ejpmr, 2024, 11(4), 205-215

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

REVIEW ARTICLE ON GLYCEROSOMES

Vinutha Naik*, Shripathy D. and A. R. Shabaraya

Srinivas College of Pharmacy, Valachil, Farangipete Post, Mangalore, Dakshina Kannada, Karnataka, India 574143.

***Corresponding Author: Vinutha Naik**

Srinivas College of Pharmacy, Valachil, Farangipete Post, Mangalore, Dakshina Kannada, Karnataka, India 574143.

Article Received on 18/02/2024 **Article Revised on 09/03/2024** **Article Accepted on 29/03/2024**

ABSTRACT

Although liposomes have proved to be versatile in the field of drug delivery, there are still some issues that need to be addressed mainly problems related to entrapment, penetration, fluidity and stability. Liposomes with deformable properties, including transfersomes, niosomes, ethosomes, glycerosomes, and invasomes, have been widely reported as carriers for enhanced transdermal drug delivery. This nanosystem generally comprises ~ 10–40% (v/v) short-chain alcohols, including ethanol, glycerol, propylene glycol, and isopropanol, which soften the phospholipid membrane, increase the solubility, and improve nanocarrier drug loading. Glycerosomes are sphere-shaped versatile vesicles consisting of one or more phospholipid bilayers similar to liposomes but contain a high concentration of glycerol (preferably 20 to 30% w/v), which modifies the liposome bilayer fluidity. In these preparations, glycerol acts as an edge activator and penetration enhancer. These drug delivery systems manifest improved stability, fluidity, entrapment and penetration in comparison to conventional liposomes. Increasing the concentration of glycerol by 10, 20 or 30 % leads to a drastic increase in glycerosome stability. They have capability to encapsulate both lipophilic and hydrophilic drugs and protect them from degradation. Now a days glycerosomal carrier are the most interesting area of work for researchers. This review article summarizes the structure, advantages, composition, methods of preparations, characterization and applications of glycerosomes.

KEYWORDS: Glycerosomes, Phospholipids, Glycerol, Penetration, Transdermal, Nasal delivery.

INTRODUCTION

Topical and transdermal application of active pharmaceutical ingredients to the skin is an attractive strategy being explored by formulation scientists to treat disease conditions rather than the oral drug delivery. The delivery of drugs across the skin is an arduous task due to permeation limiting barriers. It, therefore, requires the aid of external agents or carrier systems for efficient permeation. Lipid-based vesicular systems are carriers for the transport of drugs through the stratum corneum (Dermal drug delivery) and into the bloodstream for systemic action (Transdermal drug delivery) overcoming the barrier properties.[1] Over the course of decades, various nano-vesicular delivery systems have emerged and been utilized to improve the pharmacokinetic and pharmacodynamic profile of therapeutics.[2]

Vesicular drug delivery system are micelles made up of an aqueous core and generally lipid bilayer outer shell. The inner aqueous core encapsulates the hydrophilic drugs, while lipid bilayer entraps the lipophilic moieties. This dual ability makes the Vesicular drug delivery system, an excellent vehicle for delivery of both types of drugs.[3] Nanovesicles are the self-assembled nanoscale structures that are naturally formed and can be artificially manufactured. Nanovesicles have the most dynamic features and are used to administer drug molecules on both topical and systemic levels.[4] These nanovesicles have revitalized available treatment modalities as they are biocompatible, biodegradable, less immunogenic and capable of carrying high drug.[5]

The use of nano-sized vesicles is a novel approach to topical delivery to the skin because of their ability to penetrate deeper layers of the skin. They absorb at boundaries or enter the stratum corneum layer by altering its barrier characteristics, which encourages them to penetrate the skin. In addition, they can fluidize the stratum corneum due to the lipid bilayer present in the formulation and enhance the rate of penetration.[6]

Phospholipid nano-vesicular systems have aroused great interest, especially as a promising drug delivery system, which can enhance the transdermal, dermal, and transmucosal absorption of numerous drugs and evade their degradation in GIT and liver.[7] Liposomes were the first generation of phospholipid-based vesicles, comprising one or more lipidic bilayers around an aqueous core, allowing them to encapsulate a wide variety of drug molecules, either hydrophilic or lipophilic. Over the past decades, various additives like ethanol and surfactants had been used to alter the

physical, chemical, and functional properties of liposomes, and newer generations have been invented, allowing for more efficient drug delivery across the biological membranes. Invasomes, ethosomes, transferosomes, and glycerosomes are examples of these innovative systems that have been extensively investigated for dermal, transdermal, transmucosal, and rectal administration of several drugs.[8] Ethosomes, glycerosomes and transethosomes are soft vesicles containing ethanol, glycerol or a mixture of ethanol and a surfactant, respectively.[9]

In the majority of cases, nevertheless, the application of liposomes as carriers for topical administration of active principles is of modest or not significant since standard liposomes unable to cross the capillary walls. Later on, the patent on 'Composition for applying active substances to or through the skin' (U. S. Pat. No. 5, 716, 638) described new classes of deformable lipidic vesicles like ethosomes and transferosomes where the presence of specific additives modifies the chemical-physical and functional properties of conventional liposomes, enabling more efficient delivery of drugs to deeper layers of skin. However, the transport mechanism of such biodegradable vesicles might partly depend on the physicochemical characteristics of loaded drugs. Thus, the preparation of these vesicles must be optimised by case to case basis. Recently, a new vesicle structure with improved carrier characteristics for the purpose of pharmaceutical and cosmetics industry composed of phospholipids and water was developed and characterised. These vesicular structures also have huge volume of glycerol usually from 10% to 35% as per the recent patent on 'Use thereof in Pharmaceutical and Cosmetic Preparations for Topical applications' (US 8,778,367 B2).[10]

