INTRODUCTION

1st YEAR CHEMISTRY PRACTICAL COURSE, 2021

1. INTRODUCTION

In the B. Tech. Chemical Laboratory you will meet a wide range of techniques - from the simple to those using complex instrumentation - with which to study the properties of materials. During your time here, we want you to gain an understanding of experimental techniques and methods. It is not easy to work in a laboratory; making precise measurements requires practice, experience and judgment. In the practical course you will, we hope, acquire these skills, which are a vital preparation for later research.

2. WORKING HOURS

You will work in the laboratory during a 16-week period between 2 p.m. and 5:00 p.m. on Monday, Tuesday or Wednesday. As you cannot work beyond 5:30 p.m., it is necessary for you to plan in advance so that you are done by the time when the laboratory closes, without disrupting your experiment. From 9 a.m. to 1 p.m., and all day Thursdays and Fridays the laboratory remains closed for experiments, but you are permitted to record UV spectra and melting points to characterize the samples which you will synthesize.

3. STARTING AN EXPERIMENT

Prepare fully for an experiment by reading in advance the instructions and any appropriate background material. If the experiment lies in an area not yet covered in lectures, you may need to do some further reading, but in any case proper preparation before starting an experiment is always time well spent. You will normally work on experiments as one of a pair.

7. APPARATUS

Major apparatus for experiments is set out in various places in the laboratory. Glassware and required chemicals are set out on the benches; for other requirements, see the technical superintendents. Clean the apparatus and bench area when you finish an experiment. Rinse out glassware and leave on the benches. If the apparatus is faulty in any way, report this to a technical superintendent, even if you caused the problem. If not reported, a problem may remain undetected until the next students attempt the experiment. Do not leave unlabelled liquids in beakers or flasks - these may cause a hazard.

8. WEB SITE

A web site is available for the practical course in chemistry at the following URL: http://172.16.1.3/download/manuals/manuals.html. This contains safety and other information which you may find of value.

9. QUESTIONS?

Help on any aspect of the practical course in the Department of Chemistry, IIT Patna is always available from Dr. Prolay Das (Room No. 103, Science Building, prolay@iitp.ac.in,

10. SAFETY

You will need to work carefully in the B.Tech. Laboratory of IIT Patna, as in any other laboratory. Special hazards associated with an experiment are explained in the instructions, but laboratories are dangerous places, so you must always be alert.

If in doubt - ASK

Our experiments are drafted to take the regulations into account. If any particularly unpleasant or dangerous chemical is used during an experiment, detailed precautions are given in the instructions. However, remember that the prime responsibility for safety rests with you. The guidelines are given separately in the website; PLEASE READ THEM BEFORE STARTING WORK! Before starting work for the first time, check the location of emergency facilities. There are two exits from the laboratory - make sure you know where they are. The laboratory has a nearby emergency shower - find it! Check that you know where fire extinguishers are kept in case you need to use one in case you discover (or create!) a fire. If you suffer from any condition which may lead to sudden illness, or if you must take, even for a short time, drugs which may affect your performance in the laboratory, obtain a medical record sheet from the medical officer. In case of emergency, it may help if this has been completed and stuck inside your laboratory data book.

SAFETY REGULATIONS

Before you begin any work in the laboratory, you should read and understand the rules below. If you do not understand a rule, insist on a satisfactory explanation from your instructor or a faculty member. Keep a copy of this document in your lab manual and refer to the safety rules frequently.

I have read and understand the safety rules (1-33) below and have had an opportunity to question my instructor about them. I agree to follow these regulations when I am in the chemistry laboratory.	
Date of Safety Training:	Your Signature:

Chemistry Department Safety Rules	Comments and Examples
GENERAL RULES	
1. Do not work in the laboratory unless your instructor is present to supervise your work.	1. A qualified person must be present to: (a) see that only safe procedures are used, and (b) provide immediate aid in case of an accident.
2. Do not carry out any unauthorized experiment.	2. Perform only those experimental steps in the printed manual, or those given directly to you by your instructor.
3. Do not work under any condition that you believe to be unsafe for you or others.	3. If such a condition exists (e.g., overcrowded area, unsafe actions by another student), report it immediately to your instructor or to a faculty member in charge.
EYE PROTECTION	
4. Wear approved eye protection <u>at all times</u> in the laboratory. Approved eye protection means safety goggles with indirect venting.	4. Eyes are very susceptible to chemical injury and must be fully protected all the time. Even when you are not working, a person nearby may be carrying out a chemical procedure that might affect you.
5. Contact lenses should not be worn in the laboratory.	5. All types of contact lenses may trap a chemical against the eye tissue and cause permanent eye damage. Check with your instructor if needed.
6. Do not work with a chemical above or near your face.	6. For example, holding a beaker up to look at what is in the bottom, or filling a burette which is higher than eye-level, can result in a splash down onto your face.
HANDLING CHEMICALS	
7. Many chemicals are toxic and/or corrosive.	7. Chemical reagents require careful handling.

8. Do not taste or ingest any chemical in the laboratory. Do not take or keep food or drink items at your lab bench.	8. It may be toxic. Even NaCl may be contaminated and be unsafe. For the same reason, you can not bring food or drink into the laboratory, or eat in the laboratory (no chewing gum, tobacco, candy, bottled water or drinks, etc.)
9. Never pipet by mouth.	9. Drawing up a liquid (e.g., into a pipet) should be done only with a rubber bulb or water aspirator.
10. Never pipet directly from a reagent bottle.	10. Transfer only necessary amount of liquid reagents to a secondary container, such as a clean, dry beaker.
11. Avoid skin contact with any chemical.	11. Keep the outside of reagent containers, all of your equipment and the desk top, free from chemical spills. Wear gloves if instructed to do so.
12. Do not inhale reagent fumes.	12. Odour tests are to be made only when specifically directed to do so. Use a waving motion of your hand to bring the vapour near your nose (this is wafting).
13. Fume hoods must be used whenever toxic or corrosive vapours are released during the work you are doing.	13. Use the hood when directed to do so. If fumes develop unexpectedly, cover the container and take it to the hood at once. Work with concentrated hydrochloric, nitric, or acetic acids, or with bromine, chlorine, or hydrogen sulphide should be done only in a fume hood.
14. Alkalis are particularly corrosive. Contact with NaOH and other alkaline (basic) chemicals must be avoided. Work with solid sodium or potassium hydroxide, or with solutions of these more concentrated than 0.1 molar, should be carried out only under the direct supervision of the instructor.	14. Strong bases must be handled with great caution because they attack tissues so rapidly. Using 0.1 M sodium hydroxide in a titration also requires great care. Your instructor will demonstrate proper techniques in handling a base in the laboratory.
15. Do not heat a test tube containing a liquid over an open flame or directly on a hot plate.	15. To heat a test tube, hold it in a beaker of hot water. Liquids heated over an open flame may erupt violently and splash onto you or a person nearby.
16. Do not add water to a concentrated reagent, especially concentrated sulfuric acid.	16. Keep the mixture as dilute as possible; **add the reagent to water**. Addition of concentrated sulfuric acid to water causes much heat formation and may result in spattering of this corrosive reagent.
17. Handle liquid reagent containers with care.	17. When pouring a liquid, grasp each container so that drops cannot contact your fingers. When using a flexible polyethylene bottle, think first; do not pour from it or squeeze it in any manner that might result in a stream of liquid getting on you, or someone nearby.
18. Dry all wet glassware before storing or returning it. If you drop a beaker, for example, do not reach to catch it since this usually leads to getting cut.	18. Keep a cloth towel for drying glassware and one for wiping your hands. You may bring a towel from home.

