PES's

MODERN COLLEGE OF PHARMACY (FOR LADIES), MOSHI, PUNE-412105

Class- Third Yr. B. Pharm *Subject-Pharmaceutical Analysis IV*

Subject Incharge-Mr.P.R.Jadhav *Lecture Synopsis No.1*

Name of topic/lesson – Electrophoreis

Point-Theory

- *Electrophoresis is a method whereby charged molecules in solution, chiefly proteins and nucleic acids, migrate in response to an electrical field.*
- *Their rate of migration through the electrical field, depends on the strength of the field, on the net charge, size, and shape of the molecules, and also on the ionic strength, viscosity, and temperature of the medium in which the molecules are moving.*
- *As an analytical tool, electrophoresis is simple, rapid and highly sensitive.*
- *It can be used analytically to study the properties of a single charged species or mixtures of molecules. It can also be used preparatively as a separating technique*
- *Electrophoresis is usually done with gels formed in tubes, slabs, or on a flat bed.*
- *In many electrophoresis units, the gel is mounted between two buffer chambers containing separate electrodes, so that the only electrical connection between the two chambers is through the gel.*

References:. 1. Principles of Instrumental Analysis by Skoog, 5th edition,p.no.946

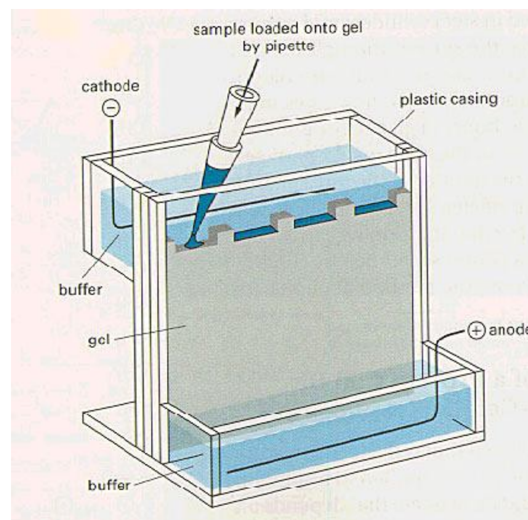
Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis IV***

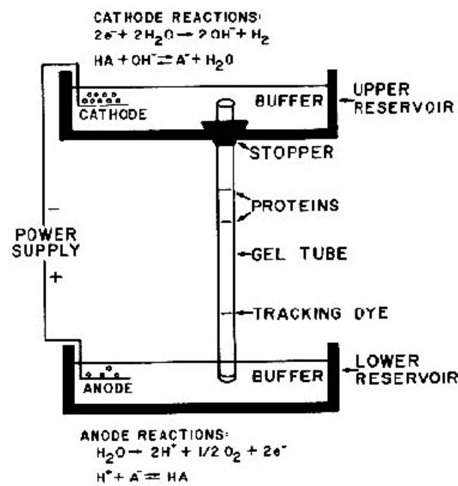
Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.2***

Name of topic/lesson – Electrophoreis

Point-Instrmentation

In most electrophoresis units, the gel is mounted between two buffer chambers containing separate electrodes so that the only electrical connection between the two chambers is through the gel.





Reference: *1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no.948*

PES's
MODERN COLLEGE OF PHARMACY (FOR LADIES), MOSHI, PUNE-412105

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis IV***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.3***

Name of topic/lesson – Electrophoreis

Point-Principle

Two basic electrical equations are important in electrophoresis

- ***The first is Ohm's Law, I = E/R***
- ***The second is P = EI***
- ***This can also be expressed as P = I^2R***

In electrophoresis, one electrical parameter, either current, voltage, or power, is always held constant

Under constant current conditions (velocity is directly proportional to current), the velocity of the molecules is maintained, but heat is generated.

Under constant voltage conditions, the velocity slows, but no additional heat is generated during the course of the run

Under constant power conditions, the velocity slows but heating is kept constant

Proteins are amphoteric compounds, that is, they contain both acidic and basic residues

Each protein has its own characteristic charge properties depending on the number and kinds of amino acids carrying amino or carboxyl groups

Nucleic acids, unlike proteins, are not amphoteric. They remain negative at any pH used for electrophoresis

References: *1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no.946*

Name of topic/lesson – *Chromatography:*

Introduction: ***Chromatography*** *(from Greek word chroma, colour) is the collective term for a family of laboratory techniques for the separation of mixtures. It involves passing a mixture which contains the analyte through a Stationary Phase, which separates it from other molecules in the mixture and allows it to be isolated.*

History:

It was the Russian botanist Mikhail Semyonovich Tiswet who invented the first chromatography technique in 1901 during his research on chlorophyll. He used a liquid-adsorption column containing calcium carbonate to separate plant pigments. The method was described on December 30 1901 at the XI Congress of Naturalists and Doctors in St. Petersburg. The first printed description was in 1903, in the Proceedings of the Warsaw Society of Naturalists, section of biology. He first used the term chromatography in print in 1906 in his two papers about chlorophyll in the German botanical journal, Berichte der Deutschen Botanischen Gesellschaft.
Chromatography terms

- *The analyte is the substance which is to be purified or isolated during chromatography*
- ***Analytical chromatography*** *is used to determine the identity and concentration of molecules in a mixture*
- *A chromatogram is the visual output of the chromatograph. Different peaks or patterns on the chromatogram correspond to different components of the separated mixture*
- *A chromatograph takes a chemical mixture carried by liquid or gas and separates it into its component parts as a result of differential distributions of the solutes as they flow around or over the stationary phase*
- *The mobile phase is the analyte and solvent mixture which travels through the stationary phase*
- *Preparative Chromatography is used to purify larger quantities of a substance*
- *The retention time is the characteristic time it takes for a particular molecule to pass through the system*

References: 1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no.836

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.566

Name of topic/lesson – *Chromatography:*

Introduction

Defination:

-A technique for identifying the components of chemical mixtures separated by preferential adsorption on an adsorbent medium, as a column of silica, a strip of filter paper, or a gel.

-A technique used to separate the components of a chemical mixture by moving the mixture along a stationary material, such as gelatin. Different components of the mixture are caught by the material at different rates and form isolated bands that can then be analyzed.

The stationary phase is the substance which is fixed in place for the chromatography procedure and is the phase to which solvents and the analyte travels through or binds to. Examples include the silica plate in thin layer chromatography.

TYPE	STATIONARY PHASE	MOBILE PHASE
paper	solid (filter paper)	liquid
thin layer (tlc)	solid (silica)	liquid
column	solid (silica)	liquid
high pressure liquid (hplc)	solid (silica)	liquid
gas liquid (glc)	solid or liquid	gas

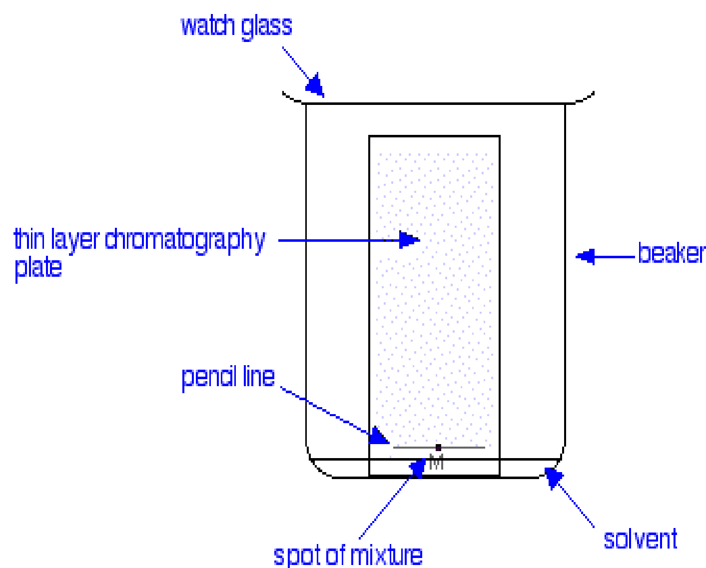
References: 1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no.836

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.566

3. Practical Pharmaceutical chemistry by beckett & stenlake, 4th edition, Part two,P.No. 85

Name of topic/lesson – *Chromatography:*

Theory: Thin Layer Chromatography



Chromatographic separations take advantage of the fact that different substances are partitioned differently between two phases, a mobile phase and a stationary phase. You have already had some experience with gas chromatography where the mobile phase is an inert gas, usually helium, and the stationary phase is a high boiling liquid coating absorbed on the surface of a granular solid in a column. In thin layer chromatography, or TLC, the mobile phase is a liquid and the stationary phase is a solid absorbent.

