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Glycerosomes: A comprehensive review on noval liposomal drug delivery system for efficient delivery of therapeutics

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Abstract
In recent years the topical drug delivery system has gained more interest as compared to oral and parenteral drug delivery. But only a small number of drug molecules can be delivered this way because of the skin's outermost layer barrier function. Encapsulating the medication within glycerosomes is therefore one way to potentially solve this problem. Glycerosomes are new vesicular drug delivery vehicles that can be applied topically or systemically. Glycerosomes can be primarily prepared from water, phospholipids and high concentration of glycerol (from 10 to 30 %) these components are safe and suitable for use in topical preparation. Glycerosomes are improved liposomes, designed for transdermal and topical medication administration. Despite the fact that liposomes are a flexible drug delivery method, there are certain issues still need to be resolved, primarily those associated with entrapment, penetration, fluidity and stability. Compared to traditional liposomes, these drug delivery methods exhibit enhanced stability, fluidity, entrapment and penetration. In glycerosomes penetration and stability dramatically increases by using phospholipids and when glycerol concentration is raised up to 10, 20, or 30. Glycerosomes have the ability to encapsulate both hydrophilic and lipophilic drug to prevent from degradation. Glycerosomes carriers are currently the most attractive research topic for scientists. This review article provides brief overview on advantages and disadvantages, methods of preparation, characterization and evaluation, applications of glycerosomes in various fields based on previously published research papers.

Keywords: Glycerosomes, liposomes, phospholipids, hydrophilic and lipophilic

Introduction

This review article discusses a novel strategy for improving vesicular characteristics including drug entrapment and penetration by altering the fluidity of the liposomal bilayer. These vesicular systems, known as glycerosomes, were mostly composed of phospholipids, water, and glycerol (10-30%) [1, 2]. Similar to other vesicular systems, these carriers are safe and widely approved for topical treatments, which makes them appropriate for skin that is highly sensitive. This is because they are free from any harmful compounds, like ethanol, as a structural component [3]. The primary application for glycerosomes has been topical preparation. The term "topical drug delivery" describes the medical application of medication dosage forms to the skin, eyes, nose, and vagina [4, 5]. However, as topical drug administration primarily targets the skin, it is important to fully understand the structure of skin before entering deeply into the study of glycerosomes [6, 2].

To improve topical delivery of drugs, techniques like iontophoresis, sonophoresis, electrophoresis, microneedles, and vesicular drug delivery devices are employed. Aqueous amphiphiles (lipids and surfactants) surround an aqueous core to form concentric bilayer structures known as vesicles. These colloidal particles have the ability to encase medications that are both hydrophilic and hydrophobic. Vesicular drug delivery appears to be the most advantageous and helpful of these for topical drugs delivery [2].

When compared to traditional dosage forms, topical medication distribution is preferable because it provides more effective patient compliance and prevents first pass hepatic metabolism [7]. The term "conventional drug delivery" describes the traditional approach to giving a medication to the body. It has numerous drawbacks, and innovative drug delivery systems (NDDS) are currently the preferred option. NDDS increases medication efficacy and transports the medication to the necessary site [8]. The innovative glycerosomes, liposomes, transferosomes, ethosomes, and nanoparticles are a few instances of NDDS [2]. The unique vesicular structures known as glycerosomes that combine water, phospholipid and large concentrations of glycerol. Since glycerol is safe to use, it is not harmful, toxic, or irritating. It is included in vesicular preparations because it makes vesicles more stable and fluid. Glycerosomes' increased fluidity helps them penetrate the skin's surface more effectively than traditional liposomes [2, 3, 7]. These adaptable vesicular carriers can be made using any phospholipid, synthetic or natural, that is used to make traditional liposomes. They can also be generated using any of the usual liposome synthesis methods. Moreover, glycerosome medication delivery effectiveness can be increased by utilising essential oils as penetration enhancers because of their high penetration efficiency and minimal toxicity [9].

Glycerosomes can produce unilamellar or multilamellar vesicles, depending on their composition and manufacturing technique. Through homogenization or sonication, glycerosomes' multilamellar vesicles can become unilamellar. Phospholipon 100 (p100) polyene phosphotidylcholine, Phospholipon® 90 (p90) lecithin, and other mixtures of hydrogenated and non-hydrogenated phospholipids derived from soybeans are examples of natural or synthetic sources of phospholipid components. One or more phospholipids can be selected from these sources [1, 3]. Another crucial element of the cell membrane that keeps its appropriate permeability and fluidity is cholesterol. To alter the formulation's electric charge, other ingredients like stearylamine may be added [3]. Due to their

superior qualities over traditional vesicular structures, ease of manufacture, and harmlessness, these nanostructures have recently attracted attention from all around the world [2, 3].

