

# 7<sup>th</sup> Analytical Sp 1 Past Paper

(2020)

(i)

## Advantages of HPLC:

HPLC has following advantages:

- Higher resolution and speed of analysis
- HPLC columns can be reused without repacking or regeneration
- Greater reproducibility due to close control of the parameters affecting the efficiency of separation
- Easy automation of instrument operation and data analysis
- Adaptability to large-scale, preparative procedure

(ii)

## Flame ionization detector:

It is the most frequently used detector in GC. The operation of the FID is based on the detection of ions formed during combustion of organic compound in hydrogen flame. The generation

of these ions is proportional to the concentration of organic species in the sample gas stream. FID compatible carrier gases include N<sub>2</sub>, He and Ar. Most organic compounds on burning in a flame is produce ions and electrons due to which the electrical conductivity of hydrogen flame is changed in an electrical field. This is the basic principle of extremely used sensitive detectors.

(iii)

### Advantages of Ag /AgCl:

Ag /AgCl electrode has following advantages:

- It has better thermal stability.

- Less toxicity and environmental problems with consequent cleanup and disposal difficulties.

- Ag /AgCl electrode can be used at temperatures > 60°C

## Disadvantages of Ag/AgCl:

Ag/AgCl electrode has following disadvantages:

- Ag reacts with more ions, plugging of the junction between electrode (Ag) and analyte solution
- It is more difficult to prepare than SCE.
- AgCl in the electrode has large solubility in saturated KCl.

(iv)

## Gradient Elution:

A separation method where the components are distributed between two phases, one of which is stationary, while the other moves in a definite direction (the mobile phase)

Gradient elution is commonly used when a mixture of solutes with a wide range of retention factors is to be separated.

(v)

### UV detector in HPLC:

ultraviolet (UV)-visible detector is most common and relatively inexpensive detector which provides better sensitivity based on the ability of substance to absorb light. During analysis, sample goes through a clear color-less glass cell, called flow cell. When UV light is irradiated on the flow cell, sample absorbs a part of UV light. There are two kinds of UV detectors

i-Fixed wavelength    ii-variable wavelength

(vi)

### Glass transition temperature:

Glass transition temperature is the temperature at which an amorphous polymer changes from a hard/glassy state to a soft/leathery state or vice versa.  $T_g$  is directly related to a material's strength and capabilities

in any given end-use application.

Glass transition temperature ( $T_g$ ) are in the range of  $150^\circ\text{C}$  upto  $315^\circ\text{C}$ .

(vii)

### Alkaline error:

At a value above pH 10 the gel layer of the glass membrane of a measurement electrode is subject to certain changes which lead to a measuring inaccuracy, known as alkaline error or sodium ion error.

This issue is caused by the presence of a high concentration of alkaline ions, especially sodium ion ( $\text{Na}^+$ ).

(viii)

### Gas sensing probes:

Gas sensors are devices that help us understand the amount of gas in the environment and the natural state of its movement.

Gas sensors (also known as gas detectors) are electronic devices that detect and identify different types of gases. They are commonly used to detect toxic or explosive gases and measure gas concentration.

(ix)

TGA used to evaluate thermal stability of material:

TGA (Thermogravimetric analysis) is an analytical technique used to determine a material's thermal stability and its fraction of volatile components by monitoring the weight change that occurs as a sample is heated at a constant rate. Many solids undergo reactions that evolve gaseous byproducts. In TGA, these gaseous byproducts are removed and changes in the remaining mass of the sample are recorded.

(X)

## Theory of DTA:

Le-châtelier described a new technique for the study of clays and minerals by examination of their temperature-time curves.

Later, Robert Austen improved this technique by introducing thermocouples, one of them was placed in sample and other in reference block in furnace and the data was interpret related to the reference block.

