INTRODUCTION

PREAMBLE:

The Pharmaceutical Industry worldwide caters to the specific health needs of the patients suffering from various diseases by designing and supplying suitable dosage forms which are popularly called as Formulations in the parlance of the Pharmacists. An urge for preparing specialized drug delivery systems as emanated in order to enhance Bioavailability and deliver the drugs at the desired specific sites of the human body. Trans-dermal drug delivery systems are very novel. They are exemplified by Phytosomes, Nanoparticles, Lyposomes, Transferosomes, Ethosomes, Liposomes, Niosomes, Cubosomes, Virosomes, Nanoemulsions, Cochleates etc. Among these Ethosomes are in the forefront which improves the Bioavailability and delivery the drug at the desired specific site. They also have excellent patient compliance and can be used either in paediatrics or geriatrics.

1. INTRODUCTION:

The most frequently used dosage form is administered orally Even though this route has prominent pros but it also has significant cons namely getting metabolized in the first stage and destroyed in the GI tract. To rectify these defects an innovative dosage form has been contemplated. Through recent researches it has been put forth that the skin plays a pivotal role as a very important medium of drug delivery into the systemic circulation.

The formulations intended for topical application/administration, controlled & continuous delivery of active ingredients into the blood stream for circulation are covered under TDDS. Permeating into the skin the drug gets distributed by the help of the TD patch. The TD patch is sticky patch which is kept in the skin. The TD patch on its interior contains a high dose of drug. This is to be placed on the skin for defined time period.

The drug is diffused to lower concentration from the higher concentration area i.e., from patch to the blood stream until it reaches the equilibrium. The drug diffuses through the blood uninterrupted over a time period there by maintaining a constant blood concentration. The TDDS can be compared to an instrument which can be applied on the skin which helps in systemic drug delivery in sufficient concentration. Hence transdermal administration can abstain from this additional limitation which is found in oral drug delivery.
Advantages over the Oral dosage forms:

- It circumvents problems related with GI absorbed due to GI pH, drug-food interactions, enzymatic activity etc.
- It is alternate route to giving drugs orally, which is not suitable in vomiting diarrhea etc.
- It passes up hepatic “first pass” effect.
- It avoids the hazards and difficulty of parenteral route.
- It develops control of the concentration of drug with short therapeutic index.
- It reduces inter and intra patient variation.
- It offers suitability for self-administration.
- It reduces dose frequency, recovering patient compliance.
- It extends action of drugs which have small plasma half-life via the drug reservoir nearby controlled release characteristics.
- It rapidly terminates action of drug by eradication of dosage form from the skin surface.
- It reduces hazards and difficulty of I.M. injections or I.V. infusions.
- It increases therapeutic effect, decreases adverse effect as it helps in establishment of drug concentration in blood -time profile and elimination of pulse entry of drugs into the systemic flow.
- It supplies conventional action over extended duration of time and ability to approximate zero-order kinetics.

Limitation of trans-dermal delivery:

- It has need of optimal physicochemical properties for the medicament to go through stratum corneum and small dose of medicament needed for therapeutic value.
- It is not easy and sometimes it may not be possible. Normally, drugs with beneficial dose less than 5 mg/day are chosen to be transported through trans-dermal route.
- Skin Membrane irritation and contact dermatitis have been reported sometimes due to some of the adjuvant and penetration enhancers which are another limitation for such delivery.
Before a Trans-dermal Dosage form is developed during the investigational drugs development, careful study is needed.

- The drugs which are somewhat efficacious are suitable for this type of delivery through trans-dermal system due to the impermeable nature of the skin\(^5\).
- The use of this system may lead to the occurrence of contact dermatitis due to the components resulting in midway discontinuation\(^6\).
- This system is not preferable in such cases where there is a requirement of higher blood levels\(^7\).
- This system may be exorbitant in cost.

**Scope of Ethosomes:**\(^4\,5\,6\)

- The Ethosomal delivery systems skip the enzymatic degradation thus decrease the food-drug interaction.
- These are helpful in the disorders like vomiting and diarrhea for conventional drug administration without loss of drug.
- Ethosomes deliver the drug direct into the systemic circulation without entering into the portal circulation and also it skips mechanical and chemical digestion thus drug loss is minimized.
- The Ethosomal system of drug delivery is non-invasive, is convenient compared to that parenteral route which is inconvenient..
- As they provide continuous drug supply, thus frequency of dose can be avoided.
- Thus Single application gives better therapy than other dosage forms.
- They act as drug reservoir and help to increase the half life or the continuous, controlled release of drug leading to better therapy.
- Due to their physical features, they can be rapidly identified in emergencies (for example, patients who are unconscious, unresponsive and in coma).
1.2 ANATOMY OF HUMAN SKIN:

Skin is the largest organ with different functions since they are achieved due to its unique anatomy that consists of different skin layers which can be described as follows.

- Outer most layer of skin is of epidermis that consists of:
  - A. Stratum corneum (outer most layer)
  - B. Viable dermis.
- Dermis.
- Sub cutis or subcutaneous fatty tissue.

In the human body skin is found to be an extensive organ which occupies an area of nearly 2m². The skin is present in the form of multilayer with an inflow of one third of the total which circulates in the body. Human skin in general makes up into three prominent layers of tissues which are mutually dependant on each other.

- Stratified, vascular, cellular epidermis.
- Connective tissue is the second layer under the stratified tissue.
- Subcutaneous layer of hypodermis.

Each layer comprises of their independent function and importance that gives the structure and integrity to the whole body.

