RECENT ADVANCEMENT AND PROSPECTIVE OF NANOCARRIERS LIKE ETHOSOME, LIPOSOME, TRANSFEROSOME FOR THERAPEUTIC APPLICATIONS WITH TOPICAL DRUG DELIVERY SYSTEM

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Abstract

For the past decades nanocarriers show Drug delivery systems are the innovative methods of drug administration that target particular situses inside the organism, in order to lower overall toxicity and boost bioavailability. Each medication delivery method has special features including morphological, chemical, and physical variances. This review describes the various types of nanocarriers like liposome, ethosome and transferosome with different fabrication method thin Film Hydration Technique/Rotary Evaporation-Sonication method, Vortexing-Sonication Method, Modified Handshaking Process, Centrifugation Process, Reverse-Phase Evaporation Method, Ethanol Injection Method etc. With this respect therapeutic effect and pharmacokinetic effect of nanocarriers describe briefly with their evaluation parameter Vesicle Morphology, Vesicle size and size distribution, entrapment efficiency, Zeta potential, Measurement of surface tension, Physical stability, In-vitro drug release, In-vitro skin permeation studies etc.

Keywords: Nanocarriers, Thin film hydration, vesicle size, entrapment efficiency, zeta potential

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DOI: 10.48047/ecb/2023.12.si5a.0450

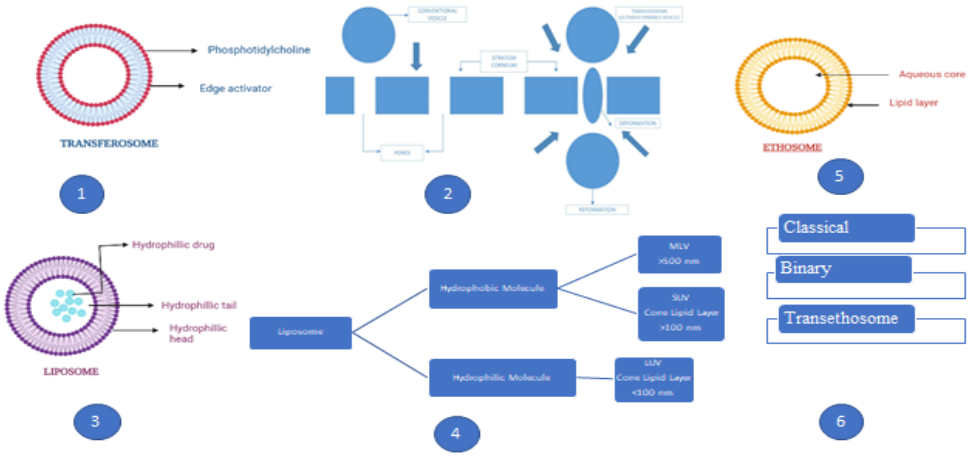


Fig no 1 *Graphical Abstract (Structure of Lipospome, Ethosome and Transferosome with their types penetration in skin)*

1.INTRODUCTION

1.1 Nano carriers

Compared to free medications, nanocarriers have a number of benefits. With the encapsulation of hydrophobic pharmaceuticals into the core of the nanocarriers, they aid in enhancing medication effectiveness. They may prevent the drug from prematurely degrading, prevent the drug from prematurely interacting with the biological environment, improve cellular penetration, manage the drug's pharmacokinetics and tissue distribution profile, and increase the drug's absorption in a specific tissue (for instance tumor).

1.2Skin Layer

The skin is the largest organ in the body and serves as a barrier against invaders. The subcutaneous layer, the dermis, the epidermis, the stratum corneum, and the appendages are only a few of the layers that make up human skin.

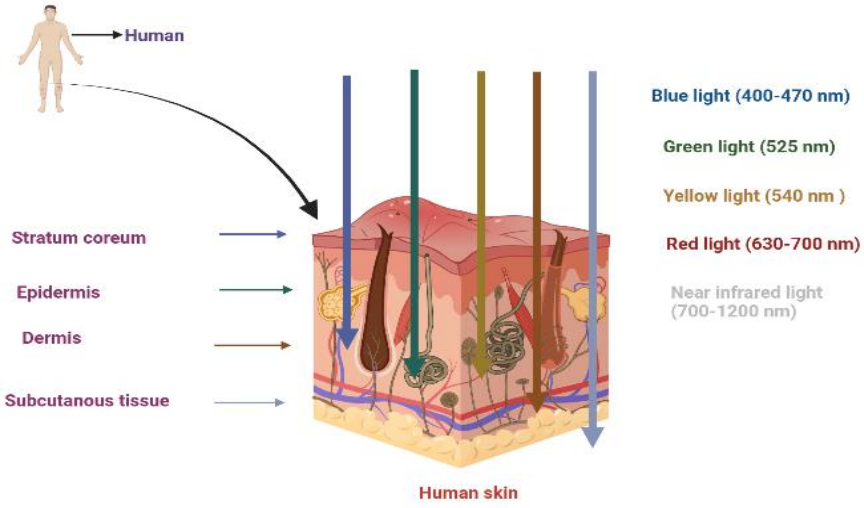


Fig 2*Transdermal penetration of skin with range*

These layers each have an impact on how well topical medications are absorbed. The stratum corneum, the outermost layer of skin, must be crossed by the topical medication when it is administered to the skin. The appendages, another component of the skin, are referred to as the "shortcut" for topical medication.[1]

1.3Topical Drug Delivery System

To treat skin diseases such as eczema, topical drug delivery (TDD) is a method of drug administration that enables the topical formulation to be administered over the skin upon application.

