

Mass of sintered glass crucible with calcium oxalate monohydrate (w_1) = -----g

Mass of sintered glass crucible (w_2) = --- g

Mass of calcium oxalate monohydrate (w_1) - (w_2) = (w_3)= ----- g

146.1g of calcium oxalate monohydrate contain 40.08 g of Ca

Mass of Ca in -----g (w_3)of Calcium oxalate monohydrate = 40.08 x (w_3)/146.1

Mass of Cain the whole of the given solution = -------x 5 g

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4. ESTIMATION OF CALCIUM AS CALCIUM OXALATE

AIM

To estimate gravimetrically the amount of calcium present in the whole of the given calcium chloride solution by precipitating it as calcium oxalate.

REQUIREMENTS

EQUIPMENTS

Hot oven, Digital balance, Suction pump

CHEMICALS

Calcium chloride, ammonium oxalate, ammonium hydroxide

GLASSWARES

G4 sintered crucible, desiccator, water bath, tripod stand, gravimetric beaker, police man rod, watch glass

PRINCIPLE

Calcium in a solution is precipitating as calcium oxalate by treating the hot hydrochloric acid solution of Ca with ammonium oxalate solution and slowly neutralizing with NH_4OH till calcium oxalate is filtered, washed and dried at 105^0C to constant weight and weighed as calcium oxalate monohydrate.

$$CaCl_2 + (NH_4)_2C_2O_4 \longrightarrow CaC_2O_4 \downarrow + NH_4Cl$$

PROCEDURE

- The given calcium salt solution is made up to 100 ml in a standard measuring flask.
- 20 ml of it is pipetted out in a clean beaker provided with watch glass and glass rod, added a drop of methyl orange indicator and then NH_4OH in drops till the pink color just turns yellow.
- 1 ml of dilute HCl is added and diluted to 100 ml.
- The solution is then heated to about 80^0 C. To the hot solution about 20 ml of saturated ammonium acetate slowly with constant stirring.
- Then dilute NH_4OH is added at once in drops, directly to the solution and not along the sides of the beaker till the solution is distinctly alkaline.
- The solution is heated in a boiling water bath for about ½ an hour and allowed the precipitate to settle. It is tested for the completion of precipitation by adding a few drops of ammonium oxalate along the sides of the beaker.
- The clear supernatant liquid is decanted through a weighed sintered crucible G-4.

- The precipitate in a beaker is washed with ammonium oxalate till it is free from chloride.
- The precipitate is transferred into the crucible and then dried in an air oven at 100^0Cfor one hour.
- It is cooled in a desiccator and weighed. Heating, cooling and weighing are repeated to getconstant weight.
- A duplicate is also carried out. From the weight of $CaC_2O_4.H_2O$ the weight of calcium is calculated knowing that 146.1g of $CaC_2O_4.H_2O$ contains 40.08 g of calcium.

RESULT

Mass of ca in the whole of the given solution = ----- g.

INFERENCE

As the specific precipitant ammonium oxalate precipitate calcium ion alone. Hence white colour calcium oxalate is obtained.

NO. OF BENEFICIARIES:43

OUTCOME:

Students can able to

- Determine the weight of calcium as calcium oxalate by gravimetric method.

5. ESTIMATION OF COPPER AS COPPER THIOCYANATE

OBSERVATION

Mass of sintered glass crucible with copper thiocyanate (W_1) = -----g

Mass of sintered glass crucible (W_2) = --- g

Mass of copper $(W_1) - (W_2) = (W_3)$ = ----- g

CALCULATION

121.54g / mol of copper thiocyanate contain 63.54g of Copper

Mass of copper in -----g (C) of CuCNS = 63.54 x (C) / 121.54

Mass of the Copper in the whole of the given solution = -------x 5 g

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5. ESTIMATION OF COPPER AS COPPERTHIOCYANATE

AIM

To estimate gravimetrically the amount of copper present in the whole of the given copper sulphate solution by precipitating it as copper thiocyanate

REQUIREMENTS

EQUIPMENTS

Hot oven, digital balance, suction pump

CHEMICALS

Copper (II)sulphate, ammonium thiocyanate, sulphuric acid, sulphurous acid, ethanol

PRINCIPLE

Copper is precipitated as copper thiocyanate in acid medium by the addition of Ammonium thiocyanate. The Copper thiocyanate (CuSCN) precipitate is filtered in a sintered crucible, washed, dried at 120^0 and weighed as CuSCN.

$$2CuSO_4 + 2NH_4CNS + 2H_2O + SO_2 \longrightarrow 2CuCNS + (NH_4)_2SO_4 + H_2SO_4$$

PROCEDURE

- The given $CuSO_4$ solution is made up to 100 ml in a standard measuring flask.
- 20 ml of it is pipetted out into a beaker provided with a watch glass and glass rod, diluted to 100 ml
- Add 5 ml of 1 M H_2SO_4, followed by about 25 to 30 ml of H_2SO_3 which has been freshly prepared, by passing SO_2 through distilled water.
- This solution is diluted to about 150 ml and heated to boiling. Add about 10 ml of 10 % NH_4CNS in small quantities to the warm solution with constant stirring. The NH_4CNS should be in excess.
- Weigh empty G-4 sintered crucible and the supernatant liquid is filtered through the G-4 sintered crucible, CuCNS is washed with cold water and ethanol till the complete removal of excess thiocyanate,
- Dry the crucible at 110^0C -120^0C for one hour and is allowed to cool then weighed.
- The precipitate is reheated cooled and weighed to constant weight. A duplicate is also carried out.
- From the weight of CuCNS the weight of Cu is calculated, knowing that 121.54g/molof CUCNS contain 63.54 g of Cu.

