Model Answers AR-7144

M.SC. (Fourth semester) Examination, 2013, Chemistry (1)

Paper: Separation techniques (CHM-402A)

Section A

1. (i) Retension time: the time it takes for a compound to travel through the column (from when an analyte is injected to when it reaches the detector) is known as the retention time (t_r).

Retension time is very important factor, because with the help of retension time, we can determine the unknown molecule by comparing the retension time of compound of standard with unknown molecule.

(ii) Size exclusion chromatography: this is a type of Chromatography in which the separation of molecules is based on the molecular size and shape through the stationary phase in column. The smaller size molecules can enter into the pores of stationary phase and hardly long time to elute from the column. However, bigger size eluted due to the bigger size of particles (pore size)

(iii)

Ascending chromatography	Descending chromatography
(a) The solvent front move from ~~down~~ side to up side and takes solute molecules to the same direction of the flow	(a) The solvent move (front) from upside to down side and takes solute molecule to same direction of flow
(b) Ascending chromatography is generally is more use ~~of~~ because of comfortably	(b) This chromatography is not simple.

(iv) Available soft gels: dextran (sephadex), polyacrylamide (Biogel P), Agarose (Biogel A), polystyrene (Biobeads) Starch, rubber

(v) TLC	HPTLC
(a) the particle size of stationary phase is bigger than HPTLC	(a) The size of particles are smaller
(b) Larger volume of sample is required (μL)	(b) in HPTCC, the volume of sample is in nL
(c) the number of theoritical plates are less	(c) Number of theoritical plates are more.
(d) This is not sensitive technique than HPTLC	(d) This is sensitive technique than TLC

(vi) Applications of HPLC: most widely used analytical technique

(a) Pharmaceutical: Pharmaceutical quality control, determination of drugs in urine and blood.

(b) Environmental: Phenol in drinking water, pesticides in water and soil

(c) Forensic science: Determination of cocaine and metabolites in clinical samples

(d) Food and Flavor: Sugar analysis in fruits

(vii) Advantages of liquid-liquid solvent extraction

(a) The analyte present in large volume of aqueous phase can be concentrated in small volume of organic solvent.

(b) Remove the interferences present in the aqueous solution while preconcentrating into organic solvent.

(viii) Purpose of ultrafiltration:

Ultrafiltration is process to used to fractionate the solutes in solution based on their size or molecular weight difference. Size or the molecular weight difference of the macrosolute retained by the membrane depends upon the pore size of the membrane.

(ix) Zone refining: It is method of purifying the solid crystal, in which a narrow region of a crystal is passed through the heated zone. The molten region is impure solid and leaves a pure material solid behind. Eg transistors are purified by this technique.

(x) Name of column used in GC

packed / capillary tubular column

OV-1, SE-30 (stationary phase – polydimethyl siloxane)

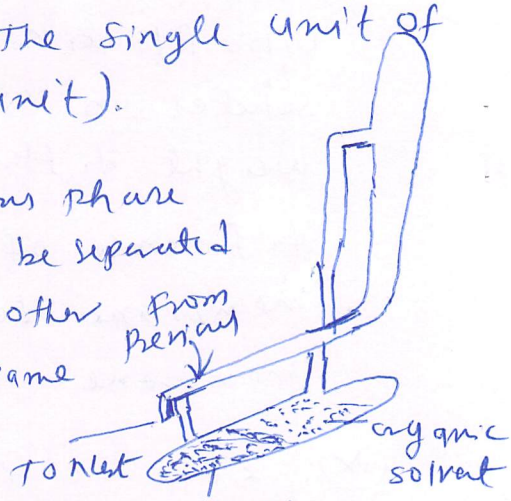
OV-3, SE-52 (stationary phase – 5% phenyl - polydimethyl siloxane)

other columns: OV-17, OV-210, OV-275

Section B

2. Craig counter distribution: This is a method of multiple liquid-liquid extraction, which permits the separation of substances with different distribution coefficient ration. this method is developed by Craig, so it is called as a Craig counter distribution. craig apparatus consists of glass tubes (r: 0, 1, 2 ...) that are disigned and arranged such that the lighter liquid phase is transferred from one tube to next.

The liquid-liquid extractions are taking place simultaneously in all the tubes of the apparatus. The single unit of Craig apparatus is shown (Single unit).