HANDLING GLASS TUBING AND SHARPS	
19. Carry glass tubing and glass thermometers only in an upright position.	19. On impact, glass tubing can snap and become a dagger. Do not run with it (or any other chemical equipment).
20. To insert glass tubing or a thermometer into a rubber stopper: (a) If needed, fire polish the ends of the tubing. (b) Lubricate the stopper hole with water or glycerine. (c) Insert the tubing cautiously, using a towel to protect your hands.	20. (a) After heating glass tubing, set it aside in a place where you will remember that it is hot. (b) **There should <u>not be</u> more than two inches between the stopper and your fingertips on the tubing or thermometer.** (c) If handled improperly glass tubing can break and become razor sharp when inserted into a stopper.
21. All broken glass laboratory waste must be placed into the special black glass disposal tubs in the lab.	21. Only paper products go into the regular bins. There are several bins available near the slab in the chemistry laboratory.
22. Waste "Sharps" must be placed in the special black tub provided in the lab.	22. Examples of "Sharps" are: syringes, syringe needles, razor blades, and scalpels.
IN CASE OF ACCIDENT	
Learn the basic laboratory first-aid measures.	
23. (a) **If a chemical splashes into your eye, <u>get help immediately</u>. Shout out, "I have chemical in my eye!"**	23. (a) Seconds count! Immediate removal of the chemical is necessary to prevent possible damage to the eye.
23. (b) If someone nearby gets a chemical in his/her eye, <u>you</u> should: (1) shout for help from the instructor, (2) provide help if the instructor is not there immediately.	23. (b) A person who has just gotten a chemical in his/her eye usually is frightened, confused, and may be unable to help himself/herself.
23. (c) **Wash the eye thoroughly with a stream of water from the eye wash fountain, or any other water source. Hold the eyelids open.**	23. (c) **After thorough washing (15 minutes is the recommended time)** the affected person must be taken to get professional medical attention.
24. Any chemical that comes in contact with your skin should be washed off with water right away.	24. This is especially important for concentrated reagents and organic liquids.
25. Know the location of fire extinguishers, fire blankets, and safety showers, in case of fire. Keep acetone and any other organic liquid at least ten feet from an open flame.	25. Use a wet towel to extinguish a small fire or the fire blanket if a person's clothes catch fire.
26. Proceed cautiously when handling hot objects. Use a towel as a hot pad when handling hot objects. Hot glass looks just like cold glass.	26. In case of burn, immerse in water immediately. Notify your instructor. Apply clean moist cloth or bandage. Seek medical attention if any question about treatment.
27. Know the exit route from your lab.	27. When the fire alarm sounds, stop what you are doing and immediately exit the lab, go down the stairs and exit the building. Wait outside for instructions.

28. Immediately report any accident to your instructor no matter how minor it may seem to you.	28. Cuts, burns, chemical burns, and inhalation or ingestion of chemicals should be treated as soon as possible by a professional medical person. Neither students nor chemistry staffs are qualified to make medical decisions.
CLOTHING IN THE LAB	
29. You must be covered continuously from shoulders to below the knees and must wear shoes that cover your feet. Bare feet, sandals, shorts, sleeveless shirts, short shirts, and short skirts are UNSAFE and must not be worn to laboratory. For fire safety, flammable materials, loose clothes, ties should not be worn, and long hair should be tied back.	29. Full coverage by (cotton) clothing and leather shoes offers the best protection against chemical spills and fire. Older clothing is advised, as is the use of lab coats or aprons. Open-toed shoes or sandals should not be worn in the chemistry laboratory.
CHEMICAL WASTE DISPOSAL	
30. Only neutral aqueous solutions go down the sink drain. Waste determinations and disposal are done by faculty and staff. Check with your instructor before disposing of any chemical.	30. All chemical waste is to be sorted into the appropriate waste container and the identity and amount must be logged onto the accompanying inventory sheet. Check with your instructor for specific details.
CLEANING GLASSWARE	
31. Clean all the glassware which you have used a. Clean general glassware by using detergent and brush kept near sink. b. Clean the glassware with stains by using chromic acid mixture kept in fume cupboard. c. Rinse with water and keep on drying racks.	31. Although it may not seem that important, cleaning glassware is one of the most important tasks that you will do in lab – contaminated glassware (along with contaminated solvents) are the two biggest causes of reactions going bad!
LEAVING LABORATORY	
32. Clean your work bench with a damp sponge. Neutralize all acid spills with sodium bicarbonate and wash with a wet sponge. Shut gas jets completely. Wash your hands.	32. Leave the area safe for the next person.
33. Do not take any chemical out of the laboratory for any reason. It is illegal!	33. You may be liable if another person is injured by a chemical (or unauthorized equipment) that you remove from the laboratory.

List of Experiments

EXP. NO.	EXPERIMENT TITLE
1	SPECTROPHOTMETRIC DETERMINATION OF STOICHIOMETRY OF A COMPLEX BY JOB'S METHOD
2	DETERMINATION OF CONCENTRATIONS OF HYDROCHLORIC ACID AND ACETIC ACID IN A MIXTURE CONDUCTOMETRICALLY
3	DETERMINATION OF THE CONCENTRATION AND THE DISSOCIATION CONSTANT OF A WEAK ACID (USING pH METER)
4	STANDARDISATION OF $KMNO_4$ SOLUTION BY OXALIC ACID
5	DETERMINATION OF THE TOTAL HARDNESS OF WATER BY COMPLEXOMETRIC TITRATION
6	DETERMINATION OF NUMBER OF COMPONENTS IN AN ORGANIC MIXTURE AND R_f OF EACH COMPONENT USING THIN LAYER CHROMATOGRAPHIC TECHNIQUE
7	SYNTHESIS AND STUDY OF SILVER NANOPARTICLES
8	QUANTITATIVE ESTIMATION OF PROTEIN BY BIURET METHOD
9	SYNTHESIS AND CHARACTERISATION OF TRIS(ACETYLACETONATO)MANGANESE (III)

EXPERIMENT NO.: 1

Spectrophotometric Determination of Stoichiometry of a Complex by Job's Method

1. Aim

In this experiment you will determine the composition of the complex formed by thiocyanate and ferric ions, using Job's method of continuous variation.

