Model Answer

M. Sc. (Third Semester), 2013, Chemistry (AS-2155)

CMT-304 (A): Microanalytical Techniques

Section-A (2 marks)

1. **(i)** Microanalysis: It is an quantitative identification of chemical substances of very small amount generally less than 10 mg or 1 mL or small surface of material less than 1 cm^2.

(ii) ppm = parts per million ≈ 1 mg/L; ppb = parts per billion ≈ 1 µg/L, ppt =part per thousand

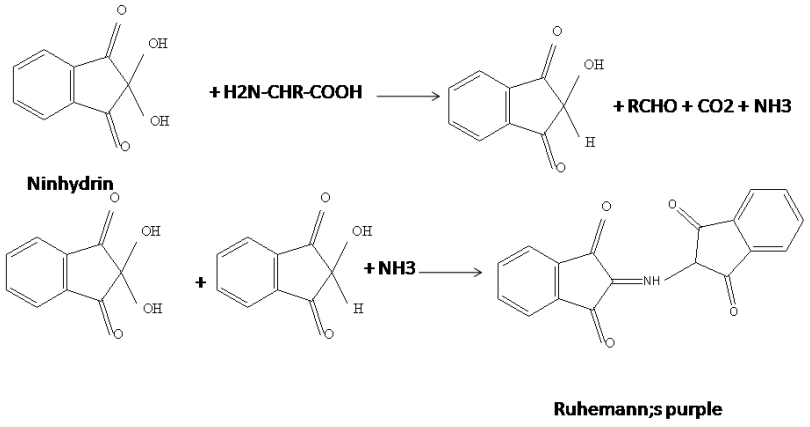
(iii) Metal ions that can be extracted from the aqueous sample using cryptands are: sodium, potassium, calcium, magnesium, iron, mercury, zinc, copper, nickel, strontium, etc.

(iv) Compound or molecule with three or more potential donor atoms that can coordinate to metal center. e.g. Cryptands and crown ethers.

(v) Crown ethers by Charles Pederson & Cryptands by Jean-Marie Lehn.

(vi) Ribose $\xrightarrow{\text{Hot HCl}}$ furfural $\xrightarrow{FeCl_3/\text{orcinol}}$ green color (Absorbance at 665 nm)

(vii) Analysis of amino acid:



Ruhemann's complex gives the absorbance at 570 nm.

(viii) Reaction for the estimation of nitroso group:

p-NO. C_6H_4. $N(CH_3)_2$ + $4Ti^{3+}$ + $4H^+$ —> p-NH_2. C_6H_4. $N(CH_3)_2$ + $4Ti^{4+}$ + H_2O

(ix) Iodine value is defined as the gram of iodine absorbed in 100 g of the fat or oil. (i) It is a useful parameter in studying oxidation rancidity of oils/fat, since higher the unsaturation greater the possibility of the oils to be rancid. (ii) Determine the amount of unsaturation in organic compounds.

(x) Classification of kinetic method of analysis: Kinetic methods are classified by a number of criteria. Broadly it can be classified as catalytic and non-catalytic methods. The catalytic methods are further divided according to the type of reactions involved. The non-catalytic reactions have been classified according to whether they are applied to the determination of single species or of several components in a mixture. The classification of kinetic methods is shown below in Table:

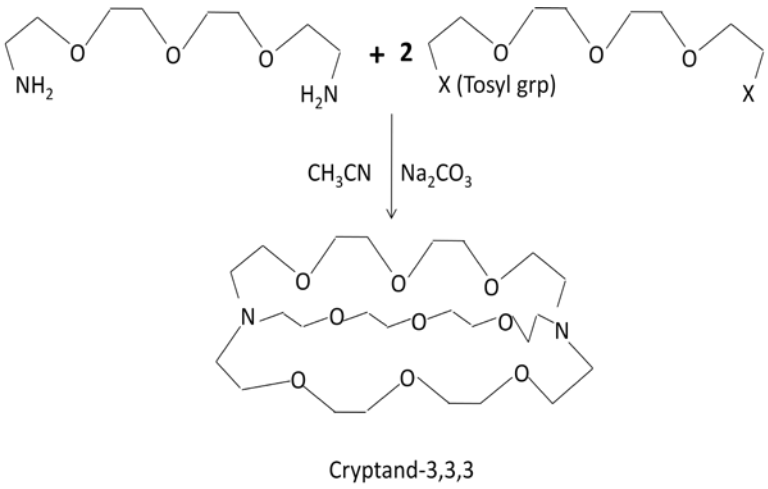
Catalytic Methods		
Non-enzymatic (Homogeneous)	Types of Reactions	Redox and Chemiluminescence Complexation
	Modified effects	Activation Inhibition Catalytic titration
Enzymatic	Homogeneous Heterogeneous(immobilized enzymes)	
	Modified effects	Activation Inhibition
Electrochemical (Heterogeneous)		
Non-catalytic methods	Determination of single species Determination of mixtures (Differential kinetics)	