PUACP

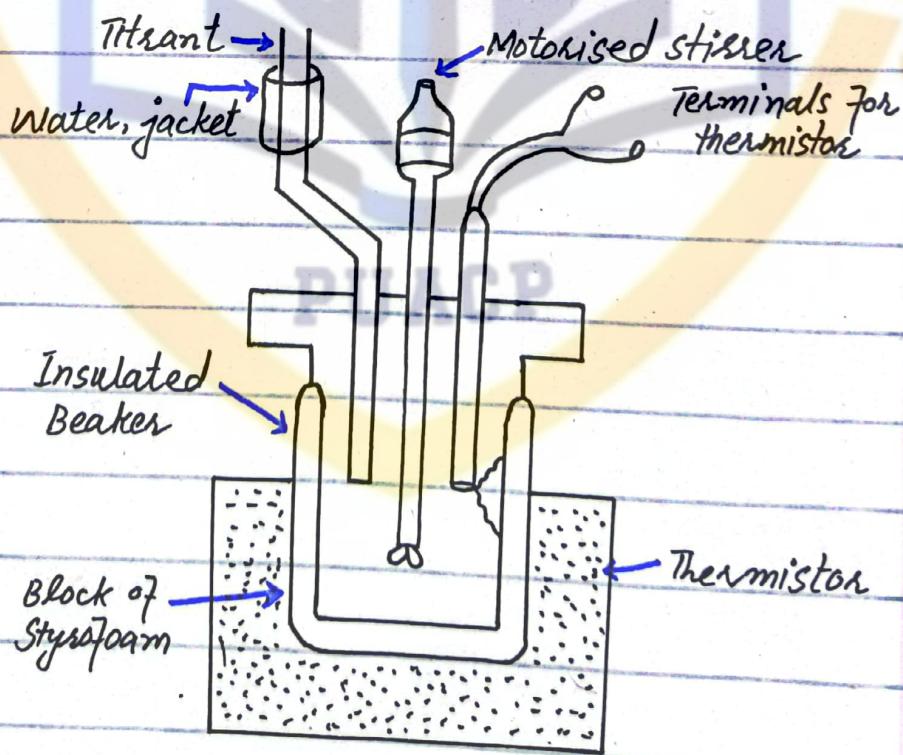
# Long Questions

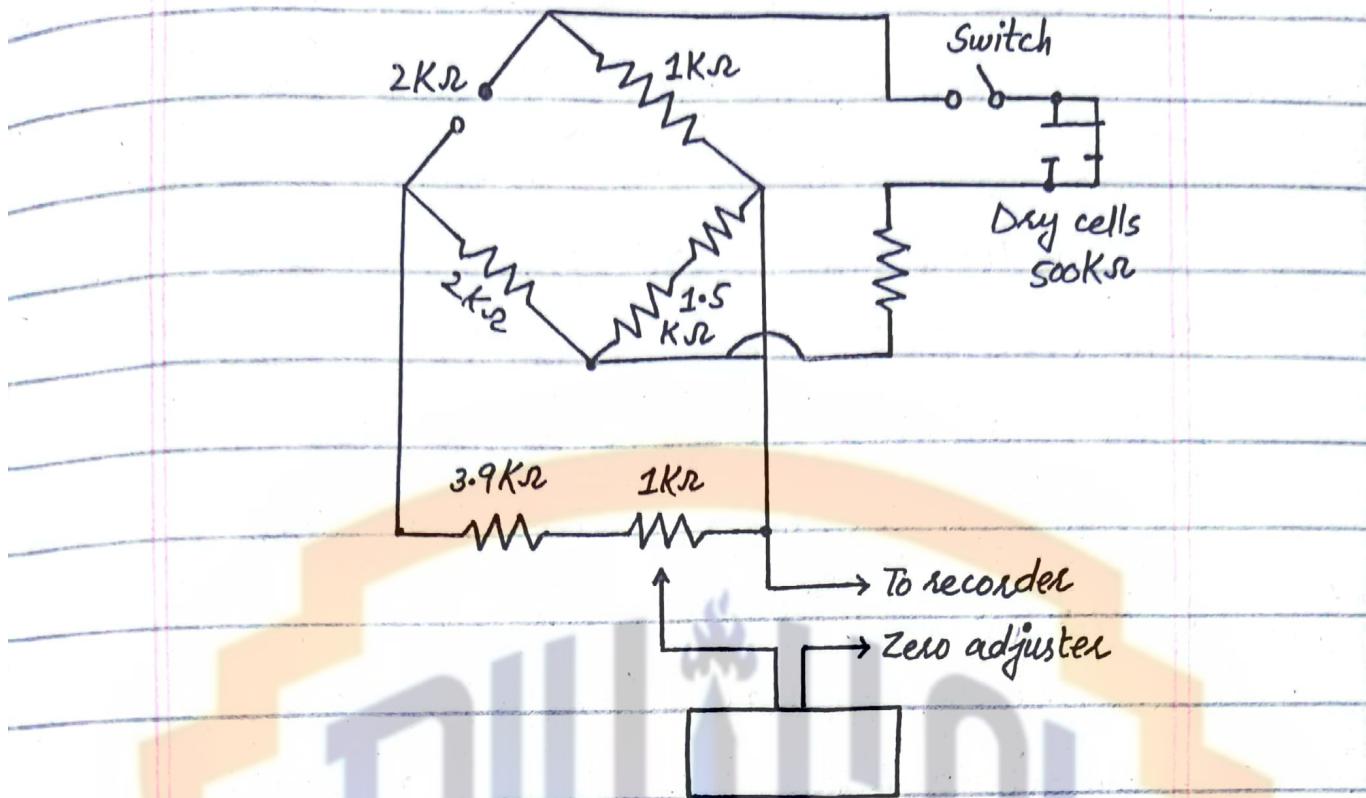
Q. 3(a)

## Instrumentation used for Thermometric titrations

The essential parts of thermo-metric titration (TT) are:

- i - A motor driven burette
- ii - A thermally insulated beaker or
- iii - Dewar flask (Styrofoam)
- iv - A thermister bridge assembly
- v - A recorder





### ⇒ Schematic Thermometric Titration

Assembly complete with a Bridge-circuit

Above figure represents the schematic thermometric titration assembly complete with a bridge-circuit. To minimise heat transfer losses from the solution by its immediate surroundings, the thermometric titrations are usually carried out in an isolated-beaker tightly closed with a stopper having provision for a burette-tip,

a motorized-glass stirrer, and a temperature-monitoring arrangement.

### Procedure:

1- Introduce the titrant from a burette that is duly mounted in a thermostated-water-jacket to maintain the temperature of the titrant within  $\pm 0.05^{\circ}\text{C}$ .

2- Experimental parameters are predetermined in such a fashion such that the volume of titrant needed for each titration must lie between 1-3 mL.

3- Automated device delivering reagent at a steady and constant rate of 600  $\mu\text{l}$  per minute usually permits recording.

4- Constant speed motorized stirrer at 600 rpm is employed to effect uniform mixing of solution.

5- Variations in temperature are measured with the help of a sensitive

thermister - sensing - element with fast response , that is sealed completely in glass and immersed in solution.

6- In the course of a thermometric titration, the thermister attached to the insulated-beaker is connected to one arm of the Wheatstone Bridge as displayed in figure.

7- The heat of reaction is either absorbed or generated upon addition of the titrant to the beaker , thereby unbalancing the Wheatstone Bridge caused by simultaneous variations in the temperature in the insulated-beaker thermister. Thus the Bridge unbalance potential is promptly plotted by the recorder.

Q.3(b)

## Factors affecting DTA

Following three factors effect the DTA curves.

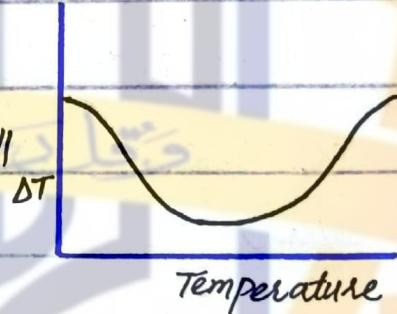
### 1- Environmental factor:

The gas which is provided outside the sample material usually inert gas is provided (e.g:  $N_2$ ).

