Nano Vesicular Systems: Innovations Driving Smarter Drug Delivery

Suryaprathap T[1], Haranath C[2]*. Nishanth Goud GV[3], Sameer T[4] and Lakshmi Sampada J[5]

[1,3,4,5] Student, Department of Pharmaceutics, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur, Andhra Pradesh, 515721

[2] Professor, Department of Pharmaceutics, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur, Andhra Pradesh, 515721

*** Corresponding author**

E mail: *haranathriper@gmail.com*

Abstract

Over the past few decades, there has been an increasing interest in nanoparticle vesicular systems such liposomes, niosomes, and transfersomes, as evidenced by recent scientific and patent literature. When compared to their bulk equivalents, nanomaterials which are characterised by their nanoscale dimensions and structures frequently display unexpected features. Self-assembled nanostructures are important drug delivery vehicles that provide benefits for a variety of administration methods. Drugs are effectively encapsulated in these micro- and nano-sized containers, allowing for targeted and prolonged release. Drug type, solubility, pH sensitivity, re0lease kinetics, additives, and—most importantly—the shape of the carrier vehicle all affect how well drugs are delivered. This study focusses on formulation processes, applications of nano vesicular systems in augmenting therapeutic efficacy and minimising medication adverse effects, and nanoparticles utilised as efficient drug carriers within delivery systems. By improving drug stability, allowing targeted administration to certain tissues or cells, and providing precise control over drug release patterns, nano vesicular devices show their flexibility in pharmaceutical applications. Nano vesicular systems offer encouraging prospects for resolving contemporary issues with medication formulation and improving therapeutic results in medicine.

Keywords: *Nano vesicular systems, Nanoparticles, Liposomes, Niosomes, Transferosomes, Nanomaterials.*

Introduction

Nano vesicular systems have emerged as key actors in pharmaceutical research and drug delivery throughout the last decade. These systems, distinguished by their nanoscale size and distinct structural features, include liposomes, niosomes, transfersomes, and other types. The appeal of these nanostructures stems from their capacity to encapsulate both hydrophilic and hydrophobic pharmaceuticals within their lipid bilayers or surfactant assemblies, providing various benefits in terms of drug solubility, stability, and targeted administration *[1]*.The excitement with nanomaterials arises from their capacity to impart unique properties to traditional medicinal formulations. These nano vesicular devices use self-assembly principles to provide precise control over drug release kinetics and targeted distribution to specific tissues or cells. This skill not only improves treatment efficacy but also reduces side effects by guaranteeing optimal medication concentrations at the target location.This study examines the design concepts, formulation methodologies, and many uses of nano vesicular systems in current pharmaceutical sciences. Furthermore, it investigates current advances in surface modification approaches aiming at improving circulation times and targeting capabilities. By thoroughly studying the growing landscape of nano vesicular systems, this article hopes to shed light on their tremendous influence on drug delivery systems and pave the way for future therapeutic medicine advances *[2]*.

Various carrier-based dosage forms:

1. Nanoparticles
2. Liposomes
3. Ethosomes
4. Bilosomes
5. Glycerosomes
6. Transferosomes

1. Nano Particles

Nanoparticles (NPs) are a basic component of nanotechnology. Nanoparticles are particulate materials having at least one dimension less than 100 nm. They can be composed of carbon, metal, metal oxides, or organic material. Nanoparticles (NPs) can take many various shapes, sizes, and structures, including spherical, cylindrical, tubular, conical, hollow core, spiral, flat, and wire. It can also have an uneven form. The surface of nanoparticles might be uniform or uneven. They can also occur in crystalline and amorphous forms, with single or many crystal solids. Multi-crystalline solids can be loose or agglomerated. The size and form of these NPs have a significant impact on their physiochemical characteristics. Because of their unique physical and chemical characteristics, NPs have found significant success in a wide range of applications in disciplines such as medicine, the environment, energy-based research, imaging, chemical and biological sensing, gas sensing, and so on. Researchers are more interested in nanotechnology since it is regarded as one of the key aspects for a clean and sustainable future *[3]*.

1.1 Methods of preparation: *[4]*.

1.1.1 Top- Down Approach: The bottom-up or constructive technique involves the assembly of material from atoms to clusters to nanoparticles. The most prevalent bottom-up processes for nanoparticle creation are sol-gel, spinning, chemical vapour deposition (CVD), pyrolysis, and biosynthesis.

1.1.1.1 Sol- gel:

It is a wet-chemical process in which a chemical solution serves as a precursor to an integrated system of discrete particles *[5]*. The precursor is then distributed in a host liquid by shaking, stirring, or sonication, resulting in a system with both a liquid and a solid phase. To recover the nanoparticles, a phase separation is performed using processes such as sedimentation, filtering, and centrifugation, and the moisture is then removed by drying *[6]*.

1.1.1.2 Spinning:

A spinning disc reactor (SDR) is used for nanoparticle production. It has a revolving disc within a chamber/reactor that allows physical factors like temperature to be regulated. The reactor is often filled with nitrogen or other inert gases to eliminate oxygen and prevent chemical reactions *[7]*. The disc rotates at varying speeds as the liquid, i.e. precursor and water, is poured in. The spinning causes the atoms or molecules to fuse together, which are then precipitated, collected, and dried. The features of nanoparticles synthesised from SDR are determined by operational parameters such as liquid flow rate, disc rotation speed, liquid/precursor ratio, feed position, disc surface, and so on *[8]*.

1.1.1.3 Chemical Vapour Deposition (CVD):

Chemical vapour deposition refers to the formation of a thin layer of gaseous reactants on a substrate. The deposition occurs in a reaction chamber at ambient temperature by mixing gas molecules. When a heated substrate comes into touch with the combined gas, it causes a chemical reaction *[9]*. This reaction results in a thin coating of product on the substrate surface, which is recovered and utilised *[10]*.