2. Safety

Do not pipette by mouth. Solutions of Hydrochloric acid at which you will be using it (0.004M) present a negligible risk. Ferric nitrate nonahydrate is harmful if swallowed or inhaled, causes irritation to skin, eyes and respiratory tract and affects the liver. Sodium thiocyanate is harmful if swallowed, may be harmful by inhalation or through skin contact and is an irritant.

3. Requirements

UV-Vis Spectrophotometer, 10 test tubes, test tube stand, 2 burettes (50mL), 2 stands, 2 burette clamps, wash bottle with distilled water, a pair of cuvettes, 4×10^{-3} M Ferric nitrate and 4×10^{-3}M Sodium thiocyanate in 4×10^{-3}M Hydrochloric acid solution, tissue paper.

4. Theory

Job's method of continuous variation is a simple method for finding the stoichiometry of a complex. It is most effective when only a single complex is formed. The success of a Job's method experiment depends upon how well Beer's law is followed. The absorbance A of a sample (the fraction of incident light that it absorbs) is defined in terms of the light incident upon it, I_o, and that transmitted, I.

$A = \log_{10} I_o / I$

The absorbance is related to concentration of the solution, c, through Beer's law, which is

$A = \varepsilon.c.l.$

ε is the molar extinction coefficient for a species and l is the optical path length.

Job's method can be used to find the stoichiometry of the compound formed by two reacting species. The spectra of solutions containing both species in varying proportions are recorded, each solution containing the same total reagent concentration. In this experiment the reagents are Fe^{3+} and SCN^-, so

$[SCN^-] + [Fe^{3+}] = \text{constant}$ (1)

A wavelength at which the complex absorbs strongly is selected and the absorbance of each solution at this wavelength is determined. As the concentration of one of the reactants, say the

Fe^{3+}, increases from zero, so does the amount of complex, so that the absorbance rises. Absorbance reaches a maximum in the solution in which metal ion and ligand are in the same ratio as in the complex, since this solution will contain the highest concentration of complex. Further additions of metal ion are balanced by a reduction in the amount of ligand because of the need to satisfy equation (1), so absorbance due to the complex then falls.

A plot of absorbance as a function of the amount of added ligand should give two straight lines, provided Beer's law is obeyed. Extrapolation of the two lines (in the direction away from zero concentration of each species) gives the stoichiometry of the complex directly, since, where the two lines cross, ligand and metal are in the correct proportion to give maximum complex formation.

5. Procedure

Prepare the following solutions which will be used throughout the experiment.

A solution of 4×10^{-3}M Fe^{3+} made by dissolving appropriate amount of $Fe_3(NO_3)_3.9H_2O$ in 4×10^{-3}M HCl.

A solution of 4×10^{-3}M SCN^- made by dissolving appropriate amount of NaSCN in 4×10^{-3}M HCl.

Keep these solutions in two different burettes, after washing and rinsing. Mix them well as follows in the test tubes.

Test Tube No.	1	2	3	4	5	6	7	8	9
Volume of $Fe(NO_3)_3$	1mL	2mL	3mL	4mL	5mL	6mL	7mL	8mL	9mL
Volume of NaSCN	9mL	8mL	7mL	6mL	5mL	4mL	3mL	2mL	1mL

Allow to stand for half an hour. Using the the solution in test tube number 5 (the 5+5mL solution) determine the wavelength of maximum absorption λ_{max} by obtaining the spectra 350 and 550nm. At λ_{max} determine the absorbance of each of the solutions prepared as in the above mentioned table.

1. Experimental Readings

Temperature of the experimental solution =
Strength of $Fe(NO_3)_3.9H_2O$ =
Strength of NaSCN =
λ_{max} =

Test tube no.	Volume of Fe^{3+} solution (A)	Volume of SCN^- solution (B)	Ratio of volumes(=A/B)	Absorbance
1.	1mL	9mL	0.111	
2.	2mL	8mL	0.250	
3.	3mL	7mL	0.429	
4.	4mL	6mL	0.667	
5.	5mL	5mL	1.000	
6.	6mL	4mL	1.500	
7.	7mL	3mL	2.333	
8.	8mL	2mL	4.000	
9.	9mL	1mL	9.000	

2. Calculation

Plot the absorbance as a function of the composition (that is ratio of volumes of Fe^{3+} and volumes of SCN^-). The ratio of the reagents corresponding to the maximum absorption in the curve gives the stoichiometry of the complex.

3. Results

Ratio of metal ion and the thiocyante ligand in the complex =

Expected formula of the complex formed =

EXPERIMENT NO.: 2

Determination of Concentrations of Hydrochloric acid and Acetic acid in a mixture conductometrically

1. Aim

In this experiment you will determine the concentrations of hydrochloric acid and acetic acid in a mixture by titrating it against a solution of sodium hydroxide of known concentrations and measuring the conductivities with the help of a conductivity meter.

2. Safety

Hydrochloric acid and acetic acids are corrosive in nature; do not allow the chemicals to come in contact of your skin. Do not pipette by mouth.

