B.Pharm 3rd Year

Q1 **Discuss the following physicochemical factors in preformulation studies**

I. Particle size

II. Particle shape

Ans. Bulk flow, formulation homogeneity, and surface-area controlled processes such as dissolution and chemical reactivity are directly affected by size, shape and surface morphology of the drug particles. In general, each new drug candidate should be tested during preformulation with the smallest particle size as is practical to facilitate preparation of homogeneous samples and maximize the drug's surface area for interactions. Various chemical and physical properties of drug substances are affected by their particle size distribution and shapes. The effect is not only on the physical properties of solid drugs but also, in some instances, on their biopharmaceutical behavior. It is generally recognized that poorly soluble drugs showing a dissolution-rate limiting step in the absorption process will be more readily bioavailable when administered in a finely subdivided state rather than as a coarse material. In case of tablets, size and shape influence the flow and the mixing efficiency of powders and granules. Size can also be a factor in stability; fine materials are relatively more open to attack from atmospheric oxygen, the humidity, and interacting excipients than are coarse materials

Microscopy: Optical microscopy is generally used as the first tool to see and measure sizes of particles ranging in size from 0.2 microns to 100 microns.

Advantages

- Easy and convenient
- A size-frequency distribution curve can be plotted by counting the number of particles in a size range
- Can detect the presence of agglomerates and particles of more than one component

Disadvantages

- Diameter is obtained from only two dimensions - length and breadth
- No estimation of the depth (thickness) of particle is available
- The number of particles that must be counted to get a good estimate of the distribution makes the method slow and tedious

Sieving:

- This method utilizes a series of standard sieves calibrated by the National Bureau of Standards.
- Sieves are generally used for grading coarser particles.
- Sieves produced by photoetching and electroforming techniques are now available with apertures from 90 microns down to as low as 5 microns.

Method:

According to the method of the U.S. Pharmacopoeia for testing powder fineness, a definite mass of sample is placed on the proper sieve in a mechanical shaker. The powder is shaken for a definite period of time, and the material that passes through one sieve and is retained on the next finer sieve is collected and weighed.

Sedimentation:

A number of classical techniques based on sedimentation methods, utilizing devices such as the Andreasen pipette or recording balances that continuously collect a settling suspension, are known. However, these methods are now in general disfavor because of their tedious nature

Q2. **Discuss the following physicochemical factors in preformulation studies**

I. Powder flow

II. Solubility

Ans When limited amounts of drugs are available Power flow properties can be evaluated by measurements of bulk density and angle of repose. Changes in particles size and shape are generally very important an increase in crystal size or a more uniform shape will lead to a small angle or repose and a smaller Carr's index.

Bulk Density :-

Knowledge of absolute and bulk density of the drug substance is Very useful in Having some idea as to the size of final dosage form the density of solids also of affects their flow Properties Carr's compressibility index can be used to predict the flow properties based on density measurement.

$$\text{Carr's index (\%)} = \frac{\text{Tapped density} - \text{Pored density} * 100}{\text{Tapped density}}$$

A similar index has been defined by Hausner :

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Pored density}}$$

Angle of repose:- The maximum angle which is formed b/w the surface of a pile of powder and horizontal surface is called the angle of repose.

Relationship between flow, angle of repose, Carr's index fee power flow

Flow	Angle of repose	Carr's index (%)
Excellent	<25	5-15
Good	25-30	12-16

Fair to passable	30-40	18-21
Poor	> 40	23-35
Very Poor		33-38
Extremely Poor		>40

Solubility:

The solubility of drug is an important physicochemical property because it affects the bioavailability of the drug, the rate of drug release into dissolution medium and consequently, the therapeutic efficiency of the pharmaceutical product.

The solubility of the molecules in various solvents is determined as a first step. This information is valuable is developing a formulation. Solubility is usually determined in variety of commonly used solvents and some oils if the molecules are lipophillic.

The solubility of material is usually determined by the equilibrium solubility method, which employs a saturated solution of the material, obtained by stirring an excess of material in the solvent for a prolonged until equilibrium is achieved.

Q2. **Discuss about the role of chemical properties in preformulation studies.**

- **Hydrolysis**
- **Solvolysis**
- **Oxidation**
- **Photolysis**
- **Metal chelating**

Ans **Hydrolysis**

Water play a major role. Nucleophilic attack of water on labile group. Drugs containing ester, amide, lactam, imide or carbamate groups are susceptible to hydrolysis. Hydrolysis can be catalysed by hydrogen ions (specific acid catalysis) or hydroxyl ions (specific base catalysis). Ex:- Penicillin

Oxidation involves the removal of an electropositive atom, radical or electron, or the addition of an electronegative atom or radical. Oxidative degradation can occur by autooxidation, and proceeds quite slowly under the influence of molecular oxygen, Antioxidant act by provide electron, accept by free radical & terminate reaction Ex:- Steroid, simavastatin. Exposed to radiation absorb light & decom.