Liposomes with deformable properties, including transfersomes, niosomes, ethosomes, glycerosomes, and invasomes, have been widely reported as carriers for enhanced transdermal drug delivery. In particular, ethosomes are transdermal delivery nanocarriers that were first reported by Touitou E in 1999. This nanosystem generally comprises ~ 10–40% (v/v) short-chain alcohols, including ethanol, glycerol, propylene glycol, and isopropanol, which soften the phospholipid membrane, increase the solubility of lipid-soluble drugs, and improve nanocarrier drug loading. Moreover, short-chain alcohols can improve transdermal permeation. However, conventional ethanol-containing ethosomes have poor stability due to the volatility of ethanol and can cause skin irritation. To circumvent these issues, the ethanol in ethosomes has been replaced with glycerol to form "glycerosomes," providing improved physical stability and biocompatibility.[11]

Glycerosomes

The term "glycerosome" was first acquainted by Manca and associates for topical delivery of diclofenac. It is a versatile drug delivery carrier system which is a modification of liposomes. They are small or large unilamellar or multilamellar lipid vesicles composed of phospholipids, water, and varying concentrations of glycerol (preferably 10 to 30% w/v). Additionally, they are nontoxic and accepted for topical application. The lipid layer in Glycerosomes is more flexible and possess high fluidity suitable for topical and transdermal drug delivery. The glycerol in these vesicles improves deformability index and thus increases skin permeation and penetration of therapeutics. Cholesterol enhances the stability of Glycerosomes as well as maintains the lipid membrane integrity by causing barrier to the aqueous phase. When Glycerosomes dispersed in aqueous phase, phospholipid rapidly arranges themselves as bi-layer vesicles.[12]

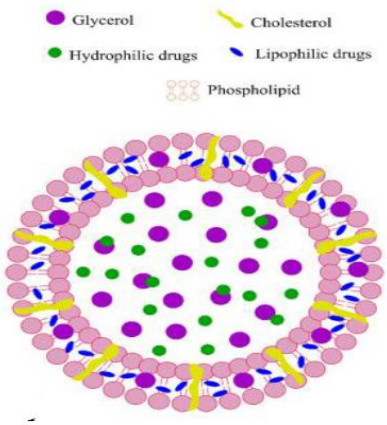


Fig. No. 1: Structure of glycerosome.[13]

Regarding the deformability of the vesicular bilayers, as evaluated by extrusion through polycarbonate membrane, glycerosomes containing 10% glycerol were as deformable as the control liposomes. On the other hand, the vesicle elasticity improved as glycerol content increased; empty and drug-loaded glycerosomes with 30% glycerol were two-fold more deformable than the control. This small decrease is similar to the effect of surfactants on the Tm values of phospholipid.[14]

Manca et al. investigated phospholipid nanovesicles containing glycerol (10–30% v/v), which they call glycerosomes. These vesicles also contain cholesterol. The authors claim that this trihydric alcohol may increase the fluidity of the vesicles' bilayer, thus improving the ability of the carrier to enhance molecule penetration of the skin.[15] It is noteworthy that compared with ethosomes, in which the use of phospholipids with relatively high content of phosphatidylcholine was mostly reported, glycerosomes were prepared using Dipalmitoylphosphatidylcholine (DPCC), hydrogenated soy phosphatidyl choline (PL90H) and 1,2-dimyristoyl-sn-glycero-3 phosphatidylcholine (DMPC).[16]

Composition of glycerosomes

Glycerosomes are new vesicular systems composed of phospholipids and cholesterol just like conventional liposomes. When dispersed in water, phospholipids quickly assemble themselves as bilayer vesicles. Only difference is that in addition to the excipients present in liposomes, these also contain glycerol and water in concentrations of 10, 20, 30, 40 and 50 %. Glycerosomes have till now been suggested for topical and skin drug delivery. It is a completely harmless and non-toxic method of drug delivery.[17]

Glycerol

Glycerol is a well-known, FDA-approved molecule posing three hydroxyl groups. The physical property of glycerol makes it useful as an additive in the pharmaceutical and cosmetic industries, e.g., as a plasticizer, thickener, emollient, demulcent, humectant, bodying agent, lubricant, etc. Glycerol was traditionally derived from the saponification, hydrolysis or transesterification of triglycerides and microbial fermentation. But in the last decade, biodiesel production emerged as a major source of crude glycerol.[18] Some studies evidently revealed that Glycerol particles or vesicles can be directly prepared from a cost-effective and easily accessible glycerol molecule and the prepared particles exhibited good biocompatibility, hemocompatibility, and non-toxicity. Therefore, Glycerol particles or vesicles were found as promising vehicles for drug delivery systems in terms of their higher loading and release capability as well as for sustained long term release profiles. It was even reported that Glycerol can be used in blend films for antibacterial cotton gauze as due it's plasticization and hydrophilic properties.[19] Glycerol can also be applied to various skin ailments because of the moisture-controlling features. It was demonstrated that the imiquimod mouse model of psoriasis was improved by Glycerol and pro-inflammatory cytokine expression was inhibited upon application topically.[20] In the antibacterial biomedical applications, chitosan acetate films were treated with Glycerol to improve their elasticity. Glycerol was used to dissolve ibuprofen (drug) to create silicone based wound dressing membranes. Moreover, it has been shown that topical application of materials containing Glycerol improves the skin properties of patients with diseases characterized by xerosis and impaired epidermal barrier function.[21]

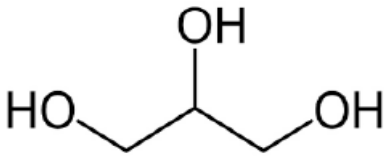


Fig. No. 2: Structure of glycerol.[22]

Phospholipids

Phospholipids are unique and versatile molecules. They are of natural occurrence and the main components in cellular membranes. Arranged as a lipid bilayer, phospholipids play a significant role in the structure and functionality of biological membranes. They are amphiphilic and consist of a hydrophilic headgroup and a lipophilic/hydrophobic tail and alcohol group. Like conventional liposomes, both natural and synthetic phospholipids can be employed for the preparation of glycerosomes.[23]