RESULT

Mass of the Copper in the whole of the give solution = ----- g.

INFERENCE:

As the specific precipitant ammonium thiocyanate precipitate copper ion alone. Hence copper thiocyanate precipitate is obtained.

NO. OF BENEFICIARIES:43

OUTCOME:

Students are able to

- Know the basic gravimetric precipitation process
- Determine the weight of copper as copper thiocyanate by gravimetric method.

ORGANIC PREPARATION

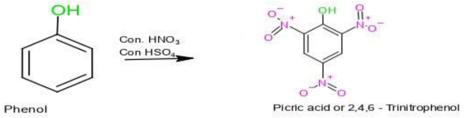
6. NITRATION REACTION PREPARATION OF 2,4,6 TRINITROPHENOL OR PICRIC ACID FROM PHENOL

AIM

To prepare 2, 4, 6 trinitrophenol from phenol as nitration reaction

PRINCIPLE

Phenol is sulphonated to get phenol sulphonic acid by concentrated H_2SO_4acid. The phenol sulphonic acid on nitration with concentrated HNO_3 acid gives picric acid or symmetric trinitrophenol.



REQUIREMENTS

EQUIPMENTS

Digital balance

CHEMICALS

1. Phenol- 5 ml
2. Con H_2SO_4 – 6ml
3. Con HNO_3 -18ml

GLASSWARES

1. Round bottom flask, air condenser
2. Buchner funnel, water bath, ice bath
3. Heating mantle

PROCEDURE

- Add 5ml of phenol in a conical flask and add 6 ml of concentrated H_2SO_4 in drops with shaking
- Heat the mixture in a water bath for about 30 minute still it dissolved
- The syrupy sulphonic acid thus formed is added in drops into 18 ml of concentrated Nitric acid in a round bottom flask, cooled in ice water with constant shaking.
- This mixture is heated in a water bath fitted with an air condenser for 90 minutes.
- Separate the yellow crystals of picric acid. Pour this solution into 100 ml of ice cold water. Filter at the suction pump and dried.

- Note the yield and 1g is recrystallized from hot water contain small amount of HCl

RESULT

Amount of picric acid obtained = ---g.

NO. OF BENEFICIARIES:43

OUTCOME

Students are able to

- Synthesis picric acid from phenol
- Do the recrystallization process.

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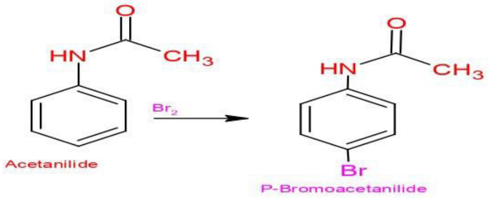
7. BROMINATIONP-BROMOACETANILIDE FROM ACETANILIDE

AIM

To prepare p-bromo acetanilide from acetanilide as brominating reaction

PRINCIPLE

Acetanilide dissolved in glacial acetic acid is brominated by bromine in glacial acetic acid. Then p- bromo- acetanilide is formed.



REQUIREMENTS

EQUIPMENTS

Digital balance

CHEMICALS

1. Acetanilide -1 g
2. Glacial acetic acid – 5mL
3. Bromine in glacial acetic acid -6.5 mL
4. Ethyl alcohol

GLASSWARES

Round bottom flask, air condenser, buchner funnel, water bath, ice bath, heating mantle

PROCEDURE

- Dissolve about 1 g of acetanilide in 5 ml of cold glacial acetic acid in a conical flask. Add 6.5 ml of Bromine in glacial acetic into conical flask slowly with constant shaking.
- Keep it to stand at room temperature for 15 minutes and pour the pale reddish range solution into a large excess of cold water when the p-bromoacetanilide rapidly crystallise out
- Stir the crystals thoroughly to eliminate acetic acid and unchanged bromine and then filtered using suction pump.

- Wash with cold water and filter at the suction pumps and dried. Note the yield and 1g is recrystallized from ethanol.

RESULT

Amount of p-bromo acetanilide obtained = ---g.

NO. OF BENEFICIARIES:43

OUTCOME

Students are able to

- Synthesis p-bromo acetanilide from acetanilide
- Do the recrystallization process.

8 . ESTER HYDROLYSIS-AROMATIC ACID FROM AN ESTER

AIM

To prepare benzoic acid from ethyl benzoate by ester hydrolysis reaction

PRINCIPLE

Esters are hydrolysed to sodium salt of the acid by boiling with NaOH. The acids are obtained from their sodium salts by acidification reaction with mineral acids.

