

Experiment: 01

To Determine the pH, Acidity and Alkalinity of Different Water Samples

Aim

To determine the pH, acidity and alkalinity of different water samples using standard laboratory methods.

Apparatus and Glassware

pH meter with glass electrode, Beakers, Conical flasks, Burette, Pipette, Measuring cylinder

Chemicals and Reagents

Standard buffer solutions, preferably pH 4.00, 7.00 and 9.20/10.00, Distilled/deionized water, Standard sodium hydroxide (NaOH) solution, e.g. 0.02 N, Phenolphthalein indicator, Methyl orange indicator, if required, Standard hydrochloric acid (HCl) or sulfuric acid solution, e.g. 0.02 N,

Theory

pH:

The pH of a water sample indicates its acidic or basic nature and is defined as the negative logarithm of hydrogen ion activity:

$$\text{pH} = -\log[\text{H}^+]$$

The pH is determined using a calibrated pH meter.

Acidity:

Acidity is the capacity of water to neutralize a strong base. It is mainly due to dissolved carbon dioxide, mineral acids and acidic salts. Acidity is determined by titrating the water sample against a standard alkali using an appropriate indicator.

Alkalinity:

Alkalinity is the capacity of water to neutralize acids. It is mainly due to hydroxide (OH^-), carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) ions. It is determined by titration with a standard acid using phenolphthalein and methyl orange indicators.

5. Procedure

A. Determination of pH

1. Switch on the pH meter and allow it to stabilize according to the manufacturer's instructions.
2. Calibrate the pH meter using suitable standard buffer solutions.
3. Rinse the electrode thoroughly with distilled/deionized water and gently blot it dry.
4. Transfer sufficient quantity of the water sample into a clean beaker.
5. Immerse the electrode into the sample, ensuring that the sensing bulb is completely immersed.
6. Stir gently or allow the reading to stabilize.
7. Record the pH displayed on the instrument.
8. Rinse the electrode with distilled water before measuring the next sample.
9. Repeat the measurement for all water samples.

B. Determination of Acidity

Procedure

1. Rinse the burette with the standard NaOH solution and fill it, ensuring that there are no air bubbles in the burette tip.
2. Pipette a known volume, usually 50.0 mL, of the water sample into a clean conical flask.
3. Add 2–3 drops of phenolphthalein indicator.
4. Titrate the sample against standard NaOH solution with continuous swirling.
5. Continue the titration until a faint pink colour persists for about 30 seconds.
6. Note the burette reading.
7. Repeat the titration to obtain concordant readings.
8. If required, perform a separate titration using methyl orange to determine acidity to the methyl-orange endpoint.

Observation Table

S.No.	Volume of water Sample (mL)	Initial Burette Reading (mL)	Final Burette Reading (mL)	Volume of NaOH Used (mL)
Rough	50.0			
I	50.0			
II	50.0			
III	50.0			

Calculation

Acidity as mg/L as CaCO_3 :

$$\text{Acidity} = \frac{V \times N \times 50,000}{V_s}$$

- V = volume of standard NaOH used (mL)
- N = normality of NaOH
- V_s = volume of water sample (mL)
- 50,000 = equivalent weight factor for CaCO_3 in mg/L

C. Determination of Alkalinity

Step 1: Phenolphthalein Alkalinity (P-Alkalinity)

1. Pipette 50.0 mL of the water sample into a clean conical flask.
2. Add 2–3 drops of phenolphthalein indicator.
3. If the solution remains colourless, P-alkalinity is zero.
4. If a pink colour develops, titrate with standard $\text{HCl}/\text{H}_2\text{SO}_4$ solution until the pink colour just disappears.
5. Record the volume of acid consumed.

Step 2: Total Alkalinity (M-Alkalinity)

1. To the same titrated sample, add 2–3 drops of methyl orange indicator.

2. Continue titration with standard acid until the colour changes from yellow to orange/red at the endpoint.
3. Record the total volume of acid consumed.
4. Repeat the titration to obtain concordant readings.

Observation Table

S.No.	Sample Volume (mL)	Acid Reading for P-Alkalinity(mL)	Total Acid Reading(mL)
Rough	50.0		
I	50.0		
II	50.0		
III	50.0		

Calculation

Phenolphthalein alkalinity:

$$P = \frac{V_P \times N \times 50,000}{V_s}$$

$$T = \frac{V_T \times N \times 50,000}{V_s}$$

Where:

- V_p = volume of acid required to reach phenolphthalein endpoint (mL)
- V_t = total volume of acid required to reach methyl-orange endpoint (mL)
- N = normality of standard acid
- V_s = volume of water sample (mL)

Results are expressed as mg/L (ppm) as CaCO_3 .

Precautions

1. Use clean and properly rinsed glassware for all measurements.
2. Calibrate the pH meter before measuring unknown samples.
3. Do not touch the pH electrode bulb with fingers or wipe it roughly.
4. Rinse the electrode between different samples to avoid cross-contamination.
5. Use freshly prepared or properly standardized titrant solutions.
6. Perform titrations in duplicate or triplicate and use concordant readings.
7. Do not leave the pH electrode dry; store it according to the manufacturer's instructions.
8. Handle acids and alkalis carefully and wear appropriate personal protective equipment.

Result

The pH, acidity and alkalinity of the given water samples are determined and reported as follows:

(mL)

S.No.	Sample	pH Acidity (mg/L as CaCO ₃)	Alkalinity (mg/L as CaCO ₃)
1	Sample 1		
2	Sample 2		
3	Sample 3		
4	Sample 4		

Experiment No: 2

Estimation of Iron (II) Using Standardized Potassium Permanganate (KMnO₄)

Aim

To determine the concentration of iron(II) ions in the given sample solution by titration against a standardized potassium permanganate (KMnO₄) solution in acidic medium.

Apparatus

Burette, Pipette, Conical flask, measuring cylinder Volumetric flask, Beaker, Funnel, Glass rod and Retort stand and clamp

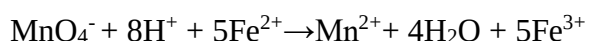
Chemicals

Given Fe(II) sample solution, Standardized KMnO₄ solution, Dilute H₂SO₄, Distilled/deionized water

Theory

Iron (II) ions are oxidized to iron (III) ions by potassium permanganate in an acidic medium. Potassium permanganate acts as a strong oxidizing agent and also serves as its own indicator. The endpoint is indicated by the appearance of permanent pink colour due to a slight excess of KMnO₄.

The reaction is:



Procedure

Titration of Fe(II) with Standardized KMnO₄

1. Rinse the pipette with the given Fe(II) solution.
2. Pipette 20.0 or 25.0 mL of the Fe(II) sample into a clean conical flask.
3. Add approximately 10–20 mL of dilute H₂SO₄ to provide the required acidic medium.
4. Add distilled water if necessary to obtain a convenient volume for titration.
5. Place the conical flask on a white tile.
6. Titrate the Fe(II) solution with standardized KMnO₄ from the burette.

7. Shake the conical flask continuously during titration.
8. Initially, KMnO_4 may be added relatively quickly, but near the endpoint it should be added dropwise.
9. The purple colour of KMnO_4 will disappear as it reacts with Fe^{2+} .
10. Continue titration until the pink colour persists for approximately 30 seconds.
11. Record the final burette reading.
12. Repeat the titration until concordant readings are obtained.

Observations

S.No.	Volume of Fe(II) Solution (mL)	Initial Burette Reading (mL)	Final Burette Reading (mL)	Volume of KMnO_4 Used (mL)
Rough	25.0			
I	25.0			
II	25.0			
III	25.0			

Concordant titre: Average volume of standardized KMnO_4 used = _____ mL

Calculations

Applying Normality Equation

$$N_1 V_1 = N_2 V_2$$

$$N_{\{\text{KMnO}_4\}} V_{\{\text{KMnO}_4\}} = N_{\{\text{Fe}^{2+}\}} V_{\{\text{Fe}^{2+}\}}$$

N_1 = Normality of standardized KMnO_4

V_1 = Volume of KMnO_4 used (mL)

N_2 = Normality of Fe(II) solution

V_2 = Volume of Fe(II) solution taken (mL)

Result

The concentration/strength of Fe(II) in the given sample solution was determined by titration with standardized KMnO_4 and found to be:

Strength of Fe(II) = _____ g/L

Precautions

1. Use dilute H_2SO_4 only for acidification.
2. Do not use HCl because chloride ions may be oxidized by KMnO_4 .
3. Do not use HNO_3 because it is an oxidizing acid and can oxidize Fe^{2+} before the titration.
4. Rinse the burette with KMnO_4 solution before filling it.
5. The endpoint should be the first permanent pale pink colour; do not add excess KMnO_4 .
6. Protect KMnO_4 solution from contamination and strong light and use a properly standardized solution.

Experiment No. : 3

Estimation of Iron (II) with Standardized $\text{K}_2\text{Cr}_2\text{O}_7$ Using Diphenylamine as Internal Indicator

Aim

To determine the concentration/amount of iron (II) ions (Fe^{2+}) in the given solution by titrating it against a standardized potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) solution .

Apparatus

Burette (50 mL), Pipette (20 or 25 mL), Conical flask (250 mL), Measuring cylinder, Volumetric flask, Funnel, Beaker, Wash bottle, Glass rod, White tile

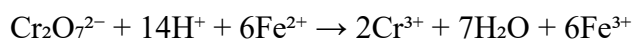
Chemicals

Given Fe^{2+} solution, Standardized $\text{K}_2\text{Cr}_2\text{O}_7$ solution, Dilute H_2SO_4 , Diphenylamine indicator, Distilled/deionized water

Theory

Iron(II) is quantitatively oxidized to iron(III) by potassium dichromate in an acidic medium.

The ionic reaction is:



Thus, one mole of $\text{K}_2\text{Cr}_2\text{O}_7$ reacts with six moles of Fe^{2+} .

Diphenylamine is used as an internal indicator. Near the equivalence point, after all Fe^{2+} has been oxidized to Fe^{3+} , the first slight excess of dichromate oxidizes the indicator, producing a violet/blue-violet end point.

Procedure

A. Burette Preparation

1. Wash and rinse burette with a small quantity of standardized $\text{K}_2\text{Cr}_2\text{O}_7$ solution.
2. Fill the burette with the standardized $\text{K}_2\text{Cr}_2\text{O}_7$ solution.
3. Pipette accurately 20.00 or 25.00 mL of the given Fe^{2+} solution into a clean conical flask.
4. Add approximately 10–20 mL of dilute H_2SO_4 to provide the required acidic medium.

5. Add 2–3 drops of diphenylamine indicator.
6. Place the flask on a white tile.
7. Titrate with standardized $\text{K}_2\text{Cr}_2\text{O}_7$ solution while continuously swirling the flask.
8. As the end point is approached, add the dichromate solution slowly, preferably dropwise.
9. The end point is indicated by the appearance of a permanent violet/blue-violet colour.
10. Record the burette reading.
11. Repeat the titration until at least two concordant readings are obtained.

Observations

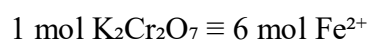
S.No.	Initial Burette Reading (mL)	Final Burette Reading (mL)	Volume of $\text{K}_2\text{Cr}_2\text{O}_7$ Used (mL)
Rough			
I			
II			
III			

Concordant titre: Average volume of $\text{K}_2\text{Cr}_2\text{O}_7$ used = _____ mL

8. Calculations

- M_1 = Molarity of standard $\text{K}_2\text{Cr}_2\text{O}_7$
- V_1 = Volume of $\text{K}_2\text{Cr}_2\text{O}_7$ used
- M_2 = Molarity of Fe^{2+} solution
- V_2 = Volume of Fe^{2+} solution taken

From the balanced equation:



$$M_1V_1 \times 6 = M_2V_2$$

Alternatively, using normality:

$$N_1V_1 = N_2V_2$$

Result

The amount/concentration of Fe^{2+} in the given solution is _____ g L^{-1} (or _____ mol L^{-1}).

Precautions

1. Use only sulfuric acid to provide the acidic medium.
2. The $\text{K}_2\text{Cr}_2\text{O}_7$ solution must be accurately standardized.
3. Add dichromate slowly near the end point.
4. Do not add excessive indicator because diphenylamine itself can consume a small amount of oxidant.
5. Read the burette at eye level to avoid parallax error.
6. Use clean and properly rinsed glassware.

Experiment No. : 4

Preparation of Hand Sanitizer

Aim

To prepare a hand sanitizer containing an appropriate concentration of alcohol and suitable supporting ingredients for hygienic hand disinfection.

Apparatus and Glassware

1 L volumetric flask or suitable graduated container, Measuring cylinders, Beakers, Glass rod, Funnel, Measuring pipette, Clean storage bottles with tight-fitting caps, , Labels, Alcoholmeter, if available

Chemicals and Reagents

Ethanol 96% v/v or suitable concentration of isopropyl alcohol, Hydrogen peroxide 3%, Glycerol 98%, Sterile/distilled water

Theory

Hand sanitizers are alcohol-based formulations designed to reduce microorganisms on the hands. Ethanol or isopropyl alcohol is used as the principal active ingredient because alcohols can disrupt microbial membranes and denature proteins.

For effective alcohol-based hand sanitization, the final formulation should contain an appropriate alcohol concentration. A commonly used laboratory formulation is based on ethanol 80% v/v or isopropyl alcohol 75% v/v, with hydrogen peroxide and glycerol as supporting ingredients.

Hydrogen peroxide helps control microbial contamination of the formulation, while glycerol acts as a humectant and helps prevent excessive drying of the skin.

Procedure

1. Clean and dry all glassware and containers.
2. Label the bottles with the product name, date of preparation and batch number.
3. Measure 833.3 mL of 96% v/v ethanol using a suitable measuring cylinder

4. Transfer the ethanol carefully into a clean 1 L volumetric flask or suitable mixing container.
5. Measure 41.7 mL of 3% hydrogen peroxide
6. Add it carefully to the ethanol and Mix gently.
7. Measure 14.5 mL of 98% glycerol.
8. Transfer it into the mixture.
9. Add sterile/distilled water to bring the total volume to 1000 mL.
10. Mix gently and thoroughly.
11. Transfer the prepared sanitizer into clean, labelled containers.
12. Close the containers immediately and Keep the containers tightly closed when not in use.
13. Store in a cool, well-ventilated area away from heat, sparks, flames and direct sunlight.

Precautions

1. Ethanol and isopropyl alcohol are highly flammable; keep them away from flames, sparks and heat sources.
2. Perform preparation in a well-ventilated area.
3. Minimize evaporation by keeping alcohol containers closed as much as possible.
4. Use clean and preferably sterilized containers for the final product.
5. Do not substitute technical or contaminated alcohol for an appropriate pharmaceutical/laboratory-grade alcohol.
6. Hydrogen peroxide should be handled carefully and kept in its appropriate container.
7. Do not add perfumes, dyes or other ingredients unless they have been validated as compatible with the formulation.

Result

A clear, homogeneous alcohol-based hand sanitizer was prepared using ethanol, hydrogen peroxide, glycerol and sterile/distilled water.

Final volume: _____ mL

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Experiment No. : 01

Qualitative Analysis of Adulterants in Common Food Items

Aim

To detect qualitatively the presence of common adulterants in selected food items such as milk, sugar, jaggery, turmeric, chilli powder, edible oils, tea, coffee, coriander powder and pulses by using simple chemical and physical tests.

Apparatus

Test tubes, Beakers, Conical flasks, Measuring cylinders, Droppers, Glass rods, Watch glasses, Filter paper, Funnel

Chemicals

- Dilute hydrochloric acid (HCl), Concentrated hydrochloric acid (HCl), where specifically required, Dilute sulfuric acid (H_2SO_4), where specifically required, Iodine solution, Iodine–potassium iodide solution, Alcohol/ethanol, Sodium hydroxide solution, Ferric chloride solution, Hydrochloric acid, Nitric acid, Acetic acid, Petroleum ether/appropriate laboratory solvent, where required

Theory

Food adulteration involves the addition or substitution of inferior, cheaper, or undesirable substances in food products. Different adulterants can be detected by characteristic physical, chemical or colour reactions.

The tests described in this SOP are screening/qualitative tests. A positive result indicates suspected adulteration and, where required, should be confirmed by an appropriate validated analytical method.

Sample Collection and Labelling

1. Collect representative samples of the food items.
2. Place each sample in a clean, dry and properly labelled container.
3. Record: Sample name, Brand/source, Batch number, if available, Date of collection

4. Keep samples free from cross-contamination.
5. Use separate spatulas/dropper for different samples whenever possible.

Qualitative Tests

A. MILK (Detection of Starch)

Procedure:

1. Take approximately 5 mL of milk in a test tube.
2. Add 2–3 drops of iodine solution.
3. Mix thoroughly.

Observation:

- Blue/blue-black colour → starch may be present.
- No blue colour → starch is not detected.

Inference:

Development of blue/blue-black colour indicates possible starch adulteration.

B. SUGAR (Detection of Chalk/Carbonate-Type Mineral Adulterants)

Procedure:

1. Dissolve approximately 2–3 g of sugar in 10 mL distilled water.
2. Filter the solution and Examine the residue on the filter paper.
3. If necessary, treat the residue cautiously with dilute HCl.

Observation:

- Insoluble residue → suggests presence of an insoluble mineral adulterant.
- Effervescence on treatment with dilute HCl → indicates a carbonate-type material may be present.

Inference:

Presence of significant insoluble residue with carbonate-type reaction indicates suspected mineral adulteration.

C. JAGGERY (Detection of Chalk/Other Insoluble Mineral Matt

Procedure:

1. Take approximately 5 g of jaggery.
2. Dissolve it in 20–30 mL distilled water.
3. Allow the solution to stand and filter it.
4. Examine the residue and treat residue carefully with dilute HCl.

Observation:

- Insoluble residue → suspected insoluble adulterant.
- Effervescence with dilute HCl → possible carbonate-containing adulterant.

Inference:

Presence of such residue/reaction indicates suspected mineral adulteration

D. TURMERIC POWDER (Detection of Metanil Yellow/Added Synthetic Colour)

Procedure:

1. Take a small quantity of turmeric powder in a test tube.
2. Add approximately 5 mL of water and mix.
3. Observe the colour of the aqueous extract.
4. For a screening test, add a few drops of concentrated HCl under a fume hood and with appropriate PPE.

Observation:

- Development of an unusual/persistent pink-red colour may indicate the presence of certain synthetic colourants such as metanil yellow.

Inference:

A positive colour reaction indicates suspected artificial colour adulteration and should be confirmed by an appropriate instrumental/chromatographic method.

E. CHILLI POWDER (Detection of Artificial Colour)

Procedure:

1. Take approximately 1 g chilli powder in a test tube.
2. Add 10 mL water and Mix and allow the sample to stand.
3. Observe the colour of the solution and any coloured material settling separately.

Observation:

- Rapid development of intense colour in the water may indicate added water-soluble artificial colour.

Inference:

Abnormally strong colour transfer suggests possible artificial colour adulteration.

F. EDIBLE OILS (Detection of Mineral Oil)

Procedure:

1. Take approximately 5 mL of the oil sample in a clean test tube.
2. Carry out an appropriate validated laboratory screening test for mineral oil using a suitable solvent/extraction procedure.
3. Compare the behaviour of the sample with an authentic reference oil.

Observation:

Any characteristic positive response according to the validated method indicates suspected mineral-oil contamination/adulteration.

Inference:

Positive screening result → suspected mineral oil.

G. TEA (Detection of Added/Used Tea Leaves or Coloured Leaves)

Procedure:

1. Spread a small quantity of tea leaves on a clean, white filter paper or watch glass.
2. Examine the leaves for unusual colour or appearance.
3. Transfer approximately 1 g tea to a beaker containing warm water.
4. Observe the colour released into the water.

Observation:

- Very rapid release of an intense artificial colour may indicate added colouring matter.
- Suspiciously uniform or unusually coloured leaves require further examination.

Inference:

Abnormal colour release indicates possible artificial colouring/adulteration.

H. COFFEE (Detection of Chicory)

Principle:

Chicory behaves differently from coffee when placed in water and tends to settle more rapidly.

Procedure:

1. Take a small quantity of powdered coffee.
2. Sprinkle it gently over the surface of cold water in a beaker.
3. Observe the behaviour of the particles and colour release.

Observation:

- Coffee generally floats for a longer period, whereas chicory tends to absorb water and sink more rapidly, releasing colour.

Inference:

Rapid sinking with characteristic colour release suggests possible chicory adulteration.

I. CORIANDER POWDER (Detection of Sawdust/Foreign Plant Material)

Procedure:

1. Take approximately 2 g coriander powder.
2. Add it to a beaker containing water.
3. Stir gently and allow it to settle.
4. Examine floating and settled material.

Observation:

- Light fibrous material floating separately from the normal coriander suspension may indicate addition of sawdust or other plant material.

Inference:

Presence of abnormal fibrous material indicates suspected adulteration.