References: *1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no.836*

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.566

Name of topic/lesson – *Chromatography:*

Theory: Thin Layer Chromatography

In thin layer chromatography, a solid phase, the adsorbent, is coated onto a solid support as a thin layer (about 0.25 mm thick). In many cases, a small amount of a binder such as plaster of Paris is mixed with the absorbent to facilitate the coating. Many different solid supports are employed, including thin sheets of glass, plastic, and aluminum. The mixture (A plus B) to be separated is dissolved in a solvent and the resulting solution is spotted onto the thin layer plate near the bottom. A solvent, or mixture of solvents, called the eluant, is allowed to flow up the plate by capillary action. At all times, the solid will adsorb a certain fraction of each component of the mixture and the remainder will be in solution. Any one molecule will spend part of the time sitting still on the adsorbent with the remainder moving up the plate with the solvent. A substance that is strongly adsorbed (say, A) will have a greater fraction of its molecules adsorbed at any one time, and thus any one molecule of A will spend more time sitting still and less time moving. In contrast, a weakly adsorbed substance (B) will have a smaller fraction of its molecules adsorbed at any one time, and hence any one molecule of B will spend less time sitting and more time moving. Thus, the more weakly a substance is adsorbed, the farther up the plate it will move. The more strongly a substance is adsorbed, the closer it will stays near the origin. Several factors determine the efficiency of a chromatographic separation. The adsorbent should show a maximum of selectivity toward the substances being separated so that the differences in rate of elution will be large. For the separation of any given mixture, some adsorbents may be too strongly adsorbing or too weakly adsorbing. Table 1 lists a number of adsorbents in order of adsorptive power.

References*: 1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no.837*

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.599

3. Practical Pharmaceutical chemistry by beckett & stenlake, 4th edition, Part two,P.No. 86

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis IV***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.8***

Name of topic/lesson – *Chromatography:*

Chromatographic adsorbents:. it depends upon the substance being adsorbed, and the solvent used for elution.

Most Strongly Adsorbent Alumina Al2

Charcoal C

Florisil MgO/SiO2 (anhydrous)

Least Strongly Adsorbent Silica gel SiO2

The eluting solvent should also show a maximum of selectivity in its ability to dissolve or desorb the substances being separated. The fact that one substance is relatively soluble in a solvent can result in its being eluted faster than another substance. However, a more important property of the solvent is its ability to be itself adsorbed on the adsorbent. If the solvent is more strongly adsorbed than the substances being separated, it can take their place on the adsorbent and all the substances will flow together. If the solvent is less strongly adsorbed than any of the components of the mixture, its contribution to different rates of elution will be only through its difference in solvent power toward them. If, however, it is more, strongly adsorbed than some components of the mixture and less strongly than others, it will greatly speed the elution of those substances that it can replace on the absorbent, without speeding the elution of the others.

number of common solvents *in approximate order of increasing adsorbability, and hence in order of increasing eluting power. The order is only approximate since it depends upon the nature of the adsorbent. Mixtures of solvents can be used, and, since increasing eluting power results mostly from preferential adsorbtion of the solvent, addition of only a little (0.5-2%, by volume) of a more strongly adsorbed solvent will result in a large increase in the eluting power. Because water is among the most strongly adsorbed solvents, the presence of a little water in a solvent can greatly increase its eluting power. For this reason, solvents to be used in chromatography should be quite dry. The particular combination of adsorbent and eluting solvent that will result in the acceptable separation of a particular mixture can be determined only by trial.*

References: 1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no.836

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.566

3. Practical Pharmaceutical chemistry by beckett & stenlake, 4th edition, Part two,P.No. 85

Class- Third Yr. B. Pharm **Subject-Pharmaceutical Analysis IV**

Subject Incharge-Mr.P.R.Jadhav **Lecture Synopsis No.9**

Name of topic/lesson – *Chromatography:*

Eluting solvents for chromatography

Least Eluting Power (alumina as adsorbent) Petroleum ether (hexane; pentane)

Cyclohexane ,Carbon tetrachloride ,Benzene ,Dichloromethane , Chloroform

Ether (anhydrous) , Ethyl acetate (anhydrous) ,Acetone (anhydrous) , Ethanol , Methanol

Water , Pyridine

Greatest Eluting Power (alumina as adsorbent) Organic acids

If the substances in the mixture differ greatly in adsorbability, it will be much easier to separate them. Often, when this is so, a succession of solvents of increasing eluting power is used. One substance may be eluted easily while the other stays at the top of the column, and then the other can be eluted with a solvent of greater eluting power. Table 3 indicates an approximate order of adsorbability by functional group.

Adsorbability of organic compounds by functional group

Least Strongly Adsorbed Saturated hydrocarbons; alkyl halides

Unsaturated hydrocarbons; alkenyl halides , Aromatic hydrocarbons; aryl halides ,Polyhalogenated hydrocarbons , Ethers ,Esters ,Aldehydes and ketones , Alcohols

Most Strongly Adsorbed Acids and bases (amines)

References*: 1.Principles of Instrumental Analysis by Skoog, 5th edition,p.no.838*

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.601

3. Practical Pharmaceutical chemistry by beckett & stenlake, 4th edition, Part two,P.No. 115

Class- Third Yr. B. Pharm **Subject-Pharmaceutical Analysis IV**

Subject Incharge-Mr.P.R.Jadhav **Lecture Synopsis No.10**

Name of topic/lesson – Chromatography:

Technique of Thin-layer Chromatography

The sample is applied to the layer of adsorbent, near one edge, as a small spot of a solution. After the solvent has evaporated, the adsorbent-coated sheet is propped more or less vertically in a closed container, with the edge to which the spot was applied down. The spot on the thin layer plate must be positioned above the level of the solvent in the container If it is below the level of the solvent, the spot will be washed off the plate into the developing solvent. The solvent, which is in the bottom of the container, creeps up the layer of adsorbent, passes over the spot, and, as it continues up, effects a separation of the materials in the spot ("develops" the chromatogram). When the solvent front has nearly reached nearly the top of the adsorbent, the thin layer plate is removed from the container

***Thin layer chromatography** (TLC) is a chromatography technique used to separate non-volatile mixtures.[1] Thin layer chromatography is performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel,aluminium oxide, or cellulose. This layer of adsorbent is known as the stationary phase.*

After the sample has been applied on the plate, a solvent or solvent mixture (known as themobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved.

***References**: 1.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.600*

2. Practical Pharmaceutical chemistry by beckett & stenlake, 4th edition, Part two,P.No. 115

Name of topic/lesson – Chromatography:

Procedure of TLC

1 Plate preparation

2 Techniques

2.1 Separation Process and Principle

3 Analyses

4 Applications

1.Thin layer chromatography (TLC) is used frequently to visualize components of a mixture. The most common TLC plate is typically a rectangular piece of glass (2.5 cm x 7.5 cm) coated with silica powder. Silica (SiO2) is a solid with an extended structure of tetrahedral silica atoms bridged together by bent oxygen atoms. On the surface of the silica particles, the solid terminates in very polar silanol (Si-O-H) groups. The silica is the stationary phase because it remains adhered to the glass plate and does not move during the chromatography process.

- *The thin layer chromatography plates are commercial pre-prepared ones with a silica gel layer on a glass,plastic, or aluminum backing. Use the wide plates for spotting several compounds on the same plate. This allows for more precise comparison of the behavior of the compounds.*

- *The samples are spotted on the thin layer plates using fine capillaries drawn from melting point capillaries. You will need to draw several spotters. Your teaching assistant will demonstrate the technique of drawing capillaries.*

- *Samples for spotting are prepared by dissolving approximately 0.1 g (the amount on the tip of a spatula) of the compound in less than 0.5 mL of a solvent (ethyl acetate, dichloromethane, or ether work well).*

***References**: 1.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.602*

2. Practical Pharmaceutical chemistry by beckett & stenlake, 4th edition, Part two,P.No. 117

***Name of topic/lesson* – Chromatography**:

Procedure of TLC

- *When spotting samples on the TLC plates, it is a good idea to check if enough sample has been spotted on the plate. Allow the solvent to evaporate and then place the plate under a short wavelength ultraviolet lamp. A purple spot on a background of green should be clearly visible. If the spot is faint or no spot is apparent, more sample will have to be applied to the plate.*

- *The chromatograms are developed in a 150-mL beaker or jar containing the developing solvent. The beaker is covered with a small watch glass. A wick made from a folded strip of filter paper is used to keep the atmosphere in the beaker saturated with solvent vapor.*

- *When the plates are removed from the developing solvent, the position of the solvent front is marked, and the solvent is allowed to evaporate. The positions of the spots are determined by placing the plates under a short wavelength ultraviolet lamp. The silica gel is mixed with an inorganic phosphor which fluoresces green in the UV light. Where there are compounds on the plates, the fluorescence is quenched and a dark purple spot appears.*

Application of the Sample

References: *1.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.604*

2. Practical Pharmaceutical chemistry by beckett & stenlake, 4th edition, Part two,P.No. 117

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis IV***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.13***

Name of topic/lesson – Chromatography:

Procedure of TLC: Application of sample:

The sample to be separated is generally applied as a small spot (1 to 2 mm diameters) of solution about 1 cm from the end of the plate opposite the handle. The addition may be made with a micropipet prepared by heating and drawing out a melting point capillary. As small a sample as possible should be used, since this will minimize tailing and overlap of spots; the lower limit is the ability to visualize the spots in the developed chromatogram. If the sample solution is very dilute, make several small applications in the same place, allowing the solvent to evaporate between additions. Do not disturb the adsorbent when you make the spots, since this will result in an uneven flow of the solvent. The starting position can be indicated by making a small mark near the edge of the plate.