Liposomes

Liposomes are phospholipid-containing spherical bilayer vesicles [10]. These were completely comprehended and acknowledged in 1970, despite Bangham's initial description of them in 1960 [11, 12, 13]. The components of phospholipids are a hydrophilic head and a hydrophobic tail. When these phospholipids come into contact with water, their hydrophobic acyl chains cause the phospholipids to form spherical structures. Van der Waals and hydrogen bonding are two chemical forces that promote liposome formation in addition to its thermodynamic stability. Liposomes have the ability to encapsulate both polar and non-polar substances because they are amphiphilic [2].

Glycerosomes

Glycerosomes are innovative vesicular drug delivery vehicles that can be used topically or systemically. These noninvasive carriers have the ability to reach deeper skin layers than a free medication should. Phospholipids, water, and a significant amount of glycerol (20–40%) make up glycerosomes, which are safe and suitable components for topical formulations. Increased hydrogen bonding and vesicle flexibility in the presence of high glycerol concentration favourably impact medication penetration [14, 15, 16]. Liposomes may encapsulate both polar and non-polar substances because of their amphiphilic nature [17]. Despite being utilised for topical and transdermal drug delivery, liposomes are not thought to be useful since they only stay in the superficial tissues and are unable to penetrate deeper into tissues [18]. These are utilised for a number of different types of drug delivery, including targeted drug delivery and sustained release, in addition to topical and transdermal drug delivery. Liposomes' stability issue restricts its application [19, 20]. Vesicular systems with enhanced properties are required. Glycerosomal carriers are currently the most fascinating research topic for scientists [3].

Glycerosomes are multifunctional vesicles in the shape of spheres that are made up of one or more phospholipid bilayers, much like liposomes, but with a high glycerol concentration that changes the fluidity of the liposome bilayer. Glycerosomes are a promising medication delivery vehicle since they can encapsulate both hydrophilic and hydrophobic medicines. The majority of the glycerosome formulations that were created were intended for topical administration, specifically the cutaneous route, where they have demonstrated encouraging outcomes. These vesicles have better spreadability and penetrability because of their high glycerol content, and they are biocompatible. Therefore, it is essential to investigate alternative topical drug delivery methods, including ophthalmic, vaginal, nasal, and rectal [21].

Difference between other vesicular drug delivery system and glycerosomes

There are a variety of vesicular drug delivery systems available, including "liposomes, microemulsions, nanoemulsion, nanogel, nanoparticles, nanostructured lipid carriers, nanocapsules, microspheres, nanocrystals, and cyclodextrins." These systems have also evolved to achieve improved solubility, a better stability profile, increased bioavailability, a prolonged effect, deeper penetration into skin tissues, and the prevention of undesirable effects [22]. Of all these systems, glycerosomes are the most preferred and advantageous because they are vesicular-based and emulsion-based, and therefore more stable and fluid than liposomes [23-25]. By raising liposomal bilayers' deformability index, the glycerol found in these vesicular systems promotes epidermal penetration [26, 14]. Glycerosomes have the following additional benefits over liposomes:

- Vesicles' a greater permeability at the target location [2]
- More drugs are being encapsulated in vesicles [27]
- Enhanced stability and fluidity of lipid vesicles [28]
- These vesicular structures do not require phospholipids to reach a transition temperature, in contrast to liposomes. These are setup at standard temperatures [22, 23].
- This has two unique properties. Two things about glycerosomes: first, they can be made at room temperature without the need for a different temperature, and second, thermolabile material can be added to them [3].

Structure and Composition of Glycerosomes

Like traditional liposomes, phospholipids and cholesterol make up the novel vesicular structures known as glicosomes. Phospholipids rapidly organise into bilayer vesicles when dissolved in water [29]. The only distinction is that these additionally contain water and glycerol at concentrations of 10, 20, 30, 40, and 50% in addition to the excipients found in liposomes. Up to now, lipidosomes have been proposed for topical and cutaneous medication administration. It is an entirely safe and non-toxic way to provide medication [14, 30, 31-35].