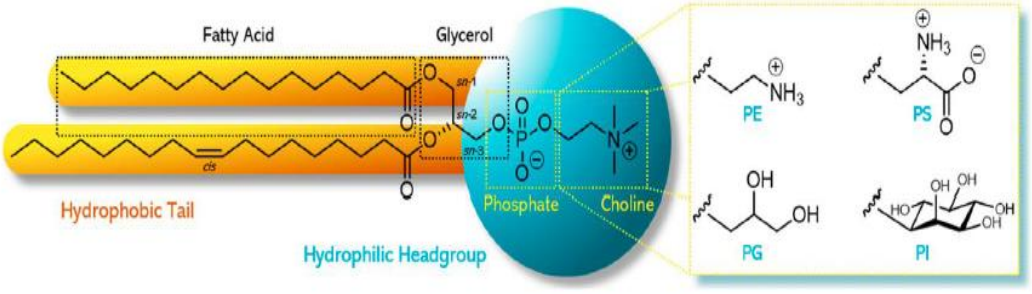


Fig. No. 3: Structure of phospholipid.[23]

Types of phospholipids[24]

Glycerophospholipids

Which are the main phospholipids in eukaryotic cells, refer to the phospholipids in which glycerol is the backbone. All naturally occurring glycerophospholipids possess a-structure and L-configuration. The chemical structures of glycerophospholipids can be classified by the head group, the length and the saturation of hydrophobic side chains, the type of bonding between the aliphatic moieties and glycerol backbone, and the number of aliphatic chains. Variation in the head group leads to different glycerophospholipids, such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidic acid, phosphatidylinositol, phosphatidylglycerol, cardiolipin. The length of the apolar moieties leads to different glycerophospholipids, e.g. dipalmitoyl, dimyristoyl, distearoyl phosphatidylcholine. The saturation of aliphatic groups characterizes different glycerophospholipids, such as dioleoyl, distearoyl phosphatidylcholine. The type of bonding (Ester or ether) between aliphatic chains and glycerol determines different glycerophospholipids, such as plasmalogen. The number of aliphatic chains is different, for example, lysophospholipids have only one acyl group at the glycerol backbone.

Sphingomyelins

Obtained from animal cell walls, these phospholipids differ from glycerophospholipids in the fact that these consist of sphingosine backbone whereas the latter consists of glycerol backbone. These not only differ in chemical structures but also differ in the number of groups present in acyl chains. Sphingophospholipids are considered asymmetric and glycerophospholipids are considered symmetric. Acyl groups present in naturally occurring sphingomyelins exceeds 20 whereas the residues of paraffin consist of fewer groups compared to the natural one. Thus it is referred to as asymmetric. Chain length in case of phosphatidylcholine (example of a glycerophospholipids) is equal and thus they are termed as symmetric molecules.

Cholesterol

Animal cell membrane is mostly made up of cholesterol. It is known to affect various properties of membranes. Rigidity, thickness, stability and fluidity of cell membranes depend on cholesterol in one or another way. In glycerosomes, the purpose of adding cholesterol is to enhance stability. Cholesterol mainly makes contact with liposomes inner cavity due to its hydrophobic nature, which renders it stable. The amount of cholesterol that can be added in vesicular systems has been reported to be 50 mol percent. Lipid and cholesterol ratio that gives rise to efficient liposomes has been reported to be 2:1. However, the reason behind this ratio is not well established. Effect of incorporation of cholesterol in vesicular structures has been widely studied and researchers have concluded that cholesterol plays role in the following. It makes the membrane impermeable to water and electrolytes and hence is included in liposome formation. It increases the ordering and packing efficiency of lipid bilayers. It helps in the de-aggregation of lipid vesicles. It improves rigidity of lipid bilayers by changing the fluidity of vesicles.[24]

Advantages of glycerosomes[25-27]

- This new vesicular system is a harmless and nontoxic topical drug delivery system.
- Glycerosomes do not depend on transition temperatures for their formation.
- They can be formed at room temperature (30 or 25°) unlike conventional liposomes.
- They improve penetration of drug in stratum corneum and deliver it to the inner layers of skin by acting as edge activator and penetration enhancer.
- These vesicles are with improved entrapment, fluidity and stability.
- Stability is improved by changing fluidity of lipid bilayer and by forming viscous preparations. Also, forms deformable and flexible vesicles.
- Glycerol, being viscous in nature homogenously spreads on skin and also there occurs no leakage of active pharmaceutical ingredient unlike conventional liposomes.
- Glycerosomes exhibit the property of changing the configuration in which hydrophilic chains of phospholipids are arranged and they can change the way in which other vesicles of the system interact with each other. This is possible since the dielectric constant of system can change in glycerosomes.
- Glycerosomes also change the plasticity of the skin layer and improve it.
- These increase the water content in the stratum corneum and minimise the obstacles in transdermal drug delivery.
- These vesicles are unique in the sense that these can act as penetration enhancing vesicles as well as the elastic ones.

Method of preparation of glycerosomes

Thin film hydration method

Weighted lipidic components (Phospholipids, cholesterol, hydrophobic active principles or ionic lipids such as DCP or stearyl amine) were dissolved in 5 ml of organic solvent (Chloroform or dichloromethane) using a 50 ml round bottom flask containing 4-5 ml of 1 mm diameter glass beads. Solvent was removed using a rotary evaporator under vacuum at 37° C for 2 hours to get on the wall of the flask lipidic film free of any trace of solvent. 5 ml of glycerol aqueous solution (glycerol concentration between 20% and 35%) also containing, if required, water soluble active principles were then added and the mixture was maintained under mechanical agitation at 10,000 rpm for 1 hour at 25° C. using a crescent-shaped plastic stirring shaft in close contact with the internal round bottom flask Surface. The emulsion, consisting of multilamellar vesicles, was maintained at rest for 1 hour and then homogenized at

high-pressure for 10 minutes at 25°C using a homogenizer set at 60,000-65,000 kPa.[28]

