RAYAT SHIKSHAN SANSTHA'S

MAHATMA PHULE ARTS, SCIENCE AND COMMERCE COLLEGE, PANVEL.

DISTRICT - RAIGAD, MAHARASHTRA. 410 206

Best College Award by University of Mumbai
Accredited at 'A+' Grade by NAAC (Third Cycle CGPA Score 3.29)
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Recipient of DBT Star College Grant

DEPARTMENT OF CHEMISTRY

MANUAL OF THE PRACTICALS CONDUCTED UNDER DBT- STAR COLLEGE SCHEME IN THE ACADEMIC YEAR: 2024-25

RAYAT SHIKSHAN SANSTHA'S

MAHATMA PHULE ARTS, SCIENCE AND COMMERCE COLLEGE,

PANVEL, DIST-RAIGAD

ACADEMIC YEAR: 2024-25

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DEPARTMENT OF CHEMISTRY
EXPERIMENT UNDER DBT STAR COLLEGE SCHEME
Academic Year: 2024-25

Class : F.Y.B. Sc. **Date:**
Name of the Student: **Roll Number:**

AIM : RAPID DETECTION OF CHEMICAL ADULTERANTS OF MILK (UREA BY COLORIMETER)

THEORY: Urea is a natural constituent of milk and it forms a major part of the non-protein nitrogen of milk. Urea concentration in milk is variable within herd. Urea is one of the ingredients of synthetic milk along with caustic soda, detergent, sugar and foreign fats. Adulteration of natural milk with synthetic milk increases the level of urea to such an extent that on consumption of this adulterated milk causes toxicological hazards. Estimation of urea concentration in milk may serve as a tool for checking the menace of adulteration of natural milk with synthetic milk.

REQUIREMENTS:

- **INSTRUMENT/APPARATUS/GLASSWARES :**

Colorimeter, Weighing balance, 250 ml Glass beaker, 100 ml Volumetric flask, 10 ml Glass cylinder, Glass rod, Glass dropper.

- **Reagent(s) required:**

i. Dimethylaminobenzaldehyde (DMAB)
ii. Concentrated HCl
iii. Trichloroacetic acid (TCA)
iv. Anhydrous potassium di-hydrogen orthophosphate (KH_2PO_4) and anhydrous di-potassium mono-hydrogen orthophosphate (K_2HPO_4)
v. Urea.

PROCEDURE:

A. Take 10 ml of milk sample and add 10 ml of 24% TCA in 50 ml glass stoppered bottle. Mix the content and filter through Whatman filter paper grade 42.

B. Take 5 ml of the above filtrate in a test tube and add 5 ml of 1.6% DMAB reagent..

C. Take the absorbance of the yellow colour so obtained at 425 nm in a spectrophotometer against reagent blank.

D. For reagent blank take 5 ml of diluting reagent and add 5 ml of 1.6% DMAB reagent.

E. For standard curve preparation, take different concentration of urea solution (0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6 mg) separately in different test tubes and make the total volume to 5 ml in each case with diluting reagent. Then add 5 ml of 1.6% DMAB reagent to each test tube to

develop yellow colour. Take the absorbance at 425 nm against the reagent blank.

F. Draw a standard curve by plotting the absorbance along Yaxis and urea concentration (in mg) along X-axis

CALCULATION AND OBSERVATION TABLE:-

Standard amount of Urea in mg	**Volume of Diluting Reagent**	**Volume of 1.6 % DMAB Reagent**	**Absorbance**
Blank	5 ml	5 ml	
0.1	5 ml	5 ml	
0.2	5 ml	5 ml	
0.4	5 ml	5 ml	
0.6	5 ml	5 ml	
0.8	5 ml	5 ml	
1.0	5 ml	5 ml	
1.2	5 ml	5 ml	
1.4	5 ml	5 ml	
1.6	5 ml	5 ml	
Sample (5 ml Filtrate)	-	5 ml	

Calibration Curve: - Plot the calibration curve of concentration of urea (x-axis) corresponding to absorbance (y-axis)

Calculation:- Read from the graph the concentration of urea (mg) corresponding to absorbance of the sample. Say the absorbance for the sample be X and the corresponding concentration from the standard curve for urea is = Y mg

RESULT:

5 ml of filtrate from sample has = Y mg urea

REFERENCES:

i) IS: 1479. (1960). Methods of test for dairy industry. Part I. Rapid examination of milk. Bureau of Indian Standards, New Delhi.

ii) Bector, B.S., Ram, M. and Singhal, O.P. (1998). Rapid platform test for the detection/determination of added urea in milk. Indian Dairyman, 50(4): 59-62.

iii) Kavita, P. (2000). Studies on the levels of urea in milk. M. Sc. Thesis NDRI Karnal.

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DEPARTMENT OF CHEMISTRY
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Academic Year: 2024-25

Class : F.Y.B. Sc. **Date :**
Name of the Student: **Roll Number :**

TITLE : PHYSICO-CHEMICAL STUDY OF WATER SAMPLE OF NEARBY WATER BODIES

AIM : To determine the turbidity of the given sample of water by using the Nephelometer / turbidimeter.

THEORY : In water supply turbidity data information can be helpful to know the effectiveness and adequacy of the treatment delivered with various coagulants and the necessary doses. According to IS 10500:1991 the maximum limit of turbidity is 10 NTU and up to 5 NTU water is acceptable and above 5 NTU the consumer acceptance decreases. Turbidity is an indicator of poor treatment plant efficiency, filter run timing, contamination of distribution system, and can fix the measurements of coagulants. Turbidity is depended on the comparison of the intensity of light scattered by the sample under characterized conditions with the power of the light scattered by a standard reference suspension under similar conditions. The turbidity of the sample is thus estimated from the amount of light scattered by the sample taking a reference with standard turbidity suspension. The higher the power of scattered light the more is the turbidity.

REQUIREMENTS:

- **INSTRUMENT/APPARATUS/GLASSWARES :**
  Nephelometer / turbidimeter, Weighing balance, 250 ml Glass beaker, 100 ml Volumetric flask, 10 ml Glass cylinder, Glass rod, Glass dropper.

- **REAGENTS:**
  - ***Turbidity free water***:-
    Generally, Distilled water is considered turbidity-free water for any test.
  - ***Stock turbidity solutions***:-

Solution 1:- For making solution dissolve 1.0 grams hydrazine suplate $(NH_2)_2.H_2SO_4$ in distilled water and dilute it to 100 ml.
Solution 2:- For the 2nd Solution Dissolve 10.0 grams hexamethylenetetramine $(CH_2)_6N_4$ in minimum quantity of distilled water and dilute it to 100 ml.
Solution 3:- Now, Mix each 5ml of solution 1 and solution 2 in 100ml flask and left it stand for at least 24 hrs., then dilute it to 100ml and mix thoroughly. The turbidity of this solution is 400 NTU.

- ***Standard Turbidity Solution***:- Take 10 ml of solution 3 in 100 ml volumetric flask and dilute it upto the mark with turbid free water. The turbidity of this suspension is 40 NTU.

PROCEDURE:

1. Set the instrument at 100 with 40 NTU standard suspension. In this case, every division on the scale will be equal to 0.4 NTU turbidity.
2. Shake thoroughly the sample, and keep it for some time to eliminate the air bubbles.
3. Put the sample in the nephelometer sample tube and find out the value on the scale.
4. If sample is suspected to have more turbidity (more than 40 NTU), dilute it so that turbidity values come below 40 NTU, and take the reading.

CALCULATION:-

- **Original/Undiluted sample:**

  Turbidity = Instrument reading x 0.4

  = ----------NTU

- **Diluted sample:**

  Turbidity = Instrument reading x 0.4 x Dilution factor*
  = ---------- x 0.4 x ----------

  = ---------- NTU

*(*Dilution factor will depend on the dilution of original sample having turbidity more than 40 NTU. For example. If Original sample is diluted to its half concentration by mixing equal volumes of original sample and distilled water then dilution factor will be 2.)*

RESULT :

The turbidity of the given sample of water is ----------NTU

REFERENCES :

1. Khopkar S.M. "*Enviromental Pollution: Monitoring And Control*" New age international limited publishers, Mumbai (2004).
2. APHA, AWWA, WPCF,*Standard methods for examination of water* 14 edition, American public health association, Washington, D.C. (2019).

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Academic Year: 2024-25

Class : F.Y.B. Sc. **Date :**
Name of the Student: **Roll Number :**

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AIM: PREPARATION OF BUFFER OF DESIRED PH AND MOLARITY AND MEASUREMENT OF PH.

Requirements: -

Chemicals: Sodium carbonate, Sodium bicarbonate, Acetic Acid, Sodium Acetate, Distilled Water.

Apparatus: 100 ml volumetric flask, weighing balance, conical flask, pipette, pH meter, etc.