3. Requirements

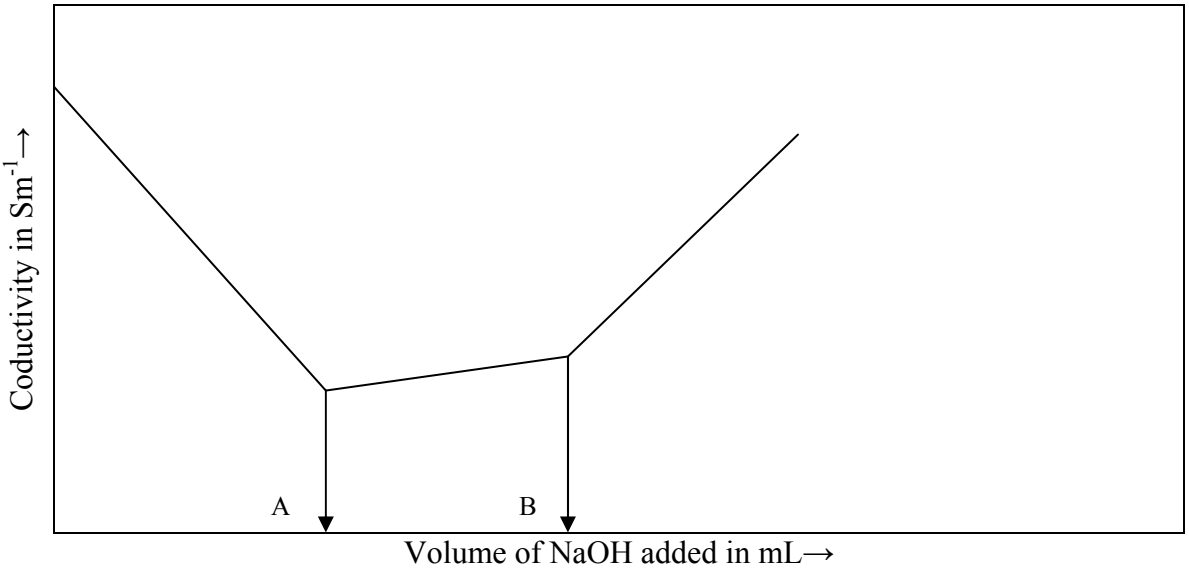
Conductivity meter with electrode, beaker (50mL), semi micro burette (10mL), stand, burette clamp, pipette (20mL), pipette pump, wash bottle with distilled water, glass rod, mixture of HCl and CH_3COOH of unknown concentration, 0.5M NaOH solution, tissue paper.

4. Theory

In a mixture of HCl and CH_3COOH, the dissociation of feebly dissociated CH_3COOH will be further suppressed due to the dissociation of HCl. So when a solution of NaOH is added to this mixture the result is the replacement of H^+ of HCl by Na^+ and the conductivity of the mixture decreases linearly as the volume of NaOH added increases.

Near about the point where the entire H^+ of HCl is removed, the dissociation of CH_3COOH slightly increases. With the addition of more NaOH, more of the completely dissociated Sodium acetate is formed. As a result the first inflexion point in rounded. Thereafter the conductivity again increased linearly. Near about the second inflexion the curve is again rounded due to the hydrolysis of CH_3COO^-.

After that, as more NaOH is added the conductivity increases rapidly and linearly due to the accumulation of free Na^+ and OH^-. As a result the experimental conductivity versus volume of NaOH added curve is expected to be of the following form:



The concentrations of the acids can be calculated by using A and B and the well known formula $N_1V_1=N_2V_2$

5. Procedure

Wash the electrode repeatedly with distilled water and dry it by tissue paper. Wash and rinse the semimicro burette with standard NaOH solution. Fill the burette with the standard NaOH solution. Wash and rinse the beaker and the pipette with the experimental solution. Pipette out 40mL of the experimental solution in the beaker and measure the conductivity of the solution. Add NaOH solution from the semi micro burette in lots of 0.5mL. After each addition mix the solution thoroughly and wait for 2 minutes to allow temperature equilibrium, and then note the conductivity. Continue adding NaOH solution and taking the conductivity readings until the three limbs of the graph are traced fully.

6. Experimental Readings

Temperature of the experimental solution =
Volume of the mixture taken =
Strength of NaOH =

Obs. No.	Total Volume of NaOH added (in mL)	Conductivity (in Sm^{-1})
1.	0.0	
2.	0.5	
3.	1.0	
4.	1.5	
5.	2.0	
6.	2.5	
7.	3.0	

8.	3.5	
9.	4.0	
10.	4.5	
11.	5.0	
12.	5.5	
13.	6.0	
14.	6.5	
15.	7.0	
16.	7.5	
17.	8.0	
18.	8.5	
19.	9.0	
20.	9.5	
21.	10.0	

7. Calculation

Plot the conductivity value against the corresponding volume of NaOH added. A curve with three limbs will be obtained. The point A corresponds to the neutralization of the strong acid; hence the volume of NaOH corresponding to the point A is the volume required for the neutralization of HCl. Similarly the point B corresponds to the volume of NaOH required to neutralize all the acids. Hence, volume corresponding to (B-A) gives the NaOH required to neutralize the weak acid.

$$\text{Concentration of HCl} = \frac{\text{Concentration of NaOH} \times A \text{ (in mL)}}{\text{Volume of experimental mixture taken (in mL)}}$$

$$\text{Concentration of } CH_3COOH = \frac{\text{Concentration of NaOH} \times (B-A) \text{ (in mL)}}{\text{Volume of experimental mixture taken (in mL)}}$$

8. Results

Concentration of HCl in the mixture =

Concentration of CH_3COOH in the mixture =

EXPERIMENT NO.: 3

Determination of the concentration and the dissociation constant of a weak acid (using pH meter)

1. Aim

In this experiment you will determine the dissociation constant of acetic acid (a weak acid) by using pH meter.

2. Safety

Do not pipette by mouth. Solutions of both acetic acid and sodium hydroxide are corrosive in nature and irritant. Special care must be taken to avoid the contact of the solutions especially with eyes.

3. Requirements

pH meter, 100mL beakers (2 nos.), pipette (20mL), burette (50mL), Sodium hydroxide (0.1M) in a regent bottle, unknown acetic acid solution, distilled water in a wash bottle, stand, burette clamp, tissue paper.

4. Theory

The aqueous solution of acetic acid dissociates as shown below

$$CH_3COOH + H_2O \longrightarrow CH_3COO^- + H_3O^+ \quad \text{……..(1)}$$

An appreciable quantity of undissociated acetic acid remains in solution. For the general weak acid HA the dissociation reaction and the dissociation constant expressions are:

$$HA(aq) + H_2O(l) \longrightarrow H_3O^+ + A^- \text{……..(2)}$$

$$K_a = \frac{[H_3O^-][A^-]}{[HA]} \quad \text{and} \quad pH = pK_a - \log \frac{[HA]}{[A^-]}$$

By titrating a weak acid with a strong base and recording the pH versus the volume of base added, the dissociation constant of the weak acid can be obtained.

5. Procedure

Wash the electrode repeatedly with distilled water and dry it by tissue paper. Wash and rinse the burette with standard NaOH solution. Fill the burette with the standard NaOH solution.

Wash and rinse the beaker and the pipette with the experimental acetic acid solution. Pipette out 20mL of the unknown acetic acid solution in the beaker and measure the pH of the solution by dipping the glass electrode in the solution. Add NaOH solution from the burette in lots of 1mL. After each addition mix the solution thoroughly and wait for 2 minutes to allow

temperature equilibrium, and then note the pH. Continue adding NaOH solution and taking the conductivity readings until a sharp change in the pH is observed. This is the neutralization point. After the neutralization point add 2mL portion of NaOH at least for 8 readings and note the pH after each addition. Plot pH versus volume of NaOH to find out the approximate volume of NaOH required for neutralization.