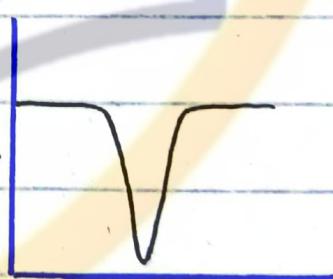
### 2- Instrumental factor:

(i) Heating rate is the main factor which effect on the DTA curve.

- If heating rate is very slow then the graph will be wider.



- If heating rate is very high then the graph with narrow peak is obtained with poor resolution.

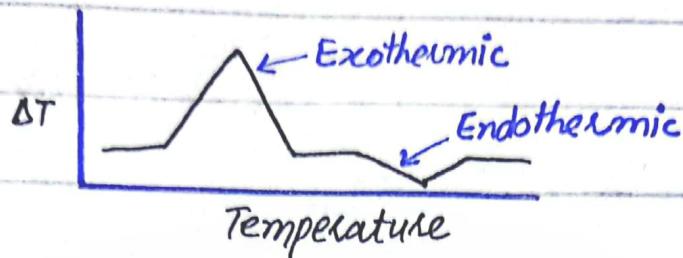


- So, we provided heating rate moderate.

(ii) Sample holder mainly are of two types

- **High thermal conductivity:** In this type we obtained following two results

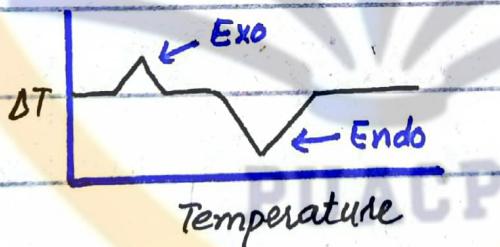
- high exothermic peak
- low endothermic peak



- Low thermal conductivity: It is opposite to the high thermal conductivity.

There are following two possibilities which are obtained as a result of low thermal conductivity

- low exothermic peak
- high endothermic peak

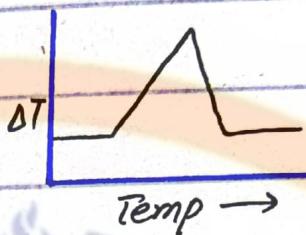


(iii) Location of thermocouples: The thermocouples should place between the sample material and reference material. This work is done to obtain the accurate graph.

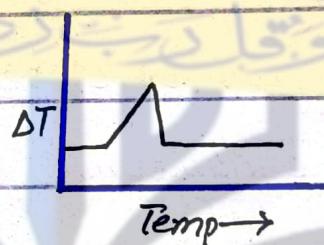
### 3- Sample characteristics:

(i) Amount of sample: We have two possibilities

- In large quantity a high peak graph is obtained.



- In small quantity graph form with lower peak.



(ii) Thermal conductivity

(iii) Size of sample The DTA curve obtain for small size molecules / particles is different from small size particles.

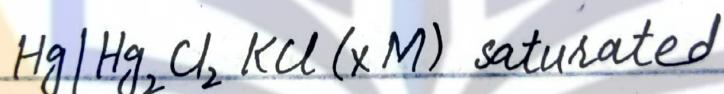
Q. 4(a)

## calomel electrode

A calomel electrode is a type of reference electrode used in electrochemical measurements to establish a stable reference potential.

### Construction:

The calomel electrode is made up of a mercury-mercurous chloride ( $\text{Hg}/\text{Hg}_2\text{Cl}_2$ ) electrode in contact with a solution of potassium chloride (KCl). It can be represented as:



The electrode reaction is:



The potential of a calomel electrode depends upon the activity of chloride in the half-cell. A calomel electrode containing a saturated solution of potassium chloride is a saturated calomel electrode; an electrode which

contain 1M potassium chloride in a normal calomel electrode (nce), and an electrode which contains 0.1M potassium in a decinormal calomel electrode (dec). The saturated calomel electrode (sce) is most widely used of the calomel electrodes.

### Working:

Commercial versions of calomel electrodes are prepared in glass or plastic tubes.

An inner tube containing the mercury which is covered with a paste of mercury-mercurous chloride ( $Hg^+ Hg_2Cl_2$ ) that is calomel

The remaining portion of the cell is filled with a solution of normal (1N) or saturated KCl.

A platinum wire sealed into a glass tube is dipped into a mercury layer and is used to provide external electrical contact.

The side tube is used for making electrical contact with a salt bridge.

Electrical connect is made through a fiber junction, a crack, a glass sleeve, a ceramic plug or by some other means at the bottom of outer tube.

The calomel electrode's potential is defined by following equation:

$$E_{\text{calomel}} = E^{\circ}_{\text{calomel}} + \frac{(RT/nF) \ln \frac{[\text{Hg}_2\text{Cl}_4]}{[\text{Hg}]}}{}$$

Where:

$E_{\text{calomel}}$  is the potential of calomel electrode.

$E^{\circ}_{\text{calomel}}$  is the standard potential of the calomel electrode.