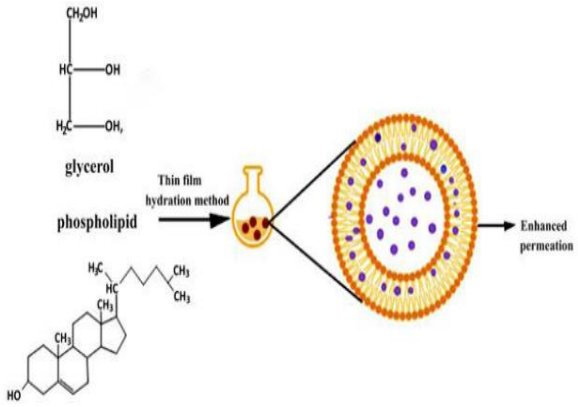


Fig. No. 4: Thin film hydration method for preparation of Glycerosomes.[29]

Injection method[30]

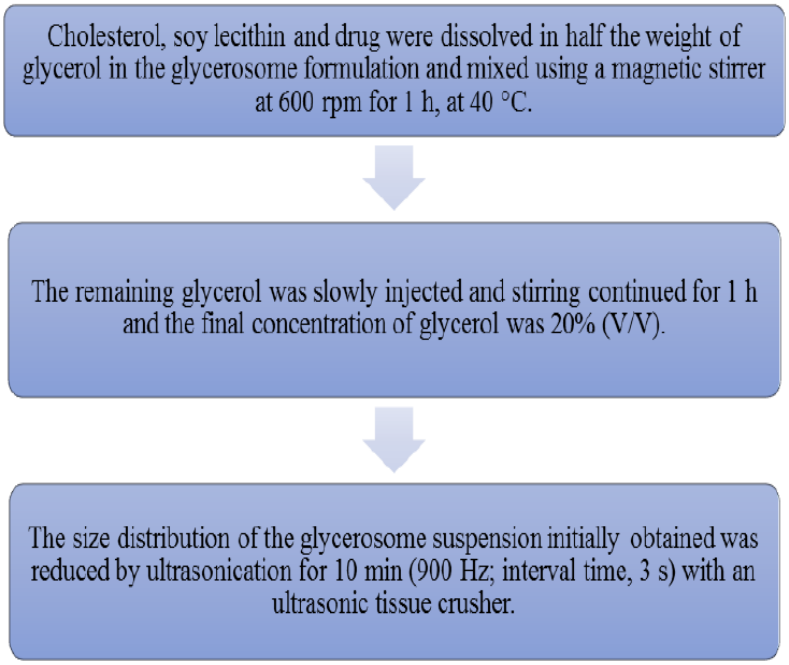


Fig. No. 5: Flow chart for the preparation of glycerosomes by injection method.

Reverse-phase evaporation method

Lipid and cholesterol were dissolved in a mixture of methanol and chloroform (3:1, v/v), while drug was dissolved in a glycerol– water solution. The aqueous phase was added to the organic phase at a ratio of 1:3 (v/v) and sonicated for 30 min in a bath sonicator to form a uniform emulsion. The organic solvents were then removed by rotary evaporation to obtain a dry film. The resulting film was hydrated with glycerol– water solution and sonicated in an ice water bath using an ultrasonic cell disintegrator for 2 min (power: 380 W, quiescent interval: 3 s). The main advantage of reverse phase evaporation method is that it results in the formation of vesicles with large aqueous to lipid ratio, entrapping more of the aqueous medium.[31]

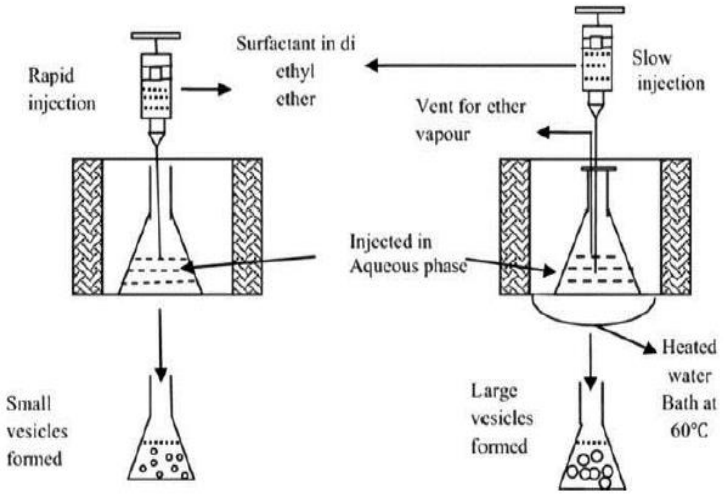
Solvent injections method[17]

1. Ether injection method

Watkins described this method, in which lipids are dissolved in diethyl ether/ether methanol mixture. These are then injected to a warm aqueous solution that contains the material to be encapsulated. The warm aqueous phase should be maintained above the boiling point of ether. Usually 55-65° temperature is maintained during encapsulation. The speed of injection should be slow and not rapid. On coming in contact with warm aqueous phase, ether evaporates giving rise to unilamellar vesicles. Disadvantages of this method include low yield of heterogenous liposomes, along with the exposure of compounds which are to be encapsulated to high temperature and organic solvents.

2. Ethanol injection method

Ethanol injection method was reported by Batzri and Korn in 1976. In this method, lipid is dissolved in ethanol and made to pass through a small orifice that may be a syringe in excess aqueous medium. The speed of injecting ethanolic lipid solution in aqueous medium should be such that complete mixing of the two is attained. Complete mixing of the two is necessary for immediate dilution of ethanol in hydration medium and for the dispersion of phospholipids in water. Main advantage of this method lies in the fact that without sonication and extrusion one can obtain small liposomes under the size range of 100 nm simply by injecting lipid solution dissolved in ether in water. Also, homogenous and dilute liposomes are obtained. Disadvantage of ethanol injection method includes the constraint due to lipid solubility in ethanol, volume of ethanol that can be added to aqueous medium is limited and thus the lipid which can be added to ethanol is limited. Ethanol remains in liposomes but can be removed by dialysis.