1.1.1.4 Laser pyrolysis:

Pyrolysis is the most popular industrial procedure for producing nanoparticles on a big scale. It entails burning a precursor with flame. The precursor is either a liquid or a vapour that is injected into the furnace under high pressure via a tiny hole and burned. The combustion or byproduct gases are then air categorised to extract the nanoparticles. Some furnaces employ lasers and plasma rather than flames to generate high temperatures for simple evaporation *[11],[12]*.

1.1.1.5 Biosynthesis:

Biosynthesis is a green and environmentally acceptable method for producing nontoxic, biodegradable nanoparticles *[13]*. Biosynthesis produces nanoparticles using bacteria, plant extracts, fungus, and other precursors rather than conventional chemicals for bio reduction and capping. The biosynthesised nanoparticles have unique and increased features that make them useful in biological applications *[14]*.

- Intracellular synthesis of nanoparticles by fungi:

  This process includes transporting ions into microbial cells and forming nanoparticles in the presence of enzymes. Nanoparticles generated within an organism are smaller in size than extracellularly reduced nanoparticles *[15]*.

- Extracellular synthesis of nanoparticles by fungi:

  Extracellular production of nanoparticles has more uses than intracellular synthesis because it eliminates unneeded cellular components from the cell. Fungi are mostly recognised for producing nanoparticles extracellularly due to their large secretory components, which are involved in nanoparticle reduction and capping *[15]*.
- Microbes for production of nanoparticles:

  Both unicellular and multicellular organisms generate inorganic compounds, either intracellularly or extracellularly. The capacity of microorganisms such as bacteria and fungus to regulate the creation of metallic nanoparticles is being used in the quest for novel materials *[16]*.

1.1.2 Bottom-Up Approach: The top-down or destructive technique involves reducing a bulk substance to nanometric-scale particles. Mechanical milling, nanolithography, laser ablation, sputtering, and thermal breakdown are among the most common nanoparticle production processes.

1.1.2.1 Mechanical milling:

Mechanical milling is employed in nanoparticle production to grind and post-anneal various components in an inert environment. Plastic deformation influences particle shape, fracture causes particle size reduction, and cold-welding causes particle size growth *[17]*.

1.1.2.2 Nanolithography:

Nanolithography is the study of creating nanometric-scale structures with at least one dimension in the size range of 1 to 100 nm *[18]*. Lithography is the technique of printing a needed form or structure on a light-sensitive substance while selectively removing a piece of the material to get the desired shape and structure *[19]*.

1.1.2.3 Laser ablation:

Laser Ablation Synthesis in Solution (LASiS) is a typical technique for producing nanoparticles from different solvents. A laser beam irradiates a metal immersed in a liquid solution, causing a plasma plume to condense and generate nanoparticles *[20]*.

1.1.2.4 Sputtering:

Sputtering is the process of depositing nanoparticles on a surface by ejecting particles when they collide with ions *[21]*. Sputtering typically involves deposition of a thin layer of nanoparticles followed by annealing. The form and size of the nanoparticles are determined by the layer thickness, temperature and annealing time, substrate type, and other factors *[22]*.

1.1.2.5 Thermal decomposition:

Thermal decomposition is an endothermic chemical breakdown caused by heat, which disrupts the chemical bonds of a substance. The nanoparticles are created by decomposing the metal at specified temperatures and undergoing a chemical process that yields secondary compounds *[23]*.

1.2 Applications

1.2.1 Medicine: Nanoparticles have made significant contributions to clinical medicine in areas such as medical imaging and drug/gene delivery. The most prevalent iron oxide particles used in biomedical applications are magnetite (Fe_3O_4) and their oxidised derivative hametite

(Fe2O3). Because of their antibacterial properties, silver nanoparticles are increasingly being employed in wound dressings, catheters, and other home goods. Gold nanoparticles are developing as prospective cancer therapies, serving as drug transporters, photothermal agents, contrast agents, and radiosensitizers *[24]*,*[25]*,*[26]*.

1.2.2 Cosmetics and Sunscreens: Traditional ultraviolet (UV) protection sunscreens lack long-term stability during use. Sunscreen with nanoparticles such as titanium dioxide gives various benefits. The UV protection properties of titanium oxide and zinc oxide nanoparticles, which are transparent to visible light while also absorbing and reflecting UV rays, have led to their application in sunscreens. Some lipsticks incorporate iron oxide nanoparticles as a pigment *[27]*.

2. Liposomes

Bangham and collegue discovered liposomes in the early 1960s. After hydration, a self-forming enclosed lipid bi-layer gave rise to the discovery of the liposome, also known as a lipid vesicle. Phospholipid vesicles, or liposomes, are spherical lipid bilayers that have the ability to ensnail lipid molecules inside the lipid bilayers or, conversely, water soluble solutes within aqueous domains. They are perfect drug carrier systems in therapeutics because they are biodegradable, biocompatible, and non-immunogenic. Phospholipids spontaneously form a closed structure with an interior aqueous environment surrounded by phospholipid bilayer membranes when they are dispersed in water. This vesicular system is known as a liposome *[28]*. Liposomes are a type of small, spherical vesicles that can be made from membrane proteins, sphingolipids, glycolipids, cholesterol, and even non-toxic surfactants. Liposomes are drug carriers that contain a wide range of compounds, including tiny chemical molecules, proteins, nucleotides, and plasmids. Lipofection is the process of introducing DNA into a host cell using liposomes *[29]*.