Section-B (8 marks)

2.

Cryptands are compound containing a bridge like structure. Cryptands are also called as "hidden" because during the complexation of metal ion, metal ion which are bound actually hidden from bulk of solvent. These compounds hold added advantages over crown ethers as they forms a three dimensional structures with strong bond between metal and ligand.

Synthesis:



Cryptand-3,3,3

Properties:

(i) These are steric cage type macrobicyclic compound with two nitrogen atoms on the bridge. The complexes are termed as cryptates. They are more stable than crown ethers.

(ii) The steric configuration of cryptates with bridge nitrogen atom are exo-exo (out-out) and exo-endo (in-in) type.

(iii) Cryptands promoted solubilization of s-block metals in organic solvents, like THF, ether and alkyl amines.

(iv) Compared to crown ethers, cryptands are more effective in complexion of anions by protonation.

(v) The hole and ion cavity size concept is applicable to cryptands for complexation.

Applications:

<u>In solvent extraction:</u>

(i) Cryptands 2,2,1 and 2,2,2 are used for the extraction of alkali metals with various counter anions.

(ii) Cryptand-2,2,2 in cyclohexane at pH 7.2-8.6 can be used for the extraction Ca(II).

<u>In extractive spectrophometry:</u>

(i) Cryptand 2,2,2 (0.01 M) in chloroform at pH 6.0 used for spectrophotometric determination of U (VI) with eosine (0.005 M) as a counter anion. It is stripped with 0.1 M perchloric acid and determined at 430 nm.

(ii) Zr (IV) can be quantitatively extracted with cryptand 2,2,2 in toluene at pH 5.0 with mineral acid and spectrophotometrically determined as a colored complex.

Chromatographic separations:

(i) Cryptands-2,2,2 is utilized as the bifunctional modifier at neutral pH for the separation of ammonium, sodium and potassium in TLC.

(ii) Inorganic anions are also separated with cryptand 2,2,1 on polystyrene divinyl benzene resin at pH 2.0 and water is used as a mobile phase.

Electroanalytical method:

(i) Lithium can be potentiometrically titrated with cryptand 2,2,2 and crptand 2,2,1.

(ii) The coulometric titration with electrogeneration of Hg(II) and formation of 1:1 complex of Hg(II) along with cryptand-2,2,2 and 2,2,1.

3.

Applications of crown ethers in solvent extractions:

(i) DB18C6 is used for the extraction of potassium and thallium from lithium solution at pH 2-4 using chloroform and bromphenol as a counter ion.

(ii) Cyclohexyl 15C5 can be used for the extraction of sodium while DC18C6 can use for the extraction of potassium in benzene in the presence of 2-ethyl-hexyl phosphoric acid.

(iii) Sr(II) can be extracted by using dibenzo or dicyclo substituted 18C6 and 24C8 in chloroform.

(iv) 0. 45 M of DB24C8 can used for the extraction of Ba(II) in nitrobenzene from 0.2M picric acid.

(v) U(VI) can be extracted by using various crown ethers, such as, DC27C9, 24C8, DC 18C6 in 8 M HCl from chloride solution.

Extractive spectrophotometry:

(i) Li (I) can be extracted by 0.02 M crown dinitrophenol azo and spectrophotometrically determine at 565 nm.