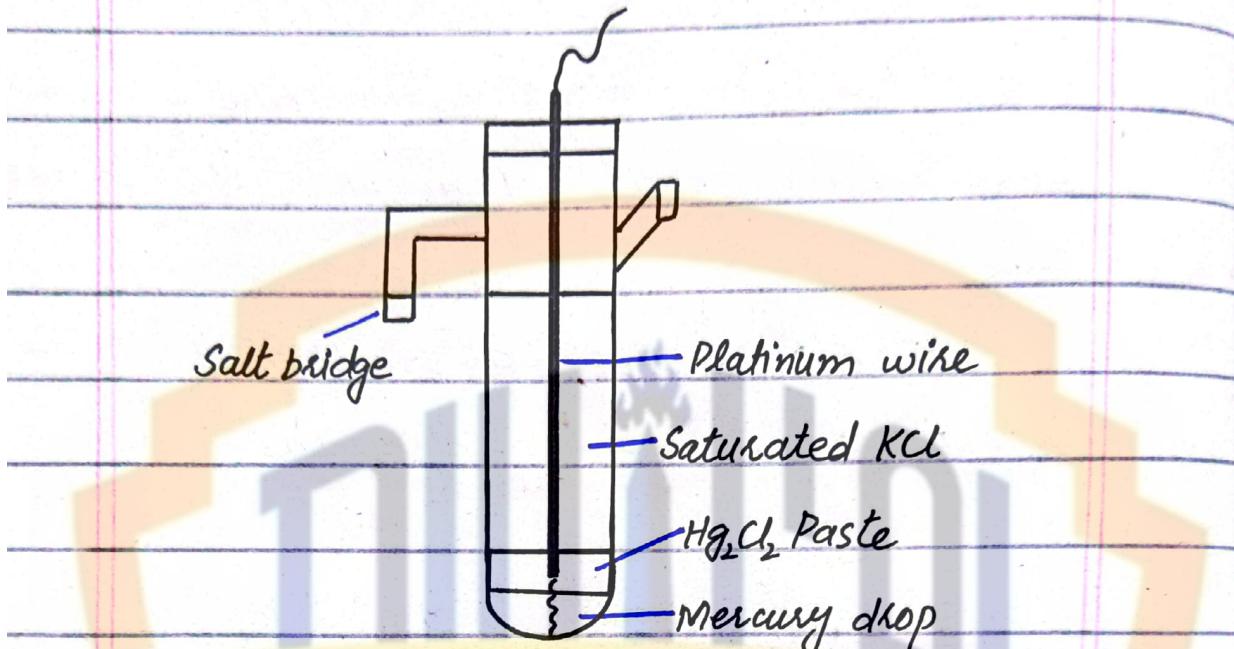
R is the gas constant

T is the temperature.

n is the number of electrons transferred in the half reaction.

F is Faraday's constant.

$[Hg_2Cl_2]$  and  $[Hg]$  are the concentrations of the mercurous chloride and mercury ions, respectively.



## Advantages:

Advantages using calomel electrodes

includes:

- Easy set-up and reproduction.
- Convenient transportation.
- Less auxiliary assets required. Calomel electrodes come with a side tube containing a KCl so no separate salt bridge is required.
- Stable potential. Potential does not change with time nor with slight temp. changes.

Q.5(b)

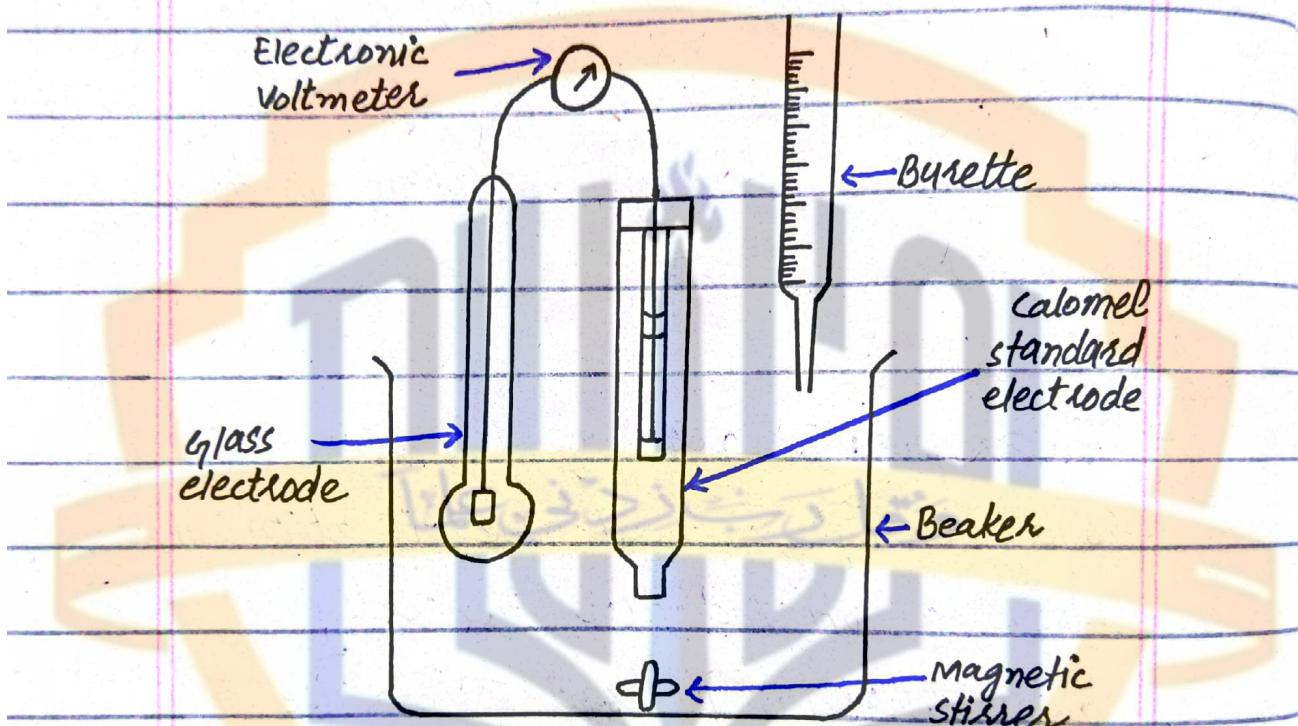
### Potentiometric acid-base titration

⇒ **Definitions:** An acid-base titration is a quantitative analysis used to determine the acid or base concentration by precisely neutralizing the acid or base with a known concentration standard solution.

⇒ **Example:** Titration of HCl with NaOH is an example of acid-base titration in which a pH indicator (generally phenolphthalein) is used to produce color by which the end point of the reaction determines. It can also be performed by potentiometric titration however it doesn't need an indicator.

⇒ **Apparatus:** The apparatus used for potentiometric acid-base titrations is:

- Burette
- Beaker
- Magnetic stirrer
- Glass electrode
- Electronic voltmeter
- calomel standard electrode



A hydrogen electrode or a glass electrode is immersed in a solution of the acid whose strength is to be determined. The glass electrode is coupled with a standard calomel electrode.