2.1 Methods Of Preparation:

There are various methods for creating liposomes. The final liposome features are greatly influenced by the phospholipid type and liposome manufacturing procedure. The methods used to create liposomes can be divided into:

2.1.1 Thin film hydration method (Bangham method): This procedure involves dissolving the hydrophobic medication and all of the lipids in an appropriate organic solvent using a round-bottom flask. The thin film layer was then produced by the organic solvent gradually evaporating under lower pressure *[30]*. After that, an aqueous buffer solution is used to hydrate the resulting thin film at a temperature higher than the utilised lipid's transition temperature (Tm). A hydrophilic medication or drugs to be inserted into the liposomes' aqueous core may be present in the hydration solution. The efficiency of drug encapsulation is dependent on the rate of hydration; the higher the encapsulation efficiency, the slower the rate of hydration. The regulation of liposome resizing, lamellarity types, and particle distributions can be achieved through two methods: using bath or probe sonicators, or extrusion through polycarbonate membranes with precise pore diameters *[31]*.

2.1.2 Reverse phase evaporation method: By creating a water-in-oil emulsion, the reverse-phase evaporation process is typically utilised as a substitute for thin-film hydration. The hydrophilic medication is first mixed directly with an aqueous buffer after the lipids have been dissolved in an organic solvent. Following the organic solvent's evaporation in a rotary evaporator operating at low pressure, lipid vesicles were formed and distributed throughout the aqueous solution. Extrusion can lower the produced vesicles' average size and polydispersity *[32]*.

2.1.3 Solvent injection method: The kind of organic solvent used to do the injections was utilised to categorise the techniques. The hydrophobic active substances and lipids were dissolved by an organic solvent that was quickly introduced into an aqueous phase. When mixing, diethyl ether allows direct solvent evaporation at a temperature higher than the solvent's boiling point. A 10-to 20-fold aqueous solution is needed when utilising ethanol for injection, and it can be vacuum-evaporated using a rotary evaporator, dialysis, or filtration *[33]*.

2.1.4 Detergent removal method: Using a round-bottom flask and an appropriate organic solvent, lipids and a high critical micelle concentration (CMC) surfactant were dissolved in this approach. After the solvent was gradually evaporated, a thin layer formed at the flask's bottom. The lipid film was then hydrated in an aqueous solution containing the drug molecules to produce a mixed micelles solution. The next steps involve dialysis, size-exclusion chromatography, adsorption onto hydrophobic beads, or dilution to remove the surfactant. After solution concentration, a LUV liposome vesicle will be created *[34]*.

2.1.5 Heating method: This approach involves immediately hydrating lipids with an aqueous solution and heating them for a minimum of one hour above the Tm of the phospholipids being employed. A 3-5% hydrating agent, such as glycerine or propylene glycol, is also added during this process. When incorporating cholesterol into the formulation, the suspension can be heated to a maximum of 100 C. In order to stop nanoparticle coagulation and sedimentation, the hydrating agents function as stabilisers and isotonizing additives *[35]*.

2.1.6 pH jumping method: The pH jumping approach is another solvent-free way to manufacture liposomes. This approach breaks down MLVs into SUVs by subjecting the aqueous solution of phosphatidic acid and phosphatidylcholine to an almost four-fold increase in pH over a brief period of time. The proportion of SUVs to LUVs produced is determined by the phosphatidic acid to phosphatidyl choline ratio *[36]*.

2.2 The Advantages Of Liposomes: *[37],[38]*.

- Biodegradable and biologically inert.
- Since phospholipids are a normal component of cell membranes, there is no risk of toxicity, antigenicity, or pyrogenicity.
- A large range of lipophilic and hydrophilic pharmacological compounds can be encapsulated by them.

- Defend the medicinal ingredients against the harmful effects of the enzymes.
- Quick distribution and durability of the active ingredient at plasma levels for an adequate amount of time.
- Lower dosages and a reduction in the frequency and severity of adverse effects are the results of the active substance's release at the level of the target organ.

2.3 The Disadvantages Of Liposomes: *[37],[38]*.

- There is a chance that hazardous effects could occur if the support or matrix breaks or disintegrates too quickly, releasing a significant amount of the medication all at once.
- The impossibility of stopping therapy abruptly in the event of a therapeutic mishap or unpleasant reaction.
- The possibility of buildup if the drug material is eliminated too slowly.
- Ineffective absorption can cause a drug's therapeutic effect to be delayed.
- The duration of the drug's passage through the gastrointestinal tract represents the period of its release.

2.4 Applications

2.4.1 Skin applications: Dermatology can make use of nanoparticles for both therapeutic and cosmetic purposes (Nanobase or Cutanova bases, for instance). The application of lipid nanoparticles in dermatological therapies offers several benefits. Because of their lipid-rich composition, the therapeutic compounds are retained in the dermis and can penetrate deeply, resulting in a greater effect with less substance. Because an occlusive layer is formed on the skin's surface, these nanoparticles will help maintain the skin's moisture content *[39]*.

2.4.2 Vaccine carriers: The incorporation of viral strains into liposomes has been the subject of much research in vaccine technology in recent years. Thus, liposomes can contain DNA, RNA, or other viral peptides with diameters up to 150 nm. This includes the protection of these embedded components in liposomes, enabling the vaccine to be administered intramuscularly. As a result, the vaccination is more effective since the liposome functions as an independent immunological adjuvant *[40]*.

2.4.3 Heart disease: The plant D. moldavica is a member of the Lamiaceae family. The plant extract has a number of pharmacological actions, the most significant of which is its impact on the myocardium, which affects coronary heart disease, atherosclerosis, and hypertension. Because of the flavonoid content, all of these processes occur. Because the active components bioavailability and solubility are significantly reduced when given in a traditional pharmaceutical form, the extract was intended to be included in lipid nanoparticles. Therefore, oral administration of these flavonoid-containing nanoparticles can be used to treat cardiac ischaemia *[41]*.

2.4.4 Pulmonary disease: In contrast to administering the extract directly, Yun Zhao Yue-Xing et al. showed that Yuxingcao extract encapsulated in solid nanoparticles has a higher pulmonary recurrence in vivo and, thus, exhibits more quick effect. As a result, nebulising solid lipid nanoparticles containing Yuxingcao extract can be used to treat a variety of respiratory conditions and has a more potent and prolonged bronchodilator effect *[42]*.