Development of thin layer plates: *The chamber used for development of the chromatogram (Figure 26) can be as simple as a beaker covered with a watch glass, or a cork-stoppered bottle. The developing solvent (an acceptable solvent or mixture of solvents must be determined by trial) is poured into the container to a depth of a few millimeters. The spotted plate is then placed in the container, spotted end down; the solvent level must be below the spots (see figure below). The solvent will then slowly rise in the adsorbent by capillary action.In order to get reproducible results, the atmosphere in the development chamber must be saturated with the solvent. This can be accomplished by sloshing the solvent around in the container before any plates have been added. The atmosphere in the chamber is then kept saturated by keeping the container closed all the time except for the brief moment during which a plate is added or removed.*

Visualization*: When the solvent front has moved to within about 1 cm of the top end of the adsorbent (after 15 to 45 minutes), the plate should be removed from the developing chamber, the position of the solvent front marked, and the solvent allowed to evaporate. If the components of the sample are colored, they can be observed directly. If not, they can sometimes be visualized by shining ultraviolet light on the plate or by allowing the plate to stand for a few minutes in a closed container in which the atmosphere is saturated with iodine vapor. Sometimes the spots can be visualized by spraying the plate with a reagent that will react with one or more of the components of the sample. As the chemicals being separated may be colorless, several methods exist to visualize the spots.*

References*: 1.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.600*

2. Practical Pharmaceutical chemistry by beckett & stenlake, 4th edition, Part two,P.No. 119

Name of topic/lesson – Chromatography:

Principle of TLC

Different compounds in the sample mixture travel at different rates due to the differences in their attraction to the stationary phase, and because of differences in solubility in the solvent.[6] By changing the solvent, or perhaps using a mixture, the separation of components (measured by the R_f value) can be adjusted. Also, the separation achieved with a TLC plate can be used to estimate the separation of a flash chromatography column

Because the distance traveled by a substance relative to the distance traveled by the solvent front depends upon the molecular structure of the substance, TLC can be used to identify substances as well as to separate them. The relationship between the distance traveled by the solvent front and the substance is usually expressed as the Rf value:

Rf value = distance traveled by substance/distance traveled by solvent front

The Rf values are strongly dependent upon the nature of the adsorbent and solvent. Therefore, experimental Rf values and literature values do not often agree very well. In order to determine whether an unknown substance is the same as a substance of known structure, it is necessary to run the two substances side by side in the same chromatogram, preferably at the same concentration.

Applications:

In organic chemistry, reactions are qualitatively monitored with TLC. Spots sampled with a capillary tube are placed on the plate: a spot of starting material, a spot from the reaction mixture, and a cross-spot with both. A small (3 by 7 cm) TLC plate takes a couple of minutes to run. The analysis is qualitative, and it will show if the starting material has disappeared, i.e. the reaction is complete, if any product has appeared, and how many products are generated (although this might be underestimated due to co-elution).

References: *1. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.613*

2. Practical Pharmaceutical chemistry by beckett & stenlake, 4th edition, Part two,P.No. 115

Class- Third Yr. B. Pharm **Subject-Pharmaceutical Analysis IV**

Subject Incharge-Mr.P.R.Jadhav **Lecture Synopsis No.15**

Name of topic/lesson – Chromatography

Paper chromatography

Introduction

Paper chromatography is one method for testing the purity of compounds and identifying substances. Paper chromatography is a useful technique because it is relatively quick and requires small quantities of material.Separations in paper chromatography involve the same principles as those in thin layer chromatography. In paper chromatography, like thin layer chromatography, substances are distributed between a stationary phase and a mobile phase. The stationary phase is usually a piece of high quality filter paper. The mobile phase is a developing solution that travels up the stationary phase, carrying the samples with it. Components of the sample will separate readily according to how strongly they adsorb on the stationary phase versus how readily they dissolve in the mobile phase.

Chromatography is a technique that is used to separate and to identify components of a mixture. This analytical technique has a wide range of applications in the real world since many substances are mixtures of chemical compounds. In this lab, you will explore two applications of chromatography - identification of an unknown ink sample and the separation of food colorings.

References: 1.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.588

2. Practical Pharmaceutical chemistry by beckett & stenlake, 4th edition, Part two,P.No. 106

Class- Third Yr. B. Pharm *Subject-Pharmaceutical Analysis IV*

Subject Incharge-Mr.P.R.Jadhav *Lecture Synopsis No.16*

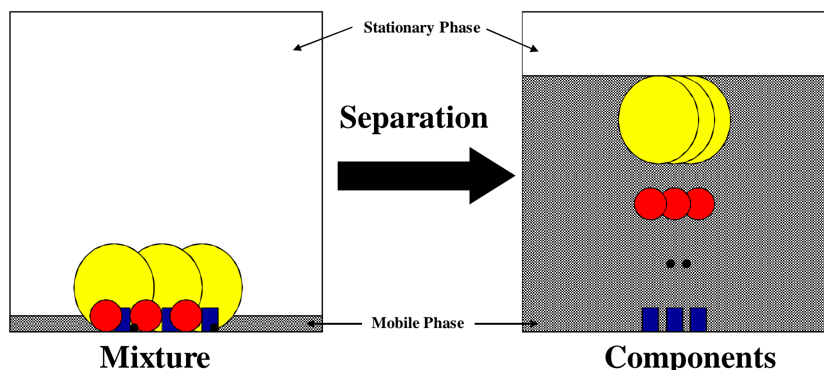
Name of topic/lesson – Chromatography

Principle

- *Capillary Action – the movement of liquid within the spaces of a porous material due to the forces of adhesion, cohesion, and surface tension. The liquid is able to move up the filter paper because its attraction to itself is stronger than the force of gravity.*
- *Solubility – the degree to which a material (solute) dissolves into a solvent. Solutes dissolve into solvents that have similar properties. (Like dissolves like) This allows different solutes to be separated by different combinations of solvents.*

Separation of components depends on both their solubility in the mobile phase and their differential affinity to the mobile phase and the stationary phase.

Illustration of Chromatography



Components	Affinity to Stationary Phase	Affinity to Mobile Phase
Blue	----------------	Insoluble in Mobile Phase
Black	✓✓✓✓✓✓	✓✓
Red	✓✓	✓✓✓✓✓
Yellow	✓	✓✓✓✓✓✓✓✓✓✓✓

***References**: 1. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.588*

2. Practical Pharmaceutical chemistry by beckett & stenlake, 4th edition, Part two,P.No. 106

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis IV***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.17***

Name of topic/lesson – Chromatography

Procedure

In paper chromatography, the sample mixture is applied to a piece of filter paper, the edge of the paper is immersed in a solvent, and the solvent moves up the paper by capillary action. Components of the mixture are carried along with the solvent up the paper to varying degrees, depending on the compound's preference to be adsorbed onto the paper versus being carried along with the solvent. The paper is composed of cellulose to which polar water molecules are adsorbed, while the solvent is less polar, usually consisting of a mixture of water and an organic liquid. The paper is called the stationary phase while the solvent is referred to as the mobile phase. Performing a chromatographic experiment is basically a three-step process: 1) application of the sample, 2) "developing" the chromatogram by allowing the mobile phase to move up the paper, and 3) calculating Rf values and making conclusions.

In order to obtain a measure of the extent of movement of a component in a paper chromatography experiment, we can calculate an "Rf value" for each separated component in the developed chromatogram. An Rf value is a number that is defined as:

$$Rf = \frac{\text{distance traveled by component from application point}}{\text{distance traveled by solvent from application point}}$$

The distance traveled by the spot is measured to the MIDDLE of the spot.