Structure of glycerol

Glycerol is both an alcohol and a viscous liquid. It has three hydroxyl groups, which give it hydrophilic qualities. Vegetable and animal fats contain glycerol, a triglyceride. It can be extracted as a by-product of making biodiesel or as part of the process of making soap. It functions as an emulsifier, lubricant, humectant, and edge activator in medicinal preparations [36]. Phospholipids: Both synthetic and naturally occurring phospholipids can be used to make glycerosomes, much like with ordinary liposomes [11]. Various hydrophilic head groups, hydrophobic tails, and alcohol groups contribute to the variety of phospholipids. These are compatible with practically all ingredients in addition to being amphiphilic. Phospholipids aggregate into various forms when dissolved in water. The ability of the phospholipids to self-assemble in a hydration media is dependent on their characteristics. Based on the differences in their backbone structures and the alcohol moiety they contain, there are many varieties of phospholipids.

Types of Phospholipids

Glycerophospholipids

Eukaryotes are the source of glycerophospholipids. The primary component of these lipids is glycerol. Phosphatidylcholine, phosphatidylserine, cardiolipin, and other lipids are formed when these lipids' hydrophilic head group changes. However, these lipids undergo acyl chain changes that lead to the creation of dimyristoyl and dipalmitoyl phosphatidylcholine [3].

Sphingomyelins

Sourced from the cell walls of animals, these phospholipids are not the same as glycerophospholipids because the former have a glycerol backbone while the latter have a sphingosine backbone. Not only do these have different chemical structures, but their acyl chain compositions vary in terms of the number of groups. It is believed that glycerophospholipids are symmetric while sphingophospholipids are asymmetric. Naturally occurring sphingomyelins have more than 20 acyl groups, although paraffin residues have less groups than the natural ones. As a result, it is called asymmetric. Because phosphatidylcholine, an example of a glycerophospholipid, has an equal chain length, they are referred to be symmetric molecules [2].

Qualities that allow phospholipids to form glycerosomes

The phospholipids must be able to create vesicles that are both safe and able to contain therapeutic substances. Vesicles that are naturally degradable must arise from the phospholipids. The vesicles that the phospholipids produce must be biocompatible with the other ingredients in the formulation [2].

Cholesterol

Cholesterol makes up the majority of an animal cell membrane. It has been shown to impact a number of membrane characteristics. Cholesterol influences the stiffness, thickness, stability, and fluidity of cell membranes in many ways. The goal of adding cholesterol to glycerosomes is to increase their stability. The hydrophobic property of cholesterol makes it stable and mostly comes into touch with the interior cavity of liposomes. It has been found that vesicular systems allow for the addition of 50 mol percent of cholesterol. According to reports, the ratio of lipid to cholesterol that produces effective liposomes is 2:1. The rationale underlying this ratio is not entirely clear, though. Numerous studies have examined the impact of cholesterol incorporation in vesicular structures, and the findings indicate that cholesterol is involved in the following processes. It is involved in the production of liposomes because it renders the membrane resistant to water and electrolytes. It improves lipid bilayer packing and ordering efficiency. It facilitates the lipid vesicles' disintegration. It modifies the movement of vesicles to increase the stiffness of lipid bilayers [2].

Method of preparation of glycerosomes

Phospholipids and liposomes that resemble cholesterol make up lipidosomes. The presence of water and glycerol in different amounts ranging from 10% to 50% is the only difference. The same routine techniques that are often used to generate liposomes can also be used to prepare glycerosomes [36, 11]. Despite this, the thin film hydration approach has been widely employed by researchers for similar purposes [36, 40]. The following is a description of the many preparation techniques.

Thin film hydration technique

This is the simplest method for making glycerosomes that Bangham *et al*. have provided [3] The thin film is produced by dissolving the phospholipid in a solvent that is organic and drying it. Next, aqueous phase (a solution of water and glycerol) is added to this thin film, and the produced dispersion is sonicated using a high-intensity ultrasonic sonicator. The advantage of this process is that, in comparison to other ways, the formulation created has better physical properties including spherical shape and smooth texture, as well as higher encapsulation efficiency. [37, 38-41]. When adding a medicine to be encapsulated, aqueous hydration buffer is used if the drug is hydrophilic, and lipid film is used if it is lipophilic [2].