2.4.5 Cancer therapy: Antineoplastic active drug doxorubicin is used to treat solid tumours, leukaemias, and lymphomas, among other forms of cancer. Oral ulcers, baldness, and bone marrow depression are the side effects of its therapy. The encapsulation of DXR in liposomes reduces the material's toxicity while preserving or even enhancing its anti-tumor efficacy. In the US, the first licensed liposomal product is doxorubicin hydrochloride, or DOXIL *[43]*.

2.4.6 Eye disease: The precorneal region and corneal inflammation have also been treated with a variety of anti-inflammatory drugs that have been included into liposomal systems. These include triamcinolone, indomethacin, diclofenac, ibuprofen, flurbiprofen, and indomethacin, all of which have reduced liposomal adverse effects and improved absorption. Liposomal systems containing timolol have been investigated in glaucoma *[44]*.

3. Ethosomes

Ethosomes are a unique carrier method for drug administration with modest penetration into biological membranes, particularly the skin. Ethosomes are a small variation of the well-known drug carrier liposome. Ethosomes are lipid vesicles that contain phospholipids, alcohol (ethanol and isopropyl alcohol in rather high concentrations), and water. Ethosomes are soft vesicles composed of phospholipids, ethanol (in larger quantities), and water. The size of ethosomes can range from tens of nanometres to microns. Ethosomes infiltrate the skin layers more quickly and have a substantially greater transdermal flow than typical liposomes. However, the specific process by which ethosomes improve absorption into deeper skin layers remains unknown. The synergistic effects of combining phospholipids with a high concentration of ethanol in vesicular formulations have been proposed to be responsible for deeper distribution and penetration in skin lipid bilayers. Unlike traditional liposomes, which are recognised primarily for delivering medications to the outer layers of skin, ethosomes can improve permeability through the stratum corneum barrier. Ethosomes can entrap medicinal molecules with a variety of physicochemical properties, including hydrophilic, lipophilic, and amphiphilic *[45]*,*[46]*.

3.1 Methods Of Preparation:

Ethosomal formulations can be produced either hot or cold, as indicated below. Both procedures are convenient, do not need complicated equipment, and are easily scaled up to industrial levels.

3.1.1 Cold Method: In this approach, phospholipids, drugs, and other lipid components are dissolved in ethanol in a covered jar at room temperature with vigorous stirring using a mixer. While the mixture is swirling, propylene glycol or another polyol is added. This combination is cooked in a water bath to 300 degrees Celsius.

Water heated to 300°C in a separate vessel is added to the mixture, which is then agitated for 5 minutes in a closed container. The vesicle size of an ethosomal formulation can be reduced to any desired amount via probe sonication or extrusion. Finally, the formulation is kept refrigerated *[47]*.

3.1.2 Hot Method: In this approach, phospholipid is dispersed in water by heating in a water bath at 400°C until a colloidal solution is formed. In a separate vessel, ethanol and propylene glycol are combined and heated to 400°C. Once both combinations have reached 400 degrees Celsius, the organic phase is introduced to the aqueous one. The medication is dissolved in water or ethanol, depending on whether it has hydrophilic or hydrophobic qualities. The vesicle size of an ethosomal formulation can be reduced to any desired amount via probe sonication or extrusion *[47]*.

3.2 Advantages *[48]*.

Compared to other transdermal and dermal delivery methods,

1. Ethosomes increase medication penetration through skin transdermal and dermal administration.
2. It is feasible to deliver big molecules such as peptides, proteins, or molecules.
3. In terms of number and depth, ethosomal systems transmit fluorescent probes (quantum dots) to the skin far more efficiently.
4. Low risk profile - The toxicological profiles of the ethosome components are extensively described in the scientific literature, therefore there is no risk of large-scale drug development.
5. Excellent patient compliance-The ethosome medicines are given in a semisolid form (gel or cream), which results in excellent patient compliance. In contrast, iontophoresis and phonophoresis are more difficult to utilise, which will impact patient compliance.
6. Products that use proprietary technology have a high commercial appeal. Ethosomes may be produced quite cheaply, with no complex technical inputs necessary.
7. The ethosomes system is passive, non-passive, and ready for rapid commercialisation.

3.3 Application Of Ethosomes

3.3.1 Transdermal Delivery of Hormones: Hormone delivery orally has been linked to issues such as high first pass metabolism, limited oral bioavailability, and a number of dose-dependent adverse effects. Each missing medication increases the probability of treatment failure. Touitou et al. evaluated the skin penetration capability of testosterone ethosomes (Testosome) over rabbit pinna skin to a commercially available transdermal testosterone patch (Testoderm patch; Alza). They discovered that the ethosomal formulation resulted in roughly 30 times more testosterone skin penetration than the commercial formulation *[49]*.

3.3.2 Delivery of anti-parkinson's drugs: Researchers created an ethosomal version of the psychoactive substance trihexyphenidyl hydrochloride (THP) and compared its distribution to that of traditional liposomal formulations. THP is an M1 muscarinic receptor antagonist used to treat Parkinson's disease. The results revealed that the ethosomal-THP formulation had a

higher skin penetration potential and may be used to effectively control Parkinson's disease *[50]*.

3.3.3 Delivery of Antibiotics: Antibiotics are more effective when delivered topically. Conventional oral medication generates a variety of allergic responses and negative effects. Conventional external preparations have poor penetration to the deep skin layers and subdermal tissues. Ethosomes can solve this problem by releasing an adequate amount of antibiotic into deeper layers of skin. Ethosomes penetrate the epidermis quickly, delivering a significant amount of medications to the deeper layers of skin and suppressing infection at its source *[50],[51]*.