REQUIREMENTS

EQUIPMENTS

Digital balance

CHEMICALS

1. Ethyl benzoate -3 ml
2. 10% Na OH – 20 ml
3. Concentrated hydrochloric acid

GLASSWARES

1. Round bottom flask, Liebig condenser
2. Buchner funnel, wire gauze
3. Heating mantle

PROCEDURE

- Take about 3 ml of ethyl benzoate in a round bottom flask. 20 ml of 10% NaOH is added to it.
- The flask in fitted with Liebig condenser and heated on wire gauze for about 45 minutes.
- When oily drops disappear, pour the mixture into a beaker and acidified with dilute hydrochloric acid.
- Wash white precipitates of benzoic acid with cold water and filters at the suction pumps and dried.
- Note the yield.

RECRYSTALLIZATION

If the solution is coloured, add a small amount of activated carbonand filter the hot solution into a conical flask. Heat the solution until the solute is completely dissolved and then allows it to cool to room temperature. Cool for 10-15 minutes on an ice bath to complete the recrystallization and collect the crystals by filtration. While the solution cools, that water is an appropriate solvent for recrystallization by performing three solubility tests. 1g of benzoic acid is recrystallized from hot water.

RESULT

Amount of benzoic acid obtained is = ---g.

NO. OF BENEFICIARIES: 43

OUTCOME

Students are able to

- Synthesis benzoic acid from ethyl benzoate
- Do the recrystallization process.

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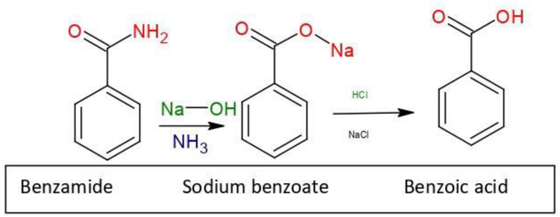
9. AMIDEHYDROLYSIS- AROMATIC ACID FROM AN AMIDE

AIM

To prepare benzoic acid from benzamide by amide hydrolysis reaction

PRINCIPLE

Amides are hydrolysed to sodium salt of the acid by boiling with NaOH. The acids are obtained from their sodium salts by acidification reaction with mineral acids.



REQUIREMENTS

EQUIPMENTS

Digital balance

CHEMICALS

1. Benzamide -3 g
2. 10% Na OH – 20 mL
3. Dilute hydrochloric acid

GLASSWARES

1. Round bottom flask, Liebig condenser
2. Buchner funnel, wire gauze
3. Heating mantle

PROCEDURE

- Take about 3 g of benzamide in a Round bottom flask. 20 ml of 10% NaOH is added to it.
- The flask is fitted with an air condenser and heated on wire gauze for about 45 minute.
- Ammonia is evolved and sodium salt of benzoic acid is formed in solution, pours the mixture into a beaker and acidified with concentrated hydrochloric acid.
- Wash white precipitates of benzoic acid with cold water and filters at the suction pumps and dried.
- Note the yield

RECRYSTALLIZATION

1g of benzoic acid is recrystallized from hot water

RESULT

Amount of benzoic acid obtained is = ---g.

NO. OF BENEFICIARIES:43

OUTCOME:

Students are able to

- Synthesis of benzoic acid from Benzamide
- Do the recrystallization process.

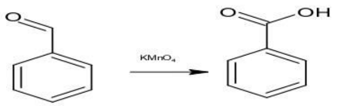
10. OXIDATION REACTION- PREPARATION OF BENZOIC ACID FROM BENZALDEHYDE

AIM

Preparation of benzoic acid from benzaldehyde

PRINCIPLE

Aldehydic group in benzaldehyde is oxidised to carboxylic group by alkaline potassium permanganate. Alkaline potassium permanganate is good oxidising agent. Thus benzaldehyde is oxidised into benzoic acid by alkaline permanganate



Benzaldehyde Benzoic acid

REQUIREMENTS

EQUIPMENTS

Digital balance

CHEMICALS

1. Benzaldehyde -6 ml
2. Anhydrous sodium carbonate 6 g/60 ml water
3. Potassium permanganate, Sodium bi sulphite, dilute H_2SO_4

APPARATUS

1. Round bottom flask, Reflex condenser
2. Buchner funnel, wire gauze
3. Heating mantle

PROCEDURE

- Take about 6ml of benzaldehyde in a round bottom flask. 6g of anhydrous sodium carbonate is added.
- The flask is fitted with reflex condenser and add Potassium permanganate solution on the top of the condenser in small quantity at a time slowly into round bottom flask and heated on wire gauze for about 45 minute.
- If there is no smell of benzaldehyde it may be concluded that the reaction is completed.

- Benzoic acid is formed in solution, pours the mixture into a beaker and adds 5g of sodium bi sulphite and acidified with dilute sulphuric acid with constant stirring until all the manganese dioxide dissolved and the solution is acidic.
- Wash white precipitate of benzoic acid with cold water and filters at the suction pumps and dried.
- Note the yield.