Topical medication formulations fall into four categories: corticosteroids, antibiotics, antiseptics, and antifungals. [2] Topical drug delivery has improved as a result of the widespread usage of

topical medications. By using chemical and physical factors, these developments improve topical medicine delivery to the skin.[3].To improves the absorption of topical medications for chemical agents, carriers like liposomes and nanotechnologies are utilised. On the other side, physical substances such as microneedles are another method for improving absorption.[4]

1.4Liposomes

Phospholipids from either the natural or manufactured world may make up the liposome bilayer. Based on the net properties of the component phospholipids, including permeability, charge density, and steric hindrance, a liposome's main physical and chemical characteristics are determined.[2] Due to interactions between water molecules and the hydrophobic phosphate groups of the phospholipids, the lipid bilayer constricts in on itself. Since the amphiphilic liposome production process is spontaneous.[5]

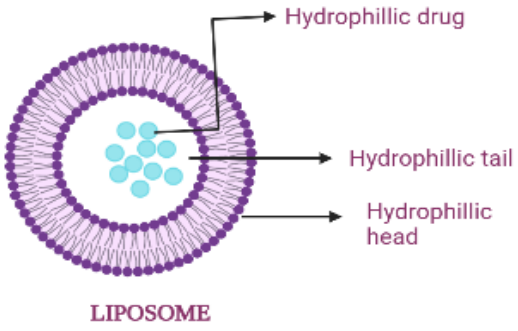


Fig 3*Structure of Liposome*

1.5Transportation through liposome mechanism

The interactions of liposomes with cells and their ultimate fate in vivo after injection determine both the drawbacks and advantages of liposome drug carriers. Studies of the contacts between cells in vitro and in vivo have shown that the primary interaction of liposomes with cells is either straightforward adsorption (by interactions with particular cell-surface components, electrostatic forces, or by non-specific weak hydrophobic) or endocytosis (by phagocytic cells of the reticuloendothelial system, for example macrophages and neutrophils)**.**[6]–[12]

1.6 Ethosomes

Ethosomes are lipid vesicles that are soft and flexible and are mostly made of phospholipids, water, and alcohol (ethanol or isopropyl alcohol) in quite high concentrations (20–45%).These vesicular phospholipids are the vesicle-forming component of the ethosomal system according to the physicochemical properties of ethosomes[13]-[14].

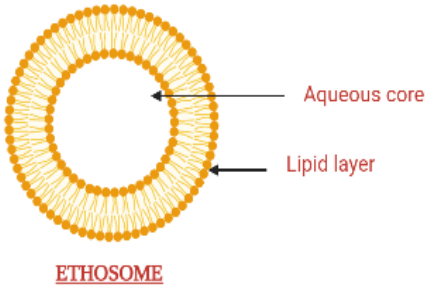


Fig 4*Structure of Ethosome*

Phospholipids may come from soya, eggs, semi-synthetics, or synthetic materials. Soya phospholipids like Lipoid S100 and Phospholipid 90 are among the most desired phospholipids (PL-90).[15]-[17].

1.7Composition of ethosomes

There are several different types of phospholipids, including phosphatidyl choline (PC), hydrogenated PC, phosphatidyl ethanolamine (PE), phosphatidyl glycerol (PPG), phosphatidyl inositol (PI), and hydrogenated PC[18]-[20].

1.8 Ethosomal drug delivery advantages

Nevertheless, the precise process for comparison to other dermal and transdermal delivery methods is as follows:

1) Improved skin penetration of drugs for transdermal medication administration.
2) It is feasible to deliver big molecules (peptides, protein molecules).
3) Its formulation uses harmless raw materials.
4) High patient compliance because the semisolid gel or cream form in which the ethosomal medication is delivered results in high patient compliance [21]-[23].

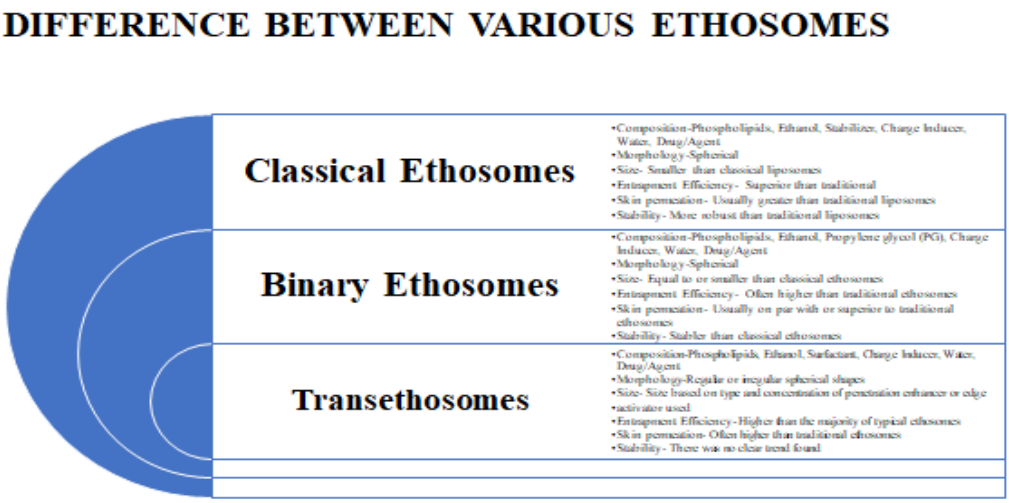


Fig 5*Difference between various types of ethosomes*

1.9Drug Penetration Mechanism of ethosomes

The improved drug penetration of ethosomes over liposomes is their principal benefit. There is no known mechanism for how drugs are absorbed from ethosomes. The following two steps are most likely involved in medication absorption:[24]

The impact of ethanol

1-Ethosomes impact

2-The impact of ethanol

Via the skin, ethanol enhances permeation. Its penetration-enhancing action has a well-known mechanism. Ethanol permeates intercellular lipids, increasing their fluidity and decreasing the density of the cell membrane's multilayer of lipids.[25]The ethanol of ethosomes increases the fluidity of cell membrane lipids, which increases skin permeability. In order to deliver the medications into the deep layers of skin, the ethosomes fused with skin lipids and penetrated the deep skin layers extremely easily.