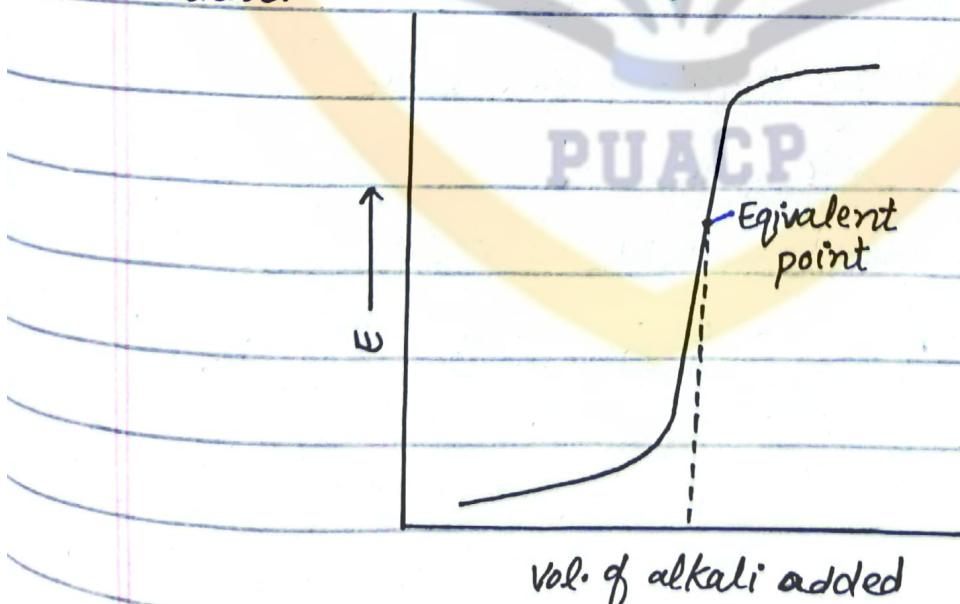
The cell thus formed is connected

to the potentiometer or electronic voltmeter. When alkali is added, pH of the solution changes. The emf of the cell also changes with pH of the solution in accordance with the relation.

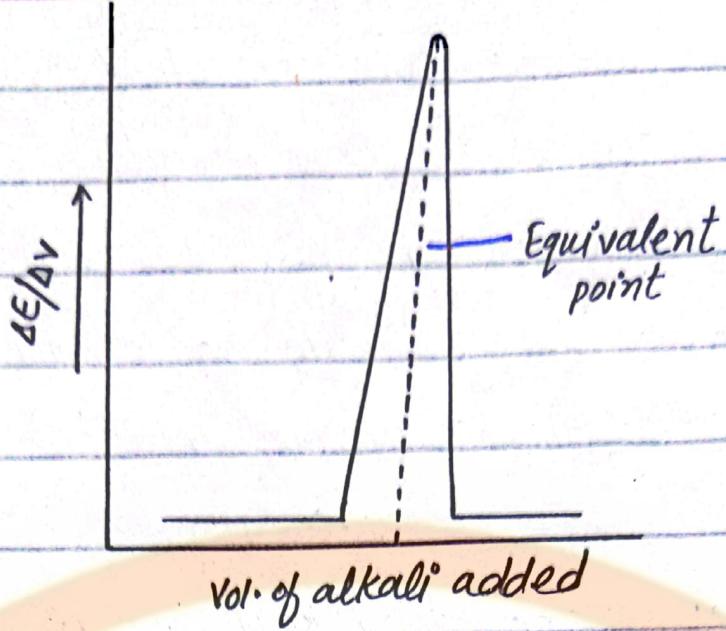
$$E = E^{\circ} + 0.0591 \text{ pH}$$

The standard alkali solution is then added from the burette in small volumes. After each addition, the emf of the cell is recorded.

The emf is then plotted against the volume of alkali added. The shape of the curve for the titration of a strong acid against strong base or alkali.



Potentiometric titration curve of strong acid or base



## Potentiometric titration curve of weak acid or base

⇒ Types of acid-base titration

1- Strong acid - Strong base

⇒ e.g.: HCl and NaOH

2- Weak acid - Strong base

⇒ e.g.: CH<sub>3</sub>COOH & NaOH

3- Strong acid - Weak base

⇒ e.g.: HCl and NH<sub>3</sub>

4- Weak acid - Weak base

⇒ e.g.: CH<sub>3</sub>COOH and NH<sub>3</sub>

Q.1

—(2022)—

## Short Questions

(i)

### Van Deemter Equation:

The Van Deemter equation is the relation between height equivalent to a theoretical plate (HETP) which is a measure of column efficiency, and linear velocity ( $\mu$ ).

Formula:

$$HETP = A + \frac{B}{\mu} + (C_s + C_m) \cdot \mu$$

HETP = Height Equivalent to a Theoretical Plate

The term involved

A = Eddy diffusion

B = longitudinal diffusion coefficient

C = the mass transfer coefficient

C is divided into  $C_s$  and  $C_m$  contribution from stationary & mobile phase respectively.

$\mu$  = linear velocity

(ii)

## Retention time:

It is the time from the point of injection of the sample to the time of emergence of the separated component from the column.

As we know,

$$V_R = t_R F_c$$

In this formula  $t_R$  is called the retention time.

## Factors of dependence:

The retention time depend upon

- the flow rate,  $F_c$  of carrier gas

- column temperature,  $T_c$

- the weight of the liquid phase

- the affinity between sample

component and liquid phases

comparing the stationary phase.

(iii)

### Normal Phase chromatography

- In normal phase chromatography the stationary bed is strongly-polar in nature and mobile phase is non-polar.

- Evolved in 1970s in the form of liquid.

- Use a non-polar, non-aqueous solvent as the mobile phase which is mainly chloroform.

- Non-polar mobile phase increases the retention time.

- Column is easy to damage.

### Reverse Phase chromatography

- In reverse phase chromatography the stationary bed is non-polar in nature and the mobile phase is polar solvent.

- A recently evolved form of HPLC.

- Use a polar mobile phase which is mainly water, methanol or acetonitrile.

- Polar mobile phase increases the retention time.

- Column is difficult to damage.