Reverse Phase Evaporation

It was Szoka and Papahadjopoulos who initially described this technique. This causes water to develop in the oil emulsion, as opposed to the spherule method. The primary benefit of the reverse phase evaporation approach is that a greater amount of the aqueous medium is trapped when vesicles that have large aqueous to lipid ratios are formed. The process entails filling a 50 ml round-bottom flask with the lipids. The fats can be added on their own or combined with other fats, including cholesterol. Controlled evaporation is used to remove the solvent. Following this stage, nitrogen gas is used to purge the dried lipid layer, and it is then redissolved in an organic solvent. The creation of inverted micelles occurs in this particular organic solvent. In order to remove the organic solvent, the dispersion is subsequently added to a rotary evaporator that is set up to operate at 200 rpm, decreased pressure, and 20–25°C. Aqueous suspension is produced when the combination transforms from viscous gel, which begins to foam during evaporation. The suspension is allowed to evaportate at 20° for over 15 minutes after adding more aqueous buffer. After dialyzing and passing through a Sepharose 4B column, the final product can either be centrifuged or eliminated. To extract non-encapsulated material, centrifugation or transit through a column are needed. Using an organic solvent such as ethanol, isopropyl ether, or a combination of isopropyl ether and chloroform with an aqueous glycerol solution, a two-phase system comprising cholesterol and phospholipids is first sonicated to create the water-in-oil emulsion in this approach. When organic solvent is extracted under low pressure, a thick gel is created. The process of continuous rotating evaporation under lower pressure prepares the glycerosomes by eliminating any remaining solvent. This technique can encapsulate big macromolecules with a high encapsulation efficiency and is used to generate huge uni- and multi-lamellar vesicles. Being introduced of the materials to organic solvents and brief bursts of sonication is the primary disadvantage of this technique [3]

Solvent Spherule Method

Using this technique, precisely weighed phospholipids are dissolved in an organic solvent and then water is added. Following an approximate one-hour vacuum agitation period, the resulting mixture is turned into liquid spherules, which are subsequently evaporated using a water bath to remove the organic solvent. The high drug content of the glycerosomal formulation and homogeneous size distribution provided by this approach are advantages [29].

Ultrasonic technique.

With a diameter of between 15 and 25 nm, these tiny unilamellar vesicles are prepared using this technique. There are two ways to ultrasonically disperse lipids in glycerol: one method involves using sonication techniques.

Probe sonication: The glycerosomal dispersion is immediately approached by the sonicator's tip. With this approach, there is a significant energy input into lipid dispersion. The vessel holding the formulation needs to be submerged in an ice/water bath because the coupling of energy at the probe's tip causes localised heat. Titanium sloughs off easily from sonicator tips, contaminating the solution and necessitating centrifugation to remove.

Bath Sonication: Bath sonication involves inserting a test tube including glycerosomal dispersion into the sonicator and sonicating the suspension for five to ten minutes over the lipid's critical solution temperature (Tc). When using this technology instead of a probe sonicator, temperature control is simple [3].

Freeze thaw Method

The freezing and thawing process is used in this method. The freeze-thaw approach can only be applied to crude phospholipids or to mixes of charged phospholipids, that is, phospholipids that exhibit both positive and negative charge. Tiny unilamellar liposomes are quickly frozen and then thawed. Then, they undergo sonication to produce LUVs. As a result of this process, bilayers of SUVs fuse together during freezing and thawing, forming LUVs. Liposomal production using this approach is inhibited by increasing ionic strength or liposome concentration. There is a drawback to the method: it is unable to be used to extract biological components, which need a lengthy and temperature-sensitive process [29].

Microfluidization method

This technique makes use of a microfluidizer that can continually produce a huge volume of liposomes with a high aqueous volume. Using a microfluidizer, Mayhew *et al*. produced

liposomes and proposed that they had characteristics similar to small extruded multilamellar liposomes. The liposomes manufactured with a microfluidizer produced smaller liposomes with a uniform size distribution when compared to typical multilamellar liposomes. According to Mayhew *et al*., the procedure entailed adding aqueous lipid suspensions to a reservoir. Fluid is pumped via filters after the suspension travels from the reservoir to the pump. High pressure must be used to pump the fluid through a 5 µm filter. The streams are then split into two and sent to the interaction chamber, where they converge at a breakneck speed in tiny channels. The liposomes are examined after processing the outflow. Micro fluidization-produced formulation has a number of benefits, such as easier manufacture, quicker dissolving, increased stability, and smaller particle size [3].

The Fusion Method Induced by Calcium

Using this process, SUVs are fused with calcium (Ca2+) to generate LUVs, which are then transformed into cochleate cylinders. To maintain the membrane's elasticity and to restore the negative charge, EDTA is also implemented [27].