RECRYSTALLIZATION

1g of benzoic acid is recrystallized from hot water

RESULT

Amount of benzoic acid obtained is = ---g.

NO. OF BENEFICIARIES: 43

OUTCOME:

Students are able to

- Synthesis of benzoic acid from benzaldehyde
- Do the recrystallization process.

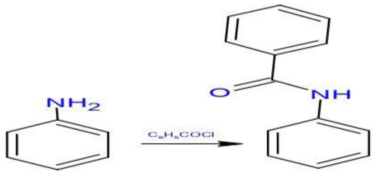
11. BENZOYLATION REACTION- PREPARATION OF BENZANILIDE FROM ANILINE

AIM

Preparation of benzanilide from aniline as Schotten –Baumann reaction

PRINCIPLE

The compounds containing an active hydrogen atom can be benzoylated in the presence of dilute NaOH . This method of benzoylation is known as Scotten Bowmann reaction.



Aniline Benzanilide

REQUIREMENTS

EQUIPMENTS L: Digital balance

CHEMICALS REQUIRED

1. Aniline	- 3 ml
2. 10% NaOH	- 15 ml
3. Benzoyl chloride	- 3 ml
4. Ethanol	- 5ml

APPARATUS REQUIRED

Conical flask, Buchner funnel

PROCEDURE

- Take about 3ml of aniline in a conical flask with rubber cork. Add 15 ml of 10% sodium hydroxide into it.
- Add 3 ml of benzoyl chloride into the conical flask at minimum intervals of time and shaking vigorously for 30 minutes. Release the rubber cork then and there to release the air.
- Solid benzanilide separates out.
- Wash white precipitates of benzanilide with cold water and filters at the suction pumps and dried.
- Note the yield.

RECRYSTALLIZATION

1g of benzanilide is recrystallized from ethanol.

RESULT

Amount of benzanilide obtained is = ---g.

NO. OF BENEFICIARIES:43

OUTCOME

Students are able to

- Synthesis benzanilide from aniline
- Do the recrystallization process.

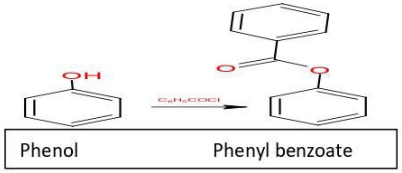
12. BENZOYLATION REACTION - PREPARATION OF PHENYL BENZOATE FROM PHENOL

AIM

Preparation of phenyl benzoate from phenol as Schotten –Baumann reaction

PRINCIPLE

The compounds containing an active hydrogen atom can be benzoylated in the presence of dilute NaOH . This method of benzoylation is known as Scotten Bowmann reaction.



REQUIREMENTS

EQUIPMENTS

Digital balance

CHEMICALS

1. Phenol	- 3 g
2. 10% NaOH	- 15 ml
3. Benzoyl chloride	- 3 ml
4. Ethanol	- 5ml

GLASSWARES

1. Conical flask
2. Buchner funnel

PROCEDURE

- Take about 3g of phenol in a conical flask with rubber cork. Add 15 ml of 10% sodium hydroxide into it.
- Add 3 ml of benzoyl chloride with small addition at a time and shaking vigorously for 30 minutes. Release the rubber cork then and there to release the air.
- Solid phenylbenzoateseparates out.
- Wash white precipitates of phenylbenzoate with cold water and filters at the suction pumps and dried.
- Note the yield.

RECRYSTALLIZATION

1g of phenyl benzoate is recrystallized from ethanol.

RESULT

Amount of phenylbenzoateobtained is = ---g.

NO. OF BENEFICIARIES:43

OUTCOME

Students are able to

- Synthesis phenyl benzoate from phenol
- Do the recrystallization process.

---

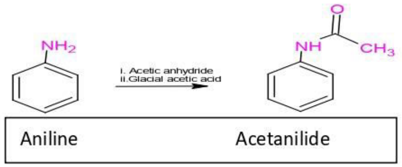
13. ACETYLATION REACTION - PREPARATION OF ACETANILIDE FROM ANILINE

AIM

Preparation of acetanilide from anilineas organic preparation

PRINCIPLE

Aniline reacts with acetic anhydride to form acetanilide by nucleophilc substitution reaction and the reaction is called acylation reaction. In this reaction aniline acts as a nucleophile and acyl (CH_3CO-) group from acetic anhydride act as an electrophile. Hence the hydrogen atom of NH_2 group is replaced by the acyl group. Zinc is used to prevent the oxidation of aniline during the chemical reaction.



REQUIREMENTS

EQUIPMENTS: Digital balance

CHEMICALS

1. Aniline	- 5 g
2. Glacial acetic acid	- 15 ml
3. Acetic anhydride	- 10 ml
4. Zn dust	- 15 ml
5. distilled water	- 100 ml
6. Ethanol	- 5ml

GLASSWARES

Conical flask, Buchner funnel, reflux condenser

PROCEDURE

- Wash all the apparatus with distilled water before starting the experiment.
- Prepare a mixture of 10 ml of glacial acetic acid and 10 ml acetic anhydride in a beaker.