Theory: pH of a solution is defined as the negative logarithm of the hydrogen ion concentration to base 10.

A buffer system is a mixture of a weak acid or a weak base and its salt (conjugate base or conjugate acid, respectively) that permits solutions to resist large changes in pH upon the addition of small amounts of acid or base. It means a buffer helps maintain a near constant pH upon the addition of small amounts of acid or base to a solution. The acidity and basicity of a solution is expressed in terms of H^+ and OH^- ion concentration.

The pH of the sample solution can be measured suitably using pH meter. It uses a combination of two electrodes, a glass electrodes and saturated calomel electrode.

Reagents preparation:

- Sodium carbonate solution 0.2M: Dissolve 2.12gm of anhydrous sodium carbonate in 100ml Distilled water.
- Sodium bicarbonate solution 0.2M: Dissolve 1.68gm of sodium bicarbonate in 100 ml of distilled water.
- Acetic Acid 0.2 M: Glacial acetic acid 1.5 ml is made up to 100 ml by using distilled water.

- Sodium Acetate Solution 0.2M: 0.64 gm of sodium acetate in 100 ml distilled water

Procedure:

1) **CARBONATE- BICARBONATE BUFFER:**

   Pipette out exactly 27.5ml of sodium carbonate (Na_2Co_3) solution. To this add 22.5ml of sodium bicarbonate solution and made up to 100ml with distilled water which corresponds to 0.2 M sodium carbonate and bicarbonate buffer. Standardise pH meter and measure the pH of required buffer. This gives the Carbonate- bicarbonate buffer pH 10.2.

2) **ACETIC ACID- SODIUM ACETATE BUFFER:**

   Pipette out exactly 36.2ml of sodium acetate solution into 100ml of standard flask and add 14.8ml of glacial acetic acid, make the volume 100ml using distilled water using distilled water. This gives 0.2 M of acetic acid and sodium acetate buffer. The pH is measured with pH meter. The pH meter is first standardised with pH buffer. Wash electrode with distilled water and introduced into 0.2M acetic acid-sodium acetate buffer prepared, the pH of solution is 4.6.

Observations table:

Sr. No.	Sample solution	Experimental pH
1	Sample of buffer solution of pH 10.2	
2	Sample of buffer solution of pH 4.6	

Result:

1. The pH of 0.2 M of carbonate- bicarbonate buffer solution is _____.
2. The pH of 0.2 M of acetic acid-sodium acetate buffer solution is _____

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References:

- Rabara, B. P. (2012). pH Measurement and Buffer Preparation.

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Academic Year: 2024-25

Class : F.Y.B. Sc. **Date :**
Name of the Student: **Roll Number :**

TITLE: MEASUREMENT OF pKa VALUES OF AMINO ACID SAMPLE.

AIM: TO DETERMINE THE ACIDIC AND BASIC DISSOCIATION CONSTANTS OF AN AMINO ACIDS.

REQUIREMENTS:

A) Chemicals: 0.1 M Amino acid (Glycine) Sample, Buffer Solution (pH= 4 & 10), 0.1 M NaOH solution, 0.1 M HCl solution, distilled water.

B) Glassware's and Instrument: pH meter, Burette, 10 cm^3 and 25 cm^3 Pipettes, 100 cm^3 Beaker, 250 cm^3 Standard Measuring flask etc.

THEORY: Amino acids possess both acidic (carboxyl group) and basic (amino group) functional groups, each of which can ionize, resulting in different charged forms of the molecule depending on the pH of the solution. The pKa value is the pH at which the amino acid is equally distributed between its protonated and deprotonated forms. Amino Acid have ability to neutralize both stronger acids and stronger base. - NH_3 acts as proton donor while COO^- acts as proton acceptor. The pH at which an amino acid exhibits no migration in an electric field is called as isoelectric pH or isoelectric point.

REACTION:

1. $^+H_3N{-}R{-}COO^-$ + H_3O^+ $\rightleftharpoons$ $^+H_3N{-}R{-}COOH$ + H_2O

2. $^+H_3N{-}R{-}COO^-$ + OH^- $\rightleftharpoons$ $H_2N{-}R{-}COO^-$ + H_2O

PROCUDURE:

1. Standardize the supplied pH meter.
2. Pipette out 50 cm^3 of 0.1 M amino acid solution in 250 cm^3 standard measuring flask and dilute of up to the mark with distilled water. Use 50 cm^3 of the solution for Part I and Part II.

Part I- pH metric titrations with strong acid

1. Wash and rinse burette with 0.1 M HCl solution and fill it up.

2. In a clean, dry 100 cm^3 beaker take 50 cm^3 of diluted amino acid solution. Dip combined electrode of pH meter into it such that the glass bulb of the electrode dips completely into the solution.

3. Note the initial pH of the solution.
4. Add 10 cm^3 of 0.1 M HCl solution from the burette. Stir the solution well and record the pH of solution when it attains steady value.
5. Repeat the step no (4), till finally 10 cm^3 of HCl solution is added, with every time adding 1.0 cm^3 of HCl solution
6. Calculate the concentration of amino acid reacted and the amount remain unreacted after every step. Find dissociation constant K_1 or pK_1.

Part II- pH metric titrations with strong base

1. Wash and rinse burette with 0.1 M NaOH solution and fill it up.
2. In a clean, dry 100 cm^3 beaker take 50 cm^3 of diluted amino acid solution. Dip combined electrode of pH meter into it such that the glass bulb of the electrode dips completely into the solution.
3. Note the initial pH of the solution.
4. Add 10 cm^3 of 0.1 M NaOH solution from the burette. Stir the solution well and record the pH of solution when it attains steady value.
5. Repeat the step no (4), till finally 10 cm^3 of NaOH solution is added, with every time adding 1.0 cm^3 of NaOH solution
6. Calculate the concentration of amino acid reacted and the amount remain unreacted after every step. Find dissociation constant K_2 or pK_2.

OBSERVATIONS:

Part I- pH metric titrations with strong acid

Equivalent of amino acid, C= (50 ×0.1) ÷ 250 = 0.02 M.

Volume of 0.1 M HCl solution added in cm^3	pH	$a = \frac{(M_{HCl} \times V_{HCl})}{V_{Total}}$	$[H^+]$ = Antilog (-pH)	$x = \{C \div (a - [H^+]\} - 1$	log x	pK_1 = pH – log x
0.0						
1.0						
2.0						
3.0						
4.0						
5.0						

6.0						
7.0						
8.0						
9.0						
10.0						

Mean pK1 = .

Part II- pH metric titrations with strong base

Equivalent of amino acid, C= (50 ×0.1) ÷ 250 = 0.02 M.

Volume of 0.1 M NaOH solution added in cm^3	pH	b= $\frac{(M_{NaOH} \times V_{NaOH})}{V_{Total}}$	$[OH^-]$ = Antilog (pH-14)	x_2 = {C÷(b-$[OH^-]$} -1	log x_2	pK_2 = pH – log x_2
0.0						
1.0						
2.0						
3.0						
4.0						
5.0						
6.0						
7.0						
8.0						
9.0						
10.0						

Mean pK2 = .

RESULTS:

1. Acidic dissociation constant of given amino acid, K_1 = (or pK_1=)
2. Basic dissociation constant of given amino acid, K_2 = (or pK_2=)

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***REFERENCES*:**

1. **T.Y.B.Sc. practical handbook, university of Mumbai.**
2. **https://egyankosh.ac.in/bitstream/123456789/68526/1/Experiment-4.pdf**

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DEPARTMENT OF CHEMISTRY
ACADEMIC YEAR: 2024-25
EXPERIMENT UNDER DBT STAR COLLEGE SCHEME

Class : F.Y.B. Sc. **Date:** **Time:**
Name of the Student: **Roll Number:**

TITLE: Separation of amino acid mixture by Paper Chromatography

AIM: To perform paper chromatography for the separation of amino acids present in the given sample.

THEORY:

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

Retention (or) retardation factor (Rf):

Retention factor is defined the ratio of the distance travelled by the solute to the distance travelled by solvent. The distance travelled relative to the solvent is called the Rf value.

Rf value = Distance travelled by solute from origin / Distance travelled by solvent from origin

REQUIREMENTS:

Apparatus:
Glass beakers, Whatmann filter paper, Petridishes, Measuring cylinder, Developing chamber and capillary tubes etc.

Chemicals:
n-butanol, Glacial acetic acid, Distilled water (4:1:5), Amino acids (Tryptophan and threonine), Ninhydrin reagents.