Repeat the process explained in the previous paragraph, this time adding NaOH solution in 2mL portions till the volume of NaOH solution added is 2mL less than required to reach the neutralization point. Then add 0.5mL portions of NaOH so that the sharp change in pH could be noted.

Plot pH versus volume of NaOH added. Determine the neutralization point from the curve and the pH of the solution at half-neutralisation point. This pH value corresponds to the pK_a of the acid. Calculate the dissociation constant constant.

6. Experimental Readings

Temperature of the experimental solution =
Strength of NaOH =
Volume of unknown acetic acid solution = 20 mL

Titration No.	Volume of NaOH added (in mL)	pH(observed)
1.		
2.		
3.		

7. Calculation

Plot the pH as a function of the volume of NaOH solution added. Find the neutralization point from the graph.

Let the volume of NaOH added till the neutralization point= V

Then, concentration of Acetic acid=Concentration of NaOH

$$Concentration\ of\ Acetic\ Acid = \frac{Concentration\ of\ NaOH \times Volume\ of\ NaOH}{Volume\ of\ Acetic\ acid}$$

$$Concentration\ of\ Acetic\ acid = \frac{0.1M \times V}{20mL}$$

Also, the pH at half of neutralization point (V/2) is equal to pK_a.

So, Acid dissociation constant,K_a=antilog(-pK_a)

8. Results

Concentration of Supplied Acetic acid solution =
Dissociation constant of Acetic acid =

EXPERIMENT NO.: 4

Standardization of $KMnO_4$ solution by Oxalic acid

1. Aim

In this experiment you will determine the molar concentration of Potassium permanganate solution by titrating it against standard oxalic acid solution.

2. Safety

Do not pipette by mouth. Solution of potassium permanganate is irritant. It is readily absorbed through skin and is harmful if swallowed. Sulphuric acid is extremely corrosive, causes serious burns, it is highly toxic, harmful by inhalation, ingestion and through skin contact. Ingestion may be fatal. Skin contact can lead to extensive and severe burns. Chronic exposure may result in lung damage and possibly cancer. Oxalic acid is harmful if swallowed and in contact with the skin. May cause burns on contact with the eyes. Special care must be taken to avoid the contact of the solutions specially with eyes.

3. Requirements

Burette (50mL), stand, burette clamp, 250ml conical flask (2 nos.), 10ml pipette, test tube, pipette bulb, water bath, 0.1 (N) Oxalic acid in reagent bottle, unknown $KMnO_4$ in volumetric flask, H_2SO_4 (1:2) in a reagent bottle.

4. Theory

Commercially available potassium permanganate generally contains impurity. Thus it cannot be used as primary standard. In order to make standard $KMnO_4$ solution, it requires to be standardized by a primary standard.

Equivalent weight of $KMnO_4$ = [2 $KMnO_4$/10] = 31.606,

which can be derived from the equation 2 $KMnO_4$= K_2O. 2MnO ;

i.e. $Mn^{7+} + 5e^- \longrightarrow Mn^{2+}$

5. Procedure

Rinse a clean burette (capacity 50 mL) thrice with 5 mL portions of the $KMnO_4$ solution. Fill up the burette with $KMnO_4$ solution up to the zero mark and note the upper meniscus. Examine that the jet of the burette is completely filled with the solution and no air bubble is left behind.

Pipette out 10 mL of standard oxalic acid (0.1 M) into a 250 mL conical flask. Add 10-15mL (i.e., ¾ of a test tube) of H_2SO_4 (1:2) and then add boiling water to dilute it to about 100 mL. Now titrate the solution with $KMnO_4$ solution. At first add $KMnO_4$ solution small quantities at a time with stirring; the pink colour of $KMnO_4$ will take some time to discharge its colour at the beginning. So initial addition should be very slow, when some $KMnO_4$ solution has been

added, the pink colour will be discharged quickly. Now add $KMnO_4$ solution more quickly with stirring. Near the end point when the rate of disappearance of the pink colour slows down, add $KMnO_4$ solution drop wise with stirring until one drop makes the whole solution pink (the pink colour persists for 30 seconds, after which the colour may be discharged again)

Note the volume of $KMnO_4$ solution added. Repeat the operation thrice.

6. Experimental Readings

Temperature of the experimental solution =
Strength of Oxalic acid = 0.1N
Volume of Oxalic acid solution = 10 mL

Titration No.	Volume of $KMnO_4$ added (in mL)	Concurrent volume (in mL)
4.		
5.		
6.		
7.		

7. Calculation

Let volume of $KMnO_4$ solution = VmL
Strength of oxalic acid = N_1

Therefore, strength of $KMnO_4$ solution $= \frac{N_1 \times 10mL}{V}$

$= \frac{0.1N \times 10mL}{VmL}$ (N)

8. Results

Concentration of Supplied $KMnO_4$ solution =

EXPERIMENT NO.: 5

Determination of the total hardness of water by Complexometric Titration

1. Aim

In this experiment you will determine the total hardness of normal tap water available in laboratory by complexometric titration method.

2. Safety

Do not pipette by mouth. Solution of EDTA is an eye irritant. Eriochrome Black-T is harmful if swallowed. Avoid its contact with eyes, skin and cloth. Eye contact with ammonia containing buffer can lead to serious eye damage. It is toxic if swallowed and harmful if inhaled.

3. Requirements

50mL burette, stand, burette clamp, 25mL pipette, pipette bulb, 250mL conical flask, Eriochrome Black-T in dropping bottle, standard EDTA solution in volumetric flask, wash bottle, NH_4Cl-NH_4OH buffer in reagent bottle, 5mL measuring cylinder, conical funnel.

4. Theory

The hardness of water is generally due to dissolved calcium and magnesium salts mainly as bicarbonates and can be determined by complexometric titration.

Complexometric titrations are those titrations in which the concentration of a metal ion is determined titrimetrically by forming complex with a strong multidentate chelating ligand (e.g. ethylenediaminetetraacetate = EDTA). In these kind of titrations, a metal ion is in the form of a complex with an organic dye (indicator) having a characteristic colour. During the course of titration, the ligand forms complex with the metal ion thereby setting the dye free from metal ion. At the end point, when all the dye molecules are set free, the solution assumes the colour characteristic of the dye.

When calcium ions are titrated with di-sodium salt of EDTA, a relatively stable complex is formed.

$$Na_2H_2Y = 2Na^+ + H_2Y^{2-}$$
$$Ca^{2+} + H_2Y^{2-} = CaY^{2-} + 2H^+$$

This titration is performed in alkaline buffer medium. In alkaline medium, the reaction is driven towards the forward direction in which the H^+ ions are consumed. Erichrome black-T is

used as indicator. It is red when it forms complex with Ca^{2+} and blue in the free state. The end point thus is indicated by the blue colour of the solution. The hardness of water is expressed in parts per million unit.