Photodecomposition may occur not only during storage, but also during use of the product. (in case topical drug**).** Product can be protecting from photolysis by storage in amber colored bottle & dark place. Tablet is protected by coating with polymer contain UV light absorbing substance. Ex:- prednisolone riboflavine.

Metal chelating agent form complex with drug (EDTA, Ethylene diamine) Ex:- Ciprofloxacin. Isomerisation is the process of conversion of a drug into its optical or geometric isomers, which are often of lower therapeutic activity Ex:-Adrenaline (epinephrine) tetracycline .

Q3. **Write a note on ICH guidelines**

• Purpose of stability testing is to provide evidence how quality varies with time under influence as temperature, humidity and light,
• establish re-test period for drug substances
• establish shelf life for drug products
• recommend storage conditions
• Test conditions based on analysis of effects of climatic conditions in the three regions of the EC, Japan, USA
• mean kinetic temperature can be derived from climatic data
• world can thereby divided into four climatic zone I-IV
• This guideline addresses climatic zones I and II
• Stability information generated in one of the three regions is mutually acceptable to the other two provided:
- information is consistent with this guideline,
- labelling is in accord with national/ regional requirements.

The four Climatic Zones

Climatic Zone Definition Storage conditions

I Temperate climate21°C/ 45% r.h
II Subtropical and Mediterranean climate25°C/60%r.h.
III Hot, dry climate30°C/35%r.h
IV Hot, humid climate30°C/70%r.h.

Mean kinetic temperature:

If a mean temperature is calculated and the difference between two temperatures is > 5°C the mean kinetic temperature should be calculated instead of the arithmetic mean temperature. This derives from the fact that the temperature dependency is not linear but logarithmic according to the Arrhenius equation.

1. Guidelines Drug Substance Drug Product

2.1.2 Stress Testing

Stress Testing

□□ help identify likely degradation products
but only those which are formed under accelerated and long term storage conditions
□□ establish degradation pathway
□□ establish intrinsic stability of molecule
□□ validate indicating power of analytical procedure
□□ depends on individual drug substance and type of drug product
□□ carried out on a single batch
□□ should include effect of
• temperature e.g. 50°C, 60°C, 70°C etc.
• humidity e.g. 75% or greater
• oxidation
• hydrolysis across a wide range of pH
• photostability as described in ICH Q1B

Results from these studies form an integral part of information provided to regulatory authorities

2.1.3 Selection of Batches

Data from formal stability studies should be provided

• a least three primary batches
• manufactured to a minimum of pilot scale
• same synthetic route
• method of manufacture and procedure should simulate final process
• quality representative of quality to be made on production scale

Other supporting data can be provided

2.1.4 Container Closure System

□□ Container closure system same or simulates packaging proposed for storage and distribution

2.1.5 Specification

□□ **Specification:**

• list of tests,
• reference to analytical procedure,
• proposed acceptance criteria

□□ **Test Attributes**

• attributes that are susceptible to change during storage,
• influence quality, safety and/or efficacy
• Should cover physical, chemical, biological, microbiological attributes

□□ **Analytical procedures**

• validated stability indicating
• replication depending on results from validation studies

The following requirements for replication can be fixed:

RSD ≤ 1% single analysis
RSD > 1% 3fold analysis

The initial assay at time point 0 should be always analysed 3fold

2.1.6 Testing Frequency

□□ General: every 3 months first year, every 6 months
second year, than annually through proposed re-test period: e.g. 0, 3, 6, 9, 12, 18, 24, 36, 48, 60 months

□□ Accelerated storage condition: 0, 3, 6 months.

Where expectation to approach significant change,
increasing testing necessary: adding samples at final
time point or forth time point in study design: 0, 3, 2 x 6 or 0, 1, 3, 6 months

2.1.7 Storage Conditions

Long term testing should cover a minimum of 12 months duration on at least three primary
batches at time of submission and should be continued sufficient to cover the proposed retest
period.

2.1.9 Evaluation

□□ **Re-test period**

Purpose of stability studies is to establish a re-test period applicable to all further batches of the drug substance manufactured under similar circumstances. It is based on results of physical, chemical, biological and microbiological tests from three batches.

□□ **No formal statistical analyses**

The data may show so little degradation and so little variability that it is apparent from looking at the data that the requested re-test period will be granted. Under these circumstances normally unnecessary to go through the formal statistical analyses; Providing a justification for the omission should be sufficient.

□□ **Statistical evaluation**

Data on a quantitative attribute that change with time: Determination of the time at which the 95% one sided confidence limit for the mean curve intersects the acceptance criterion etc,

2.1.9 Evaluation

□□ **Extrapolation**

Limited extrapolation of the real time data beyond the observed range to extend the re-test period can be undertaken at approval time, if justified.

Justification should be based on

• Knowledge on mechanism of degradation
• results of accelerated testing,
• goodness of fit of mathematical model
• existence of supporting data and batch size

2.1.11 Statements/Labelling

□□ **Storage Statement**

Storage statement established for labelling should be in accordance with national/regional requirements. Statement based on stability evaluation

□□ **Re-test date**

Re-test date derived from stability information.