PROCEDURE:

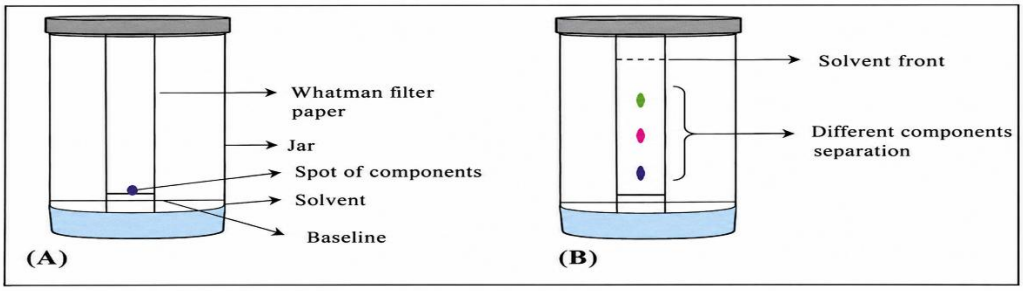
Solvents system and its preparation methods:

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

Paper chromatography:

4. The chromatography paper is cut into rectangular strips and marks a line on the paper with pencil at about 2 cm from the bottom.
5. With the help of capillary tube, the samples are applied at different points on the starting line.
6. Now, place the chromatography paper in the developing chamber, which contains the mobile phase.
7. While placing the paper, it is important that the solvent level should not reach the starting line or the sample spots and paper shouldn't touch the walls of the developing chamber.
8. After sometime the solvent rises up the paper or the stationary phase by capillary action and dissolves the sample.
9. The components of the sample move along with the solvent in upward direction. Check if the solvent has reached near the top level of chromatography paper which is called Solvent front.
10. Then the paper is removed when it reaches the top and marked the level with pencil.
11. By using UV light, ninhydrin or iodine vapors examined the different spots of varied colors.

SCHEMATIC REPRESENTATION:



OBSERVATIONS AND CALCULATIONS:

Sr. No.	Observation	Value
(1)	Distance travelled by tryptophan	
(2)	Distance travelled by threonine	
(3)	Distance travelled by the unknown mixture	
(4)	Distance travelled by the solvent	

RESULT:

Sr. No.	Result	Value
(1)	Rf value of tryptophan	
(2)	Rf value of threonine	
(3)	Rf value of unknown mixture	

REFERENCES:

1. http://www.chemguide.co.uk/analysis/chromatography/paper.html.
2. http://en.wikipedia.org/wiki/Ninhydrin.
3. http://wiki.answers.com/Q/Disadvantages_of_using_paper_chromatography.
4. Dr. S. Ravi Shankar, 2010, *Text book of Pharmaceutical Analysis*, Page no: 15.8
5. http://www.buzzle.com/articles/paper-chromatography.html.

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Class : S.Y.B. Sc. **Date:** **Time:**
Name of the Student: **Roll Number:**

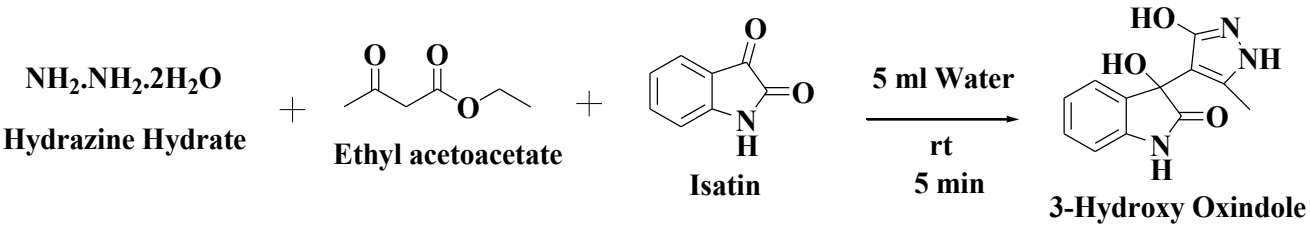
TITLE: GREEN SYNTHESIS OF DIVERSELY FUNCTIONLIZED OXINDOLE

AIM: Green Synthesis of 3-hydroxy oxindoles by using one pot, 3-component reaction of hydrazine hydrate, ethyl acetoacetate and Isatin in water.

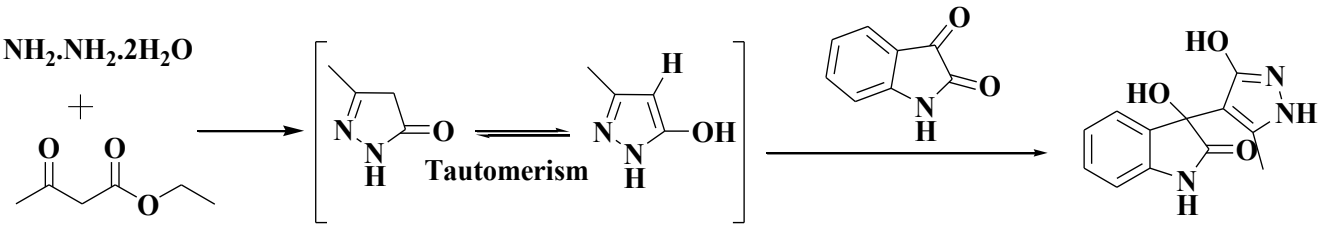
REQUIREMENTS: Hydrazine hydrate, Ethyl acetoacetate, Isatin, Distilled water, Test tube, Glass rod, funnel, Filter papers etc.

THEORY: The term "Green Chemistry" was coined by Paul Anastas in 1991. It is defined as "The invention, design and application of chemical products and processes to reduce or to eliminate the use and generation of hazardous substances". Goal of Green Chemistry is to create better, safer chemicals while choosing the safest, most efficient ways to synthesize them and to reduce waste. In the context of the Green Chemistry, now a day's use of Multi-component reactions (MCRs) for the synthesis of biologically imperative heterocyclic compound become highly important tool in organic synthesis. 3-Substituted 3-hydroxy oxindole is important molecular framework found in range of natural products and pharmaceutically important compounds. In this context, green synthesis of 3-hydroxy-3-(3-hydroxy-5-methyl-1H-pyrazol-4-yl)indolin-2-ones molecules by one pot, 3-component reaction of Hydrazine hydrate, Ethyl acetoacetate and Isatin using water at room temperature is desirable.

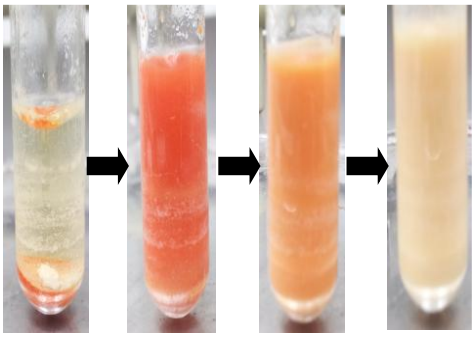
REACTION:



MECHANISM:



PROCUDURE:



1. In a Test tube, take 5 ml of distilled water.
2. To the above test tube, add 0.5 ml of Hydrazine hydrate, 0.5 ml of Ethyl acetoacetate and mix it well using glass rod. (*The test tube will become warm at this stage*)
3. Then add 0.5 gm of Isatin to the above solution.
4. Now mix it using glad rod till the buff white precipitate is formed.
5. Filter the precipitate, wash with 10 ml distilled water and dry it.
6. Measure the weight of the compound and record the melting point.

OBSERVATIONS:

Supplied weight of the reactant (Isatin) : ---------- gm (**A**)

Observed weight of the product 3-Hydroxy Oxindole) : ---------- gm (**B**)

Melting Point of Compound: ---------- °C (Expected 226-228 °C)

CALCULATIONS:

Theoretical Yield:

147 gm of Isatin = 245 gm of 3-Hydroxy Oxindole

So, **A** gm of Isatin = _____ gm of 3-Hydroxy Oxindole

$$\textbf{Theoretical Yield} = \frac{245 \times (A)}{147} = \frac{245 \times \_\_\_\_}{147} = \text{------ gm (C)}$$

Percentage Yield:

$$\textbf{Percentage Yield} = \frac{\text{Observed weight of Compound (B)}}{\text{Theoretical Yield (C)}} \times 100$$

$$\textbf{Percentage Yield} = \frac{(B)}{(C)} \times 100 = \text{---------- X 100}$$

Percentage Yield = ---------- % (D)

RESULTS:

- **Observed Yield (B) = ---------- gm**
- **Theoretical Yield (C) = ---------- gm**
- **Percentage Yield (D) = ---------- %**
- **Melting Point = ----------°C**

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***REFERENCES*:**

1. Thakur, P. B., Meshram, H. M. *RSC Advances* 4 (2014): 6019-6026
2. Thakur, P. B. *The Patent office Journal No, 52/2020 (201921024078)*

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DEPARTMENT OF CHEMISTRY
EXPERIMENT UNDER DBT STAR COLLEGE SCHEME
Academic Year: 2024-25

Class: S.Y.B.Sc. **Roll number:** **Date:**

Name of the student:

TITLE : ANALYSIS OF MICRONUTRIENTS OF SOIL SAMPLE.

