SLT INSTITUTE OF PHARMACEUTICAL SCIENCES
GURU GHASIDAS VISHWAVIDYALAYA, BILASPUR (C.G)
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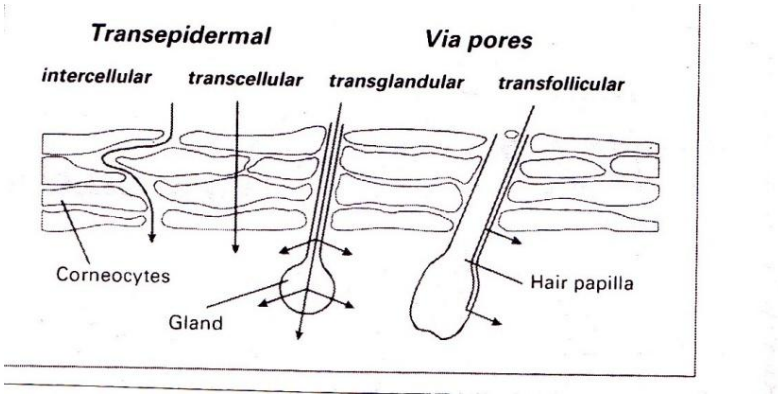
PRODUCT DEVELOPMENT (AS-2550)
MODEL ANSWERS

SECTION A

1. i) (a) Phenolic
   ii) Saccharin, aspartame,glycerine, sorbitol, cyclamate
   iii) (c) 0.05-0.1
   iv) mottling
   v) Sampling plan is the decision of planning for the number of samples to be charged to the stability chambers and the plan of sampling from the batch so that the whole batch is covered.
   vi) components of occusert are : transparent polymer membrane, drug reservoir, rate controlling ethylene vinyl acetate copolymer,energy source, delivery portal.

   vii) Schematic diagram for pathways of skin penetration



   viii) The systemic absorption of drugs from the rectum depends on the height at which drug release occurs in the rectum. Drug absorbed by the middle or inferior vein goes directy into the circulation via the inferior vena cava and by pass the liver.
   ix) The USP test procedure for evaluating toxcicity of plastics consists of three phases-
      a) implanting small piece of plastic material intramuscularly in rabbit.
      b) Injecting elutes using sodium chloride injection with and without alcohol, intravenously in mice
      c) Injecting elutes using polyethylene glycols and sesame oil intraperitoneally in rabbit. The reaction from the test sample must not be significantly greater than non reactive control samples.
   x) Four important functions of pilot plant are-
      a) review of the product formula to evaluate and determine its ability to withstand batch scale modifications.
      b) selection, approval and validation of raw material specification.

c) selection and validation of appropriate processing equipments.
d) evaluation and validation of process and production controls.
e) transfer of developed technology/process to shop floor for routine manufacturing.

xi) Methods to prepare resealed erythrocytes are- membrane perturbation method, electro- encapsulation method,dillutional haemolysis,isotonic osmotic lysis,dialysis, entrapment by endocytosis.

xii) liposomes are lipid vesicles made of amphiphillic molecules (phospholipids and mixture of lipids containing phospholipids) capable of self organization properties. They form lipidic bilayer and this encapsulates small volume of hydrophilic molecules In the internal aqueous phase whereas amphiphillic molecules are incorporated in the bilayer . Transfersomes are composed of a natural amphipath- soya phosphatedylcholine with an edge active molecule eg sodium cholate and therefore ultradeformable with high skin penetrating potential than liposomes. They can deliver low and high molecular weight drugs and show absence in lag phase in absorption.

xiii) segregation of particles takes place due to difference in size and density. In non homogenous mixture, the smaller particles fall through the voids between larger particles and concentrate at the base with larger ones above, resulting in segregation. Therefore a homogenous blend with uniform size particle has less tendency to segregate.

Ans2

In vitro evaluation techniques Tablets are evaluated by a variety of methods.

1. ***Analytical determination of tablet content:*** *The weight variation of the tablets can be measured by weighing each individual tablets and determining the percent difference from the intended amount. Guidelines in the USP 24/NF19 Supplement 1 indicate that each tablet "shall be not less than 90% and not more than 110% of the theoretically calculated weight for each unit."*
2. ***Tablet hardness:*** *The tablets must be hard enough to withstand mechanical stress during packaging, shipment, and handling by the consumer. Section <1216> of the USP 24/NF19 outlines a standard tablet friability test applicable to manufactured tablets. Most compounding pharmacy would not have the apparatus specified in Section <1216>. However, there are several hand operated tablet hardness testers that might be useful. Examples of devices are the Strong Cobb, Pfizer, and Stokes hardness testers. The principle of measurement involves subjecting the tablet to an increasing load until the tablet breaks or fractures. The load is applied along the radial axis of the tablet. Oral tablets normally have a hardness of 4 to 8 or 10 kg; however, hypodermic and chewable tablets are much softer (3 kg) and some sustained release tablets are much harder (10-20 kg).*

3. ***Tablet disintegration:*** *The USP XXI tablet disintegrator supports a 10 mesh screen about 2 inches above the bottom of a 1000 ml beaker. Fill the beaker with 1000 ml of water, add a stirring bar, and place the beaker on a magnetic stirring plate. Stir at a moderate speed. Drop the tablets onto the mesh screen and record*

the time needed for the tablets to disintegrate. A reasonable disintegration time should be between 15 and 30 minutes, although the time will depend on the product, the stirring speed, etc.