![Figure 1: Proposed mechanism of drug absorption through skin](image)
Stratified vascular cellular epidermis. This layer of tissue differs in its thickness and this variation is mainly due to the size of the cell and the layers of cells comprised in

![Figure 2: Structure of human skin](image)

It which varies at various places like it is 0.06mm on eyelids and is very thick at soles and palm i.e; of 0.8mm. It is comprised with an outer layer known as stratum corneum and succeeded by epidermis. Epidermis is comprised with an active basal epithelial cell population and is found to be of the thickness 150 micrometers. During the cell division and tissue differentiation old and dead cells move towards outer and peripheral regions from inner layers of skin. Underneath this layer there are other layers which constitute to form viable epidermis, which consist of: stratum lucidum, stratum granulosam, stratum spinosum and stratum germinativum.

**a. Stratum corneum:**

The peripheral layer of the skin also called as Horney layer. It has an approximate thickness of about 10mm in dry state but may swell several times of this thickness when in hydrated state. It comprises of cells in10-25 layers which are dead and made up of keratin known as corneocyte. It is flexible in nature but comparatively impermeable. As this layer consists of Keratin cells irrigated with lipid “motar” that act as barrier for the drug penetration.
b. Viable epidermis:

This layer varies in its thickness from 0.06 to 0.8mm i.e.; in eyelids and in soles because of the various layers thickness varies that are in this layer, which are replaced by new cells that are proliferated by Mitosis. 7.

Epidermis:

This outer layer that is thickest of all the layers is due to the presence of keratin cells. It is tough and acts as the cornier layer of the body. It does not allow the penetration of water, liquids and microorganisms into the body. The keratin cells are generated in the inner layer of the epidermis that travels towards the peripheral and replace the old dead cells with new cells. The outer most layer of epidermis is called as stratum corneum, this layer at some areas of the body consists of melanin to become tougher and provides more thickness to avoid damage to the internal organs and blood vessels.

Due to presence of melanin, sunlight that can damage DNA cannot penetrate into the body. Thus it protects from harmful UV radiation, leading to cancer. There are some special cells called Langerhans cells that provide skin's immune system by identifying and destroying foreign microorganisms causing disorders.

B. Dermis:

This layer is lying beneath the epidermis and consists of sebaceous glands, sweat glands that help in controlling the body temperature, fluid balance and keep the skin moisture. Based on the temperature difference the sweat glands secrete sweat that consists of salts, ions, electrolytes, water and other chemicals that helps cool the body. Apocrine sweat glands secrete an oily, thick and odorous sweat. To keep the body moist and to avoid the foreign substances from entering into the body, the sebaceous glands secrete sebum into hair follicles. Sebum is oil, which keeps the skin moist and soft and acts as a barrier against foreign substances.

Hair also plays a vital role in body temperature regulation, body appearance and in protection of body. The hairs are of different type based on the origin of hairs the hair follicles produce different types of body hair. Follicles have stem cells that help in the growth of damaged epidermis.

The nutrients to the dermis are provided by the blood vessels that carry blood and nutrients to the dermis. They also help to maintain body temperature. Temperature helps in blood vessels enlargement to ease heat by allowing more blood to skin. In cold temperature,
blood vessels constrict to avoid body heat of blood to get eliminated. The number of hairs, sebaceous glands, hair follicles, blood vessels and nerve endings vary at different parts of the body, for example on soles of palms and feet hairs are absent where as they are many on head.7.

C. Subcutaneous:

It lies beneath the dermis and epidermis and acts as a supporting tissue. It is also known as subcutaneous fat tissue which stores fat in it. It plays a prominent role in regulation of body temperature, energy storage and mechanical protection. It helps in supplying of nerves and blood vessels to the skin that makes the skin pressure sensory organ. In TDDS drug passes through via all these layers and then it reaches the systemic circulation but in topical DDS the drug has to pass stratum corneum and its retention in skin layers is desirable7.

1.3 ROUTES OF PENETRATION:

There are two possible major options for the diffusing drugs to enter into the systemic circulation.

(a) Via epidermis

(b) Shunt pathway (Diffusion)

The two major routes of drug penetration are through sweat ducts, hair follicles and sebaceous glands.
Figure 4: A- multilayer skin model showing sequence of Transdermal permeation of drug for systemic delivery, B-Intracellular verses transcellular diffusion
Trans-corneal penetration:

Intra cellular penetration:

The drug passes via the cells of stratum corneum. Usually hydrophilic drugs undergo this process. Water is accumulated on the filament of protein peripherally whenever the stratum corneum gets hydrated and facilitates the movement of polar molecules.

Intercellular penetration:

The drug molecules dissolve and get diffused into the non-aqueous area present between the protein filaments. No polar substances follow this route of penetration.

Trans-appendageal penetration:

This is otherwise known as shunt pathway\(^{11}\) in which drug gets transfused through the hair follicle. In the aqueous pathway sweat glands play vital role and pilo sebaceous glands play key role for drug transfusion by sebaceous\(^{10}\). Due to its comparatively smaller area which is less than 0.1% compared to the surface of area the trans-appendageal pathway is of least importance but preferable for large polar compounds.

The route of penetration depends on the physicochemical properties of drug and partition through the various layers of the skin. This can be explained in the form of series of steps.

- The drug adsorption and penetrating on S.Corneum surface.
- Drug Diffusion through viable epidermis and S.Corneum.
- And lastly through the papillary dermis into micro circulation.
Figure 5: Simplified representation of skin showing routes of penetration: 1. through the sweat ducts; 2. directly across the stratum corneum; 3. via the hair follicles.

1.4 OPTIMIZATION TECHNIQUES USED RECENTLY TO IMPROVE TDDS:

A. Enhancement Techniques Based on the Structure\textsuperscript{13,14,15,16}:

1. Micro fabricated Micro needles:

This practical method is used to increase the penetration of TDDS. It is on a boom in recent years. It uses physical pathway via upper epidermis. These devices are a combination of transdermal patch and hypodermic needle where in the micro dimensional needles are inserted into the surface of the skin.
Figure 6: Micro-needle Delivery Device Design

(A) Hollow Micro-needles  (B) Solid Micro-needles

Micro needles are little lustrous devices which are made by the techniques of silicon etching and micro-mechanical system. Various methods are available for administering the drug by use of Transdermal drug delivery system. They are as follows:

![Image of micro-needle delivery device design]

**Figure 7: The basic design of micro-needle delivery devices.** Needles of approximately with or without centre hollow channels are placed onto the skin surface so that they penetrate the stratum corneum and epidermis without reaching the nerve endings present in the upper dermis.