J. PULSES (Detection of Artificially Coloured/Polished Pulses)

Procedure:

1. Take a small quantity of pulses in a clean beaker.
2. Add approximately 20 mL water.
3. Shake gently and Observe the colour of the water.

Observation:

- Noticeable colour released into the water may indicate added water-soluble colouring matter.

Inference:

Significant colour transfer indicates possible artificial colouring.

Result

The given food samples were subjected to qualitative screening tests for common adulterants. Based on the observed physical and chemical reactions, the presence or absence of suspected adulterants was recorded.

Precautions

1. Avoid cross-contamination between food samples.
2. Use freshly prepared reagents wherever required.
3. Perform colour tests against a suitable blank/control whenever possible.
4. Handle concentrated acids only under the supervision of a qualified instructor and preferably inside a fume hood.
5. Dispose of chemical waste according to institutional laboratory-waste procedures.
6. A positive screening test should be reported as “suspected adulteration” unless confirmed by a validated method.

Conclusion

Qualitative analysis provides a simple and useful approach for the preliminary screening of common food adulterants. Physical tests such as sedimentation, flotation and magnetic separation, together with suitable chemical tests, can help students understand the principles of food-quality analysis. Definitive regulatory conclusions require validated analytical methods and appropriate reference standards.

Experiment No. : 02

Aim

To identify and distinguish carbohydrates qualitatively using Molisch's, Benedict's, Fehling's and Barfoed's tests.

Apparatus Required

Test tubes, Measuring cylinder, Beaker, Water bath, Pipettes

Chemicals/Reagents Required

Molisch's Reagent, Benedict's Reagent, Fehling's Solution, Barfoed's Reagent, Test Samples

Theory

a) Molisch's Test

Carbohydrates are dehydrated by concentrated sulphuric acid to form furfural or hydroxymethyl furfural derivatives. These react with α -naphthol to produce a violet/purple ring at the junction of the two layers.

b) Benedict's Test

Reducing sugars reduce Cu^{2+} ions present in Benedict's reagent to cuprous oxide (Cu_2O) on heating. Depending on the concentration of reducing sugar, the colour changes from blue $\rightarrow$ green $\rightarrow$ yellow $\rightarrow$ orange $\rightarrow$ brick-red precipitate.

c) Fehling's Test

Reducing sugars reduce the alkaline copper(II) complex of Fehling's solution to brick-red cuprous oxide (Cu_2O) on heating.

d) Barfoed's Test

Monosaccharides reduce Cu^{2+} ions in the mildly acidic Barfoed's reagent rapidly on heating,

producing a red Cu_2O precipitate. Disaccharides generally react much more slowly. The test therefore helps distinguish monosaccharides from disaccharide

5. Procedure

A. Molisch's Test

1. Take about 2 mL of the carbohydrate solution in a clean test tube.
2. Add 2–3 drops of Molisch's reagent and Mix thoroughly.
3. Incline the test tube and carefully add about 1–2 mL concentrated H_2SO_4 along the side of the test tube so that it forms a separate lower layer.
4. Do not shake the tube.
5. Observe the junction between the two layers.

Observation

Formation of a violet/purple ring at the interface indicates the presence of carbohydrate.

B. Benedict's Test

1. Take 2 mL Benedict's reagent in a test tube.
2. Add approximately 0.5–1 mL carbohydrate solution and Mix well.
3. Heat the mixture in a boiling water bath for about 2–5 minutes.
4. Allow it to stand briefly and observe the colour/precipitate.

Observation

Observation	Interpretation
Blue solution remains	Negative
Green colour/precipitate	Low concentration of reducing sugar
Yellow precipitate	Moderate amount
Orange precipitate	Higher amount
Brick red	A strong positive test

Result

Formation of coloured precipitate, particularly orange/brick-red Cu_2O , indicates a reducing sugar.

C. Fehling's Test

1. Take 1 mL Fehling's Solution A in a test tube.
2. Add 1 mL Fehling's Solution B.
3. Mix immediately before use.
4. Add about 1 mL carbohydrate solution.
5. Heat the mixture in a boiling water bath for approximately 2–5 minutes.
6. Observe the colour and precipitate.

Observation

The initial blue solution changes to a brick-red precipitate of Cu_2O if a reducing sugar is present.

Result

Brick-red precipitate → Fehling's test positive → reducing carbohydrate present.

D. Barfoed's Test

1. Take approximately 2 mL Barfoed's reagent in a clean test tube.
2. Add about 1 mL carbohydrate solution and Mix well.
3. Heat in a boiling water bath.
4. Observe the formation of a red precipitate, preferably within approximately 2–3 minutes.
5. Do not prolong heating unnecessarily because some disaccharides may also give a positive reaction on prolonged heating.

Observation

Red ppt formation indicates Monosacharides presence

Result

Rapid formation of red Cu_2O precipitate $\rightarrow$ monosaccharide present.

Precautions

1. In Molisch's test, add concentrated H_2SO_4 carefully along the wall of the test tube; never add water to concentrated acid.
2. Handle concentrated H_2SO_4 only under appropriate laboratory supervision and preferably in a fume hood.
3. For Benedict's, Fehling's and Barfoed's tests, use a water bath rather than directly heating the test tube whenever possible.
4. In Barfoed's test, observe the reaction within the specified heating period; prolonged heating can cause disaccharides to give misleading positive results.
5. Fehling's A and B should be mixed freshly before performing the test.
6. Dispose of copper-containing waste according to institutional chemical-waste procedure.

Experiment No. : 03

Aim

To detect the presence of proteins and identify characteristic amino acid residues in protein samples using Biuret, Millon's and Xanthoproteic tests.

Apparatus Required

Test tubes, Droppers, Pipettes, Measuring cylinder, Beakers, Glass rod, Water bath, Fume hood

Chemicals/Reagents Required

Biuret Reagent, Millon's Reagent, Xanthoproteic Reagent, Test Sample

Theory

A. Biuret Test

Proteins and compounds containing two or more peptide bonds react with Cu^{2+} ions in an alkaline medium to form a violet/purple coloured coordination complex. The test is therefore used as a general test for proteins and peptide bonds.

B. Millon's Test

Millon's reagent reacts mainly with the phenolic group of tyrosine residues present in proteins. On heating, a white precipitate that may become brick-red/red indicates the presence of tyrosine-containing proteins.

C. Xanthoproteic Test

Proteins containing aromatic amino acids such as tyrosine, tryptophan and, to a lesser extent, phenylalanine undergo nitration with concentrated nitric acid. A yellow colour is produced, which may become orange on treatment with alkali.

PROCEDURE

A. Biuret Test

Procedure

1. Take approximately 2 mL of protein solution in a clean test tube.
2. Add about 2 mL of 10% NaOH solution and Mix thoroughly.
3. Add 2–3 drops of dilute CuSO_4 solution slowly with shaking.
4. Observe the colour developed.

Observation

Development of a violet or purple colour indicates a positive Biuret test.

Result

Violet/purple colour → Protein containing peptide bonds is present.

B. Millon's Test

Procedure

1. Take approximately 2 mL of protein solution in a clean test tube.
2. Add about 1 mL of Millon's reagent carefully.

3. Mix gently.
4. Heat the test tube carefully in a hot-water bath.
5. Observe the colour and precipitate formed.

Observation

A white precipitate, which changes to pink, red or brick-red on heating, indicates a positive Millon's test.

C. Xanthoproteic Test

Procedure

1. Take approximately 2 mL of protein solution in a clean test tube.
2. Carefully add about 1 mL concentrated HNO_3 and Mix gently.
3. Heat carefully in a water bath if required.
4. Observe the development of colour.
5. After cooling, carefully add dilute NaOH solution or dilute ammonia solution.

Observation

- Yellow colour develops after treatment with nitric acid.
- On addition of alkali, the colour changes to orange or deep yellow.

Result

Yellow/orange colour → Aromatic amino acid residues are present in the protein.

Precautions

1. Millon's reagent contains mercury compounds and strong acid and must be handled with suitable PPE and preferably in a fume hood.
2. Concentrated HNO_3 is highly corrosive and should be handled with gloves, safety goggles and laboratory coat.
3. Avoid direct heating of test tubes containing concentrated acids. Use a suitable water bath under supervision.

4. Dispose of mercury-containing Millon's test waste as hazardous heavy-metal waste; it must not be poured into the sink.

Experiment No. : 04

Aim

To determine the water/moisture content of a food sample by measuring the loss in mass after drying the sample in a hot-air oven under controlled conditions.

Apparatus Required

Hot-air oven, Moisture/porcelain weighing dishes with lids, Desiccator containing a suitable drying agent, Mortar and pestle/grinder, watch glass, Sample container

Materials/Reagents

No chemical reagent is normally required.

Food sample: Approximately 2–5 g of a representative sample.

Preparation of Sample

1. Select a representative portion of the food sample.
2. If the sample is solid or coarse, grind it to obtain a reasonably uniform material.
3. Avoid excessive grinding that may cause heating and loss of moisture. then Mix the sample thoroughly.
4. Perform the analysis promptly after sample preparation to minimize moisture exchange with the atmosphere.

Theory

Most food samples contain water that can be removed by heating at a controlled temperature. In the oven-drying method, a known quantity of food sample is weighed accurately and dried in a hot-air oven until a constant mass is obtained.

The decrease in mass after drying is assumed to represent the water/moisture content of the sample.

$$\% \text{ Moisture} = (\text{Loss in mass during drying}) / (\text{Mass of sample taken}) * 100$$

$$\text{Loss of mass during drying} = W_1 - W_2$$

$$\text{Mass of sample taken} = W_1 - W_0$$

Where:

- W_0 = Mass of empty, dry weighing dish (g)
- W_1 = Mass of weighing dish + sample before drying (g)
- W_2 = Mass of weighing dish + dried sample (g)

The percentage of dry matter is:

$$\% \text{ Dry Matter} = 100 - \% \text{ Moisture}$$

PROCEDURE

1. Clean and dry the weighing dish and lid.
2. Place the empty dish with its lid in the hot-air oven.
3. Dry at approximately 105 °C for 30–60 minutes.
4. Remove the dish using tongs.
5. Place it in a desiccator and allow it to cool to room temperature.
6. Weigh the empty dish accurately.
7. Repeat drying, cooling and weighing if necessary until a constant mass is obtained. Record the mass as W
8. Accurately weigh approximately 2–5 g of the prepared food sample into the dried weighing dish.
9. Spread the sample uniformly over the bottom of the dish to facilitate efficient drying.
10. Place the lid on the dish and Weigh the dish containing the sample accurately. Record the mass as W_1 .
11. Remove the lid slightly or place it as appropriate according to the laboratory's drying procedure.

12. Transfer the dish containing the sample to a preheated hot-air oven.
13. Dry the sample at approximately 105 °C. Initially dry for approximately 2–3 hours.
14. Remove the dish using tongs and immediately cover it with the lid.
15. transfer it to a desiccator.
16. Allow it to cool to room temperature.
17. Weigh accurately and record the mass.
18. Return the dish to the oven for an additional 30–60 minutes.
19. Cool in the desiccator and reweigh.
20. Repeat the drying, cooling and weighing cycle until constant mass is obtained. Record the final mass as W_2 .

.Calculation

$$\% \text{ Moisture} = (\text{Loss in mass during drying}) / (\text{Mass of sample taken}) * 100$$

$$\text{Loss of mass during drying} = W_1 - W_2$$

$$\text{Mass of sample taken} = W_1 - W_0$$

Where:

- W_0 = Mass of empty, dry weighing dish (g)
- W_1 = Mass of weighing dish + sample before drying (g)
- W_2 = Mass of weighing dish + dried sample (g)

The percentage of dry matter is:

$$\% = \text{Dry Matter} = 100 - \% \text{ Moisture}$$

13. Precautions

1. Use a representative and well-mixed sample.
2. The weighing dish must be clean, dry and free from residual moisture before use.
3. Do not handle the weighing dish directly with bare hands; use tongs or suitable forceps.

4. Maintain the oven at the prescribed temperature throughout the experiment.
5. Avoid overheating the sample, as decomposition or charring can lead to an erroneous moisture value.
6. Weigh the sample accurately using a calibrated analytical balance.
7. Keep the weighing dish covered during transfer from the oven to the desiccator and during weighing.
8. Do not open the hot-air oven unnecessarily because this can cause temperature fluctuations.

Experiment No. : 05

Aim

To assess the quality and degree of rancidity of edible oil samples by determining: Acid Value (AV) and Peroxide Value (PV) – an indicator of primary oxidation products formed during oxidative rancidity.

Apparatus Required

Burette, 25 or 50 mL, Pipettes, Conical flasks, 250 mL, Measuring cylinders, Beakers, Glass stopper/test tube stopper, Amber-coloured bottle for peroxide-value reagents, Water bath, if required, Funnel

Chemicals and Reagents

For Acid Value (Edible oil sample, Standardized alcoholic KOH solution, approximately 0.1 N, Neutralized ethanol, Phenolphthalein indicator)

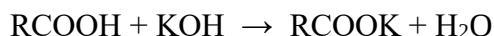
For Peroxide Value (Edible oil sample, Standardized sodium thiosulphate solution, approximately 0.01 N, Acetic acid, Chloroform or another validated suitable solvent, Saturated potassium iodide (KI) solution, Freshly prepared starch indicator, Distilled water)

Theory

A. Acid Value

During deterioration of fats and oils, triglycerides may undergo hydrolysis, producing free fatty acids (FFAs). The acid value is defined as the number of milligrams of KOH required to neutralize the free fatty acids present in 1 g of oil.

The oil is dissolved in a suitable neutralized solvent mixture and titrated against standardized alcoholic KOH using phenolphthalein as indicator.



A higher acid value generally indicates greater hydrolytic deterioration and poorer oil quality.

B. Peroxide Value

Oxidative rancidity results in the formation of hydroperoxides and other primary oxidation products.

In the peroxide-value method, the peroxides present in the oil oxidize iodide ions to iodine in an acidic medium. The liberated iodine is titrated with standardized sodium thiosulphate solution.

A higher peroxide value indicates greater primary oxidative deterioration.

PROCEDURE

Determination of Acid Value

7.1 Titration

1. Accurately weigh approximately 5.0 g of oil sample into a clean, dry 250 mL conical flask.
2. Add approximately 50 mL of neutralized ethanol.
3. Warm gently if necessary to dissolve the oil completely.
4. Add 2–3 drops of phenolphthalein indicator.
5. Titrate with standardized 0.1 N alcoholic KOH while swirling continuously.
6. Continue titration until a faint pink colour persists for approximately 30 seconds.
7. Record the burette reading.
8. Perform a reagent blank determination using the same procedure without the oil sample.

7.2 Calculation of Acid Value

$$\text{Acid Value} = ((V - B) * N * 56.1) / W$$

Where:

- V = Volume of KOH used for sample titration (mL)
- B = Volume of KOH used for blank (mL)
- N = Normality of KOH
- W = Mass of oil sample (g)
- 56.1 = Molecular mass of KOH in mg/mmol

Unit

mg KOH per g of oil

B. Determination of Peroxide Value

7.3 Titration

1. Accurately weigh approximately 5.0 g of oil sample into a dry glass-stoppered conical flask.
2. Add approximately 30 mL of acetic acid–chloroform solvent mixture.
3. Swirl gently until the oil dissolves.
4. Add approximately 0.5 mL freshly prepared saturated KI solution.
5. Immediately stopper the flask and mix gently.
6. Keep the flask in the dark for approximately 1 minute.
7. Add approximately 30 mL distilled water.
8. Titrate the liberated iodine with standardized 0.01 N sodium thiosulphate solution.
9. Continue titration until the yellow colour becomes pale.
10. Add approximately 0.5–1 mL starch indicator.
11. Continue titration until the blue colour disappears.
12. Record the burette reading.

13. Perform a blank determination using the same reagents but without the oil sample.

7.4 Calculation of Peroxide Value

$$\text{Peroxide Value} = ((V - B) * N * 1000) / W$$

Where:

- V = Volume of sodium thiosulphate used for sample (mL)
- B = Volume of sodium thiosulphate used for blank (mL)
- N = Normality of sodium thiosulphate
- W = Mass of oil sample (g)

Unit

milliequivalents of active oxygen per kilogram of oil (meq O₂/kg)

Precautions

1. Accurately standardize the KOH and sodium thiosulphate solutions before use.
2. Perform blank titrations for both methods.
3. Protect peroxide-value samples and reagents from unnecessary exposure to light.
4. Use freshly prepared KI and starch indicator where appropriate.
5. Do not expose oil samples to excessive heat before analysis.
6. Avoid prolonged exposure of oil to air during sample preparation.
7. Perform titrations at approximately the same temperature.
8. In the peroxide-value test, perform the iodine liberation step under the prescribed dark conditions.
9. Add starch near the endpoint rather than at the beginning of the titration.

Experiment No. : 06

To design a basic Hazard Analysis and Control Plan (HACCP) for a selected food product.

Selected Food: Pasteurized Milk

Aim

To identify potential biological, chemical and physical hazards associated with the production and handling of a selected food and to develop a basic Hazard Analysis and Critical Control Point (HACCP) plan for controlling these hazards.

Materials Required

HACCP worksheet, Food process flow chart, Pen/pencil, Calculator, Food safety reference material, temperature records, where available, Sample food product/process information

Theory

HACCP (Hazard Analysis and Critical Control Point) is a systematic preventive approach used to identify, evaluate and control hazards that may affect food safety.

The three major categories of food hazards are:

A. Biological Hazards

These include: Pathogenic bacteria, Viruses, Parasites, Yeasts and moulds

Examples relevant to milk include Salmonella, pathogenic Escherichia coli, Listeria monocytogenes and Staphylococcus aureus.

B. Chemical Hazards

These may include: veterinary drug residues, Pesticide residues, Cleaning and sanitizing chemical residue, Lubricants, Naturally occurring toxins, Undeclared allergens where applicable

C. Physical Hazards

These include: Glass fragments, Metal pieces, Stones, Plastic pieces, Hair or other foreign materials

Procedure

1. Select a food product for analysis.
2. Describe the Food
3. Construct the Process Flow Diagram

The basic flow diagram for pasteurized milk is:

Raw Milk Collection



Transportation



Reception & Inspection



Filtration



Pasteurization — CCP 1



Rapid Cooling



Packaging



Cold Storage — CCP 2



Distribution

Identification of Critical Control Points (CCPs)

A Critical Control Point (CCP) is a step at which control can be applied and is essential to prevent, eliminate or reduce a food-safety hazard to an acceptable level.

For this simplified example, the major CCPs are:

CCP-1: Pasteurization

Main hazard: Survival of pathogenic microorganisms.

CCP-2: Cold Storage

Main hazard: Growth of microorganisms due to inadequate temperature control.

Other steps may be classified as prerequisite controls or CCPs depending on the actual process, hazard analysis and validated HACCP study.

HACCP CONTROL PLAN

CCP-1: Pasteurization

Hazard

Survival of pathogenic microorganisms due to inadequate heat treatment.

Preventive Control

Controlled pasteurization using a validated time-temperature combination.

For example, a commonly used milk pasteurization condition is:

$\geq 72^{\circ}\text{C}$ for ≥ 15 seconds

or another legally/technically validated equivalent process.

The exact legal requirement should be taken from the applicable food-safety standard for the product and jurisdiction.

Critical Limit

The prescribed validated pasteurization temperature and holding time must be achieved.

Monitoring

Continuously or routinely monitor temperature, Record holding time, Check the pasteurizer/temperature-recording system, Maintain processing records.

Corrective Action

If the critical limit is not achieved:

1. Stop or hold the affected product.
2. Do not release the product as pasteurized.
3. Identify and segregate the affected batch.
4. Investigate the cause of deviation.
5. Reprocess only where permitted by the validated process and applicable regulations.
6. Repair or recalibrate equipment if required.
7. Document the corrective action.

Verification

- Review temperature/time records.
- Calibrate temperature-measuring equipment.
- Periodically verify the effectiveness of pasteurization.
- Review microbiological testing where applicable.

Records

- Pasteurization temperature record
- Holding-time record
- Equipment calibration record
- Corrective-action record

CCP-2: Cold Storage

Hazard

Growth of microorganisms due to inadequate refrigeration.

Preventive Control

Maintain the product under the validated and legally applicable refrigerated storage conditions.

Critical Limit

Maintain the specified storage temperature throughout storage and distribution.

For a basic practical example:

The milk should be stored at a temperature of 5°C or lower.

The exact limit should be established according to the applicable food standard, product specification and validated shelf-life process.

Monitoring

- Monitor refrigerator/storage temperature regularly or continuously.
- Record temperature at defined intervals.

- Check refrigerator performance.

Corrective Action

If the specified temperature is exceeded:

1. Identify the affected product.
2. Record the duration and maximum temperature reached.
3. Assess product safety according to the validated procedure.
4. Restore proper refrigeration.
5. Investigate equipment or handling failure.
6. Dispose of or otherwise control affected product when safety cannot be demonstrated.
7. Record the corrective action.