1 mL of 0.01 M EDTA = 1 mg of $CaCO_3$

5. Procedure

Fill the cleaned burette with standard EDTA solution (supplied) after rinsing with same.

Pipette out 25 mL of water sample (supplied) into a 250 mL conical flask. Add 5 mL buffer solution followed by 4 to 5 drops of Erichrome black-T solution. Titrate the solution with EDTA solution from the burette till the colour of the solution changes purple red to blue. Note down the volume of EDTA consumed.

Repeat the steps explained in the previous paragraph, till three concurrent readings are obtained.

6. Experimental Readings

Temperature of the experimental solution =
Strength of EDTA =
Volume of water sample taken =
Density of water =

Observation No.	Volume of Water sample taken	Burette Readings			Concurrent Reading
		Initial	Final	Difference	
1.	25mL				
2.	25mL				
3.	25mL				
4.	25mL				

7. Calculation

Let the volume of EDTA required for 25mL water is V, and density of water is d g/mL

Amount of $CaCO_3$ in 25mL of water = V mg

$$Concentration\ of\ CaCO_3\ in\ supplied\ water(in\ ppm) = \frac{V}{25 \times d} \times 10^3$$

8. Results

Concentration of $CaCO_3$ in sample of water =

EXPERIMENT NO.: 6

Determination of the number of components in an organic mixture and R_f of each component using thin layer chromatographic technique

1. Aim

In this experiment you will learn the technique of thin layer chromatography and determine the number of components in a mixture and R_f values of few compounds.

2. Safety

Dust of silica gel affects respiratory system and causes irritation to eyes. Wash hands (or exposed area) with soap and water after use. Ethyl acetate is irritant (specially to eyes) and harmful if swallowed. Its vapours may cause drowsiness. It is highly inflammable.

3. Requirements

250mL beakers of tall form (4 nos.), petri dishes (4nos.), test tubes (3 nos.), capillary tubes (3nos.), test tube stand, TLC Plates coated with slurry of silica gel (4nos.), unknown organic compounds, solvents (S_1, S_2 and S_3), Iodine crystals, 5mL measuring cylinder.

4. Theory

Liquid chromatography is a highly efficient technique used to identify number of components in a mixture as well as to monitor the progress of a reaction. By this method, sharper separation and detection of very small quantity of components can be achieved in a short time. In thin layer chromatography (TLC), the stationary phase (silica gel or alumina mixed with a binder like $CuSO_4$) is mixed with a solvent to form a slurry and in then applied on a glass or plastic plate to make a coating. The prepared thin layer on glass is called a chromoplate.

A small capillary tube is dipped inside the mixture and then taken out. A spot is made, at one end of the chromoplate, with the mixture remaining inside the capillary tube. The chromoplate is then placed inside a chamber containing the solvent. The solvent (eluent) moves up, along the chromoplate, by capillary action through the spot. Solvent used for this purpose should be low boiling so that the plate can dry quickly after removing from the chamber.

The ratio of the distance travelled by a compound (from the spot) to travelled by the solvent front is called Rf value of the compound.

$$R_f = \frac{\text{Distance moved by the compound}}{\text{Distance moved by the solvent}}$$

The R_f value of a compound is a characteristic property of the compound for the particular solvent used. This property is used to identify each component in a mixture. In general, a polar

compound has a lower R_f than a non polar one. Written below are the trends of R_f values of some compounds with increasing polar character.

Saturated hydrocarbon < alkenes, alkynes, aromatic hydrocarbons < esters, aldehydes and ketones < amines, alcohols, thiols < carboxylic acids.

For a particular compound, the R_f value increases with increasing polarity of the solvent. The polarity orders of some commonly used solvents are:

Hexane< Cyclohexane< Carbon tetrachloride< Trichloroethylene < Toluene, Dichloromethane < Chloroform < Diethyl ether < Ethyl acetate < Acetone < Propanol < Ethanol < Methanol.

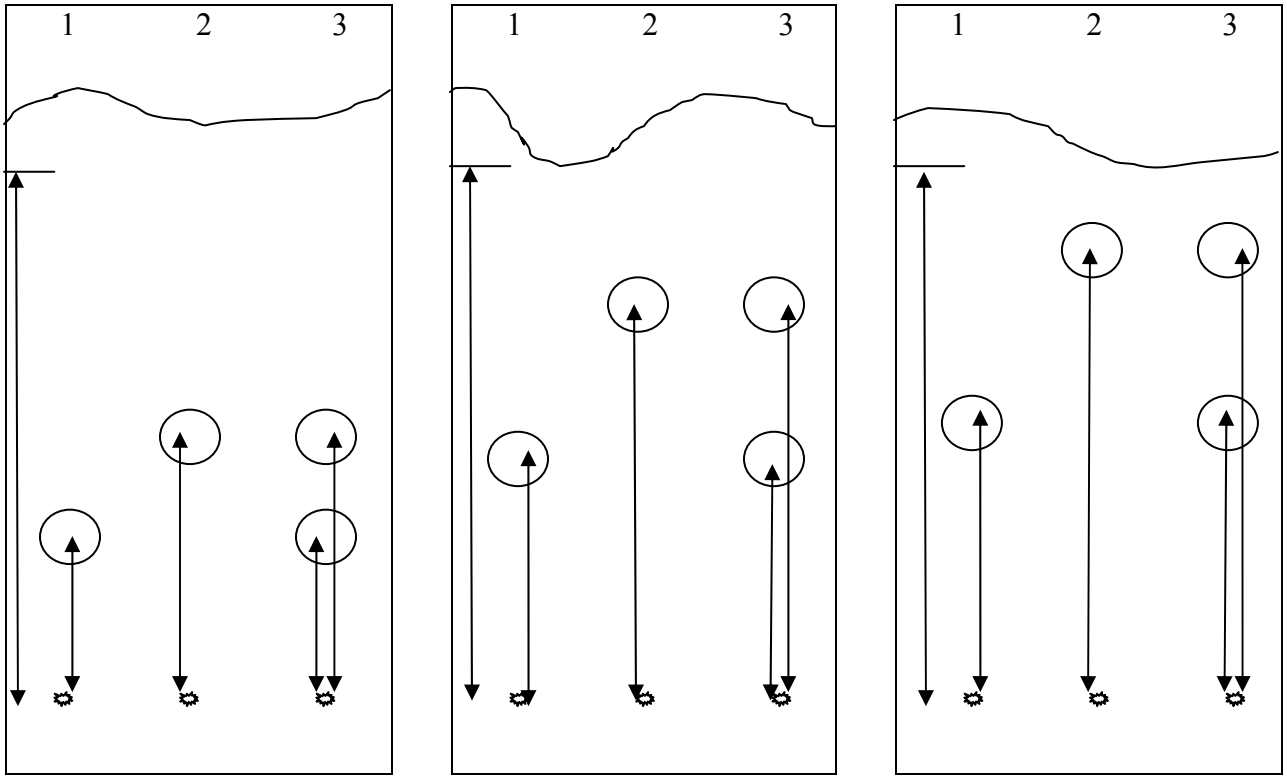
To detect a spot on the plate several methods, such as visualisation under UV lamp (if the compound is UV active), iodine vapour chamber, spraying with 10% sulfuric acid in methanol etc. are used.