(ii) 18C6 in dichloromethane is used for the estimation of Sb(III) as a iodo-complex using 1 M H_2SO_4 and 0.1 M KI solution.

(iii) 18C6 in toluene is used to extract Sr(II) at pH 3.0 from picric acid and sripped with 1 M HCl and analyzed at 670 nm.

(iv) DC18C6 in dichloromethane used for the extraction of Sn(VI) in 1.5 M H_2SO_4 and 1.6 M KI and determined as iodo-complex at 410 nm.

(v) Cu(II) is extracted with DB18C6 in butanol with zincon as the counter anion to form ion paired complex and estimated directly at 610 nm.

4.

Determination of ascorbic acid by calorimetrically

It includes two steps:

(1) Ascorbic acid $\xrightarrow[\text{Activated charcoal or + HPO3 + } CH_3COOH \text{ solution}]{\text{Shaking}}$ dehydroascorbic acid

(2) Dehydroascorbic acid + 2,4 dinitrophenyhydrazine

↓

Osazone (dissolved in H_2SO_4 and gives orange red coloration, absorbance at 540 nm)

Reagents:

(i) Metaphosporic acid: Dissolve 15 g HPO_3 in a mixture of 40 ml acetic acid and 450 ml of water.

(ii) 2% 2,4 dinitrophenyhydrazine: dissolve 2g 2,4 dinitrophenyhydrazine in 0.5 N 100 ml H_2SO_4 and filter.

(iii) 10% thiourea solution

(iv) 85% H2SO4 prepared by adding 85 ml conc. H_2SO_4 to 100 ml H_2O

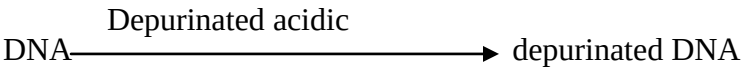
(v) Standard ascorbic acid solution: Dissolve 100 mg ascorbic acid in 4% oxalic acid

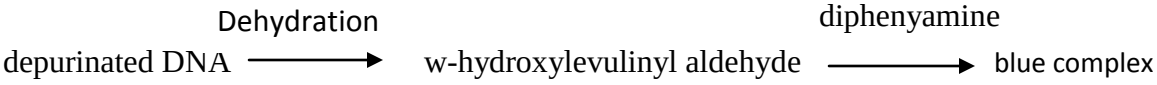
Procedure:

Grind the 10 g sample material in sufficient quantity of reagent. (i) Filter and cooled the filtrate. 15 ml of this filtrate is mixed with 0.75 g of activated charcoal. Shake and filter. Filtrate consists of DHAA. 1 drop of 10% thiourea and 1 ml of 2,4-dinitrophenyhydrazine is added to 4 ml of this DHAA. Mix thoroughly and place the test tube in a water bath at 37 ^{O}C for 3 hrs. Run an appropriate blank without the reagent. Cool the reaction mixture in ice. Add 5 ml sulphuric acid drop wise with stirring. Measure the absorbance at 540 nm. Plot the absorbance Vs concentration and calculate the ascorbic content in the sample.

5.

Estimation of DNA by reaction with diphenylamine:





Reagents:

(i) DNA standard: 0.5 mg/ml

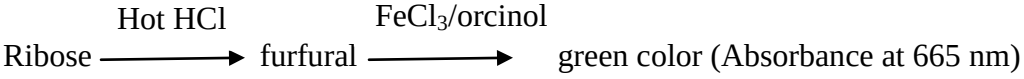
(ii) Saline citrate: (0.15 mL NaCl in 0.015M sodium citrate)

(iii) Diphenylamine: Mix 5 g in 500 ml glacial acetic acid + 12.5ml H_2SO_4.

Procedure:

Take the 1, 2, 3, ml of isolated DNA dissolved in standard saline citrate in different test tubes and similar aliquot of 0.5 mg DNA/ml std. Make up the total volume in each test tube up to 3 ml with distilled water. Add 6 ml diphenylamine to each test tube. Heat the solution until the boiling followed by the cooling. Plot the graph between absorbance (at 595 nm) Vs quantity of DNA. Calculate the concentration of DNA dissolved in saline citrate solution.