4. ***Tablet dissolution:*** *A tablet can have a rapid disintegration time yet be biologically unavailable. The dissolution rate of the drug from the primary particles of the tablet is the important factor in drug absorption and for many formulations is the rate-limiting step. Therefore, a dissolution time is more indicative of the availability of a drug from a tablet than the disintegration test. It measures the amount of time required for a given percentage of drug in a tablet to go into solution.It is done using USP dissolution test apparatus.*
5. ***Friability:*** *Tablets are subjected to uniform tumbling motion for specified period (25 rovolutions per minute for 4 min.).Weight loss from tablet should not be more than 1%.*
6. ***Thickness:*** *It needs to be controlled to ensure proper dose.It can be measured by a calipers.*
7. ***Stability Study-****accelarated temp. stability study at 75% RH, helps to determine the influence of storage conditions on shelf life.*

Suspensions-

1. ***Sedimentation volume-*** *Ratio of ultimate height to initial height.The larger the ratio, better the suspendability.*
2. ***Viscosity-*** *taken out by a Brookfield viscometer mounted on a helipath stand. The T bar spindle descends slowly into the susp. And the resistence is measured. Better susp. Show lesser rate of increase of resistence with spindle turns.*
3. ***Particle size Analysis:****Susp. Are subjected to Freeze Thaw cycle causing particle growth. Particle size is measured by microscopy.*
4. ***Zeta Potential:****It is measured by microelectrophoresis and speed of movement of particlesacross the field is determined.*

Emulsions-

1. ***Physical parameters-***
   Particle size and size distribution.
   Phase separation and coalescence
   Viscosity
   Zeta potential

2.Evaluation of emulsion shelf life

Temperature stress- Some tests cycle the product for 24 hr between –-5 to 40^{0}C while others use 12 hr cycle between 5-35^{0}C.
Centrifugation stress- It may involve a 5 min. test using high speed centrifuge whereas 20 min. test using a 20 min. centrifuge.

Parenterals-

1. ***Freedom from particulate matter-*** *by using visual inspection or by a fiber optic light system or by light scattering.USP allows 6000 particles /container of SVP and not more than 25/ml of LVP.*
2. ***Sterility test –*** *Performed by either direct inoculation or membrane filtration technique.The probability microbial survival is called sterility assurance level (SAL) .Acceptable SAL is 10^{-6}.*
3. ***Pyrogens and bacterial endotoxins-*** *Rabbit test involves rise in rectal temp. of rabbits after iv injection of a test solution and is designed for products that can be tolerated by a test rabbit in a dose not to exceed 10 ml/Kg within a period not more than 10 min.*
   Limulus Amoebocyte Lysate test uses the lysate of aqueous extract of circulating amoebocytesof horseshoe crab. This test has greater sensitivity, lesser variation.
4. ***Package integrity test-*** *Following are the commonly used leak tests- Visual inspection, Bubble test, Pressure/Vacuum decay/,dye test, chemical tracer test.*

Topical Products-

1. ***Physical and Chemical stability-****Discoloration, variable rheologic behavior,viscosity,loss of volatile phase on storage.*
2. ***In vitro skin permeation-****The apparatus consists of- i) a donor comp.where the formulation is applied uniformly ii) the membrane (skin) through which the drug permeates. iii) receptor solution. Examples of such models are- Franz diffusion cell, flow through diffusion cell.*
3. ***Amount of drug deposited into skin-***
4. ***Skin sensitivities-*** *draise test and non Draise animal test.*

In vivo evaluation methods

1. ***X ray studies for monitoring GI transit –*** *Barium sulphate tablets of 8 mm dia. Are prepared in different types of polymers and administered to ten rabbits with 25 ml water . At different intervals, rabbits are Xray photographed and observed for nature and position of tablets.*
2. ***Drug bioavailability-****To assure the therapeutic conc. Of drug in the blood, samples are analysed at specific time intervals, and pharmacokinetic parameters Cmax and AUC are determined . The apparent in vivo drug release profile can also be obtained from urinary drug level studies for drugs excessively excreted unchanged in urine.*

Ans 3 The two important characteristics of a tablet granulation are : fluidity and compressibility.

Particle size and shape-This affects the average tablet weight, weight variation, DT, friability, Flowability and drying rate.The shape coefficient for a sphere is 6. It increases as particles Become irregular. For cube it is 6.8.

Surface area – It is important for drugs having limited water solubility. SA in such cases signif-Cantly affects dissolution rate. two methods of determining SA are gas adsorption method in Which amount of gas adsorbed to form a monolayer is measured. In the air permeability Method, the rate at which air permeates a bed of powder, is used to calculate SA.

Density –It may influence compressibility, porosity, dissolution,etc.Dense hard granules may Require higher compressible loads to produce a cohesive compact which may increase DT and Dissolution time.Granule density is determined by mercury displacement or benzene displace-ment method. $D= M/V_p – V_i$ where,

V_p = Total volume of pyknometer

V_i = Volume of intrusion fluid containing the mass of granules M

Bulk density is used to describe packing of granules.

$Þ_b= M/ V_b$ M is the mass of particles and V_b is the total volume of packing.

Compressibility $C= p_b - p_u (100)/ p_b$ where p_u is the untapped bulk density.

Strength and Friability – The strength of a wet granule is mainly due to surface tension of the Of liquid and capillary forces.These forces are responsible for initial aggregation of wet powder. Upon drying the granule has strong bonds resulting from fusion or crystallization and curing of Adhesive or binder. Under these conditions Vander Waals forces are of sufficient strength to produce strong dry granules. Granule strength and friability affect changes in particle size distribution and therefore compressibility. Granule strength can be measured by placing the granule between anvils and the force required to break the granules is measured.