**a. Poke with patch approach:**

Initially the skin is pierced which is succeeded by the patch of drug which is applied at the site of treatment.
b. **Poke and Coat approach:**

The drug is applied like a coat on needle which is then pierced into the skin wherein further dissolution of the medicament occurs.

c. **Biodegradable micro needles:**

The biodegradable polymeric micro needles encapsulate the drug which is further inserted into the skin.

d. **Hollow needles:**

A hollow shaped bore is used to inject the drug via need.

2. **Macro flux:**

This method constitutes a micro projector array made of titanium by which a superficial pathway is made via the layer of skin. It is mainly comprised of a disk made of titanium which is fixed to polymeric adhesive material. The titanium disk measures about $8\text{cm}^2$ and constitutes of the drug coated tiny, titanium made tooth like micro projections. 300 micro projections are present for every centimetre length where as a single micro projection has a length of 200 micro meter.

The layer of dead cells of stratum corneum comprising of length 10-25micro meter is penetrated where 'holes' known as micro channels are created which allows the passage of large sized molecules into the further most inner layers of epidermis. The titanium made micro projections are tiny enough to induce pain. Thus this is a comfortable method to deliver the trans-dermal passage of larger molecular weight drugs, for example: Insulin, vaccines and several peptide hormones. This novel system allows patients to receive drugs for about 12 week’s duration.

**Macro flux have been designed in three different models, they include:**

1. **Dry-coated macro flux system:**

   In this type delivery takes place in short period. It is constituted of drug coated micro projection array which is attached to an elastic adhesive backing made of polymer.
2. **D-TRANS Macro flux system:**

   It consists of a combination of micro projection array and reservoir of drug which is used for the short time period administration.

3. **E-TRANS Macro flux system:**

   This system is used when there is demand and is a combination of micro projection array and electro transport system.

3. **Trans-dermal Metered- Dose Spray (TMDS):**

   This system is for topical purpose which is in the form of solution constituting of vehicle which is volatile in nature, where as the drug is totally dissolved in solution form. The permeation of drug is satisfactory and sustained level is approached.

**The pros of TMDS are as follows:**

- It is acceptable.
- It is flexible to use.
- Manufacture is simple.
- The non-occlusive property aids in improving delivery with absence of irritation of skin.

**B. Electrically- Based Enhancement Techniques:**

1. **Iontophoresis:**

   In this type of technique the placement of medicament is over the skin beneath two electrodes with a passage of (<0.5mA) current which drives the drug into the skin away from the electrode\textsuperscript{13}.
Figure 8: The basic principle of ionto-phoresis. A current passed between the active electrode and the indifferent electrode-repelling drug away from the active electrode and into the skin.

Figure 9: The basic principle of phonophoresis. Ultrasound pulses are passed through the probe into the skin fluidizing the lipid bilayer by the formation of bubbles caused by cavitation.
2. **Ultrasound:**

   This technique promotes the trans-dermal route transportation of medicament via skin of wrist watch sized which is known as sonophoresis or phonophoresis. A coupling agent (gel or cream or ointment) is mixed with the drug which acts as a medium for transferring ultrasonic energy to the skin from the device. There is an involvement of breakage of the lipid molecules of stratum corneum thus promoting permeation of drug through the layers. It utilizes ultrasound to understand the property of stratum corneum\textsuperscript{14,15}.

3. **Photomechanical Waves:**

   A change in the lacunars system is produced by usage of the mechanism of photochemical wave resulting in transient channel formation via stratum corneum through the mechanism of permeabilization\textsuperscript{16,17}.

4. **Electroporation:**

   Using the 100-1000 volt/cm of short electrical pulses the two layers of lipids are perforated to produce aqueous pores. It may also bemuse as a combination with Iontophoresis to increase the peptide permeation\textsuperscript{17}.
Figure 10: The basic principle of electroporation. Short pulses of high voltage current are applied to the skin producing hydrophilic pores in the intercellular bilayers via momentary realignment of lipids.

5. Electro- Osmosis:

It is defined as the volume flow or bulk fluid flow, when there is a different voltage is applied to a charged porous membrane\textsuperscript{18}.

1.5 VELOCITY BASED ENHANCEMENT TECHNIQUES \textsuperscript{19,14,16}:

Needle - Free Injections:

a. Jet syringe
b. Iject
c. Implaject
d. Intraject
e. Mini-ject
f. Crossject
g. Jet syringe

2. Powder ject devices:

Solid particles of size (20-100 mm) are fired into the inner layers of skin through S.corneum by the aid of helium gas in the form of a supersonic shock.

Other Enhancement Techniques:

1. Liposomes:

Concentric bimolecular layers of colloidal particles with a capability of encapsulating the drugs are known as liposomes. They also have the capability to penetrate epidermis by enclosing a liquid volume along with the drug\textsuperscript{13}.

2. Transferosomes:

They are modified liposomes in combination with edge activator like sodium cholate. These forms enter the subcutaneous layers of skin by leaving the usual route of cutaneous capillaries because of their large size. The carriers of transferosomes can make a depot of drug with a higher concentration of drug into the systemic circulation\textsuperscript{14}. 
3. Skin abrasion:

In this method the superficial layer of skin is removed so as to deliver the drug. Generally the impermeable upper layers of the skin are eroded for the formation of micro channels with the help of a very small particle size\(^{15}\).

4. Medicated Tattoos:

The temporary tattoo is modified to form Med Tats which comprises of the active ingredient of the drug for delivery through transdermal route. Usually this route is preferred in children who cannot use the common dosage forms\(^{17}\).