Verification

- Review temperature records.
- Calibrate thermometers.
- Inspect refrigeration equipment.
- Periodically evaluate product shelf life and microbiological quality.

Records

- Refrigerator temperature log
- Calibration record
- Corrective-action record
- Product-disposition record

Result

A basic HACCP-based hazard analysis and control plan was successfully designed for pasteurized milk.

The major hazards identified were biological, chemical and physical hazards, and the principal control points in the simplified process were pasteurization and refrigerated storage.

The HACCP plan provides a preventive approach for controlling significant food-safety hazards through defined critical limits, monitoring, corrective actions, verification and documentation.

Precautions

1. The HACCP plan should be based on the actual process flow of the selected food.
2. Every processing step should be evaluated for biological, chemical and physical hazards.
3. Critical limits should be based on validated scientific evidence and applicable food-safety regulations.
4. Do not designate a step as a CCP merely because a hazard exists; use a systematic HACCP decision process.
5. HACCP should be supported by effective GMP, GHP and sanitation programmes.
6. The HACCP plan should be reviewed whenever there is a change in ingredients, processing conditions, equipment, packaging or intended use.
7. The example temperature limits in this SOP should not replace legally applicable or validated limits for a commercial process.

Experiment No.7

Aim: To demonstrate different methods of water purification, namely filtration, boiling, distillation and activated charcoal adsorption, and to understand their principles, procedures, applications and limitations.

Apparatus: Beakers, Conical flasks, Measuring cylinders, Funnel, Filter paper, Filtration stand (if required), Glass rod, Evaporating dish, Distillation flask, Condenser, Receiving flask, Heating mantle or suitable heating source, Thermometer, Activated charcoal, Wash bottle, Tripod stand and wire gauze, Tongs, Clean sample bottles, Distilled/deionized water.

Chemicals: Raw/turbid water sample, Activated charcoal, Filter paper, clean cotton or sand, Distilled/deionized water, Suitable indicator or test reagents

Theory:

Filtration: Filtration is a physical method of water purification in which suspended and insoluble impurities are removed by passing water through a porous filtering medium. Depending on the filter used, filtration can remove suspended solids, turbidity and some microorganisms.

Boiling: Boiling is a thermal method of water purification. Heating water to boiling temperature for an adequate period destroys most disease-causing microorganisms, including many bacteria, viruses and protozoa. However, boiling does not remove dissolved salts, heavy metals or most chemical contaminants.

Distillation: Distillation purifies water by converting it into vapour through heating and subsequently condensing the vapour back into liquid water. Most non-volatile dissolved salts, suspended matter and many contaminants remain behind in the boiling vessel.

Activated Charcoal Adsorption: Activated charcoal has a highly porous structure and a large surface area. It removes or reduces certain dissolved organic substances, colour, taste and odour by **adsorption** onto its surface. It is not a complete method for removing all microorganisms, salts or inorganic contaminants.

Procedure:

A. Filtration

1. Take the turbid water sample in a beaker.
2. Place moistened filter paper in a funnel.
3. Pour the sample slowly through the filter paper.
4. Collect the filtrate and observe the reduction in suspended particles/turbidity.
5. Clean the apparatus after use.

B. Boiling

1. Take the water sample in a heat-resistant beaker.
2. Heat until boiling and maintain boiling for the prescribed time.
3. Cool the water in a covered container.
4. Observe and compare the treated and untreated samples.

C. Distillation

1. Assemble the distillation flask, condenser and receiving flask.
2. Add the water sample and start cooling-water circulation.
3. Heat the sample to produce vapour.
4. Collect the condensed water (distillate).
5. Stop heating before the flask becomes dry and compare the samples.

D. Activated Charcoal Adsorption

1. Add activated charcoal to the water sample.
2. Stir and allow sufficient contact time.
3. Filter the mixture to remove charcoal.
4. Collect and compare the treated water with the untreated sample.

Observation:

Observation table for filtration

Water Sample	Appearance Before Filtration	Appearance After Filtration	Suspended Matter Removed
Raw water			
Sample 1			
Sample 2			

Observation table for boiling

Parameter	Before Boiling	After Boiling
Appearance		
Odour		
Turbidity		
Microbial status, if tested		

Observation table for distillation

Parameter	Original Water	Distilled Water
Appearance		
Turbidity		
Odour		
Dissolved solids, if tested		

Observation table for Activated Charcoal Adsorption

Parameter	Before Treatment	After Activated Charcoal Treatment
Colour		
Odour		

Turbidity		
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Precautions

- Use clean apparatus and labelled samples.
- Handle hot water and heated glassware carefully.
- Never seal the distillation setup; ensure condenser cooling water flows.
- Do not allow the distillation flask to boil dry.
- Handle activated charcoal carefully to avoid inhalation.
- Use clean filter paper and filter slowly.
- Do not consume experimental water.
- Clean apparatus after use.
- Wear lab coat, goggles and gloves.

Result:

The different methods of water purification—filtration, boiling, distillation and activated charcoal adsorption were successfully demonstrated.

Experiment No.8

Aim: To separate and identify Fe^{3+} and Al^{3+} ions present in a given mixture by paper chromatography using a suitable solvent system and detecting reagents.

Apparatus: Chromatography paper/Whatman filter paper, Chromatography chamber or wide-mouth glass jar with lid, Capillary tubes, Beakers, Measuring cylinder, Pipette/dropper, Glass rod, Pencil, Ruler, Forceps, Watch glass, Developing chamber, Distilled/deionized water, Hot air oven or drying arrangement (if required).

Chemicals:

For Sample Preparation: Given mixture containing Fe^{3+} and Al^{3+} ions, Distilled/deionized water
Dilute hydrochloric acid, if required

For Chromatographic Development: Suitable developing solvent/mobile phase as prescribed by the laboratory method

For Detection of Fe^{3+} : Potassium thiocyanate (KSCN) solution or another suitable Fe^{3+} detecting reagent

For Detection of Al^{3+} : Suitable aluminium ion detecting reagent, such as aluminon reagent, according to the prescribed laboratory method

Theory: Paper chromatography is a separation technique based on the different distribution of components between a stationary phase and a mobile phase. In this experiment, the mixture containing Fe^{3+} and Al^{3+} ions is applied as a small spot on chromatography paper. When the paper is developed in a suitable solvent, the ions migrate at different rates due to differences in their distribution, solubility and interaction with the stationary phase. The separated ions are identified using suitable colour-forming reagents.

The movement of each component is expressed by its R_f (retardation factor) value:

$$R_f = \text{Distance travelled by the solute} / \text{Distance travelled by the solvent front}$$

The R_f value is characteristic of a particular ion under specified experimental conditions.

Procedure:

- Cut chromatography paper and draw a pencil baseline **1–2 cm above the lower edge**; mark positions for Fe^{3+} , Al^{3+} and standards.
- Add the developing solvent to the chamber, keeping it below the baseline, and close the chamber.
- Apply a small, concentrated spot of the Fe^{3+} – Al^{3+} mixture on the baseline and dry it.
- Place the paper vertically in the chamber and allow the solvent to rise by capillary action.
- Remove the paper, immediately mark the solvent front and allow it to dry.
- Develop the spots using suitable detecting reagents; Fe^{3+} may give a red/blood-red colour with thiocyanate, while Al^{3+} is detected using a suitable aluminium reagent.
- Observe the separated spots and compare them with standards.
- Calculate Rf values

Observation Table**Chromatographic Observation**

Component	Colour of Spot After Detection	Distance Travelled by Spot (cm)	Distance Travelled by Solvent Front (cm)	Rf Value	Identification
Fe^{3+}					
Al^{3+}					

Standard and unknown comparison

Solution	Spot Observed	Colour	Rf Value
Standard Fe^{3+}			
Standard Al^{3+}			

Calculations:

The retardation factor (Rf) for each ion is calculated using:

$R_f = \text{Distance travelled by the component} / \text{Distance travelled by the solvent front}$

For Fe^{3+} :

$R_f(\text{Fe}^{3+}) = \text{Distance travelled by } \text{Fe}^{3+} \text{ spot} / \text{Distance travelled by solvent front}$

For Al^{3+} :

$R_f(\text{Al}^{3+}) = \text{Distance travelled by } \text{Al}^{3+} \text{ spot} / \text{Distance travelled by solvent front}$

Precautions:

- Use clean, dry apparatus and handle paper only by its edges.
- Use pencil, not ink, for markings.
- Keep sample spots small, concentrated and dry between applications.
- Keep the solvent below the sample spot.
- Keep the chamber closed and paper vertical during development.
- Mark the solvent front immediately after removal.
- Use identical conditions for standards and unknowns.
- Wear PPE and avoid direct contact with chemicals.
- Dispose of chemical and paper waste properly.

Result:

Ion	Colour/Detection	Rf Value	Result
Fe^{3+}			Separated/Identified
Al^{3+}			Separated/Identified

Experiment No. 9

Aim: To separate and identify different plant pigments, mainly chlorophyll and carotenoids, present in green leaves using paper chromatography, and to determine their relative migration by calculating their R_f values.

Theory: Plant leaves contain several pigments, including chlorophyll a, chlorophyll b, carotenoids and xanthophylls. These pigments differ in their chemical properties, polarity, solubility and affinity towards the stationary and mobile phases. In paper chromatography, the pigments are separated because they migrate through the chromatography paper at different rates when carried by a suitable solvent.

The major pigments can be identified based on their characteristic colours:

- Chlorophyll a – blue-green
- Chlorophyll b – yellow-green
- Xanthophylls – yellow
- Carotenes/carotenoids – yellow to orange

The movement of each pigment is expressed using the R_f (retardation factor):

$$R_f = \text{Distance travelled by pigment} / \text{Distance travelled by solvent front}$$

The R_f value depends on the solvent system, chromatography paper, temperature and other experimental conditions.

Apparatus: Fresh green leaves, preferably spinach or other green leafy material, Mortar and pestle, Scissors, Chromatography paper/Whatman filter paper, Chromatography chamber or wide-mouth glass jar with lid, Capillary tubes, Beakers, Test tubes, Measuring cylinder, Pipette/dropper, Glass rod, Pencil, Ruler, Forceps, Funnel, Filter paper, Watch glass, Distilled/deionized water.

4. Chemicals and Reagents

- Fresh green leaves

- Suitable organic solvent for pigment extraction, such as **acetone or ethanol**, according to the laboratory protocol
- Suitable chromatography developing solvent/mobile phase, such as a validated petroleum ether–acetone or other appropriate solvent mixture
- Anhydrous sodium sulfate, if required for drying the pigment extract

Procedure:

- Select fresh green leaves, wash, dry and cut into small pieces.
- Grind the leaves with a suitable solvent and filter the green extract.
- Cut chromatography paper and draw a pencil baseline 1–2 cm above the lower edge.
- Mark the sample spot on the baseline and avoid using ink.
- Add developing solvent to the chamber, keeping its level below the baseline, and close the chamber.
- Apply the pigment extract as a small concentrated spot and allow it to dry.
- Place the paper in the chamber and allow the solvent to rise by capillary action.
- Remove the paper, mark the solvent front and dry the chromatogram.
- Observe and record the separated coloured pigment bands.
- Calculate R_f for each pigment.

A

Observation Table

Pigment Band	Colour Observed	Distance Travelled by Pigment (cm)	Distance Travelled by Solvent Front (cm)	R _f Value	Identification
Band 1					
Band 2					
Band 3					
Band 4					

Typical Observation

Pigment	Expected Colour	Relative Migration
Chlorophyll a	Blue-green	Moderate to high
Chlorophyll b	Yellow-green	Lower than chlorophyll a
Xanthophyll	Yellow	Higher than chlorophylls

Carotene	Yellow-orange/orange	Usually highest
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Calculation:

The retardation factor (Rf) of each pigment is calculated using:

$$R_f = \text{Distance travelled by pigment} / \text{Distance travelled by solvent front}$$

Precautions

- Use fresh leaves and protect the extract from strong light.
- Use pencil, not ink, for marking.
- Keep the sample spot small and concentrated.
- Keep the solvent below the baseline.
- Keep the chromatography paper vertical.
- Mark the solvent front immediately.
- Handle organic solvents carefully in a ventilated area.
- Wear appropriate PPE throughout the experiment.

Result:

Pigment	Colour Observed	Rf Value
Chlorophyll a		
Chlorophyll b		
Xanthophyll		
Carotenoid/Carotene		

Experiment No. 10

Aim: To estimate the concentration of the important soil macronutrients potassium (K), calcium (Ca) and magnesium (Mg) in given soil samples using flame photometry after suitable extraction of the nutrients from the soil.

Theory: Flame photometry is an analytical technique based on the measurement of the intensity of light emitted by certain metal ions when their atoms are excited in a flame. When a solution containing metal ions is introduced into a flame, the solvent evaporates and the metal atoms are excited to higher energy levels. As the excited atoms return to their ground state, they emit light of characteristic wavelengths. The intensity of emitted radiation is proportional to the concentration of the respective element within the working range of the instrument.

- Potassium (K): produces a characteristic emission in the red/violet region.
- Calcium (Ca): produces a characteristic emission in the orange-red region.
- Magnesium (Mg): is not measured reliably by all routine flame photometers because its emission is relatively weak and may require a suitable instrument, wavelength setting or an alternative analytical method.

Therefore, K and Ca are commonly estimated directly by flame photometry, while Mg estimation should be performed only if the available flame photometer and validated laboratory method support magnesium measurement. The concentration of each nutrient is determined by comparing the emission of the unknown soil extract with a series of standard solutions.

Apparatus: Flame photometer with appropriate filters/wavelength settings, Flame photometer accessories and burner assembly, Air compressor or gas supply, Volumetric flasks, Beakers, Conical flasks, Measuring cylinders, Pipettes, Test tubes, Funnel, Filter paper, Whatman filter paper, Electronic balance, Soil sieve (preferably 2 mm), Mortar and pestle, Mechanical shaker, Glass rod, Wash bottle, Sample bottles, Distilled/deionized water.

Chemicals:

Air-dried soil samples, Soil samples passed through a suitable sieve, Suitable soil-extracting solution, such as 1 N ammonium acetate (NH_4OAc), pH 7.0, for exchangeable K and Ca/Mg estimation, Standard potassium (K) solution, Standard calcium (Ca) solution, Standard

magnesium (Mg) solution, Distilled/deionized water, Dilute acid or alkali, Appropriate blank solution

Procedure:

- Collect, clean, air-dry, sieve and label the soil sample.
- Weigh 5.0 g soil, add 25 mL ammonium acetate extractant (pH 7) and shake for the prescribed time.
- Filter the extract and dilute if necessary; record the dilution factor.
- Prepare K, Ca and Mg standards along with a reagent blank.
- Calibrate the flame photometer using the blank and standards.
- Select the appropriate filter/wavelength for K, Ca and Mg.
- Aspirate the soil extract and record stable emission readings.
- Determine the concentration of each nutrient from the calibration curve.
- Apply the extraction and dilution factors to calculate the K, Ca and Mg content in the soil sample.

Observation:

A. Potassium Standards

Standard No.	K Concentration (mg/L)	Flame Reading
Blank	0	
1		
2		
3		

B. Calcium Standards

Standard No.	K Concentration (mg/L)	Flame Reading
Blank	0	
1		

2		
3		

C. Magnesium Standards

Standard No.	K Concentration (mg/L)	Flame Reading
Blank	0	
1		
2		
3		

D. Soil Sample Readings

Soil Sample	K Reading	Ca Reading	Mg Reading	Dilution Factor
Sample 1				
Sample 2				
Sample 3				

Calculations:

A. Concentration from Calibration Curve

The concentration of K, Ca or Mg in the soil extract is obtained from the respective calibration curve:

Concentration of nutrient in extract = Instrument/calibration-curve concentration $\times$ dilution factor

B. Conversion to Nutrient Content in Soil

If:

- C = concentration of nutrient in final extract (mg/L)
- V = total volume of extracting solution (L)
- DF = additional dilution factor, if any
- W = weight of soil taken (kg)

Then:

$$\text{Nutrient content (mg/kg soil)} = (C \times V \times DF) / W$$

Since 1 mg/kg = 1 ppm, the result may also be reported as ppm.

Precautions:

- Use clean, labelled glassware and properly prepared soil samples.
- Remove stones, roots and plant residues before analysis.
- Accurately weigh samples and measure extraction volumes.
- Use fresh, standardized standards for calibration.
- Calibrate the flame photometer before analysis.
- Keep the burner and nebulizer clean and unblocked.
- Filter soil extracts before aspiration.
- Dilute samples above the calibration range.
- Rinse the system between samples to prevent carry-over.
- Record dilution factors and take duplicate/triplicate readings.
- Follow proper instrument and laboratory safety procedures.

Result:

Concentration of K, Mg and Ca.....mg/Kg

Experiment No. 11

Aim: To determine the amount of iron (Fe) present in a given vitamin/dietary tablet using a spectrophotometric method and to compare the experimentally determined iron content with the value declared on the product label.

Theory: Spectrophotometric determination of iron is based on the formation of a coloured complex of iron with a suitable colour-developing reagent. The intensity of the colour produced is proportional to the concentration of iron in the solution within the working range of the method. In a commonly used method, iron in the tablet is converted to a suitable oxidation state and reacted with 1,10-phenanthroline to form an orange-red iron complex. The absorbance of the coloured solution is measured using a spectrophotometer at the appropriate wavelength, commonly around 510 nm. A series of iron standard solutions of known concentration is used to prepare a calibration curve of absorbance versus iron concentration. The concentration of iron in the tablet solution is obtained from this calibration curve. The general relationship is based on the Beer–Lambert law:

$$A = \epsilon bc,$$

Where:

- A = absorbance
- ϵ = molar absorptivity
- b = path length of the cuvette
- c = concentration of the absorbing species

Apparatus: UV-Visible spectrophotometer, Glass or quartz cuvettes, Analytical balance, Mortar and pestle, Beakers, Conical flasks, Volumetric flasks, Measuring cylinders, Pipettes, Micropipette, Test tubes, Test-tube rack, Funnel, Filter paper, Glass rod, Wash bottle, Dropper, Weighing boat, Magnetic stirrer or hot plate.

Chemicals: Iron standard solution, 1,10-Phenanthroline solution, Hydroxylamine hydrochloride, Acetate buffer, Dilute hydrochloric acid, Distilled/deionized water, Reagent blank solution.

Procedure:

- Record the tablet details and average tablet mass.
- Grind the tablets to a uniform powder and accurately weigh the required amount.
- Extract iron using dilute HCl, warm gently if required, cool, transfer to a volumetric flask and make up the volume.
- Filter and dilute the extract to fall within the calibration range.
- Prepare iron standards and a reagent blank.
- Add reducing agent, 1,10-phenanthroline and buffer to standards and sample; allow colour to develop.
- Measure absorbance at approximately 510 nm after blank correction.
- Prepare the calibration curve by plotting iron concentration vs. absorbance.
- Obtain the calibration equation and use it to determine iron concentration in the tablet sample.

Observation:

A. Iron Standard Solutions

Standard No.	Iron Concentration (mg/L)	Absorbance
Blank	0.00	
1		
2		
3		

B. Tablet Sample

Sample	Dilution Factor	Absorbance	Iron Concentration from Calibration Curve (mg/L)
Tablet Sample			
Duplicate, if required			

Calculations:

From the calibration curve:

$$C = (A - b) / m$$

$$\text{Iron (mg/g)} = (C \times V \times DF) / W$$

$$\text{Iron per tablet (mg/tablet)} = \text{Iron in weighed powder} \times (\text{Average tablet mass} / \text{Mass of powder taken})$$

$$\% \text{ Label Claim} = (\text{Experimental iron per tablet} / \text{Labelled iron per tablet}) \times 100$$

Precautions:

- Use clean, dry glassware and accurately weigh the tablet powder.
- Ensure complete extraction of iron.
- Use accurately prepared standards and reagent blank.
- Maintain the same reagent concentrations and colour-development time.
- Use clean, dry cuvettes and the specified wavelength.
- Ensure absorbance falls within the calibration range.
- Perform duplicate/triplicate measurements when required.
- Record dilution factors accurately.
- Handle acids and reagents with proper safety precautions.

Result:

Iron content = _____ mg/tablet

% Label Claim = _____ %

Experiment No. 12

Aim: To identify and determine the concentration of caffeine and benzoic acid present in a given soft drink sample using UV-Visible spectrophotometry and appropriate calibration standards.

Apparatus: UV-Visible spectrophotometer, Quartz cuvettes, Beakers, Volumetric flasks, Measuring cylinders, Pipettes, Micropipette, Test tubes, Test-tube rack, Glass rod, Funnel, Filter paper, Wash bottle, Sample bottles.

Chemicals: Soft drink sample, Caffeine standard solution, Benzoic acid standard solution, Distilled/deionized water, Suitable solvent, Suitable buffer or dilute acid/base (if required), Blank solution.