Fig, No. 6: Solvent injection method of preparation of glycerosomes.

Critical parameters of glycerosomes

Particle size analysis

The particle size and polydispersity index (PDI) of glycerosomes were analyzed using dynamic light scattering (DLS) technique with a Malvern Autosizer Nano ZS90 inspection system (Malvern Instruments Ltd, Malvern, UK) at 25°C and an angle of 90°.[32]

Morphological analysis

The vesicular structures of glycerosomes were confirmed by observing under trinocular microscope at 45x. Sample was set on slide (drop form) and was covered with coverslip. Excess sample was wiped off and the samples were then observed.[33]

Entrapment efficiency

Various methods are used for finding the entrapment efficiency. One of the method of determination of entrapment efficiency is ultracentrifuge. The formulations were placed in centrifuge and were rotated for 50 mins at 45000 rpm. The supernatant was collected after 50 mins. It was then analyzed spectrophotometrically.[34]

$$\text{Formula} = \frac{\textit{Total drug content} - \textit{Free drug content}}{\textit{Total drug content}} \times 100$$

Manca et al. have used the method of dialysis. They passed the glycerosome formulation from dialysis tube of 12,000 to 14,000 Dalton and then separated entrapped formulations from the non-entrapped one. These were then assayed for drug content. Entrapment is measured on the basis of percentage of drug which was obtained after dialysis to that which was taken before dialysis.[17] Formula=obtained drug content / initial drug content×100.

Deformability of glycerosomes

Deformability of glycerosomes was measured by extrusion, using the LipoFast-Basic extruder (Avestin Europe GmbH; Mannheim, Germany). The samples were extruded through a 19 mm polycarbonate membrane with 50 nm pore size (Avestin Europe GmbH; Mannheim, Germany), at a constant pressure of 7 bar, for 5 min. The extruded sample was collected in a syringe, meanwhile vesicle size and Polydispersity index were monitored by Dynamic light scattering (DLS) analysis, before and after extrusion. Finally, deformability of vesicles was calculated according to the following Equation,[35]

D = average size (nm) be f ore extrusion / average size (nm) after extrusion

Determination of penetration

This evaluation parameter helps in determining the extent to which glycerosomes has penetrated the skin layer. This can be performed by ex vivo means. It helps in determining drug delivery through the skin layer. Franz diffusion cell is used for this purpose. The dorsal surface of rat skin was excised with fatty layers removed surgically, washed with alcohol and temporary stored at

−80 ◦C. Before commencing the permeation study, the stored rat skin was equilibrated in buffer solution for 2 h at room temperature. The Franz diffusion cells decorated the skin specimens securely between donor and receptor compartments with the stratum corneum side facing the donor compartment. Prior to the study, receptor compartment was filled with 7.5 mL of PBS solution and stirred continuously with a small magnetic bead at 500 rpm, maintained at a temperature of 37 ± 0.5 ◦C. Glycerosomal preparation is placed onto the surface of skin and at regular intervals of time, i.e., 0, 1, 2, 3, 4, 6, 8, 12, 14, 16, 20 and 24 h, 1 mL of solution was withdrawn from the receiving compartment and same amount was replaced with fresh solution. The drug content is analysed by suitable method (UV or HPLC).[12]

Stability of glycerosomes

For stability evaluation, glycerosomes were stored either at refrigeration temperature (4°C±2°C) or at room temperature (25°C±2°C) for 1 month. After 0 day, 7 days, and 30 days, their vesicle size distribution and Entrapment efficiency were determined in order to estimate the effect of storage temperature on the physical and chemical stability of glycerosomes.[36]

Applications of glycerosomes

Topical delivery with glycerosomes

Zhu C *et al*., have done study on optimizing glycerosome formulations via an orthogonal experimental design to enhance transdermal triptolide delivery. In this study, glycerosomes were prepared as the transdermal vehicle to enhance the transdermal delivery of triptolide. With entrapment efficiency and drug loading as dependent variables, the glycerosome formulation was optimized using an orthogonal experimental design. Phospholipid-to-cholesterol and phospholipid-to-triptolide mass ratios of 30:1 and 5:1, respectively and a glycerol concentration of 20 % (V/V) were used in the optimization. The glycerosomes prepared with the optimized formulation showed good stability, with an average particle size of 153.10 ± 2.69 nm, a zeta potential of –45.73 ± 0.60 mV and an entrapment greater than 75 %. Finally it is concluded that glycerosomes significantly increased the transdermal delivery of triptolide compared to conventional liposomes. As efficient carriers for the transdermal delivery of drugs, glycerosomes can potentially be used as an alternative to oral triptolide administration.[30]

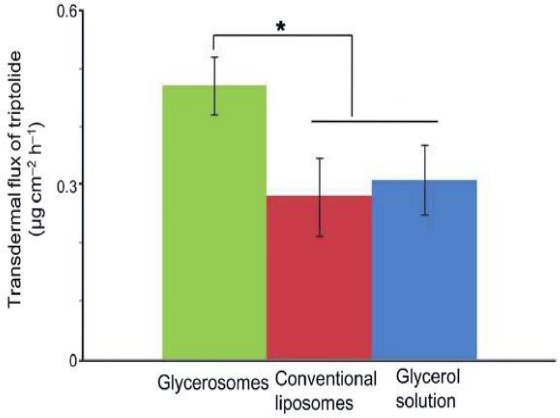


Fig. No. 7: Ex vivo transdermal flux of triptolide in glycerosomes, conventional Liposomes and 20 % glycerol solution (*p < 0.05; n = 5).