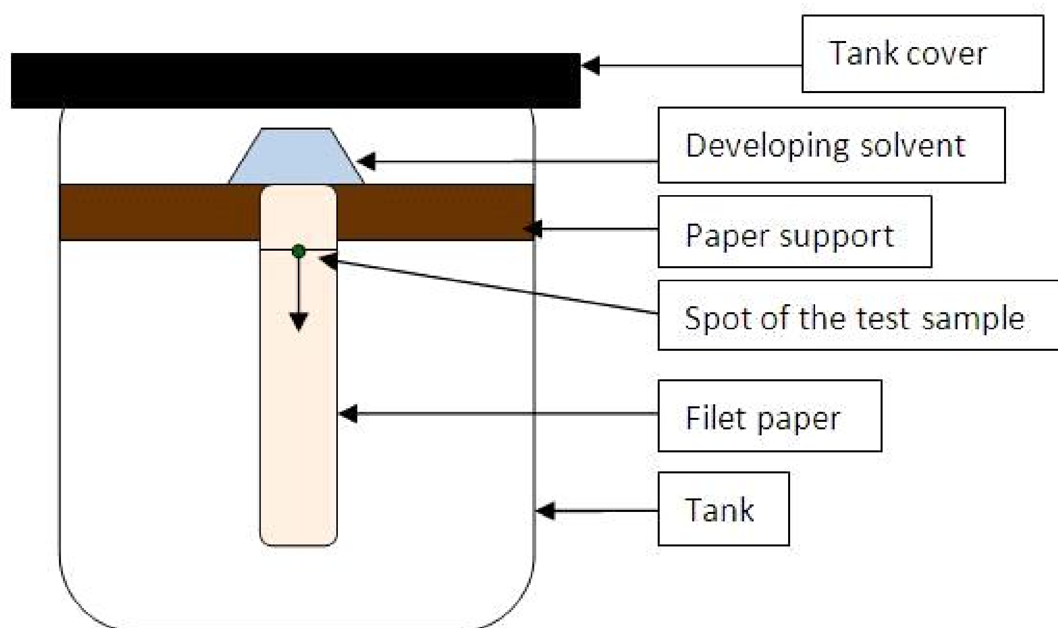
***References**: 1.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.592*

2. Practical Pharmaceutical chemistry by beckett & stenlake, 4th edition, Part two,P.No. 108

Name of topic/lesson – Chromatography

Types of Paper Chromatography

- ***Descending Paper Chromatography***-*In this type development of chromatogram is done by allowing the solvent to travel down the paper is called Descending Chromatography. Here the mobile phase is present in the upper portion.*



- ***Ascending Paper Chromatography***-*Here the solvent travel upward direction of the Chromatographic paper. Both the Descending and Ascending Paper Chromatography are used for separation of Organic and Inorganic substances.*

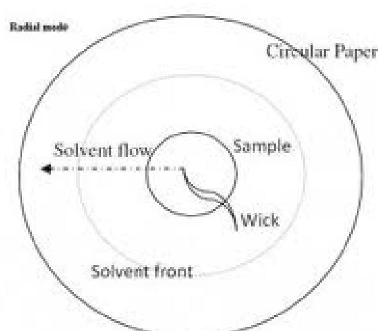
1. ***Ascending-Descending Paper Chromatography***-*It is the hybrid of both the above technique. The upper part of the Ascending chromatography can be folded over a rod and allowing the paper to become descending after crossing the rod.*

References: *1.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.588*

2. Practical Pharmaceutical chemistry by beckett & stenlake, 4th edition, Part two,P.No. 106

Name of topic/lesson – Chromatograph

4. Radial Paper Chromatography*-It is also called as Circular chromatography. Here a circular filter paper is taken and the sample is given at the center of the paper. After drying the spot the filter paper tied horizontally on a Petridish containing solvent. So that Wick of the paper is dipped inside the solvent. The solvent rises through the wick and the component get separated in form of concentrate circular zone.*



5. Two-Dimensional Paper Chromatography*-In this technique a square or rectangular paper is used. Here the sample is applied to one of the corner and development is performed at right angle to the direction of first run.*

TECHNIQUES:

AVOID EXCESSIVE HANDLING OF PAPER

1. The chromatography paper is cut in about 2.5 x 10 cm strips. Along one of the shorter sides, draw a horizontal line in pencil (lead will not move) about 1.5 cm from the edge of the strip. This will be your "base line", the starting line where the samples will be spotted.

2. Apply a dot of ink from each black felt tip pen on the baseline. Make sure dots are evenly spaced on the baseline and not too close to the edge. It may be helpful to use a pencil to mark the spots before placing the ink on the paper. See the diagram below.

3. Label each spot in pencil so that you know what each spot is.

4. Stand the paper in the eluting solution. Taping the top of the strips to the side of the beaker may be helpful.

Base Line

References*: 1.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.588*

2. Practical Pharmaceutical chemistry by beckett & stenlake, 4th edition, Part two,P.No. 106

Name of topic/lesson – Chromatography

Column chromatography in chemistry is a method used to purify individual chemical compounds from mixtures of compounds. It is often used for preparative applications on scales from micrograms up to kilograms. The main advantage of column chromatography is the relatively low cost and disposability of the stationary phase used in the process. The latter prevents cross-contamination and stationary phase degradation due to recycling.

The classical preparative chromatography column, is a glass tube with a diameter from 5 mm to 50 mm and a height of 5 cm to 1 m with a tap and some kind of a filter (a glass frit or glass wool plug – to prevent the loss of the stationary phase) at the bottom. Two methods are generally used to prepare a column: the dry method, and the wet method.

Types of column packing: 1. Dry method 2. Wet method

*1. For the **dry method**, the column is first filled with dry stationary phase powder, followed by the addition of mobile phase, which is flushed through the column until it is completely wet, and from this point is never allowed to run dry.*

*2. For the **wet method,** a slurry is prepared of the eluent with the stationary phase powder and then carefully poured into the column. Care must be taken to avoid air bubbles. A solution of the organic material is pipetted on top of the stationary phase. This layer is usually topped with a small layer of sand or with cotton or glass wool to protect the shape of the organic layer from the velocity of newly added eluent. Eluent is slowly passed through the column to advance the organic material. Often a spherical eluent reservoir or an eluent-filled and stoppered separating funnel is put on top of the column.*

***References**:*

1. *Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.646*
2. *Practical Pharmaceutical chemistry by beckett & stenlake, 4th edition, Part two,P.No. 86*
3. *Principles of Instrumental Analysis by Skoog, 5th edition,p.no .893*

Class- Third Yr. B. Pharm **Subject-Pharmaceutical Analysis IV**

Subject Incharge-Mr.P.R.Jadhav **Lecture Synopsis No.21**

Name of topic/lesson – Chromatography

HPTLC

- *Introduction*
- *Principle of HPTLC*
- *Difference between HPTLC & TLC*
- *Features of HPTLC*
- *Instrumentation*
- *Steps involved in HPTLC*
- *Rf value*
- *Factor affecting HPTLC*

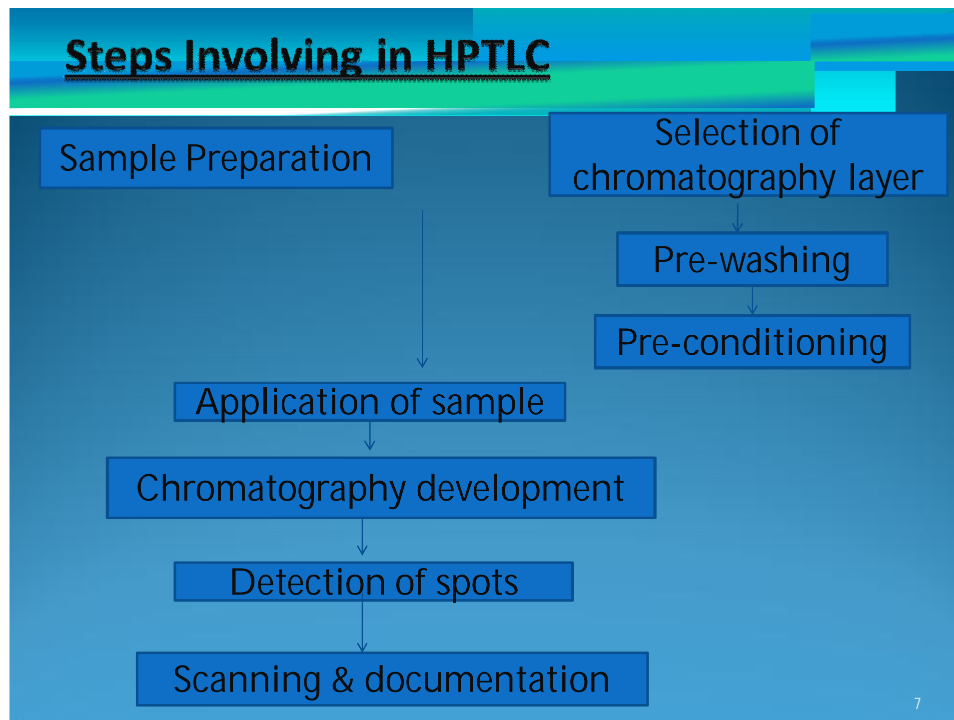
Principle

- *Same theoretical principle of TLC.*
- *Separation may result due to adsorption phenomenon.*

Reference: *1.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.615*

2.Pharmaceutical Analysis modern methods by Munson,Part Two,155-167

Name of topic/lesson – Chromatography



Factors affecting HPTLC

- *Type of stationary phase*
- *Mobile phase*
- *Layer thickness*
- *Temperature*
- *Mode of development*
- *Amount of sample*
- *Dipping zone*

Reference: *1. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,p.no.2.616*

2. Pharmaceutical Analysis modern methods by Munson,Part Two,155-167

Name of topic/lesson – Chromatography

Scanning & Documentation

- *HPTLC plates are scanned at selected UV regions WL by the instrument & the detected spots are seen on computer in the form of peaks.*
- *The scanner converts band into peaks & peak height or area is related to the concentration of the substance on the spot.*

Applications.