- Place (5 gm) of aniline in a round bottom flask and carefully add equal amount of acetic anhydride and glacial acetic acid mixture (equal volumes) and add a zinc dust.
- Set up the reflux condenser with the round bottom flask.
- Heat the reaction mixture gently for about 15–20 minutes on water bath.
- Reaction mixture quickly crystallize.
- Pour the hot reaction mixture in a beaker containing ice cold water with constant stirring.
- Carefully stir the reaction mixture vigorously to hydrolyse excess of acetic anhydride.
- Crude product of acetanilide is precipitated, collect and filter of the colourless crystals at the suction pump, next time wash thoroughly with water. Dry the crude product of acetanilide

RECRYSTALLIZATION

1g of acetanilide is recrystallized from ethanol.

RESULT: Amount of acetanilide obtained is = ---g.

NO. OF BENEFICIARIES: 43

OUTCOME

Students are able to

- Synthesis acetanilide from aniline
- Do the recrystallization process.

14.CHROMATOGRAPHIC SEPARATION OF POTASSIUM PERMANGANATE AND POTASSIUM CHROMATE

AIM

To separatepotassium permanganate and potassium dichromate from their mixture using column chromatography.

PRINCIPLE

This technique is based on the principle of differential adsorption where different molecules in a mixture have different affinities with the adsorbent present in the stationary phase. The molecules having higher affinity remain adsorbed for a longer time decreasing their speed of movement through the column. However, the molecules with lower affinity move faster, thus allowing the molecules to be separated in different fractions.

Here, the stationary phase in the column chromatography also termed the adsorbent is a solid and the mobile phase is a liquid that allows the molecules to move through the column smoothly. The type of interaction between the stationary phase (adsorbent) and the solute is reversible in nature.

In this experiment, alumina is used as the adsorbent. Dilute nitric acid and dilute sulphuric acid are used as the mobile phases to elute potassium permanganate ($KMnO_4$)and potassium dichromate ($K_2Cr_2O_7$) respectively. On developing the column, $KMnO_4$ and $K_2Cr_2O_7$areeluted as pink and yellow coloured solutions, respectively.

REQUIREMENTS

EQUIPMENTS

1. Digital balance

CHEMICALS

1. Alumina
2. Nitric acid
3. Sulphuric acid
4. Potassium permanganate
5. Potassium dichromate
6. Cottonglass wool

GLASSWARES

1. Chromatography column or a 25 ml burette
2. Beakers 100 ml capacity
3. Conical flasks 100 ml capacity
4. Funnel.

PREPARATION OF REAGENTS

1. 0.5 M NITRIC ACID SOLUTION

0.5m HNO_3 solution is prepared by diluting astock solution of HNO_3, 32 ml of the commercial acid should be diluted to 1 litre to make a 0.5 m solution.

2. 1.0 M SULPHURIC ACID SOLUTION

1.0m H_2SO_4 solution is prepared by diluting a stock solution of H_2SO_4, 56 ml of commercial acid should be diluted to 1 litre to make a 1.0m solution.

3. SOLUTION CONTAINING A MIXTURE OF $KMN0_4$ & $K_2Cr_2O_7$:

14.7 g of $K_2Cr_2O_7$ and 7.9 g of $KMnO_4$ are dissolved in 1 litre of distilled water to make a solution which is 0.05 M with respect to both $K_2Cr_2O_7$ and $KMnO_4$.

PROCEDURE

1. PREPARATION OF COLUMN

A glass column or a burette of about 20cm long and 1-2cm in diameter is taken. Some cotton glass wool is placed in the bottom of the column. Nearly 10 ml of hot water is poured and air is removed from the wool. Some water is allowed to flow by opening the stop cock. About 20 gof alumina is taken in a beaker containing 100 ml of water and is boiled to remove the dissolved air. Now a small quantityof alumina slurry is transferred to the glass column with the help of a funnel and a glassrod. The column is tapped with a glass rod covered with 5 cm rubber tubing. The process of pouring slurry and tapping is continued till a column of about 15 cm length is formed. The excess water is drained off leaving a water layer of about 5 mm thick above the alumina surface. Now a small disc of filter paper (diameter equal to that of glass column) is cut and placed into the column at the top surface of alumina to ensure that the column packing is not disturbed when more liquid is added to the column.

Alternatively, a wad of cotton or glass wool can also be used. When preparing and operating the column, it is ensured that the liquid level is above thetop of the alumina bed. Failure to maintain the liquid level can lead to trapping of air-bubbles in the alumina which could seriously impair the efficiency of the column and affect the separations of the two coloured components.

2. To ensure that the column is free from all reducing impurities, 1 ml of laboratory $KMn0_4$ solution (about 0.02 M*)* is first placedon the column with the tap closed and washed thoroughly with 0.5 M HNO_3 until the washings are colourless. This would require to run off about 10 ml of coloured solution.