Estimation of RNA:



Reagents:

(i) Standard RNA solution: Dissolve 50 µg/ml in ice chilled 10 ml tris-acetate buffer containing 1 mM EDTA.

(ii) Acid reagent for orcinol: Add 10%, 2 ml solution of $FeCl_3.6H_2O$ to 400 ml conc. HCl.

6% alcoholic Orcinol: Dissolve 6 g orcinol in 95% 100 ml ethanol.

Procedure:

Dissolve the isolated RNA in buffer to an approximate concentration of 50 µg/ml. Take 0.5, 1, 1.5,3 ml of isolated RNA in a series of test tubes and same volume of 50 µg/ml in another set of tube. Make up the volume up to 3 ml with water. Add the 6 ml acid reagent (ii) to each tubes followed by the addition of 65, 0.4 ml of alcoholic Orcinol. Shake the tube to mix and cover the

tubes with aluminum foil and then heat in boiling water bath for 20 minutes. Cool and read the absorbance of blue color at 665 nm. Draw a standard graph between absorbance Vs concentration and calculate the amount in isolated RNA solution from this standard curve.

6.

Principle for the estimation of ester in organic compounds:

Ester present in organic sample can be estimated by hydrolysis method with standard alkali solution. The hydrolysis of ester requires excess of alkali and back titration is done with standard acid using phenolphthalein as an indicator.

$$RCOOC_2H_5 + NaOH \rightarrow RCOONa + C_2H_5OH$$

Preparation of reagents:

(i) N/2 N NaOH: 5 g NaOH is dissolved in 250 mL DW.

(ii) N/10 N HCl: 2.1 mL of concentrated HCl is made up to 250 mL DW.

(iii) Phenolphthalein: 0.1% phenolphthalein in ethanol is used as indicator.

Procedure: Weigh accurately 0.8-1.3 g of substance in a round bottom flask equipped with a water condenser and add 50 mL of standard alkali (N/2 NaOH solution). Water is used as a solvent for dissolving water soluble compound and organic solvent is used for organic soluble compounds. Heat the reaction mixture under reflux until hydrolysis complete (1 hour). This is indicated by the disappearance of fruity smell of ester. Cool the solution and transfer into a 250 mL volumetric flask and dilute the solution to the mark. Determine the amount of unused alkali by titrating by 25 mL of dilute solution against standard acid (N/10 HCl) using phenolphthalein as an indicator.

Calculation for the amount ester in sample:

Weight of substance = w_g

The amount of alkali initially taken in flask = 50 mL N/2 NaOH = 25 mL N NaOH (V_1)

25 mL dilute solution = V_2 mL of N/10 N HCl

Thus, the amount of alkali unused would be equivalent to 10 V_2 of N/10 HCl= V_2 of N HCl=V_2 mL of NaOH

The amount of alkali used in the hydrolysis = (25-V_2) mL of N NaOH

Amount of ester in 1000 mL N alkali

$= \frac{Mx(25-V2)}{1000}$

7.

Principle for the estimation of carbonyl group by hydroxylamine hydrochloride method:

A weighed amount of sample is treated with excess of hydroxylamine hydrochloride in the presence of pyridine to yield oxime and pyridine hydrochloride is estimated with NaOH solution.

$R_2C=O + NH_2OH.HCl \rightarrow R_2C=NOH + HCl + H_2O$

The rate of oxime formation depends on the nature of R and R' (propianaldehyde, furfuraldehyde, benzaldehyde and acetone) react quantitatively about an hour to complete the reaction. The pyridine HCl so formed is reacted with standard solution of NaOH using bromophenol as indicator.

$C_5H_5N.HCl + NaOH \rightarrow C_6H_5N + NaCl + H_2O$

Preparation of reagents:

(i) Hydroxylamine hydrochloride: Dissolve hydroxylamine hydrochloride (17.5 g) in 500 mL DW.

(ii) Bromophenol indicator: 4% alcoholic solution of bromophenol (1 mL) in pure pyridine (5mL) and dilute to 250 mL with ethanol.

(iii) Methanolic NaOH (0.5 N): Dissolve 5 g NaOH in 25 mL DW and then dilute up to 250 mL methanol and standardize with 0.5 N HCl.