The re-test date should be displaced on the container label

2.2 Drug Product

□□ **2.2.1 General**

Design of the formal stability studies should be based on

• knowledge and properties of drug substance,
• experience gained from clinical formulation studies.

□□ **2.2.2 Photostability Testing**

One primary batch, standard conditions according to ICH Q1B

2.2.3 Selection of Batches

□□ Required are at least three primary batches.

• Same formulation and in same container closure system as proposed for marketing.
• Manufacturing process should simulate that applied to production batches.
• Same quality and meeting specifications as that intended for marketing.

□□ Two of the three batches at least pilot scale third can be smaller

• for solid oral dosage forms pilot scale is generally on tenth that of full production scale or
• 100000 tablets or capsules, whichever is larger.

□□ Drug products should be manufactured by using different batches of the drug substance.

□□ Stability studies should be performed on each individual strength and container size

of the drug product unless bracketing or matrixing is applied.

□□ Other supporting data can be provided.

2.2.4 Container Closure System

□□ Container closure system proposed for marketing (if appropriate any secondary packaging and container label)

□□ Supporting information:

• results of open storage of stress testing
• studies in other packaging materials

2.2.5 Specification

□□ **Specification is a list of**

• tests, test attributes
• reference to analytical procedures
• proposed acceptance criteria release and shelf life

□□ **Test attributes**

• attributes susceptible to change during storage
• may influence quality, safety and/or efficacy
• should cover physical, chemical, biological, microbiological attributes.

□□ **Analytical procedures**

• fully validated and stability indicating
• Replication will depend on results of validation studies:

Following requirements were fixed:

RSD ≤ 1.5% single analysis

RSD > 1.5% 3fold analysis

Initial analysis generally 3fold

ICH Q1AR2 C 27

□□ **Acceptance Criteria**

• based on all available stability information
• differences between release and shelf life acceptance criteria justified
• difference for antimicrobial preservative content supported by validated correlation of chemical content and preservative effectiveness
• Single primary batch should be tested for antimicrobial preservative effectiveness at proposed shelf life

2.2.6 Testing Frequency

□□ **Long term studies**

• first year every three months. 0, 3, 6, 9, 12
• second year every six months: 12, 18, 24
• third year and longer annually: 24, 36, 48, 60

□□ **Accelerated studies**

• general minimum three time points: 0,3,6 months
• expectation of significant change increases testing adding samples at final time point or forth time point: 0, 3, 2x6 or 0, 1, 3, 6 months

□□ **Intermediate storage condition studies**

Minimum four time points, including initial and final e.g.: 0,6,9,12 months, at time of submission 0,6 months

□□ **Reduced design**

Matrixing or bracketing for reduction of testing frequency if justified

Q4.**Write a note on**

a. **GMP**
b. **Quality audit**

Ans **Good manufacturing practice**" or "**GMP**" are guidance that outline the aspects of production and testing that can impact the quality of a product. Many countries have legislated that pharmaceutical and medical device companies must follow GMP procedures, and have created their own GMP guidelines that correspond with their legislation. Basic concepts of all of these guidelines remain more or less similar to the ultimate goals of safeguarding the health of the patient as well as producing good quality medicine, medical devices or active pharmaceutical products. In the U.S. a drug may be deemed adulterated if it passes all of the specifications tests but is found to be manufactured in a condition which violates current good manufacturing guidelines. Therefore, complying with GMP is a mandatory aspect in pharmaceutical manufacturing.

Although there are a number of them, all guidelines follow a few basic principles.

- Manufacturing processes are clearly defined and controlled. All critical processes are validated to ensure consistency and compliance with specifications.
- Manufacturing processes are controlled, and any changes to the process are evaluated. Changes that have an impact on the quality of the drug are validated as necessary.
- Instructions and procedures are written in clear and unambiguous language. (Good Documentation Practices)
- Operators are trained to carry out and document procedures.
- Records are made, manually or by instruments, during manufacture that demonstrate that all the steps required by the defined procedures and instructions were in fact taken and that the quantity and quality of the drug was as expected. Deviations are investigated and documented.
- Records of manufacture (including distribution) that enable the complete history of a batch to be traced are retained in a comprehensible and accessible form.
- The distribution of the drugs minimizes any risk to their quality.
- A system is available for recalling any batch of drug from sale or supply.
- Complaints about marketed drugs are examined, the causes of quality defects are investigated, and appropriate measures are taken with respect to the defective drugs and to prevent recurrence.
- GMP guidelines are not prescriptive instructions on how to manufacture products. They are a series of general principles that must be observed during manufacturing.

When a company is setting up its quality program and manufacturing process, there may be many ways it can fulfill GMP requirements. It is the company's responsibility to determine the most effective and efficient quality process

Quality assurance (**QA**) refers to the planned and systematic activities implemented in a quality system so that quality requirements for a product or service will be fulfilled [1]. It is the systematic measurement, comparison with a standard, monitoring of processes and an associated feedback loop that confers error prevention. This can be contrasted with Quality "Control". which is focused on process outputs.