1.10Transferosomes

According to some research, it may effectively distribute drugs without irritating the skin, making it a possible treatment for skin cancer. Initially, "Ceve and his coworkers" introduced transferosomes. A "carrying body" is what transferosomes are. It is made out of the terms transferre and some, which together indicate "to carry over" and "a body," respectively.

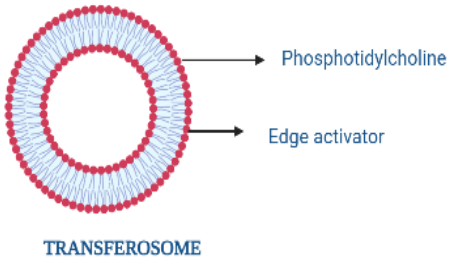
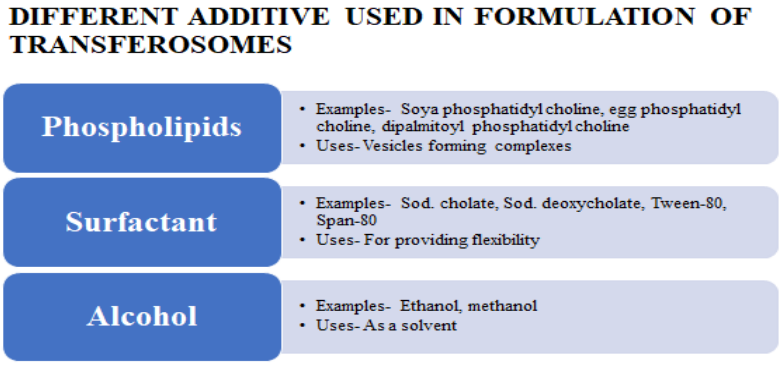


Fig 6*Structure of Transferosome*

Different additives used in transferosome



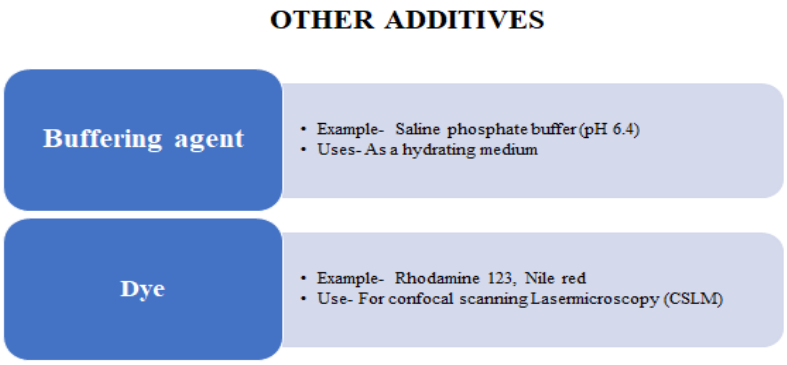


Fig 7*Different additives used in transferosome*

1.11Advantages of transferomes

The greater the degree of deformability, the better the penetration of intact vesicles, transferosomes may pass and flex through small constrictions without suffering appreciable loss.[26]

1-Around 90% of lipophillic drugs have significant levels of entrapment efficiency.

2-The architecture of transferosomes, which combines hydrophobic and hydrophilic components, may accept medicinal molecules with a broad range of solubility.[27]

3-Transferosomes serve as a vehicle for both high and low molecular weight medications.

4-Transferosomes may break down and are biocompatible.

5-Transferosomes may be used to administer medications topically and orally.

1.12Surfactant

Surfactants have a lengthy history of involvement in the advancement of human civilisation, with their presence being acknowledged as early as 2800 BC. The second half of the 20th century saw the fast growth of nanotechnology, which has resulted in notable advancements in food and medicine nanotechnology employing nanoparticles.[28]

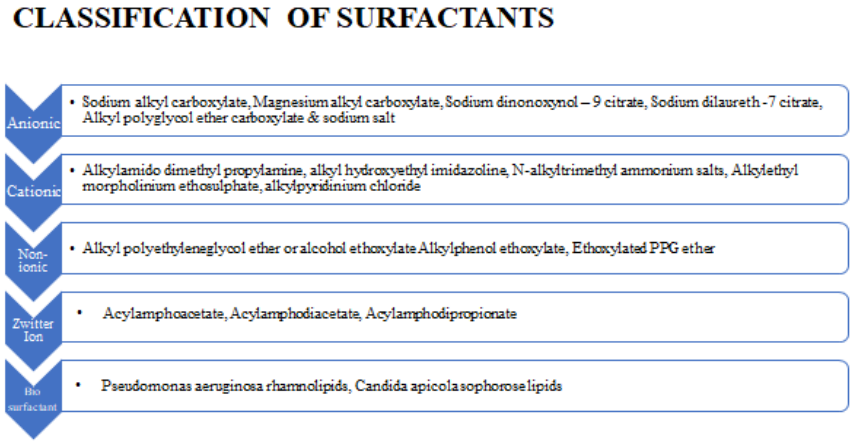


Fig 8*Classification of surfactant*

1.13Transferosome penetration mechanism

Most importantly, vesicle integrity for the underlying hydration affinity should not be disrupted while this deformation is taking place[30].As the transferosome is too huge to disperse to the surface, it must enforce and find its own path through the organ. Similar to normal endocytosis, intercellular drug transfer requires layer-by-layer diffusion of vesicle lipid with the cell membrane[29]-[31].

