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Experiment 1: Determination of Potassium content of Commercial salt sample by Flame
Photometry

Aim: To determine the Potassium content of Commercial salt sample by Flame Photometry.

Requirements: Flame photometer, Stock solutions of Potassium, 100 cm³ volumetric flasks, 10 cm³ pipette.

Theory: When a solution containing cations of sodium and potassium is sprayed into flame, the solvent evaporates and ions are converted into atomic state. In the heat of the flame (temperature about 1800°C), small fraction of the atoms is excited. Relaxation of the excited atoms to the lower energy level is accompanied by emission of light (photons) with characteristic wavelength (Na: 589 nm, K: 766 nm). Intensity of the emitted light depends on the concentration of particular atoms in flame.

Procedure:

Standard Addition Method:

1. Dilute the given solution of K to 100 cm³ with distilled water.
2. Take five 100 cm³ measuring flask numbered 1,2,3,4 and 5.
3. To each flask add 10 cm³ of the diluted sample solution.
4. Add 1.0, 2.0, 3.0, 4.0 and 5.0 cm³ of standard 100ppm stock solution of K to the above flask respectively.
5. Dilute each solution upto the mark with distilled water.
6. Measure the emission intensity for each of the above solution.
7. Plot a graph of Emission Intensity against the concentration of added standard.
8. From the X-intercept of the graph find the concentration of potassium in the sample solution.

Observations:

Flask No.	Volume of		Total volume of the solution in cm ³	Concentration of the added standard in ppm	Emission Intensity
	Sample solution in cm ³	Standard solution in cm ³			
1	10	1.0	100	1.0	

2	10	2.0	100	2.0	
3	10	3.0	100	3.0	
4	10	4.0	100	4.0	
5	10	5.0	100	5.0	

Calculations:

Concentration of K ions in the given solution = _____ ppm

∴ Amount of K ions in the given solution = _____ mg = _____ g

Result:

1. Concentration of K ions in the given sample solution = _____ ppm
2. Amount of K ions in the given sample solution = _____ g

Experiment 2: Photometric Titration of Copper (II) with EDTA

Aim: To determine the amount of Copper present in the given solution by photometric titration with EDTA.

Requirements: Spectrophotometer, 0.02 M EDTA solution, Stock solution of Cu^{+2} ions, 100 cm^3 volumetric flasks, 10 cm^3 pipette.

Theory: The reaction for this experiment is:

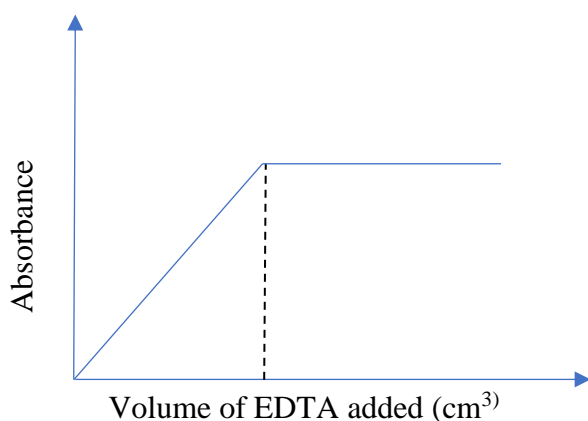


Where H_2Y^{2-} is $\text{Na}_2\text{H}_2\text{Y}$

The titration is performed at 625 nm; both the copper-EDTA chelate and the copper (I) ion absorb at this wavelength, but the molar absorptivity of the chelate is much higher. Figure 1a is a plot of absorbance (uncorrected for dilution) vs. milliliters of titrant. The points fall below the extrapolated lines in the end-point region, because the reaction is incomplete near the equivalence point. After the equivalence point, the added excess EDTA titrant forces the reaction to completion. The further addition of titrant leads to dilution; therefore, the absorbance will then decrease slightly. The pH is critical for this titration, because a large change in pH changes the effective binding constant. An acetate buffer is used to maintain the pH between 2.4 and 2.8 to avoid this problem. This low pH also permits the copper to be titrated in the presence of metal ions that form weaker complexes with EDTA.