3.3.4 Minoxidil ethosomes for hair loss: Minoxidil, a lipophilic medication, is administered topically to the scalp to cure hair loss. Minoxidil ethosomes were generated and tested in vivo in hairless rats to determine how minoxidil is targeted to pilosebaceous units via ethosomes. The results indicated minoxidil localisation in pilosebaceous units, indicating that minoxidil can be delivered more effectively utilising ethosomal carriers *[52]*.

3.3.5 Ethosomes for vaginal delivery: pH-responsive ethosomes were developed and tested for the vaginal administration of metronidazole, an antifungal drug. The in vitro permeation investigation was carried out using a Franz diffusion cell using a regenerated cellulose semi-permeable membrane and phosphate buffer pH 5.5 as a medium. The study found that the ethosomal gel effectively delivers metronidazole, with a maximum flow of 143.67±2.73 mg/cm2/h *[53]*.

4. Bilosomes

Bilosomes are closed bilayered vesicular transporters of lipids that include non-ionic surfactants and bile salts. Their size spans from 5 to 200 nm, including spherical, unilamellar, and multilamellar vesicles. Bilosomes differ from liposomes and niosomes in terms of composition, chemical stability, and storage requirements. Nanostructured lipid carriers containing amphiphilic-based bile salts aid in medication penetration across intact biological membranes such as the gut, cornea, and skin. Bilosomes were designed to minimise antigen degradation during GI transit while simultaneously improving mucosal penetration. Bile acids are produced in the liver and stored in the gallbladder, where they exist as ionised bile salts under normal circumstances. They are amphiphilic molecules with a steroid nucleus, a hydrophilic side chain containing hydroxyl groups, and a hydrophobic side chain containing a methyl group. They help emulsify and solubilise dietary lipids by forming mixed micelles. Bile salts improve the permeability of lipophilic drug molecules across the plasma membrane, resulting in increased oral bioavailability of many physiologically active compounds. Most protein/peptide or vaccines given parenterally were found to provide only systemic immunity; however, when these vaccines were encapsulated in bilosomes and administered, they demonstrated both systemic and mucosal immunity, with no interaction between pathogens and host at mucosal surfaces *[54],[55]*.

4.1 Methods of Preparation:

4.1.1 Thin-Film Hydration Technique: To prepare drug-loaded bilosomes, a specific amount of the drug, CHO, surfactant/surfactant combination (Span:Tween), or an adequate molar quantity of phospholipid was added in a round bottom flask with an organic solvent. The mixture was then attached to a rotary evaporator and exposed to low pressure and rpm (revolutions per minute) to evaporate the solvent, resulting in a dry, thin coating. The dry film was hydrated with double-distilled water or phosphate buffer saline containing bile salts to generate drug-loaded bilosomes. Furthermore, the generated bilosome dispersion was sonicated (probe sonicator/bath sonicator) or homogenised to minimise vesicle size before being kept at 4°C *[56]*.

4.1.2 Reverse Phase Evaporation Method: In this process, soybean phosphatidylcholine and bile salts are dissolved in an organic solvent, such as absolute ether, and then a buffer solution containing protein is added drop by drop. The mixture is sonicated in a water bath for 5 minutes, or until no emulsion forms. The organic solvent is removed from the emulsion by rota evaporation at a speed of 50 rpm. A buffer is then added to hydrate the dry lipids, resulting in a homogenous dispersion. Finally, this dispersion is extruded via a high pressure homogeniser before being purified by ultracentrifugation to produce drug-loaded bilosomes *[57]*.

4.1.3 Hot Homogenization Method: To prepare bilosomes using the heat homogenisation technique, lipid components such as mono palmitoyl glycerol, cholesterol, and dicetyl phosphate are melted at 140°C for 5 minutes and then hydrated with buffer solution. This mixture is then homogenised, followed by the addition of bile salt solution to create a dispersion with empty vesicles, which is then homogenised again. The antigen buffered solution is then added to the homogenate, and protein entrapment is accomplished by repeated Freeze Thaw cycles. Antigen is introduced at the end to reduce long-term exposure to homogenisation *[58]*.

4.1.4 Ethanol Injection Method: The drug, surfactant, and CHO were dissolved in an ethyl alcohol-containing beaker in a water bath at 60°C. The edge activator and bile salt were dissolved in aqueous solution. The ethyl alcohol solution was injected into a fivefold bigger vehicle of phosphate buffer saline, which was magnetically agitated at the same temperature. The solution is continuously mixed until all of the ethyl alcohol has evaporated, and the abrupt turbidity signals the development of extremely deformable bilosomes. The produced bilosome dispersion was then sonicated using a water bath sonicator and kept at 4°C *[59]*.

4.1.5 Melt Method: In a proper ratio, phospholipid (1-monopalmitoyl glycerol), CHO, and diacetyl phosphate were combined and heated to 130°C. After adding the medication and bile salt to the buffer, the solution was quickly vortexed for 2 minutes. As vesicles form, the medication becomes trapped. The unentrapped medication was then extracted using centrifugation *[60]*.

4.1.6 Solvent Evaporation Method: A certain amount of surfactant, bile salt, CHO, and medication was weighed and dissolved in an organic solvent. The resultant organic solution was then put on a magnetic stirrer (60°C) to completely evaporate the sol vent. Later, the generated residue was hydrated with phosphate buffer to create the bilosome solution *[61]*.

4.2 Advantages *[62]*.

- Bilosomes have effective emulsifying and solubilising properties, leading to increased encapsulation efficiency.
- They are non-invasive drug delivery systems with diverse therapeutic applications.
- Bilosomes exhibit low toxicity and excellent stability.
- Bilosomes improve bioavailability by improving permeability.
- They can increase medication release and duration of activity.

4.3 Disadvantages *[62]*.

- In-vivo and in-vitro correlations are poor, and ex-vivo permeation has limitations.
- The negative charge of bile salts prevents effective trapping of anionic active agents.
- Choose integrated bile salts to avoid cytotoxic side effects or slight discomfort.