5. Laser Radiation:

A laser beam is applied in a direct and controlled manner onto the skin resulting in burning of the stratum corneum without much damage to the epidermis thus enhancing lipophilic and hydrophilic drug delivery\(^{20}\).

6. Super saturation:

The use of super saturation system can raise the thermodynamic nature of the drug that increases the permeation of tropically used formulation due to the increase in unity value. The permeation through the skin is dependent on the saturation degree and not dependent on the absolute concentration value of the drug\(^{17}\).

7. Magnetophoresis:

The magnetic field increases when the applied strength is increases that is applied on drug diffusion flux\(^{15}\).

1.6. Types of Trans-dermal patches:

a. Single layered drug with adhesive:

They contain drug in a monolayer which is in between the adhesive materials in a temporary liner to release the drug in the skin\(^{21}\).

b. Multi-layer drug in adhesive:

It constitutes one layer of immediate release of drug and other layer of controlled drug along with the permanent backing and temporary liner in adhesive so as to release the drug\(^{22}\).
c. Vapour patch:

They constitute an adhesive layer which plays a role in both adhering the other layers and also in releasing of vapour. These patches are novel type for the release of essential oils in the problem of decongestion. These are various models in the market to manage the sleep quality and cigarette cessation\(^2\).

d. Reservoir system:

In backing layer the drug reservoir is encapsulated, that is a rate controlling membrane and impervious in nature, which may micro-porous or non-porous. The drug may be modified and can be formulated as a gel, suspension, solution and can also be dispersed in solid matrix polymer within the drug reservoir. Drug compatible with polymeric hypo-allergic adhesive membrane can be applied externally\(^2\).

e) Matrix system:

i. Drug-in-adhesive system:

Drug-in-adhesive system involves in the drug to disperse in the adhesive polymer and then it is spread by melting or casting the solvent on a backing layer which is impervious in nature. This is together known as the drug reservoirs which are coated with unmediated type of adhesive layers made of polymer for the purpose of protection.

ii. Matrix dispersion system:

In a polymer matrix of hydrophilic and lipophilic nature there is a homogenous dispersion of the drug. The backing layer which is impermeable to the drug is fabricated in a compartment constituting an occlusive base plate on which polymer disk containing drug is fixed on it. Strip formation is done by the adhesive is spread on the circumference of the drug reservoir.

f. Micro reservoir system:

This system comprises form of combined matrix-dispersion system and the reservoir. The drug suspension in a liquid polymer solution soluble in water and then dispersion of the solution in a homogenous manner in a polymer of lipophilic nature to form many microscopic spheres of reservoirs of drugs which are thousands in number.
Stabilization of unstable thermodynamically dispersion is achieved by cross-linking polymer with aid agents used for cross linking.

1.7 TDDS PREPARATION BY VARIOUS METHODS:

a. TPX membrane Asymmetric method:
A polyester film of type 1009 and of 3m which is heat sealable with a 1cm diameter of concave can be used in the form of backing membrane; by this the fabrication of patch of prototype can be done. The concave membrane which is encapsulated by a TPX [poly (4-methyl-1-pentene)] membrane which is asymmetric in nature and enclosed with an adhesive material has the dispersion of the sample of drug in it.25

b. Teflon Circular mould method:
In a half of the organic solvent with different ratio of polymers is utilized to mix with calculated amount of drug and in the rest of the solvent enhancers are added as solvent. Enhancers in concentrations of different amount are mixed in the other remaining half quantity of organic solvent and then addition is done in it. A plasticizer like Di-N-butyl phthalate is an extra addition in the polymer solution of the drug.

These contents are put in to the mould after stirring for 12 hrs and are put into the mould made of circular Teflon. The moulds are then kept on to the surface which are levelled properly and then it has a covering of funnel which is inverted in nature to put a control on the vaporization of solvent in a model of laminar flow hood with 0.5 meter per second of air speed.

Evaporation of solvent is done for about 24hrs and the films which are dried are needed to be stored for another 24hrs at a temperature of about 25+/− 0.5degree centigrade. This storage is done in silica gel based desiccators prior to evaluation for the elimination of effects of ageing. These films need to be evaluated within duration of 1 week of its preparation.26

c. Mercury substrate method:
This technique involves the introduction of drug in the mixture comprising of polymer solution and plasticizer. Stirring of the resultant mixture should be done for about 10-15mins to get a resultant dispersion which is homogenous in nature and then this resultant dispersion
is poured into a mercury surface which is levelled and an inverted funnel is used to cover it to prevent the evaporation of solvent\textsuperscript{27}.

\textbf{D. IPM membranes method:}

Involves the use of Carbomer 940 polymer in blend of propylene glycol and water in which using magnetic stirrer drug is dispersed, by stirring periodically for 12 hrs. This dispersion's viscosity is enhanced and its neutralization is done by the addition of the substance triethanolamine\textsuperscript{27}.

\textbf{E. EVAC membranes method:}

For the preparation of final transdermal route therapeutic system, the rate controlling membranes which can be used are 1% reservoir gel of Carbopol, polyethylene, ethylene vinyl acetate copolymer. The use of propylene glycol can be preferred for water insoluble drugs.

Drug is dissolved in propylene glycol by further addition of Carbopol resin and then neutralization of the solution takes place by adding 5% w/w sodium hydroxide solution and then on a strip of backing layer the gel constituting the drug is placed. Rate controlling membrane is applied on the gel and the borders are sealed using heat and hence a device which is leak proof is obtained\textsuperscript{29}.