Flow Properties – Forces that exist between particles are-

Frictional forces, surface tension forces, mechanical forces caused by interlocking of particles Of irregular shape, electrostatic forces, cohesive or Vander Waals forces.These affect granule Properties such as particle size, size distribution,Particle shape surface texture or roughness. With fine powders less than 150 um, frictional and VanderWaals forces predominate. For particles Larger than these, frictional forces predominate. Flow properties can be determined by two Methods-

Angle of repose- $\tan\alpha = H/R$

Value of α less than 30 shows free flowing granulation.

Hopper flow rate – T he instrument continuously monitors the flow of material out of conical Hoppers into a recording balance device.

Compaction – Tablet presses are affixed with transducers to measure the forces applied during Compression. Signals produced by transducers are monitored by computer.

METHODOLOGY OF TABLET GRANULATION

1. Pneumatic Dry Granulation (PDG)

- Is based on a pneumatic dry granulation process, anovel dry method for automatic or semi-automaticproduction of granules,
- Enables flexible modification of drug load,disintegration time and tablet hardness,
- Can achieve:○ High drug loading, even with ‘difficult’ APIs and combinations, Taste masking Excellent stability,
- Is compatible with other technologies, such as sustained release, fast release, coating,
- Is suitable for heat labile and moisture sensitive drugs, and
- Is the subject of a number of patent applications.

The PDG Technology produces porous granules with
excellent compressibility and flowability characteristics

2. Freeze granulation Technology

Swedish Ceramic Institute (SCI) has adopted and developed an alternative technique – freeze granulation (FG) – which enables preservation of the homogeneity from suspension to dry granules By spraying a powder suspension into liquid nitrogen, the drops (granules) are instantaneously frozen. In a subsequent freeze-drying the granules are dried by sublimation of the ice without any segregation effects as in the case of conventional drying in air. The result will be spherical, free flowing granules, with optimal homogeneity. FG provides optimized condition for the subsequent processing of the granules, for example easy crushing to homogeneous and dense powder compacts in a pressing operation. High degree of compact homogeneity will then support the following sintering with minimal risks for granule defects.

3. Foamed Binder Technologies (FBT)

Foamed binder technology can help to achieve faster, simpler, and safer wet granulation processing. Using familiar, proven METHOCEL polymers, this technology greatly improves binder distribution in the formulation mix and yields a remarkable array of processing advantages. Compared to conventional spray processing, foamed binder technology can shorten processing times by reducing water requirements. It can improve reproducibility through more uniform binder distribution. Moreover, it eliminates spray nozzles and their many variables in granulation processing equipment. Foam processing also offers better end point determinations and reduced equipment clean-up time. While foamed binder processing offers many advantages, this technology doesn't demand new equipment or radical changes in processing techniques.

4. Melt Granulation Technology

Melt granulation is processes by which granules are obtained through the addition of either a molten binder or a solid binder which melts during the process. This process is also called melt agglomeration and thermoplastic granulation. Principle of melt granulation: The process of granulation consists of a combination of three phases:

I. Wetting and nucleation,
II. Coalescence step,
III. Attrition and breakage.

5. Steam Granulation

• It is modification of wet granulation. Here steam is used as a binder instead of water.
• In this method of granulating particles involves the injection of the required amount of liquid in the form of steam.
• This steam injection method, which employs steam at a temperature of about 150° C., tends to produce local overheating and excessive wetting of the particles in the vicinity of the steam nozzles, thereby causing the formation of lumps in the granulated product.

6. Moisture Activated Dry Granulation (MADG)

• In this method moisture is used to activate the granules formation but the granules drying step is not necessary due to moisture absorbing material such as MCC.
• The moisture-activated dry granulation process consists of two steps, wet agglomeration of the powder mixture followed by moisture absorption stages.

• A small amount of water (1–4%) is added first to agglomerate the mixture of the API, a binder, and excipients. Moisture absorbing material such as MCC and potato starch is then added to absorb any excessive moisture.
• After mixing with a lubricant, the resulting mixture can then be compressed directly into tablets. Hence, this process offers the advantage of wet granulation is that eliminates the need for a drying step.
• MCC, potato starch, or a mixture of 50% of each can be used as moisture absorbing material.

8. Thermal Adhesion Granulation Process (TAGP)

It is applicable for preparing direct tabletting formulations. TAGP is performed under low moisture content or low content of pharmaceutically acceptable solvent by subjecting a mixture containing one or more diluents and/or active ingredients; a binder; and optionally a disintegrant to heating at a temperature in the range from about 30ºC to about 130ºC in a closed system under mixing by tumble rotation until the formation of granules. This method utilizes less water or solvent than traditional wet granulation method . It provides granules with good flow properties and binding capacity to form tablets of low friability, adequate hardness and have a high uptake capacity for active substances whose tabletting is poor. In thermal adhesion granulation, granules are formed during mixing of the moist powder under ontinuous tumble rotation, as the heated powder mass flows within the container and agglomerates with the aid of the binder. Drying and milling to form the desired granules are unnecessary in the present invention due to the low amount of moisture introduced to the tabletting mixture.