1.14Comparison of ethosomes, liposomes and transferosomes

Ethosomes and transfersomes, two forms of nanovesicles, were examined and contrasted with traditional liposomes. We generated and characterized transfersomes with various edge activators, such as sodium deoxycholate, sodium cholate, and sodium taurocholate, and ethanolosomes with ethanol concentrations of 10, 30, and 50%. The produced vesicles had negative

zeta potential values (45.40 to 86.90 mV), nanometric vesicle sizes (453.10-796.80 nm), and high entrapment efficiencies (46.73-65.99%). [32]

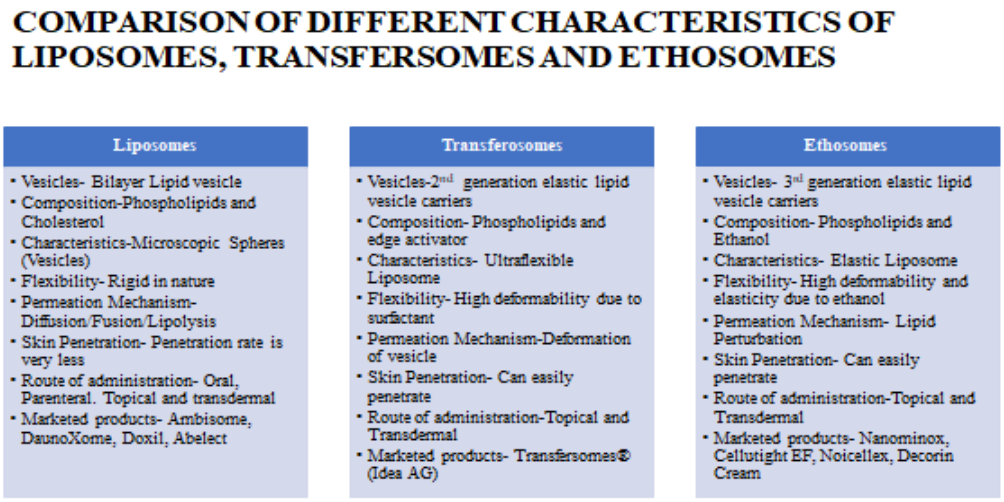


Fig 9 *Comparison of different charcterstics of nanocarriers (Liposome, Ethosome and transferosome*

1.15Nanocarrier loaded gel

Nanoparticles have already had extraordinary success in developing effective treatment plans for diseases like cancer. Drugs now have novel pharmacological effects thanks to nanotechnologies, which greatly rely on the nanocarriers utilised [33].Improved pharmacokinetic and biodistribution profiles result in a higher drug therapeutic index thanks to benefits including extended systemic circulation, passive or active targeting, high drug-loading yield, and the potential for medication combination.

Nanomedicines, originally used to treat cancer, are currently being developed for various illnesses, including atherosclerosis and diabetes, as well as other medical uses, such in vivo imaging and tissue engineering.[34]-[35]. ideal topical vehicle for ocular nasal vaginal intestinal and dermal drug delivery. Indeed, gels are drug-delivery platforms. The three-dimensional polymer network of the gel offers an ideal opportunity for prolonged release of therapeutic molecules, such as proteins and gel-based techniques are being developed to improve their administration using stimuli-responsive in situ gelation[36].

An important innovation has been the development of hybrid materials: bio-synthetic scaffolds with cell-loaded gels. The gel mimics extracellular media and influences and guides cell development or differentiation. Nanocarriers-loaded gels provide promising avenues to develop new therapeutic strategies, combining the advantages of both nano- and gel technologies[37][38]

2. DIFFERENT METHOD USED IN PREPARATION OF NANOCARRIERS

2.1Fabrication process of nanocarrier (Transferosome)

For this process, despite the fact that there are several patented Transferosome preparation techniques. In order to produce the most suitable carriers with the best deformability, drug carrying capacity, and stability, the best preparation conditions and vesicles compositions must be identified, designed, and optimised through conducting individually designed experimental procedures for each therapeutic agent.The following broad descriptions apply to each method.[39]

2.1.1Thin Film Hydration Technique/Rotary Evaporation-Sonication Method

An appropriate (v/v) ratio of chloroform and methanol, for instance, is used to dissolve the phospholipids and edge activator (the components that form the vesicles) in a round-bottom flask. This process may integrate the lipophilic medication. A rotating hoover evaporator is used to evaporate the organic solvent above the lipid transition temperature while under lowered pressure in order to create a thin layer. Keep it under suction to get rid of any remaining solvent residue.

2.1.2 Vortexing-Sonication Method

In a phosphate buffer, the medication, edge activator, and phospholipids are combined. After that, the mixture is vortexed to create a milky transfersomal suspension. After being sonicated for the appropriate amount of time at room temperature using a bath sonicator,

It is then extruded via polycarbonate membranes (example: 450 and 220 nm) [40][41]

2.1.3Modified Handshaking Process

The modified handshaking technique and the rotary evaporation-sonication method have the same fundamental ideas. The lipophilic drug, the phospholipids, the edge activator, and the organic solvent are all introduced to a round-bottom flask for the modified handshaking procedure. The solvent should thoroughly liquefy all of the excipients, producing a clear, transparent solution. The organic solvent is then eliminated using handshaking evaporation rather than rotary hoover evaporation.[42]

2.1.4Suspension Homogenization Method

A suitable quantity of edge activator is added to an ethanolic phospholipid solution to create Transferosomes. After that, the produced suspension and buffer are combined to produce a total lipid concentration. The resultant mixture is then twice to three times sonicated, frozen, and then thawed [43]

2.1.5Centrifugation Process

The organic solvent dissolves the phospholipids, edge activator, and lipophilic drug. A rotary evaporator is then used to extract the solvent at the appropriate temperature and lowered pressure. Under hoover, any leftover solvent is cleaned up. By centrifuging the formed lipid film at room temperature, the proper buffer solution is soaked into it. This step allows for the introduction of hydrophilic drugs. The resultant vesicles are enlarged at room temperature.[44]

Further sonication is performed at ambient temperature on the resulting multilamellar lipid vesicles.[45]