\textbf{f. Aluminium backed adhesive film method:}

Unstable matrices may be obtained in the transdermal delivery system in cases where the loading dose exceeds 10mg. In such cases adhesive films which are backed with aluminium are found to be suitable and preferable. To prepare such films the solvent to be used is chloroform as almost many drugs and adhesives easily get dissolved in it. Initially the drug is mixed in the chloroform and a material which behaves as an adhesive in addition to the solution of drug and mixed properly. A custom made of aluminium former enclosed with foil of aluminium and its edges are blanked off with the aid of cork blocks which are tightly fit into it\textsuperscript{30}.

\textbf{g. Preparation of TDDS by using pro Liposomes:}

With the usage of film deposition technique preparation of pro liposomes are done by carrier method. By taking the earlier reference the medicament and lecithin can be taken in the ratio of 0.1: 2.0 as an optimized option. For the preparation of pro liposome’s 5mg of powder of Mannitol is taken in a round bottomed flask of 100ml and kept at a temperature of
60-70 degrees centigrade and then rotation of the flask is done at the rate of 80-90 rotations per minute, using vacuum drier Mannitol powder is dried for about 1/2 hour. The temperature of water bath is adjusted to 20-30° after the completion of the drying. Drug and lecithin is dissolved in mixture of suitable organic solvent and 0.5ml of this solution is taken in RBF at 37degree centigrade, then 0.5ml of sol.

Is mixed after completion of the drying. After final loading the flask containing pro liposome is connected to the lyophilizer and the Mannitol powder with the drug is kept in desiccators for a night and then the resultant product is sieved via 100 meshes.

The resultant powder is put into a bottle made of glass and it is stored for freezing until it is characterized30,31.

h. By using free film method:

By using the casting method a cellulose acetate free film is made by producing polymer sol. With chloroform, casting is done on surface of mercury. Plasticizers of 40%w/w of polymer also incorporated. A glass ring is taken and kept on the mercury surface already available in a glass petridish to this a quantity of 5 ml polymer solution is added. An inverted funnel is placed on the petridish to control solvent evaporation the solvent is totally evaporated. The mercury surface is observed for the formation. The film is allowed to dry and is removed and placed between wax paper sheets in desiccators until its further use. If we require free films with varied thickness they are obtained by adding different polymer solutions.32
1.8 Classification of TDDS:

Figure 11: Classification of Trans-dermal drug delivery system
1.6.1 Nano particles:

The colloidal particles which have varied size of about 10-1000 nm diameters are known as nanoparticles. They are prepared by the aid of 5-10 polymers which are biodegradable and in which an active pharmaceutical ingredient is entrapped/ absorbed/ chemically coupled. 33-38

Nano particle classification:

Category Example:

- Nano- tubes carbon, (fullerenes).
- Nano wires- Metals, semiconductor, oxides, sulphides, nitrides.
- Nano crystals- Quantum dots insulators, semiconductor, metals, and magnetic materials.
- Other nano particles - ceramic oxides, metals.
- Nano bots bio chips, nubots

1.6.1.2 TRANSFEROSOMES:

The German company by name IDEA AG has registered a trademark name known as transferosomes (latin word transfere that means carry across and soma means body) to refer to this drug delivery technique.

The transferosomes vehicle is an artificial carrier resembling with the cell vesicle involved in exocytosis and thus it is suitable for the delivery of the drug which is controlled and targeted potentially. 39, 40

PROS:

- The permeability of the drug via skin is much more due to the flexible nature of its membranes 3.
- These constitute moieties which are hydrophobic as well as hydrophilic in nature and thus have the capacity for the accommodation of the drug molecules which have a solubility nature of wide range.
- The drugs which have large as well as small molecular weight can also be carried in it. For example: Different drugs like analgesics, anaesthetics, albumin, gap junction protein, sex hormone, anti cancer and corticosteroid 4.
• More amount of drug can also be carried in these transfersomes. 90% of lipophilic natured drug can be carried.
• Thus the medicament is protected from the degradation by the atmosphere.
• Natural phospholipids are used for the preparation of transfersomes thus making its nature biodegradable as well as biocompatible.
• The specific therapy can be cited by the selection of the composition and mode of administration which is suitable. It can be applicable for both delivery of the drug as well as systemic delivery of the drug.
• The release of the contents can be slow and gradual due to its depot form.
• The method is simple so it can be scaled up.

Limitations:

• Due to its oxidative degradation nature, these transfersomes are found to have chemically unstable nature.
• The natural phospholipids do not have purity so it acts as a limitation to accept these as carriers for delivery of the drug.
• The preparation of transfersomes is costly.

1.6.1.3 Phytosome:

It is a technology which is patented in nature and which is manufactured by a leading company which manufactures both drugs and nutraceuticals. It is used for the incorporation of extracts of plants which are standardized in nature (or) the constituents extracted from plants which are soluble in water for the production of complexes of molecules which are compatible with lipids known as Phytosome. Thus it improves the bioavailability nature and absorption nature. The parameters of pharmacokinetics and pharmacology are present in improvised format thus can be preferred for the treatment of liver diseases of both acute and chronic nature which have a nature of origin of infection as well as degeneration.

It is also beneficial for anti-inflammatory action and in composition of pharmaceuticals and cosmetic nature. The Phytosome is derived by the reaction of soy phospholipids and derivatives of botanical origin in an opportune solvent. Due to their characteristics of spectroscopy as well as physical-chemical nature these are taken into consideration as new entities.
Pros of Phytosomes:

The absorption of polar constituents derived from the plant which are not soluble in lipid is enhanced via both the routes oral and topical administration thus showing improved bioavailability and hence improvised benefit in the therapeutic nature is obtained\(^\text{13}\).

When the bioavailability of the active ingredient is enhanced, then there is a reduction in the requirement of its dose. The ingredient used in the preparation of Phytosomes is phosphatidylcholine which has the nature of hepatoprotective and also acts as a vehicle. Hence it gives an effect of synergism when other drugs of hepatoprotective nature are given\(^\text{14}\).

The stability is maintained as there is a formation of chemical bonds binding both phytoconstituent as well as phosphatidyl molecule. Phospholipids add an extra benefit of nutrition.