Requirements: A Stock Solution of K containing 1 X 10-3 g/cm3 of the metal can be prepared by dissolving 0.477 g of KCl in 250 cm^3 of distilled water in standard measuring flask (i.e. Strength of stock solution is 1000 ppm.) Sample solution, Potassium Chloride, Distilled water, Flame Photometer, 250 & 100 cm^3 standard measuring flask, 10 & 25 cm3 calibrated pipette, 100 & 250 cm3 beaker etc.

Theory: Flame Photometer is concerned with the emission of radiation in flame by individual elements & correlation of the emission intensity with the concentration of these elements. It is also called as flame emission spectroscopy. Because of a flame to provide the energy of excitation to atoms introduced into the flame.

The sample is usually presented for analysis in the form of solution. It is transformed in to a mist of fine droplets & sprayed in to the flame in a reproducible & constant manner. The series of complex processes that occur in the flame being with the evaporation of the solvents vaporization of the solute & dissociation of the solute compounds. The excited atom which are unstable, quickly emit photons & return to lower energy state, eventually reacting the unexcited state. The measurements of emitted photon i.e.radition form the basis of flame photometry.

Procedure: (Calibration curve method)

1.Dilute 10 cm^3 of the supplied stock solution i.e.1000 ppm. concentration to 100 cm^3 in a standard measuring flask with distilled water. This gives standard solution of 100 ppm.

2. Dilute the supplied sample solution to 100 cm^3 in a standard measuring flask with distilled water.

3. Take six serially number 100 cm^3 standard measuring flasks.

4.Add 5, 10, 15, 20, & 25 of 100 ppm of K solution to the standard measuring flasks numbered from 1 to 5 Dilute each flasks to 100 cm^3 with distilled water.

5. Pipette out 10 cm^3 of the diluted sample solution of K in 100 cm^3 standard measuring flask & dilute up to the mark (flask No. 6)

6. Measure the emission intensity for each solution as described. Note the emission intensities of the different solution of known concentration & sample solution as shown in table.

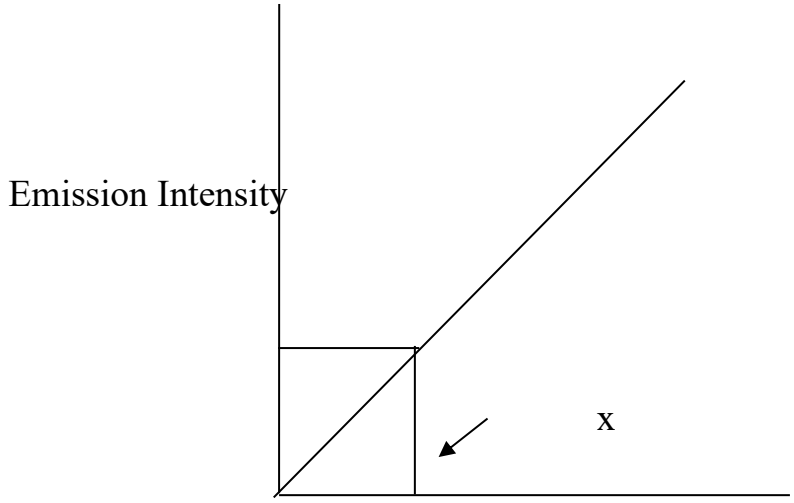
7. Plot the graph of concentration of solution (in ppm) against emission intensities. This forms the calibration curve.

8. With the help of emission intensity of the of the sample solution obtain the concentration of K from the calibration curve.

OBSERVATION TABLE:

Flask Numbers	Volume of standard solution in cm^3	Final volume of solution in cm^3	Concentration of solution in ppm.	Emission intensity (I)
1.	05	100	05	
2.	10	100	10	
3.	15	100	15	
4.	20	100	20	
5.	25	100	25	
6.	10.0(sample)	100	Unknown	

Graphs: For the concentration of calibration curve plot the graph of emission intensity for the solution against concentration of solution in ppm.



Where x = unknown concentration.

Calculations:

Concentration of K^+ ions in the given sample solution = x ppm.

Therefore, amount of K^+ ions in the given solution= x mg/L

Result: The amount of K^+ ions in the given sample solution = mg.

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DEPARTMENT OF CHEMISTRY
EXPERIMENT UNDER DBT STAR COLLEGE SCHEME
Academic Year: 2024-25

Class: S.Y.B. Sc. **Date:**
Name of the Student: **Roll Number:**

AIM: PREPARATION OF SANITIZER, SOAP, HANDWASH AND PERFUMES.

REQUIRMENTS:

Sodium lauryl ether sulphate (70%), Cocodiethanolomide, Cocomonoethanolomide, E.D.T.A.,Methyl paraben,perfumes,Stainless steel pot,burner,stirrer,dist. Water, measuring cylinder,digital balance etc.

THEORY:

Hand hygiene is one of the most important elements of infection control. Liquid hand wash is an anti-bacterial liquid that has an active substance used to inhibit, destroy, or render harmless the active bacteria on hands. Liquid hand washes are generally preferred over soaps due to ease of use and the ability of liquid soaps to maintain the skin's natural moistureuid hand wash is an anti-bacterial liquid that has an active substance used to inhibit, destroy, or render harmless the active bacteria on hands. Liquid hand washes are generally preferred over soaps due to ease of use and the ability of liquid soaps to maintain the skin's natural moisture.

PROCEDURE:

1. Weigh 225gm Sodium lauryl ether sulphate (70%).
2. Transfer in stainless steel pot. Add 15 ml Cocodiethanolomide in 200 ml of distilled water.
3. Stir well for 20 minutes(clockwise and anti-clockwise direction). Milky solution is obtained. (Say Pot A).
4. Take 200 ml distilled water add 10 g Cocomonoethanolomide, 1g E.D.T.A. and 1 g methyl paraben in stainless steel pot.
5. Heat the above mixture on burner upto dissolve properly. (Say pot B).
6. Mix the solution from Pot B into Pot A slowly. Mix it thoroughly.
7. Cool the reaction mixture at room temperature.
8. Add colour (as per choice).
9. Add 2 ml perfume (as per choice).
10. Keep the solution in container and place a stopper on it. Liquid soap is ready to use.

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DEPARTMENT OF CHEMISTRY
EXPERIMENT UNDER DBT STAR COLLEGE SCHEME
Academic Year: 2024-25

Class: S.Y.B. Sc. **Date:**
Name of the Student: **Roll Number:**

Title : Estimation of Sugar by Folin-WU Method

Aim: To estimate the glucose level in the given glucose sample.

Principle: Glucose reduces the cupric ions present in the alkaline copper reagent to cuprous ions or the cupric sulfate is converted into cuprous oxide, which reduces the phosphomolybdic acid to phosphomolybdous acid, which is blue when optical density is measured at 420 nm.

Reagents Required:

1. Alkaline copper-reagent: Dissolve 40 gms of anhydrous sodium carbonate (Na_2CO_3) in about 400 mL of water and transfer it into a 1-liter flask. Dissolve 7.5 gms of tartaric acid in this solution and 4.5 gms of $CuSO_4$, which is dissolved in 100 mL water. Mix the solution and make it up to 1 liter.
2. Phosphomolybdic acid reagent: Add 5 gms of sodium tungstate to 35 gm of phosphomolybdic acid. Add 20 mL 10% NaOH and 50 mL distilled water. Boil vigorously for 20-30 min so as to remove the NH_3 present in molybdic acid, cool and dilute to 350 ml with water. Add 125 ml of ortho phosphoric acid and make up the volume up to 500 ml.
3. Standard glucose solution: Dissolve 100 mg of glucose in 100 ml distilled water and take 10 ml from this stock and make it up to 100 ml.

Preparation of protein free filtrate: To 1 ml Glucose sample, add 8 ml distilled water, 0.5 ml of2/3 N sulfuric acid and 0.5 ml of 10% sodium tungstate solution in a stoppered centrifuge tube and mix the contents. Then centrifuge at 3000 rpm for 10 min and collect the supernatant as sample.

Procedure:

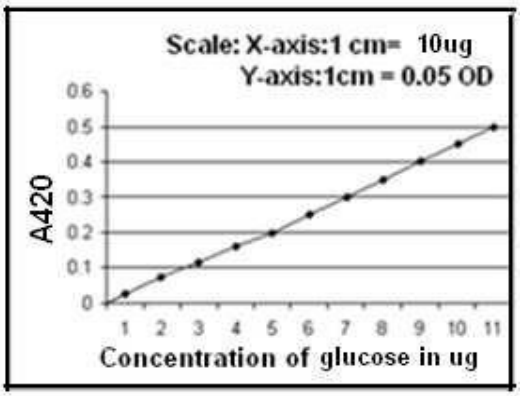
1. Pipette out 0.0, 0.4, 0.8, 1.2, 1.6 and 2 ml of working standard in to the series of labeledtest tubes.
2. Pipette out 2 ml of the given sample (protein free filtrate) in another test tube.
3. Make up the volume to 2 ml in all the test tubes. A tube with 2 ml of distilled water serves as the blank.
4. Now add 2 ml of copper sulfate solution and incubate in a boiling water bath for 8 minand cool it.