Two principles included in QA are: "Fit for purpose", the product should be suitable for the intended purpose; and "Right first time", mistakes should be eliminated. QA includes management of the quality of raw materials, assemblies, products and components, services related to production, and management, production and inspection processes.Suitable *Quality* is determined by product users, clients or customers, not by society in general. It is not related to cost and adjectives or descriptors such "High" and "Poor" are not applicable. For example, a low priced product may be viewed as having high quality because it is disposable where another may be viewed as having poor quality because it is disposable

Q5. **Discuss the following**

i. **ISO 9002**
ii. **ISO 9004**

Ans **ISO 9000** is a family of standards related to quality management systems and designed to help organizations ensure that they meet the needs of customers and other stakeholders The standards are published by ISO, the International Organization for Standardization, and available through National standards bodies. ISO 9000 deals with the fundamentals of quality management systems), including the eight management principles on which the family of standards is based. ISO 9001 deals with the requirements that organizations wishing to meet the standard have to fulfil.

Third party certification bodies provide independent confirmation that organizations meet the requirements of ISO 9001. Over a million organizations worldwide are independently certified, making ISO 9001 one of the most widely used management tools in the world today. Despite widespread use, however, the ISO certification process has been criticized as being wasteful and not being useful for all organizations The three standards (ISO 9001, ISO9002, and ISO 9003) were combined into ISO 9001 in the year 2000 revision (ISO 9001:2000) which was replaced by ISO 9001:2008.

ISO 9002 (last revised in 1994) was titled: *Model for quality assurance in production, installation, and servicing* had basically the same material as ISO 9001 but without covering the creation of new products. It was very applicable for contract manufacturing. Organizations now use ISO 9001 standard and take exception to certain clauses in section 7 of the standard which involve the design of products and/or service. So there is no longer a need for ISO9002.

The current ISO 9000 Family is:

- **ISO 9000:2005** Fundamentals and Vocabulary used in the ISO 9000 Standards
    - What is ISO 9000?
- **ISO 9001:2008** contains the actual requirements an organization must comply with to become ISO 9001 Registered. People often say "ISO 9000" certified, but what they mean is they have met the requirements of the ISO 9001 standard.
    - What is ISO 9001?
- **ISO 9004:2009** Managing for the sustained success of an organization.
    - What is ISO 9004
- Will provide your organization with guidance and support to achieve sustained success by a quality management approach. It can be used by any organization, regardless of size, type and activity.
- Promotes self-assessment as an important tool for the review of the maturity level of your organization. It covers leadership, strategy, management system, resources and processes, to identify areas of strength and weakness and opportunities for improvements and innovations.
- Provides a wider focus on quality management than ISO 9001. It addresses the needs and expectations of all relevant interested parties. ISO 9004 also provides guidance for the systematic and continual improvement of your organization's overall performance.
- Can be used alongside ISO 9001 and other management system standards, but can also be used independently.
- Is not intended for certification, regulatory or contractual use.

Q6. **Write a note on extraction and preparation of thyroid**

Ans. Synonym: Thyroid gland, Dry thyroid

Official source : The thyroid gland of sheep, pig or ox

Thyroid gland

The thyroid gland consists of two lobes on each sides of thyroid cartilage, the well marked structure lying in the middle of neck and popularly known as Adam's Apple. In man a narrow band of tissue, the isthmus, stretching across the wind pipe, unites the lower ends of the lobes. The latter is almond shaped . The gland is enclosed in a capsule to which certain muscles of neck are attached, serving to keep the thyroid in postion The gland is dark red, very vascular and abundantly supplied with blood.

Preparation of thyroid

This consist of----------

- Separate removal of the encapsulated lobes of the glands as quickly as possible

after slaughter of the animal.

- Complete separation of lobes from all superficial tissues eg the capsule and adhering muscular tissue fat and membrane.
- Rejection of lobes which are in any way abnormal eg usually large or small or encysted i.e when cut across showing sacs enclosing fatty matters
- Rapid drying of lobes, after preliminary slicing or mincing, at a temperature not exceeding 600C followed by reduction to a coarse powder.
- Removal of fats by percolation with light petroleum.
- Complete removal of moisture from defatted material by storage in a desicator, followed by reduction to a fine powder.

Moisture and fats constitute about 80% of fresh thyroid, hence approx. five parts of this yield one parts of dry thyroids.

Constituents

- Thyroxine, a complex acidic compound containing 65.4% of iodine
- Di-iodothyrosine, a complex basic substance
- Idothyroglobulin, Thyroxine produces the physiological effects of thyroid glands itself though it has not yet been proved that the whole effects of the latter is attributable to the thyroxine alone. Di –iodo tyrosine does not produce the characteristics effects of thyroid.