1.6.1.4 Niosomes:

By hydrating the surfactants which are non-ionic and synthetic in nature by either incorporating or not incorporating cholesterol (or) other lipids we can obtain niosomes. These have similarity to liposomes and are vesicular systems and can be used as vehicles for drugs with amphiphlic and lipophilic nature.

Niosomes are formed by the mixing of cholesterol and surfactants of non-ionic property by hydrating them\(^\text{2}\).

Pros of Niosomes:

- It can be applied for cosmetic and therapeutic purpose.
- The therapeutic performances of the medicament molecule are improvised by slowing the rate of clearance from the systemic circulation. By this the medicament is protected from the pharmacological environment.
- Niosomal dispersion which is present in aqueous nature is emulsified into a phase of non-aqueous nature to promote delivery.
- They are active osmotically and they have stability, they also enhance the stability nature of the drug which is enclosed in it.
• The surfactants require no special care for handling them and no special requirement for storing them.
• The permeation of drug through transdermal is improved and bioavailability of drug is enhanced which is poorly observed orally.
• The medicament reaches target site where action is to be showe, via different routes like oral, topical and parenteral.
• They have an appearance in which all the different moieties are constituted together of hydrophilic, lipophilic and amphiphlic nature and thus accommodation of drug molecules with varied solubilities takes place.
• The formulation of vesicle is of variable and controlled character. The vesicle characters can be controlled by altering composition of vesicle, lamellarity nature, size of the vesicle, surface charge of it, tapped volume and concentration.

1.6.1.5 Lipospheres:

These constitute of microscopic particles which are solid in nature and dispersible in water and have the diameter from 0.1 to 100 micrometer range. These are comprised of external layer of phospholipids which is enveloped on the internal core of triglycerides also known as hydrophobic fat core which is in solid state. This core is comprised of a material which is bioactive in nature and which is impregnated in the solid matrix.

The lipospheres are used to deliver different types of drugs like anti-inflammatory drugs, antibiotics, local anaesthetics and anti-cancer agents in a controlled manner. These also act as vehicles for vaccines and adjuvants. In recent times for delivering the drug orally, lipospheres have been opted.

Pros of Lipospheres:

They have much better pros when compared to the other systems.

• The physical stability of the substance is increased due to the absence of coalescence.
• The dispersing nature in aqueous medium is greater.
• The cost of ingredients is also low.
• Scaling up and preparation of lipospheres is also very easy.
• Drugs with hydrophobic nature are entrapped excessively.
• The particle size is controlled in nature.

The drug molecules which are incorporated have decreased mobile nature which reduces the leakage of the drug, circumvention of instabilities because of the interacting emulsifier film and drug molecules. The drug which is enclosed in it has a property of extended release after a single dose injection. Once the lipid matrix is solidified the static interface promotes the surface modification of the particles of carrier.

Cons:

There is a co-existence of different lipid modifications and species of colloids that may result in a difference in the solubility nature as well as the melting point characteristics of auxiliary and the active type of species.

• The capacity of low drug loading for compounds with hydrophilic nature.
• The kinetic properties of the distribution process are also varied.
• The degradation of the drug is induced by high pressure.
• The data for stability is insufficient.

1.6.1.6 Cubosomes:

These are nanoparticles which appear like dots square shape and they are slightly spherical with a diameter ranging from 10 to 500 nanometre. Luzzati and Husson first identified that each dot represents a pore constituting aqueous and cubic phases in a system of lipid and water by X-ray scattering technique.

Applications of Cubosomes:

• The release of solubilised substance in a controlled manner is its main application.
• The cubic phase is preferred for release of the drug in a controlled manner due to the tiny size of pore which is about 5 to 10 nanometre, its biodegradability by enzymes which are simple in nature and its nature of solubilising molecules with hydrophilic, amphiphilic and hydrophobic nature.
• Its wide range use to treat melanoma (cancer).
• For its convenient use in depositions at topical and mucosal surface and to deliver different drugs these cubic phases are made in bio adhesive nature.

1.6.1.7 Virosomes:

The carriers which carry the genes to their respective target cells are divided into two systems namely viral and non-viral vectors. The viral vectors are obtained by replacing few genes of virus with the gene of interest and are meant to be the transducing system with great efficiency.

This is related to the characteristics of proteins of viral capsid and the glycoproteins of membrane, such as their binding capacity to the receptors of cell and also to pass via or fuse to the membranes of cells. But the matter of viral vector system's safety has been given much concern. Even the issues which are related to the induction of immune responses which are undesirable and inflammation and insertion mutagenesis which is found in retroviruses, these areas are still found to be major challenging issues.

There is incorporation of physical methods for example: gene gun and Electroporation and chemical approaches for example: cationic lipids, nanoparticles, cationic polymers. The vehicles which are studied extensively are cationic liposomes. In such type of systems, the lipids of cationic nature condense the DNA via interactions which are electrostatic in characteristic with the phosphate groups of nucleic acids which are negatively charged thus resulting in the formation of lipoplexes.

With reference to in vivo use liposomal delivery system combines the characters of safety of delivery systems which are made of liposomes with the cellular interaction of viral vectors. Virosomes constitute envelopes of virus which is comprised of membranes made of lipid, viral spike glycoprotein but the viral genetic material is absent.

Pros of Virosomes:

• Virosomes are of biodegradable, biocompatible and non-toxic nature.
• The FDA has approved this Virosomes technique due to its high safety profile in humans.
• The risk for the transmission of disease is absent.
• Auto immunogenicity or anaphylaxis has no chances.
• These Virosomes have the capability to deliver the drug in the target cell's cytoplasm.
• These can be broadly applied to peptides, nucleic acids, fungicides, proteins, antibiotics and anti cancer drugs.
• The drugs are protected from getting degraded.
• These facilitate the activity of fusion in endo-lysosomal pathway.