5. Then add 2 ml of phosphomolybdic acid to all the test tubes. Dilute the solution up to 25 ml mark on the Folin-Wu tube. Mix the contents and read the absorbance at 420 nm against blank.
6. Then plot the standard curve by taking concentration of glucose along X-axis and absorbance at 420 nm along Y-axis.
7. From the standard curve calculate the concentration of glucose in the given sample

Observations and Calculations:

Volume of standard glucose (ml)	Volume of distilled water (ml)	Concentration of glucose (µg)	Volume of copper sulfate solution (ml)	Incubate In boiling water bath for 8 min and cool to R T	Volume of phospho molybdic acid (ml)	Dilute the solution up to 25 ml mark on the Folin-Wu tube.	A_{420}
0.0	2.0	00	1.5		2		0.00
0.4	1.6	40	1.5		2		
0.8	1.2	80	1.5		2		
1.2	0.8	120	1.5		2		
1.6	0.4	160	1.5		2		
2.0	0.0	200	1.5		2		
2.0 Sample	0.00	To be Estimated	1.5		2		

Standard curve



Result: The given unknown sample contains µg /ml.

References:

1. Oser B. L., (1976) *Hawk's physiological chemistry*. 14th Edn. Tata McGraw Hillpublishing Company Ltd, New Delhi, India.

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DEPARTMENT OF CHEMISTRY
EXPERIMENT UNDER DBT STAR COLLEGE SCHEME
Academic Year: 2024-25

Class: S.Y.B. Sc. **Date:**
Name of the Student: **Roll Number:**

AIM: TO STUDY ACID-BASE TITRATION CURVES BY USING PH-METRY

Requirements:

pH meter, 0.1M NaOH, 0.1M HCl, distilled water, buffer solutions of pH 4,7 and 9.2, etc.

Theory:

All acids dissociate in aqueous solution to yield H^+ ions. Some acids like HCl, H_2SO_4, HNO_3 etc. are completely ionized in aqueous medium whereas CH_3COOH, HCOOH etc. ionize to a small extent only. The former are known as strong acids and the later are known as weak acids. The pH of any solution is defined as negative logarithm to the H^+ ($-\log H^+$) and has values between 0-14. The pH < 7 indicate acidic solution, pH > 7 indicate basic solution and pH = 7 means neutral solution.

In an acid-base titration, the important information to obtain is the equivalence point. It is the point at which an equal amount of acid has been neutralised by equal amount of base or vice versa. The pH of a solution can be measured accurately with the help of a pH meter. It affords a direct method of obtaining a titration curve. Acid-Base titrations are basically of four types- I) Titration of a strong acid with a strong base, II) Titration of a strong acid with a weak base, III) Titration of a weak acid with a strong base and IV) Titration of a weak acid with a weak base.

This experiment involves the study of titration of a strong acid (i.e. HCl) with a strong base (i.e. NaOH).

Procedure:

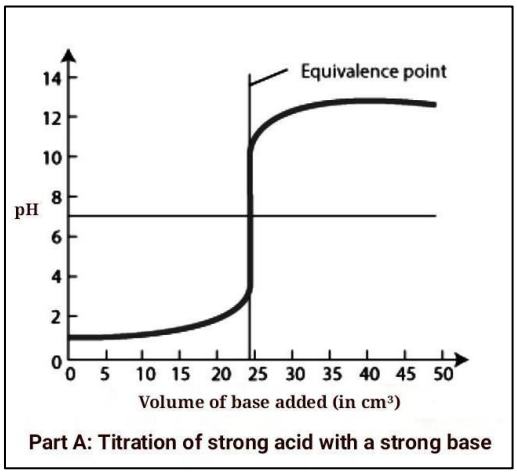
1. Standardize the pH-meter using buffer solutions of pH 4, 7 and 9.2.
2. Clean the electrode with distilled water and wipe them with tissue paper or filter paper.
3. Wash and fill the burette with 0.1M NaOH solution.
4. Take 10 cm^3 of 0.1M HCl solution in a 100 cm^3 beaker
5. Immerse the glass electrode in the above beaker and stir the solution for about one minute and measure the pH of the solution.
6. Initially add 0.2 cm^3 of 0.1M NaOH solution from the burette to the beaker containing HCl solution. Stir well and measure the pH of the solution.
7. Repeat the step no.6 and determine the pH after each addition.
8. After addition of total 6 cm^3 of 0.1M NaOH solution to the 0.1M HCl solution, increase the addition of 0.1M NaOH from 0.2 cm^3 to 0.5 cm^3.
9. Continue the addition of NaOH solution till the pH of the titrated solution reaches the value of about 10 and measure the pH of the solution.

Observation Table:

Sr. No.	Volume of base added (in cm^3)	pH
1	0.0	
2	0.2	
3	0.4	
4	0.6	
5	0.8	
6	1.0	
7	1.2	
8	1.4	
9	1.6	
10	1.8	
11	2.0	
12	2.2	
13	2.4	
14	2.6	
15	2.8	
16	3.0	
17	3.2	
18	3.4	
19	3.6	
20	3.8	
21	4.0	
22	4.2	
23	4.4	
24	4.6	
25	4.8	

Sr. No.	Volume of base added (in cm^3)	pH
26	5.0	
27	5.2	
28	5.4	
29	5.6	
30	5.8	
31	6.0	
32	6.5	
33	7.0	
34	7.5	
35	8.0	
36	8.5	
37	9.0	
38	9.5	
39	10.0	
40	10.5	
41	11.0	
42	11.5	
43	12.0	
44	12.5	
45	13.0	
46	13.5	
47	14.0	
48	14.5	
49	15.0	
50	15.5	

Graph: Plot a graph of pH against volume of base added.



Part A: Titration of strong acid with a strong base

Result:

When a strong acid is titrated with a strong base, pH at the equivalence point will be _____.

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DEPARTMENT OF CHEMISTRY
EXPERIMENT UNDER DBT STAR COLLEGE SCHEME
Academic Year: 2024-25

Class : T.Y.B. Sc. **Date :**
Name of the Student: **Roll Number :**

AIM: SYNTHESIS OF ZINC OXIDE (ZnO) NANOPARTICLES USING THE HYDROTHERMAL METHOD

THEORY:

Nanomaterials are broadly defined as material that has at least one dimension less than 100 nm. Zinc Oxide (ZnO) is a wide band gap semiconductor material. Nanoparticles of zinc oxide with a size below 100 nm have a large surface area and high catalytic activity. The exact physical and chemical properties of zinc oxide nanoparticles depend on the different ways they are synthesized, size, morphology, etc. Zinc oxide nanoparticles are useful in a variety of industries, including paints, rubber, cosmetics, and wastewater treatment.

REACTION:

$Zn(NO_3)_2.6H_2O$ + NaOH ⟶ ZnO nanoparticles

REQUIREMENT:

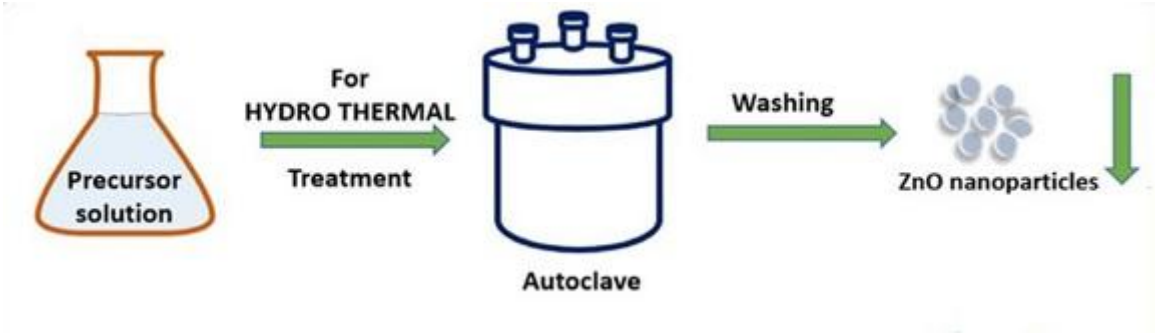
CHEMICALS: Zinc nitrate [$Zn(NO_3)_2.6H_2O$], sodium hydroxide [NaOH], distilled water.