Rani D *et al*., formulated and evaluated minoxidil bearing glycerosomes. Present study was undertaken to assess the potential of Glycerosomes as a novel drug delivery system for topical application of Minoxidil. Glycerosomes was prepared by using lipid thin film hydration method. Prepared formulations were evaluated in terms of particle size, surface analysis, zeta potential, entrapment efficiency and in-vitro drug release. Morphological investigations showed that all vesicles exhibit a spherical shape, with a mean diameter of 121.58 nm. Selection of the appropriate experimental conditions result in the production of Minoxidil loaded Glycerosomes having high entrapment efficiency (88.41 ± 0.57% w/w) and high cumulative percent drug release (88.54 ± 0.23% w/w) at 16th hour.[37]

Zhang K *et al*., 16 have done study on essential oil-mediated glycerosomes increase transdermal paeoniflorin delivery. In this study, a novel glycerosome carrier containing essential oils was prepared for topical administration of Paeoniflorin (anti-rheumatic drugs) to enhance its transdermal drug delivery and improve drug absorption in the synovium. The formulation of glycerosomes was optimized by a uniform design, and the final vehicle was composed of 5% (w/v) phospholipid, 0.6% (w/v) cholesterol, and 10% (v/v) glycerol, with 2% (v/v) Speranskia tuberculata essential oil (STO) as the transdermal enhancer. Compared with conventional preparations, STO-glycerosomes showed better percutaneous penetration properties and significantly enhanced the accumulation of Paeoniflorin in the synovium. In vivo studies also suggested that the drug concentration in the knee joint could remain

elevated at a high level even after a long time. These effects were closely related to the combination of glycerol and essential oils added in the liposomal system. So STO-glycerosomes are a potentially safe and applicable vehicle of Paeoniflorin for the treatment of Rheumatoid arthritis.[31]

Maan S *et al*., fabricated and evaluated controlled release transdermal drug delivery system of carvedilol using design expert® software for the management of hypertension. In this study Glycerosomes of carvedilol was prepared using Phospholipid E80, glycerol and cholesterol via film hydration technique. Optimization was done using Central Composite Design under Design Expert software. While PVP and EC in a ratio of 4:1 in chloroform (5 mL) with plasticizer dibutyl phthalate (30%) were used for preparing Matrix type transdermal patch containing Carvedilol loaded glycerosomes. Optimized glycerosomes containing Carvedilol showed 115.7 nm particle size, and -16.4 mV zeta potential. Transmission electron microscopy analysis also showed similar vesicle size and reveals globular structure of glycerosomes containing carvedilol. Nearly 0.31 mm thickness, 0.14 % g weight variation, 99.13% flatness and 98.72% drug content was found in Matrix type transdermal patch having glycerosomes of carvedilol Transdermal patch with Franz diffusion cell showed approx. 90% release upto 48 hr during in-vitro permeation studies. In-vivo pharmacological assessment was done for efficacy estimation of glycerosomes transdermal patch by N-nitro-L-arginine methyl ester which produced significant hypertension in rats. The application of Carvedilol loaded glycerosomes transdermal patch resulted in a gradual decrease in BP, with the maximum effect from the patch observed at 10 hr ($p < 0.001$), the effect continued for 48 hr clearly indicating the gradual release of drug by transdermal patch for a long period. It is finally concluded that, developed novel transdermal patch having Carvedilol loaded glycerosomes may be considered as a promising approach for controlled release of drug with effective hypertension management.[38]

Nasal drug delivery using glycerosomes

MJ Naguib *et al*., have investigated the potential of utilizing glycerosomes as a novel vesicular platform for enhancing intranasal delivery of lacidipine. Lacidipine is a potent dihydropyridine calcium channel blocker used for management of hypertension and atherosclerosis. The drug has low and fluctuating oral bioavailability owing to its extensive hepatic first-pass metabolism and reduced water solubility. Accordingly, this work aimed at overcoming the aforementioned challenges through the formulation of intranasal nano-sized lacidipine glycerosomes. Box-Behnken was successfully employed for the formulation and in vitro optimization of the glycerosomes. Statistical analysis revealed that cholesterol concentration exhibited a significant effect on the vesicle size, while Phospholipon® 90G and glycerol concentrations exhibited significant effects on both entrapment efficiency and deformability index. The optimized formulation showed spherical shape, good deformability, vesicular size of 220.25 nm, entrapment efficiency of 61.97%, and enhanced *ex vivo* permeation by 3.65 fold compared to lacidipine suspension. Confocal laser scattering microscope revealed higher penetration depth via nasal mucosa for rhodamine labelled glycerosomes (up to 60 µm) in comparison to rhoadamine dye solution (26 µm). In addition, the optimized lacidipine glycerosomes caused significant reduction in methylprednisolone acetate-induced hypertension in rats for up to 24 h in comparison to oral drug suspension. Histopathological assessment showed intact nasal mucosal epithelial lining with no signs of inflammation or necrosis confirming the safety and tolerability of the proposed glycerosomes. The declared results highlights the potential of utilizing the proposed glycerosomes as safe and effective platform for intranasal delivery of lacidipine.[39]

Melis V *et al*., have done study on inhalable polymer-glycerosomes as safe and effective carriers for rifampicin delivery to the lungs. Rifampicin loaded glycerosomes, vesicles composed of phospholipids, glycerol and water, were combined with trimethyl chitosan chloride (TMC) to prepare TMC-glycerosomes or, alternatively, with sodium hyaluronate (HY) to obtain HY-glycerosomes. These new hybrid nanovesicles were tested as carriers for pulmonary delivery of rifampicin. Glycerosomes without polymers were also prepared and characterized. All vesicles were similar: they were spherical, multilamellar and able to incorporate good amount of rifampicin (EE% ~ 55%). The addition of the polymers to the formulations allowed an increase of mean diameter. All the glycerosomes, in particular HY-glycerosomes, were able to deliver the drug to the furthest stages of the Next Generation Impactor and the aptitude of the vesicles to be nebulized was always higher than that of drug dispersion. Rifampicin nano incorporation in vesicles reduced the *in vitro* drug toxicity on A549 cells, as well as increased its efficacy against *Staphylococcus aureus*. Finally, the *in vivo* biodistribution and accumulation, evaluated after intra-tracheal administration to rats, confirmed the improvement of rifampicin accumulation in lungs.[40]