Theory: UV-Visible spectrophotometry determines compounds based on their absorption of UV/visible radiation at characteristic wavelengths. Caffeine and benzoic acid show different absorption maxima and can therefore be identified and quantified. Their concentrations are determined using the Beer–Lambert law ($A = \epsilon bc$) and calibration curves prepared from standard solutions.

Procedure:

- Degas the soft drink sample and filter if necessary.
- Dilute the sample appropriately with the selected solvent.
- Prepare standard solutions of caffeine and benzoic acid.
- Prepare a suitable blank for spectrophotometer correction.
- Record the UV spectra of the standards and sample to determine λ_{max} .
- Measure the absorbance of the standards and construct calibration curves.
- Measure the absorbance of the diluted soft drink sample.
- Determine caffeine and benzoic acid concentrations from the calibration curves.
- Apply the dilution factor to calculate their concentrations in the original sample.
- For simultaneous analysis, use two selected wavelengths and the corresponding calibration equations.

Observation:

Sample	λ_{max} (nm)	Absorbance	Identification
Caffeine standard	_____	_____	Caffeine
Benzoic acid standard	_____	_____	Benzoic acid
Soft drink sample	_____	_____	Caffeine + Benzoic acid

Calibration & Sample Results

Parameter	Caffeine	Benzoic Acid
Calibration range (mg/L)	_____	_____
Sample absorbance	_____	_____
Concentration (mg/L)	_____	_____

Calculation:

A. Concentration from Calibration Curve

For either caffeine or benzoic acid:

$$A = mC + b$$

Therefore:

$$C = (A - b) / m$$

Where:

- **A** = absorbance of the sample
- **C** = concentration of analyte in the measured solution
- **m** = slope of calibration curve
- **b** = intercept

B. Correction for Sample Dilution

$$C_0 = C \times DF$$

Where:

- C_0 = concentration in the original soft drink
- C = concentration obtained from the calibration curve
- DF = total dilution factor

C. Caffeine Content

Caffeine concentration in original sample (mg/L) = Concentration from calibration curve $\times$ Dilution factor

D. Benzoic Acid Content

Benzoic acid concentration in original sample (mg/L) = Concentration from calibration curve $\times$ Dilution factor

Precautions:

- Use clean, dry glassware and quartz cuvettes.
- Degas soft drinks carefully to remove CO_2 and avoid foaming.
- Prepare accurate standards, samples and suitable blank.
- Calibrate the spectrophotometer before measurements.
- Keep cuvettes clean, scratch-free and handle only by the sides.
- Wipe cuvettes and maintain the same orientation.
- Keep absorbance within the calibration range.
- Apply dilution factors accurately.
- Take duplicate/triplicate readings when required.
- Avoid contamination and filter cloudy samples.
- Follow proper safety precautions while handling chemicals.

Result:

Caffeine: λ_{max} = ____ nm; Concentration = ____ mg/L; Identified/Determined.

Benzoic Acid: λ_{max} = ____ nm; Concentration = ____ mg/L; Identified/Determined.

Experiment No. 13

Aim: To carry out an aldol condensation reaction under solvent-free conditions using a grinding technique, and to demonstrate a simple, environmentally friendly method of organic synthesis with reduced use of organic solvents.

Apparatus: Mortar and pestle, Spatula, Weighing balance, Weighing boat/watch glass, Beakers, Conical flask, Measuring cylinder, Droppers/pipettes, Glass rod, Funnel, Filter paper, Büchner funnel and suction filtration apparatus, Wash bottle, Watch glass, Melting-point apparatus, Capillary tubes, Desiccator

Chemicals: Benzaldehyde, Acetone, Sodium hydroxide (NaOH), Distilled/deionized water, Washing solvent, Ethanol

Theory: Aldol condensation is a C–C bond-forming reaction between carbonyl compounds. Benzaldehyde reacts with acetone in the presence of a base to form dibenzalacetone. Under solvent-free conditions, grinding in a mortar and pestle promotes the reaction.

Procedure:

- Clean and dry the mortar and pestle.
- Accurately measure benzaldehyde, acetone and NaOH.
- Add NaOH, benzaldehyde and acetone to the mortar.
- Grind the mixture thoroughly for the prescribed time.
- Transfer the mixture to a beaker and add distilled water slowly.
- Allow the solid product to precipitate.
- Filter and wash the product with cold distilled water.
- Dry and weigh the crude product.
- Recrystallize the product using a suitable solvent, if required.
- Filter, dry and weigh the purified crystals.
- Note the appearance and determine the melting point, if required.

Observation:

S. No.	Parameter	Observation
1	Appearance of reactants	_____

2	Colour before grinding	_____
3	Colour during grinding	_____
4	Texture during reaction	_____
5	Reaction time	_____
6	Appearance of product	_____
7	Colour of product	_____

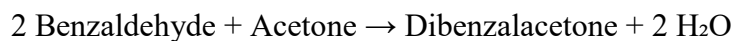
Calculations:

A. Theoretical Yield

First calculate the number of moles of each reactant:

$$\text{Moles} = \text{Mass} / \text{Molar mass}$$

For the reaction:



Determine the limiting reagent from the stoichiometric quantities.

Then:

Theoretical yield of product (g) = Moles of limiting reagent $\times$ Stoichiometric factor $\times$ Molar mass of product

B. Percentage Yield

$$\% \text{ Yield} = (\text{Actual yield} / \text{Theoretical yield}) \times 100$$

Where:

- Actual yield = mass of purified product obtained experimentally
- Theoretical yield = maximum calculated mass of product

Precautions:

- Use clean, dry apparatus and accurately weigh reactants.
- Handle benzaldehyde and acetone carefully in a well-ventilated area.
- Handle NaOH carefully as it is corrosive.

- Mix and grind the reagents gently to avoid splashing.
- Do not add solvent unless prescribed.
- Wash the product thoroughly to remove residual base.
- Dry the product completely before weighing.
- Handle hot solvents carefully during recrystallization.
- Wear appropriate personal protective equipment (PPE).

Result:

The Aldol condensation was successfully carried out under solvent-free grinding conditions.

Mass of crude product = ____ g

Mass of purified product = ____ g

Theoretical yield = ____ g

Percentage yield = ____ %

Melting point = ____ °C

Experiment No. 14

Aim: To synthesize Adipic acid from a suitable cyclohexene/cyclohexanol-based starting material using an environmentally preferable green oxidizing agent, and to demonstrate the principles of green chemistry, particularly the use of safer oxidants and reduction of hazardous waste.

Apparatus: Round-bottom flask, Reflux condenser, Heating mantle/water bath, Magnetic stirrer, Measuring cylinder, Volumetric flask, Pipettes, Beakers, Conical flasks, Dropping funnel, Thermometer, Glass rod, Funnel, Filter paper, Büchner funnel, Watch glass, Ice bath, Analytical balance, Melting-point apparatus, pH paper/pH meter, Wash bottle

Chemicals: Cyclohexene or cyclohexanol, Hydrogen peroxide (H_2O_2), Suitable catalyst, Distilled water, Acid/base, Ice, Suitable recrystallization solvent

Theory: Adipic acid is an important dicarboxylic acid used in the manufacture of nylon-6,6. In this experiment, cyclohexene or cyclohexanol is oxidized to adipic acid using hydrogen peroxide (H_2O_2) as a greener oxidant. The reaction involves oxidation and cleavage of the cyclic substrate, producing adipic acid. The product is isolated by precipitation/crystallization, filtered, dried and characterized.

Procedure:

- Clean and dry the apparatus and add the required starting material, water and catalyst.
- Assemble the flask with a reflux condenser and start stirring.
- Add hydrogen peroxide slowly and carefully.
- Maintain the prescribed temperature and reaction time.
- Observe changes in colour, temperature and precipitation.
- Cool the reaction mixture and adjust the pH if required.
- Allow adipic acid to precipitate/crystallize.
- Filter and wash the product with cold distilled water.
- Dry and weigh the crude product.
- Recrystallize the product if required, then filter and dry.

- Record the mass, appearance and melting point of the purified adipic acid.
- Observation Table

Observation:

S. No.	Parameter	Observation
1	Starting material	_____
2	Oxidant used	_____
3	Catalyst, if used	_____
4	Initial appearance	_____
5	Reaction temperature	_____
6	Reaction time	_____
7	Colour change	_____

6. Calculations:

A. Moles of Starting Material

Moles of starting material = Mass of starting material / Molar mass of starting material

B. Theoretical Yield of Adipic Acid

Determine the stoichiometric relationship between the selected starting material and adipic acid.

Theoretical yield (g) = Moles of limiting reagent $\times$ Stoichiometric factor $\times$ Molar mass of adipic acid

The molar mass of adipic acid ($C_6H_{10}O_4$) is approximately 146.14 g/mol.

C. Percentage Yield

% Yield = (Actual yield / Theoretical yield) $\times$ 100

Where:

- Actual yield = mass of purified adipic acid obtained experimentally
- Theoretical yield = maximum calculated mass of adipic acid

8. Precautions:

- Measure all reagents accurately.

- Handle hydrogen peroxide carefully as an oxidizing agent.
- Add H_2O_2 slowly to control heat and foaming.
- Use a condenser and maintain proper water circulation.
- Monitor the reaction temperature carefully.
- Keep H_2O_2 away from combustible and incompatible materials.
- Handle acids and bases carefully during pH adjustment.
- Cool the mixture before filtration.
- Dry the product completely before weighing.
- Handle hot solvents carefully during recrystallization.
- Wear appropriate PPE.

Result:

Adipic acid was successfully synthesized using a green oxidation method.

Mass of crude adipic acid = ____ g

Mass of purified adipic acid = ____ g

Theoretical yield = ____ g

Percentage yield = ____ %

Melting point = ____ °C

Experiment No 15.

Extraction of Natural Pigments (Chlorophyll, Curcumin and Anthocyanin) Using Ethanol or Water as Solvent Instead of Conventional Organic Solvents

Aim

To estimate the natural pigments—chlorophyll, curcumin, and anthocyanin—extracted from suitable plant sources using ethanol or water as eco-friendly solvents, and to compare their extraction efficiency with that of conventional organic solvents.

Apparatus

Beakers, test tubes, measuring cylinders, conical flasks, glass stirring rods, funnel, mortar and pestle, droppers, water bath or hot plate, digital balance, centrifuge, UV–Visible spectrophotometer, quartz/glass cuvettes, thermometer.

Chemicals

Fresh leaves (spinach or other green leaves) for chlorophyll, turmeric powder or fresh turmeric for curcumin, red cabbage or purple flower petals for anthocyanin, ethanol, distilled water, hydrochloric acid (dilute), sodium hydroxide solution (dilute), buffer solutions, distilled water for washing and dilution.

Theory

Natural pigments such as chlorophyll, curcumin and anthocyanin are colored compounds present in plants. They can be extracted using polar solvents such as ethanol or water, which are safer and more environmentally friendly than many conventional organic solvents. The extracted pigments can be identified and estimated by measuring their absorbance using UV–Visible spectrophotometry at their characteristic wavelengths.

Procedure

1. Take suitable fresh plant material for each pigment: green leaves for chlorophyll, turmeric for curcumin, and red cabbage or purple flower petals for anthocyanin.
2. Wash the material thoroughly with distilled water and cut it into small pieces.
3. Grind the plant material separately using a mortar and pestle.
4. Add a measured quantity of ethanol or distilled water to the ground material and mix well.
5. Allow the mixture to stand for 15–30 minutes with occasional stirring.
6. Warm the mixture gently in a water bath, if required, to enhance pigment extraction.

7. Filter the extract using filter paper and collect the clear filtrate.
8. Dilute the extracts suitably with the respective solvent.
9. Use the appropriate solvent as a blank and measure the absorbance of each extract using a UV–Visible spectrophotometer at the characteristic wavelength of the respective pigment.
10. Record the absorbance values and calculate the concentration/amount of each pigment using the appropriate formula.

Observation

S.No.	Pigment	Plant Source	Colour of Extract	λ_{max} (nm)	Absorbance
1	Chlorophyll	Green leaves	Green	~662	_____
2	Curcumin	Turmeric	Yellow	~425	_____
3	Anthocyanin	Red cabbage / purple petals	Red/Purple	~520	_____

Result

The natural pigments chlorophyll, curcumin, and anthocyanin were successfully extracted from their respective plant sources using ethanol/water as safer alternative solvents. The extracted pigments showed characteristic colours and absorbance maxima, demonstrating that ethanol and water can be effectively used for the extraction and spectrophotometric estimation of natural pigments.

Precautions

1. Use fresh and clean plant material for extraction.
2. Wash the plant material properly before grinding.
3. Use clean and dry apparatus to avoid contamination.
4. Use the same quantity of plant material and solvent for comparative studies.
5. Avoid excessive heating, as natural pigments may degrade at high temperatures.

6. Protect the extracts from direct sunlight, especially anthocyanin and chlorophyll extracts.
7. Filter the extracts properly before spectrophotometric measurement.
8. Use the appropriate solvent as the blank in the UV–Visible spectrophotometer.
9. Ensure that the cuvettes are clean and free from air bubbles before taking absorbance readings.
10. Handle ethanol and dilute acids/alkalis carefully and follow appropriate laboratory safety practices.

Experiment No 16.

Preparation of biodiesel from vegetable oil (transesterification). Green Analytical Techniques

Aim

To prepare biodiesel from vegetable oil by the process of transesterification using green analytical technique

Apparatus

Beaker, conical flask, measuring cylinder, pipette, glass rod, magnetic stirrer or hot plate, thermometer, separating funnel, funnel, filter paper, weighing balance, test tubes, test tube stand, water bath, spatula

Chemicals

Vegetable oil, methanol or ethanol, sodium hydroxide or potassium hydroxide, distilled water, anhydrous sodium sulfate

Theory

Biodiesel is a renewable fuel produced from vegetable oils or animal fats. Vegetable oils mainly contain triglycerides, which react with an alcohol such as methanol or ethanol in the presence of a base catalyst such as NaOH or KOH. This reaction is called transesterification. During transesterification, triglycerides are converted into fatty acid alkyl esters (biodiesel) and glycerol as a by-product.



The process reduces the viscosity of vegetable oil and produces a fuel with properties suitable for use as a diesel substitute. The experiment demonstrates a green analytical technique by utilizing a renewable raw material and producing a biodegradable alternative fuel.

Procedure

1. Take about 50 mL of vegetable oil in a clean, dry beaker and warm it gently to about 50–60°C.
2. Prepare the catalyst solution by dissolving a small quantity of NaOH or KOH in methanol with continuous stirring.
3. Add the prepared methanolic catalyst solution slowly to the warm vegetable oil.
4. Stir the mixture continuously for about 20–30 minutes while maintaining the temperature around 50–60°C.
5. Transfer the reaction mixture to a separating funnel and allow it to stand undisturbed for several hours.
6. Two layers will form: the upper layer is biodiesel (fatty acid methyl esters) and the lower layer is glycerol.
7. Carefully drain off the lower glycerol layer from the separating funnel.
8. Wash the biodiesel gently with a small quantity of warm distilled water to remove residual catalyst, methanol and impurities.
9. Allow the layers to separate and remove the wash water carefully.
10. Dry the biodiesel using anhydrous sodium sulfate and filter it to obtain clear biodiesel.
11. Record the appearance, colour and yield of the prepared biodiesel.

Observation

S. No.	Observation	Result
1	Appearance of vegetable oil before reaction	Clear to pale yellow, viscous liquid
2	Reaction mixture after transesterification	Two distinct layers formed
3	Upper layer	Biodiesel (fatty acid alkyl esters), comparatively clear and less viscous
4	Lower layer	Glycerol, darker and more viscous
5	Appearance of purified biodiesel	Clear to pale yellow liquid
6	Odour	Mild characteristic oily/fuel-like odour
7	Physical state of biodiesel	Liquid at room temperature

Result

Biodiesel was successfully prepared from vegetable oil by the transesterification process using an alcohol and a suitable base catalyst. The formation of a separate biodiesel layer and glycerol layer confirmed the conversion of triglycerides into fatty acid alkyl esters (biodiesel).

Precautions

1. Handle methanol/ethanol and NaOH/KOH carefully, as they can be hazardous.
2. Perform the experiment in a well-ventilated area or fume hood and avoid inhaling vapours.
3. Do not heat methanol directly over an open flame because it is highly flammable.
4. Maintain the reaction temperature around 50–60°C and avoid overheating.
5. Use clean and dry apparatus to prevent contamination and unwanted side reactions.
6. Add the catalyst solution slowly to the vegetable oil with continuous stirring.
7. Handle the separating funnel carefully and release pressure periodically if required.
8. Allow sufficient time for complete separation of the biodiesel and glycerol layers.
9. Wash the biodiesel carefully to avoid excessive mixing and emulsion formation.
10. Wear lab coat, safety goggles and suitable gloves throughout the experiment.

Experiment No 17.

Determination of acetic acid in vinegar using green titration (minimum solvent)

Aim

To determine the amount of acetic acid present in a vinegar sample by acid–base titration using a green titration method that minimizes the use of solvents and reduces chemical waste

Apparatus

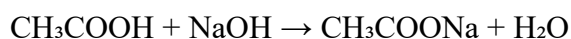
Burette, burette stand, conical flask, measuring cylinder, pipette, funnel, beaker, glass rod, wash bottle, dropper, weighing balance

Chemicals

Vinegar sample, standard sodium hydroxide (NaOH) solution, phenolphthalein indicator, distilled water

Theory

Vinegar contains acetic acid (CH₃COOH), a weak monoprotic acid. Its concentration can be determined by titrating it against a standard solution of sodium hydroxide (NaOH). The reaction is a neutralization reaction:



Phenolphthalein is used as an indicator, which changes from colourless to pale pink at the endpoint. In the green titration approach, the analysis is performed using a minimum amount of solvent, thereby reducing chemical consumption, waste generation, and environmental impact. The amount of acetic acid is calculated from the volume and concentration of NaOH required for neutralization.

Procedure

1. Rinse the burette with standard sodium hydroxide (NaOH) solution and fill it with the same solution.
2. Take a small, accurately measured volume of vinegar sample in a clean conical flask.
3. Add 2–3 drops of phenolphthalein indicator to the vinegar sample.
4. Add only a small quantity of distilled water, if required, to facilitate mixing, following the minimum-solvent approach.
5. Titrate the vinegar sample with standard NaOH solution while swirling the flask continuously.
6. Continue the titration until a faint, permanent pink colour appears.
7. Note the burette reading at the endpoint.
8. Repeat the titration until concordant readings are obtained.
9. Calculate the concentration of acetic acid in the vinegar using the volume and concentration of NaOH consumed.

Observations

S. No.	Volume of Vinegar Sample (mL)	Initial Burette Reading (mL)	Final Burette Reading (mL)	Volume of NaOH Used (mL)
I	5.0			
II	5.0			
III	5.0			

Calculations

Applying	Normality	Equation
		$N_1 V_1 = N_2 V_2$
N_1	= Normality of standardized NaOH	
V_1	= Volume of NaOH used (mL)	
N_2	= Normality of vinegar solution	

V_2 = Volume of vinegar solution taken (mL)

Result

The amount of acetic acid present in the given vinegar sample was determined by green titration using a minimum quantity of solvent.

Precautions

1. Rinse the burette properly with the NaOH solution before filling it.
2. Use accurately measured vinegar and standard NaOH solution.
3. Add only a minimum quantity of distilled water.
4. Add only 2–3 drops of phenolphthalein indicator.
5. Add NaOH slowly near the endpoint.
6. Take the endpoint as the first permanent pale-pink colour.
7. Perform titration carefully to obtain concordant readings.

Experiment No 18.

Estimation of Vitamin C in fruit juices by a greener redox titration

Aim

To estimate the amount of Vitamin C (ascorbic acid) present in a fruit juice sample by a greener redox titration method, using an environmentally friendly oxidizing agent and minimizing the use of hazardous chemicals and waste generation

Apparatus

Burette, burette stand, pipette, conical flask, measuring cylinder, volumetric flask, beaker, funnel, glass rod, wash bottle, dropper, analytical balance, fruit juice sample container

Chemicals

Fruit juice sample, standard iodine solution, potassium iodide, dilute sulphuric acid, distilled water, starch indicator

Theory

Vitamin C (ascorbic acid) is a water-soluble reducing agent that readily undergoes oxidation to dehydroascorbic acid. In the redox titration, vitamin C reduces iodine to iodide while itself being oxidized. The amount of iodine consumed is directly proportional to the amount of vitamin C present in the fruit juice. The endpoint is detected by the appearance of a persistent blue colour with starch indicator.