INSTRUMENTS/APPARATUS/GLASSWARE: Autoclave, oven, beaker, glass rod, magnetic stirrer.

PROCEDURE:

(1) Take 20 mL of 0.5 M zinc nitrate solution in a 100 mL beaker.
(2) Add 20 mL of 5 M sodium hydroxide solution to another 100 mL beaker.
(3) Then after, the above NaOH solution is added dropwise to the zinc nitrate solution under continuous stirring until the P^H of the reactants/solution becomes 12.
(4) This solution mixture was transferred into a Teflon-lined sealed stainless-steel autoclave and kept in an oven at a temperature of 100°C for 2 h.
(5) After the completion of the reaction, the stainless-steel autoclave was taken outside and allowed to cool naturally to room temperature.
(6) The resultant solution was filtered, washed with distilled water, and kept for drying under the lamp.
(7) After proper drying take the weight of the obtained material and records the yield.

SCHEMATIC REPRESENTATION:



OBSERVATIONS AND CALCULATIONS:

(1)	Weight of the obtained precipitate	----------grams.

RESULT:

Weight of the obtained ZnO nanomaterials	----------grams.

REFERENCES:

(1) Mohan, S.; Vellakkat, M.; Aravind, A.; U, R. Hydrothermal Synthesis and Characterization of Zinc Oxide Nanoparticles of Various Shapes under Different Reaction Conditions. *Nano Express* **2020**, *1* (3), 030028. https://doi.org/10.1088/2632-959X/abc813.

(2) Liu, B.; Zeng, H. C. Hydrothermal Synthesis of ZnO Nanorods in the Diameter Regime of 50 Nm. *J. Am. Chem. Soc.* **2003**, *125* (15), 4430–4431. https://doi.org/10.1021/ja0299452.

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DEPARTMENT OF CHEMISTRY
EXPERIMENT UNDER DBT STAR COLLEGE SCHEME
Academic Year: 2024-25

Class: T.Y.B. Sc. **Date:**
Name of the Student: **Roll Number:**

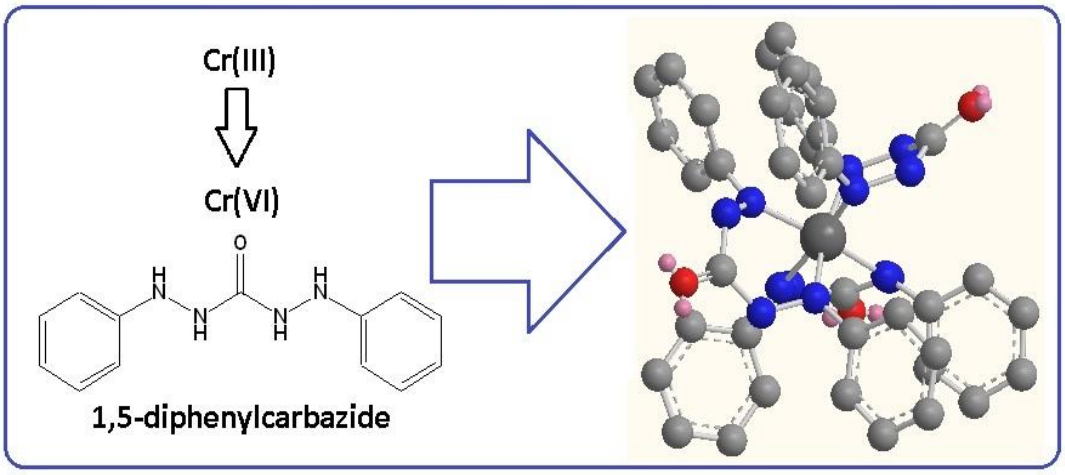
Title: Heavy metal analysis of Industrial waste water Cr (VI) by UV-Visible spectrophotometer

Aim: TO DETECT THE Cr (VI) PRESENT IN INDUSTRIAL WASTE WATER BY UV-VISIBLE SPECTROPHOTOMETER.

Requirements: 100 cm3 beakers, 250 cm3 beakers, 100 cm3 volumetric flask, quartz cuvettes, 50 cm3 measuring cylinder, glass rods, Industrial waste water, distilled water, methanol, $K_2Cr_2O_4$, diphenyl carbazide, phosphoric acid, ammonium hydroxide buffer, weighing balance.

Theory: Chromium is a heavy metal which occurs in the environment primarily in two valence states, trivalent Cr(III) and hexavalent Cr(VI). Chromium (III) is an essential micro-nutrients for the human body, while the Cr(VI) is highly toxic and carcinogenic. Toxic chromium enter into the water system, derived from nature and from waste or industrial waste such as metal industrial waste water, electroplating, wood preservation, fertilizers, leather preservation industry. Cr(III) is in the form of hydroxide compounds as Cr(OH)n (3-n)+.

Reaction:



Procedure:

1. Cr(VI) sample (2 cm^3) was transferred to a beaker.
2. Sulphuric acid (0.2 M, 1 cm3) and 1,5-diphenylcarbazide (0.5% w/v, 1 cm3) were added, and the mixture was gently shaken and left for five minutes.
3. Likewise different concentrations of Cr(VI) samples were prepared from the stock solution.
4. The absorbance was measured for different concentrations of Cr(VI) samples in nm against reagent blank.

Observations and Calculations:

1. The absorbance of the 10 ppm Cr(VI) sample = _____
2. The absorbance of the 50 ppm Cr(VI) sample = _____
3. The absorbance of the 100 ppm Cr(VI) sample = _____

Results:

1. λ_{max} was observed at _____ nm

References:

Annija Lace, David Ryan , Mark Bowkett and John Cleary Chromium Monitoring in Water by Colorimetry using Optimised 1,5-Diphenylcarbazide Method, International Journal of Environmental Research and Public Health 2019, https://doi.org/10.1155/2016/3468635

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EXPERIMENT UNDER DBT STAR COLLEGE SCHEME
Academic Year: 2024-25

Class: T.Y.B. Sc. **Date:**
Name of the Student: **Roll Number:**

TITLE: - SYNTHESIS OF MIXED LIGAND COMPLEXES OF LANTHANUM.

Aim: Synthesis of mixed ligand complexes of La(III) with 1-nitroso-2-naphthol (1N2N) and L-valine amino acid (HL).

Requirements: -

Chemicals: Lanthanum Chloride [$LaCl_3.7H_2O$], 1-nitroso-2- naphthol, L-valine, Ethyl Alcohol, diluted ammonia, Distilled Water.

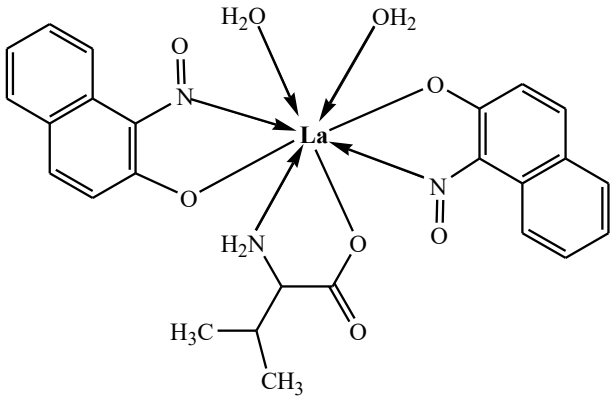
Apparatus: 100cm^3 Beakers, 250 cm^3 beaker, Glass rod, pH meter, water bath, Clay pipe triangle etc.

THEORY: La(III) ions form green colored mixed ligand complex when treated with 1-nitroso-2-napthol and L-valine. The 1-nitroso-2-naphthol is yellowish brown solid and act as a bidentate ligand. Similarly, L-valine is a colorless bidentate ligand contains -N and or -O donor atoms. Both ligands react with $LaCl_3$.$7H_2O$ and forms mixed ligand complex at neutral medium.

Reaction:

$$LaCl_3 .7H_2O + 2(1N2N) + HL \rightarrow [La\ (1N2N)_2(L).2H_2O] + 3HCl + 5H_2O$$

Structure:



Structure of [La(1N2N)$_2$(Val)·2H_2O

Procedure:

(1) Dissolve 1.113 g or the supplied sample of $LaCl_3.7H_2O$ in a 10 cm^3 distilled water in a 100 cm^3 beaker

(2) Weigh 1.038 g of 1-nitroso-2-naphthol in a 20 cm^3 ethyl alcohol in another 100 cm^3 beaker. If required, heat it on a boiling water bath to dissolve it.