Procedure:

1. Turn on the spectrometer to allow it to warm up. Set the wavelength to 625 nm and zero the meter with distilled water.
2. Dilute the given solution of Cuprous ions upto the mark with distilled water.
3. Add 10 cm^3 of diluted Cu^{2+} solution and 10 cm^3 of the acetate buffer solution to all 9 the beakers.
3. Add 0.2M EDTA to all 9 the beakers with these volumes (0, 2, 3, 4, 4.5, 5, 5.5, 6, 7).
4. Record the absorbance of all solution at 625 nm
5. Plot the absorbance of the solution against the volume of the EDTA, determine the endpoint by extrapolating the two linear portions of the curve to an intersection point.

**Calculations:**

10 cm³ of diluted Cu²⁺ solution required x cm³ of 0.02M EDTA solution.

1000cm³ of 1M EDTA = 63.546 g of Cu

$$\therefore x \text{ cm}^3 \text{ of } 0.02 \text{ M EDTA} = A = \frac{63.546 \times 0.02 \times x}{1000} \text{ g of Cu}$$

$$\therefore \text{Amount of Cu}^{+2} \text{ present in the stock solution} = 100A = \text{_____ g}$$

Result:

Amount of Copper present in given solution = _____ g

Experiment 3: To prepare benzilic acid from benzil using Green approach. (Microwave)

Aim: To prepare benzilic acid from benzil using Green approach.

Requirements: 100 ml conical flask, 200ml beakers, filter paper, oven, 0.5 g benzil, 20% alc KOH, dil. Sulfuric acid, benzene for recrystallisation

Procedure:

1. Take 0.1 g of benzil in a 100 ml conical flask.
2. To this add 12 ml of alc. KOH (20%).
3. Cover the conical flask with funnel and place it in the microwave oven.
4. Place 100 ml beaker containing 50 ml water next to conical flask in microwave oven. This acts as a heating source for reaction mixture.
5. Heat the reaction mixture in microwave at 60% (540W) intensity for one minute.
6. Cool the reaction mixture, fine crystals of pot. benzillate separate out. Filter the crystals and dissolve in 30 ml of distilled water. Filter again to remove any insoluble impurities.
7. To the clear filtrate, add dil. H_2SO_4 dropwise till the solution becomes acidic and benzilic acid separates out as ppt.
8. Let the reaction mixture cool down for complete pptn.
9. Filter the ppt. Wash with hot distilled water.
10. Dry the ppts and recrystallize from benzene and note the yield.
11. Determine the m.pt (150 degrees) of pure product.

Results:

1. Yield of the product = _____ g
2. Percentage Yield = _____ %
3. Melting point of the product = _____

Reaction:

Calculation of Theoretical Yield:

Calculation of Percentage Yield:

Experiment 4: To determine pKa and pI of amino acids by pH metry.

Aim: To determine pKa and pI of amino acids by pH metry.

Requirements:

0.1 M amino acid solution, 0.1N NaOH, 0.1N HCl, pH-meter, combination electrode.

Theory:

An amino acid will contain both -NH₂ and -COOH group.

At low pH, the amino acid is protonated at both the amine and carboxyl functions. At this pH, the amino acid carries a positive charge and will migrate to the cathode.

At high pH, both the carboxyl and amine groups are deprotonated. At these pH values, the negatively charged amino acid will migrate to the anode.

At some intermediate pH, the amino acid is a zwitterions, and carries no net charge. This is called the isoelectric point of the amino acids, and is designated pI, although pI is also commonly seen.

For an amino acid with only one amine and one carboxyl group, the pI can be calculated from the mean of the pKas of this molecule.

$$pI = \frac{(pK_1 + pK_2)}{2}$$

Procedure:

PART-I:

1. Dilute 50cm³ of the given amino acid to 250 cm³ in a standard flask using distilled water.
2. Pipette out 50cm³ of this diluted solution in a 100cm³ beaker, immerse the combination electrode and record the pH.
3. Fill 0.1N HCl in the burette, add 1.0 cm³ of 0.1 N HCl at a time, stir, record the pH. Calculate pK₁.