Q7. **Write a note on extraction and preparation of liver**

Ans Liver is the largest hetrocrine or mixed gland of the body which is located in upper and right side of the abdominal cavity below the diaphragm. The liver consist four lobes; Left central lobes, left lateral lobe, right central lobe and caudate lobes on right side.The liver cells are called hepatocytes which secret bile. Bile is carried to the duodenum by bile duct, bile contain no digestive enzyme.

Liver preparation

- Oral extracts: the first extract prepared were rapidly filtrates from a maceration in water of minced liver. Cohn made a purified extract by heating the filtrate to 85^0C to ppt. inert matter, filtering and concentrating filtrate under reduced pressure. The product was called cohn Fraction-D. Later he introduces a further purification by ppt of more inert matter with 70% alcohol. The filterate from this concentrated under reduced pressure and mixed with 90% alcohol gave a ppt of high potency.
- Proteolysed liver refinement although producing a potent extract effective in pernicious anaemia.
- Injectable extracts A purification of the cohn fraction G using 95% alcohol produced a water soluble products which was used as an intravenous injection.In spite of its apparent purity reaction occurred in patients when injection was given by this route
- Folic acid This substance is present in liver in small amounts but the dose necessary to give a full erythropoietic response.

Q8. Discuss the formulation and evaluation of Shampoos

A shampoo is a preparation of a surfactant (i.e. surface active material) in a suitable form – liquid, solid or powder – which when used under the specified conditions will remove surface grease, dirt, and skin debris from the hair shaft and scalp without adversely affecting the user.

- Requirements of a Shampoo:

1. It should effectively and completely remove dust or soil, excessive sebum or other fatty substances and loose corneal cells from the hair.
2. It should produce a good amount of foam to satisfy the psychological requirements of the user.
3. It should be easily removed on rinsing with water.
4. It should leave the hair non-dry, soft, lustrous with good manageability and minimum fly away.
5. It should impart a pleasant fragnance to the hair.
6. It should not cause any side-effects / irritation to skin or eye.
7. It should not make the hand rough and chapped.

Formulation of shampoo

CONDITIONING SHAMPOOS	
Steryl dimethyl benzyl ammonium chloride	5.5%
Ethylene glycol monostearate	2%
Cetyl alcohol	2.5%
Water	Upto 100%
Color, perfume, preservative	q.s

Evaluation of Shampoos

1. Foam and foam stability:

- The Ross-Miles foam column test is accepted. 200 ml of surfactant solution is dropped into a glass column containing 50ml of the same solution. The height of the foam generated is measured immediately and again after a specified time interval, and is considered proportional to the volume.
- Barnett and Powers developed a latherometer to measure the effect of variables such as water hardness, type of soil and quantity of soil on foam speed, volume and stability.
- Fredell and Read titrated actual standard oiled heads of hair with additive increments of shampoo until a persistent lather end point appeared.

2. Detergency and cleaning action:

- Cleansing power is evaluated by the method of Barnet and Powers
- 5gm sample of soiled human hair is placed at 35°c in 200 cc of water containing of 1 gm of shampoo.
- The flask is shaken 50 times a minute for 4 minutes. Then washed once again with sufficient amount of water, then after filter the hair dried and weighed.
- The amount of soil is removed under these condition is calculated.

Wetting Action:

- Canvas disk sinking test: A mount veron cotton duck # 6 canvas disk 1 inch in diameter, is floated on the surface of a solution, and the time required for it to sink is measured accurately.

Rinsing:

- Skilled beauticians are employed to make comparisons on the performance of several shampoos.

Conditioning Action:

- Conditioning action is a difficult property to assess. This is because it is basically dependent on subjective appraisal.
- No method has been published for measuring conditioning action.
- The degree of conditioning given to hair is ultimately judged by shampoo user who is making the evaluation on the basis of past experience and present expectations.

. Microbiological assay:

- PREPARATION OF PRE-INOCULUM Take the loopful culture of staphylococcus aureus (ATCC6532) aseptically and transfer to sterilized and cooled 100 ml SCDM (broth).
- Mix well. Incubate the broth at 37oC for 24 hrs.
- PREPARATION OF MEDIA Soya bean casein digests medium, soya bean casein digest agar and nutrient agar.
- PREPARATION OF POUR PLATES Sterilized SCD agar (100 ml) is cooled to 40°C and mixed with 5 ml of 24 hrs old pre inoculated culture.
- This is immediately poured in plates (340 ml each) and allows to set.
- MAKING THE WELLS ON AGAR PLATES The wells are dig on agar plates with sterilised well digger aseptically.
- Take 100µml of each sample, add to well aseptically. Incubate the plates at 37oC for 24 hrs to 48 hrs.
- Observe the effectiveness of sample on culture growing on the agar plate and we can see the effectiveness of sample in the form of zone of inhibition around each well containing different sample.