Ethanol Injection Method

In 1976, Batzri and Korn reported on the ethanol injection method. This approach involves dissolving lipid in ethanol and forcing it through a tiny hole—possibly a syringe—in an excess of aqueous media. When infusing ethanolic lipid solution into an aqueous media, the speed at which the two are completely mixed should be achieved. For the phospholipids to disperse in water and for ethanol to be diluted immediately in hydration medium, the two must be well mixed. The primary benefit of this technique is that it can produce small liposomes less than 100 nm in size without the need for sonication or extrusion by injecting a lipid solution dissolved in ether into water. Moreover, diluted and uniform liposomes are produced. The limitation resulting from lipid solubility in ethanol limits the amount of lipid that can be added to ethanol and, consequently, the quantity of ethanol that can be applied to aqueous medium. This is one drawback of the ethanol injection method. Although dialysis can eliminate it, ethanol is still present in liposomes [2].

Solvent Injections Method, Ether Injection Method

This procedure, which dissolves lipids in a combination of diethyl ether and ether methanol, was described by Watkins. Subsequently, these are introduced into a heated aqueous solution containing the substance to be encapsulated. It is important to keep the heated aqueous phase above ether's boiling point. During encapsulation, a temperature of 55–65° is typically maintained. Injections should be made slowly rather than quickly. Ether evaporates when it comes into touch with a heated aqueous phase, creating unilamellar vesicles. This approach has drawbacks, such as a limited yield of heterogeneous liposomes and the exposure of the chemicals that need to be encapsulated to high temperatures and organic solvents. One advantage of this technique is that it prevents oxidative lipid degradation [29].

Double Emulsion Evaporation

This procedure generates a double emulsion of type W1/O/W2. It is composed of two aqueous phases: inner and outer. Each oil globule in the outer aqueous phase contains a small droplet of the inner aqueous phase, while the outer phase is made up of scattered individual oil globules. The steps involved in double emulsion evaporation are as follows:

The drug's aqueous phase, which is dissolved in water, is combined with an organic solvent that contains lipids. This creates the water in oil emulsion, which is subsequently homogenised appropriately to form the primary emulsion (W1/O). Double emulsion is created when the primary emulsion is mixed with an outer aqueous phase that contains stabilizer. One advantage of the double emulsion solvent evaporation method is that it can produce formulation using simple tools, is appropriate for controlling process variables, and is comparatively simple to use. This method works well for encapsulating proteins and peptides, which are very water-soluble materials [29].

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 technology [37]. Alkyl maltosides' function as detergents in the dialysis process of liposome production was examined by Alpes *et al*. According to a report, the essential

micelle concentration of the detergent and the dialysis membrane's penetration rate determine how quickly detergent is removed. It was also noted that the critical micelle concentration is primarily determined by the alkyl chain length. Decyl maltoside was found to be the best detergent for preparing vesicles using the detergent removal method because of how quickly it forms vesicles [2].

French Pressure Cell Method

This method prepares multilamellar 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 [29].

Critical parameters of glycerosomes

Particle size analysis

Utilising a zetasizer, one may assess the particulate size analysis and polydispersity index (PI) of the glycerosome formulation. It is also referred to as photon correlation spectroscopy [36, 40] or dynamic laser light scattering [39, 41]. This is often accomplished using a Malvern zetasizer.

Development of vesicles

The use of cryo-TEM, TEM, and SEM (scanning electron microscopy) can verify the development of vesicles [2, 39]. Before being examined under an electronic microscope in a TEM, the samples are first stained with 1% phosphotungstic acid. Using a carbon rod coated with samples, ethane is poured to its melting point for cryo-TEM analysis. Subsequently, these are examined using TEM.

Determination of deformation index

The process of determining the deformation index involves passing the glycerosomal formulations through a membrane extruder that has a specified pore size. This is done at an appropriate pressure using an extruder. It is expected that the membrane's hole size will be less than the average phospholipid vesicle size. Flexible vesicles are necessary for penetration via skin because they may readily fit into skin pores. This kind of vesicles are thought to be formed by glycerol; therefore, the deformation index is computed to determine if glycerol can generate vesicles that may modify shape [28].

Calculating entrapment efficiency (%)

There are various methods for figuring out entrapment effectiveness Manca *et al*. [36, 39, 40] have employed the dialysis technique. Following the passage of the glycerosome preparation from a dialysis tube containing 12,000 to 14,000 Dalton, the entrapped and non-entrapped formulations were separated. The drug content of these was then tested. The formula for measuring entrapment is acquired drug content/initial drug content×100, which compares the percentage of drug ingested prior to dialysis to that produced after dialysis [2].

