Sub:- Pharmaceutical Analysis - II

Time - 2 hrs. Full Marks - 20

Question No. 1 is Compulsory.
Answer four Questions.
All Questions Carry equal marks.

Q.1.

(A) (iv) (All)
(B) (i) (Chromone Moiety)
(C) (iii) (Both)
(D) (ii) (Benzene)
(E) (ii) (5-7)

Q.2.

(a) Preparation of KF-reagent:-

The process of Karl fischer Reagent is as follows

→ Add 125 g of Iodine to a solution containing 670 ml of methanol and 170 ml of pyridine and cool
→ Place 100 ml of pyridine in a 250 ml graduated cylinder and keeping the pyridine cold in an ice bath, pass in dry sulphur dioxide until the volume reaches 200 ml.
→ Slowly add this solution with shaking to the cooled iodine mixture. Shake to dissolve the iodine, transfer the solution to the apparatus and allow the solution to stand overnight before standardizing.
→ One ml of this solution when freshly prepared is equivalent to approximately 5 mg of water.
→ Protect from light while in use. Store bulk stock of reagent in a suitably sealed glass stoppered containers and under refrigeration.
→ For the determination of trace amount of water, it is preferable to use a reagent with a water equivalency factor of not more than 2.0 which will lead to the consumption of a more significant volume of titrant

When reacting with water, the bromine iodine is reduced to colorless iodine. At the end point of the titration when all the water is consumed the color of the solution increasingly from yellow to brown. As there is no sharp color change and the coloration differs in non polar solvent (such as DMF) and polar solvent (eg. methanol) it is not easy to determine the end point visually. For this reason, the end point of the titration is usually determined electrometrically with a double platinum wire electrode. There are two ways to electrometrically detect the end point

(A) Bi amperometric indicator
(B) Bi voltametric indicator.

(A) Bi amperometric indicator :-
A constant voltage of approximately 500mV is applied to the wire of the electrode and the resulting current is measured. As long as there is water in the sample, no free iodine is present in solution. When the end point of the titration has been reached, the following reaction occurs at the wires of the electrode

Cathode- $I_2 + 2e^- \rightarrow 2I^-$

Anode- $2I^- - 2e \rightarrow I_2$

When the end point has been reached the current flows increases from almost nil to a few μA.

(B) Bivoltametric indication :-
A small current (normally in the range of 1 to 50 μA) is applied between the electrodes and the voltage required is measured.

electrode then direct current. The voltage required to maintain the current is in the range of several 100 mV as long as an excess of water is present in the sample. When the end point of the titration is reached, free iodine is available in the solution and the voltage drops to 100 mV or less.

2 (c) Limitations of Karl fischer Titration :-

→ Manual volumetric KF titration requires reloading for each determination and hence has a high solvent consumption.

→ The margin of error is relatively large when manual volumetric KF-titration is applied to material that contain starch.

→ Manual titration requires considerable operator input.

→ KF titration is a destructive technique.

→ Coulometric KF titration is suitable only for samples containing small amounts of water, and larger amounts may overwhelm the reagent capacity and thus false result, besides taking excessively long period for the determination.

→ KF titration depends upon a redox reaction and thus any component of the sample which is an active redox chemical (such as dimethyl sulfoxide) will react with the iodine in the reagent and generate false result.

→ Ketones, aldehydes, boric acids and metal peroxides as well as salicylic and strong acids are not suitable for this titration with modification, as their reaction with methanol-bound water, resulting in a varnishing end point and falsely high water content requiring the use of methanol-free reagents with such

(4) → Carbonates, oxides and hydroxides also undergo side reaction and are not suitable for titration.

Q. 3:

Electrodes

Potential of a single electrode cannot be measured. It is therefore coupled with another electrode whose potential is known, and this is called reference electrode. The two electrodes that is experimental electrode whose potential is to be found out and the reference electrode constitute a cell (X) whose emf can be measured by potentiometer, thus two electrodes are (A) Reference electrode (B) Indicator electrode

(A) Reference electrode

A reference electrode is an electrode which has a stable and well known electrode potential. The high stability of the electrode potential is usually realised by employing a redox system with constant concentration of each participant of the redox reaction.

There are many reference electrodes are used. The simplest is when the reference electrode is used as a half cell to build and electrochemical cell. This allows the potential of the other half cell to be determined.

Common reference electrodes and potential with respect to standard hydrogen electrode are—

→ Standard hydrogen electrode (SHE) ($E = 0.000$ V) activity of H^+ = 1 Molar

→ Normal hydrogen electrode (NHE) ($E = 0.000$ V) Concentration H^+ = 1 Molar

→ Reversible hydrogen electrode (RHE)

$E = 0.000V - 0.0591 * pH$

electrode.