Ear drug delivery using glycerosomes

Magdy M *et al*., have done studies on engineered triamcinolone acetonide loaded glycerosomes as a novel ear delivery system for the treatment of otitis media**.** In this study, the fabrication of glycerosomes was explored to boost triamcinolone acetonide delivery to the middle ear *via* the otic application to improve treatment of otitis media. Opting a D-optimal design, triamcinolone acetonide glycerosomes were formulated and optimized using ethanol injection method. The optimized formula was assessed for morphology, viscosity, *ex vivo* tympanic membrane permeation and deposition and physical stability. Moreover, otitis media induction in rats using lipopolysaccharides was conducted, histological

and biochemical investigations were performed to assess the therapeutic potential of triamcinolone acetonide glycerosomes and their tolerability as well. The optimized formula displayed a nanosized value (106.1 ± 2.82), low polydispersity index (0.079 ± 0.04), satisfactory drug entrapment efficiency (80.62 ± 4.41 %), shear thinning behavior and excellent physical stability. *Ex-vivo* tympanic membrane permeation and deposition monitoring for 24 h demonstrated greater flux and deposition compared to free drug. More importantly, the *in vivo* studies demonstrated the supremacy of glycerosomes with respect to tolerability and efficacy in alleviating otitis media following ototopical application compared to marketed drug. Such therapeutic modality represents a promising option to boost the efficacy of otic drugs, awaiting clinical translation.[41]

Oral drug delivery using glycerosomes

Zaki RM *et al*., have performed central composite optimization of glycerosomes for the enhanced oral bioavailability and brain delivery of quetiapine fumarate**.** This study aimed to formulate and statistically optimize glycerosomal formulations of Quetiapine fumarate (QTF) to increase its oral bioavailability and enhance its brain delivery. The study was designed using a Central composite rotatable design using Design-Expert® software. The independent variables in the study were glycerol % *w*/*v* and cholesterol % *w*/*v*, while the dependent variables were vesicle size, zeta potential, and entrapment efficiency percent. The numerical optimization process resulted in an optimum formula composed of 29.645 (*w*/*v*%) glycerol, 0.8 (*w*/*v*%) cholesterol, and 5 (*w*/*v*%) lecithin. It showed a vesicle size of 290.4 nm, zeta potential of −34.58, and entrapment efficiency of 80.85%. The optimum formula was further characterized for Differential scanning calorimetry (DSC), X-Ray diffraction study (XRD), Transmission electron microscopy (TEM), in-vitro release, the effect of aging, and pharmacokinetic study. DSC thermogram confirmed the compatibility of the drug with the ingredients. XRD revealed the encapsulation of the drug in the glycerosomal nanovesicles. TEM image revealed spherical vesicles with no aggregates. Additionally, it showed enhanced drug release when compared to a drug suspension and also exhibited good stability for one month. Moreover, it showed higher brain C_{max}, AUC_{0-24}, and $AUC_{0-\infty}$ and plasma AUC_{0-24} and $AUC_{0-\infty}$ in comparison to drug suspension. It showed brain and plasma bioavailability enhancement of 153.15 and 179.85%, respectively, compared to the drug suspension. It is concluded that, the optimum glycerosomal formula may be regarded as a promising carrier to enhance the oral bioavailability and brain delivery of Quetiapine fumarate.[42]

REFERENCE

1. Chacko IA, Ghate VM, Dsouza L, Lewis SA. Lipid vesicles: A versatile drug delivery platform for dermal and transdermal applications. Colloids and Surfaces B: Biointerfaces, 2020; 195: 111-262.
2. Elsharkasy OM, Nordin JZ, Hagey DW, de Jong OG, Schiffelers RM, Andaloussi SE *et al*.
3. Extracellular vesicles as drug delivery systems: Why and how? Advanced drug delivery reviews, 2020; 159: 332-43.
4. Azeez NA, Deepa VS, Sivapriya V. Phytosomes: emergent promising nano vesicular drug delivery system for targeted tumor therapy. Advances in Natural Sciences: Nanoscience and Nanotechnology, 2018; 9(3): 033001.
5. Nayak AK, Hasnain MS, Aminabhavi TM, Torchilin VP. Nanovesicular systems in drug delivery. InSystems of Nanovesicular Drug Delivery. Academic Press, 2022; 1(1): 1-15.
6. Neupane YR, Mahtab A, Siddiqui L, Singh A, Gautam N, Rabbani SA *et al*. Biocompatible nanovesicular drug delivery systems with targeting potential for autoimmune diseases. Current Pharmaceutical Design, 2020; 26(42): 5488-502.
7. Imam SS, Gilani SJ, Zafar A, Jumah MN, Alshehri S. Formulation of Miconazole-Loaded Chitosan–Carbopol Vesicular Gel: Optimization to In Vitro Characterization, Irritation, and Antifungal Assessment. Pharmaceutics, 2023; 15(2): 581-94.
8. Salem HF, Ali AA, Rabea YK, El-Ela FI, Khallaf RA. Glycerosomal thermosensitive in situ gel of duloxetine HCl as a novel nanoplatform for rectal delivery: in vitro optimization and in vivo appraisal. Drug Delivery and Translational Research, 2022; 12(12): 3083-103.
9. Ahmed OA, Badr-Eldin SM. Development of an optimized avanafil-loaded invasomal transdermal film: Ex vivo skin permeation and in vivo evaluation. International journal of pharmaceutics, 2019; 570: 118-657.
10. Touitou E, Natsheh H. Topical administration of drugs incorporated in carriers containing phospholipid soft vesicles for the treatment of skin medical conditions. Pharmaceutics, 2021; 13(12): 2129-50.
11. Mohammed YB, Alqahtani A, Lakshmi S, Gnanaprakash K, Kumarappan CT. Studies on formulation development and evaluation of tolnaftate-loaded glycerosomes. Materials Research Innovations, 2021; 25(4): 233-42.
12. Ruan H, Shen L, Hou X, Li J, Guo T, Zhu C *et al*. Stratum corneum lipidomics analysis of the transdermal permeability achieved by phytosterol-mediated glycerosomes combined with peppermint oil. Drug delivery and translational research, 2023; 1-25.
13. Md S, Alhakamy NA, Aldawsari HM. Plumbagin-loaded glycerosome gel as topical delivery system for skin cancer therapy. Polymers (Basel), 2021; 13(6): 923.
14. Jha A, Kumar M, Bharti K, Mishra B. Glycerosomes: a new tool for effective drug delivery. InSystems of Nanovesicular Drug Delivery, 2022; 1: 277-291.