4.4 Applications Bilosomes

4.4.1 Treatment of Type 2 Diabetes: Type 2 diabetes is distinguished by high blood glucose levels and symptoms such as frequent urination, hunger, and weight loss, which can result in serious consequences. Apigenin is a natural bioactive molecule classified as flavones that is used to treat type 2 diabetes. It was loaded into a nanoelastic bilosome vesicular carrier. The established pharmacokinetics and intestinal permeability investigations indicated increased systemic bioavailability and drug penetration *[63]*.

4.4.2 Delivery of Antiviral Drug: The ex vivo absorption investigation, which evaluated intestinal permeation using the everted gut sac technique, found that the created and optimised formulation had much greater apparent permeability coefficients than the acyclovir suspension and commercialised formulation. Similarly, the penetration coefficient was significantly greater than in the commercial formulation and ACV solution *[56]*.

4.4.3 Delivery of Antibiotics: Researchers created bilosome formulations for levofloxacin and doxycycline to improve antibiotic efficacy. The release characteristics of levofloxacin and doxycycline bilosomes in synthetic stomach juice followed unique patterns. It also showed antibacterial activity in vitro against B. pseudomallei that was equivalent to unformulated antibiotics. Furthermore, in a murine model, doxycycline and levofloxacin vesicular formulations showed increased efficacy, higher survival rates, and longer action against B. pseudomallei, demonstrating the developed bilosomes' potential as a promising drug delivery vesicular platform for mitigating significant side effects *[60]*.

4.4.4 Delivery of Antiarrhythmic Drug: Dronedarone hydrochloride (DRN), a new class III antiarrhythmic drug used to treat atrial fibrillation, has low bioavailability. Researchers investigated the transdermal method to increase DRN skin penetration and bioavailability, therefore addressing first-pass metabolism and boosting systemic absorption. The generated DRN-encapsulated bilosomes were integrated into carboxymethylcellulose, resulting in an increased average flow that allowed the medication to pass through tiny paracellular gaps into the skin layers *[64]*.

4.4.5 Delivery of Antifungal Drug: Researchers created butenafine-loaded bilosomes (BN-BSo) to boost antifungal activity via the transdermal method. The optimised formulation was loaded into Carbopol 940 (1% w/v) gelling agent to generate (BN-BSog) butenafine loaded bilosome gel, which was then tested for pH, viscosity, in vitro drug release, diffusion, antifungal activity, and irritation. In vitro, BN-BSo released more drug than BN-BSog or pure butenafine *[65]*.

4.4.6 Treatment of Acne: Researchers investigated bilosomes as a possible vesicular carrier for delivering dapsone via the skin. The ex vivo investigation found that the bilosome-treated formulation retained 1.5 times more dapsone than the dapsone alcoholic solution. Furthermore, the in vivo histopathology research demonstrated the safety of the created topical formulation, with no evidence of inflammation or abnormalities *[66]*.

5. Glycerosomes

Glycerosomes are modified liposomes that have a greater glycerol and phospholipid content (10-50%, v/v). Compared to existing vesicular systems such as liposomes and niosomes, which both have low entrapment effectiveness and penetrability, these bilayer vesicles are believed to represent a novel technique for enhancing penetrability. The glycerol found in glycerosomal formulation creates creates the hydration and lipid fluidisation of skin, which is the process by which glycerosomes penetrate the skin. Glycerosomes were first hypothesised by Manca et al. Subsequently, they were discovered to be a unique vesicular transporter for medication administration. Here, the glycerol section modifies the smoothness of the vesicle film and includes a number of materials, including cholesterol, which enhance the lipidic bilayer’s durability. Additionally, It may also include fundamental or acidic lipid particles, which alter the electrical charge of vesicular surfaces and inhibit liposome aggregation. Their remarkable capabilities include the capacity to efficiently transfer cosmeceutical medicine components to the skin, hence improving its beauty features *[67],[68]*.

5.1 Method Of Preparation:

5.1.1 Solvent/Ether Injection Method: Watkins suggested this approach. Lipids are dissolved in diethyl ether or an ether-ethanol combination using this technique. Subsequently, it is added to a warm, 55–65° aqueous solution containing dissolved medication. The method's drawbacks include a wide variety of sizes and a relatively low yield of useful unilamellar vesicles. One advantage of this technique is that it prevents oxidative lipid degradation *[69]*.

5.1.2 Ethanol Injection Method: In 1973, Batzri and Korn proposed an injectable technique for ethanol. Using a syringe, ethanolic lipid solution is injected into excess aqueous medium in this procedure to enable phospholipid dispersion in water and full ethanol mixing in the hydration medium. The benefit of this technology is that it can produce liposomes that are smaller than 100 nm without the need for sonication or extrusion. Furthermore, homogeneous diluted liposomes can be produced. In addition, there are other drawbacks to the approach, such as the restricted solubility of lipids in ethanol *[70]*.

5.1.3 French Pressure Cell Method: This method prepares multi lamellar vesicles (MLVs) to flow through a small aperture at 20000 pressure and 4°. When compared to liposomes created using the sonication method, the methodology produces larger liposomes. Sustaining the conditions necessary for vesicle preparation is an extremely challenging endeavour. A pre-processing phase (usually by sonication) is necessary to ensure that the input cell volume is free of big cell clumps, which is a limitation of the approach *[71]*.

5.1.4 Detergent Removal Method: It is the method most commonly employed to ensnare living things and protein molecules. Vesicles are generated using detergents with high critical micelle concentrations, which can be anionic, cationic, or non-ionic. Detergents are combined with phospholipids, and detergents can be extracted via gel chromatography. The benefits of fast replication and uniform vesicle production are provided by this approach *[72]*.