Ans 4

Emulsions are thermodynamically dispersed Systems with Liquid immiscible Phases
Dispergere (Latin): to remove, to redistribute and Emulgere (Latin): to milk out
Emulsions can be o/w or w/o types. The diameter of the dispersed phase is in range of 0.1-10 um an emulsion droplet interface has at any point the same interfacial tension (in contrast to many suspension particles) sometimes emulsions are subdivided arbitrarily regarding the droplet size (macro-, mini-, microemulsions) .

Tests for Identification of Emulsion Types:

• Dilution test: emulsion can be diluted only with external phase
• Dye test: water or oil soluble dyes
• CoCl2/filter paper test: filter paper impregnated with CoCl2 and dried (blue) changes to pink when o/w emulsion is added
• Fluorescence: some oils fluoresce under UV light
• Conductivity: for ionic o/w emulsions
(o/w emulsions conduct electric current)

Work of emulsion formation-

The increase in the energy of an emulsion compared to the nonemulsified components is equal to ΔW.This amount of energy can be considered as a measure of the thermodynamic instability of an emulsion.

$\Delta W = \sigma \cdot \Delta A$

ΔW is the free energy of the interface and corresponds to the reversible work brought permanently into the system during the emulsification process .This makes an emulsion very prone to coalescence processes which lead to a decrease in ΔA and subsequently in ΔW.

The conclusion is straightforward that ultimate stability against coalescence processes is only achieved if σ approaches zero

Theories of emulsification

surface tension theory
oriented wedge theory
interfacial film theory

Emulsifying agents

Surfactants – ionic and non ionic

Non ionic surfactants are effective over pH range of 3-10, cationic surfactants over 3-7 and anionic Surfactants require ph of 8. Surfactants with HLB 3-6 are lipophillic,and form w/o emul., surfactants with HLB 8-18 form o/w emul.For optimal stability of o/w emul. Its phase inversion temp. should be 25-70 degree higher than its storage temp.

Hydrophillic colloids – they position themselves between o/w interface but do not lower the interfacial tension but form multimolecular film at the interface. Eg clays, bentonite, polysaccharides.

finely divided solids- They are wetted by both oil and water and concentrate at the interface to form particulate film. Eg heavy metal hydroxides, glyceryl tristearates.

Antimicrobial Preservatives

A combination of water soluble methyl parabens and oil soluble propyl paraben has good preservative action.Phenolic preservatives are susceptible to interaction with polyoxyethylethylene group compounds.

Antioxidants

Eg lecithin, propyl gallate, ascorbic acid,, BHT, BHA, alkyl gallates

Equipments for emulsification

1. Mechanical stirrers
2. **Homoginizers**
3. Ultrasonifiers
4. Colloid mills

Signs of instability

1. Creaming
2. Coalescense

Evaluation

Physical parameters-

Particle size and size distribution

Phase separation and coalescence

Viscosity

Electrophoretic properties

Shelf Life- It takes long time for development of signs of instability. To accelerate the stability program, Emulsion is subjected to various stress conditions –

Temperature stress- Product is cycled for 24 hrs between -5 to 40^0 C or for 12 hr between 5 to 35 ^{0}C .

Centrifugation stress- For 5 min using high speed centrifuge, or 20 min using low speed centrifuge.

Microemulsions- They have globule size 10-100 nm, have excellent kinetic stability and appear transparent or translucent but surfactant is used in higher concentration 6-8%

Multiple Emulsions

Ans 5

The following preparations are designed solely for parenteral use only after addition of drugs that require dilution or must be dissolved in an aqueous vehicle prior to injection:

Water

Since most liquid injections are quite dilute, the component present in the highest proportion is the vehicle. The vehicle of greatest importance for parenteral products is water. Water of suitable quality for compounding and rinsing product contact surfaces, to meet United States Pharmacopeia (USP) and other compendia specifications for Water for Injection (WFI), may be prepared either by distillation or by reverse osmosis. Only by these two methods is it possible to separate various liquid, gas, and solid contaminating substances from water.

Sterile Water for Injection, USP is a sterile, nonpyrogenic preparation of water for injection, which contains no bacteriostat, antimicrobial agent or added buffer and is supplied only in single-dose containers to dilute or dissolve drugs for injection. For I.V. injection, add sufficient solute to make an approximately isotonic solution. Sterile Water for Injection, USP must be made approximately isotonic prior to use. Intravenous administration of Sterile Water for Injection without a solute may result in hemolysis.

Water for Injection, USP is chemically designated H_2O.

10% Dextrose Injection, USP is a sterile, nonpyrogenic solution of dextrose in water for injection.Each mL contains dextrose, hydrous 50 or 100 mg. 10% Dextrose Injection, USP is hypertonic. They contain no bacteriostat or antimicrobial agent or added buffer and are supplied only in single-dose containers to dilute or dissolve drugs for injection. See Table for other characteristics. Although Dextrose Injection, USP is classified only as a fluid and nutrient replenisher, the small volumes supplied for diluting or dissolving other drugs are used only as pharmaceutic aids for parenteral injection of other drugs. 10% Dextrose Injection, USP may also be used to increase the specific gravity of an anesthetic solution utilized in hyperbaric spinal anesthetic techniques. When used for this purpose, the volume used should be governed by the manufacturer's recommendation for the drug to be administered.

0.9% Sodium Chloride Injection, USP is a sterile, nonpyrogenic, isotonic solution of sodium chloride and water for injection. Each mL contains sodium chloride 9 mg. It contains no bacteriostat or antimicrobial agent or added buffer and is supplied only in single-dose containers to dilute or dissolve drugs for injection. The solution may contain hydrochloric acid or sodium hydroxide for pH adjustment.