PART-II:

1. Pipette out 50cm³ of the diluted solution in a 100 cm³ beaker, immerse the combination electrode and record the pH.
2. Fill the burette with 0.1 N NaOH, add 1.0 cm³ of 0.1N NaOH at a time, stir, record the pH. Calculate pK₂.

Observations:

Table-I:

$$\text{Equivalents of amino acid } c = \frac{50 \times 0.1}{250} = 0.02 \text{ M}$$

a = analytical conc. of HCl added

Vol. of 0.1N HCl added in cm ³	pH	$a = \frac{N_{HCl} \times V_{HCl}}{V_{total}}$	$[H^+] = \text{antilog}(-\text{pH})$	$x = \frac{c}{a - [H^+]} - 1$	$\log x$	$\text{pK}_1 = \text{pH} - \log x$
0						
1						
2						
3						
4						
5						
6						
7						
8						
9						

Table-II:

$$\text{Equivalents of amino acid } c = \frac{50 \times 0.1}{250} = 0.02 \text{ M}$$

b = analytical conc. of NaOH added

Vol. of 0.1N NaOH added in cm ³	pH	$b = \frac{N_{NaOH} \times V_{NaOH}}{V_{total}}$	$[OH^-] = \text{antilog}(\text{pH} - 14)$	$x = \frac{c}{a - [OH^-]} - 1$	$\log x$	$\text{pK}_2 = \text{pH} + \log x$
0						

1						
2						
3						
4						
5						
6						
7						
8						
9						

Calculations:

$$\text{Isoelectric point } \text{pH}_{\text{iso}} = \frac{(\text{p}K_1 + \text{p}K_2)}{2}$$

Results:

(1) $\text{p}K_1$ of given amino acid =

(2) $\text{p}K_2$ of given amino acid =

(3) Isoelectric point pH_{iso} =

Amino acid	pKa₁	pKa₂	pKa₃	pI
Glycine	2.34	9.60	---	5.97
Alanine	2.34	9.69	---	6.00
Valine	2.32	9.62	---	5.96
Leucine	2.36	9.60	---	5.98
Isoleucine	2.36	9.60	---	6.02
Methionine	2.28	9.21	---	5.74
Proline	1.99	10.60	---	6.30
Phenylalanine	1.83	9.13	---	5.48
Tryptophan	2.83	9.39	---	5.89
Asparagine	2.02	8.80	---	5.41
Glutamine	2.17	9.13	---	5.65
Serine	2.21	9.15	---	5.68
Threonine	2.09	9.10	---	5.60
Tyrosine	2.20	9.11	---	5.66
Cysteine	1.96	8.18	---	5.07
Aspartic acid	1.88	9.60	3.65	2.77
Glutamic acid	2.19	9.67	4.25	3.22
Lysine	2.18	8.95	10.53	9.74
Arginine	2.17	9.04	12.48	10.76

MICROSCALE EXPERIMENTS IN CHEMISTRY

INTRODUCTION:

The rising cost of chemicals and decreased flow of funds are causing great concerns to the chemistry teachers. A tug - of - war had also been going on in recent years, to balance the budget of running practical courses and the standards of experiments to be carried out by the students. Since the economy is always the winner, the number of experiments had been the losers. A group of scientists from University of Pune and Fergusson College, Pune are striving hard to maintain the standards of experiments at a friendly budget i.e., by carrying out the organic reactions in capillaries / tiles, using semimicro test tubes for heating experiments (instead of the conventional test tubes) and adopting to microscale preparations. Their attempts serve many purposes.

(i) cuts down the cost of chemicals.

(ii) experiments have become environment friendly.

(iii) less time consuming

(iv) less hazardous to the teachers, students and lab, assistants. What more it becomes affordable.