In the beginig, tube #0, contains aqueous phase containg the mixture of substances to be separated in the heavier solvent and all the other tubes contain equal volume of the same same solvent. The lighter solvent is added to tube #0, extraction equalibrium take ~~solvent~~ place and the phases are allowed to seperate. The upper phase of tube #0 is then transferred to tube #1 and fresh solvent is added to tube #0 and the phases are again equilibrated. The upper layer of tube #0 and #1 are simultaneously transferred to tube #1 and #2, respectively. This process is repeated several times to get maximum extraction of substances present in the sample.

The distribution ratio (D) of substance in organic phase to aqueous phase is $D = \frac{(C_A)_{org}}{(C_A)_{aq}} = \frac{p}{q}$ $\left[p+q=1,\ p=\frac{D}{D+1},\ q=\frac{1}{D+1}\right]$

The total fractions of solutes in n number of tubes of r number

$$f_{nr} = \frac{n!}{r!\,(n-r)!} \cdot \frac{D^r}{(D+1)^n}$$

similarly, the chromatographic seperation of solute molecules in column is supposed to be same as the craig counter distribution. Here, the column is made up of different theoritical plates, and each plates the equalibration of solute molecules between stationary phase

of theoritical plate and mobile phase. This process of seperation of solutes (mixture of substances) is sames as the seperation of substances in different craig apparatus (in tubes). In craig apparatus, depends on the number of tubes used in the separation. similarly, in chromatography separation depends upon the number of theoritical plates present in the column.

3. Principle of paper chromatography:

This chromatography is based on partition chromatography in which the substances are distributed between two liquids — one is stationy phase (water) which is held in the fibers of paper and called as stationary phase; the other is moving liquid or mobile phase. the components of the mixture to be seperated migrate at different rates and appear as spot at diffent portion of the paper.

$$Rf = \frac{\text{Distance travelled by the substance}}{\text{Distance travelled by the solvent}}$$

Experimental steps for paper chromatography:

(i) choice of paper chromatography:
Ascending / descending / radial / Ascending & descending

(ii) choice of filter paper:
qualitative / quantitative, for hydrophilic / lipophilic neutral, charged molecules

Characteristics of chromatography paper
Fast / slow / medium

(iii) Developing solvents: Based on polarity increasing polarity
n-Hexane < cyclohexane < carbon tetrachloride < Benzene < Toluene < Diethyl ether < chloroform < Ethyl acetate < n-butanol < n-Propanol < Acetone < Ethanol < methanol < water.

(iv) Preparation of sample: Substances are dissolved in perticular solvent depending upon the solubility of substance.

(v) Spotting: Micro syringe or Capillary tube is used for spotting on the edge of paper of volume 10 - 20 µL

(vi) Drying / Developing: spots on the filter paper after putting the paper in developing solvents and dried at room temperature.

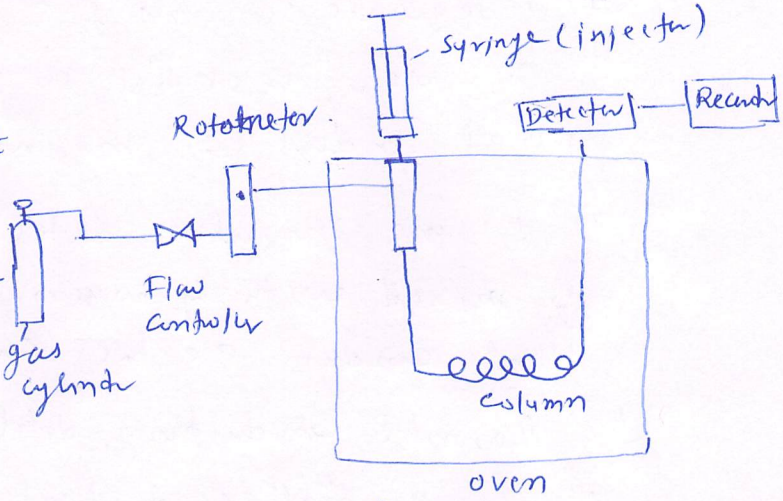
(vii) visualizing: chemicals/reagents are used to develope colored spots on the chromatographic paper. Physically it can be seen by UV lamp also

(viii) Rf calculation: The value of Rf is calculated. The different substances have different values which is used to identify the substances present in the sample.