Water-Miscible Vehicles

A number of solvents that are miscible with water have been used as a portion of the vehicle in the formulation of parenterals. These solvents are used to solubilize certain drugs in an aqueous vehicle and to reduce hydrolysis. The most important solvents in this group are ethyl alcohol, liquid polyethylene glycol, and propylene glycol. Ethyl alcohol is used in the preparation of solutions of cardiac glycosides and the glycols in solutions of barbiturates, certain alkaloids, and certain antibiotic .Such preparations are given intramuscularly. There are limitations with the amount of these co-solvents that can be administered, due to toxicity concerns greater potential for hemolysis, and potential for drug precipitation at the site of injection.

Non-Aqueous Vehicles

The most important group of non-aqueous vehicles is the fixed oils. The USP provides specifications for such vehicles, indicating that the fixed oils must be of vegetable origin so they will metabolize, will be liquid at room temperature, and will not become rancid readily. The USP also specifies limits for the free fatty acid content, iodine value, and saponification value (oil heated with alkali to produce soap, i.e., alcohol plus acid salt). The oils most commonly used are corn oil, cottonseed oil,peanut oil, and sesame oil. Fixed oils are used as vehicles for certain hormone (e.g., progesterone, testosterone, deoxycorticicosterone) and vitamin (e.g., Vitamin K,

Vitamin E) preparations. The label must state the name of the vehicle, so the user may beware in case of known sensitivity or other reactions to it.

- **Finished product quality control test**

✓ Leaker test
✓ Pyrogen test
✓ Particulate test
✓ Sterility test.
✓ Uniformity of weight.
✓ Uniformity of content

- **Leak test**

• To detect incompletely sealed ampoules.

Principle

10% methylene blue or 0.1% FDC red one or red two. Generally combined with autoclave.

Disadvantage

Leakage of 15 micron in diameter or smaller is not detected. Vial and bottles are not subjected to this test.

- **LAL test**

Limulus Amoebocyte Lysate test or bacterial Endotoxin test for the validation of depyrogenation process.

Reagent - LAL reagent (limulus Polyphemus)

Reaction - In presence of Endotoxin a firm gel is formed within 60 min when incubated at 37^0 C.

- **characteristics**

• Test tube scale.
• Only pyrogen of gram negative bacteria detected.
• Semi quantitative test.
• Sensitivity in terms of Endotoxin unit.
• In-vitro test.
• Doesn"t measure fever producing potential of Endotoxin.
• Sensitivity varies with different microbial source of LAL.

- **Pyrogen test- Fever response of rabbit**

• **Sham test** is performed to select the proper animals for the main tests.
• **Rabbit test** - Qualitative fever response test.

Procedure

• Test solution is injected into the vein of rabbit. Temperature elevation is seen for 3 hrs.

Disadvantage

✓ Biological variation
✓ Expensive
✓ Laborious
✓ Dose dependent.
✓ Not for anti pyretic drug

- **Particulate test USP**

 Visually inspected all (WHITE AND BLACK)
 Any with visible particle is discarded.

• Large volume parental

- 50 particles of 10µm
- 5 particulates of 25 µm per ml

Particulate count is done by:

1. Light obscuration particle count test
2. Microscopic particle count test

Sterilization "The act or process, physical or chemical, that destroys or eliminates all viable microbes including resistant bacterial spores from a fluid or a solid."
Examples of sterilization methods are : steam treatment at 121℃, dry heat at 230℃, flushing with a sterilizing solution such as hydrogen peroxide (H_2O_2) or ozone (O_3), irradiation, and filtration.

■ **Sterility**

"The reduction of anticipated levels of contamination in a load to the point where the probability of survival is less than 10^{-6}."

BIO-INDICATORS FOR VALIDATION OF STERILIZER

- Bio-indicators are preparations of microorganisms innoculated into the product, adsorbed onto paper strips or glass beads, or suspended in a liquid medium and sealed in ampoules for steam sterilization.

Procedures	**Species**
Steam	B. stearothermophilus
	Cl. Sporogenes
Dry heat	B.Subtills var. niger
Gas	B. subtilis var. niger
Radiation	B. pumilus,
	B. cereus,
	B. Sphaericus

Ans 6

Advantages of controlled drug delivery

1. Controlled administration of a therapeutic dose at a desirable rate of delivery.
2. Maintenance of drug concentration within an optimal therapeutic range for prolonged duration of treatment.
3. Maximization of efficacy-dose relationship.
4. Reduction of adverse side effects.
5. Minimization of the needs for frequent dose intake.
6. Enhancement of patient compliance.

Disadvantages of controlled drug delivery systems:

1. Decreased systemic availability
2. Poor invitro-invivo correlations
3. Chances of dose dumping
4. Dose withdrawal is not possible.
5. Higher cost of formulation

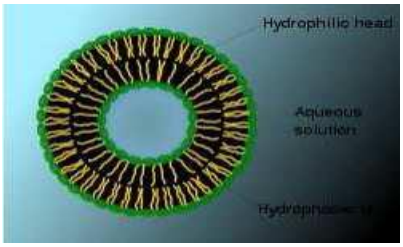
Applications of controlled drug delivery

1. Mucoadhesive drug delivery system
2. Colon drug delivery system
3. Pulmonary drug delivery system
4. Ocular drug delivery system