2.1.6Reverse-Phase Evaporation Method

In a round-bottom flask, the phospholipids and edge activator are added, and the combination of organic solvents is used to dissolve them (example: diethyl ether and chloroform). This process may integrate the lipophilic medication. The lipid films are then obtained by rotary evaporating the solvent. In the organic phase, which is mostly made up of isopropyl ether and/or diethyl ether, the lipid coatings are once again dissolved. Using a rotary evaporator, the organic solvent is slowly evaporated to create a viscous gel that later transforms into a vesicular suspension. [46]

2.1.7 High-Pressure Homogenization Technique

In PBS or distilled water including alcohol, the phospholipids, edge activator, and drug are equally disseminated before being concurrently swirled and ultrasonically shaken. The mixture is then shaken intermittently using ultrasonic energy. A high-pressure homogenizer is subsequently used to homogenise the resultant mixture. The Transferosomes are then kept under the proper conditions.[47],[48]

2.1.8 Ethanol Injection Method

The phospholipid, edge activator, and lipophilic drug are each dissolved in ethanol with magnetic stirring for the appropriate amount of time, until a clear solution is achieved, to create the organic phase. The water-soluble materials are dissolved in the phosphate buffer to create the aqueous phase. This step allows for the introduction of hydrophilic drugs. Up to 45ºC–50ºC are applied to both solutions. [49]

2.2Fabrication process of nanocarrier (Ethosome)

The literature describes a variety of ethosome preparation techniques, some of which have been listed in the preceding text.[50]

2.2.1 Hot method

When the phospholipid dispersion in water is at 40°C, the medication is dissolved in a solution of ethanol and propylene glycol and added to the solution. The preparation is sonicated using the Probe Sonicator for three cycles of five minutes each at 4°C, with a rest period of five minutes. To get nano-sized ethosomes, the formulation is then homogenized three times at 15,000 psi pressure using a high pressure homogenizer. [51]

2.2.2 Cold method

This technique for ethosomal preparation is the most popular and commonly utilised one. The medication, phospholipids, and other lipid components are dissolved in ethanol with vigorous stirring at room temperature. In a water bath, the mixture is heated to 30°C. In a separate saucepan, the water is heated to 30°C before being added to the mixture above and stirring for five minutes. If desired, the ethosomal formulation's vesicle size may be reduced by sonication or extrusion in

order to increase its extensibility. Lastly, the mixture has to be adequately refrigerated kept.[52]

2.2.3 Classic Mechanical Dispersion Method

In a round bottom flask, soyaphosphotidylcholine is dissolved in a solution of chloroform:methanol (3:1). A thin lipid layer is formed on the flask wall when the organic solvents are evaporated using a rotating hoover evaporator above the lipid transition temperature. The deposited lipid layer is then cleaned of any remaining solvent combination by placing the container's contents under a hoover for the night. By rotating the flask at the appropriate temperature, hydration is accomplished using various concentrations of a hydroethanolic mixture containing the medication. [53]

2.2.4Classic approach

The medication and phospholipid are dissolved in ethanol and heated in a water bath to 30°C + 1°C. In a closed vessel, the lipid mixture is added to with double-distilled water in a thin stream while being constantly stirred at a speed of 700 rpm. By three rounds of passing over a polycarbonate membrane using a manual extruder, the resultant vesicle solution is homogenised. [54]

2.3. Fabrication process of nanocarrier (Liposome)

2.3.1 Thin film hydration method

One of the popular techniques for creating liposomes is this one. and. Multilamellar vesicles (MLV) are made by solubilizing phospholipid, either natural or synthetic, in ethanol, dichloromethane, chloroform, or a 3:1:2:9:1 combination of chloroform and methanol. The next step is the hydration procedure, which involves hydrating a totally dried thin film for 1-2 hours at 60ºC–70°C with an aqueous phase phosphate buffer solution with a pH of 7.2.[55]

2.3.2 Injection methods

2.3.2.1 **Ether injection method**

Here, the lipid mixture is continuously stirred to dissolve in ether or diethyl ether before being injected into a PBS or aqueous phase. Which under injection pressure induces the elimination of practically all organic solvent that finally forms liposomes, Moreover, the heterogeneous liposomal formulation flaw affects this approach.[56]

2.3.2.2 **Ethanol injection method**

In the ethanol injection technique, the lipid mixture is dispersed in ethanol while being continuously stirred, and then it is injected into either distilled water or a warmed TRIS-HCl buffer. A drug's hydrophobicity and hydrophilicity determine how much of it enters a liposomal vesicle. It benefits from utilizing non-toxic materials and ethanol and is simple to scale up.[57]

2.3.3 Sonication method

It is the approach of creating small unilamellar vesicles that is most frequently acknowledged (SLV). SLV are created by solubilizing natural or synthetic phospholipid in a 3:1, 2:1, or 9:1 v/v ratio of either methanol or chloroform to ethanol, dichloromethane, or ethanol. When this mixture is rotated and dried in a rota-evaporator under vacuum at a temperature of around 45ºC to 60°C, a homogenous thin film develops.

Overnight, Layers is dried under nitrogen. After that, the thin film is hydrated by being submerged in an aqueous phase phosphate buffer solution with a pH of 7.2 for 1-2 hours at 60ºC-70°C. The bath sonicator is further used to change the size of vesicles. To eliminate any titanium particles that may have been introduced owing to overheating during the sonication process, the liposomes are then centrifuged. The main disadvantage of such vesicles is the reduced encapsulating space.[58]

2.3.4 High-pressure extrusion method

By dissolving natural or synthetic phospholipid in chloroform, dichloromethane, ethanol, or a 3:1, 2:1, or 9:1 v/v solution of chloroform and methanol, liposomes may be created. When this mixture is rotated and dried in a rota-evaporator under vacuum at a temperature of around 45–60 °C, a homogenous thin film develops. Overnight, Layers is dried under nitrogen..[59]

2.3.5 Reverse-phase evaporation method

Here, the lipid mixture is dissolved under continuous stirring in organic solvents such as ether, diethyl ether, or a mixture of diethyl ether and chloroform (1:1 v/v); a mixture of methanol-chloroform (1:2 v/v); before being added to a PBS solution or an aqueous phase that contains citric acid and Na2HPO4 to increase the formulation's overall effectiveness. [60]

2.3.6 Calcium-induced fusion

In this instance, acidic phospholipids are employed to produce SUV by thin film hydration, which is then followed by the addition of calcium, which leads to fusing and the formation of MLV.