5. Procedure

Take a 5×10 cm coated TLC plate (Chromoplate). Make three spots 1cm above from the bottom of the plate and make 1, 2 and 3 respectively on the top of the plate with a pin. Take 10 mg each of compounds A and B into two separate test tubes and dissolve in 2mL ethyl acetate. In the third test tube, mix equal amounts of the solutions of A and B (1mL each). Take a thin capillary tube and dip into the solution of A. Transfer the liquid from the loaded capillary to spot number 1 by just touching the plate for a second. Repeat it for compound B into spot number 2 and for the mixture (A+B) for spot number 3. Put the TLC plate into a supplied solvent chamber containing 50mL of solvent (labelled as S_1), until the solvent level rises to 1 cm less than the top of the plate. Remove the plate, quickly mark the upper limit of the solvent front with a pin and allow it to dry at room temperature. Put the dry TLC plate into a chamber containing a few crystals of Iodine. Leave it for a few minutes until Iodine vapours are adsorbed by the compounds and distinct spots are visible on the plate. Remove the TLC plates from the chamber and carefully encircle each darkened spot. Mark their mid points and measure the distance between the midpoint of each spot and the starting point (that is spots 1, 2 and 3). Find out the R_f values of the spots.

Repeat the above procedure for solvent systems S_2 and S_3 respectively. Find out the number of components in A and B individually and compare with the number of components in the mixture. Remember that the number of spots generated from each starting spot is equal to the number of components in the mixture. You need to find out which one of the solvent system gives better resolution. Determine the R_f value of each component.

6. Experimental Readings



S_1 S_2 S_3

Sovent System	Distance travelled by					R_f			
	Solvent front	Alone		Mixture		Alone		Mixture	
		A	B	A	B	A	B	A	B
S_1									
S_2									
S_3									

7. Results

Number of components in the mixture =
R_f of A in S_1 =
R_f of A in S_2 =
R_f of A in S_3 =
R_f of B in S_1 =
R_f of B in S_2 =
R_f of B in S_3 =

EXPERIMENT NO.: 7

Synthesis and study of Silver Nanoparticles

4. Aim

In this experiment you will synthesize silver nano particles and study its characteristics like colour, spectrum and stability.

5. Safety

$AgNO_3$ is poisonous, skin and eye irritant and can lead to deposition of black silver stains on the skin. Sodium boro hydride is toxic by ingestion and harmful if inhaled and in contact with skin. The dilute solutions of both reagents do not cause any harm.

6. Requirements

Silver Nitrate solution (10 mL of 1 mM), $NaBH_4$ solution (ice cooled) (30 mL of 2 mM), magnetic plate & magnetic bar, 50mL round bottomed flask, 50mL measuring cylinder, 100mL Beaker (2 nos.), dropper (1 no.), test tube (2 nos.), test tube stand (1 no.), spectrophotometer (Common to all).

7. Theory

Nanotechnology deals with processes that take place on nanometer scale, that is, from approximately 1 to 100 nm. Properties of metal nanoparticles are different from those of bulk materials made from the same atoms. For example, the striking effect of nanoparticles on color has been known since antiquity when tiny metal particles were used to colour glass in church windows. Silver particles stained the glass yellow, while gold particles were used to make ruby-colored glass. In performing the experiment described here, you will observe the bright yellow colour of silver nanoparticles compared to colorless silver nitrate solution and metallic bulk silver.

The chemical reaction is the sodium borohydride reduction of silver nitrate:

$AgNO_3 + NaBH_4 \rightarrow Ag + \frac{1}{2}H_2 + \frac{1}{2}B_6H_6 + NaNO_3$

The method produces 12 ± 2 nm particles. The plasmon absorbance is near 400 nm. The wavelength of maximum absoption, λ_{max}, depends upon the size of the particle. The particle size and corresponding λ_{max} are given below.

Sl. No.	Paricle size (in nm)	λ_{max} (in nm)
1.	10-14	395-405
2.	35-50	100-110
3.	60-80	140-150

8. Procedure

Clean a 50mL round bottomed flask and a magnetic stirring bar. Take 30mL of 2.0mM $NaBH_4$ solution in the round bottomed flask and chill it in an ice bath. Add 10mL 1.0 mM silver nitrate drop wise (about 1 drop/ second) to the solution taken in the round bottomed flask, kept over magnetic stir plate. Continue stirring the reaction mixture vigorously. The solution will turn light yellow after the addition of 2mL of silver nitrate and a brighter yellow when all of the silver nitrate will be added. The entire addition should take about three minutes, after which stop the stirring and remove the stirring bar. Note down the colour of the colloid.

Store the colloid in a transparent vial and observe the time for breakdown of colloid. If the colloid doesn't breakdown even after 45 minutes, it may be considered stable. Meanwhile, take about 3mL of the colloid in a clean cuvette and observe the Spectrum in 350-600nm range. Note down the wavelength of maximum absorbance, λ_{max}, and predict the approximate size of the particles.

9. Experimental Readings

Colour of the colloid =
λ_{max} =
Corrosponding size of the particles =
Breakdown time of the colloid =

EXPERIMENT NO.: 8

Quantitative Estimation of Protein by Biuret Method

1. Aim

In this experiment you will determine the amount of protein by using Biuret method.

2. Safety

Biuret reagent is a strong base (pH >12) and toxic due to the copper. Whenever you work with Biuret Reagent solutions you must: wear eye protection, avoid skin contact, clean up any spills, follow directions for proper disposal.

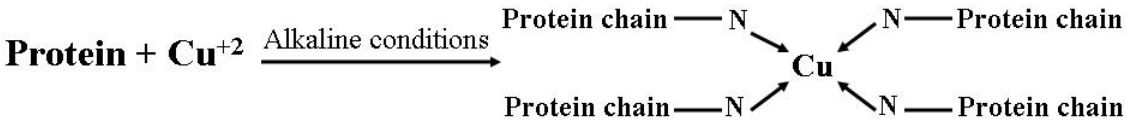
The protein standard solutions containing only BSA are harmless.