- *HPTLC rapidly gaining importance in several fields of science like*
- *Pharmaceuticals analysis*
- *Biochemistry*

. Pharmacokinetics studies

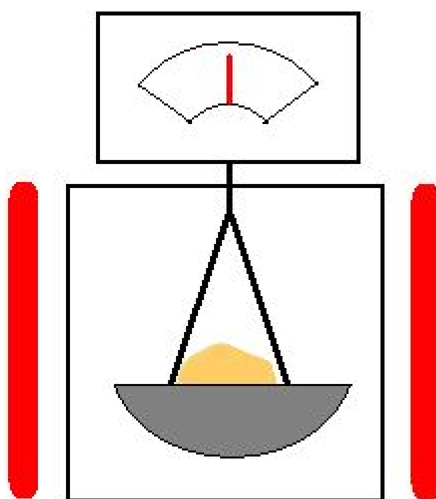
Reference: *1.Pharmaceutical Analysis modern methods by Munson,Part Two,155-167*

Name of topic/lesson – Thermal Analysis:

Thermogravimetric Analysis
(TGA):

Introduction

- *A technique measuring the variation in mass of a sample undergoing temperature scanning in a controlled atmosphere*
- *Thermobalance allows for monitoring sample weight as a function of temperature*
- *The sample hangs from the balance inside the furnace and the balance is thermally isolated from the furnace*



Reference: *1.Pharmaceutical drug analysis by Ashutosh Kar,2nd edition,P.No. 257*

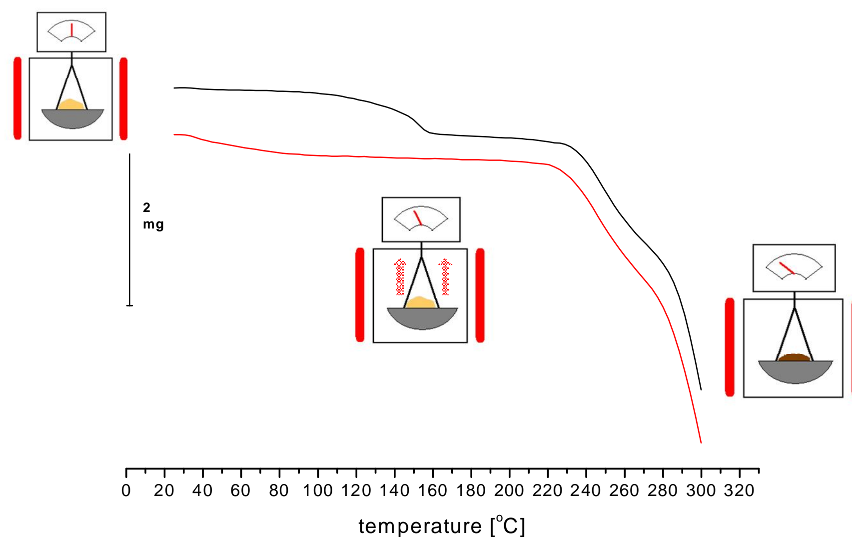
2.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,P.No. 2.702

Class- Third Yr. B. Pharm ***Subject-Pharmaceutical Analysis IV***

Subject Incharge-Mr.P.R.Jadhav ***Lecture Synopsis No.23,24***

Name of topic/lesson – *Thermal Analysis:*

Principle



- *Modern instrumentation used for thermal analysis usually consists of the following parts:*
  - *sample holder/compartment for the sample*
  - *sensors to detect/measure a property of the sample and the temperature*
  - *an enclosure within which the experimental parameters (temperature, speed, environment) may be controlled*
  - *a computer to control data collection and processing*

Reference: *1. Principles of Instrumental Analysis by Skoog, 5th edition,p.no .982*

2.Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,P.No. 2.749

Name of topic/lesson – Thermal Analysis:

Differential Scanning Calorimetry (DSC)

- *DSC measures the heat absorbed or liberated during the various transitions in the sample due to temperature treatment*
  - ***Differential:*** *sample relative to reference*
  - ***Scanning:*** *temperature is ramped*
  - ***Calorimeter:*** *measures heat*
- *DSC measurements are both **qualitative** and **quantitative** and provide information about physical and chemical changes involving:*
  - *Endothermic processes – sample absorbs energy*
  - *Exothermic processes – sample releases energy*
  - *Changes in heat capacity*
- ***Polymorphism*** *- the ability of a compound to crystallise in more than one crystal form*
- ***Pseudopolymorphic forms (solvated forms)*** *- crystalline solids containing solvent molecules as an integral part of their crystal structure*
- ***Amorphism*** *- the absence of regular or crystalline structure in a body solid; amorphous materials do not possess three-dimensional long-range molecular order*

Reference: *1. Principles of Instrumental Analysis by Skoog, 5th edition,p.no .982*

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,P.No. 2.750

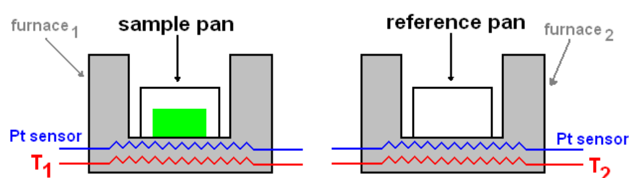
Class- Third Yr. B. Pharm **Subject-Pharmaceutical Analysis IV**

Subject Incharge-Mr.P.R.Jadhav **Lecture Synopsis No.27**

Name of topic/lesson – Thermal Analysis:

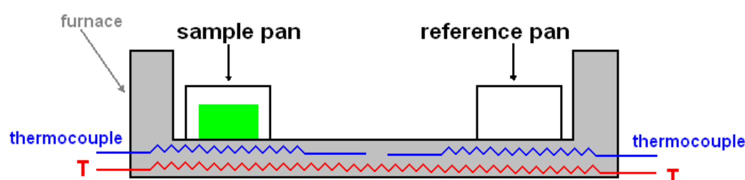
Principle of DSC-

- *Power Compensation DSC*



  - *High resolution / high sensitivity research studies*
  - *Absolute specific heat measurement*
  - *Very sensitive to contamination of sample holders*

- *Heat Flux DSC*



  - *Routine applications*
  - *Near / at line testing in harsh environments*
  - *Automated operation*
  - *Cost-sensitive laboratories*

Reference: 1. *Principles of Instrumental Analysis by Skoog, 5th edition,p.no .982*

2. *Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,P.No. 2.751*

Name of topic/lesson – Thermal Analysis:

DSC Analysis-

- ***Melting points*** – *crystalline materials*
- ***Desolvation*** – *adsorbed and bound solvents*
- ***Glass transitions*** – *amorphous materials*
- ***Heats of transitions*** – *melting, crystallisation*
- ***Purity determination*** – *contamination, crystalline/amorphous phase quantification*
- ***Polymorphic transitions*** – *polymorphs and pseudopolymorphs*
- ***Processing conditions*** – *environmental factors*
- ***Compatibility*** – *interactions between components*
- ***Decomposition kinetics*** – *chemical and thermal stability*

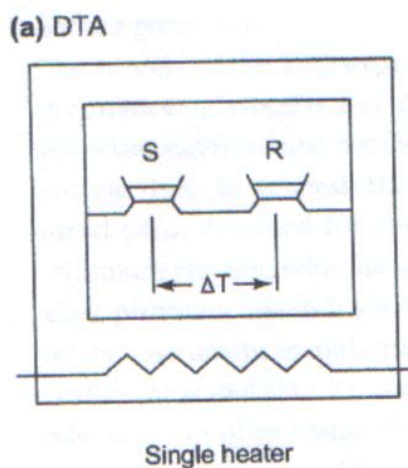
Pharma applications:

- *Enhanced analysis of polymorphism*
- *Detection of low level amorphous content*
- *Suppression of decomposition – "true" melting points*
- *Detection of low energy transitions*
- *Characterisation close to processing conditions*
- *Separation of overlapping events*

Reference: *1. Principles of Instrumental Analysis by Skoog, 5th edition,p.no .982*

2. Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,P.No. 2.750

Name of topic/lesson** – **Thermal Analysis:



difference in temperature between the sample and reference is plotted against sample temperature.

Principle:

Endothermic: *Fusion, vaporization, sublimation, desorption, reduction, decomposition, degradation. Glass transition (e.g. baseline shift).*

Exothermic: *Crystallization, condensation, solidification, adsorption, precipitation, oxidation, degradation, curing of resins.*

Applications:

- *Glass transitions,Melting and boiling points,Crystallisation time and temperature,Percent crystallinity*
- *Heats of fusion and reactions, Specific heat capacity, Oxidative/thermal stability*
- *Rate and degree of cure, Reaction kinetics, Purity*

***Reference**: 1. Principles of Instrumental Analysis by Skoog, 5th edition,p.no .982*

2. *Instrumental methods of Chemical Analysis by Chatwal,Anand, 5th edition,P.No. 2.752*

Name of topic/lesson – Radiochemical methods

Neutron source

__Neutron source__ is a neutron source used for stable and reliable initiation of nuclear chain reaction in nuclear reactors, when they are loaded with fresh nuclear fuel, whose neutron flux fromspontaneous fission is insufficient for a reliable startup, or after prolonged shutdown periods. Neutron sources ensure a constant minimal population of neutrons in the reactor core, sufficient for a smooth startup. Without them, the reactor could suffer fast power excursions during startup from state with too few self-generated neutrons (new core or after extended shutdown).