Procedure

1. Filter the fruit juice sample to remove pulp and suspended particles.
2. Pipette a known volume of the fruit juice into a clean conical flask.
3. Add a small quantity of distilled water and dilute the sample appropriately.
4. Add a few drops of starch indicator near the expected endpoint.
5. Fill the burette with the standard iodine solution and note the initial burette reading.
6. Titrate the fruit juice sample with iodine solution while continuously swirling the flask.
7. Continue the titration until a permanent faint blue colour appears, indicating the endpoint.
8. Note the final burette reading and calculate the volume of iodine solution consumed.
9. Repeat the titration to obtain concordant readings.
10. Calculate the amount of Vitamin C (ascorbic acid) present in the given fruit juice from the titre value.

Observation

S. No.	Parameter	Observation
1	Sample	Given fruit juice
2	Titrant	Standard iodine solution

S. No.	Parameter	Observation
3	Indicator	Starch solution
4	Initial colour of sample	Colourless/light coloured
5	Colour at endpoint	Persistent faint blue
6	End point	Appearance of permanent faint blue colour

Result

The Vitamin C (ascorbic acid) present in the given fruit juice sample was successfully estimated by greener redox titration using standard iodine solution.

Precautions

1. Use clean and properly rinsed apparatus to avoid contamination.
2. Filter the fruit juice properly before titration.
3. Use freshly prepared fruit juice sample to minimize oxidation of Vitamin C.
4. Rinse the burette with iodine solution before filling it.
5. Add the iodine solution slowly near the endpoint while continuously shaking the flask.
6. Add starch indicator near the endpoint to obtain a sharp colour change.
7. Observe the endpoint carefully to avoid over-titration.
8. Perform the titration under similar conditions for concordant results.

Experiment No 19.

Preparation of biodegradable polymer film

Aim

To prepare a biodegradable polymer film from a natural polymer and study its film-forming properties as an eco-friendly alternative to conventional plastic.

Apparatus

Beaker, measuring cylinder, glass rod, weighing balance, hot plate or heating mantle, thermometer, Petri dish or glass plate, spatula, funnel.

Chemicals

Starch, glycerol, distilled water, and acetic acid

Theory

Biodegradable polymer films can be prepared from natural polymers such as starch, which consists mainly of amylose and amylopectin. When starch is heated with water, its granules swell and gelatinize, forming a polymeric matrix. Glycerol acts as a plasticizer, reducing intermolecular forces and making the film flexible. A small amount of acetic acid helps improve the film-forming process. On drying, the mixture forms a thin, flexible film that can naturally degrade by the action of microorganisms, making it an environmentally friendly alternative to conventional plastic.

Procedure

1. Take the required amount of **starch** in a beaker and add distilled water.
2. Add a small quantity of **glycerol** as a plasticizer and mix thoroughly.
3. Add a few drops of **acetic acid** and stir the mixture well.
4. Heat the mixture gently with continuous stirring until a uniform, thick and gelatinous paste is obtained.
5. Remove the beaker from the heat and allow the mixture to cool slightly.

6. Pour the warm mixture onto a clean Petri dish or glass plate and spread it uniformly to form a thin layer.
7. Allow the film to dry at room temperature or in a drying oven at low temperature.
8. Carefully peel the dried biodegradable polymer film from the surface.
9. Observe the appearance, flexibility and physical characteristics of the prepared film.

Result

A biodegradable polymer film was successfully prepared from starch using glycerol as a plasticizer. The obtained film was thin, flexible, and suitable as an eco-friendly alternative to conventional plastic.

Precautions

1. Use clean and dry apparatus throughout the experiment.
2. Weigh the starch accurately.
3. Heat the mixture gently and stir continuously to avoid burning or lump formation.
4. Do not overheat the starch mixture.
5. Spread the mixture uniformly to obtain a film of even thickness.
6. Allow the film to dry completely before peeling it from the surface.
7. Handle the dried film carefully to prevent tearing.

Experiment No 20.

Photocatalytic degradation of a dye using TiO_2 nanoparticles.

Aim

To study the photocatalytic degradation of a dye using titanium dioxide (TiO_2) nanoparticles under UV/visible light irradiation and evaluate their effectiveness as a photocatalyst.

Apparatus

Beaker, measuring cylinder, glass rod, magnetic stirrer, magnetic bar, UV/visible light source, test tubes or quartz reaction vessel, pipette, funnel, filter paper, centrifuge or filtration assembly, stopwatch, and UV–Visible spectrophotometer.

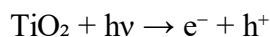
Chemicals

Titanium dioxide (TiO_2) nanoparticles, suitable dye solution (e.g., methylene blue), distilled water, and dilute hydrochloric acid or sodium hydroxide solution for pH adjustment.

Theory

Photocatalysis is a process in which light energy activates a semiconductor catalyst to produce reactive species that can break down organic pollutants. TiO_2 nanoparticles act as an effective photocatalyst because of their suitable band gap and high surface area.

When TiO₂ is exposed to UV/visible light of sufficient energy, electron–hole pairs are generated:



The photogenerated holes and electrons react with water and dissolved oxygen to form highly reactive species such as hydroxyl radicals ($\bullet\text{OH}$) and superoxide radicals ($\text{O}_2\bullet^-$). These reactive species attack the dye molecules and gradually degrade them into simpler, less harmful products such as CO₂ and H₂O.

The decrease in dye concentration can be monitored using a UV–Visible spectrophotometer by measuring the absorbance at the dye's maximum absorption wavelength (λ_{max}). Thus, the experiment demonstrates the potential of TiO₂ nanoparticles for the treatment of dye-contaminated wastewater.

Procedure

1. Prepare a dilute solution of the selected dye, such as methylene blue, using distilled water.
2. Transfer a measured volume of the dye solution into a clean beaker.
3. Add a known quantity of TiO₂ nanoparticles to the dye solution and stir well to obtain a uniform suspension.
4. Keep the suspension in the dark for a short period with continuous stirring to establish adsorption–desorption equilibrium.
5. Expose the suspension to a UV/visible light source while continuously stirring.
6. At regular time intervals, withdraw small portions of the reaction mixture.
7. Separate the TiO₂ nanoparticles by centrifugation or filtration.
8. Measure the absorbance of the clear solution using a UV–Visible spectrophotometer at the dye's λ_{max} .
9. Record the absorbance values and compare them with the initial absorbance of the dye solution.
10. Determine the extent of dye degradation from the decrease in absorbance with irradiation time.

Result

The dye solution showed a noticeable decrease in colour intensity upon exposure to UV/visible light in the presence of TiO₂ nanoparticles, confirming photocatalytic degradation. Thus, TiO₂ nanoparticles were found to be effective photocatalysts for the degradation of the dye under light irradiation.

Precautions

1. Use clean and dry glassware throughout the experiment.
2. Prepare the dye solution and TiO_2 suspension accurately.
3. Maintain a uniform concentration and amount of TiO_2 nanoparticles in each trial.
4. Ensure proper and uniform exposure of the reaction mixture to UV/visible light.
5. Avoid direct exposure of eyes and skin to UV radiation.
6. Keep the reaction mixture well stirred to ensure proper contact between TiO_2 and the dye.
7. Allow sufficient time for TiO_2 nanoparticles to settle or separate before measuring absorbance, if required.
8. Handle TiO_2 nanoparticles carefully and avoid inhalation of the fine powder.

Experiment No 21.

Convert fruit peels into natural cleaners or pH indicators

Aim

To convert fruit peels into eco-friendly natural cleaners or natural pH indicators and study their effectiveness as sustainable alternatives to conventional chemical products.

Apparatus

Beakers, measuring cylinder, glass rod, funnel, filter paper, mortar and pestle, knife, chopping board, weighing balance, test tubes, test tube stand, dropper, Petri dishes, pH paper or universal indicator, and distilled water.

Chemicals

Fruit peels (orange/lemon), distilled water, vinegar (acetic acid), baking soda (sodium bicarbonate), and a mild liquid soap.

Theory

Fruit peels contain natural compounds such as organic acids, essential oils, flavonoids, and pigments. These compounds can be extracted using water and used for eco-friendly applications. Citrus peels, such as orange and lemon, contain citric acid and essential oils, which help in cleaning and removing grease. Some fruit peels contain natural pigments that change colour depending on the pH of a solution. These pigments can therefore act as natural pH indicators. The colour change can be observed when the peel extract is added to acidic, neutral, or basic solutions. Thus, fruit peels can be converted into useful natural cleaners or biodegradable pH indicators, reducing dependence on synthetic chemicals and promoting waste utilization.

Procedure

1. Collect fresh fruit peels such as orange or lemon peels and wash them thoroughly with water.
2. Cut the peels into small pieces and grind them using a mortar and pestle.
3. Add the crushed peels to a beaker containing distilled water and heat gently for 10–15 minutes.
4. Allow the mixture to cool and filter it using filter paper and a funnel. Collect the filtrate as the fruit-peel extract.
5. For preparing a natural cleaner, use the citrus-peel extract and mix it with a small quantity of vinegar and mild liquid soap.
6. For preparing a natural pH indicator, add a few drops of the fruit-peel extract separately to acidic, neutral, and basic solutions.
7. Observe and record the colour changes produced by the extract in the different solutions.
8. Test the prepared natural cleaner on a greasy or stained surface and observe its cleaning action.
9. Compare the observed cleaning and indicator properties and assess the usefulness of fruit peels as eco-friendly alternatives.

Result

The fruit peels were successfully converted into an eco-friendly natural cleaner and pH indicator. The citrus-peel extract showed cleaning action due to its natural acidic compounds and essential oils, while the indicator extract exhibited visible colour changes in solutions of different pH, demonstrating its potential as a natural pH indicator.

Precautions

1. Use **fresh and clean fruit peels** free from dirt and pesticides.
2. Wash the peels thoroughly before extraction.
3. Handle the knife and heating apparatus carefully.
4. Avoid overheating the fruit-peel extract, as it may destroy some natural compounds.
5. Use clean and dry glassware to prevent contamination.
6. Do not mix the prepared indicator with strong acids or bases.
7. Label all test tubes properly to avoid confusion between samples.
8. Store the prepared extract in a clean, closed container and use it within a short period.

Experiment No 22.

Monitoring the esterification of acetic acid with ethanol (acid-catalysed) and identification of reactants and product (ethyl acetate).

Aim

To study the acid-catalysed esterification of acetic acid with ethanol to form ethyl acetate, and to monitor the reaction by identifying the reactants and the ester product.

Apparatus

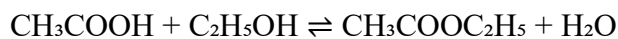
Test tubes, test tube stand, measuring cylinder, beakers, glass rod, pipette/dropper, conical flask, water bath, thermometer, funnel, filter paper, and test-tube holder.

Chemicals

Acetic acid (CH_3COOH), ethanol ($\text{C}_2\text{H}_5\text{OH}$), concentrated sulphuric acid (H_2SO_4) as catalyst, sodium bicarbonate solution, and distilled water.

Theory

Esterification is a chemical reaction in which a carboxylic acid reacts with an alcohol in the presence of an acid catalyst to form an ester and water. In this experiment, acetic acid reacts with ethanol in the presence of concentrated sulphuric acid to produce ethyl acetate, which has a characteristic fruity smell. The reaction is:



Concentrated H_2SO_4 acts as a catalyst and also helps remove water, favouring ester formation. The reaction is reversible and reaches equilibrium. The formation of ethyl acetate can be identified by its characteristic fruity odour and by its physical properties.

Procedure

1. Take about 5 mL of acetic acid and 5 mL of ethanol in a clean test tube.
2. Carefully add 2–3 drops of concentrated sulphuric acid and mix gently.
3. Place the test tube in a warm water bath for about 10–15 minutes. Do not heat directly.
4. Allow the mixture to cool and carefully pour it into a beaker containing a small amount of sodium bicarbonate solution.
5. Observe the formation of an oily layer and note its characteristic fruity odour carefully by wafting, not by directly inhaling.
6. The fruity-smelling product indicates the formation of ethyl acetate ($\text{CH}_3\text{COOC}_2\text{H}_5$).
7. Record the observations and identify the reactants and product of the esterification reaction.

Result

Ethyl acetate was successfully prepared by the acid-catalysed esterification of acetic acid with ethanol. The formation of ethyl acetate was indicated by its characteristic fruity odour, confirming the esterification reaction.

Precautions

1. Handle concentrated sulphuric acid carefully and avoid contact with skin.
2. Do not heat the reaction mixture directly; use a water bath.
3. Use clean and dry apparatus to avoid contamination.
4. Add sulphuric acid slowly and carefully.
5. Do not inhale the vapours directly; identify the ester by gentle wafting.

6. Handle ethanol carefully as it is highly flammable.
7. Allow the reaction mixture to cool before further testing.
8. Perform the experiment in a well-ventilated area.

23)Prepare 2,4-dinitrophenyl hydrazones of acetone, 2-butanone, hexan-2,3-one and separate the mixture of hydrazone by paper chromatography. Measure the R_f value in each case

Principle

Aldehydes and ketones react with 2,4-dinitrophenylhydrazine (2,4-DNPH) in an acidic medium to form crystalline 2,4-dinitrophenylhydrazones. The different hydrazones have different polarities and therefore travel different distances on chromatography paper when developed with a suitable solvent.

Chemical

Acetone, 2-Butanone, Hexan-2,3-one, 2,4-Dinitrophenylhydrazine reagent, Suitable acidic/alcoholic reaction medium, Ethanol, Chromatography paper, Developing solvent appropriate for the laboratory's chromatography system

Apparatus

Test tubes, Droppers/capillary tubes, Beaker or chromatography chamber, Pencil and ruler, Water bath, Filter paper

Theory

2,4-Dinitrophenylhydrazones are crystalline derivatives formed by the condensation reaction of aldehydes and ketones with 2,4-dinitrophenylhydrazine (2,4-DNP reagent) in an acidic medium, with elimination of water. In this experiment, acetone, 2-butanone, and hexan-2,3-one react with 2,4-dinitrophenylhydrazine to form their corresponding yellow/orange 2,4-dinitrophenylhydrazones. The mixture of these hydrazones can be separated by paper chromatography because the different compounds have different affinities for the stationary phase (paper) and the mobile phase (solvent), causing them to travel different distances.

Procedure

A. Preparation of individual hydrazones

1. Label three clean test tubes as A, B, and C for acetone, 2-butanone, and hexan-2,3-one, respectively.

2. Add a small quantity of the respective ketone to each test tube.
3. Add the required amount of 2,4-dinitrophenylhydrazine reagent to each tube.
4. Mix thoroughly and allow the reaction to proceed in the acidic medium.
5. If necessary, warm the tubes gently in a water bath according to the laboratory protocol.
6. Formation of a yellow to orange precipitate indicates formation of the corresponding 2,4-dinitrophenylhydrazone.
7. Allow the products to cool and collect the precipitates by filtration.
8. Wash the precipitates with a small quantity of cold solvent and allow them to dry.
9. Dissolve a small amount of each hydrazone in a suitable volatile solvent to prepare solutions for chromatography.

B. Preparation of the mixture

10. Mix small quantities of the three hydrazone solutions to obtain a single mixture.
11. Keep the individual hydrazone solutions as reference samples.

C. Paper chromatography

12. Cut a strip of chromatography paper of suitable size.
13. Using a pencil, draw a straight baseline approximately 1–2 cm from one end of the paper.
14. Mark separate positions on the baseline for the mixture and, if required, the individual hydrazone standards.
15. Apply small spots of the samples using a capillary tube. Allow each spot to dry before applying another small portion so that compact spots are obtained.
16. Pour the selected developing solvent into the chromatography chamber to a depth below the baseline.
17. Place the paper in the chamber, ensuring that the solvent does not directly cover the sample spots.
18. Close the chamber and allow the solvent to move up the paper.
19. Remove the paper when the solvent front has travelled a suitable distance.
20. Immediately mark the solvent front with a pencil.
21. Allow the chromatogram to dry.
22. Locate the separated yellow/orange hydrazone spots and mark their centres.

Result

The mixture of the three 2,4-dinitrophenylhydrazones is separated into distinct chromatographic spots. The (Rf) value of each hydrazone is calculated from its distance travelled relative to the solvent front.

Precautions

1. 2,4-Dinitrophenylhydrazine (2,4-DNP) reagent should be handled carefully and contact with skin or eyes should be avoided.
2. Perform the reaction in a well-ventilated area or fume hood, as the reagents and solvents may produce harmful vapours.
3. Use clean and dry apparatus to avoid contamination of the hydrazone products.
4. Add the ketone samples and 2,4-DNP reagent in the specified quantities to obtain proper derivatives.
5. During paper chromatography, apply small and concentrated spots on the baseline without damaging the paper.

24) Preparation of silver nanoparticles using green synthesis

MATERIALS & Chemicals: Silver Nitrate AgNO₃ 1mM solution, Fresh plant leaves 10g, Distilled water, Ethanol for washing

Apparatus: Beakers 250mL, Bunsen burner/Hot plate, Whatman filter paper No.1, Magnetic stirrer, Centrifuge, UV-Vis Spectrophotometer, pH meter, Microwave/Oven, Aluminum foil.

Theory

Ag⁺ ions from AgNO₃ are reduced to Ag⁰ by phytochemicals present in plant extract like flavonoids, phenols, terpenoids.

These phyto chemicals also act as capping agents to prevent aggregation.

Color change from colorless → yellow → brown indicates AgNP formation due to Surface Plasmon Resonance.

Reaction: $\text{Ag}^+ + \text{Phytochemical} \rightarrow \text{AgO NPs} + \text{Oxidized phytochemical}$

Common plants used: Azadirachta indica Neem, Ocimum sanctum Tulsi, Aloe vera, Green tea, Citrus peel³.

PROCEDURE

Step 1: Preparation of Plant Extract

1. Collect fresh leaves. Wash thoroughly 3-4 times with distilled water to remove dust.
2. Chop 10g leaves into small pieces. Add to 100 mL distilled water in 250mL beaker.
3. Boil at 60-80°C for 10-15 min on hot plate.
4. Do not boil vigorously. Cool and filter through Whatman filter paper. Collect clear filtrate. This is plant extract. Store at 4°C. Use within 24hr.

Step 2: Synthesis of Silver Nanoparticles

1. Prepare 1mM AgNO₃ solution: Dissolve 0.0169g AgNO₃ in 100 mL distilled water.
2. Store in amber bottle/cover with foil. Light sensitive.
3. Take 90 mL of 1mM AgNO₃ solution in 250mL beaker. Add 10 mL plant extract dropwise to AgNO₃ solution with continuous stirring at 500 rpm.
4. Ratio: 9:1 ratio of AgNO₃ : Plant extract. Can vary 1:9 to 9:1 for optimization. Maintain pH 8-10 by adding 0.1M NaOH.
5. Alkaline pH gives faster reduction. Stir at room temp 25-30°C for 30-60 min OR heat at 60°C for 15-20 min. Observation: Solution color changes from colorless → pale yellow → reddish brown → dark brown. This confirms AgNP formation.

Step 3: Purification Centrifuge

1. the reaction mixture at 8000-10000 rpm for 15 min. Discard supernatant.
2. Collect pellet of AgNPs. Wash pellet 2-3 times with distilled water and ethanol to remove impurities.
3. Dry pellet in hot air oven at 60°C for 4-6 hours OR lyophilize.

Observation

Parameter	observation
Initial colour of AgNO ₃	Colourless
After adding extract	Pale yellow
After 30 min	Reddish brown
Final colour	Dark brown
UV –Vis maximum wavelength	420-440

Precautions

1. Use clean, dry, and properly sterilized glassware to avoid contamination during synthesis.
2. Prepare the plant extract freshly and filter it properly to remove suspended particles.
3. Use accurately measured quantities and concentrations of silver nitrate solution and plant extract.
4. Handle silver nitrate carefully, as it can cause stains and irritation to the skin and eyes.
5. Add the plant extract to the silver nitrate solution slowly with continuous stirring to obtain uniform nanoparticles.

25) Synthesis of ZnO nanoparticles by precipitation method.

Chemicals Required

Zinc acetate dihydrate, or another suitable soluble zinc salt ,Sodium hydroxide (NaOH)
,Distilled/deionized water ,Ethanol, if required for washing

Apparatus

Beakers ,Magnetic stirrer with hot plate ,Magnetic stir bar ,Measuring cylinder or volumetric flask
,Dropping pipette/burette , pH meter , Centrifuge or vacuum filtration setup , Drying oven , Ceramic crucible , Muffle furnace, Mortar and pestle, Analytical balance

Theory

ZnO nano particles can be prepared by precipitating a zinc hydroxide precursor from an aqueous zinc salt solution using a base such as sodium hydroxide. The precipitate is subsequently washed, dried, and calcined to convert the precursor into ZnO nanoparticles.

5. Procedure

A. Preparation of zinc precursor solution

1. Accurately weigh the required amount of zinc acetate dihydrate.
2. Dissolve it in distilled/deionized water to prepare the desired concentration of zinc precursor solution.
3. Stir the solution continuously until a clear solution is obtained.