. Evaluation of eye irritancy:

- The test calls for dropping 0.1 ml of liquid shampoo in the conjunctiva sac of one eye of the rabbit , the other eye serving as control.
- In the case of the first three animals, the treated eye remains unwashed. Since washing the eye may or may not alleviate symptoms of injury.
- The six remaining animals are divided into two equal groups.
- In the first of these groups eyes instilled with the substances are washed with 20 ml of lukewarm water two seconds after treatment and in the second group after instillation.
- Readings are then made at 24, 48 and 72 hr and again four and seven days after treatment.
- If the lesions have not cleared up in seven days the test material is considered as severe irritant.

8. Viscosity:

- Viscosity of the liquid shampoo is determined using a Brookefield viscometer
- 100 mL of the shampoo is taken in a beaker and the spindle is dipped in it for about 5 min and then the reading is taken.

Q9. Give an account about the structure and function of skin

Skin is a flexible, self-repairing capsule that separates the internal environment of the body from the external environment The cutaneous membrane covers the external surface of body in surface area and weight. In adult skin covers an area about 2 square meters and weigh 4.5- 5 kg, about 16% of total body weight. It ranges in thickness from o.5mm on eyelid to 4mm on heels. However most of body it is 1-2 mm thick. Skin forms the body's protection against the entry of foreign substances, pathogens, and radiation and prevents the loss of endogenous contents, including water. The advantages of drug delivery through the skin include easy accessibility, convenience, prolonged therapy, avoidance of liver first pass metabolism and a large surface area.

Skin is composed of two layers, the epidermis and the dermis, separated by a basement membrane zone. Beneath the dermis there is a layer of adipose tissue and sweat glands.

Epidermis

The keratinocytes which is a part of epidermis (50-100 um thick) migrates outward from the basal cell into highly differentiated non dividing cells. This forms 90% of epidermal cell. During proliferation, keratinocytes renovate from polygonal cell to spinous cells, flattened granular cells, and finally to flattened polyhedral dead corneocytes full of the protein keratin. Epidermis can be divided into

I) Stratum basale(SB),
ii) Stratum spinosum,(SS),
iii) Stratum Granulosum (SG) and
iv) Stratum Corneum.

The epidermal cell layers are interconnected by desmosomes. Stratum basale is a thick layer made up of polygonal cells superficially and columnar or cubiodal epithelial cells in the deeper parts. Here, new cells are constantly formed by mitotic division. Newly formed cells move continuously towards the stratum corneum. The stem cells, which give rise to new cells, are known as keratinocytes. From this, some projection extends downwards to the dermis

Prickle cell layer is composed of several layers of polygonal prickle cells or squamous cells. The layers become flat as they near the surface so that their long axis appears parallel to the skin surface. These cells possess intracellular bridges or tonofilaments. These intercellular cytoplasmic tonofilaments contain PAS-positive material that is precursor of keratin. Granular cell layers consist of 1 to 3 layers of flat cells containing keratohyaline basophilic granules which are PAS-negative. Granular cell layer is much thicker in palms and soles. Stratum lucidum is present exclusively in palms and soles as a thin homogenous, eosinophilic, non-nucleate zone. Horny layer (Stratum corneum) is also normally devoid of nuclei and consists of eosinophilic layers of keratin. Intra epidermal nerve endings are present in the form of market cells which are touch receptors

DERMIS

The dermis is tough and elastic. It is formed from connective tissue and matrix contains collagen fibres interlaced with elastic fibres. Rupture of elastic fibre occurs when the skin is over stretched, resulting in permanent striae, that may be found in pregnancy and obesity. A collagen fibre binds water and gives the skin its tensile strength, but as this ability decline with age, wrinkle develops. Fibroblast, Macrophages and mast cells are found in the dermis. Underlying its deepest layer there is areolar tissue and varying amount of adipose fat.

The dermis consists of 2 parts- the superficial *parspapillaris or papillary dermis*, and the deepar *pars reticularis* or *reticular dermis*. The dermis is composed of fibrocollagenic tissue containing blood vessels, lymphatics and nerves. In the skin of fingers, arteriovenous shunts or *glomera* are normally present. The specialized nerve endings present at some sites perform specific functions. These are as under:

- *Pacinian corpuscles* concerned with pressure are present in deep layer of skin.
- *Meissner corpuscles* are touch receptor, located in the papillae of skins of palms, soles, tips of fingers and toes.
- *Ruffini corpuscles* are cold receptors found in the external genitalia.
- *End-bulbs of Krause* are cold receptors found in the external genitalia.

Beside these structures, the dermis contains cutanious appendages or adnexal structures. These are sweat glands, sebaceous glands, hair follicles, arrectores pilorum and nails.