1.6.1.8Nano emulsions:

Nano emulsions are defined as isotropic substances which is made by dispersing oil in water. They are stable thermodynamically and may be transparent to translucent, which are stabilized using surfactant molecules which act as an interfacial film with the size of droplet of about 20 to 500 nm.

The preparation and scaling up of these nano emulsions is easy. As these substances have increased stability and bioavailability so they have gained researcher's attention. The different advantages of nano emulsions are as follows:

• To gain improved water solubility and the total bioavailability of drugs which have lipophilic nature, nano emulsion is the system of choice.
• The primary reason in sustained and targeted delivery of the drug is the nano sized particle which leads to the enormous interfacial areas linked with nano emulsions would affect drug's transport property.
• Nanoemulsions have made the reproducible increase in bioavailability and concentrations of plasma drug.
• These oil droplets which are of fine size get emptied rapidly from the stomach thus facilitating wide range distribution of the drug throughout the tract of intestine thus resulting in minimization of irritation which is usually observed when there is an extended contact of the drug with the intestinal wall.
• The capacity of solubilisation is greater in nano emulsions when compared to the micellar solutions which are simple in nature where as the thermodynamic stability of the nano emulsion is much better when compared to the unstable dispersion,

For example- emulsions, suspensions due to their manufacture with very little energy but longer shelf life.
• They even offer larger o/w interfacial areas and ultra low interfacial layer tension.
• They provide better facility when compared to the already present self-emulsifying system in the parameters of rapid onset of action and a decreased inter subject variability in the fluid volume of GIT.
• Nano emulsions have similarity to micro emulsions in two characteristics i.e. both are having optical transparency and higher kinetic stability.
• Most of the nano emulsions appear to be transparent optically though it is given at large loading dose due to its structure which is much smaller when compared with the visible wavelength.
• Peptides which are prone to be subjected to enzymatic hydrolysis in the GI tract are also delivered by the nano emulsions.

Cochleates:

The tiny uni lamellar liposomes which are charged negatively are condensed and an array of bilayers of lipid are formed which give a presentation of cigar shaped micro structures known as cochleates. Large sheets are formed by the fusion of small liposomes made of phosphatidylserine (PS) in the presence of the substance calcium.

The surface of the sheets is hydrophobic in nature and to prevent its interaction with water they get rolled into cigar shaped cochleates. A typical cylinder of cochleates which is featured by tightly packed bi layers and an elongated structure is revealed by freeze fracture electron microscopy.18,19

Applications:

The nano cochleates based ApoA1 formulation is developed to treat atherosclerosis and various other coronary heart diseases like hypercholesterolemia in which the low density lipoproteins are present in higher levels and the high density lipoproteins are present in lower levels and which is the major risk factor for other cardiovascular diseases as well as atherosclerosis. There is an inverse prominently between the HDL levels and heart diseases.

HDL will enhance the removal of cholesterol from peripheral cells after the cholesterol esterification mediated by enzyme these cholesterol esters are transported into the body. A naturally occurring lipoprotein ApoA1 is a HDL which is most important
in cholesterol esterification mediated by enzyme and its transportation to the liver thus providing protection to the vessels from atherosclerosis.

HDL’S capability to enhance cholesterol transformation to liver and provide protection from atherosclerosis is due to the intraperitoneal administration or infusion of ApoA1. One of its major limitations is that ApoA1 cannot be given through parenteral route due to its protein nature and its rapid degradation by the GIT enzyme and thus it is not reached to the blood in the form of an intact molecule.

Thus nano cochleates encourages the delivery of ApoA1 by oral route and are a beneficiary tool in treating atherosclerosis and other heart disorders which are caused due to variations in cholesterol and LDL levels.

- The type of nano cochleates which are able to stabilize and give protection to extended varieties of micro nutrients and also help in increasing the nutritional value of processed foods are bio geode nano cochleates.
- In the application of vaccine and gene therapy proteins, peptides and DNA re supplied by nano cochleates.
- A potential anti fungal agent known as Amphotericin B is potentially delivered by nano cochleates both through oral and parenteral route with a good safety profile and the cost of treatment is also found to be less.
- These preparations of Amphotericin B even at low doses found to be very stable and efficacious. They even found to have improved patient compliance. Investigation on the advantages of cochleates constituting Amphotericin was performed by Delmas et al., murine model by taking orally administered doses 0-40 mg/kg of B/W/day/of about 14 days in of systemic aspergillosis.
- On administering CAMB oral dose 20 mg/kg/day and 40mg/kg/day the survival rate was found to be 70% where as the colony counts was reduced to more than 2 logs in livers, kidneys and lungs.
- The treatment of Aspergillosis can be done successfully by orally administered CAMB.
- The bactericidal activity is improved and toxicity is also reduced by the use of cochleates. For aminoglycosides and peptides which have linear and cyclic nature cochleates must allow administration via oral route. The achievement of the proof of principle of the anti-TB cochleates efficacy was done by the use of clofazimine as an antibacterial drug model.
• Omega-3 fatty acids can be delivered to cakes, muffins, pasta, cookies and soups, without any changes in the product's odour and taste by the nano cochleates.

• The nano cochleates which have been developed by the bio delivery sciences international can be used for delivering the nutrients such as vitamins, omega fatty acids much efficiently to the cells and lycopens without any change in the taste and colour of the food which makes the super foodstuff's theory a reality and these offer various advantages including improved cognitive functions, increased energy, anti ageing benefits and better immune function.

1.6.1.9 Liposomes:

A liposome is a small vesicle or bubble which comprises the same material as that of the membrane of the cell. These can be used for delivering the anti-cancer drugs as well as drugs for treating other diseases by filling them in the liposomes.