(iv)

## Split mode injectors

- Only a small portion of sample enters the column, and the rest is sent to waste.
- This is used when the analytes are in high concentration and would over-load the column.
- It has following characteristics:
  - High temperature
  - High linear velocity
  - Rapid transfer
  - Bulk of sample wasted
  - Split ratio important
  - Linear geometry

## splitless mode injectors

- One can inject higher percentage of the sample to the column.
- This is used for trace analysis.
- Use cold trapping and solvent effects to focus bands.
- It has following characteristics:
  - High temperature
  - Low linear velocity
  - Slow transfer
  - Bulk of sample and solvent to column

(iv)

## Liquid junction potentials:

The phenomenon that occurs when two solutions of electrolytes of different concentrations are in contact with each other is called liquid junction potential.

The more concentrated solution will have a tendency to diffuse into the comparatively less concentrated one.

(vii)

## Primary reference electrode      Secondary reference electrode

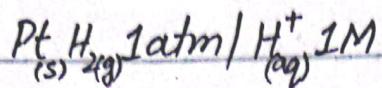
A primary reference electrode is one whose potential under saturated conditions is to be defined to be exactly zero.

e.g.: SHE (saturated hydrogen electrode)

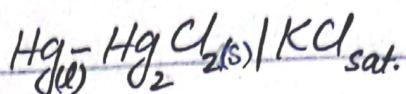
A secondary reference electrode is one whose potential is calculated by connecting to the standard hydrogen electrode.

e.g.: Calomel electrode, Ag/AgCl electrode

The cell is represented as:



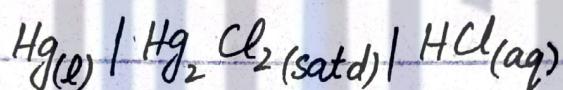
The <sup>saturated</sup> calomel electrode (SCE) is represented as:



(vii)

### calomel electrode:

calomel electrode is represented as:



Its  $E^\circ$  value is +0.24V.

(viii)

### Saturated electrode potential:

It is defined as the potential of the electrode when all of the ion concentrations are 1.00M, the temperature is 25°C, and any gases that are involved in the cell reactions are at a pressure of 1atm. It is denoted by the symbol  $E^\circ$ .

(ix)

## Thermocouple:

A thermocouple is a device for measuring temperature. It is also called thermal junction, thermometer or thermal. It comprises two dissimilar metallic wires joined together to form a junction. When the junction is heated or cooled, a small voltage is generated in the electric circuit of the thermocouple which can be measured, and this corresponds to temperature.

### Types:

Primarily there are 8 types of thermocouples:

1-B-Type Thermocouple

2-E-Type Thermocouple

3-J-Type Thermocouple

4-N-Type Thermocouple

5-K-Type Thermocouple

6-R-Type Thermocouple

7- T-Type Thermocouple

8- S-Type Thermocouple

(X)

### DSC

- DSC is Differential Scanning calorimetry.

- Heat flow is measured against temperature change at particular time.

- Used to analyze  
→ proteins  
→ antibodies etc.

- Sample is always a liquid.

### DTA

- DTA is Differential Thermal Analysis.

- Temperature difference developed between sample & reference compound is measured at identical heat treatments.

- Used to analyze  
→ thermal properties of minerals  
→ for the characterization of polymers & biological materials.

- Sample can be used as a solid substance.

(xi)

## Thermal analysis:

Thermal analysis is a general term defining a technique used to analyze the time and temperature at which physical change occurs when a substance is heated or cooled

### Types:

There are several types of TA.

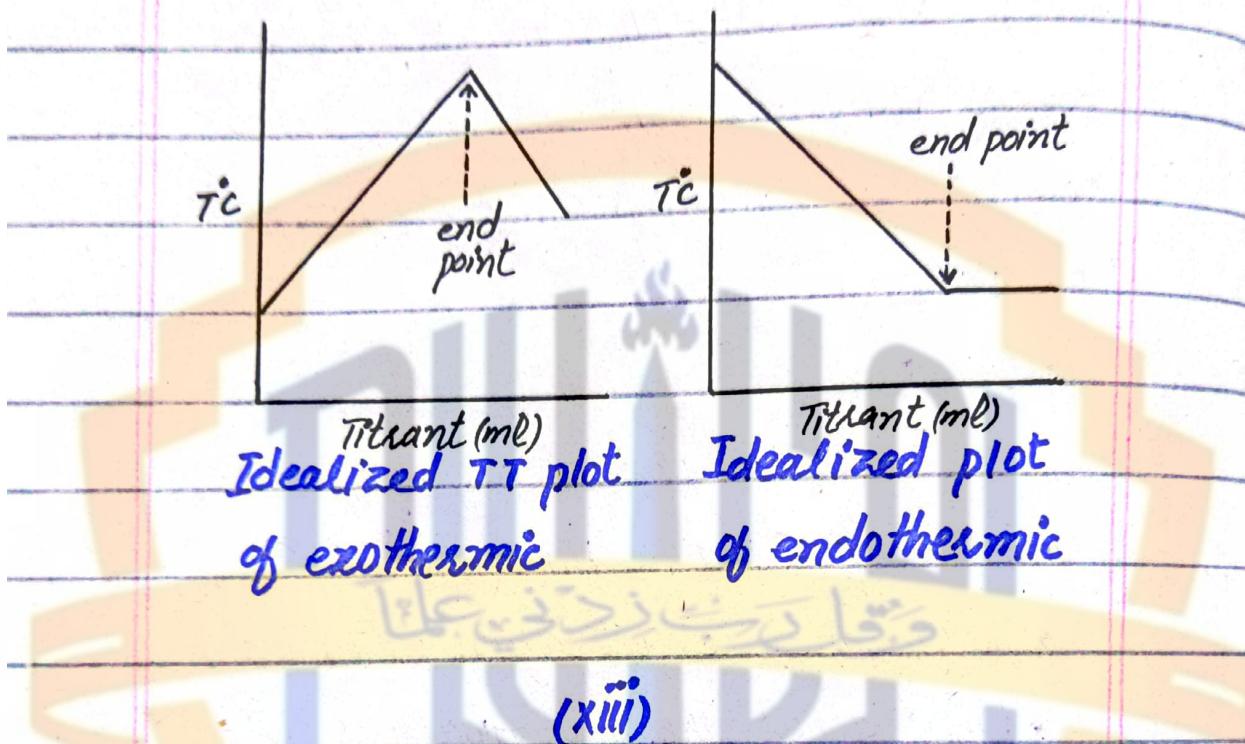
- Thermogravimetric Analysis (TGA)
- Thermometric titration (TT)
- Differential Thermal Analysis (DTA)
- Differential Scanning Calorimetry (DSC)