1. **Sweat glands:** These are of 2 types- eccrine and apocrine.
   - i) **Eccrine glands:** They are present all over the skin but are most numerous on the palms, soles and axillae. They are coiled tubular glands lying deep in the dermis. Their ducts pass through the epidermis on the surface of the skin as pores via which they empty their secretion i.e sweat. The glands are lined by two main types of secretory cells: basal, acidophilic, clear or chief cells. The secretory cells are surrounded by myoepithelial cells.
   - ii) **Apocrine glands:** Apocrine glands are encountered in some areas only- in the axillae, in the anogenital region, in the external ear as modified glands called ceruminous glands, in the eyelid as Moll's glands, and in the breast as mammary glands. Apocrine glands are also tubular glands but have larger lumina. Apocrine glands have a single layer of secretory cells which contain acidophilic, PAS-positive, prominent granular cytoplasm. The type of secretion in apocrine glands is decapitation secretion as if the cytoplasm of the secretory cells is pinched off (apo = off).
2. **SEBACEOUS (HOLOCRINE) GLANDS:** Sebaceous glands are found everywhere on the skin except on the palms and soles. They are often found in association with hair but can be seen in a few areas devoid of hair as modified sebaceous glands such as in the external auditory meatus, nipple and areola of male and female breast, labia minora, prepuce, and meibomian glands of the eyelids. Sebaceous glands are composed of lobules of sebaceous cells containing small round nuclei and abundant fatty, network-like cytoplasm.
3. **Hair:** The hair grows from the bottom of the follicle. It has therefore, an intra cutaneous portion present in the hair follicle and the shaft. The hair follicle consists of epithelial and connective tissue components. The hair shaft is made up of an

outer sheath and pigmented cortex and inner medulla.

4. **ARRECTORES PILORUM:** These are small bundles of smooth muscles attached to each hair follicle. When the muscle contracts, the hair becomes more erect, the follicle is dragged upward so as to become prominent on the surface of the skin producing what is known as *'goose skin'*.
5. **Nails:** The nails are thickenings of the deeper part of the stratum corneum that develop at specially modified portion of the skin called nail bed. The nail is composed of clear horny cells, resembling stratum lucidium but are much more keratinized.

Q10. Give an account about the structure and function of hair

Hair serves many functions. The most obvious is to serve as insulation, but it's also used to provide camouflage (for example, the spot pattern on a fawn deer mimics the play of light and shade on a forest floor), for sex recognition (male mammals often have ruffs, manes, or beards as secondary sex characteristics) and even for social purposes, such as aggressive display (as when a dog's "hackles rise" or a cat elevates her fur on the approach of a dog). In cross sections not all the hairs are cut on the same level, and some of the sheaths which end before the surface is reached will not be seen if you happen to have a section that is too high up. Use the presence or absence (and the size) of sebaceous glands as a rough indicator of where you are with respect to the "deep" or "shallow" parts of the follicle.. This longitudinal section shows the outer root sheath and the cortex and medulla of the hair shaft very well; and the inner root sheath can be made out too. The inner root sheath doesn't go all the way up the side of the hair follicle: it ends about halfway to the surface. The outer root sheath, however, is continuous with the surface epidermis at the top of the follicle.

The outermost layer of the hair is a cuticle like bark on a tree, more easily seen in cross sections (see below). The cuticle of the hair is so closely pressed against the hair shaft and the adjoining cuticle of the root sheath (see below) that the two are indistinguishable in your sections. Inside the cuticle most hairs (not all) have two distinct regions: a cortex in which the dead keratinized cells are very densely packed, and a medulla, in which they aren't so densely packed. These will be easy to tell apart, especially in slide 35. The cortex looks like a rind on a melon, with the medulla as the "meat" inside. Some hairs have no medulla; these are the curly "wool hairs" as opposed to the long "guard hairs" seen in furbearing animals. The "glassy membrane" is the basement membrane of the follicle, separating it from the CT around it. This hair has been cut in cross section, at a level of the follicle where the inner (IRS) and outer (ORS) root sheaths are both visible. The inner sheath has several layers, and in this field the translucent tricohyaline granules of Huxley's layer are easily visible. These granules are markedly similar to the keratohyaline granules of the epidermal keratinocytes: more proof that hair production is a similar process. In this picture the deeply pigmented cortex and less pigmented medulla of the hair are visible. in the follicle where the inner root sheath terminates. Only the outer root sheath and the hair proper are visible. Notice the small sebaceous gland profile at lower right. Some hairs are hollow. Instead of a cellular medulla, there's an air space inside. This is pretty common in

Arctic animals, most notably the polar bear. By having an air space *inside* the hair, its insulation value is greatly increased. This eons-old natural solution to the problem of keeping out the cold even has a human made equivalent: the commercial fiber "Holofil" used in sleeping bags and low cost insulated jackets. It's basically imitation polar bear hair, a thin acrylic filament with a hole through the middle. Imitation is indeed the sincerest form of flattery.