5. Oral thin films
6. Nasal drug delivery system
7. Gastro retentive drug delivery system
8. Vaginal drug delivery system
9. Resealed erythrocytes

liposomes

A liposome is a tiny bubble (vesicle), made out of the same material as a cell membrane. Liposomes can be filled with drugs, and used to deliver drugs for cancer and other diseases.Liposomes were first described by British haematologist Dr Alec D Bangham FRS in 1961 (published 1964), at the Babraham Institute, in Cambridge. They were discovered when Bangham and R. W. Horne were testing the institute's new electron microscope by adding negative stain to dry phospholipids. The resemblance to the plasmalemma was obvious, and the microscope pictures served as the first real evidence for the cell membrane being a bilayer lipid structure. The name liposome is derived from two Greek words: 'Lipos' meaning fat and 'Soma' meaning body. Structurally, liposomes are concentric bleeder vesicles in which an aqueous volume is entirely enclosed by a membraneous lipid bilayer. Membranes are usually made of phospholipids, which are molecules that have a hydrophilic head group and a hydrophobic tail group. The head is attracted to water, and the tail, which is made of a long hydrocarbon chain, is repelled by water.



Scheme of a liposome formed by phospholipids in an aqueous solution.

In nature, phospholipids are found in stable membranes composed of two layers (a bilayer). In the presence of water, the heads are attracted to water and line up to form a surface facing the water. The tails are repelled by water, and line up to form a surface away from the water. In a cell, one layer of heads faces outside of the cell, attracted to the water in the environment, and another layer of heads faces inside the cell, attracted by the water inside the cell. The hydrocarbon tails of one layer face the hydrocarbon tails of the other layer, and the combined structure forms a bilayer.When membrane phospholipids are disrupted, they can reassemble themselves into tiny spheres, smaller than a normal cell, either as bilayers or monolayers. The bilayer structures are liposomes. The monolayer structures are called micelles. The lipids in the plasma membrane are chiefly phospholipids like phosphatidyl ethanolamine and phosphatidylcholine. Phospholipids are amphiphilic with the hydrocarbon tail of the molecule being hydrophobic; its polar head hydrophilic. As the plasma membrane faces watery solutions on both sides, its phospholipids accommodate this by forming a phospholipid bilayer with the hydrophobic tails facing each other. Liposomes can be composed of naturally-derived phospholipids with mixed lipid chains (like egg phosphatidylethanolamine), or of pure surfactant components like DOPE (dioleoylphosphatidylethanolamine). Liposomes, usually but not by definition, contain a core of aqueous solution; lipid spheres that contain no aqueous material are called micelles, however, reverse micelles can be made to encompass an aqueous environment.

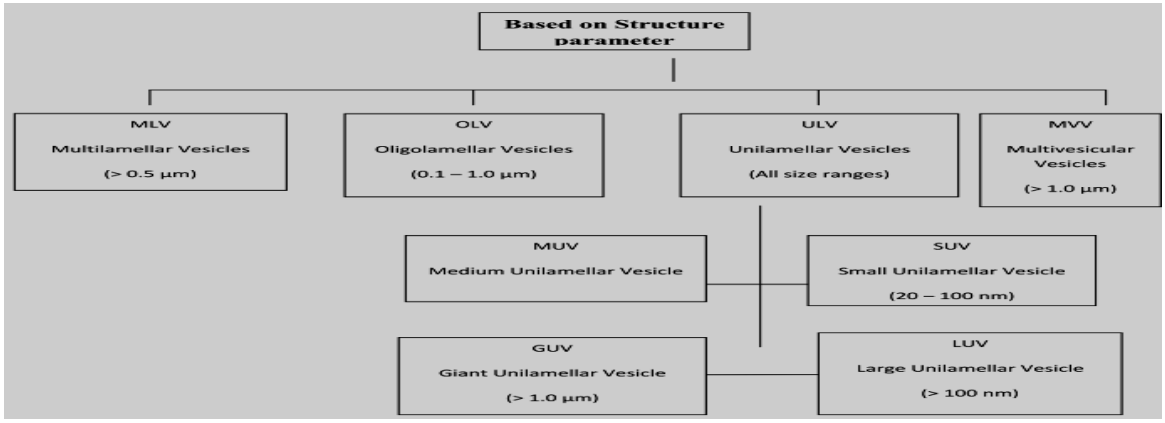
ADVANTAGES

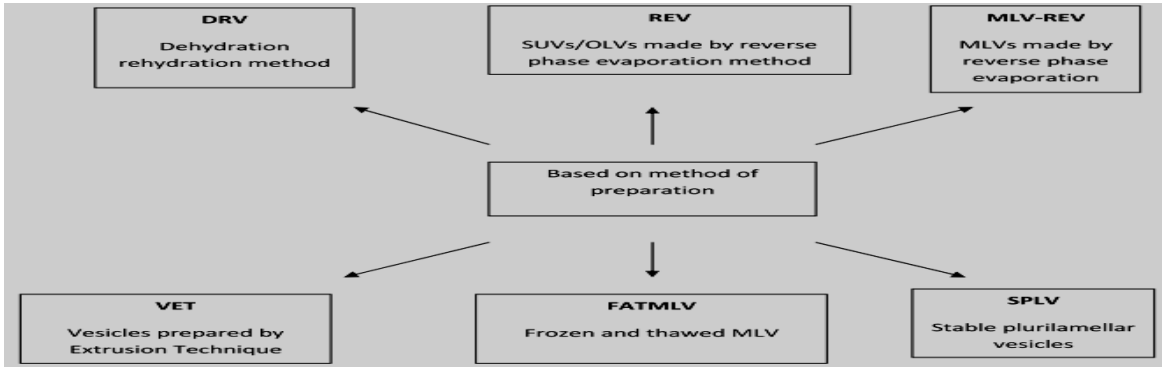
Some of the advantages of liposome are as follows:

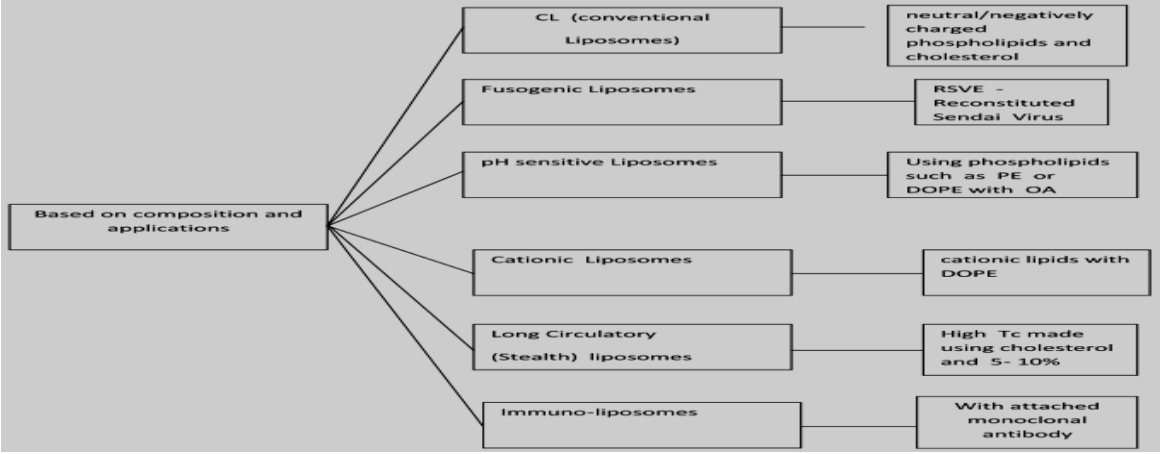
- Provides selective passive targeting to tumor tissues (Liposomal doxorubicin).
- Increased efficacy and therapeutic index.
- Increased stability via encapsulation.
- Reduction in toxicity of the encapsulated agents.
- Site avoidance effect.
- Improved pharmacokinetic effects (reduced elimination, increased circulation life times).
- Flexibility to couple with site specific ligands to achieve active targeting.

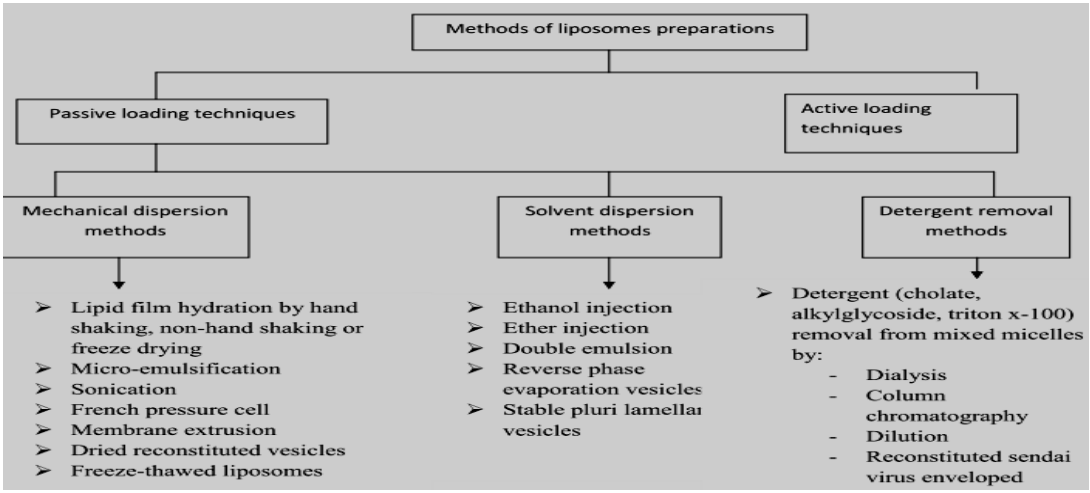
Types of liposomes - Liposomes are classified on the basis of

1) Structural parameters
2) Method of preparation
3) Composition and applications









Methods of liposome preparation: General methods of preparation
All the methods of preparing the liposomes involve four basic stages:
1. Drying down lipids from organic solvent.
2. Dispersing the lipid in aqueous media.
3. Purifying the resultant liposome.
4. Analyzing the final product.

Method of liposome preparation and drug loading
The following methods are used for the preparation of liposome:
1. Passive loading techniques
2. Active loading technique.

Passive loading techniques include three different methods:
1. Mechanical dispersion method.
2. Solvent dispersion method.
3. Detergent removal method (removal of nonencapsulated material).

Mechanical dispersion method
The following are types of mechanical dispersion methods:

1.1. Sonication.
1.2. French pressure cell: extrusion.
1.3. Freeze-thawed liposomes.
1.4. Lipid film hydration by hand shaking, non-hand shaking or freeze drying.
1.5. Micro-emulsification.
1.6. Membrane extrusion.
1.7. Dried reconstituted vesicles.