The startup sources are typically inserted in regularly spaced positions inside the reactor core, in place of some of the fuel rods.

The sources are important for safe reactor startup. The spontaneous fission and cosmic rays serve as weak neutron sources, but these are too weak for the reactor instrumentation to detect; relying on them could lead to a "blind" start, which is an unsafe condition.[1] The sources are therefore positioned so the neutron flux they produce is always detectable by the reactor monitoring instruments. When the reactor is in shutdown state, the neutron sources serve to provide signals for neutron detectors monitoring the reactor, to ensure they are operable.[2] The equilibrium level of neutron flux in a subcritical reactor is dependent on the neutron source strength; a certain minimum level of source activity therefore has to be ensured in order to maintain control over the reactor when in strongly subcritical state, namely during startups.[3]

PES's

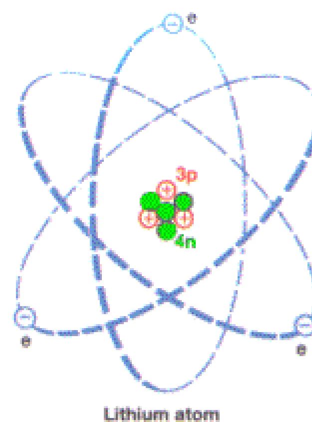
MODERN COLLEGE OF PHARMACY (FOR LADIES), MOSHI, PUNE-412105

Class- Third Yr. B. Pharm *Subject-Pharmaceutical Analysis IV*

Subject Incharge-Mr.P.R.Jadhav *Lecture Synopsis No.34*

Name of topic/lesson – Radiochemical methods

Detection & Measurement of Radioactivity



Lithium atom

All substance are made of atoms. These have electrons (e) around the outside, and a nucleus in the middle. The nucleus consists ofprotons (p) and neutrons (n), and is extremely small. (Atoms are almost entirely made of empty space!). In some types of atom, the nucleus is unstable, and will decay into a more stable atom. Thisradioactive decay is completely spontaneous. You can heat the substance up, or subject it to high pressure or strong magnetic fields - in fact, do whatever you like to it - and you won't affect the rate of decay in the slightest. When an unstable nucleus decays, there arethree ways that it can do so. It may give out:-

- *an **alpha particle** (symbol α)*
- *a **beta particle** (symbol β)*
- *a **gamma ray** (symbol γ)*

Many radioactive substances emit α particles and β particles as well as γ rays.

Alpha Particles:

*Alpha particles are made of **2 protons and 2 neutrons**. This means that they have a **charge** of **+2**, and a**mass** of **4** (the mass is measured in "atomic mass units", where each atomic mass units", where each proton & neutron=1). Alpha particles are relatively **slow** and **heavy**. They have a **low penetrating power** - you can stop them with just a sheet of **paper**. Because they have a large charge, alpha particles ionize other atoms strongly and have a range of only a few centimetres in air.*

*Alpha particles are made of 2 protons with 2 neutrons. This means that when a nucleus emits an alpha particle, it loses 2 protons and so its **atomic number decreases by 2**. Also, when a nucleus emits an alpha particle, its **atomic mass decreases by 4** (that's 2 protons plus 2 neutrons). So Americium-241 (an α -source used in smoke detectors), which has an atomic number of 95 and an atomic mass of 241 will decay to Neptunium-237 (which has an atomic number of 93 and an atomic mass of 237).*

Name of topic/lesson – Radiochemical methods

*Gamma rays are **waves, not particles**. This means that they have **no mass** and **no charge**. Gamma rays have a **high penetrating power** - it takes a thick sheet of metal such as **lead**, or **concrete** to reduce them significantly. Gamma rays do not directly ionise other atoms, although they may cause atoms to emit other particles which will then cause ionisation.*

*Gamma rays (γ) are electromagnetic waves, rather like X rays and radio waves. Thus gamma rays have **no mass** and **no charge**.*

*After a nucleus has emitted an α-particle or a β-particle, it may still have too much energy: we say it is in an "excited state". It can get rid of this energy by emitting a pulse of very high frequency electromagnetic radiation, called a gamma ray. Gamma rays do not pull electrons off atoms they pass, as α-particles and β-particles do. This means that they do not lose much energy as they travel, as they do not interact as much with the matter they pass. Therefore, gamma rays have a **high penetrating power**, and a **very long range**.*

It's worth noting that there is no such thing as a pure γ-ray source. Gamma rays are given off by most α-emitters and β-emitters. If we want a source of pure gamma rays, we can get it by using a substance that emits both β and γ, and simply keep it in an aluminium container that stops the β-particles.

Name of topic/lesson – Radiochemical methods

Measurement of Radioactivity

For measuring radioactivity, three types of devices are available:

1. *Gas-filled tube counters e.g. the Geiger Muller Counter*
2. *Scintillation Counters*
3. *Semi-conductor Detectors*

1. ***The Geiger Counter:*** *A potential difference just below that required to produce a discharge is applied to the tube (1000 V). Any atoms of the gas struck by the γ-rays entering the tube are ionized, causing a discharge. Discharges are monitored and counted by electronic circuitry and the output is reported as counts/sec or Rontgens/hr or mR/hr.*

2. ***Scintillation Counters:*** *Crystals of certain substances e.g. cesium fluoride, cadmium tungstate, anthracine and sodium iodide emit small flashes of light when bombarded by γ-rays. The most commonly used phosphor in scintillation counters is NaI with a minute quantity of thallium added. In the instrument, the crystal is positioned against a photocell which in turn is linked to a recording unit. The number of flashes produced per unit time is proportional to the intensity of radiation. Small portable scintillation counters are available.*

3. ***Semi-Conductor Detectors:*** *A semi-conductor is a substance whose electrical conductivity is between that of a metal and an insulator. It is noted that Ge(Li) semi-conductors ate excellent detectors of γ-rays with a resolution ten times higher than NaI (Th) scintillometers. The main disadvantage of these is a lower efficiency for higher energy x-rays. Besides, Ge(Li) semi-conductors need to be cooled by liquid nitrogenand are hence cumbersome and not suitable as field instruments.*

Besides the above there are instruments known as γ-ray spectrometers, which can distinguish different energy peaks and hence make it possible to identify the source of radiation.

A Geiger counter will record "counts per minute", but this doesn't tell us what the radioactive substance is actually doing, merely what is reaching the detector. It also tells us nothing about the amount of damage being done to you. Thus we need several different units in order to measure radioactivity.

Units of Measuring Radioactivity:

There are three measurement units for radioactivity: the Becquerel measures radioactivity, the Gray measures the absorbed dose and the Sievert measures the biological effects.

- *1. The becquerel (Bq) is the SI derived unit of radioactivity. One becquerel is defined as the activity of a quantity of radioactive material in which one nucleus decays per second. The activity of a source is measured in bacquerels.*

  This is a very small unit, and multiples are often used:

  1 MBq = 1 mega Becquerel = 1,000,000 Bq

  1 GBq = 1 giga Becquerel = 1,000,000,000 Bq

  1 TBq = 1 tera Becquerel = 1,000,000,000,000 Bq

  The radioactivity of an environment, a material or a foodstuff is given in Becquerels per kilogram or per liter.

- *2. The gray (Gy) is defined as the absorbed dose of radiation per unit mass of tissue. One gray is the absorption of one joule of radiation energy per kilogram of matter. The amount of radiation your cells absorb is measured in grays.*

  1 Gy = 1 joule per kilogram

  Sub-multiples are often used:

  1 mGy = 1 milligray = 0.001 Gy

  1 µGy = 1 microgray = 0.000001 Gy

  1 nGy = 1 nanogray = 0.000000001 Gy

Name of topic/lesson – Radiochemical methods

Radioisotopes in Industry

- ***Science and industry use radioisotopes in a variety of ways to improve productivity and, in some cases, to gain information that cannot be obtained in any other way.***
- ***Sealed radioactive sources are used in industrial radiography, gauging applications, and mineral analysis.***

The attributes of naturally decaying atoms, known as radioisotopes, give rise to their multiple applications across many aspects of modern day life (see also information paper on ***The Many Uses of Nuclear Technology****).*

Industrial tracers

Radioisotopes are used by manufacturers as tracers to monitor fluid flow and filtration, detect leaks, and gauge engine wear and corrosion of process equipment. Small concentrations of short-lived isotopes can be detected whilst no residues remain in the environment. By adding small amounts of radioactive substances to materials used in various processes it is possible to study the mixing and flow rates of a wide range of materials, including liquids, powders, and gases and to locate leaks.