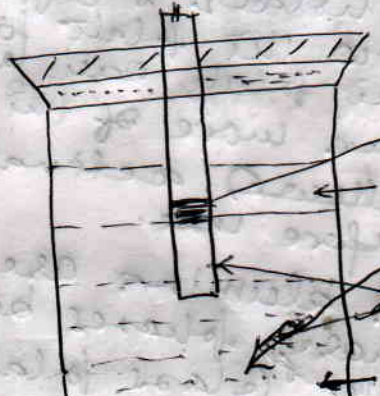
(b) Indicator Electrode:

Indicator electrode is used to measure the potential of analyte solution by comparing with that of the reference electrode. The potential is directly proportional to the concentration of ion of the hydrogen electrode. Indicator electrodes are mainly of two types metal indicator electrode and ion selective electrode. In metal indicator electrode redox reaction occurs on the metal surface. Pt and Cu are used as metal indicator electrode

Examples of Reference electrode is Saturated Calomel Electrode.

It consists of porous disc at the base of the electrode which is clogged. Above it glass tube is filled with Potassium chloride crystals. Above it is filled with Calomel paste which is prepared by grinding mercury chloride with pure mercury and minute millilitre of Saturated potassium chloride solution. Finally a layer of mercury is placed in the electrode vessels. The reaction of Saturated Calomel Electrode

$$Hg_2Cl_2 + 2e^- \longrightarrow 2Hg(l) + 2Cl^-$$



When sample contains several components, its analysis becomes complicated. for analysing such sample, a method should be available to determine a particular component in presence of others. There are two ways to deal with such problems. The interference of a particular substance can be eliminated by adding a complexing agent which reacts selectively with the interfering substances so that now it does not disturb the main reaction: this technique is known as masking. Another approach is to isolate the interfering species from the sample mixture by some separative technique such as solvent extraction, ion exchange or chromatography.

Chromatography is a separative process which is very useful for separating molecular mixture. In this technique advantage is taken of different adsorption of different components of a mixture by an adsorbent.

In thin layer chromatography an adsorbent coated on a glass plate acts as the stationary phase. The mobile phase percolates the adsorbent and carries along with it various components of a mixture which move with different speeds and thus get separated. Silica gel or cellulose powder is very often used as adsorbent. The adsorbent should be such that different components should have different values of Rf in it.

I Preparation of thin layer plate

For coating five 20 x 20 plates about 30 g. TLC silica gel to 60 ml water is needed. It is mixed to form slurry. Once made, then the time available to put in the application and to pull across the glass plates is limited since the binder will hydrate and the slurry will no longer be flowing liquid. For a stronger layer polyvinyl alcohol can be added to a TLC grade silica gel. After coating the plate, the plate holder is tilted allowed to remain flat. Allow the plate to air dry overnight the next day, the plate can be activated if desired to remove more of the adsorbed water. The plate is then allowed activated for 30 minute at 110°C just before use.

II Silica gel and alumina are among the most common stationary phases but others are available as well. Glass plates are chemically [illegible] and reactive stains

Contd.

Silica gel can be exclusively used for amino acid and hydrocarbons. It is also important to note that silica gel is acidic. Therefore silica gel offers poor separation of basic samples and can cause a deterioration of acid-labile molecule. This would be true for alumina plates in acidic solution as well. It is important to note that there are differences between silica gel and alumina. Alumina is basic and will not separate sample dye as large as silica gel would at a given layer thickness. Also alumina is more reactive chemically than silica gel. These care would avoid decomposition and rearrangement of the sample.

III Proper solvent selection is perhaps the most important aspect of TLC and determining the best solvent may require a degree of trial and error. As with plate selection, keep in mind the chemical properties of the analytes. A common starting solvent is 1:1 hexane: ethyl acetate. Varying the ratio can have a pronounced effect of Rf. Rf value range from 0 to 1 with 0 indicating the solvent polarity is very low and 1 indicating that the solvent polarity is very high. When performing experiments you do not want your value to be 1 or 0 because components that is to be separated have different polarity. If the value is 0 it is needs to increase your solvent polarity If the value is 1 you need to decrease solvent polarity.