3. Pipetted out accurately 5 ml of the mixture of $KMnO_4$ and $K_2Cr_2O_7$ and is dripped into the column in the form of thin layer of solution at alumina surface, keeping the stopcock closed.

4. The mixture is allowed to run evenly into the adsorbent until the liquid level isjust above the top of alumina.

5. Then added 1 ml of 0.5M HNO_3 and again the column is allowed to run until the liquid meniscus is just above the top of the column.

6. The column is first developed with 0.5M HNO_3 to elute $KMnO_4$. Thefraction of effluent is collected which is pink in colour in a conical flask and is marked as number 1

7.Next the column is developed with 1M H_2SO_4 to elute $K_2Cr_2O_7$. The fraction of effluent containing dichromate (light yellow) is collected in a second conical flask.

OBSERVATION

The two components are separated in the form of two different coloured zones and are collected in different fractions.

RESULT

The mixture is separated into its components. Potassium permanganate is collected as a pink coloured solution where as potassium dichromate is collected as yellow-coloured solution.

NO. OF BENEFICIARIES:45

OUTCOME:

The students are able to

- Separate compound mixtures
- Explain the technique of column chromatography
- Remove impurities and isolate metabolites from biological fluids

15.CHROMATOGRAPHIC SEPARATION OF AMINO ACIDS

AIM

The aim of the experiment is to perform Ascending paper chromatography for the separation of amino acids present in the given sample.

PRINCIPLE

Chromatography is used to separate mixtures of substances into their individual components. All forms of chromatography work on the same principle. They all have basic requirements of *stationary phase* (a solid or a liquid supported on a solid) and a *mobile phase* (a liquid or a gas). The mobile phase flows through the stationary phase and carries the components of the mixture with it. Different components travel at different rates based on their affinities toward stationary phase and mobile phase.In paper chromatography, the stationary phase is a very uniform adsorbent paper. The mobile phase is a suitable liquid solvent or mixture of solvents.

The Retention (or) retardation factor (R_f) value is calculated for identifying the spots in qualitative analysis.

Retention factor is defined as the ratio of the distance travelled by the solute to the distance travelled by solvent.

$$\mathbf{R_f} = \frac{\text{distance travelled by the solute}}{\text{distance travelled by the solvent}}$$

The R_f value ranges from 0 to 1. The ideal value is 0.3 to 0.8. The R_f value is constant for every compound in a particular combination of stationary and mobile phase. When the R_f value of a sample and reference compound is same, the compound is identified by its standard. When the R_f value differs, the compound may be different from its reference standard.

REQUIREMENTS

APPARATUS

1. Glass beakers
2. Whatman filter paper
3. Petridish
4. Measuring cylinder
5. Developing chamber and capillary tubes etc.

CHEMICALS

1. n-butanol
2. Glacial acetic acid
3. Distilled water (4:1:5)
4. Amino acids (Tryptophan and Threonine)
5. Ninhydrin reagents.

SOLVENT SYSTEM AND ITS PREPARATION METHOD

n-butanol and water are taken in 4:5 ratios in a conical flask and is allowed to saturate for 24 hours. By using separating funnel n-butanol and water are separated. The saturated n-butanol and glacial acetic acid are taken in the ratio of 4:1 which can be used as a solvent system (or) mobile phase.

PROCEDURE

The chromatography paper is cut into rectangular strips and a line is marked on the paper with pencil at about 2 cm from the bottom. With the help of capillary tube, the samples are applied at different points on the starting line and marked as A, B and C. Now, the chromatography paper is placed in the developing chamber, which contains the mobile phase. While placing the paper, it is important that the solvent level should not reach the starting line or the sample spots and paper shouldn't touch the walls of the developing chamber. After sometime the solvent rises up the paper or the stationary phase by capillary action and dissolves the sample. The components of the sample move along with the solvent in upward direction.

It has to be checked whether the solvent has reached near the top level of chromatography paper. Then the paper is removed when it reaches the top and the level is marked with pencil. This level (or) height is called the "solvent front". By using UV light, ninhydrin or iodine vapours the different spots of varied colours are examined. Each spot represents a specific component of the sample.

INFERENCE

From the R_f value, unknown mixture is found to be -------- and -------- amino acids.

RESULT

The distance moved by tryptophan and threonine is …… cm and ….. cm respectively, and the solvent is ……cm

R_f value of tryptophan is ……

R_f value of threonine is ……..

R_f value of unknown mixture is ….. & …..

NO. OF BENEFICIARIES:45

OUTCOME: The students are able to

- Learn the techniques on separation of mixtures using chromatography techniques
- Identify and separate colored mixtures like pigments.
- Identify unknown organic and inorganic compounds from a mixture.

16. CHROMATOGRAPHIC SEPARATION OF LIPIDS

AIM

The aim of the experiment is to perform thin layer chromatography for the separation of lipids present in the given sample.

PRINCIPLE

Thin Layer Chromatography can be defined as a method of separation or identification of a mixture of components into individual components using finely divided adsorbent coated or spread over a chromatographic plate. The mobile phase solvent flows through because of capillary action (against gravitational force). The components move according to their affinities towards the adsorbent. The component with more affinity towards the stationary phase travels slower. The component with lesser affinity towards the stationary phase travels faster. Thus, the components are separated on a thin layer chromatographic plate based on the affinity of the components towards the stationary phase.