(3) Transfer the solution of 1-nitroso-2-naphthol in a solution of $LaCl_3.7H_2O$ with constant stirring and reflux it in water bath for 10 minutes.
(4) To this refluxed solution add 10 cm^3 solution of L-valine (0.351 g) in distilled water. Stir the solution vigorously and warm it in water bath for 10 minutes.
(5) Then raise the pH of the above reaction mixture up to 7.0 by slow addition of diluted ammonia solution for complete precipitation of the complex.
(6) Filter the precipitates through Whatman filter paper no. 41 using Buchner funnel at the suction pump.
(7) Wash the complex with distilled water 2-3 times and then with 2-3 cm^3 ethyl alcohol.
(8) Dry the complex in an electric oven at 110°C.
(9) Weigh the product to obtain the yield (x g).

Observations and Calculations:
Yield of the Complex:
(A) Theoretical yield of the complex
271 g of $LaCl_3.7H_2O$ gives 635.39 g of the complex

1.113 g $LaCl_3.7H_2O$ = $\frac{635.39 \times 1.113}{271}$

= 2.60 g of the complex

(B) Observed yield of the complex (x g) = _____
(C) Percentage yield of the complex:
2.60 g of the complex = 100 % yield

x g of the complex = $\frac{x \times 100}{2.60}$

= 38.46 x %

(D) Magnetic Behavior of Complex:
The $[La(1N2N)_2(Val)\cdot 2H_2O$ mixed ligand complex contains La(III) metal ion.
The electronic configuration of La is [Xe] $4f^0\ 5d^1 6s^2$.
La^{3+} = [Xe] $4f^0\ 5d^0 6s^0$
The number of unpaired electrons in La^{3+} metal ion = ----------electron
Therefore magnetic behavior of the complex = ----------

Result:
(1) Yield of the complex:
(A) Theoretical = ----------g
(B) Observed = ----------g
(C) Magnetic behavior of the complex = ----------

References:

1. N.K. Fayad, T.H. Al-Noor and F.H. Ghanim, Chem. Mater. Res., 2(5) 18-29, (2012).
2. G. A. Thakur, U. N. Dhaigude and P. B. Thakur, Orient. J. Chem. 36(4), 632-639, (2020)

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DEPARTMENT OF CHEMISTRY
EXPERIMENT UNDER DBT STAR COLLEGE SCHEME
Academic Year: 2024-25

Class: T.Y.B. Sc. **Date:**
Name of the Student: **Roll Number:**

TITLE : EXTRACTION OF CHEMICAL CONSTITUENTS FROM MEDICINAL PLANTS.

AIM : Extraction of Chemical constituents from medicinal plants present in college campus.

THEORY : Extraction involves the separation of medicinally active constituents from complex matrix of plant by using selective solvent in standard extraction condition. Active constituent extracted from different parts of plant such as leaves, root, stem and fruits. The present experiment primarily concerns with basic extraction procedure by using Rotary shaker for estimation of amount of chemical constituents present in a particular part of plant sample.

REQUIREMENT:

CHEMICALS:
Methanol, Ethanol, Ethyl Acetate, Dichloromethane, N-Hexane, Pet-Ether, Chloroform.

INSTRUMENTS/APPARATUS/GLASSWARES:
Rotary Shaker, Mortal Pestal, Electric water bath, conical flask.

PROCEDURE:

1. Freshly collected sample of plant is crushed by using mortal pastel for extraction.
2. Place 5g crushed sample in a conical flask & add 50 ml of solvent in it
3. Then Keep the flask on rotary shaker for 2 hours.
4. Then the contents of the flask were filtered separate it through Whatman filter paper no. 41 in pre- weighed dry beaker and solvent was evaporated to dryness on a water bath.
5. The dried residue then weighed and percentage extract were calculated.

CALCULATION:

1. % Extract = $\frac{W-W1}{W2}\ x\ 100$

   = ------ %

Where:
W1 = Weight of empty beaker in g.
W = Weight of empty beaker with extract in g.
W2 = Weight of sample in g.

RESULT :

The Percentage extract of chemical constituent in the given plant sample is ----------%.

REFERENCES :

1. Harborne.J.B, "Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis." 2nd Edition, Chapmann and Hall Publishers, London, 1998.

2. Handa SS, Khanuja SPS, Longo G, Rakesh DD. Extraction Technologies for Medicinal and Aromatic Plants. International centre for science and high technology, Trieste, 2008, 21-25

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DEPARTMENT OF CHEMISTRY
EXPERIMENT UNDER DBT STAR COLLEGE SCHEME
Academic Year: 2024-25

Class: T.Y.B. Sc. **Date:**
Name of the Student: **Roll Number:**

TITLE: ESTIMATATION OF PROTEIN USING BIURET METHOD.

Aim: TO ESTIMATE THE PROTEIN USING BIURET METHOD.

Theory / Principle: The -CO-NH- bond (peptide) in polypeptide chain reacts with copper sulphate in alkaline medium to give a purple colour which can be measured at 540 nm.

Apparatus: Test tubes, Pipettes, Colourimeter, thermostat etc.

Chemicals: 1. Protein standard (5 mg BSA/ ml)

2. Biuret Reagent: Dissolve 3 gm of copper sulphate

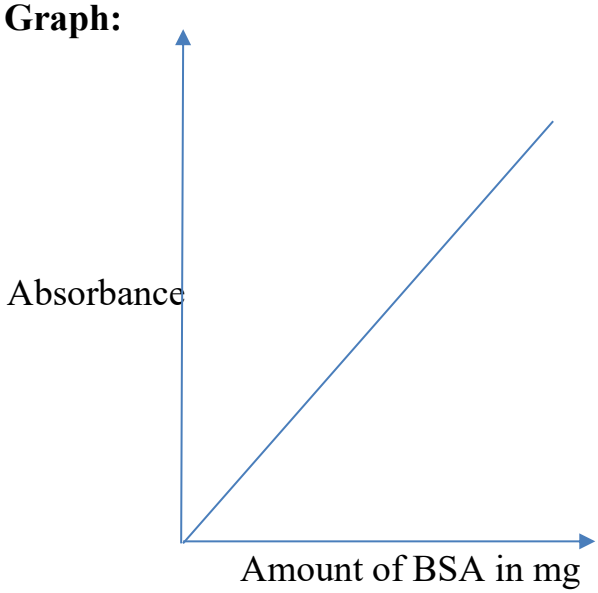
($CuSO_4.5\ H_2O$) and 9 gm of sodium potassium tartarate in 500 ml of 0.2 mol/ litre sodium hydroxide , add 5 gm of potassium iodide and make up to 1 litre with 0.2 mol/litre sodium hydroxide.

Procedure:
1. Pipette out 0.0, 0.2, 0.4, 0.6, 0.8, and 1 ml of working standard into the series of labeled test tubes.
2. Pipette out 1 ml of the given sample in another test tube.
3. Make up the volume to 1 ml in all test tubes. A test tube of 1 ml of distilled water serves as blank.
4. Now add 3 ml of Biuret reagent to all the test tubes including the test tubes labeled as 'blank' and 'unknown'
5. Mix the contents of the tubes by overtaxing / shaking the tubes and warm at 37^0C for 10 minutes.
6. Now cool the contents to room temperature and record the absorbance at 540 nm against blank
7. Then plot the standard curve by taking concentration of protein along X- axis and absorbance at 540 nm along Y-axis.
8. Then from this standard curve, calculate the concentration of protein in the given sample.

Observation Table:

Volume of standard BSA (ml)	Volume of Distilled water (ml)	Concentration of protein (mg)	Volume of Biuret reagent (ml)		Absorbance
0.0	1.0	00	3	Incubate at 37 ^{0}C for 10 minute and cool	0.0
0.2	0.8	1	3		
0.4	0.6	2	3		
0.6	0.4	3	3		
0.8	0.2	4	3		
1.0	0.0	5	3		
Unknown	0.0	To be estimated	3		

Nature of the Graph:



Result: The given unknown sample contains …… mg protein/ml.

References:

1. A manual Laboratory Techniques, 1983. Editors: Raghuramulu N. Nair, National Institue of Nutrition, Hydrabad , India.

2. An Introduction of Practical biochemistry , 3rd Edition, Tata Mc Graw Hill publishing Company, New Delhi, pp 159.

Student Signature **Teacher Signature**

Rayat Shikshan Sanstha's
Mahatma Phule Arts, Science and Commerce College,
Panvel, District- Raigad (Maharashtra)
DEPARTMENT OF CHEMISTRY
ACADEMIC YEAR: 2024-25
EXPERIMENT UNDER DBT STAR COLLEGE SCHEME

Class : T.Y.B. Sc. **Date:**
Name of the Student: **Roll Number:**

TITLE: ESTIMATION OF SUGAR BY DNSA METHOD.

AIM: TO ESTIMATE THE AMOUNT OF REDUCING SUGARS PRESENT IN THE GIVEN SAMPLE BY DNSA METHOD.