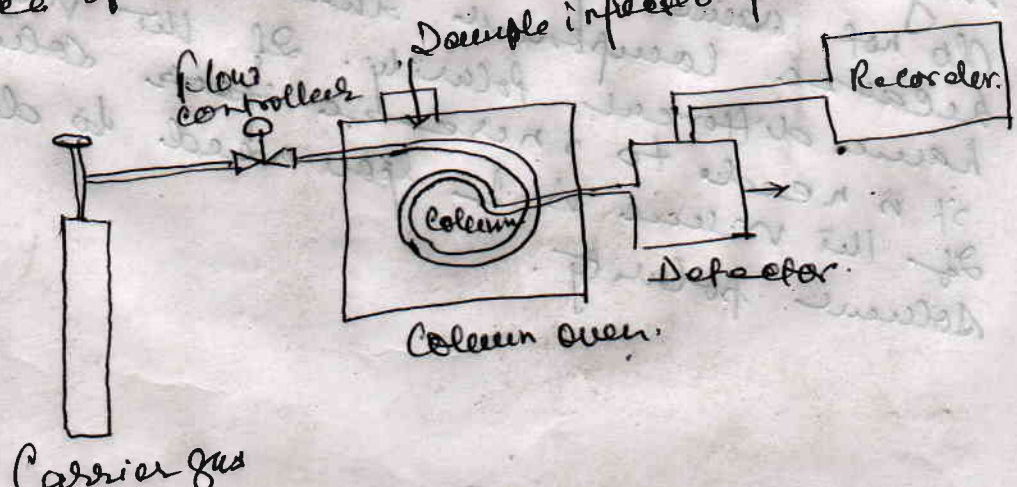
IV. Activation of the plate

Before loading the sample on the TLC plate the plate needs to be activated. Activation means to heat it and remove all the moisture. As the stationary phase is an excellent adsorbent, it absorbs moisture from surrounding that hinders the Rf value. The activation is carried out by placing the plate in a hot air oven for 1 hr at 111°C temperature.

Sample application can be done using a fine capillary. Very less amount of sample is loaded. A large sample may lead to diffusion of sample and streak bands are formed. On the plate while spotting the sample. Apart from the sample size, the concentration is also important factor. Same needs to be diluted for a distinct spot or concentrated again leads to the formation of long streak bands

Q. 5.

Gas chromatography specifically gas liquid chromatography - involves a sample being vaporised and injected onto the head of the chromatographic column. The sample is transported through the column by the flow of inert gaseous & mobile phase. The column itself contains a liquid stationary phase which is adsorbed onto the surface of a inert solid.



Carrier gas :- The carrier gas must be chemically inert. Commonly used gases include nitrogen, helium, argon and Carbon dioxide. The choice of gas is often dependent upon the type of detector which is used. The carrier gas system also contains a molecular sieve to remove water and other impurities.

Sample Injection port :-

→ For optimum column efficiency, the sample should not be too large and should be introduced onto the column as a plug of vapour - slow injection of large samples causes band broadening and loss of resolution.

→ The most common injection method is where a microsyringe is used to inject sample through a rubber septum into a flash vaporiser port at the head of the column.

→ The temp of the port is usually kept about 50°C higher than BP of the least volatile component of the sample.

→ Packed column, sample size ranges from tenth of a microliter upto 20 microliters.

→ Capillary column need much less sample typically around 10^{-3} μl.

Column :- There are two general types of column, packed and capillary. Packed column contains a finely divided inert solid support material (commonly used on diatomaceous earth) coated with liquid stationary phase. Most packed column are 1.5 - 10 meter in length and have an internal diameter of 2 - 4 mm.

Capillary column have an internal diameter of a few tenth of a millimeter. There are two types wall coated

(16)

Column temperature :-

for precise work the column temperature must be controlled to within tenths of a degree. The optimum column temperature is dependent upon the BP of the sample. As a rule of thumb, a temperature slightly above the average BP of the sample results in an elution time of 2-30 minutes. Minimal temp. gives good resolution but increases elution time. If a sample has a wide boiling range, then temperature programming can be useful. The column temp is increased (either continuously or in steps) as separation proceeds.

Detectors :- There are many detectors which can be used in gas chromatography. Different detectors will give different types of selectivity. A non-selective detector responds to all compound except the carrier gas, a selective detector responds to a range of compounds with common physical or chemical property and a specific detector responds to a single chemical compound.

Detectors can also be grouped into concentration dependent detectors and mass flow dependent detectors. The signal from a concentration dependent detector is related to the concentration of solute in the detector. Mass flow dependent detectors usually destroy the sample and the signal is related to the rate at which solute molecules enter the detectors.

Working :- A very simple overview of what happens in the gas chromatography process is as follows

→ The eluent is introduced from a gas cylinder outside the machine. It is called the carrier because that is exactly what it does - carry the sample being studied through the machine.