The production of large unilamellar vesicles (LUV) is caused by the final addition of ethylene diamine tetra acetic acid (EDTA) to MLV.[61]

3.CHARACTERIZATIONS OF ETHOSOMES:

3.1. Vesicle Morphology

Transmission electron microscopy (TEM) and scanning electron microscopy (SEM), which stain the samples negatively with an aqueous solution, are used to examine the morphology of the ethosomal system. The solution of ethosomes was stained using a tiny carbon-coated grid. Blotting is used to remove the extra solution. It is subsequently dried, and TEM or SEM is used to see the vesicles.[62]–[64]

3.2. Vesicle size and size distribution

Vesicles in the ethosome formulation are typically evaluated with PCS (photon correlation spectroscopy) and dynamic light scattering (DLS) using a computerized system[65]. The formulation's ingredients have an impact on the range of diameters between microns and nanometers.

3.3 Percent entrapment efficiency: Determining entrapment efficiency is crucial since it gives ethosomes their delayed release properties [66]. The following methods are often used to calculate the entrapment efficiency.[67]-[68].

3.4 Zeta potential:

The Zeta potential is the development of charge at the interface between a liquid medium and its solid surface. With a zeta meter or zeta sizer, the zeta potential is evaluated. Milli Volts[69], and Zeta Potential Unit are used to represent it [70].

3.5Permeation distinctiveness:

The penetration capabilities of ethanol have long been recognized. Two effects are attributed to the penetration properties of which are enhanced by the creation of flexible ethosomal characteristics and are provided by the synergistic interaction between the lipids in skin, vesicles, and ethanol, demonstrating that the penetration enhanced by ethosomes is significantly superior to the penetration enhanced by ethanol alone. The following are the two results.

3.5.1 Push effect: Increase in thermodynamic activity brought on by ethanol vaporization
3.5.2 Pull effect: Ethanol lowers the skin's natural barriers, causing the drug's penetration to be increased. [71]

3.6 Measurement of surface tension: The Du Novy ring tensiometer is a ring-based technique for determining a drug's surface tension.

3.7 Physical stability: Cholesterol plays a crucial role in the dispersion of ethosomes throughout the body, and aggregation occurs when cholesterol is absent. Because of the high level of ethanol in ethosomal formulation and the sensible amount of cholesterol that ensures its stability, cholesterol is discovered to be stabilized in the bilayer when ethosomes are preserved in the gel form. This flexibility of ethosomal vesicles is also made certain. The freeze drying method [72]-[73].

3.8 Transition temperature: Differential scanning calorimetry is utilized to find theethosomal system's transition temperature.

3.9 Vesicle - skin interaction study: Fluorescence microscopy, scanning electron microscopy, and transmission electron microscopy may all be used to examine the interaction between the skin and vesicle. Similar to SEM and TEM, fluorescence microscopy is carried out using the same procedure.

Table no:1evaluation parameter

S.no	Test	Technique/Instrument
1	Particle shape	Scanning Electron Microscopy, Transmission Electron Microscopy
2	Particle size analysis	Optical Microscopy
3	Drug Content	High Performance Liquid Chromatography/UV.
4	Drug Entrapment Efficiency	Ultra centrifugation technique.
5	In Vitro drug release study	Franz Diffusion cell
6	In Vitro skin permeation study	Franz Diffusion cell
7	Transition Temperature	Differential scanning calorimetry

3.10 CHARACTERIZATION OF LIPOSOMES:

3.10.1 Percentage Yield: The weight of the dried finished product in relation to the beginning weight of the drug and polymer used to make the liposomes has been utilized to determine the percentage yield of various liposome

formulations. The formulation below is how this calculation is done.

Yield percentage = (yield in practice divided by the total yield in theory) / 100.

3.10.2 Particle Size: Optical microscopy is used to gauge the size of liposomes. The liposomes of various formulations are evaluated while the eye piece and stage micrometers are calibrated. At least 30 liposomes have been identified.[76]

3.10.3 Liposome Surface Morphology

A method of imaging that produces photographic pictures and basic data. Particle system (nano system) (micro system),An electron beam scans the sample in a predetermined pattern after positioning it in an evaluation chamber. A multitude of physical events are created when the electron beam interacts with the specimen, and when they are identified, they may be utilized to create pictures and reveal fundamental details about the specimens. [77]

3.10.4 Zeta potential analysis

With the Zetasizer, the surface charge of drug-loaded vesicles is determined (Malvern Instruments, Malvern, UK). The average zeta potential of the vesicles is obtained after an analysis that lasts 60 second.[78]

3.10.5Analyzing drug efficiency

The centrifugation method is used to determine the loading effectiveness of liposomes. A 2ml microcentrifuge tube contains 2mg of lyophilized formulation that has been precisely measured and weighed. 2 ml of the ethanol-PBS combination are added to it, and the formulation is vortexed to suspend it. Next it is centrifuged at 15000 rpm for 15 minutes after being sonicated for a few minutes. The absorbance of the clear supernatant solution is measured in a UV/VIS Spectrophotometer against a blank at a wavelength of 432 nm after centrifugation. The standard curve is used to calculate the drug content.

3.10.6 Drug loading: The liposomes' efficacy in loading drugs is calculated using the method below:

(Weight of the medication in a formulation) X 100/Total formulation weight is the percentage loading)[79]

3.10.7 Investigation on drug release from vitro membranes made of cellophane

For this work, cellophane membrane (0.45 m, purchased from Sigma Chemicals) was employed. 1g of the preparation is put into a cellophane membrane that has been pre-soaked in the release medium for an overnight period. At 37°C 0.5°C, the loaded membrane is securely positioned in the dissolving media (phosphate buffer, pH 7.4). The samples are spectrophotometrically analyzed at the chosen max, and the drug concentration is calculated using the calibration curve that was previously created. Every data point was the average of three conclusions. During a period of 12 hours, in vitro release experiments are recorded.[80].