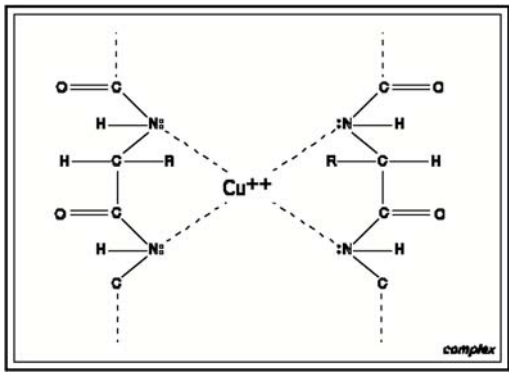
3. Requirements

Test tubes (6 nos.), test tube stand, a pair of cuvettes, semi micro burette (2 nos.), 50 mL burette, stands (3 nos.), burette clamps (3 nos.), UV-Vis spectrophotometer, Biuret solution in reagent bottle, BSA solution.

4. Theory

Proteins are known to be polymers of amino acids, which are linked serially by peptide bonds (-CO-NH-). The Biuret method is the simplest method for estimating protein concentrations, and is based on the fact that the –CO-NH- group (present in all proteins) can form a colored complex with copper(II) ions in an alkaline medium, whose absorbance value reached a maximum at 562 nm. The intensity of the color produced is proportional to the number of peptide bonds present in the sample. The cupric ions cause chelation of the peptide bonds of proteins under alkaline conditions resulting in the production of purple colored complex. Thus, molecules containing 2 or more peptide bonds associate with the cupric ions to form a coordination complex that imparts a purple color to the solution with λ_{max} = 562 nm. The purple color of the complex can be measured independently of the blue color of the reagent itself with a spectrophotometer or colorimeter.





5. Procedure

Preparation of Biuret Reagent

A formula for biuret reagent is (per liter final volume) 9g Sodium potassium tartrate (f.w. 282.22), 3g $CuSO_4.5H_2O$ (f.w. 249.68), 5g Potassium iodide (f.w. 166.0), all dissolved in order in 400 ml 0.2 M NaOH (f.w. 40.0) before bringing to final volume of 1 liter. The volume can be scaled up or scaled down according to requirement. Discard a black precipitate, if formed.

Assay

Warm up the spectrophotometer 15 min. before use. Prepare stock solution of Bovine Serum Albumin (BSA) containing 5 mg/ml in distilled water or millipore water. Set up a series of BSA solutions in clean test tubes using 0.1, 0.2, 0.4, 0.6, 0.8 and 1 ml of the stock solution and using distilled water, make up the volume to 4 ml. For the blank use distilled water. Prepare varying solutions of the unknown as indicated in the table and take 4 ml of the diluted solution separately in test tubes. Add 6 ml of Biuret solution in each tube, mix and incubate at 37°C or room temperature for 10 minutes. The appearance of the purple color indicates the presence of a protein in the sample. Read the absorbance of the individual solutions at 562 nm after setting to zero absorbance value with the blank. Calculate the protein concentration in the test sample by comparing its absorbance value with those of the standard curve.

6. Experimental Readings

Temperature of the experimental solution =

Serial No.	Concentration ug/ml	BSA stock solution(ml)	Distilled water (ml)	Biuret soln. (ml)	A_{560}
1	125	0.1	3.9	6.0	
2	250	0.2	3.8	6.0	
3	500	0.4	3.6	6.0	
4	750	0.6	3.4	6.0	
5	1000	0.8	3.2	6.0	
6	1250	1.0	3.0	6.0	
UNKNOWN SAMPLE					
7	Unknown	Unknown	Unknown	6.0	

7. Calculation

Prepare a standard curve of absorbance versus micrograms protein (or *vice versa*), and determine amounts from the curve. Determine concentrations of original samples from the amount of protein, volume/sample, and dilution factor, if any.

8. Results

Concentration of BSA in unknown solution =

EXPERIMENT NO.: 9

Synthesis and Characterization of Tris(acetylacetonato) Manganese (III)

1. Aim

In this experiment you will synthesize tris(acetylacetonato)manganese(III), characterize it by determining melting point.

2. Safety

$KMnO_4$ is harmful if swallowed, irritant and readily absorbed through skin. Acetylacetone is harmful if swallowed or inhaled and is irritant. It is flammable therefore it should be kept away from flame.

3. Requirements

100mL beaker, buchner funnel setup, watch glass, 5mL measuring cylinder, dropper, spatula,glass rod, capillary tube, wash bottle, thermometer, melting point apparatus, filter paper, $KMnO_4$, Acetylacetone.

4. Theory

The complex manganese tris(acetylacetonate) or tris(acetylacetonato)manganese(III) is synthesised by reacting $KMnO_4$ with acetylacetone.

$KMnO_4+CH_3COCH_2COCH_3 \rightarrow [Mn(acac)_3]+(CH_3CO)_2CH\text{-}CH(COCH_3)_2+K^+ + H_2O$

The reaction is based on electron-transfer between Mn(VII) and acacH. No buffer is required. The compound is obtained as dark brown-black crystals. $Mn(acac)_3$ is monomeric in nature.

The oxidation state of manganese in the compound can be determined iodometrically by reduction of a known amount of the compound with acidified potassium iodide solution followed by titration of the liberated iodine with standard sodium thiosulphate solution. The redox titration can be also used for quantitative determination of manganese content of the compound.

5. Procedure

Take finely powdered $KMnO_4$ (0.5 g, 6.4 mmol) in 5 mL of distilled water into a 100 mL beaker. Dissolve it by slightly warming on a water-bath. To this solution add acetylacetone (2.3

mL, 44 mmol) in several portions in dropwise with time to time swirling. Stir the reaction mixture for 5 min. Keep it again on a hot water-bath until dark brown-black shiny crystals appear from the reaction mixture. Remove the reaction mixture from the water-bath and allow the mixture to cool for 20 min. Filter the reaction mixture with suction on a Buchner funnel and wash the solid material with small amount of cold acetylacetone-water mixture (1:1, v/v, 1 mL). Collect the dark-brown black crystals and dry in vacuo. Weigh the solid material. Calculate its yield. Find out the melting point of the compound and submit the sample.

6. Experimental Readings

Weight of the sample prepared =
Melting point of the compound =

7. Calculation

Calculate the theoretical yield, and find out the percentage yield of the product.

8. Results

Weight of the sample =
Percentage yield =
Melting point =
Wavelength of maximum absorbance=