ANALYSIS OF AN ORGANIC COMPOUND

A) Preliminary Tests:

EXPERIMENT	OBSERVATION	INFERENCE
1. Test For Aromaticity: a. Substance is introduced into the flame using a nickel spatula.	i. Burns with a smoky flame ii. Burns with a non smoky flame	i. Presence of an aromatic compound ii. Presence of an aliphatic compound
b. Substance is added to a mixture of 3 drops of con. sulphuric acid & 3 drops of con. nitric acid and warmed on a water bath for about 10 minutes. The solution is poured, then into water.	An yellow solution or precipitate is formed	Presence of an aromatic compound The yellow colour is due to the formation of nitro compounds by the nitration of aromatic compounds.
2. Test For Unsaturation a. A drop of the substance is taken on a porcelin tile and add adrop of con. Bromine water to it.	i. Decolouration ii. Decoloration followed by tubidity	i. Presence of unsaturation ii. Presence of aniline/ phenol
b. To a pinch of substance add, a drop of dil. KMnO_4	Decolouration	Presence of unsaturation/ easily oxidisable compound
3. Solubility Tests: i. water	i. Soluble	i. Presence of urea, carbohydrates etc.

ii. 5 % Na ₂ CO ₃ iii. 5% NaOH iv. 5% HCl	ii. Soluble iii. Soluble iv. Soluble	ii. Presence of acids iii. Presence of acids, phenols iv. Presence of amines
5. Action on Litmus: Moistened litmus paper is brought into contact with the substance	i. Blue litmus paper turns red ii. Red litmus paper turns blue iii. Neutral	i. Presence of acids, phenols ii. Presence of amines iii. Presence of carbohydrates, esters, carbonyls etc.
6. Action of Sulphuric Acid 5 mg. of the substance is warmed with 3 drops of con. H ₂ SO ₄	Chars with a smell of burnt sugars	Presence of carbohydrate

B) Solubility test

EXPERIMENT	OBSERVATION	INFERENCE
1. About 5mg of the compound is taken on a watch glass. One or two drops of water are added.	a. The compound partially dissolves. b. The mixture remains undissolved	Presence of carbohydrates, urea amine salts, salts of carboxylic acids, sulphanilic acid dicarboxylic acid etc b. Absence of all the above
2. In another watch glass 5mg of compound is taken and then tested with a drop of the saturated bicarbonate solution	Brisk effervescence. The solid acid reappears on addition of a drop of 8N HCl	Presence of an acid confirmed.

Theory:

The carboxylic acids react with bicarbonate to form sodium salts of the acids. Alkali metal salts being water soluble comes along with the aqueous layer. On acidification the free acid is generated as a solid.



3. In a watch glass 5mg of compound is taken and then	Solubility observed and a Turbidity or oil is formed	presence of phenolic compound confirmed.
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tested with a drop of 2N sodium hydroxide solution.	upon adding a drop of 8N HCl	
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Theory:

Phenolic compounds react with sodium hydroxide to form sodium phenolate. Since alkali metal salts are soluble in water, phenolate ion comes along with the aqueous layer. Upon acidification, liberates the phenol.



4. In another watch glass 5 mg of compound is taken and then tested with a drop of 2N hydrochloric acid solution.	Solubility observed and a Turbidity or oil is formed upon adding a drop of 10N NaOH.	presence of amino compound confirmed.
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Theory:

The amines form amine hydrochlorides with hydrochloric acid. The hydrochloride being water soluble comes along with the aqueous layer. Upon neutralization the free amine is liberated.



If all the above tests are negative then the given compound is **Neutral** in nature.

C) Detection of C, H, O, N, S and X⁻

(Lassaigne's Test)

Preparation of Sodium fusion Extract:

Two small cut pieces of sodium are fused in a semi-micro hard glass tube. About 5mg of the substance is added to it and fused again. The tube is cooled to room temperature. Keeping the tube in a slanting position 4 drops of water are added (the first drop of water is allowed to react with excess of sodium. The second drop is added after the initial reaction is over. Then the third and the fourth drops of water are added slowly). This is the sodium fusion extract. The following tests are performed with it.