Applications Of Liposomes

Liposomes are used for drug delivery due to their unique properties. A liposome encapsulates a region on aqueous solution inside a hydrophobic membrane; dissolved hydrophilic solutes cannot readily pass through the lipids. Hydrophobic chemicals can be dissolved into the membrane, and in this way liposome can carry both hydrophobic molecules and hydrophilic molecules. To deliver the molecules to sites of action, the lipid bilayer can fuse with other bilayers such as the cell membrane, thus delivering the liposome contents. By making liposomes in a solution of DNA or drugs (which would normally be unable to diffuse through the membrane) they can be (indiscriminately) delivered past the lipid bilayer. Liposomes are used as models for artificial cells. Liposomes can also be designed to deliver drugs in other ways. Liposomes that contain low (or high) pH can be constructed such that dissolved aqueous drugs will be charged in solution. As the pH naturally neutralizes within the liposome (protons can pass through some membranes), the drug will also be neutralized, allowing it to freely pass through a membrane. These liposome's work to deliver drug by diffusion rather than by direct cell fusion. Another strategy for liposome drug delivery is to target endocytosis events. Liposomes can be made in a particular size range that makes them viable targets for natural macrophage phagocytosis. These liposomes may be digested while in the macrophage's phagosome, thus releasing its drug. Liposomes can also be decorated with opsonins and ligands to activate endocytosis in other cell types.

- The use of liposomes for transformation or transfection of DNA into a host cell is known as lipofection.
- In addition to gene and drug delivery applications, liposomes can be used as carriers for the delivery of dyes to textiles, pesticides to plants, enzymes and nutritional supplements to foods and cosmetics to the skin.

The use of liposomes in nano cosmetology also has many benefits, including improved penetration and diffusion of active ingredients, selective transport of active ingredients; longer release time, greater stability of active ingredients, reduction of unwanted side effects and high biocompatibility. Another interesting property of liposomes is their natural ability to target cancer. The endothelial wall of all healthy human blood vessels is encapsulated by endothelial cells that are bound together by tight junctions. These tight junctions stop any large particle in the blood from leaking out of the vessel. Tumour vessels do not contain the same level of seal between cells and are diagnostically leaky. This ability is known as the Enhanced Permeability and Retention effect. Liposomes of certain sizes, typically less than 400nm, can rapidly enter tumour sites from the blood, but are kept in the bloodstream by the endothelial wall in healthy tissue vasculature. Both hydrophilic and hydrophobic drugs can be encapsulated in liposomes. Liposomes are also relatively non-toxic and biodegradable.

Protection against enzyme degradation of drugs

Liposomes are used to protect the entrapped drug against enzymatic degradation whilst in circulation. The basis is that the lipids used in their formulation are not susceptible to enzymatic

degradation; the entrapped drug is thus protected while the lipid vesicles are in circulation in the extracellular fluid.

Drug targeting

The approach for drug targeting via liposomes involves the use of ligands (e.g., antibodies, sugar residues, apoproteins or hormones), which are tagged on the lipid vesicles. The ligand recognises specific receptor sites and, thus, causes the lipid vesicles to concentrate at such target sites. By this approach the otherwise preferential distribution of liposomes into the reticuloendeothelial system RES (liver, spleen and bone marrow) is averted or minimized.

Topical drug delivery

The application of liposomes on the skin surface has been proven to be effective in drug delivery into the skin. Liposomes increase the permeability of skin for various entrapped drugs and at the same time diminish the side effect of these drugs because lower doses are now required.

Treatment of human immunodeficiency virus(HIV) infections

Several antiretroviral nucleotide analogues have been developed for the treatment of patients suffering from the acquired immunodeficiency syndromes (AIDS). These include antisense oligonucleotide, which is a new antiviral agent that has shown potential therapeutic application against HIV-1.

Enhanced antimicrobial efficacy/ safety

Antimicrobial agents have been encapsulated in liposomes for two reasons. First, they protect the entrapped drug against enzymatic degradation. For instance, the penicillins and cephalosporin are sensitive to the degradative action of J-lactamase, which is produced by certain microorganisms. Secondly, the lipid nature of the vesicles promotes enhanced cellular uptake of the antibiotics into the microorganisms, thus reducing the effective dose and the incidence of toxicity as exemplified by the liposomal formulation of amphotericin B.

EVALUATION

a. Estimation of Entrapped Drug

Drug entrapped within the liposomes was estimated after removing the unentrapped drug, which was separated by collecting the supernatant after subjecting the dispersion to centrifugation in a cooling centrifuge at1000 rpm at a temperature of 4_0C for 30 min, where upon the pellets of liposomes were washed again with buffer to remove any unentrapped drug and the washing was combined with supernatant and was analyzed for drug content at 252nm.

b. Entrapment Efficiency Percentage

= Entrapped drug/Total drug × 100

Surface Characterization by SEM studies

***c. In Vitro*drug release study**

d. Release Kinetics and Mechanisms

The rate and mechanism of release of drug from the prepared Liposomes can be analyzed by fitting the release data into following

i) The zero order equation can be represented as

$$C = C_0 – K_0t$$

Where

C is the amount of drug released at time t

K_0 is the release rate

ii) The first order equation can be represented as

$$LnC = LnC_0 – K_1t$$

Where

K_1 is the release rate constant

iii) The Higuchi equation can be represented as

$$Q = K_2t_{1/2}$$

Where
K_2 is the diffusion rate constant
The release profile data was substituted in different kinetic models viz… Zero order kinetics, first order kinetics and Higuchi kinetics.

Dr ALPANA RAM
ASSOC. PROF
PHARMACY