The Retention (or) retardation factor (R_f)value is calculated for identifying the spots in qualitative analysis.

Retention factor is defined as the ratio of the distance travelled by the solute to the distance travelled by solvent.

$$\mathbf{R_f} = \frac{\text{distance travelled by the solute}}{\text{distance travelled by the solvent.}}$$

The R_f value ranges from 0 to 1. The ideal value is 0.3 to 0.8. The R_f value is constant for every compound in a particular combination of stationary and mobile phase. When the R_f value of a sample and reference compound is same, the compound is identified by its standard. When the R_f value differs, the compound may be different from its reference standard.

REQUIREMENTS

APPARATUS

1. TLC plates
2. TLC Jar (An alternative is a beaker covered with a watch glass or aluminium foil)
3. Spotting capillaries
4. Measuring cylinder
5. Screw-cap Jar

CHEMICALS

1. Silica gel G
2. Petroleum ether

3. Diethyl ether
4. Glacial acetic acid
5. Coconut / any other lipid as standard
6. Iodine

PROCEDURE

1. PREPARATION OF TLC PLATES (STATIONARY PHASE):

i) BY DIPPING

A known volume of 33 ml of methanol and 67 ml of chloroform are taken in a 150mlscrew-cap jar and added 35 g of silica gel *G* to the jar. The jar is closed with the cap and shaken vigorously for about a minute toprepare a slurry of silica gel G. Two clean microscope slide plates, by holding them together at the top are dipped into the slurry for about two seconds. Touched the bottom of the stacked slides to the mouth of the jar to drain off the excess slurry. Let them be air dried for a minute. Then the plates are activated by drying them for 30 minutes in an oven at 100^{O}C.

ii) BY SPREADING:

A clean glass slide plate is taken. About10 g of silica gel G is mixed with 20 ml of water. The mixture is stirred and shaken well to remove any lumps and is poured on to the glass slide plate. Let the plate air dry for ten minutes and then dried at 100^{O}C in an oven for 30 minutes to activate the adsorbent.

2. PREPARATION OF MOBILE PHASE

For the purpose of lipid separation, particularly for neutral lipids such as triglycerides, an organic, largely nonpolar solvent mobile phase consisting of petroleum ether: diethylether: acetic acid at a ratio of 84:15:1 is prepared.

EXPERIMENT

Two clean TLC plates are placed on the hot plate silica side up for approximately three minutes to reactivate silica. Following, removed the TLC plates from heat. The origin (bottom) of the plate is marked with a pencil and labelled each lane. The standard is spotted in the first lane slowly and the spot is allowed to dry. Next, the lipid mixture is slowly spotted in the designated lanes and allowed to dry. The TLC plate is placed in solvent tank and made sure that the solvent does not touch the product sample. Closed the lid of the chamber and allowed the plate to develop. Once the solvent front nears the top of the plate, the plate is removed from the TLC chamber, the solvent front is quickly marked with a pencil, and allowed to dry. The plate is placed into the iodine chamber for visualization and secured the lid. Once adequately stained (about 3-5 minutes), removed the plate and lightly outlined the spots.

INFERENCE

The solvent mixture is intended to capture both nonpolar and polar species in the lipid mixture. The polarity index for petroleum ether (0.1) positions this solvent component as extremely nonpolar, and will allow the most nonpolar lipid in the mixture to be “dissolved.” Where as acetic acid, which has a high polarity index (6.2), is much more polar since it has the capacity to ionize, and serves as a solvent for the more polar species in this mixture. However, the components in this solvent are not provided in equal quantities; it is both the polarity index as well as the relative amount of each solvent component that indicates how it will carry specific lipid species up the TLC plate.

Solvent Component	Polarity Index	Ratio
Petroleum Ether	0.1	84
Diethyl Ether	2.8	15
Acetic Acid	6.2	1

RESULT

R_f value of triglyceride is ……
R_f value of cholesterylester is ……..
R_f value of free fatty acid is ……

NO. OF BENEFICIARIES:45

OUTCOME

The students are able to

- Learn the technique of thin layer chromatography
- Detect a particular compound present in a mixture.
- Establish that two compounds from a different origin are the same.
- Determine the number of compounds present in a mixture.

17.CHROMATOGRAPHIC SEPARATION OF CARBOHYDRATES

AIM

The aim of the experiment is to perform paper chromatography for the separation of carbohydrates present in the given sample.

PRINCIPLE

Distribution of solute (sugar) between the stationary and mobile phases, that is the partition process is the major factor in the PC separation of sugars. Their partition coefficients are substantially in favour of the aqueous phase. Therefore, withnon-aqueous developers, sugars appear on the paper chromatogram with low R_f values, where as with developer containing larger aqueous ratio, the R_f values of sugars are much higher. This is because a sugar molecule containing larger numberof hydroxyl groups which is readily soluble in water and makes the partition coefficient in favour of the aqueous phase. Further, the R_f values of sugars are affected by their structural formulae, their molecular mass, the number of-OH groups, and presence of other kinds of groups such as aldehydes or ketones etc.