B. Precipitation

4. Prepare an aqueous NaOH solution of suitable concentration separately.
5. Place the zinc precursor solution on a magnetic stirrer and begin stirring.
6. Add the NaOH solution slowly and dropwise to the zinc precursor solution while maintaining continuous stirring.
7. Monitor the pH during addition. Continue addition until the required alkaline pH is reached, typically around pH 10–12, according to the laboratory protocol.
8. Continue stirring for approximately 30–60 minutes to promote complete precipitation and obtain a uniform precursor.
9. Allow the suspension to age for several hours, or overnight if specified by the experimental protocol.

C. Collection and washing

10. Collect the precipitated zinc hydroxide precursor by centrifugation or filtration.
11. Wash the precipitate several times with distilled/deionized water to remove soluble salts and excess alkali.
12. If required, perform a final wash with ethanol.
13. Continue washing until the washings are substantially free of residual soluble impurities.

D. Drying

14. Transfer the washed precipitate to a clean drying dish.
15. Dry it in an oven at approximately 80–100 °C until a constant mass is obtained.
16. Gently grind the dried material using a clean mortar and pestle.

E. Calcination

17. Transfer the dried precursor into a suitable ceramic crucible.
18. Place the crucible in a muffle furnace.
19. Calcine the material at approximately 400–500 °C for about 2–3 hours, or according to the validated laboratory protocol.
20. Allow the furnace and sample to cool to room temperature before removing the crucible.
21. Grind the resulting material gently to obtain a fine ZnO nanopowder.
22. Store the ZnO nanoparticles in a clean, dry, properly labelled container.

Result

ZnO nanoparticles are synthesized successfully by the precipitation method. The final product is obtained as a fine ZnO powder after precipitation, washing, drying, and calcinations.

Precautions

1. Use clean and dry glassware to prevent contamination of the ZnO nanoparticles.
2. Accurately measure all chemicals, especially the zinc salt and precipitating agent, to maintain the required concentration.
3. Add the precipitating agent slowly with continuous stirring to obtain a uniform precipitate.
4. Maintain the required pH and temperature throughout the precipitation process.
5. Avoid unnecessary exposure of the prepared nanoparticles to dust and other contaminants.

26) Preparation of copper oxide nano particle

Chemicals:

Copper Sulphate pentahydrate $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 5g, Sodium Hydroxide NaOH 2g, Distilled water, Ethanol

Apparatus: Beakers 250mL, Magnetic stirrer + stir bar, Burette, pH meter, Funnel, Filter paper/Buchner funnel, Hot plate, Centrifuge, Muffle furnace 300-500°C, Mortar pestle, Measuring cylinder.

Theory

Copper(II) salt reacts with base NaOH to form Cu(OH)₂ precipitate. On heating/calcination, Cu(OH)₂ decomposes to black CuO nanoparticles.



PROCEDURE

Step 1: Preparation of Solutions

- 1) Dissolve 5g CuSO₄·5H₂O in 100 mL distilled water.
- 2) Stir to get clear blue solution.
- 3) Dissolve 2g NaOH in 100 mL distilled water. Stir to get clear solution.

Step 2: Precipitation of Cu(OH)₂

- 1) Take Solution A in 250mL beaker on magnetic stirrer at 500 rpm.
- 2) Heat to 60-70°C. Add Solution B dropwise using burette with continuous stirring. Maintain pH 10-12.
- 3) Blue precipitate of Cu(OH)₂ forms immediately. Continue stirring for 30 min at 60°C for uniform particle size.
- 4) Age the precipitate for 1 hour at room temp.

Step 3: Washing & Drying

- 1) Filter the blue precipitate using Buchner funnel.
- 2) Wash 3-4 times with hot distilled water to remove Na₂SO₄ and excess NaOH.
- 3) Wash once with ethanol to reduce agglomeration.
- 4) Dry the precipitate in hot air oven at 80°C for 4-5 hours. You get blue Cu(OH)₂ powder.

- 5) Calcination to CuO NPs.
- 6) Transfer dried powder to a crucible.
- 7) Heat in muffle furnace at 350-400°C for 2 hours.
- 8) Color changes from Blue → Black.
- 9) This is CuO NPs.
- 10) Cool to room temp in desiccators .
- 11) Grind gently in mortar pestle to get fine black powder

Yield & Observation;

Stage	Colour	Product
After Precipitation	Light blue	Cu(OH) ₂
After calcinations	black	CuO nanoparticle

Theoretical Yield – 1.6g CuO from 5g CuSO₄·5H₂O

Particle Size – 20-50 nm

Precautions

1. Use clean and dry glassware to avoid contamination of the copper oxide nanoparticles.
2. Accurately measure the copper salt and other reagents according to the prescribed procedure.
3. Add the precipitating/reducing agent slowly with continuous stirring to obtain a uniform product.
4. Maintain the required pH, temperature, and reaction time throughout the synthesis.

27) Photocatalytic Effect using silver ZnO and Copper oxide nanoparticles

Chemical

Silver (Ag) nanoparticles ,Zinc oxide (ZnO) nanoparticles ,Copper oxide (CuO) nanoparticles ,Selected dye solution, e.g. methylene blue ,Distilled/deionized water

Appratus

Beakers or conical flasks ,Measuring cylinders or volumetric flasks ,Magnetic stirrer,Magnetic stir bars,Pipettes ,Centrifuge or filtration setup ,UV–Visible spectrophotometer ,Light source appropriate for the selected photocatalyst ,Aluminium foil or other light-blocking material

Principle

Photocatalytic degradation occurs when semiconductor nanoparticles absorb light of suitable energy and generate electron–hole pairs. These charge carriers react with water and dissolved oxygen to produce reactive oxygen species such as hydroxyl radicals, which can oxidatively degrade organic dye molecules. Silver nanoparticles can also exhibit photocatalytic/plasmon-assisted activity under suitable illumination. The extent of degradation is determined by measuring the decrease in dye concentration, generally using UV–Visible spectroscopy.

4. Preparation of Dye Solution

1. Prepare a known concentration of the selected dye using distilled/deionized water.
2. Prepare sufficient solution for the control and nanoparticle experiments.
3. Record the initial absorbance of the dye solution at its wavelength of maximum absorption ($\lambda_{\max}$).

5. Experimental Groups

Prepare separate reaction systems:

System	Dye	Nanoparticle
Control	Dye solution	None
A	Dye solution	Ag nanoparticles
B	Dye solution	ZnO nanoparticles
C	Dye solution	CuO nanoparticles

Keep the dye concentration, solution volume, nanoparticle concentration, irradiation time, and other experimental conditions identical for meaningful comparison.

6. Procedure

A. Dark adsorption equilibrium

1. Transfer an equal volume of dye solution into four labelled reaction vessels.
2. Add the same predetermined mass/concentration of Ag, ZnO, and CuO nanoparticles to the respective vessels.
3. Keep one vessel without nanoparticles as the control.
4. Stir all nanoparticle suspensions in the dark for approximately 30 minutes, or for the period established by the laboratory protocol.
5. This dark period allows adsorption of dye onto the nanoparticle surface to approach equilibrium.
6. Collect a sample from each suspension and separate the nanoparticles by centrifugation or filtration.
7. Measure the absorbance of the clear supernatant/filtrate at ($\lambda_{\max}$).

B. Photocatalytic irradiation

8. Place the reaction vessels at a fixed distance from the selected light source.
9. Start irradiation while maintaining continuous stirring.
10. Keep the irradiation conditions identical for Ag, ZnO, and CuO experiments.
11. Withdraw aliquots at predetermined time intervals, for example **0, 15, 30, 45, 60, 75 and 90 minutes**.
12. Separate the nanoparticles from each aliquot by centrifugation or filtration before spectrophotometric measurement.
13. Measure the absorbance of each clear sample at the dye's ($\lambda_{\max}$).
14. Record all absorbance values.

Conclusion

The photocatalytic activities of Ag, ZnO, and CuO nanoparticles are evaluated by monitoring the decrease in dye absorbance during light irradiation. The nanoparticle showing the highest percentage degradation and/or highest apparent rate constant exhibits the greatest photocatalytic activity under the conditions used

Precautions

1. Use clean and dry glassware to avoid contamination of the photocatalytic reaction.
2. Accurately weigh the required amounts of Ag, ZnO, and CuO nanoparticles before use.
3. Handle nanopowders carefully and avoid inhalation or direct contact with the particles.
4. Use the same concentration and volume of the test solution for comparative experiments.
5. Maintain constant pH, temperature, irradiation time, and stirring conditions throughout the experiment.

28) Water Purification Test using silver ZnO and Copper oxide nanoparticles

MATERIALS & APPARATUS Chemicals: Synthesized Ag NPs, ZnO NPs, CuO NPs, Contaminated water sample / Pond water, Nutrient Agar, Sterile distilled water

Apparatus: Beakers 250mL, Magnetic stirrer, UV lamp/Visible light, Micropipette, Petri plates, Laminar air flow, Incubator 37°C, Autoclave, Spectrophotometer/NTU meter, Filter paper, Whatman No.14.

Theory

Nanoparticles have high surface area and release ions/ROS that kill bacteria and adsorb impurities.
Ag NPs: Release Ag⁺ ions → damage bacterial DNA, cell membrane
ZnO NPs: Generate ROS under light → kill microbes + photocatalytic degradation
CuO NPs: Release Cu²⁺ ions → protein denaturation + antibacterial
Efficiency is checked by: Bacterial count reduction and Turbidity/Color removal.

PROCEDURE

Part A: Treatment of Water with NPs -- Sample Collection: Collect 500 mL contaminated water. Note initial pH, turbidity, odor.

Setup 4 Beakers 100mL each:

Beaker 1: Control - 100mL water, No NPs

Beaker 2: 100 mL water + 10 mg Ag NPs

Beaker 3: 100mL water + 10 mg ZnO NPs

Beaker 4: 100mL water + 10 mg CuO NPs

Treatment:

- 1) Stir all beakers at 300 rpm for 60 min at room temp.
- 2) For ZnO: Expose to UV/ sunlight for 30 min for photocatalysis.
- 3) Ag and CuO work in dark also.
- 4) Separation: After treatment, filter each sample through Whatman filter paper to remove NPs.

Part B: Test 1 - Antibacterial Activity by Plate Count Method

- 1) Take 1mL of treated water from each beaker. Do serial dilution up to 10^{-4} in sterile water. Spread 100 μ L of 10^{-3} and 10^{-4} dilution on Nutrient Agar plates. Incubate at 37°C for 24 hours.

Count CFU:

- 1) Count colonies and calculate CFU/mL = No. of colonies x Dilution factor x 10
- Part C: Test 2 - Turbidity & Physicochemical Parameters Measure Turbidity of each sample using NTU meter or by absorbance at 600 nm. Measure pH and Color change visually. % Removal = $[(\text{Initial} - \text{Final})/\text{Initial}] \times 100$

RESULT INTERPRETATION Best Antibacterial: Usually Ag NPs > CuO NPs > ZnO NPs

Best for dye/turbidity: ZnO NPs due to photo catalysis

Safe Limit: Treated water should have <100 CFU/mL for drinking as per WHO

Precautions

1. Use clean and dry glassware to prevent contamination of the water samples.

2. Collect the water samples in clean, properly labelled containers and avoid external contamination.
3. Accurately measure the required amounts of silver, ZnO, and CuO nanoparticles.
4. Handle nanoparticle powders carefully and avoid inhalation or direct contact with them.
5. Maintain the same volume of water sample, nanoparticle dosage, pH, temperature, and treatment time for comparative tests.

29)Antibacterial Activity of Nanoparticles

Chemicals Nutrient Agar, Sterile Distilled water, Ethanol 70%, Nanoparticle suspension in water/DMSO, Positive control: Standard antibiotic disc/Streptomycin 10µg/mL, Negative control: Solvent used for NPs

Apparatus: Petri plates sterile, Micropipette + sterile tips, Cork borer 6mm, Inoculation loop, Sterile cotton swabs, Laminar air flow, Incubator 37°C, Autoclave, UV-Vis for NP characterization⁴.

PRINCIPLE - Nanoparticles diffuse from the well into agar medium and inhibit bacterial growth. The zone of inhibition formed around the well is directly proportional to antibacterial activity. Larger zone = higher activity. Common test organisms:

PROCEDURE

Step 1:

- 1) Preparation of Media & Plates Prepare Nutrient Agar as per manufacturer.
- 2) Sterilize by autoclaving at 121°C, 15 psi for 15 min.
- 3) Pour 20-25 mL molten agar into sterile petri plates under laminar air flow.
- 4) Let it solidify. Label plates.

Step 2: 1) Preparation of Bacterial Inoculum Take 18-24 hr fresh bacterial culture in nutrient broth. Adjust turbidity to 0.5 McFarland standard $\sim 1.5 \times 10^8$ CFU/mL using sterile saline/UV-Vis

at 600nm. Dip sterile cotton swab into inoculum. Streak evenly on agar surface 3 times, rotating plate 60° each time. Swab rim last.

Step 3: Well Loading Make 4-6 wells of 6mm diameter in agar using sterile cork borer. Wells should be 2cm apart from each other and from edge. Load wells with:

Well 1: Test - Nanoparticle suspension 50µL [e.g. 1mg/mL]

Well 2: Test - Nanoparticle suspension 100µL [e.g. 1mg/mL]

Well 3: Positive Control - Standard antibiotic 50µL

Well 4: Negative Control - Solvent used for NPs 50µL Let the liquid diffuse for 30 min at room temp.

Step 4: In cubation Invert plates and incubate at 37°C for 24 hours in incubator.

Step 5: Observation & Measurement After 24hr, measure diameter of clear zone of inhibition around each well in mm using ruler/vernier caliper. Include diameter of well in measurement .No zone in negative control confirms activity is due to NPs only.

Conclusion

The photocatalytic activities of Ag, ZnO, and CuO nanoparticles are evaluated by monitoring the decrease in dye absorbance during light irradiation. The nanoparticle showing the highest percentage degradation and/or highest apparent rate constant exhibits the greatest photocatalytic activity under the conditions used.

Precaution

- 1) Use sterile glassware, Petri plates, pipettes, and other instruments to prevent contamination.
- 2) Handle bacterial cultures using aseptic techniques throughout the experiment.
- 3) Use the required nano particle concentration accurately for each test sample.
- 4) Prepare the bacterial suspension with a uniform and appropriate cell concentration for reliable comparison

30) Qualitative Analysis of soil, fertilizers and pesticide samples Analysis of organic carbon, available nitrogen, phosphorus and potassium

Apparatus

- Beakers and conical flasks ,Measuring cylinders ,Volumetric flasks,Pipettes ,Funnels and filter paper, Test tubes, Shaker ,pH meter ,Spectrophotometer/colorimeter, Flame photometer
- Burette and titration apparatus ,Hot plate or digestion/distillation apparatus, where required

Principle

Soil fertility is assessed by determining important chemical parameters:

- **Organic carbon:** estimated by oxidation of soil organic matter using a dichromate-based method and measuring the residual/produced chromium species.
- **Available nitrogen:** extracted or converted into a measurable nitrogen form and estimated by a suitable colorimetric or distillation method.
- **Available phosphorus:** extracted from soil using an appropriate extractant and determined colorimetrically after formation of a phosphor molybdate complex.
- **Available potassium:** extracted using an appropriate ammonium salt extractant and measured using a flame photometer.

Qualitative analysis of fertilizers and pesticide samples involves characteristic chemical tests to identify major nutrients or functional chemical groups.

3. Sample Collection and Preparation

Soil sample

1. Collect representative soil samples from the selected sampling area using clean tools.
2. Remove stones, roots, leaves, and other visible debris.
3. Mix the samples thoroughly to obtain a representative composite sample.
4. Air-dry the sample in a clean area.
5. Crush gently and pass through an appropriate soil sieve, commonly **2 mm** for routine soil analysis.

6. Store the prepared sample in a clean, labelled container.

Fertilizer sample

7. Collect a representative fertilizer sample.
8. Grind lumps gently and mix thoroughly.
9. Store in a labelled, airtight container.

5. Reagents

The exact reagents and concentrations should follow the laboratory's validated soil-testing method.

Typical reagents include:

- Potassium dichromate and concentrated sulfuric acid for organic carbon determination
- Appropriate reagents for available nitrogen determination
- Sodium bicarbonate or another validated phosphorus extractant, depending on soil type
- Ammonium acetate extractant for available potassium
- Ammonium molybdate and related reagents for phosphorus color development
- Standard potassium solution for flame-photometer calibration
- Standard solutions appropriate for nitrogen determination
- Distilled/deionized water

A. Qualitative Analysis of Soil

6. Physical Examination

1. Record the colour, texture, odour, and general appearance of the soil.
2. Determine the soil pH using a suitable soil-water suspension.
3. Record the observations.

7. Qualitative Tests for Major Nutrients

1. Prepare an aqueous or appropriate extract of the soil sample.

2. Perform qualitative tests for nitrogen, phosphorus, and potassium using the laboratory's standard reagent procedures.
3. Compare the test response with appropriate blanks/standards.
4. Record the results as positive, negative, or trace where applicable.

Determination of Organic Carbon

8. Principle

Soil organic carbon is oxidized by an excess of dichromate in strongly acidic conditions. The amount of oxidant consumed is related to the organic carbon present and is determined by titration or another validated measurement procedure.

9. Procedure

1. Accurately weigh the prescribed quantity of air-dried, sieved soil into a clean flask.
2. Add the specified volume of standard potassium dichromate solution.
3. Carefully add concentrated sulfuric acid according to the validated laboratory procedure.
4. Mix and allow the reaction to proceed for the specified time.
5. Dilute with distilled water as required.
6. Add the specified indicator.
7. Titrate the excess dichromate with the standardized reducing solution.
8. Perform a reagent blank under identical conditions.
9. Calculate the organic carbon content from the difference between blank and sample titration values.

Determination of Available Nitrogen

Theory

Available nitrogen in soil is converted/extracted into a measurable nitrogen form and quantified using an appropriate standardized method, such as alkaline permanganate distillation followed by titration.

Procedure

1. Accurately weigh the prescribed amount of soil into the distillation apparatus.
2. Add the required reagents according to the validated nitrogen-analysis method.
3. Distil the liberated ammonia into a suitable receiving solution.
4. Continue distillation until the prescribed amount of distillate has been collected.
5. Determine the amount of ammonia by titration with standardized acid.
6. Run a reagent blank.
7. Calculate the available nitrogen content and express the result in the required units, commonly kg N/ha.

Determination of Available Phosphorus

Principle

Available phosphorus is extracted from soil using an appropriate extractant. The extracted phosphate reacts with molybdate-based reagents to form a coloured complex. The intensity of colour is measured spectrophotometrically and compared with a standard calibration curve.

Procedure

1. Accurately weigh the prescribed quantity of soil.
2. Add the specified phosphorus extractant.
3. Shake the suspension for the required time.
4. Filter the extract to obtain a clear solution.
5. Pipette a measured aliquot of the extract into a volumetric flask or test tube.
6. Add the specified colour-developing reagents.
7. Make up to the required volume and allow the colour to develop for the specified time.
8. Measure the absorbance at the wavelength specified by the validated method.
9. Prepare a phosphorus standard calibration curve.
10. Determine the phosphorus concentration of the soil extract from the calibration curve.
11. Express the result in the required units, commonly kg P/ha.

Determination of Available Potassium

Principle

Available potassium is extracted from soil using an appropriate extracting solution, commonly neutral ammonium acetate. The potassium concentration in the extract is measured using a flame photometer.

Procedure

1. Weigh the prescribed quantity of air-dried soil.
2. Add the required volume of ammonium acetate extractant.
3. Shake the suspension for the specified time.
4. Filter the extract.
5. Prepare potassium standards covering the expected concentration range.
6. Calibrate the flame photometer using the blank and potassium standards.
7. Aspirate the soil extract into the flame photometer.
8. Record the potassium reading.
9. Calculate the available potassium concentration and express it in the required units.

Qualitative Analysis of Fertilizer Samples

Procedure

1. Record the fertilizer type and label information.
2. Dissolve an appropriate amount of the fertilizer in distilled water or prepare the recommended extract.
3. Filter if necessary.
4. Perform suitable qualitative tests for the claimed nutrient components.
5. Compare the observations with appropriate positive controls.
6. Record the identified nutrient components.

Qualitative Analysis of Pesticide Samples

Procedure

1. Record the pesticide formulation and active ingredient information from the product label, if available.
2. Prepare the sample only using the laboratory's approved pesticide-analysis procedure.
3. Use appropriate extraction and separation techniques where required.
4. Identify the active ingredient using a validated analytical method such as TLC, UV–Visible spectroscopy, FTIR, HPLC, or GC, depending on the pesticide.
5. Compare the analytical response with an authenticated reference standard.
6. Record the result.