4. Instrumentation of gas chromatography (GC)

Main components of GC

(i) Carrier gas (ii) sample injecter, (iii) Column, (iv) oven (v) Detector.



(i) carrier gas:- which must be chemically inert, include helium, nitrogen and nitrogen. Carrier gas should not react with sample and Stationary phase.

(ii) sample injection system: Common method of sample injection involves the use of micro syringe to inject a liquid or gaseous sample through a self-sealing, Silicone-rubber diaphragm or septum. Sample volume – 1-10 µL

(iii) column: column can be made from copper, stainless steel, aluminium or glass. two types of column:-

(a) packed column: 2-6 mm (id), 3-10 mm (od) and coating of silica particles < 100-300 µm)

(b) Capillary / open tubular column: They are again two types: wall-coated tubular (WCOT): 30-90 meters id (0.025-0.075 mm)

support-coated open tubular (SCOT): coating of 30 µm particle size.

(iv) ovens: Column oven temperature is important variable that must be controlled for the seperation of compounds in GC. the temperature of the oven is keep near to the Boiling point of compound that should be analysed.

(iv) Detectors: the ideal detector for GC Chromatography should have following characteristics —

* Adequate sensitivity * Good stability and reproducibility * a linear response to solute that extends over a several orders of magnitude.

(a) Flame ionization detector: the effluent from the column is mixed with hydrogen and air, then ignited electrically that conduct electric current is measured.

(b) Thermal conductivity detector (TCD): Based on the changes in the thermal conductivity of the gases stream brought about by the presence of analyte molecules.

(c) Electron Capture detector: organic molecules capture electrons and there is decrease in the current is measured.

5. solvent extraction solvent systems: —

(i) Extraction by chelation: chelating ligands play an important role in extraction of metals because they comprise of an impressive array of extracting and sequesting - metal chelate represents a type of coordinate compound in which a metal combines with a poly-functional base capable occupying two or more positions in the coordination sphere of metal ion to form a cyclic compound. $M^{2+} + 2\left[\begin{matrix} B_1 \\ (X)_y \\ B_2 \end{matrix}\right] \rightleftharpoons \left[(X)_y \begin{matrix} B_1 \\ B_2 \end{matrix} M \begin{matrix} B_1 \\ B_2 \end{matrix} (X)_y\right]$

Eg: Extractant
Trienoyltrifluoroacetone (TTA) used for Extraction of U(VI) at 3.4 — 8.0 PH in the presence of Th & Ce in the presence of Benzene solvent

(ii) Extraction by ion pair formation: most of the high molecular weight amines are used for

the extraction of metal ions by ion pair formation. The mechanism of extraction can be described as follows:

$$R_3N_{org} + H^+ + A^- \rightleftharpoons R_3NH^+A^-_{org} \text{ (Extraction)}$$

$$R_3NH^+A^-_{org} + B^- \rightleftharpoons R_3NH^+B^- + A^-_{org} \text{ (anion Exchange)}$$

Eg. of extracting agents: Trioctylamine (TOA)
triiso octylamine (TIOA) amberlite LA-1, LA-2
Primene TMT

Extraction of iron in chloride media:

$$Fe^{3+} + 4Cl^- \rightleftharpoons FeCl_4^- \text{ (complex formation)}$$

$$R_3N_{org} + H^+ + Cl^- \rightleftharpoons [R_3NH^+_{org}\, Cl^-_{org}]$$

$$[R_3NH^+Cl^-_{org}] + [FeCl_4^-] \rightleftharpoons [R_3NH^+FeCl_{4\,org}^-] + Cl^-$$

(salt formation)

(iii) Extraction by solvation: the solvent molecule participated in the extraction of metal ions and acts as an extractant. the basic character of the oxygen atom enables the solvent molecule in the coordination sphere of the metal ion to form a solvated complex.
Eg: Co^{2+} can be extracted in octanol (R-OH) in the presence of perchlorate ion as $Co(R-OH)\cdot 2ClO_4^-$.
Such solvation is generally possible with solvent like alcohol and ketones.

(iv) Synergic solvent extraction: such extractions are involved by using two extracting agents. The extractants may be chelating agent and solvation solvent or two chelating agents or two solvation solvents.