→ ~~The flow in GC~~ The carrier gas is the mobile phase. The flow rate of the carrier gas is carefully controlled to give clearest separation of the components in the sample

→ The carrier enters the machine through an inlet port which

→ The gas that make up the sample separate out as they move along the column which contains the stationary phase. The column is a very thin tube, sometimes as much as 30-60 m coiled and entirely contained inside an oven that keeps it a high enough temperature to ensure that the sample remains in gas form. The temp. of the oven can be carefully controlled.

→ As the sample separates out and its constituent gases travel along the column at different speeds, a detector senses and record them. Various different detectors can be used including flame ionisation detectors, thermal conductivity detectors and mass spectrometers.

→ The data analyzer/recorder attached to the machine draws a chromatogram (chart) which peaks corresponding to the relative amounts of the different chemicals in the sample.

Q. 6.

→ High performance liquid chromatography is a widely used analytical technique that many industries and research fields rely heavily on.

→ It is used to quantify, identify and isolate the components of non-volatile liquid mixture. Solvent is pumped from a solvent reservoir and mixed with the liquid sample. The solvent sample mixture passes through HPLC column and into a detector, where an electronic output is given as a chromatograph. The waste collects in a vessel outside the machine.

→ There are 3 main categories of HPLC, normal phase HPLC, reverse phase HPLC and Ultra-HPLC (UHPLC)

Schematic instrumentation of HPLC

Solvent reservoir:— Mobile phase contents are contained in a glass reservoir. The mobile phase, or solvent in HPLC is usually a mixture of polar and non polar liquid components whose respective concentration are varied depending the composition of the sample.

Pumps:— A pump aspirate the mobile phase from the solvent reservoir and force it with through the system column and detector. Depending on a number of factors including column dimension, particle size, of the stationary phase, the flow rate and composition of the mobile phase, operating pressures of upto 42000 KPA about can be generated.

Sample injector:- The injector can be a single injection or an automated injection system. An injector for a HPLC should

Column:- They are usually made of polished stainless steel are between 50 and 300 mm long and have internal diameter of between 2 and 5 mm.

Detector:- The HPLC detector located at the end of column detect the analytes as they elute from the chromatographic column.

The essential features of HPLC instrumentation are the following.

1) A solvent delivery system
2) Sample injection system
3) A chromatographic column
4) A detector

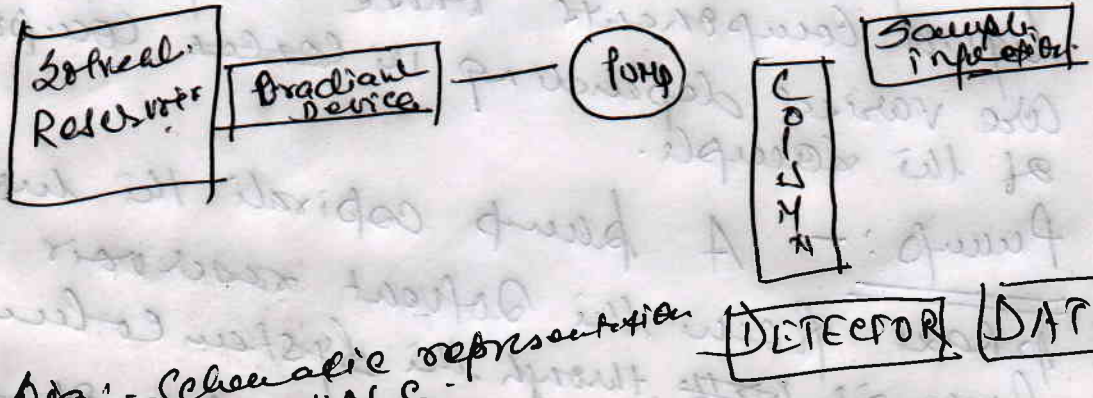


Fig:- Schematic representation of HPLC

Operation:-

1) The sample mixture to be separated and analyzed is introduced in a discrete small volume into the stream of mobile phase.
2) The components of the sample move through the column at different velocities - which depends upon chemical nature or nature of different types of column etc.
3) Many

4) The use of smaller particle size packing materials requires the use of higher operational pressure and typically improves chromatographic resolution of sorbent particles may be hydrophobic or polar in nature.

5) Common mobile phase used include any miscible combination of water with various organic solvent. Some techniques include water free mobile phase.

The choice of mobile phase components additive and gradient condition depends on nature of the column and sample components. Often a series of trial runs performed with the sample in order to find the HPLC method which gives adequate separation.