Determination of Penetration

This one aids in figuring out how much of the skin layer has been penetrated by glycerosomes. Ex *vivo* methods can be used to accomplish this. It aids in figuring out how drugs are delivered via the skin layer. For this, a Franz diffusion cell is employed. The stratum corneum of the animal skin is oriented towards the donor side when it is placed between the donor and receptor compartments. Following the application of glycerosome formulations to the stratum corneum at specific intervals, the medium gathered within the receptor compartment is removed and substituted with new medium. It is examined for drug content using an appropriate technique (HPLC or UV). Following the administration of each sample, the skin separates from the Franz diffusion cell. The epidermis and dermis are separated, and the medication quantity is then calculated by sonicating the layers [28].

Measuring drug release *In vitro*

Glycerosomes drug release *in vitro* is measured using a dialysis bag. This process involves filling a dialysis bag with one millilitre of glycerosome preparation and submerging the bag in phosphate buffer. Samples are removed at predetermined intervals and examined using an HPLC or UV technique [2].

Evaluation of fluidity

The fluidity of lipid bilayers is assessed by investigations using differential scanning colorimetry (DSC). With the aid of this technique, one can ascertain the phospholipids' transition temperature. Transition temperatures of phospholipids have been observed to show how they interact with exogenous chemicals. Accordingly, a shift in the transition temperature upon the addition of glycerol is noted, which further supports the idea that the glycerosomes are more fluid. The hydration impact of glycerol on lipid bilayers lowers the melting point of the acyl chain of phospholipids, hence reducing the transition temperature of phospholipids. Amphepathic drugs and cholesterol have an effect on the phospholipid's transition temperature. By stopping the pretransition peak from growing over the main transition peak, cholesterol disrupts the bilayer packing. The effects of amphiphilic diclofenac sodium were described by Manca *et al*. According to reports, diclofenac decreased the transition temperature via increasing the glycerol-phospholipid bilayer contact. This is because diclofenac is amphipathic and enters lipid bilayers through insertion [36, 40]. Salem *et al*. described using glycerosomes and their gel to administer celecoxib and cupferron topically. The phospholipid they employed was soybean phosphatidyl choline. Unlike Manconi *et al*. [36], who documented the influence on vesicles by correlating phospholipids Tm before and after forming them in glycerosomes, they demonstrated the effect on fluidity of vesicles through the comparison the transition temperatures of celecoxib and cupferron. Celecoxib and cupferron in their pure forms had sharp DSC peaks of 162 and 177°, Salem *et al*. observed, while the glycerosomal formulations produced slightly lower temperature peaks at 145 and 164°. More vesicle fluidity and improved encapsulation efficiency were demonstrated by this.

Determination of Stability

Zeta potential can be found using a zetasizer, which also provides information about the stability of the manufacturing process and any charges that may be present on the surface of glycerosomes as a result of the addition of

charged species during formulation. The formulation is more stable when the zeta potential is negative. Glycerosomes have reportedly been shown to be more stable than liposomes. Glycerosomes did not exhibit any changes in vesicle size or PI during the stability study period, according to research findings [36, 40]. Nonetheless, glycerosome vesicle size changes were noted by several researchers. The production of curcumin polymer glycerosomes and curcumin glycerosomes was described by Manca *et al.* [39]. The above formulations were kept for ninety days at room temperature in order to undertake stability experiments. Glycerosomes grew in size, according to the data. It took 30 days to notice this alteration, and until the stability investigations were finished, nothing changed. In contrast to trimethyl chitosan, glycerosomes containing hyaluronan exhibited no change in size after 60 days. Speranskia tuberculata essential oil was added to paeniflorin glycerosome formulations, according to Zhang *et al*., to enhance skin delivery. Glycerosome-containing oil was kept both at room temperature and as low as 4º for the purpose of conducting stability investigations. On certain days, formulas were examined. Findings revealed that there were differences in particle size in glycerosomes kept at room temperature. While the glycerosomes that were kept at 4°C exhibited no modifications in particle size, the size of the particles increased [14]. Glycerosomes did not exhibit any change in size at the conclusion of stability testing, although liposomes did, leading Manca *et al*. to hypothesise that glycerol functioned as a non-aggregating agent [2].