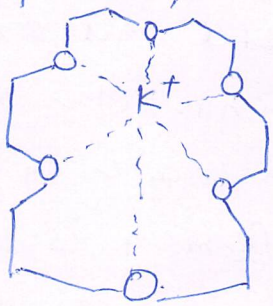
Eg: Dialkyl phosphoric acid in conjunction with neutral organophosphorus esters. The optimum conditions for such extraction are that the chelating ligands should neutralise the metal charge by chelating, the solvent solvent should coordinate less strongly than chelaty agent.

$$M^{n+}_{aq} + nHR_{org} \rightleftharpoons MR_{n\,org} + nH^+$$

$$MR_{n\,org} + nB_{org} \rightleftharpoons MR_nB_{n\,org} \text{ (adduct)}$$

(v) Extraction by crown ethers: These form stable complexes with a number of metal ions - with alkali metals (Na^+, K^+, Cs^+). Complexation is considered to result mainly from electrostatic ion - dipole attraction between the metal ion situated in the cavity of the ring and oxygen atom surrounding it.

Eg: Extraction of Na^+, K^+ and Ca^{2+} ions with crown 6 forming a ring.



18C6

6. Theory of capillary electrophoresis:

Electrophoresis is a seperation method based on the differential rate of migration of charged species in an applied dc electric field.

Capillary electrophoresis: is high speed and high resolution Separations of small volume of samples (0.1 to 10nL). The separated species eluted from end of the capillary.

The separation of molecules depends on the:

(i) Migration rates in CE: the migration rate if ion is depend on the electric field -

the electric field is in turn proportional to the magnitude of the applied voltage (V); $v = \mu_e \times \frac{V}{L}$ [v - migration rate μ_e — electrophoretic mobility, V — applied voltage, L - length]

(ii) Plate height in CE: the plate number in CE is $N = \frac{\mu_e V}{2D}$ [D - is diffusion coefficient]

Resolution increases as the plate count increases, it is desirable to use high applied voltages to achieve high resolution separations.

(iii) Electroosmotic flow: When a high voltage is applied across a fused-silica capillary tube containing a buffer solution, electroosmotic flow usually occurs, in which the bulk liquid migrates towards the cathode.

As shown in Fig., the cause of electroosmotic flow is the electric double layer that develops at the silica solution interface. At pH = 3, the silica capillary contains negative charged because of Sinanol group (Si–OH). Buffer cations congrate around the electrical double layer adjacent to the negative surface of capillary tube. The cations in the diffuse layer migrate toward the cathode (negative), because they are solvated, they drag in bulk solvent as in Fig (2)

Fig (1) Charge distribution at the silica capillary interface and resulting electroosmotic flow.

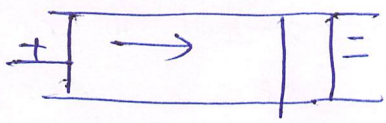


Fig (2) Electroosmotic Flow

The rate of electroosmotic flow is generally greater than electroosmotic migration velocity of individual ions. The analytes migrate according to their charges within

the capillary, the electroosmotic flow rate is usually sufficient to sweep all positive, neutral and negative species toward the same end of the capillary, so all can be detected as they pass by common point.

Applications of CE:

(i) CE can be used for pharmaceutical anaysis of pharmaceutical drugs and related substances

(ii) Protein characterization: The charged species of protein can be separated in CE and used for protein identification

(iii) CE is good separality instrument for DNA sequency and purity.

(iv) Also used for the separation of neutral compounds such as steroids.

(v) clinical anaysis: Extensively used in clinical purpose for the separation of serum proteins such as albumin, α-1 globulin, α-2 glabulin, β-1 and β-2 globulin and γ-globulin

7. Cation exchange chromatography:—

A cation exchanger is a high molecular weight, cross linked polymer having sulphonic, carboxylic, phenolic etc. are integral part of the resin and an equivalent amount of cation. Cation exchangers posses negatively charged groups and these will attract positively charged molecules.