5.1.5 Double Emulsion Evaporation: This process starts with the preparation of the primary emulsion and ends with the creation of the double emulsion (w/o/w). An aqueous phase distributed within a lipid phase makes up the primary emulsion. The advantages of the double emulsion solvent evaporation method include its ease of use, suitability for controlling process variables, and ability to produce formulation using simple instruments. This method works well for encasing proteins and peptides, which are very water-soluble materials *[73]*.

5.2 Advantages Of Glycerosomes *[74],[75]*.

- This novel vesicular medication delivery technology is safe and non-toxic for topical use.
- The generation of glycerosomes is independent of transition temperatures. Unlike traditional liposomes, they can be generated at room temperature (30 or 25°).
- They function as edge activators and penetration enhancers to boost drug penetration in the stratum corneum and transport it to the inner layers of skin.
- These vesicles have increased stability, fluidity, and entrapment. Viscosity preparations and modifications to the lipid bilayer's fluidity both increase stability. additionally creates flexible and malleable vesicles.
- Unlike traditional liposomes, glycerol is viscous by nature, thus it uniformly distributes over skin and doesn't leak active pharmaceutical ingredients.
- Glycerosomes possess the ability to rearrange the hydrophilic chains of phospholipids and modify the manner in which other vesicles within the system communicate with one another. This is feasible because glycerosomes can alter the system's dielectric constant.

- Glycerosomes also modify and enhance the skin layer's plasticity.
- These lessen the barriers to transdermal medication distribution and raise the stratum corneum's water content.
- These vesicles are special because they have the ability to function as both elastic and penetration-enhancing vesicles.

5.3 Disadvantages Of Glycerosomes *[69],[76]*.

- Less than 20% glycerol concentration results in less productive glycerosomes than 20% or higher concentrations.
- Less than 20% glycerol in vesicles results in low flexibility, low penetration, and low viscosity.
- The disruption of phospholipid orientation is another drawback.
- At room temperature, vesicles with high transition temperatures cause confusion.
- Drug release is delayed as glycerol becomes more entrapped in vesicles because it throws off the osmotic equilibrium between the donor and receptor sides.
- Glycerol increases particle size and decreases drug release when added to vesicles.
- Although glycerosome viscosity improves stability, it may also cause vesicles to take longer to emerge from the formulation and reach the skin's surface.

5.4 Applications of Glycerosomes in Pharmaceutical System

- Using diclofenac as a standard and conventional liposome as a control, Manca et al. (2013) created glycerosomes hydrated with "dipalmitoyl glycerol phosphatidylcholine-cholesterol films" and aqueous solutions of glycerol in concentrations of 10–30%, v/v. The study demonstrated how glycerosomes improve diclofenac's skin penetration and deposition *[67]*.
- In order to protect the oral mucosa, Maria Manconi et al. (2018) prepared vesicular systems for Citrus limon extract. The outcome might point to a desirable strategy for preventing bacterial and oxidative damage to the oral cavity *[77]*.
- In 2020, Naguib et al. conducted an in vitro and ex vivo permeation investigation to investigate the intranasal administration of glycerosomes loaded with lacidipine. Compared to an oral medication suspension, an optimised formulation showed a decrease in methylprednisolone acetate-induced hypertension. The findings showed that glycerosomal formulations are superior in terms of safety and efficacy when it comes to intranasal lacidipine delivery *[78]*.
- In 2021, Shadab and colleagues synthesised and assessed plumbagin-loaded glycosome gel (GM) and juxtaposed it with traditional liposomes as a potential treatment for skin cancer. Box-Behnken Design optimised the gel, and in vitro release tests were carried out. High skin was found in the results *[79]*.

6. Transferosomes

Transdermal drug delivery systems are defined as topically applied medications, such as patches or semisolids, that are applied to intact skin and penetrate the skin to enter the bloodstream at a controlled rate. Modern Transdermal Drug Delivery Systems (TDDS) have been created, and they are thought to be useful for rate-controlled drug delivery.

Transdermal systems are intended to administer medications to the systemic circulation through the skin in a controlled, continuous manner. When applied to the skin, the physicochemical qualities of medications are less permeable. One type of elastic or highly flexible vesicle is the transferosome. These are innovative delivery systems for bioactives that work well. Vesicles are made up of phospholipids and surfactants and are designed to interact closely. The ratio of specific surfactants to the total quantity of surfactants determines a vesicle's flexibility *[80]*.

6.1 Methods Of Preparation:

6.1.1 Rotary Film Evaporation Method: A different term for this strategy is the modified hand shaking method. This method involves shaking by hand a 1:1 combination of ethanol and chloroform at a temperature higher than the transition temperature of the lipid to solubilise the API, lecithin, and edge activator. The resultant liquid is then kept for evaporation to extract the organic solvent. To ensure that the organic solvent is completely removed, the thin lipid layer is left overnight. After that, the film is hydrated by spinning it with a pH 6.5 buffer at 60 RPM for an hour at room temperature. The remaining vesicles inflate at room temperature for two hours. After being sonicated at room temperature, residual vesicles were converted into small vesicles. Evaporation of Rotating Film *[81]*.

6.1.2 Reverse Phase Evaporation Method: The procedure for this method is as follows: under nitrogen purging aqueous fluids containing edge activators, lipids and organic solvents were combined in a flask with a circular bottom. The medicine is combined with either lipophilic or lipophobic medium, depending on how soluble it is. The produced material is sonicated and then allowed to sit for 30 minutes, or until it looks to be a homogenous mixture. When pressure is reduced to a minimum, the organic phase is destroyed. The material changes into a gel that is viscous and generates vesicles *[82]*.

6.1.3 Vortex or Sonication Method: In this process, phospholipids and edge activators are continuously swirled to distribute in phosphate buffer. It is sonicated in a bath sonicator to generate a milky solution, which is then extruded through polycarbonate membranes *[82]*.