Radiotracers are used widely in industry to investigate processes and highlight the causes of inefficiency. They are particularly useful where process optimization can bring material benefits, such as in the transport of sediments. Radiotracers are also used in the oil and gas industry to help determine the extent of oil fields.

Inspection

Radioactive materials are used to inspect metal parts and the integrity of welds across a range of industries. Industrial gamma radiography exploits the ability of various types of radiation to penetrate materials to different extents. Gamma radiography works in much the same way as X-rays screen luggage at airports. Instead of the bulky machine needed to produce X-rays, all that is needed to produce effective gamma rays is a small pellet of radioactive material in a sealed titanium capsule.

The capsule is placed on one side of the object being screened, and some photographic film is placed on the other side. The gamma rays, like X-rays, pass through the object and create an image on the film. Just as X-rays show a break in a bone, gamma rays show flaws in metal castings or welded joints. The technique allows critical components to be inspected for internal defects without damage.

X-ray sets can be used when electric power is available and the object to be scanned can be taken to the X-ray source and radiographed. Radioisotopes have the supreme advantage that they can be taken to the site when an examination is required – and no power is needed. However, they cannot be simply turned off, and so must be properly shielded both when in use and at other times.

The process of gamma radiography, a type of non-destructive testing (NDT), is used to validate the integrity of poured concrete and welds on fluid vessels, pipelines, or critical structural elements. The unique characteristics of gamma radiography have resulted in the technique becoming a crucial tool throughout many industries. For example, to inspect new oil or gas pipelines, special film is taped over the weld around the outside of the pipe. A machine called a 'pipe crawler' carries a shielded radioactive source down the inside of the pipe to the position of the weld. There, the radioactive source is remotely exposed.

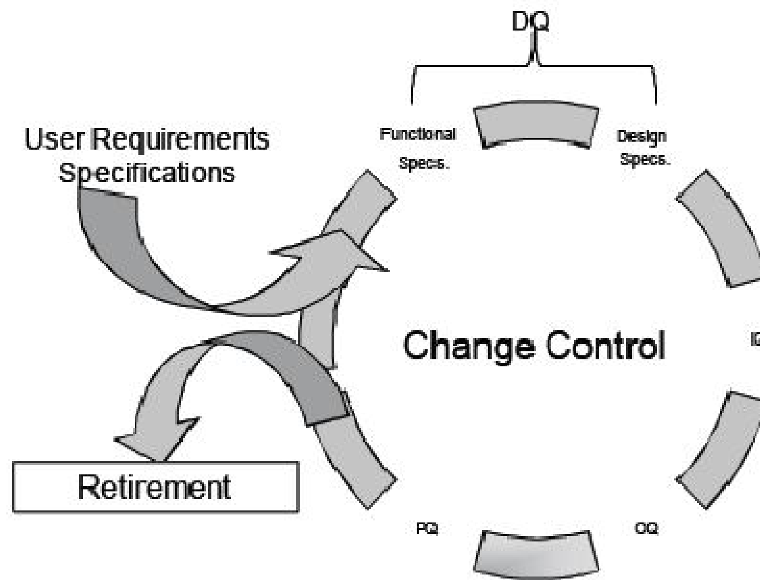
Name of topic/lesson –validation

A validation programme involves various components in pharmaceutical organisation related to process, equipment and product.
It is a regulatory requirement for pharmaceutical companies to perform Instrument Validation on all new instruments. Instrument Validation requires detailed knowledge of the instrumentation system being validated and is therefore usually
performed by the company supplying the instrument.

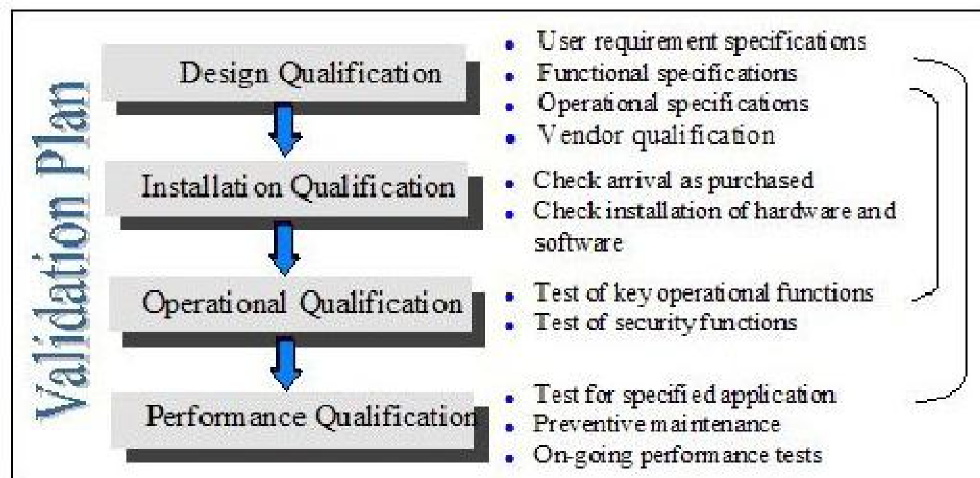
EQUIPMENT VALIDATION

*Definition (US-FDA):- Validation is the **establishment** of documentary evidence which provide a **high degree assurance** of specified **process** will consistently produce the product meeting with predetermined specification and*
quality attributes.Validation studies are performed for analytical equipment,
tests, facility systems such as air, water, steam, the manufacturing, cleaning, sterilization processes

CYCLE OF VALIDATION



VALIDATION PLAN



Name of topic/lesson –validation

PHASE-1(QUALIFICATION)

DESIGN QUALIFICATION:

"A documented review of the design, at an appropriate stage or stages in the project, for conformance to operational and regulatory expectations."

Specification of requirements for facilities, plants and equipment, mainly in connection with purchase/modification of equipment.

-User Requirement Specification (URS)Functional Specification (FS)

-Tender Specifications and Drawings

-Vendor Quality procedures and documents Purchase Specification

Phase-2

(process qualification phase)

OPERATIONAL QUALIFICATION

The documented action of demonstrating that process equipment and ancillary systems work correctly and operate consistently In accordance with established specifications.Operation Qualification (OQ) for testing the equipment in the selected user environment to ensure that it meets the previously defined functional and performance specifications.

Guidelines stress the importance of equipment qualification simulating actual production conditions, including 'worst case' situations and that "tests and challenges should be repeated a sufficient number of times to assure reliable and meaningful results." "three consecutive batches" is recommended for process validation rather than for equipment qualification. No specific number of "runs" for equipment qualification, but multiple tests to simulate actual operating ranges and to establish consistency are expected.

PERFOMANCE QUALIFICATION

Assurance that the process continues to comply with established requirements.

Performance Qualification (PQ) for testing that the system consistently performs as intended for the selected application.

Performance Qualification

Purpose*- To define testing requirements in a product/ process/performance/qualification/validation protocol.*

Name of topic/lesson –validation

Principle- *Performance qualification validation testing plan are usually process or product specific but will include the following background and result for the plan, testing method used, and a predetermined and general and/or specific acceptance criteria.*

RE-VALIDATION

Re-validation is required when the operating equipment or system has been changed in some way.

It is carried out when:

Major mechanical equipment has been replaced

Computer systems have been replaced

New products have to be run on the system.

Critical items have been replaced or repaired.

PHASE-3

(VALIDATION MAINTENANCE PHASE)

It is the maintenance of the validation which has been
already
established.

MASTER VALIDATION PROTOCOL

The Master Validation Protocol (MVP) is the outline of the
validation for a facility.

MVP :

Identifies which items are subjects to validation.

Defines the nature and extent of testing expected to be done on each
item.

Outlines the test procedures and protocols to be followed to
accomplish validation.

Describes functional responsibilities and requirements to document
the work carried out and the results obtained.

Installation Qualification

Installation qualification consists of documented verification that all key aspects of the dissolution apparatus are in working condition and have been properly installed in accordance with manufacturer's specifications in the proper operating environment.

Calibration

The installation qualification should document that specific devices contained within the dissolution apparatus (e.g., speed, time, and temperature displays)
have been calibrated to traceable standards.

INSTALLATION QUALIFICATION

Environmental

Conditions

Computerized System

Equipment Information

Preventive maintenance

SOP

Utilities

DISSOLUTION APPARATUS VALIDATION

Dissolution is defined as the process by which a known amount of drug substance goes into solution per unit of time under standardized conditions.
The primary goal of dissolution testing is to be used as a qualitative tool to provide measurements of the bioavailability of a drug as well as to demonstrate bioequivalence from batch-to-batch.
Validation is achieved by performing a series of validation activities; for a dissolution apparatus, validation is obtained through installation qualification and operational qualification.