3.10.8 Studies on stability

One of the most essential studies conducted during the development process is the stability study. The ideal product is one that remains stable in a wide variety of temperature and humidity conditions. The stability of the completed items must be ensured because they may be transported into different environmental zones.[81], [82]

3.10.9Evaluation parameter

Testing for accelerated stability is conducted on all formulations. A formulation's aesthetic appeal, clarity, pH, and percentage of medication remaining are all examined. There have been no changes in the visual appearance, clearness, pH, or percentage of medication left. After 4 weeks of stability. Although there have been some very tiny pH variations in the formulation, they still fall within the allowed range + (0.5).

3.**11 CHARACTERIZATION OF TRANSFEROSOME**

The characterisation properties of the transfersomes may be determined using a number of published techniques, including vesicle shape and size, size distribution, polydispersity index, zeta potential. Measurements of the number of vesicles per cubic millimeter, entrapment effectiveness, degree of deformability, and skin permeability are useful for optimizing the formulation of the transferosomal [81]-[82].

S.no	Evaluation parameter	Use of characterization Parameter
	Vesicle size, size distribution	Influence how effectively they are uptaken by cells
	Zeta potential	To optimize the formulations of suspensions, emulsions and protein solutions, predict interactions with surfaces, and optimise the formation of films and coatings.
	Vesicle morphology	These will help transport materials that an organism needs to survive and recycle waste materials.
	Entrapment efficiency	%Entrapment efficiency = Total amount of the drug added − Amount of the free drug Total amount of the drug added × 100
	Drug content	Drug content is an important parameter to evaluate the properties of the drug-loaded particles
	Degree of deformability	The decisive factor affecting the rolled metal deformation resistance is the total relative compression value
	Surface charge and charge density	It is a measure of how much quantity of electric charge is accumulated over a surface
	In-vitro drug release	In vitro release assays are employed to predict stability
	In-vitro skin permeation studies	In vitro permeation studies are meant to evaluate the permeation of drugs across the skin layers.
	Stability studies	To obtain information on the stability of a pharmaceutical product in order to define its shelf-life and utilization period under specified packaging and storage conditions.

3.11.1 Zeta potential, morphology, and vesicle size

While preparing transfersomes, comparing batches of products, and scaling up procedures, the vesicle size is one of the crucial variables. Regarding the formulation's physical stability during storage, the vesicle size's altering throughout storage is a crucial variable. As their bilayer membranes have a high degree of curvature, vesicles smaller than 40 nm are more likely to undergo fusion processes. In contrast, significantly bigger and electro neutral transfersomes aggregate by vander waals contacts because their membrane contact areas are substantially higher[83]. The capacity to encapsulate the medicinal molecules in transfersomes depends in part on the size of the vesicle. A high lipid-to-core ratio is preferable for lipophilic and amphiphilic agents, whereas encapsulating hydrophilic substances is favoured for greater aqueous core volumes. The vesicle diameter may often be determined using the dynamic light scattering (DLS)technique or photon correlation spectroscopy (PCS). The suspension of the vesicle can be combined with the appropriate media, and three separate measurements of the vesicular size can be made .Moreover, another method is to prepare the sample in distilled water and filter it using a 0.2 mm membrane filter [84].

3.11.2 Vesicles Per cubic millimeter Number

The optimization of the transfersome composition and other process factors depends on this parameter. Five times' worth of unsonicated transfersomal formulations are used sodium chloride, 0.9%. This sample is examined using an optical microscope and a hemocytometer. By using an optical microscope, the transfersomes with vesicles larger than 100 nm may be seen [85] We count and compute the number of transfersomes in tiny squares using the following formula:

Total transfersomes per cubic millimetre = (Total counted transfersomes x dilution factor / 4000)Number of squares tallied in total

The quantity of drug entrapped in the formulation is known as the entrapment efficiency (%EE). By extracting the unentrapped medication from the vesicles utilizing a variety of methods, including centrifugation using a small column[86].

3.11.3 Entraptment Efficiency

The entraptment efficancy can be calculated using either direct or indirect approaches in this procedure. The direct technique after ultracentrifugation would be to remove the supernatant and then break the sedimented vesicles with a suitable solvent that can lyse the sediment. The resultant solution can then be diluted and cleaned of contaminants using a syringe filter (0.22-0.45 m).

The entrapment efficiency, or percentage of drug entrapment, is represented as:

%Entraptment efficiency = amount of drug entrapped/total amount of drug added x100

Using a suitable solvent to dilute the supernatant and filtering it to eliminate contaminants is the indirect method of calculating the %EE. Using the proper analytical technique, the concentration of the drug in the supernatant is identified as the free drug.
The proportion of drug entrapment is therefore stated as:

% Entrapment efficiency= total amount of drug added- amount of free drug/ total amount of drug added x100

Deformability Level

This variable is significant since it influences how well the transfersomal formulation penetrates. The benchmark for this investigation is pure water. Several people participate in the preparation. Known microporous screens with pores between 50 and 400 nm. DLS measurements are used to record the particle size and size distribution after each pass.

The formula for expressing deformability is D = J(rv/rp)

Where D is the degree of deformability, J is the volume of solution extruded over the course of five minutes, rv is the size of the vesicle, and rp is the size of the barrier's pore.