Procedure: Take sample (w_g) and add 3 mL hydroxylamine hydrochloride and bromophenol in 250 mL iodine flask. Stop the flask and keep the solution for completion of the chemical reaction. Similarly, blank experiment is performed; and titrates with NaOH solution to blue green color.

Calculation:

Weight of the sample = w_g

Normality of NaOH solution = N

Volume of NaOH used for blank experiment = V_1 mL

Volume of NaOH used for sample experiment = V_2 mL

% carbonyl group = $\frac{(V1-V2)xNxMx100}{wx1000}$

8.

Ring oven technique is used for the determination of the metal ions:

The ring oven technique was introduced by Professor Weisz in 1954 as a means of accomplishing systematic separations on single drop of solution. Since its introduction, the technique has been adapted to quantitative procedures and dozens of fascinating applications have been evolved, of qualitative analysis of substances. This is a relatively cheap method, easy to operate and useful for separation, concentration and analysis of inorganic and organic substances at microgram and nanogram levels.

Principle:

Ring oven technique is most suitable method for carrying out analysis of metal pollutants in water and air. This technique is based upon the spotting sample at the center of circular filter paper and by capillary action different ions migrate at different rate toward the edge of circular filter paper. The rate is related to capillary action or may be due to difference of partition coefficient if solvent system is used. The paper is taken dried on ring oven, special kind of dryer and various reagents are sprayed. Different concentric rings are obtained of different intensity for different metal ions. The obtained rings are punched out carefully and compared with the rings of standard metal ions for qualitative quantitative determination. This technique is very useful for the quantitative determination of metal ions in unknown in sample.

Instrumentation:

The equipment needed for ring oven study is ring oven or electric dryer that suitable for drying filter papers. The ring oven is consists of heating ring, ordinarily 22 or 33 mm i.d. The heating area of ring oven is constructed b aluminum, cooper steel and glass. For extremely delicate analysis, gold or platinum related equipment has been used to avoid contamination.

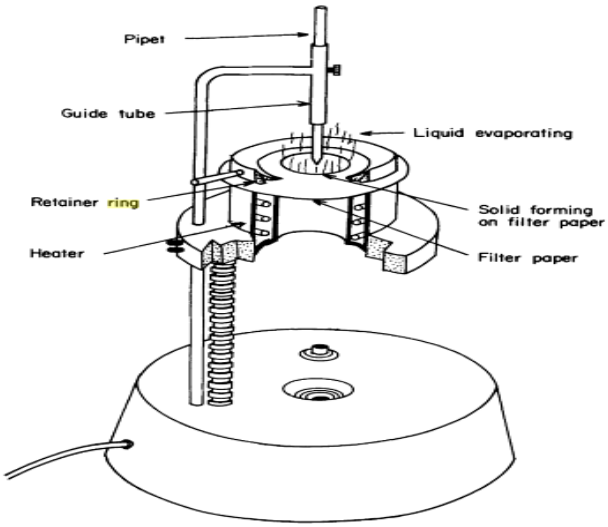


Figure 2. Essential features of ring oven.

Procedure for the estimation metal ions:

The ring oven method is special spot test procedure carried out on filter paper. The detection and determination of the constituents of pollutants is accomplished through the use of extremely sensitive organic reagents that are specific or selective. The operation consists of simply of placing a piece of filter paper on the heated surface of ring oven and introducing soluble sample material at the center of paper. The sample solution is washed through the pores of the paper by addition of 5-10 microdrops of an appropriate solvent. The sample solutes are thus transported or washed to the area of the heated ring surface. As the solution approaches the heated ring zone, the carrier solvent is evaporated, thus the depositing the solute as a sharp ring. The filter paper with ring of the deposited salts (sample) is finally removed from the surface of the ring oven and then appropriate tests are done.

The mixture of metal ions of Ag, Pb and Hg is spotted on the center of filter paper and in the presence of HCl and ethylacetate mixture, the migration of ions takes place, faster being lead and slowest being silver. Now, K_2CrO_4 solution is sprayed on filter paper, inside we get a ring of silver, then pale green ring for mercury and followed by outer ring for lead as turmeric yellow.