Name of topic/lesson –XRD

X-ray diffraction (XRD) instruments

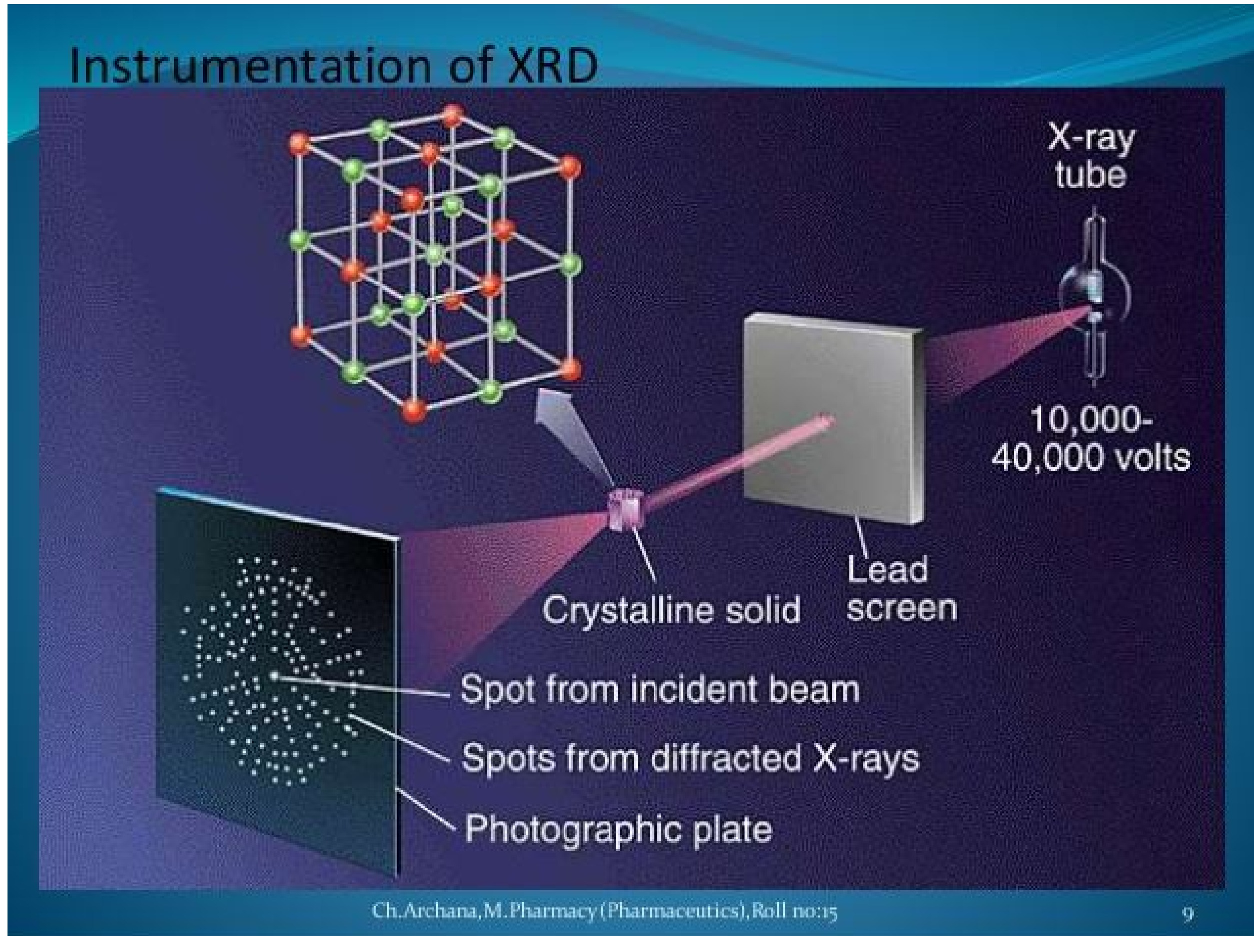
X-ray diffraction (XRD) is one of the most important non-destructive tools to analyze all kinds of matter—ranging from fluids, to powders and crystals. From research to production and engineering, XRD is an indispensable method for materials characterization and quality control. Rigaku has developed a range of diffractometers, in co-operation with academic and industrial users, which provide the most technically advanced, versatile and cost-effective diffraction solutions available today.

X-ray diffraction techniques are used for the identification of crystalline phases of various materials and the quantitative phase analysis subsequent to the identification. X-ray diffraction techniques are superior in elucidating the three-dimensional atomic structure of crystalline solids. The properties and functions of materials largely depend on the crystal structures. X-ray diffraction techniques have, therefore, been widely used as an indispensable means in materials research, development and production.

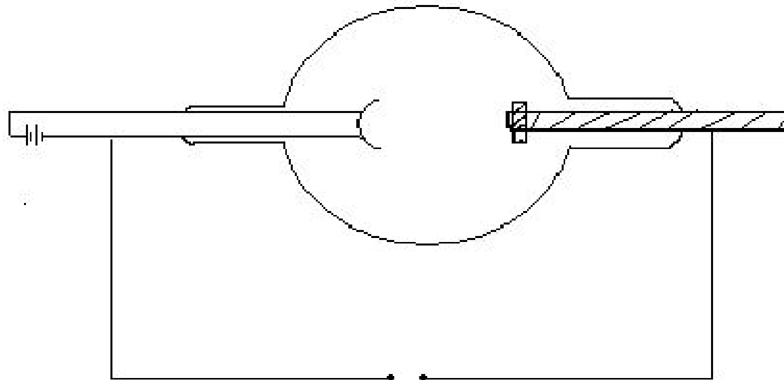
The Bragg equation, $n\lambda = 2d\sin\theta$ is one of the keystones in understanding X-ray diffraction. In this equation, n is an integer, λ is the characteristic wavelength of the X-rays impinging on the crystallize sample, d is the interplanar spacing between rows of atoms, and θ is the angle of the X-ray beam with respect to these planes. When this equation is satisfied, X-rays scattered by the atoms in the plane of a periodic structure are in phase and diffraction occurs in the direction defined by the angle θ.In the simplest instance, an X-ray diffraction experiment consists of a set of diffracted intensities and the angles at which they are observed. This diffraction pattern can be thought of as a chemical fingerprint, and chemical identification can be performed by comparing this diffraction pattern to a database of known patterns.

Name of topic/lesson –XRD

instrumentation



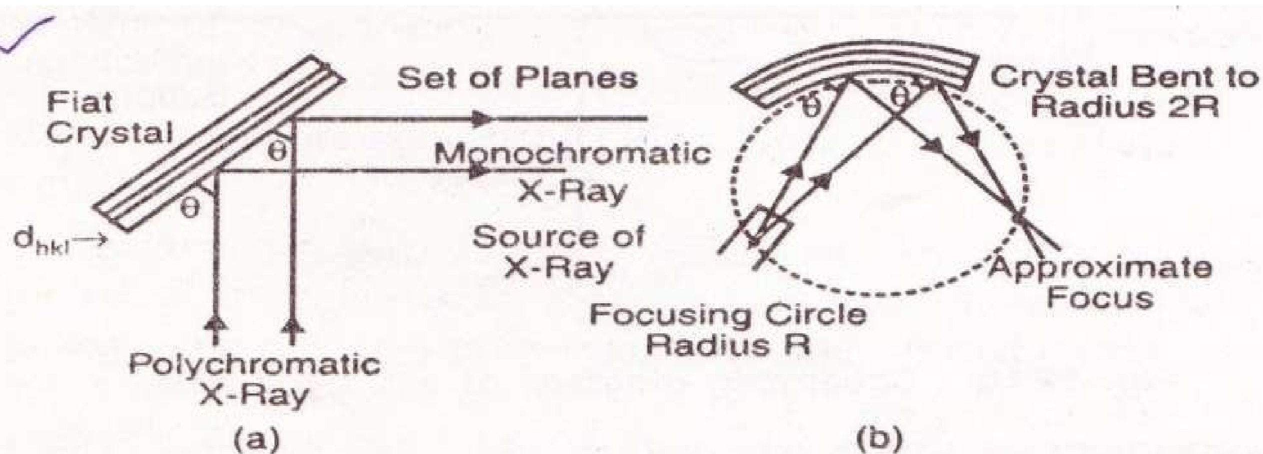
Name of topic/lesson –XRD



INSTRUMENTATION

Production ofx-rays
Collimator
Monochromator
Filter
Crystalmonochromator
Detectors
a.Photog raphic methods
b.Count er methods

Name of topic/lesson –XRD



(a) Flat crystal monochromator and, (b) Curved crystal monochromator.

Crystal monochromators is made up of suitable crystalline material positioned in the x-ray beam so that the angle of reflecting planes satisfied the Bragg's equation for the required wavelength the beam is split up into component wavelengths crystals used in monochromators are made up of materials like Nacl, lithium fluoride, quartz etc.

Name of topic/lesson –XRD

Application:

Structureofcrystals

Polymer characterisation

Stateofannealinmetals

Particle size determination

Spotcountingmethod

Broadening of diffraction lines

Low-anglescattering

methodstocomplexes

Determinationofcis- transisomerism

Determination of linkage isomerism