(xii)

## Thermometric titration:

It is an instrumental titration technique where end points are located accurately. It utilizes the enthalpy change of a chemical reaction to locate the end point. This technique is an

adiabatic system, meaning to heat transfer, that yield a plot of temperature against the volume of the titrant.



### Pre-column:

A precolumn or also called guard column protects the main column of plugging and contamination by sample and mobile phases.

It contains a packing chemically identical to that in analytical column.

Mainly used to remove impurities from the solvent & protect analytical column.

It is having large particle size.

It is having short length of 2 to 10cm,  
so does not affect separation.

(xiv)

### Derivatization in HPLC:

Derivatization or chemical structure modification is often used in bioanalysis performed by liquid chromatography technique in order to enhance detectability or to improve the chromatographic performance for the target analyte, improve thermal stability of target compound. To fulfill all these requirement, derivatization is done.

PUACP

(xv)

### Reverse phase mode in HPLC:

Reverse phase chromatography is a term of HPLC. In reverse phase chromatography the stationary phase/bed

is non-polar in nature and the mobile phase is polar solvent. This term is recently evolved in HPLC.

(Liquid) Water, methanol or acetonitrile are mainly used as mobile phase in HPLC. Polar mobile phase increases the retention time. In reverse phase chromatography the column is difficult to damage.

## Long Questions

Q.2

(a)

### Nernst Equation:

**Definition:** The Nernst equation provides a relation between the cell potential, the standard potential, temperature and reaction quotient.

Even under non-standard conditions, the cell potential of electrochemical cells can be determined with the help of

Nernst equation:

It is often used to determine the electrode potential of electrode theoretically.

Mathematical form: (Single electrode potential)

$$E_{\text{cell}} = E^{\circ} - \frac{RT}{nF} \ln Q$$

$$\text{or } E_{\text{cell}} = E^{\circ} - \frac{2.303 RT}{nF} \log Q$$

Where,

$E_{\text{cell}}$  = cell potential of the cell

$E^{\circ}$  = cell potential under standard condition

$R$  = Universal gas constant

$T$  = Temperature

$n$  = no. of electrons transferred in the redox reaction

$F$  = Faraday constant

$Q$  = reaction quotient

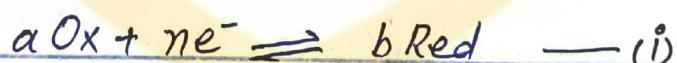
Nernst Equation at 25°C: For measurements carried out 298K, the Nernst equation can be expressed as

$$E = E^\circ - \frac{2.303 \times 25 \times R}{nF} \log Q$$

Therefore, as per the Nernst equation, the overall potential of an electro-chemical cell is dependent on reaction quotient.

### Derivation of Nernst Equation:

Consider an oxidation-reduction half reaction which is carried out by coefficients of Ox and Red in the balanced half-reaction.



Where,

Ox = Oxidized form of species

Red = Reduced form of species

n = no. of electrons  $e^-$  involved  
in half-reaction

$a^{\text{Eq}} b$  = coefficient of Ox and Red  
in balanced half-reaction.

The thermodynamic feasibility of the reaction taking place can be determined from the change in Gibbs free energy  $\Delta G$  for reaction

$$\Delta G = \Delta G^\circ + RT \ln \frac{a_{\text{Red}}^b}{a_{\text{Ox}}^a} \quad \text{--- (ii)}$$

Where,

$R$  = gas constant ( $8.314 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ )

$T$  = absolute temperature

$a_{\text{Red}}$  = activity of Red

$a_{\text{Ox}}$  = activity of Ox

$\Delta G^\circ$  = Gibbs free energy of substance  
in its standard form

Standard form for all substances is  
that (at), which occurs at  $1\text{ atm}$  pressure  
and, unless otherwise specified at  $25^\circ\text{C}$ .

The free energy change for a reaction can be related to the potential E of a reaction by:

$$\Delta G = -nFE \quad \text{--- (iii)}$$

$$\Delta G^\circ = -nFE^\circ \quad \text{--- (iv)}$$

Where,

$F$  = Faraday constant (96487 C/mol)

$E^\circ$  = Standard potential

Put the values of  $\Delta G$  &  $\Delta G^\circ$  in eq (ii)

$$-nFE = -nFE^\circ + RT \ln \frac{a_{\text{red}}^b}{a_{\text{ox}}^a}$$

$$E = E^\circ - \frac{RT}{nF} \ln \frac{a_{\text{red}}^b}{a_{\text{ox}}^a} \quad \text{--- (v)}$$

$$\therefore \ln = 2.303 \log$$

$$E = E^\circ - \frac{2.303RT \log \frac{a_{\text{red}}^b}{a_{\text{ox}}^a}}{nF} \quad \text{--- (vi)}$$

At 25°C, eq (vi) can be further simplified by substitution and combination of the constants preceding logarithmic term

of yield.

$$E = E^{\circ} - \frac{0.05917}{n} \log \frac{a_{\text{Red}}^b}{a_{\text{Ox}}^a}$$

This is called Nernst equation. It can be rewritten as:

$$E = E^{\circ} - \frac{RT}{nF} \ln \frac{[\text{Red}]^b}{[\text{Ox}]^a}$$

Where, square brackets indicate the molarity of the substances

(b)

### Applications of Nernst Equation:

The Nernst equation can be used to calculate:

- Single electrode reduction or oxidation potential at any conditions
- Standard electrode potentials
- Comparing the relative ability as a reductive or oxidative agent.
- Finding the feasibility of combining such single electrodes to produce

electric potential.