a. Test for Nitrogen A drop of the extract is placed on a tile. A drop of a con. solution of FeSO ₄ followed by a drop of 50% H ₂ SO ₄ is added to it.	Prussian blue colour is obtained	Presence of nitrogen
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<p>b. Test for Halogens: A drop of the extract is treated with con. HNO_3 followed by a drop of AgNO_3 solution</p>	<p>i. Curdy white precipitate soluble in ammonium hydroxide ii. Pale yellow precipitate sparingly soluble in ammonium hydroxide iii. Yellow precipitate insoluble in ammonium hydroxide</p>	<p>i. Presence of chlorine ii. Presence of bromine iii. Presence of iodine</p>
<p>If halogen is present, About 5 mg of the substance is warmed with 3 drops of alcoholic AgNO_3 on a water bath and acidify with a drop of con. HNO_3.</p>	<p>i. Immediate formation of a precipitate ii. No precipitate</p>	<p>i. Presence of halogen in the side chain ii. Presence of halogen in the aromatic nucleus</p>
<p>c. Test for Sulphur: A drop of the extract is mixed with a drop of sodium nitroprusside on a tile.</p>	<p>Violet colour</p>	<p>Presence of sulphur</p>

D) Determination of class of compound:

I. Under C, H, (O) group:

<p>1. Test for carboxylic acid: 1a. About 5 mg of the substance is treated with a drop of highly saturated NaHCO_3 solution on a tile</p>	<p>Brisk effervescence</p>	<p>Presence of carboxylic acid</p>
<p>1b. A drop of the substance is mixed with a drop of neutral FeCl_3 solution on a tile</p>	<p>i. Violet color ii. Flesh color</p>	<p>i. Presence of salicylic acid ii. Presence of phthalic acid</p>
<p>2. Test for ortho - dicarboxylic acid: About 5 mg. of the substance is heated with an equal amount of resorcinol and 1 drop of con. H_2SO_4 in a semi micro tube. Take a drop of it at the tip of a glass rod and dip it in very dil. NaOH in a semi micro tube.</p>	<p>Green fluorescence</p>	<p>Presence of o- dicarboxylic acid</p>

<p>3. Test for Phenol:</p> <p>a. Neutral Ferric chloride test A drop of the substance is mixed with a drop of neutral FeCl_3 solution on a tile.</p>	Violet colouration	Presence of phenol
<p>b. Phthalein Reaction: About 5 mg of the Substance is heated with about 10 mg of phthalic anhydride and a drop of con. sulphuric acid in a semimicro tube. The solution is cooled and diluted with about 1 ml of water. A drop of the solution is mixed with a drop of 50%, sodium hydroxide solution on a tile.</p>	Red, blue or green color	Presence of phenol
<p>c. Liebermann's reaction : About 5 mg of the substance is heated with about 5 mg of sodium nitrite and 2 drops of con. sulphuric acid in a semi micro tube. It is cooled and diluted with about 1 ml of water. A drop of it is placed on a tile and mixed with a drop of 10% sodium hydroxide solution.</p>	A bluish green color is produced	Presence of phenols
<p>4. Test for Carbohydrates: (Molisch's test) A drop of an alcoholic solution of α- naphthol is mixed with a drop of aqueous solution of the substance on a tile and was mixed with a drop of con. Sulphuric acid.</p>	A deep violet color	Presence of sugars
<p>5a. Test for aldehydes / ketones: Borsche's reagent test:</p>	Red orange precipitate	

A drop of the substance (if it is liquid) or a drop of an alcoholic solution is placed on a tile. A drop of the con. Solution of 2,4- DNP (Borsch reagent) is added to it.		Presence of aldehydes/ ketones
5b. Schiff's reagent test: A drop of the substance is mixed with a drop of Schiff's reagent	Pink colour	Presence of aldehyde
5c. 2 drops or about 5 mg of the substance is mixed with 2 drops of Fehling A and 2 drops of Fehling B solutions in a semimicro test tube. It is heated in a water bath for about 5 minutes.	Red precipitate	Presence of aldehyde/ Reducing sugars
6a. Test for ester: About 2 drops of the substance is heated with 2 drops of methanolic solution of hydroxylamine hydrochloride and 2 drops of 50% NaOH and cooled. A drop of it is placed on a tile. It is mixed with dil. HCl and neutral FeCl ₃ , a drop of it each.	Violet color	Presence of ester
6b. drops of the substance is heated with 2 drops of 50% NaOH solution, till it is dissolved. Cool it and then add 4 drops of 50% HCl.	White precipitate	Presence of ester
7a. Test for alcohols: Add a small piece of Na metal to 2 drops of the substance.	Effervescence	Alcohols present
7b. A drop of the substance is added to a drop of acetyl chloride in fume hood.	Violent reaction with evolution of fumes.	Alcohols present