In these cation exchangers, the hydrogen ions are mobile and exchangable with other cations. The anions ($-COO^-$, SO_3^{2-} and $-O^-$) remain attached to the resin. Eg:

$$H_nR + nNa \rightleftharpoons Na_nR + nH^+$$

(Resin) (solution) (Resin) solution

The resin having sodium ions, can exchange these ions with other cations: $2\,NanR + n\,Ca^{2+} \rightleftharpoons CanR_2 + 2\,Na^{+}$

Some commonly available cation exchangers

	Trade Name	Functional gp	Frame work material
1.	Amberlite IR-120	$-SO_3H$	styrene/ divinyl benzene
2.	Dowex	$-SO_3H$	〃
3.	Zeocit	$-SO_3H$	〃
4.	Amberlite-200	$-SO_3H$	〃
5.	SE Cellulose	$C_2H_4-SO_3H$	Cellulose
6.	Imberlite IRC-50	$-COOH$	methacrylic acid

Anion exchange chromatography: Anion exchangers have positively charged groups which will attract negatively charged molecules. An anion exchanger is polymer having amine or quaternary ammonium group as integral part of the resin and equivalent amount of anion such as Cl^-, SO_4^{2-}, OH^- ions. The exchange behaviour of these materials may be represented as follows:

$$2\,R\,Cl_n + n\,SO_4^{2-} \rightleftharpoons R_2(SO_4)_n + 2n\,Cl^-$$

(Resin) (Solution) (Resin) (solution)

$$RCl_n + n\,OH^- \rightleftharpoons R(OH)_n + n\,Cl^-$$

(Resin) (Solution) (Resin) solution

Commercial available anion exchangers

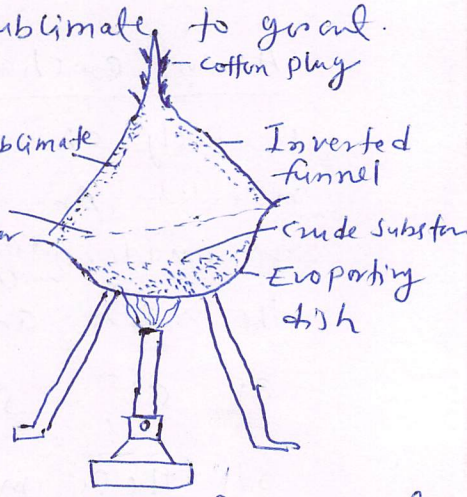
	Trade name	Functional gp	Frame work material
1.	Amberlite IRA-400	$-CH_2-N^+(CH_3)_3$	styrene / devinyl benzene
2.	Zeocit FF-IP	〃	〃
3.	Amberlit IRA-1410	$-CH_2-N^+-CH_2-CH_2$	〃
4.	QAE sephadex A-25	$-N^+-$	Dextran

8. Sublimation: is changing of substances directly from the solid to the vapour state without passing through an intermediate liquid phase. The example of such compounds are naphthalene anthracene, Camphor and indigo. The following equalibrium exists for such compounds. solid $\rightleftharpoons$ vapor

The sublimation is very useful in separating substance, which sublimes on heating (volatile solid) from non volatile impurities.

A simple apparatus for the process of sublimation is shown in fig. The substance is placed in an evaporting dish. It covered with perforted filter paper and inverted funnel is placed on the sheet. The sheet of the funnel is closed with cotton plug to avoid vapours of the sublimate to go out.

The dish is gently heated, as a result the solid substance changes to vapor from crude sample. The vapors rise up and pass through the holes in the perforated sheet and get deposited on the cooler walls of the funnel. The funnel is kept cool by wrapping it with a wet filter paper or wet cloth.

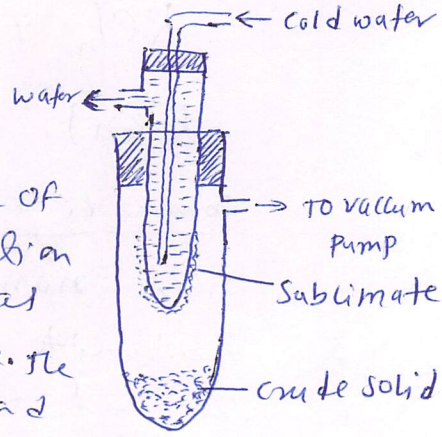


Apparatus for sublimation

Eg: Caphor contaminated with small amount of non-volatile impurities such as succinic acid can be purified by this method.

Sublimation under Reduced pressure:

some substances, decomposes under the sublimation temperature, For this type of substances, a technique called sublimation under reduced pressure is employed as follows: The apparatus is shown here. The sample is placed at the bottom tube and connected vaccum for reducing the pressure. and warm gently with water bath and inner tube is filled with water. The substance condense on the



Apparatus for sublimation under reduced pressure