- Emf of an electrochemical cell
- Unknown ionic concentrations
- The pH of solutions and stability of sparingly soluble salts can be measured with the help of the Nernst equation.

Q.3

(a)

### Derivatization in GC:

Derivatization is the process of chemically modifying a compound to produce a new compound which has properties that are suitable for analysis using a GC or HPLC.

### Why derivatization in GC?

- To permit analysis of compounds which are not directly amenable to analysis due to for example, inadequate stability and volatility.

- To improve chromatographic behaviour or detectability.
- Many compounds do not produce a useable chromatography or the sample of interest goes undetected. As a result it may be necessary to derivatize the compound before GC analysis is done.
- The main reason for derivatizing is to impart volatility to otherwise non-volatile compounds
- Derivatization is typically done to change the analyte properties for a better separate and also for enhancing the method sensitivity.
- To improve the chromatographic separation, peak shape or response of the analyte.

(b)

Types of capillary column in gas chromatography:

etc. There are following main types of open tubular columns

- Wall coated open tubular column (WCOT)
- Support coated open tubular column (SCOT)
- Porous layer open tubular column (PLOT)
- Fused silica open tubular column (FSOT)

Wall coated columns consist of a capillary tube whose walls are coated with liquid stationary phase.

In support coated columns the inner wall of the capillary is lined with a thin layer of support material such as diatomaceous earth, onto which the stationary phase has been adsorbed. SCOT column are generally less efficient than WCOT columns. Both types of capillary column are more efficient than packed columns.

Porous layer open tubular column are made by extending the inner wall of column by substances such as fused silica.

A new type of WCOT column was developed

known as Fused Silica Open Tubular column

in 1979. Fused silica is produced by purified quartz with a very low metal oxide contents.

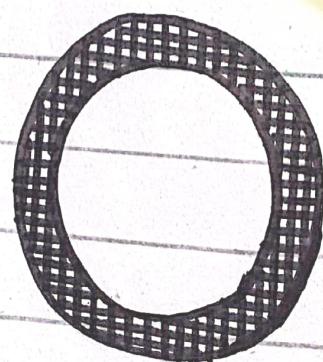
Mostly long, narrow open tubular columns are made of fused silica and coated with polyimide. These columns are flexible and can be shaped into coils.

They have the advantages of flexibility, low reactivity, physical strength and used for majority of analyses.

Advantages of open tubular columns:

Open tubular column gives

- better resolution.
- larger theoretical plate number
- greater sensitivity
- smaller sample capacity (sample load)
- decreases analysis time than packed column



Porous Layer Open  
Tubular Column (PLOT)

Particular layer thickness

5-50  $\mu\text{m}$ . Tube I.D 320-

530  $\mu\text{m}$ .

Packed capillary column

I.D < 1mm

Open Wall coated Tubular Column (WCOT)

Film thickness 0.1-0.8  $\mu\text{m}$

Tube I.D 100-530  $\mu\text{m}$

Cross section of a Fused Silica Open Tubular Column



Q.4

(a)

## Applications of TGA curves:

- TGA is primarily used to characterize materials by measuring their change in mass as a function of temperature.
- TGA can be used to determine a substance's:
  - Change in mass due to decomposition,
  - oxidation, evaporation or combustion
  - Absorbed moisture content
  - Volatilization rate of material
- TGA is used to study the kinetics of the reaction rate constant.
- From TGA, we can determine the purity and thermal stability of both primary and secondary standard.
- Used in the study of catalyst
- Analysis of dosage form
- Oxidative stability of materials
- Estimated life time of a product.

- TGA is especially useful for studying polymer-based products that need to react to high heat or rapid temperature changes in specific ways.
- The most common application of TGA in business and industry is to test the thermal resistance of products for quality assurance and safety purposes.
- The effect of reactive or corrosive atmosphere on materials.
- TGA is frequently used to test pharmacologic products before they are approved for distribution and patient use.

(b)

### Problems in using HPLC:

Despite advancements in HPLC methods and technology, there are still issues that rear their ugly head when using an HPLC system. Most common HPLC problems are:

## 1- HPLC Pressure Problems:

The primary issues with pressure include:

Abnormal pressure which generally means there is no flow because there is no power, happens when there is a leak or air trapped in the pump head, there is an issue with the controller setting or a piston is broken. If there is flow and pressure, the meter or pressure transducer could need replacement.

Pressure cycling can be caused by air in the pump, faulty valves, a system leak, seal failure in the pump, insufficient degassing, or the use of gradient elution.

## 2- HPLC Leakage Problems:

Leaks can occur anywhere within the HPLC.

Leaks at the fittings typically means the fitting needs to be tightened, cleaned,

or replaced if it stripped or damaged.

Other issues include overtightening of the fitting or using parts from different manufacturers.

Leak

Leaks at the HPLC column can be caused by a loose end-fitting that needs to be tightened.

Leaks at the pump can be due to loose fittings or loose valves that must be tightened. There could also be failure of the mixer seal, pump seal, pulse damper in which cause the faulty part needs to be repaired or replaced.

### 3- HPLC Chromatography Problems:

Possible problems that can stem from HPLC columns:

Peak tailing due to fit blockage, a column void, sample interaction with active sites, an interfering peak, the wrong

mobile phase pH, or a column that needs to be replaced.

Peak fronting due to low temperature, using the wrong sample solvent, overloading the sample, or a bad column that needs to be replaced.

Split peaks due to contamination in the column inlet or on the guard or incompatibility of the sample solvent with the mobile phase.

Distortion of large peaks due to overloading the sample.

Distortion of small peaks due to using the wrong injection solvent.

Retention time drift due to poor control of temperature or column equilibration or mobile phase changing.

Tailing of acidic or basic peaks  
due to inadequate buffering.

Peaks that are too small or too  
large due to issues with detector  
attenuation, injection size, or an  
improper recorder connection.

PUACP