Conclusion

The soil sample is qualitatively examined and its organic carbon, available nitrogen, available phosphorus, and available potassium contents are determined using standard analytical procedures. Fertilizer and pesticide samples are examined using suitable qualitative and, where necessary, instrumental methods

OPERATION OF LABORATORY CENTRIFUGE STANDARD OPERATING PROCEDURE (SOP)

AIM: To provide a standardized procedure for the safe and proper operation of a laboratory centrifuge for the separation of components of liquid samples based on differences in density.

SCOPE: This SOP is applicable to students, research scholars, laboratory staff and faculty members authorized to operate the centrifuge in the laboratory.

PRINCIPLE: Centrifugation separates suspended or dissolved components of a sample by applying centrifugal force. During rotation, particles of greater effective density move toward the bottom/outer region of the centrifuge tube, while less dense components remain closer to the top.

REQUIRED EQUIPMENT AND ACCESSORIES

- Laboratory centrifuge
- Appropriate centrifuge rotor
- Compatible centrifuge tubes
- Tube holders/adapters, where applicable

PRE-OPERATION SAFETY CHECKS

Before starting the centrifuge:

1. Place the centrifuge on a stable, level and vibration-free surface.
2. Inspect the centrifuge, rotor, buckets, adapters and rotor lid for cracks, corrosion, deformation or other visible damage.
3. Do not use damaged tubes, rotors, buckets or accessories.
4. Ensure that the rotor is correctly installed and securely locked according to the manufacturer's instructions.
5. Use only tubes and adapters approved for the particular rotor.
6. Check the maximum permitted speed/RCF and maximum sample load for the rotor and tubes.
7. Fill the centrifuge tubes with the required sample volume without exceeding their specified capacity.
8. Close sample tubes securely.
9. Balance the tubes before loading them into the rotor. Opposing tubes should be of the same type and contain equal or appropriately balanced masses.
10. Place the tubes symmetrically in the rotor.
11. Ensure that the rotor lid is properly fitted and secured before operation.

Proper symmetrical loading and balancing are essential because asymmetric loading can cause excessive vibration and damage to the centrifuge or rotor.

OPERATING PROCEDURE

1. Switch on the centrifuge.
2. Install the appropriate rotor and accessories if they are not already installed.
3. Load the balanced centrifuge tubes symmetrically into the rotor.
4. Secure the rotor lid.
5. Close the main centrifuge lid properly.
6. Set the required speed according to the experimental procedure and rotor specifications.
7. Set the required centrifugation time.
8. Set the temperature, if the centrifuge is refrigerated and temperature control is required.
9. Check all settings before starting.
10. Start the centrifuge.
11. Observe the centrifuge briefly during acceleration. Excessive vibration, unusual noise or abnormal movement indicates a possible problem.
12. If unusual vibration or noise occurs, stop the centrifuge immediately and allow the rotor to come to a complete stop before investigating.
13. Allow the centrifuge to complete the programmed run.
14. Do not attempt to open the lid while the rotor is rotating.
15. After the rotor has completely stopped, open the lid.
16. Remove the tubes carefully without disturbing the separated layers or pellet.
17. Switch off the centrifuge after completion of work, if required by the laboratory's operating practice.

Manufacturer guidance specifically emphasizes securing the rotor/rotor lid, symmetrical loading and using tubes appropriate for the rotor and maximum load.

SAMPLE REMOVAL

1. Open the centrifuge only after the rotor has completely stopped.
2. Remove tubes carefully and keep them upright where required.
3. Avoid shaking or disturbing the tubes if a clear supernatant/pellet separation is required.
4. Transfer the separated fraction according to the experimental procedure.

5. Dispose of samples and waste according to the laboratory's chemical/biological waste-disposal procedure.

EMERGENCY PROCEDURE

In case of excessive vibration or unusual noise

1. Immediately press the STOP/START-STOP button.
2. Do not open the lid until the rotor has completely stopped.
3. Do not attempt to restrain or stop the rotor manually.
4. After complete stoppage, switch off the instrument.
5. Check for tube breakage, incorrect balancing, loose rotor or other visible problems.
6. Do not restart the centrifuge until the cause has been identified and corrected.

CLEANING AND MAINTENANCE

1. Switch off the centrifuge before cleaning.
2. Remove the rotor and accessories when required by the manufacturer's instructions.
3. Wipe the rotor chamber and external surfaces with an appropriate laboratory cleaning agent.
4. Clean spills immediately using the appropriate laboratory decontamination procedure.
5. Dry the rotor and chamber thoroughly after cleaning.
6. Inspect the rotor and accessories regularly for corrosion, cracks, scratches or deformation.
7. Do not use a rotor or accessory showing significant corrosion or mechanical damage.
8. Follow the manufacturer's instructions for rotor cleaning, disinfection and maintenance.

Rotor care and inspection are important for safe operation and equipment life.

PRECAUTIONS

- Never operate the centrifuge with the lid open.
- Never open the lid while the rotor is rotating.
- Never operate an improperly balanced centrifuge.
- Never exceed the manufacturer's specified RPM/RCF.
- Never exceed the maximum permitted tube or rotor capacity.
- Never use cracked, damaged or chemically incompatible tubes.
- Never use a damaged or corroded rotor.

- Use only compatible rotors, buckets, adapters and tubes.
- Do not leave an operating centrifuge unattended when laboratory rules require supervision.
- For hazardous, infectious or aerosol-generating samples, use the appropriate sealed/aerosol-tight rotor or containment system.
- Always follow the specific manufacturer's operating manual for the centrifuge model installed in the laboratory.

SHUTDOWN

After completion of the experiment:

1. Ensure that the rotor has completely stopped.
2. Remove all samples.
3. Inspect the rotor and chamber for spills or contamination.
4. Clean and dry the equipment as required.
5. Switch off the centrifuge.
6. Leave the equipment in the condition specified by the manufacturer's instructions.

Project

Synthesis and Chemosensing Properties of Naphthalene-Based Dual-Channel Probe

Aim

To synthesize a naphthalene-based benzimidazole chemosensor by condensation of o-phenylenediamine with 2-hydroxynaphthaldehyde in acetic acid and to study its absorption and emission properties for the selective detection of Cu^{2+} ions.

Apparatus/Equipments

Round-bottom flask, reflux condenser, magnetic stirrer/hot plate, TLC apparatus, filtration setup, UV–Visible spectrophotometer, Fluorescence Spectrophotometer, quartz cuvettes, volumetric flasks and micropipettes, Magnetic stirrer, Rotary Evaporator (Rotovap).

Chemicals

2-Hydroxy-1-naphthaldehyde, Hydrazine hydrate, Absolute Ethanol, Dichloromethane (DCM), Acetonitrile, Nitrate salts of Cu^{2+} , Hg^{2+} , Zn^{2+} , etc.

Theory

The naphthalene-based benzimidazole chemosensor is synthesized through the condensation of o-phenylenediamine with 2-hydroxynaphthaldehyde under acidic conditions using acetic acid. The synthesized molecule contains nitrogen and oxygen donor sites capable of interacting with Cu^{2+} ions. Coordination of Cu^{2+} with the chemosensor alters its electronic environment, resulting in changes in its UV–Visible absorption and fluorescence emission properties. These changes are used to investigate its sensing behaviour.

Procedure:

Step 1: Condensation Reaction

1. Dissolve 1 mmol of the naphthalene precursor (e.g., 2-hydroxy-1-naphthaldehyde) in 20 mL of glacial acetic acid.
2. Add a stoichiometric amount of o-phenylenediamine to the solution.
3. Reflux the mixture for 4–6 hours at 80°C with constant stirring. Monitor the reaction progress using Thin Layer Chromatography (TLC).

Step 2: Solvent Removal (using Rotary Evaporator)

Once the reaction is complete (confirmed by TLC):

1. Transfer the reaction mixture into a round-bottom flask (RBF).
2. Mounting: Attach the RBF to the Rotary Evaporator using a Keck clip.
3. Vacuum & Rotation: Start the rotation (approx. 100-150 rpm) and gradually apply vacuum.
4. Heating: Lower the flask into the water bath heated to 45°C (appropriate for Ethanol).

5. Concentration: Evaporate the solvent until the volume is reduced to approx. 2-3 mL or until a crude solid begins to precipitate. This step is crucial for increasing the yield during the subsequent crystallization phase.
6. Venting: Stop the rotation, release the vacuum, and carefully remove the RBF.

Step 3: Purification

1. Cool the concentrated mixture in an ice bath to induce complete precipitation.
2. Filter the crude product and wash with cold ethanol.

Chemosensing Evaluation Procedure

Step 1: Stock Solution Preparation

1. Prepare a stock solution of the synthesized probe (e.g., 1.0×10^{-3} M) in an appropriate solvent (Acetonitrile).
2. Prepare stock solutions of various metal ions (1.0×10^{-2} M) using their nitrate or perchlorate salts.

Step 2: UV-Visible Absorption Studies

1. Take 3 mL of the probe solution in a quartz cuvette.
2. Record the UV–Visible absorption spectrum of the free chemosensor and note its λ_{max} .
3. Titration: Add incremental amounts (e.g., 1-10 equivalents) of the target analyte.
4. After each addition, mix thoroughly and record the spectra.
5. Record the absorption spectrum after each addition.

Step 3: Emission Studies

1. Record the fluorescence emission spectrum of the free chemosensor at a suitable excitation wavelength (absorption maxima if absorption).
2. Add successive concentrations of Cu^{2+} solution.
3. Record the fluorescence emission spectrum after each addition.
4. Note the change in fluorescence intensity and emission wavelength.
5. Compare the emission response of the free sensor with that of the sensor– Cu^{2+} system.

Selectivity Study

1. Prepare solutions of selected competing metal ions.
2. Add equal concentrations of each metal ion separately to the chemosensor solution.
3. Record the corresponding absorption and/or fluorescence response.
4. Compare these responses with the response obtained for Cu^{2+} .
5. A distinct response towards Cu^{2+} indicates selective sensing.

Expected Result

The synthesized naphthalene-based benzimidazole compound is expected to exhibit characteristic absorption and fluorescence properties. Addition of Cu^{2+} ions is expected to produce a significant change in its absorption and/or fluorescence response due to interaction of Cu^{2+} with the donor atoms of the chemosensor. The observed spectral changes demonstrate its potential for selective optical detection of Cu^{2+} ions.

Precautions:

1. Ensure the glass flask is securely attached with a Keck clip. Check for cracks in the glassware to avoid implosion under vacuum. Empty the waste solvent receiver regularly.
2. Use clean quartz cuvettes for spectroscopic measurements.
3. Maintain constant sensor concentration and solvent composition.
4. Add Cu^{2+} solution accurately in small aliquots.
5. Allow sufficient time for complex formation after each addition.

Project

Synthesis and Theoretical Analysis of Push-Pull Chromophores

Aim

To synthesize a series of substituted benzimidazole-based chromophores by condensation of o-phenylenediamine with different aromatic aldehydes under acidic conditions and to study their structural and electronic properties using theoretical calculations.

Apparatus

Round-bottom flask, condenser, magnetic stirrer/hot plate, measuring cylinders, pipettes, filtration apparatus, vacuum filtration setup, weighing balance and melting-point apparatus.

Chemicals

o-Phenylenediamine, Benzaldehyde, p-Nitrobenzaldehyde, p-Chlorobenzaldehyde, p-Aminobenzaldehyde, glacial acetic acid, Ethanol, Dichloromethane, Hexane, Distilled water

Theory

Benzimidazole-based chromophores can be synthesized by the condensation of o-phenylenediamine with aromatic aldehydes under acidic conditions. Different substituents on the aldehyde, such as $-\text{NO}_2$, $-\text{Cl}$ and $-\text{NH}_2$, modify the electronic properties of the resulting benzimidazole derivatives. The synthesized compounds can therefore be compared to establish the relationship between the nature of the substituent and the electronic/optical properties of the chromophore. The synthesized chromophores are subsequently analysed theoretically to investigate their molecular geometry, frontier molecular orbitals and electronic properties.

Procedure

Synthesis

1. Take an o-phenylenediamine (1 mmol) in a round-bottom flask.
2. Add the selected aromatic aldehyde (1 mmol) in approximately equimolar proportion.
3. Add a 10 ml of acetic acid.
4. Refluxed the reaction mixture under controlled conditions with continuous stirring, as appropriate for the selected reaction conditions.
5. Monitor the progress of the reaction by a suitable technique such as TLC, where available.
6. After completion of the reaction, allow the mixture to cool.
7. Pour the reaction mixture into water, if appropriate, to precipitate the product.
8. Filter the solid product and wash it thoroughly to remove unreacted starting materials and acid.
9. Purify the product by recrystallization using a suitable solvent.

10. Dry the purified product and determine its yield and melting point.
11. Repeat the procedure separately using the different aldehydes.
12. Note the melting point and record the UV–Visible.

Theoretical Analysis

1. Draw the molecular structures of the synthesized chromophores using suitable computational software.
2. Optimize the molecular geometries using an appropriate computational method.
3. Calculate the HOMO and LUMO energies and the HOMO–LUMO energy gap.
4. Examine the distribution of HOMO and LUMO over the donor, π -conjugated system and acceptor portions of the molecule.
5. Calculate other relevant properties such as dipole moment and molecular electrostatic potential, where appropriate.
6. Compare the calculated electronic properties of the different substituted benzimidazole chromophores.
7. Correlate the theoretical results with the experimentally observed UV–Visible absorption properties.

Data Analysis

Compare the synthesized compounds with respect to: UV–Visible λ_{max} , HOMO energy, LUMO energy, HOMO–LUMO energy gap, Dipole moment, Effect of electron-donating and electron-withdrawing substituents

The results can be used to establish a structure–property relationship.

Expected Result

Electron-withdrawing ($-\text{NO}_2$) and electron-donating ($-\text{NH}_2$) substituents are expected to produce different electronic effects, which can be evaluated through their UV–Visible spectra and calculated frontier molecular orbital properties.

Precautions

1. Handle o-phenylenediamine and substituted aldehydes with appropriate protective equipment.
2. Perform acid-mediated reactions carefully and under appropriate ventilation.

Project

A novel chemosensor for selective detection Cupric ions: Interplay between theory and experiment.

Aim

To investigate the selective sensing behaviour of a benzimidazole-based chemosensor towards Cu^{2+} ions and correlate the experimental spectroscopic response with theoretical calculations.

Equipments/Apparatus/Software Required

UV–Visible spectrophotometer, quartz cuvettes, volumetric flasks, pipettes/micropipettes and suitable, Microsoft Excel, Computational Chemistry Software (Gaussian).

Chemicals

Benzimidazole-based chemosensor, Acetonitrile (AR Grade), Cu^{2+} , Zn^{2+} , Ni^{2+} , Co^{2+} , Fe^{3+} , Mg^{2+} and Ca^{2+} as nitrates or chlorides, distilled water

Theory

Benzimidazole contains nitrogen donor atoms that can participate in coordination with metal ions. Interaction of Cu^{2+} with the benzimidazole-based chemosensor can alter its electronic structure and produce measurable changes in UV–Visible absorption. Theoretical calculations are used to identify the probable Cu^{2+} binding site and compare the electronic properties of the free sensor and its Cu^{2+} complex. The predicted interaction is then verified experimentally through spectroscopic titration and selectivity studies.

Procedure

Theoretical Study

1. Draw and optimize the molecular structure of the benzimidazole-based chemosensor using suitable computational software.
2. Identify the potential nitrogen donor sites involved in Cu^{2+} coordination.
3. Calculate the HOMO and LUMO energies and other relevant molecular properties.
4. Construct and optimize the proposed sensor– Cu^{2+} complex.
5. Compare the optimized structures and electronic properties of the free sensor and Cu^{2+} complex.
6. Use the theoretical results to propose the probable Cu^{2+} binding mode and sensing mechanism.

UV–Visible Spectroscopic Study

1. Prepare a dilute solution of the benzimidazole-based chemosensor in a suitable solvent.
2. Prepare a standard Cu^{2+} stock solution.
3. Record the UV–Visible spectrum of the sensor alone using the solvent as blank.

4. Add successive known aliquots of Cu^{2+} solution to the sensor solution.
5. Mix thoroughly and allow sufficient time for complex formation.
6. Record the absorption spectrum after each addition.
7. Note changes in absorbance and λ_{max} .
8. Continue the titration until no significant further spectral change is observed.
9. Extract the data in an excel sheet.

Selectivity Study

1. Prepare solutions of selected competing metal ions, including Zn^{2+} , Ni^{2+} , Co^{2+} , Fe^{3+} , Mg^{2+} and Ca^{2+} .
2. Add equal concentrations of each metal ion separately to identical sensor solutions.
3. Record the absorption response.
4. Compare the responses with that obtained for Cu^{2+} .
5. A significantly stronger or characteristic response towards Cu^{2+} indicates selective sensing.

Data Analysis

Plot the following graphs in Microsoft Excel: Absorbance vs. Cu^{2+} concentration; Sensor response towards Cu^{2+} and competing metal ions; Experimental spectral changes in comparison with theoretical predictions.

Expected Result

The benzimidazole-based chemosensor is expected to show a concentration-dependent optical response upon addition of Cu^{2+} ions. The observed spectral changes should correlate with the theoretically predicted coordination of Cu^{2+} through the nitrogen donor site(s) of the benzimidazole-based receptor. The combined theoretical and experimental results can therefore be used to establish the probable binding mode and sensing mechanism.

Precautions

1. Maintain constant sensor concentration and solvent composition.
2. Add Cu^{2+} solution accurately in small aliquots.
3. Allow sufficient time for complex formation after each addition.

Project

Spectrophotometric Analysis of a Coumarin-Based Dual Optical Turn-On Chemosensor for Selective Detection of Urea

Aim

To study the spectrophotometric response of a coumarin-based dual optical turn-on chemosensor towards urea and evaluate its selectivity for the detection of urea.

Apparatus

UV–Visible spectrophotometer, Fluorescence spectrophotometer, Quartz cuvettes, Analytical balance, Volumetric flasks, Micropipette/pipettes, Test tubes or sample vials, Volumetric Flasks

Chemicals

Coumarin-based chemosensor, Urea, AR Grade Acetonitrile, Distilled/deionized water.

Theory

A coumarin-based chemosensor interacts selectively with urea through molecular interactions such as hydrogen bonding or other analyte–sensor interactions. This interaction changes the electronic environment of the coumarin chromophore and produces a measurable change in its optical properties. The response can be studied by UV–Visible spectroscopy fluorescence spectroscopy. An increase in absorption and/or fluorescence intensity after addition of urea is referred to as a “turn-on” response. The response of the sensor is measured at different concentrations of urea. The change in absorbance or fluorescence intensity is then plotted against urea concentration to evaluate the sensing behaviour.

Procedure

Preparation of Sensor and Urea Solution

1. Accurately weigh the required quantity of the coumarin-based chemosensor/urea to make 1×10^{-3} M solution.
2. Dissolve sensor in AR grade acetonitrile and urea in water.
3. Transfer the solution to a volumetric flask and make up the volume with the same solvent.
4. Prepare suitable working solutions by dilution of the stock solution.

Procedure for absorption/emission Analysis

1. Switch on the UV–Visible spectrophotometer.
2. Select a suitable wavelength range covering the absorption region of the coumarin sensor.
3. Use the corresponding solvent as the blank.

- Record the absorption spectrum of the sensor in the absence of urea.
- Note the wavelength of maximum absorption (λ_{max}) and corresponding absorbance.
- Add a small, known volume of urea solution to the sensor solution.
- Mix properly and allow sufficient time for interaction between the sensor and urea.
- Record the absorption spectrum again.
- Continue the addition of successive aliquots of urea.
- Continue until the change in absorbance becomes nearly constant.

Fluorescence Analysis

- Prepare the sensor solution at an appropriate concentration.
- Record the fluorescence spectrum of the sensor in the absence of urea.
- Select a suitable excitation wavelength based on the absorption spectrum.
- Record the emission spectrum and note the fluorescence intensity.
- Add successive known amounts of urea.
- Mix and allow the required equilibration time after each addition.
- Record the fluorescence spectrum after every addition.
- Note the fluorescence intensity at the emission maximum.
- Continue until the fluorescence response reaches a plateau.

Observation Table

S. No.	Urea concentration	Absorbance/Fluorescence intensity
1		
2		
3		
4		

Calculations

Plot the following graphs:

- Absorbance vs. urea concentration.
- Fluorescence intensity vs. urea concentration,

Precautions

- Use clean quartz cuvettes for all spectroscopic measurements.
- Avoid fingerprints and scratches on the optical surfaces of the cuvette.
- Use the same solvent system throughout the experiment.
- Maintain the sensor concentration constant during the titration as far as possible.

Project

To Derive a Structure–Property Relationship by Studying the Solvatochromic Effect of Push–Pull Organic Chromophores

Aim

To study the solvatochromic behaviour of a push–pull organic chromophore in solvents of different polarity and to establish a relationship between molecular structure, solvent polarity and optical properties.

Equipments/Apparatus Required

UV–Visible spectrophotometer, quartz cuvettes, volumetric flasks, pipettes/micropipettes and analytical balance.