6.1.4 Suspension Homogenization Process: In this procedure, a suitable concentration of an edge-active chemical, such as sodium cholate, is combined with an ethanolic soybean phosphatidylcholine solution to create transferosomes. To get a total lipid concentration, this prepared solution is then combined with Triethanolamine-HCl buffer. The resultant suspension is frozen, thawed, and then sonicated two or three times *[83]*.

6.1.5 Modified handshaking Process: The "lipid film hydration technique," a modified kind of hand shaking, is used in this process to prepare the transferosomes. A 1:1 mixture of ethanol and chloroform was used to dissolve the medication, lecithin (PC), and edge activator. Evaporation with hand shaking was used to remove the organic solvent above the lipid transition temperature (43°C). With rotation, a thin lipid coating developed inside the flask wall. The thin coating was left overnight to allow the solvent to completely evaporate.

After that, the film was hydrated for 15 minutes at the appropriate temperature using phosphate buffer (pH 7.4) and moderate shaking *[84]*.

6.2 Advantages Of Transferosomes *[85]*,*[86]*.

- Because of their infrastructure, which consists of both hydrophobic and hydrophilic moieties, transferosomes can hold medicinal molecules with a variety of solubilities. They do not suffer appreciable loss when they bend and pass through thin constriction (between 5 and 10 times smaller than their own diameter).
- This system's high deformability allows intact vesicles to penetrate more effectively. Both low and large molecular weight medications, such as insulin, albumin, sex hormone, corticosteroids, analgesics, and anaesthetics, can be transported by them.
- They are similar to liposomes in that they are made of natural phospholipids, making them both biocompatible and biodegradable.
- When it comes to lipophilic drugs, their entrapment efficiency is close to 90%.
- They shield the medication within the capsule from being broken down by proteins and peptides, for example.
- They serve as depots, releasing their contents gradually and being able to be employed for topical and systemic medication administration. Because of their straightforward process and lack of needless or inappropriately pharmacological ingredients, they are simple to scale up.
- At first look, liposomes, a type of lipid bilayered vesicle, and transferosomes seem to have little in common. But in terms of functionality, transferosomes are far more versatile and flexible than liposomes, which are often used.
- Because of their membrane's remarkable flexibility, transferosomes can squeeze themselves through gaps that are much tiny than their own diameter.
- Because of their infrastructure, which consists of both hydrophobic and hydrophilic components, transferosomes may hold medicinal molecules with a broad range of solubility.
- Transferosomes exhibit demonstrable losslessness in the face of narrow constriction, ranging from five to ten times smaller than their own diameter. Better penetration of intact vesicles across tight junctions is facilitated by this high deformability.

6.3 Disadvantages Of Tranferosomes *[85]*,*[86]*,*[87]*,*[88]*.

- Transferosomes are prone to oxidative destruction, which makes them chemically unstable.
- An additional factor that works against the use of transferosomes as drug delivery vehicles is their natural phospholipid purity.
- Formulations for transferosomes are pricey.
- One of the main obstacles to the transferosomes' widespread use is their high cost.
- For therapeutic efficacy, many drugs—particularly hydrophilic ones—permeate the skin too slowly.
- Drug release can vary depending on the site of change in skin barrier function within an individual, between individuals, and as they age.
- Not recommended for large amounts of drugs.

- Skin irritation and hypersensitivity reactions can happen.
- Medicines that need elevated blood levels cannot be taken.
- Extremely vulnerable to oxidative deterioration, which causes transferosome instability
- An additional factor that makes employing transferosomes as drug delivery vehicles problematic is the purity of the natural phospholipids.

6.4 Applications Of Transferosomes:

6.4.1 Delivery of Insulin: Insulin is usually injected subcutaneously, which is a cumbersome method. The drawbacks of discomfort, bigger size (making it inappropriate for transdermal distribution using conventional method), and 50% response relative to subcutaneous injection are resolved by encapsulating insulin into transferosomes (transfersulin) *[89]*.

6.4.2 Delivery of corticosteroids: Transferosomes optimise the drug dose applied topically, hence improving both the site specificity and overall drug safety of corticosteroid delivery into skin. The corticosteroids derived from transferosomes have biological activity at dosages multiple times lower than those presently employed in the management of skin conditions *[89]*.

6.4.3 Delivery of proteins and peptides: Proteins and peptides have been transported extensively using transferosomes as the carrier. Due to their huge size, proteins and peptides are biogenic molecules that are exceedingly difficult to distribute transdermally and are totally broken down in the GI tract when taken orally *[90]*.

6.4.4 Delivery of anticancer drugs: Transferosome technology has been used to try transdermal administration of anti-cancer medications like methotrexate. The outcomes were positive. This offered a fresh method of treating cancer, particularly skin cancer *[90]*.

6.4.5 Delivery of anaesthetics: Lidocaine and tetracaine formulations based on transferosomes demonstrated permeability comparable to subcutaneous injections in less than ten minutes. Transferosomal anaesthetics have a longer-lasting impact than equivalent subcutaneous bolus injections, with a maximum resultant pain insensitivity that is almost as high (80%) *[90]*.

6.4.6 Delivery of herbal drugs: Transferosomes have the ability to enter the stratum corneum and provide the necessary nutrients locally for the skin's maintenance *[90]*.

Conclusion

Nano vesicular systems, which include Nanoparticles, Liposomes, Ethosomes, Transferosomes, and other sophisticated structures, are a potential frontier in drug delivery and treatments. Their capacity to improve the bioavailability, stability, and targeting of medicinal medicines highlights their importance in current pharmaceutical science. These methods have distinct advantages, including regulated medication release, lower toxicity, and the possibility of non-invasive delivery pathways. Despite their tremendous promise, difficulties like as scalability, stability in storage, and manufacturing complications must be solved.

Ongoing advances in nanotechnology, material science, and formulation techniques are projected to improve these systems, opening the path for wider clinical use. As research advances, nano vesicular systems have the potential to revolutionise personalised medicine by providing more effective and patient-centered treatment options.

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