<p>8. Test for Hydrocarbons: With 5mg each of the substance and picric acid prepare a saturated solution in alcohol separately (add 3 or 4 drops of alcohol). Mix The solutions, heat and Cool.</p>	Yellow precipitate	Presence of hydrocarbons

II. Under C, H, (O), N group:

<p>1. Test for primary Amines : Dye test : A drop of the substance is placed on a tile. A drop of dil. hydrochloric acid is added to it. A drop of saturated sodium nitrite solution followed by a drop of β - naphthol dissolved in 25% NaOH is added to it.</p>	Red azo dye	Presence of aromatic primary amine
<p>2. Test for Secondary Amines: 2 drops of substance is mixed with 2 drops of dil. Hydrochloric acid and 2 drops of saturated solution of sodium nitrite in a semimicro tube. A drop of con. sulphuric acid and a drop of phenol are added. Heated for a minute. A drop of this solution is mixed with a drop of dil. NaOH on a tile.</p>	A bluish green color is produced	Presence of secondary amine
<p>3. Test for tertiary amine Dye test : 2 drops of benzaldehyde and 4 drops of the substance are heated with 1 drop of con. sulphuric acid and 50 mg of lead dioxide. A drop of this solution is mixed with a drop of dil. HCl on a tile.</p>	Malachite green dye is formed	Presence of tertiary amine.
<p>4a. Test for nitro group: Reduction to amines :</p>		

<p>About 3 drops of the substance is reduced with 3 drops of con. hydrochloric acid and metallic tin or zinc in a semimicro tube, by heating for about 5 minutes. with a drop of the supernatant liquid dye test is performed on a tile.</p> <p>(as under test for amines)</p>	Red azo dye	Presence of nitro group
<p>4b. Mulliken - Barker's test: About 3 drops of the substance and 3 drops of alcohol are boiled with 3 drops of calcium chloride solution and a pinch of zinc dust. Heated to boiling and cooled. A drop of it is mixed with a drop of Tollen's reagent on a tile.</p>	A black precipitate	Presence of nitro group
<p>5. Test for Anilide: About 10 mg of the substance is heated strongly in a dry semi micro tube with soda lime. The resulting vapour is collected in another semi micro tube containing 5 drops of dil. hydrochloric acid. With a drop of the acid solution dye test is performed</p>	Scarlet Red dye	Presence of anilide
<p>6. Biuret test: About 5 mg of the substance is heated strongly in a dry semi micro tube to its melting point. Cooled and the residue is dissolved in 3 drops of water. A drop of it is mixed with a drop of dil. CuSO₄ and with a drop of dil. NaOH solution on a tile.</p>	A violet colour	Presence of diamide
<p>7. Test for Aromatic monoamide: About 5 mg of the substance is heated with 5 drops of 25% NaOH solution and cooled. A</p>	White precipitate	Presence of monoamide

drop of it is mixed with a drop of con. HCl		
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III. Under C, H, (O), N, S group:

<p>1a. Test for thiourea:</p> <p>About 5 mg of the substance is heated with 5 drops of dil. NaOH solution and cooled. A drop of it is mixed with a drop of lead acetate solution on a tile.</p>	A black / brown precipitate	Presence of thiourea
<p>1b. 5 mg of the substance is heated in a dry semi micro tube until it melts. It is cooled and the residue is dissolved in 4 drops of water. A drop of it is mixed with a drop of neutral FeCl_3 solution on a tile.</p>	Blood red coloration	Presence of thiourea