Chemical

Push–pull organic chromophore (4-Dimethylamino-4'-nitroazobenzene (DMNA)), solvents of different polarity (n-Hexane, Toluene, Dichloromethane, Chloroform, Tetrahydrofuran, Ethyl acetate, Acetonitrile, Dimethyl sulfoxide, Methanol, Ethanol), distilled water.

Theory

Push–pull organic chromophores contain an electron-donating group (donor) and an electron-accepting group (acceptor) connected through a conjugated π -system. Such molecules often exhibit solvatochromism, in which their absorption and/or emission wavelength changes with the polarity of the surrounding solvent. The change occurs because solvent polarity can stabilize the ground and excited electronic states to different extents. The resulting shift in the absorption or fluorescence maximum provides information about the electronic structure and charge-transfer character of the chromophore.

Procedure

1. Prepare a dilute stock solution of the push–pull chromophore in a suitable solvent.
2. Prepare solutions of the same chromophore concentration in selected solvents having different polarities.
3. Record the UV–Visible absorption spectrum of the chromophore in each solvent using the respective solvent as blank.
4. Note the wavelength of maximum absorption (λ_{max}) and corresponding absorbance for each solvent.
5. Compare the λ_{max} values obtained in solvents of different polarity.
6. Identify the direction and magnitude of the spectral shift (bathochromic or hypsochromic shift).

7. Relate the observed spectral changes to solvent polarity and the donor–acceptor nature of the chromophore.
8. Plot λ_{max} versus solvent polarity parameter (e.g., dielectric constant) to investigate the structure–property relationship.

Observation Table

S. No.	Solvent	Polarity parameter	Absorption λ_{max} (nm)

Result

4-Dimethylamino-4'-nitroazobenzene (DMNA) exhibited solvent-dependent changes in its UV–Visible absorption maximum when studied in solvents of different polarity. The results demonstrate that the molecular structure and solvent environment significantly influence the optical properties of the chromophore, establishing a structure–property relationship.

Precautions

1. Use clean quartz cuvettes and appropriate solvent blanks.
2. Maintain approximately the same chromophore concentration in all solvents.
3. Use dilute solutions to avoid aggregation and excessive absorbance.
4. Avoid exposure of the chromophore solutions to strong light.
5. Ensure that the chromophore is completely dissolved before recording spectra.
6. Maintain constant temperature during measurements.

Project: Glucose-Assisted Synthesis of MnFe_2O_4 Nanoparticles

To synthesize MnFe_2O_4 nanoparticles using glucose as fuel followed by calcination in a muffle furnace.

Apparatus

Erlenmeyer flasks, titration flask, magnetic stirrer, hot plate, measuring cylinder, pipette/dropper, pH paper, filter apparatus, hot-air oven, alumina crucible, muffle furnace, crucible tongs and desiccator.

Chemicals

FeSO_4 , $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, Glucose, NH_4OH , EtOH

Theory

Ferrite nanoparticles are mixed-metal oxides nanoparticles with the general composition (MFe_2O_4), where (M) may be Mn, Co, Ni, Zn, etc. MnFe_2O_4 is a spinel ferrite possessing useful magnetic, electrical and optical properties. Because of their small particle size, high surface area and tunable magnetic behaviour, ferrite nanoparticles are important in catalysis, sensors, magnetic separation, environmental remediation. In the auto-combustion method, metal salts act as oxidizing precursors while glucose act as organic fuel/complexing agents. The metal ions are first mixed with the sugars to obtain a homogeneous precursor. Addition of NH_4OH promotes formation of a metal-hydroxide/organic precursor. On heating, the concentrated precursor undergoes a rapid self-sustaining redox combustion, in which the organic fuel reacts with oxidizing metal species, producing heat and gaseous products. This combustion promotes intimate mixing of Mn and Fe at the molecular level and forms a fine oxide precursor. Subsequent calcination removes residual organic matter and promotes crystallization of the spinel MnFe_2O_4 phase.

Procedure:

1. Solution A: Dissolve 1 mmol $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ and 3 mmol glucose in 10 mL deionized water in a 100 mL Erlenmeyer flask.
2. Stir Solution A for 20 minutes at room temperature.
3. Solution B: Dissolve 2 mmol FeSO_4 and 6 mmol glucose in 10 mL deionized water in another 100 mL Erlenmeyer flask.
4. Stir Solution B separately for 20 minutes at room temperature.
5. Transfer and mix Solutions A and B in a 100 mL flask to obtain Solution C.
6. Stir Solution C for 50 minutes at room temperature to ensure homogeneous mixing.
7. Add NH_4OH solution gradually to neutralize/adjust the solution as required, followed by stirring for 1 hour.

8. Heat the resulting mixture on a hot plate at 70 °C for 1 hour to obtain the precursor.
9. Transfer the obtained product to a suitable crucible and calcine at 800 °C for 1 hour in a muffle furnace.
10. Allow the product to cool, grind it gently, and collect the resulting MnFe_2O_4 nanoparticles for further characterization.

Result

MnFe_2O_4 nanoparticles were successfully synthesized by the glucose assisted auto-combustion method followed by calcination at 800 °C for 1 hour. A fine dark brown/black powder was obtained, which was subjected to further characterization to confirm the formation and properties of MnFe_2O_4 nanoparticles.

Precautions:

1. Use a lab coat, gloves and safety goggles.
2. NH_4OH should be handled carefully and preferably in a well-ventilated area.
3. Avoid inhalation of nanoparticle powder.
4. Use crucible tongs and heat-resistant protection when handling furnace-heated materials.
5. Do not touch the hot crucible directly.

Aim: To synthesize ZnO nanoparticles by a precipitation method and study the effect of calcination temperature on their optical properties using UV-Visible spectroscopy.

Apparatus

Erlenmeyer flasks, beakers, magnetic stirrer, hot plate, measuring cylinder, pipette/dropper, pH paper or pH meter, filter apparatus, hot-air oven, alumina/porcelain crucibles, muffle furnace, crucible tongs, desiccator and UV-Visible spectrophotometer.

Chemicals

Zinc acetate dihydrate, Sodium hydroxide, Ethanol, Distilled/deionized water

Theory

ZnO is a semiconductor material with a wide direct band gap of approximately 3.3–3.4 eV at room temperature. ZnO nanoparticles have attracted considerable interest because of their optical, electronic, photocatalytic and sensing properties. Calcination is a thermal treatment in which a precursor is heated at a controlled temperature to remove volatile and organic components and promote formation and crystallization of the metal oxide.

The calcination temperature can affect the crystallinity, particle growth and defect concentration of ZnO, which in turn can influence its optical absorption. UV-Visible spectroscopy is therefore used to compare ZnO samples prepared at different calcination temperatures.

The optical band gap can be calculated as:

$$E_g = 1240/\lambda$$

where:

- E_g = optical band gap (eV)
- λ = wavelength (nm)

Procedure

1. Dissolve 2.19 g $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ in 50 mL deionized water and stir for 20 minutes.
2. Add NaOH dropwise to the zinc acetate solution with continuous stirring until pH 9–10.
3. Continue stirring for 30 minutes to obtain a white $\text{Zn}(\text{OH})_2$ precipitate.
4. Filter the precipitate and wash it several times with distilled water.
5. Dry the precipitate in a hot-air oven at 80–100 °C.
6. Divide the dried product into three approximately equal portions.
7. Calcine the portions separately at 400 °C, 500 °C and 600 °C, respectively, for 2 hours in a muffle furnace.
8. Allow the samples to cool in a desiccator and grind them gently.

9. Disperse a small amount of each ZnO sample in ethanol and record the UV-Visible absorption spectrum using ethanol as the blank.
10. Compare the absorption maxima of the three samples and calculate their approximate optical band gaps.

Observations:

S.No.	Sample Code	Calcination temperature	Absorption Maxima	Band Gap
1	Zn-1	400°C		
2	Zn-2	500°C		
3	Zn-3	600°C		

Results

ZnO nanoparticles were successfully synthesized by the precipitation method and calcined at different temperatures. The effect of calcination temperature on the optical properties of the synthesized ZnO nanoparticles was studied using UV-Visible spectroscopy.

Project: Determination and Comparative Analysis of Ash Content in Different Plant Materials

Aim: To determine and compare the ash content of different plant materials by controlled incineration using a muffle furnace.

Apparatus

Alumina crucibles, crucible tongs, hot-air oven, muffle furnace, desiccator, mortar and pestle, weighing bottle.

Chemicals

Different dry plant materials, such as: Rice husk, Wheat straw, Dry leaves

Theory

Plant materials contain organic components such as cellulose, hemicellulose, lignin, proteins and carbohydrates, along with inorganic mineral components. When a plant sample is heated strongly in the presence of air, the organic matter undergoes combustion and is converted mainly into gaseous products such as CO_2 and water vapours. The inorganic mineral constituents remain as a non-combustible residue known as ash.

The ash content provides an approximate measure of the total inorganic/mineral matter present in a plant material. Different plant materials contain different amounts of minerals; therefore, their ash contents can be compared. The muffle furnace provides controlled high-temperature heating and allows the organic matter to be completely incinerated while retaining the mineral residue. The ash is generally obtained as a grey, white or off-white residue.

Thus, comparison of ash percentage provides a simple quantitative method for comparing the inorganic mineral content of different plant materials.

Procedure

1. Collect different plant materials and remove visible dirt and foreign matter.
2. Dry the samples in a hot-air oven at approximately 105 °C until constant mass.
3. Grind each dried sample separately into small pieces or coarse powder.
4. Clean, dry and weigh empty crucibles. Record their masses as (W-1).
5. Add approximately 2 g of each dried plant sample to a separate crucible and weigh. Record as (W-2).
6. Place the crucibles in a muffle furnace and heat gradually to approximately 550 °C.
7. Maintain the temperature for about 3–4 hours, or until the sample is completely ashed.
8. Allow the crucibles to cool and transfer them to a desiccator.
9. Weigh the crucibles containing ash and record the mass as (W-3).
10. Then calculate the ash percentage.

Observations

Calculation of Ash %

$$\text{Ash (\%)} = (\text{Mass of ash} / \text{Mass of sample}) \times 100$$

Mass of sample: W-2–W-1

Mass of ash: W-3–W-1

S. No.	Plant material	Empty crucible W-1g	Crucible + sample W-2g	Crucible + ash W-3g	Ash (%)
1	Rice husk				
2	Wheat straw				
3	Dry leaves				

Result

The ash content of the selected plant materials was determined by muffle-furnace incineration, and the samples were compared on the basis of their percentage ash content.

Aim: To study the effect of calcination temperature on the formation of iron oxide prepared from an iron hydroxide precursor.

Apparatus

Beakers, magnetic stirrer, hot plate, filter funnel, hot-air oven, crucibles, muffle furnace, desiccator and crucible tongs.

Chemicals Required

Ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), Ammonium hydroxide (NH_4OH), Distilled water

Theory

Iron oxides are important inorganic materials with applications in pigments, catalysis, magnetic materials and environmental chemistry. Iron hydroxide undergo dehydration and thermal transformation on heating to form iron oxide. The extent and nature of this transformation depend on calcination temperature. In this project, the same precursor is calcined at different temperatures while keeping the calcination time constant. The resulting samples are compared through their mass and colour. This demonstrates the influence of thermal treatment on iron oxide formation.

Procedure

1. Dissolve an appropriate amount of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in distilled water.
2. Stir the solution until a clear solution is obtained.
3. Add dilute NH_4OH slowly with continuous stirring.
4. A reddish-brown precipitate of iron oxide is formed.
5. Allow the precipitate to age for 1 hour.
6. Filter and wash the precipitate with distilled water.
7. Dry at 80–100 °C until constant mass.
8. Divide the dried product into three equal portions.
9. Calcine the portions separately at 400, 500 and 600 °C for 2 hours in a muffle furnace.

10. Cool the samples in a desiccator and weigh them.

11. Record their colour and mass.

Observation

Sample	Calcination temperature (°C)	Final mass (g)	Colour
Fe-1	400		
Fe-2	500		
Fe-3	600		

Calculation

$$\% \text{Mass loss} = \frac{W_i - W_f}{W_i} \times 100$$

where W_i and W_f are the masses before and after calcination, respectively.

Result

Iron oxide was prepared and the effect of calcination temperature on its formation was studied.

Projects

Aim: To synthesize Manganese Ferrites using Fructose as a chelating agent.

Apparatus

Erlenmeyer flasks, titration flask, magnetic stirrer, hot plate, measuring cylinder, pipette/dropper, pH paper, filter apparatus, hot-air oven, alumina crucible, muffle furnace, crucible tongs and desiccator.

Chemicals

FeSO_4 , $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, Fructose, NH_4OH , EtOH

Theory

Ferrite nanoparticles are mixed-metal oxides nanoparticles with the general composition (MFe_2O_4), where (M) may be Mn, Co, Ni, Zn, etc. MnFe_2O_4 is a spinel ferrite possessing useful magnetic, electrical and optical properties. Because of their small particle size, high surface area and tunable magnetic behaviour, ferrite nanoparticles are important in catalysis, sensors, magnetic separation, environmental remediation. In the auto-combustion method, metal salts act as oxidizing precursors while glucose act as organic fuel/complexing agents. The metal ions are first mixed with the sugars to obtain a homogeneous precursor. Addition of NH_4OH promotes formation of a metal-hydroxide/organic precursor. On heating, the concentrated precursor undergoes a rapid self-sustaining redox combustion, in which the organic fuel reacts with oxidizing metal species, producing heat and gaseous products. This combustion promotes intimate mixing of Mn and Fe at the molecular level and forms a fine oxide precursor. Subsequent calcination removes residual organic matter and promotes crystallization of the spinel MnFe_2O_4 phase.

Procedure:

11. Solution A: Dissolve 1 mmol $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ and 3 mmol fructose in 10 mL deionized water in a 100 mL Erlenmeyer flask.
12. Stir Solution A for 20 minutes at room temperature.
13. Solution B: Dissolve 2 mmol FeSO_4 and 6 mmol glucose in 10 mL deionized water in another 100 mL Erlenmeyer flask.
14. Stir Solution B separately for 20 minutes at room temperature.
15. Transfer and mix Solutions A and B in a 100 mL flask to obtain Solution C.
16. Stir Solution C for 50 minutes at room temperature to ensure homogeneous mixing.

17. Add NH_4OH solution gradually to neutralize/adjust the solution as required, followed by stirring for 1 hour.
18. Heat the resulting mixture on a hot plate at $70\text{ }^\circ\text{C}$ for 1 hour to obtain the precursor.
19. Transfer the obtained product to a suitable crucible and calcine at $800\text{ }^\circ\text{C}$ for 1 hour in a muffle furnace.
20. Allow the product to cool, grind it gently, and collect the resulting MnFe_2O_4 nanoparticles for further characterization.

Result

MnFe_2O_4 nanoparticles were successfully synthesized by the glucose assisted auto-combustion method followed by calcination at $800\text{ }^\circ\text{C}$ for 1 hour. A fine dark brown/black powder was obtained, which was subjected to further characterization to confirm the formation and properties of MnFe_2O_4 nanoparticles.

Precautions:

6. Use a lab coat, gloves and safety goggles.
7. NH_4OH should be handled carefully and preferably in a well-ventilated area.
8. Avoid inhalation of nanoparticle powder.
9. Use crucible tongs and heat-resistant protection when handling furnace-heated materials.
10. Do not touch the hot crucible directly.

Project: Synthesis of Heterocycles using Manganese Iron Oxide

Aim: Synthesis of Benzimidazoles Using Manganese Ferrite (MnFe_2O_4) Nanoparticles as a Catalyst

Apparatus

Erlenmeyer flasks, titration flask, magnetic stirrer, hot plate, measuring cylinder, pipette/dropper, pH paper, filter apparatus, hot-air oven, alumina crucible, muffle furnace, crucible tongs and desiccator.

Chemicals

FeSO_4 , $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, Fructose, NH_4OH , EtOH

Theory

Benzimidazole derivatives are synthesized by the condensation of *o*-phenylenediamine with aromatic aldehydes. MnFe_2O_4 nanoparticles are used as a heterogeneous catalyst to facilitate the reaction. The magnetic nature of MnFe_2O_4 allows the catalyst to be conveniently separated from the reaction mixture using an external magnet and potentially reused.

General procedure for the synthesis of benzimidazoles

1. Weigh 0.54 g of *o*-phenylenediamine and transfer it to a suitable flask.
2. Add 5 mL of ethanol and stir until the *o*-phenylenediamine dissolves.
3. Add an aromatic aldehyde (5 mmol) to the reaction mixture.
4. Add MnFe_2O_4 nanoparticles (0.05 mmol) as the catalyst.
5. Stir the reaction mixture using a magnetic stirrer until the reaction is complete.
6. Monitor the progress of the reaction by thin-layer chromatography (TLC).
7. After completion of the reaction, separate the MnFe_2O_4 catalyst from the reaction mixture using an external magnet.
8. Wash the recovered catalyst thoroughly with ethanol.
9. Dry the recovered catalyst at 70 °C for 1 h for possible reuse.
10. Purify the crude product by recrystallization from ethanol.
11. Collect the purified benzimidazole derivative and dry it properly.
12. Record the mass, percentage yield and physical characteristics of the synthesized product.

Precautions

1. Do not touch the reaction mixture or nanoparticles directly with bare hands.

2. Ethanol is flammable; keep it away from open flames and excessive heat.
3. Dispose of chemical waste according to the laboratory's prescribed waste-disposal procedures.

Practical

Separation of Spinach Pigments via Column Chromatography using Rotary Evaporation.

Aim

To extract and isolate the primary photosynthetic pigments (Chlorophyll a, Chlorophyll b, Xanthophylls, and Carotenes) from spinach leaves (*Spinacia oleracea*) using adsorption column chromatography and to concentrate the purified fractions using a rotary evaporator under controlled thermal conditions.

Materials and Reagents

- **Biological Sample:** Fresh spinach leaves (approx. 20g).
- **Stationary Phase:** Silica gel (60–120 mesh).
- **Solvents:** Acetone (Extraction), Hexane or Petroleum Ether (Mobile phase), Methanol.
- **Equipment:** Mortar and pestle, Glass chromatography column (25x300 mm), Rotary Evaporator, Separatory funnel, UV-Vis Spectrophotometer (optional for characterization).

Theory

The separation is based on the differential adsorption of pigments on a stationary phase (Silica gel) and their varying solubilities in a mobile phase (solvent gradient). Carotenes, being the least polar, elute first, followed by Chlorophyll a, Chlorophyll b, and finally the more polar Xanthophylls. The rotary evaporator is used to concentrate these heat-sensitive pigments by lowering the boiling point of the solvent under reduced pressure, preventing thermal degradation.

Procedure

Part A: Extraction of Pigments

1. Wash and dry spinach leaves, then remove the midribs.
2. Grind the leaves in a mortar with 20 mL of Acetone and a pinch of CaCO_3 (to prevent acidification and conversion to pheophytins).
3. Filter the extract into a separatory funnel. Add 20 mL of Hexane and 20 mL of water.
4. Gently shake and allow the layers to separate. The upper Hexane layer contains the pigments.
5. Collect the Hexane layer and dry it over anhydrous Na_2SO_4 .

Part B: Column Chromatography

1. **Column Packing:** Prepare a slurry of Silica gel in Hexane and pack the column evenly. Ensure there are no air bubbles.
2. **Loading:** Carefully load the concentrated pigment extract onto the top of the column.

3. Elution:

- Fraction 1 (Carotenes - Yellow/Orange): Elute with 100% Hexane.
- Fraction 2 (Chlorophyll a - Blue-Green): Elute with Hexane:Acetone (90:10).
- Fraction 3 (Chlorophyll b - Yellow-Green): Elute with Hexane:Acetone (80:20).
- Fraction 4 (Xanthophylls - Yellow): Elute with Hexane:Acetone (70:30) or pure Acetone.

Part C: Solvent Removal (Rotary Evaporator)

1. Transfer each collected fraction into a clean, pre-weighed round-bottom flask (RBF).
2. Setup: Attach the flask to the Rotary Evaporator. Set the water bath temperature to 40°C.
3. Operation: Start the rotation (120 rpm) and gradually apply the vacuum.
4. Concentration: Monitor the solvent collection in the receiver. Stop the process once the solvent is removed and a dark pigment film or concentrated residue remains.
5. Storage: Immediately dissolve the residue in a minimum volume of Hexane/Acetone and store in a vial wrapped in foil at -20°C.

Expected Results

The column will display distinct colored bands moving at different rates. The order of elution will reflect the polarity of the pigments: Carotenes (Least polar) > Chlorophyll a > Chlorophyll b > Xanthophylls (Most polar).

Precautions

1. Hexane and Acetone are highly flammable. Keep away from open flames.
2. Pigments are light-sensitive; perform the experiment in dim light or wrap glassware in aluminum foil where possible.
3. Check for cracks in the rotary evaporator glassware to prevent implosions.