REQUIREMENTS:

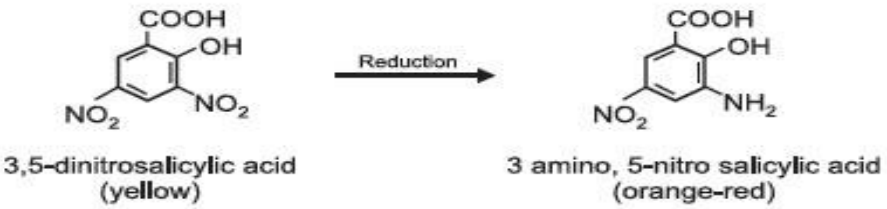
A) Chemicals:

1. Sodium potassium tartrate: Dissolve 45 gm of sodium potassium tartrate in 75 mL of H_2O.
2. 3,5-DNS solution: Dissolve 1.5 gm of DNS reagent in 30 mL of 2 M/litter NaOH.
3. 2 M NaOH: 80 gm of NaOH dissolved in 1 litter of water.
4. DNS reagent: Prepare fresh by mixing the reagents (1) and (2) make up the volume to 150 mL with water.
5. Standard sugar solution:
   (i) Stock standard sugar solution: 250 mg of glucose in water and make up the volume to 100 mL (2500 ppm).
   (ii) Working standard solution: Take 10 mL from this stock solution and make up the volume to 100 mL (250 ppm).

B) Glassware and Instrument: Test Tubes, Micropipette (1 mL and 5 mL), Spectrophotometer, etc

THEORY: Sugars with reducing property arising out of the presence of a potential aldehyde or keto group are called reducing sugars. Ex. Glucose, galactose, lactose, maltose etc. 3,5 dinitro salicylic acid (DNSA) is reduced to 3-amino- 5-nitrosalicylic acid in the presence of reducing sugars in an alkaline solution. The reducing sugar is oxidized to sugar acid and Rochelle's salt.

REACTION:



3,5-dinitrosalicylic acid (yellow) → 3 amino, 5-nitro salicylic acid (orange-red)

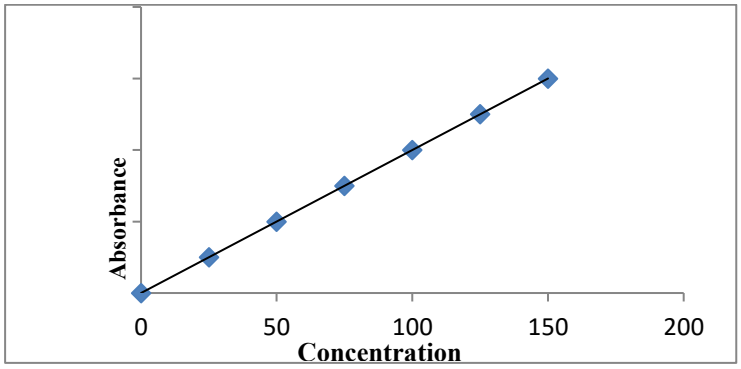
PROCUDURE:

1. Take 7 clean, dry test tubes.
2. Pipette out standard sugar solution of 0, 1, 2, 3, 4, 5, and 6 mL in different test tubes and make up the volume of all test tubes to 10 mL with distilled water concentrations ranging from 0, 25, 50, 75, 100, 125, and 150 ppm respectively.
3. Add 3 mL DNS reagent to all the test tubes and mix plug the test tube with cotton or marble and keep the test tube in a boiling water bath for 5 minute. Take the tubes and cool to room temperature.
4. Measure the absorbance at 540 nm against the blank. Please note that all the tubes must be cooled to room temperature before reading, since the absorbance is sensitive to temperature.
5. Prepare standard curves of the sugars provided and use them to estimate the concentration of the unknowns provided.

OBSERVATIONS:

Sr. No.	Concentrations (ppm)	Absorbance
1	00	
2	25	
3	50	
4	75	
5	100	
6	125	
7	150	

GRAPH:



RESULTS: The unknown solution of sugar contains ..... mg of glucose.

***REFERENCES*:**

1.https://biocyclopedia.com/index/biotechnology_methods/biochemistry/estimation_of_reducing_sugars_by_the_dinitro_salicylic_acid_dns_method.php
2.https://www.researchgate.net/profile/NeelaGanesan/post/How_can_I_prepare_the_DNS_solution_used_for_determining_the_reducing_sugar/attachment/5cc7e829cfe4a7df4aed1692/AS%3A753269991305217%401556604969441/download/dns+procedure.pdf

Signature of the Student **Signature of the Teacher**

Rayat Shikshan Sanstha's
Mahatma Phule Arts, Science and Commerce College,
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DEPARTMENT OF CHEMISTRY
EXPERIMENT UNDER DBT STAR COLLEGE SCHEME
Academic Year: 2024-25

Class: T.Y.B.Sc. **Date:**

Name of the student: **Roll Number:**

TITLE: ESTIMATION OF AMINO ACID BY NINHYDRIN METHOD

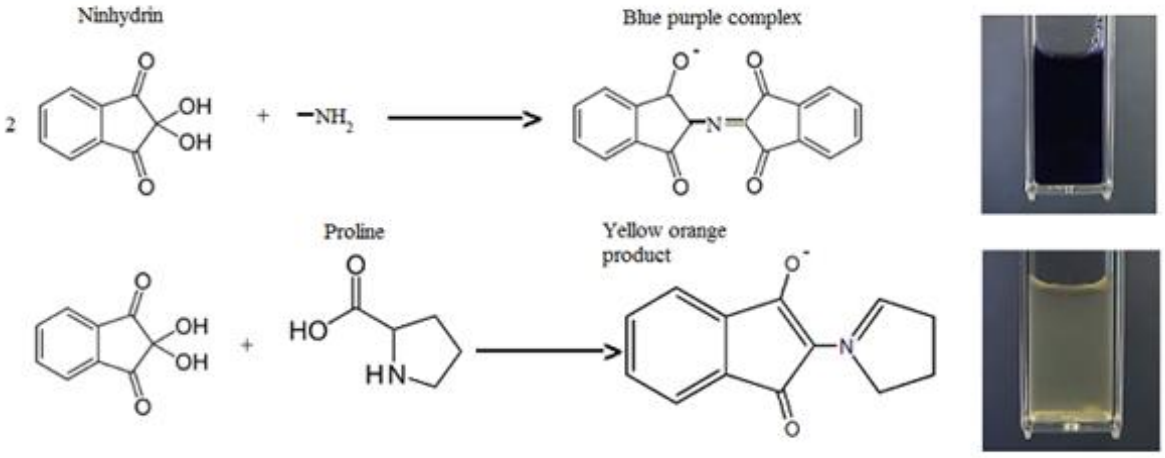
AIM- To Estimate the amount of Amino acid present in the given sample by Ninhydrin method.

REQUIREMENTS:
Chemicals: Ninhydrin, Ethanol, Standard Amino acid solution, Distilled water.
Apparatus: Test tube, Pipette, Vortex machine, Water bath, Spectrophotometer.

THEORY:

Ninhydrin (triketohydrindene hydrate) is used to assess amino acid qualitatively and quantitatively in ninhydrin reaction. All amino acids having α amino group, when heated in excess of ninhydrin give a purple-coloured product. Only proline and hydroxyproline- amino acids molecules which contain a secondary amino group produce yellow condensation product. The colour intensity is proportional to the concentration of amino acid i.e. ammonia from the amino groups.

REACTION:



PROCEDURE:

1. Take seven clean and dry test tubes and label them as 1, 2, 3, 4, 5, 6 and unknown.
2. Take a tube labelled as one as blank containing 4ml of just distilled water and the rest of the tubes labelled 2 to 6 for construction of a standard curve. Tube seven is for the unknown sample.
3. Into the tubes marked 2 to 6, add standard amino acid solution in aliquots of 0.2 to 1ml.
4. Add water to each tube to make up the volume to 4 ml.
5. To all the tubes, add 1 ml of the ninhydrin reagent including blank and unknown and mix well by vertexing.
6. Cool the tubes.
7. Cover the tubes with caps on top and keep them in boiling water bath for 15 minutes.
8. Cool the tubes to room temperature and measure the optical density of the solutions at 550 nm against a blank.
9. Prepare a standard curve of absorbance against amino acid concentration.
10. Determine the amount of amino acid in the unknown sample by plotting a standard curve of 550 nm on the Y-axis and concentration of amino acid on the X-axis.

OBSERVATIONS:

Sr. No.	Std. amino acid (ml)	Absorbance (nm)
Blank	-	
1	0	
2	0.2	
3	0.4	
4	0.6	
5	0.8	
6	1	
unknown		

RESULTS: The amount of amino acid present in a given unknown sample is _____.

REFERENCES: Tiwari A. (2015). Practical Biochemistry. LAP Lambert Academic Publishing.

Signature of the Student **Signature of the Teacher**