Application of glycerosomes

Glycerosome Uses in Cosmetic Products Triptolide-loaded glycerosomes and traditional liposomes were compared in a study conducted by Zhu *et al*. (2020). Enhanced trans-dermal permeability, stability, and biocompatibility of glycerosomes than traditional liposomes were observed in the optimisation process, which employed an orthogonal experimental design. Glycerosomes therefore have tremendous promise for use as drug delivery vehicles in the dermis and transdermal layers. In 2014, Wang *et al*. looked into the effectiveness of croton oil-induced rosacea and created hexosomes, glycerosomes, and ethosomes with "soy phospholipid, sodium deoxycholate, and tretinoin; 1, 2-hexanediol, glycerol, and ethanol" as the main ingredients. The current research showed that hexosomes have a great deal of promise for improving skin penetration. Because of this, it was discovered that the suggested procedure had a great deal of promise for treating rosacea [37].

Inhaled Drug Delivery using Glycerosomes

Because they have a few advantages over regular aerosols, including continuous release, reduced local irritation, and great stability due to their big hydrophillic core, glycerosomes may be utilised to treat a variety of respiratory illnesses. Rifampicin and curcumin have recently been effectively delivered to the lungs via the intratracheal route using glycerosomes. This led to an enhanced drug build up in the lungs [39]. According to Manca *et al*., curcumin can be delivered to the lungs by being incorporated into polymer glycerosomes. In order to create polymer glycerosomes, they combined two polymers with glycerosome formulations to create polymer glycerosomes. The findings showed that the size range of glycerosomes and polymer glycerosomes was 65–112 nm and they were unilamellar. Additionally, it was observed that both formulations showed signs of curcumin accumulation in the lungs; however, *in vitro*, polymer glycerosomes demonstrated higher biocompatibility. The formation of a network around polymer glycerosomes, as opposed to a coating, was observed by researchers when utilising hydrophilic polymers and glycerol. By forming more stable vesicles than previously and making minor adjustments to the dispersion medium, this mesh/network facilitates the effective delivery of phospholipid vesicles in *vivo*. In addition, polymers are biocompatible and safe for use, among their many other benefits. They have excellent delivery properties as well. Because polymer glycerosomes aerosolized, they were able to effectively transfer curcumin to the lungs while simultaneously enhancing its anti-inflammatory and antioxidant properties. Overall, polymer glycerosomes have been shown to be appropriate for transport to the lungs, where they are anticipated to enhance patient adherence and prevent hepatic first pass metabolism [39].

The formulation of polymer glycerosomes for lung administration to treat pulmonary disorders was reported by Melis *et al*. They contained a lot of rifampicin. Glycerosomes containing polymer and those without were made. To create polymer glycerosomes, trimethyl chitosan chloride and sodium hyaluronate were added. The findings showed that the pulmonary system's medication concentration increased as a result of polymer glycerosomes. Polymers produced stable vesicles that increased the amount of medicine deposited in the lungs and increased the mean vesicle width. These vesicles were easily aerosolized and were distributed as far as possible using a next-generation impactor [2].

Oral Delivery with Glycerosomes

Manconi *et al*. described the preparation of PEGylated Penetration Enhancement Vesicles, Glycerosomes, and Liposomes for the oral delivery of Citrus Lemon Extract. The phenol extract was made by macerating the fruit rind in ethanol. Next, various vesicular systems were loaded with this extract. These were made with the intention of improving the effectiveness of vesicular systems-based treatment for oral cavity illnesses. According to a claim, glycerosomes and PG-PEVs outperforming traditional liposomes due to their increased penetration. Both of extract-loaded and empty vesicles had their PI and zeta potential assessed. It was noted that the sizes of PEGylayed penetration boosting vesicles, glycerosomes, and empty liposomes were modest. Additionally, an increase in glycerol (25 and 50%) and glycol (12, 25 and 50%) was shown to cause vesicles to enlarge to a size of about 100 nm. Having the capacity of the solvent to alter the lipid bilayer assembly and encourage the creation of larger vesicles was the reason given for the larger size of vesicles with high concentrations of glycerol and glycol. It was said that every vesicle loaded with extract was enormous in size. The zeta potential of empty vesicles was negative, while that of full vesicles was less negative. Extract-loaded glycerosomes showed a restricted size distribution (PI<0.25), while PEGylated penetration vesicles showed a polydispersed size distribution (PI<0.39). All vesicles, regardless of kind, had an entrapment efficiency of roughly 63%. The viscous form of glycerosomes and PEGylated penetration vesicles, in contrast to normal liposomes,