REQUIREMENTS

APPARATUS

1. Boiling tubes 5
2. Measuring cylinder (100 ml) 1
3. Spotting Capillaries
4. Spraying-bottle 5
5. Whatman No.1 filter paper sheets

CHEMICALS:

1. Sugars - D-glucose/ D-fructose/ D-xylose/ L-rhamnose/ D-galactose/
2. D-mannose/ Lactose/ Maltose/ Sucrose
3. Detectors (list is given below)
4. 1-Butanol
5. Acetic acid

SOLUTIONS

1. UNKNOWN SUGAR SAMPLE SOLUTION: It can be prepared by dissolving any one or two sugars in water.

2. **DETECTOR:** Any one of the following detectors may be prepared.

DETECTOR-1: Ammoniacal silver nitrate: 5 ml of saturated aqueoussolution of silver nitrate is added to 50 ml of acetone, finally ammonia solutionis added to make the solution clear and basic in nature.

DETECTOR-2: Aniline hydrogen phthalate: 1 ml of aniline and 1.66 g of phthalic acid are dissolved in 100 ml of 1-butanol saturated with water.

DETECTOR-3: *p*-Anisidine hydrochloride in 100 ml of 1-butanol.

DETECTOR-4: (For non-reducing sugars): A solution is prepared by mixing 0.25g of sodium borate + phenol red + methanol in (1:2:7) proportion.

3. DEVELOPER: In a separating funnel 1-butanol, acetic acid and water in the proportion (4:1:5) are taken and shaken gently. The layers are allowed to settle. The lower aqueous layer is removed and the upper organic phase (layer) is taken as the developer for sugars.

PROCEDURE

PREPARATION OF SOLUTION

I) SAMPLE SOLUTIONS

The aqueous solution of any three of the following is prepared by dissolving 0.2-0.5 ***g*** of each sugar in 5 ml of water in a smalltest tube. The sugars are :D-glucose, D-fructose, D-xylose, L-rhamnose, D-galactose, D-mannose, Lactose, Maltose and sucrose.

II)PREPARATION OF MIXTURE SOLUTION OF SUGARS: Few drops of each sample sugar solution is added in a dry test tube.

III) UNKNOWN SUGAR SOLUTION: It can be prepared by dissolving any one or two sugars in water.

PAPER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SUGARS:

The chromatographic paper strips of the required size is cut. On each strip a line is drawn with a pencil at about 1cm from one end and a point is marked at the centre of the line. The sample is to be applied at this mark. The name of the particular sugar is written on the upper side of the paper with pencil. The respective sugar solution is applied to the point of application separately onthe marked strips. For each solution, a fresh capillary tube is used. The mixture solution and the unknown solutions are applied separately on other strips. The spots are dried by allowing the solvent to evaporate.

Next, clean and dry boiling tubes are taken and 10-15ml of the developer is placed in each of these tubes. The spotted and dried paper strips are suspended in the respective boiling tubes containing distilled water with upper end pinned to the cork and the lower end touching the developer. Care should be taken to see that this is done gently and the strip is vertical. The spot should always be above the developer level. Now the developer is allowed to rise along the paper and wait till the developer (solvent front) reaches near the upper end of the paper. The paper strip is removed from the boiling tube and the solvent front is marked with the help of a pencil. The strip is dried until the acetic acid odour from the strip is no more present. The strip is treated with a detector by a spraying bottle. The strip is heated at 105^0C in an oven until the coloured zones of sugars are seen. The coloured zones are encircled and marked the centre of each zone.

The R_f values are calculated and the R_f values of individual sugars are compared with that of their R_f values in mixtures to identify the sugars present in the mixture/sample solution.

OBSERVATION AND CALCULATION

Colour of the spots of various sugars is observed. The colour depends on the detector used. The distance travelled by the centre of the solute zone (ds) and the distance travelled by the solvent front (dm) on the paper chromatogram is measured.

The R_f values of each sugar is calculated by the relation R_f= ds /dm.

DISCUSSION

The colour of the sugar zones on the chromatogram depends onthe detector used. For example, using detector-1, reduction of ammoniacal silvernitrate results into metallic silver. Therefore, the reducing sugars give rise tobrown-black spots after heating to 100^0C.

A popular spraying reagent is detector-2, aniline hydrogen phthalate. The colour development depends on the following mechanism. Heating of sugar with an acid produces furfuraldehyde which can be condensed with an aromatic amine or phenolto give coloured compounds. Sugars which react can appear as red or brown spots. Itis a sensitive and popular spraying reagent for aldopentose's, aldohexoses, methyl pentoses and reducing disaccharides.

RESULT

Sugars present in the unknown sample are --------------.

NO. OF BENEFICIARIES: 45

OUTCOME

The students are able to

- Identify and separate-coloured mixtures like pigments.
- Identify unknown organic and inorganic compounds from a mixture.