3.11.4Release of drugs in Vitro

A scientific approach to formulation design can be made possible by the in vitro drug release profile, which can offer key insights on the release mechanism and kinetics. Transfersomes in vitro drug release is frequently assessed in relation to the reference product or the free drug. Many investigations have undoubtedly produced fruitful information about the medication release patterns of created transfersomes formulations. By using adhesive tape, the donor chamber is attached to the receptor chamber. A magnetic bar stirs the fluid in the receptor chamber continuously. As the average skin surface temperature is 32 ºC[87]-[88].

3.11.5In vitro studies on skin permeation

The goal of this study was to develop an original approach that combines molecular orbital (MO) calculations with artificial neural networks to estimate the skin permeability coefficient (log K (p)) of substances from their three-dimensional molecular structure. In the skin permeation investigation, Franz diffusion cells are used.[89]
The phosphate buffer saline solution in the receptor compartments of the Franz diffusion cells is agitated by a magnetic bar. The temperature of the receptor fluid should be maintained around 37 +_0.5 degree centigrade since it is utilized to simulate blood circulation beneath the skin [90].
Each donor compartment is put on the membrane with an adequate amount of the testing formulation added, and the top of the diffusion cell is opened to simulate non concluded circumstances.[91]-[92].

3.11.6 Stabilitystudies of Transfersome

By analyzing the shape and size of the vesicles in relation to time, one may ascertain the stability of transfersome vesicles. The mean size and shape of a sample may be determined using DLS and TEM.[93].

4.CONCLUSION

Nanotechnology's multidisciplinary versatility allowed for expansion and developments that raised people's standards of living. So far, the fundamentals of how nanotechnology is used in medicine and how nano drugs are made are still not well understood. As a result, substantial research in the area of nanomedicine, particularly in drug delivery systems, is required. Nanocarrier is always searching for better and more effective ways to cure illnesses. These new medicines must also be affordable, which places a strong demand on scientific research to find them. Being able to isolate the sickness and spare other healthy parts of the body from injury is a critical component of any therapy. Due to their accessibility, biocompatibility, and stability, metal nanoparticles are being used as targeted drug delivery systems with great success today. These actions generate a stronger biological impact because they enable the medications to be encapsulated and delivered directly to the targeted areas.

5. FUTURE PROSPECTIVE

In recent decades, a series of nano-carriers (nano-emulsion, nanoparticles, nanospheres, nano-micelles, etc.) have been created to deliver bioactives to specific places in regulated ways. Due to its beneficial uses in the pharmaceutical and cosmetic sectors, nano- formulations have attracted enormous demand. The potential for plant-based nano-formulated anti-aging medications with higher effectiveness in smaller dosages has been sparked by the stated uses of nanotechnology in refining the activities of plant-derived natural bioactive that are applicable in aging treatments. In order to make any conclusive statements, further pre-clinical and clinical research that takes into account the toxicological

effects of nano-carriers in the physiological environment is required. To create safe and effective phyto-nano-products for aging intervention, integrated research using nanotechnology, Ayurveda, and other traditional herbal remedies must also be conducted.

Table no: 3 recent studies of liposomes nanocarrier system for enhancement of drug delivery system

S.no	Nano carrier system	Loaded active compound	Research outcomes	References
1	High carbon containing cationic flexible liposomes	pramipexole	While increased carbon content and a higher amount of substitution can be explored for sustained therapeutic levels, low chain and mono substituted phospholipid displayed quicker and higher plasma concentration.	[85]
2	Ultradeformable liposomes	Ammonium glycyrrhizate	Ultradeformable liposomes containing ammonium glycyrrhizate reduced inflammation in human volunteers.	[86]

Table no: 5 recent studies of transferoosmenano carrier system for enhancement of drug delivery system

S.no.	category	Drug	In vitro/in vivo studies	outcomes	References
1	NSAID	Meloxicam	Skin interaction studies	The physicochemical properties of the vesicle systems and increased skin permeability have an impact on the SC barrier function.	[90]
2	Anti-cancer drug	Methotrexate	Skin studies	Enhanced linearity via transdermal EM flux with an increase.	[94]
3	Estrogen	Oestradiol	Plasma drug concentration measurement	the transdermal flux increased	[92]
4	NSAID	Curcumin	Modified Franz diffusion cell	increased entrapment effectiveness	[94]
5	Vitiligo	Corticosteroids	cellophane membrane	almost entirely in position	[85]
6	Progestin	Norgesterol	In vivo study conducted on male albino rats skin	enhanced self-penetration and higher entrapment efficiency	[94]
7	Chemotherapy	Tamoxifen	Skin studies	Improved transdermal flux	[94]
8	Hypoglycemic	Insulin	Franz diffusion cell	high effectiveness of encapsulating	[75]
9	Antihypertensive (HCN channel blocker)	IvabradineHcl	In vivo/ in vitro studies	Compared to the pure medication, showed better skin retention and permeability as well as improved pharmacokinetic characteristics.	[90]
10	NSAID	Lornoxicam	Skin intraction studies	Demonstrated high anti-inflammatory action compared to transferosomes, as well as higher flow and an apparent permeability coefficient.	[90]
11	Anti micobacterial	Rifampicin and vancomycin	Skin studies	Better skin permeability, excellent in vitro anti-lesishmanial activity, and substantially smaller lesions were all shown by transferosomes.	[94]

List of Abbrevation

BCE-Before common era
TDD-Topical drug delivery
PBS-Phosphate buffered saline
TLC-Thin layer chromatography
MLV-Multi lamellar vesicles
SUV-Small unilamellar vesicles
SCF-Super critical fluid
DLS-Dynamic light scattering
PCS-Photon correlation spectroscopy
HPLC-High performance liquid chromatography
API-Active pharmaceutical Ingredient
UV-Ultra violet
RH- Releative Humidity
ICH-International council for Harmonisation

Acknowledgement

I would like to convey my heartfelt gratitude to **Dr. Prabhakar Vishvkarma, Dr. Mojhaid ul Islam** for his tremendous support in the completion of my review paper.Your useful advice and suggestions were really helpful to me during review paper writing .In this aspect I am eternally grateful to you.

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