demonstrated good stability. The glycols made the vesicles viscous, which stopped them from fusing together. The antibacterial and antioxidant properties of the vesicles were also assessed. Since keratinocytes are derived from the oral mucosa's epithelium, they were employed to test antioxidant activity. Hydrogen peroxide was used to stress the cells, and a water-dispersed extract was selected as the standard. It was reported that the vitality of glycerosomes and PEGylated penetration vesicles improved to 100% and that of liposomes to 90%. By evaluating the zone of bacterial growth inhibition, antibacterial activity was evaluated. The reference used was an extract diluted in water. It was said that the bacterial growth was suppressed to the same degree by each vesicle. Furthermore, ensnaring the extract in vesicles and introducing it into the oral cavity has been suggested to facilitate effective dispersion of contents and enhance their release into the oral mucosa [2, 3].

Intrasynovial drug delivery:

Rheumatoid arthritis can be cured with a variety of therapies, such as corticosteroids, non-steroidal anti-inflammatory drugs, and medications that change the way anti-rheumatic illness's function. Due to their incapacity to administer the medication at the appropriate dose into the synovial cavity, none of them are able to accomplish the medication's ultimate purpose. The glycerosomal drug delivery technology can be used to alleviate this issue. Due to inadequate penetration, papeiflorin, an anti-inflammatory and immune-regulating medication primarily used to treat rheumatoid arthritis, has limited clinical use. When this medication was enclosed in glycerosomes, the synovial cavity saw a larger gathering of it [14].

Sustained Release Drug Delivery

With very little adverse effect, glycerosomes can be utilised to deliver a continuous release of medication and to keep drug levels steady for a predetermined amount of time. To achieve optimal drug release rate and sustained release in *vivo*, medications such as betamethasone and rifampicin have been encapsulated in glycerosomes [3, 11].

Intra-follicular Administration

One potential treatment option for skin conditions like hair loss is intrafollicular medication delivery. A new glycerosomal formulation makes it possible to apply minoxidil and other hard-to-absorb substances topically for localised action, namely to the sebaceous gland and hair follicles [41].

Glycerosomes for Delivery of Anti-inflammatory Agents

A wide range of adverse effects are associated with anti-inflammatory medications. Glycerosomal encapsulation is one approach that might be used to boost these medications' therapeutic efficacy. Glycerosomes have been successfully used to carry nonsteroidal anti-inflammatory medication constituents including diclofenac, celecoxib, and cupferron. Finally, studies have demonstrated that glycerosomes have a high degree of biocompatibility with human keratinocytes [42].

Enhance Antimicrobial Efficacy/Safety

the two explanations why antimicrobial drugs are enclosed in glycerosomes. They mainly guard against enzymatic breakdown of the medication contained. Additionally, because the vesicles are lipid-based, the antimicrobials are more readily absorbed by the cells of the microorganisms, lowering the toxicity level and frequency. After being successfully loaded into glycerosomes, various antimicrobial drugs, including resveratrol, gallic acid, tolnafate, and citrus lemon extract, shown superior antibacterial effectiveness against cultured plankton of various Streptococcus and Lactobacillus species [2, 3].

Ocular Drug Delivery with Glycerosomes

Glycerosomes are mainly used for topical drug delivery, recently the eye drop of antifungal drug like natamycin was prepared to enhance its entrapment efficacy and improve its penetration [2].

Conclusion and future perspectives

Taking everything into account, glycerosomes respond to a novel vesicular structure composed of varying quantities of glycerol. These structures work well for getting past skin barriers and delivering the drug to the deeper layers of the skin. Additionally, the glycerosomal drug delivery strategy can be essentially used because to its ability to increase skin penetration, enhance encapsulation effectiveness, and generate more flexible and fluidic vesicles. In this method, glycerol is employed as a carrier that modifies the skin's ability to permeate different layers. The simplicity of glycerosomal formulation at ambient temperature is an additional benefit.

Osmotic imbalance on both sides causes a delay in drug release when a larger quantity of medicines is encapsulated. However, in terms of consistency, trapping, smoothness, and stability, these configurations have shown themselves over the past few years [63]. There is a paucity of study on in *vivo* administration, despite glycerosomes' potential as drug delivery nanocarriers. Future research should focus on combining relevant biophysical investigations with *in vitro*/in *vivo* assessments, learning more about the drug release and encapsulation characteristics of these nano-self-assemblies, and investigating the possible clinical uses of these nano-self-assemblies.

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