The British haematologist Dr. Alec D Bangham FRS described about liposomes in 1961 in the Babraham institute in Cambridge which was published in 1964. This was discovered by Bangham and R.W. Horne while testing the electron microscope of the institute by the addition of negative stain to the phospholipids which were dry. It resembled to the plasma lemma and the first true evidence that the cell membrane is a lipid structure with bi layers was given by the microscopic pictures.

The derivation of the word liposome has been done from the two words of Greek are 'Lipos' which means 'fat' and 'soma' which means 'body'. In the liposomes there is an internal aqueous volume which is covered by a membrane of lipid bi layer giving it a structure of concentric bleeder vesicles. These membranes are usually comprised of phospholipids, which constitute of a head group of hydrophilic nature and a tail group of hydrophobic nature. As the head is hydrophilic in nature it gets attracted to water and the tail which is hydrophobic in nature gets repelled by water, it is made of a long hydrocarbon chain.
The stable membranes are naturally found to be made up of bi layers of phospholipids. When water is present the head group gets attracted to water and they make up to form a surface which faces the water where as the tail group is repelled by it and they form a surface away from the water. So in a cell we can observe that one layer will have its heads facing outer side of the cell i.e. attracted to the environment's water where as the other layer will be facing the inner side of the cell attracted to the water which is in the cell. Both the hydrocarbon layers face each other to give an appearance of bilayers.\textsuperscript{33}

If there is a disruption of the phospholipids membrane then they have the capability to reform into spheres, which is small when compared to a normal cell either as monolayer’s or bilayer where the bilayer structures are known as liposomes and the monolayer structures are known as micelles. The plasma membrane mainly constitutes of phospholipids for example phosphatidylycholine and phosphatidylethanolamine.

These phospholipids are amphiphilic whose nature is hydrophobic consist of tails that are hydrocarbons. Plasma membrane contains moisture (water) on this interior as well as the exterior surface thus the hydrophobic tails face each other in the phospholipids. Liposomes can be made up of phospholipids which are derived naturally with lipid chains which are mixed for example egg phosphatidylethanolamine or DOPE (dioleoyl phosphatidylethanolamine) which is a pure surfactant component.

Generally liposomes constitute an aqueous solution in the core but by definition it is not defined so. The liposomes which do not have aqueous material are known as micelles however to encompass an environment of aqueous nature reverse micelles can be made.\textsuperscript{34}
Pros:

Few of the pros are as follows:

- The targeting to tumour tissues is done in a selectively passive manner.
- The therapeutic index and efficacy is increased.
- Enhanced stability through encapsulation.
- The agents which are encapsulated have a reduced toxicity.
- The pharmacokinetic effects are improved (Enhanced circulation life times, reduced elimination).
- For the achievement of active ligands there is flexibility in coupling with site specific ligands.
- Site avoidance effect. \(^{33}\)

Varieties of liposomes:

The liposomes can be classified based upon various considerations namely:

- Structural parameters.
- Preparation method.
- Applications and composition.
Figure 13: Classification of liposomes based on Structural parameters.
Figure 14: Classification of liposomes based on method of preparation.

- **DRV**
  - Dehydration rehydration method

- **REV**
  - SUVs/OLVs made by reverse phase evaporation method

- **MLV-REV**
  - MLVs made by reverse phase evaporation

- **VET**
  - Vesicles prepared by Extrusion Technique

- **FATMLV**
  - Frozen and thawed MLV

- **SPLV**
  - Stable plurilamellar vesicles
Figure 15: Classification of liposomes based on composition and applications.
Characterizations of Liposomes:

Visualization:

Liposomes can be visualized by using transmission electron microscopy (TEM) and microscopic electronic Scanning (SEM) 51.

Zeta potential and Vesicle size:

Vesicle size and the zeta potential can be determined using photon correlation spectroscopy (PCS) and dynamic light scattering (DLS) 52.

Entrapment efficiency:

By the use of ultra centrifugation technique the efficiency of entrapment of the drug by the Liposomes can be measured53.

Transition temperature:

Using differential scanning calorimeter the determination of transition temperature of the lipid systems of particles can be done54.

Measurement of Surface tension activity:

Surface tension activity of the drug in aqueous solution can be found by using Du-Nouy ring tensiometer following ring method55.

Vesicle stability:

The stability can be determined by assessing size and structure of the particles. TEM is used to measure structure changes and DLS is used to measure mean size56.

Penetration and permeation studies:

The depth of penetration of Liposomes can be visualised by (CLSM) Confocal-laser-scanning-microscopy57.

As ethosomes are malleable vesicles, soft and potential carriers for drug transportation interest in the formulation and development is increasing tremendously. Active drug permeation can be enhanced by tailoring due to efficacy, safety, and simplicity in their preparation of ethosones. When compared to hydro-alcoholic solution and liposomes,
ethosomes are more efficiently deliver the drug to skin. Peptides, proteins, cationic drugs and hydrophilic drugs can be encapsulated in the ethosomes. For the novel therapies development ethosomal carriers provided new opportunities as well as opened challenges which can be beneficial for total health care potential of universe.

Table 1: Different Additives Employed In Formulation of Ethosomes

<table>
<thead>
<tr>
<th>Class</th>
<th>Uses</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>For gel formation</td>
<td>Carbopol D934</td>
</tr>
<tr>
<td>Dye</td>
<td>For characterization study</td>
<td>Fluorescent Isothiocyanate (FITC), 6- Carboxy fluorescence, Rhodamine red, Rhodamine-123</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>To provide vesicle membrane stability</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>Alcohol</td>
<td>As vesicle membrane softner &amp; to enhance penetration</td>
<td>Isopropyl alcohol, Ethanol</td>
</tr>
<tr>
<td>Polyglycol</td>
<td>To enhance skin penetration.</td>
<td>Transcutol RTM, Propylene glycol</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>Component used for formation of vesicles</td>
<td>Distearyl phosphatidyl choline</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dipalmityl phosphatidyl choline</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Egg phosphatidyl choline</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soya phosphatidyl choline</td>
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</tbody>
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