Q11. Discuss the following

1) Moisturising cream
2) Hair cream

Moisturising cream A cream is an emulsion of oil and water in approximately equal proportions. It penetrates the stratum corneum outer layer of skin well. Cream is thicker than lotion, and maintains its shape when removed from its container. It tends to be moderate in moisturizing tendency. For topical steroid products, oil-in-water emulsions are common. Creams have a significant risk of causing immunological sensitization due to preservatives. It has a high rate of acceptance by patients. There is a great variation in ingredients, composition, pH, and tolerance among generic brands. A great moisturizer for dry skin that helps heal, repair, and maintain soft, supple skin. Increasing skin hydration (by reducing evaporation)

E.g.: Aloe moisturizing cream

Method of formulation:

Heat coconut oil & beeswax until wax melt. Stir & cool slowly add the *aloe vera* gel drop at a time.Continue stirring & when the mixture thickens, add the vitamin oil & *chamomile* extract.

Ingredients Maximum levels (% w/w)
Silicones and volatile silicones (e.g. *cyclomethicone*) 50
Emollients (oils, waxes, etc.) 30
Moisturising agents (e.g. *glycerin*) 15
Additional ingredients (e.g. plant extracts, vitamins, UV filters) 10
Fillers (e.g. starch, *kaolin*, *talc*) 10
Emulsifying agents (e.g. *sorbitan sesquioleate*, *sorbitan stearate*) 5
Cosmetic colorants (e.g. often iron oxides) 2
Preservatives, antimicrobials 2
Viscosity controlling agents (e.g. *cellulose gum*) 1
Parfum 1
Aqua to 100

Hair cream

Materials Used In Preparation of Hair Creams

The basic composition of cream:-

1. Emulsifying agent: It is used to reduce the interfacial tension between oil and water and thus make them miscible with each other e.g. wool alcohol, lanolin stearic acid, bees wax, paraffin wax petroleum jelly.
2. Oil: Mineral oil, arachis oil, paraffin oil.
3. Water: pure water, lime water.
4. Humectant: It is used to prevent fast drying of water in cream preparation e.g.

triethanolamine,propylene glycol.

5. Perfume: It is used to give good odour and fragnance to the cream e.g. sandalwood oil, lavender oil35.

6. Colour: It is used to give good appearance to the specific cream preparation e.g. Eosin, titanium dioxide, saffron.

7. Preservative: It is added in hair cream preparation to avoid the microbial growth because the microbial growth is very high in aqueous phase e.g. propyl para-hydroxy benzoate,para-hydroxy benzoic acid.

8. Active Ingredient:-bleaching adents, anti fungal agents, preservatives, etc are added in hair cream as per requirement e.g H_2O_2, $KMnO_4$ Solution, Ammonium bisulphate, Thioglycollate.34

Hair nourshing cream:

Massage the Himalaya hair loss cream gently on the scalp with the help of your fingers3. Carefully and slowly cover the entire scalp. For best results leave the cream applied on the scalp over night and then rinse it off next morning. Use this cream twice daily.

Hair Remover Cream:

General Formula: Thioglycollic acid 6.0 gm
Calcium Carbonate 20.0 gm
Titanium dioxide 2.0 gm
Sodium lauryl sulphate 0.5 gm
Cetyl Alcohol 5.0 gm
Glycerine 5.0 gm
Water 61.5 gm
Perfume q.s.
Calcium Hydroxide q.s.

It is apply on body surface with spatula and after 5-15 min. it is remove by using spatula. It provide smoothness to body surface and remove the unwanted hair3, 36.

Hair Bleaching Cream:

General Formula: Ammonium bicarbonate 20.0 gm
Ammonium bisulphate 10.0 gm
Light magnesium carbonate 50.0 gm
Light calcium carbonate 20.0 gm

This powder mixture is to be mixed with hydrogen paroxide solution before use. It is apply on body surface with spatula generally in face. It give good appearance and light shade to the hair.

EVALUATION TESTS

1. Stability of the ingredient: This is done by normal stability study of the active ingredient at room temperature or by accelerated stability study.

2. Physical stability: This is particularly important for emulsion type preparation. This can be done by accelerated test by exposing the preparation alternatively to heat and cold.

3. Rheology: This can be studied by using viscometer to measure the viscosity.

4. Toxicity test: Generally for hair removing cream this test is essential. This test can be done on animal. For this purpose rabbit can be used. Preparation can be applied for hair removal and effect on the skin can be studied by observation or by microscopic study.

5. Dye test: The scarlet red dye is mixed with the cream. Place a drop of the cream on a microscopic slide covers it with a cover slip, and examines it under a microscope. If the disperse globules appear red the ground colourless. The cream is o/w type. The reverse condition occurs in w/o type cream i.e. the disperse globules appear colourless in the red ground.

6. Patch Test: The patch tests were applied to the upper back for 48 h, read at 72 h and most tests (85.8%) were also evaluated a second time, on days 5 – 7according to the international rules.

PACKAGING and LABELLING

The hair cream being the semisolid preparation can be filled in either collapsible tubes of metal, plastic or in wide mouthed container like jar. The container should be well closed, so as to minimize exposure to the air.

The label should bear information like name of the material its net mass ingredient and their composition direction for use, batch number license number date of manufacture and it shelf life as well as the manufacturers name and address. Any other information related to consumer for example expiry date. USFDA had set standards and specifications for packaging and labeling of containers and closures.