15. Natsheh H, Touitou E. Phospholipid vesicles for dermal/transdermal and nasal administration of active molecules: The effect of surfactants and alcohols on the fluidity of their lipid bilayers and penetration enhancement properties. Molecules, 2020; 25(13): 2959-3001.
16. Manca ML, Cencetti C, Matricardi P, Castangia I, Zaru M, Sales OD *et al*. Glycerosomes: Use of hydrogenated soy phosphatidylcholine mixture and its effect on vesicle features and diclofenac skin penetration. International journal of pharmaceutics, 2016; 511(1): 198-204.
17. Manca ML, Manconi M, Zaru M, Valenti D, Peris JE, Matricardi P *et al*. Glycerosomes: Investigation of role of 1, 2-dimyristoyl-sn-glycero-3-phosphatidycholine (DMPC) on the assembling and skin delivery performances. International Journal of Pharmaceutics, 2017; 532(1): 401-7.
18. Gupta P, Mazumder R, Padhi S. Glycerosomes: Advanced liposomal drug delivery system. Indian journal of pharmaceutical sciences, 2020; 82(3): 385-97.
19. Goyal S, Hernández NB, Cochran EW. An update on the future prospects of glycerol polymers. Polymer International, 2021; 70(7): 911-7.
20. Sharma A, Verma C, Mukhopadhyay S, Gupta A, Gupta B. Development of sodium alginate/glycerol/tannic acid coated cotton as antimicrobial system. International Journal of Biological Macromolecules, 2022; 216: 303-11.
21. Choudhary V, Kaddour-Djebbar I, Custer VE, Uaratanawong R, Chen X, Cohen E *et al*. Glycerol improves skin lesion development in the imiquimod mouse model of psoriasis: experimental confirmation of anecdotal reports from patients with psoriasis. International journal of molecular sciences, 2021; 22(16): 8749-64.
22. Sahiner M, Yilmaz AS, Ayyala RS, Sahiner N. Poly (Glycerol) Microparticles as Drug Delivery Vehicle for Biomedical Use. Pharmaceutics, 2023; 15(2): 384-98.
23. Yamada, T., A. Habuka, and I. Hatta. Moisturizing mechanism of glycerol and diglycerol on human stratum corneum studied by synchrotron X-ray diffraction. International Journal of Cosmetic Science, 2021; 43, 1: 38-47.
24. Drescher S, van Hoogevest P. The phospholipid research center: Current research in phospholipids and their use in drug delivery. Pharmaceutics, 2020; 18, 12(12): 1235-71.
25. Li J, Wang X, Zhang T, Wang C, Huang Z, Luo X *et al*. A review on phospholipids and their main applications in drug delivery systems. Asian journal of pharmaceutical sciences, 2015; 1, 10(2): 81-98.
26. Vitonyte J, Manca ML, Caddeo C, Valenti D, Peris JE, Usach I *et al*. Bifunctional viscous nanovesicles co-loaded with resveratrol and gallic acid for skin protection against microbial and oxidative injuries. European Journal of Pharmaceutics and Biopharmaceutics, 2017; 114: 278-87.
27. Barichello JM, Yamakawa N, Kisyuku M, Handa H, Shibata T, Ishida T *et al*. Combined effect of liposomalization and addition of glycerol on the transdermal delivery of isosorbide 5-nitrate in rat skin. International journal of pharmaceutics, 2008; 357(2): 199-205.
28. Manca ML, Zaru M, Manconi M, Lai F, Valenti D, Sinico C *et al*. Glycerosomes: A new tool for effective dermal and transdermal drug delivery. International journal of pharmaceutics, 2013; 455(2): 66-74.
29. Zaru M, Manca ML, Fadda AM, Orsini G, inventors; PRIGEN Srl, assignee. Glycerosomes and use thereof in pharmaceutical and cosmetic preparations for topical applications. United States patent US 8,778,367, 2014; 15: 1-14.
30. Singh R, Zeeshan F, Srivastava D, Awasthi H. A Discursive Review of Recent Development and Patents on Glycerosomes. Recent Patents on Nanotechnology, 2023; 1, 17(3): 183-9.
31. Zhu C, Zhang Y, Wu T, He Z, Guo T, Feng N. Optimizing glycerosome formulations via an orthogonal experimental design to enhance transdermal triptolide delivery. Acta Pharmaceutica, 2022; 72(1): 135-46.
32. Zhang K, Zhang Y, Li Z, Li N, Feng N. Essential oil-mediated glycerosomes increase transdermal paeoniflorin delivery: Optimization, characterization, and evaluation in vitro and in vivo. International journal of nanomedicine, 2017; 5: 3521-32.
33. Vanti G, Ntallis SG, Panagiotidis CA, Dourdouni V, Patsoura C, Bergonzi MC *et al*. Glycerosome of Melissa officinalis L. essential oil for effective anti-HSV Type 1. Molecules, 2020; 25(14): 3111-26.
34. Gupta P, Mazumder R, Padhi S, Gupta MP, Pharm M. Development of Natamycin loaded glycerosomes, a novel approach to defend ophthalmic keratitis. Indian Journal of Pharmaceutical Education and Research, 2020; 54(2): 163-72.
35. Imam SS, Gilani SJ, Zafar A, Jumah MN, Alshehri S. Formulation of Miconazole-Loaded Chitosan–Carbopol Vesicular Gel: Optimization to In Vitro Characterization, Irritation, and Antifungal Assessment. Pharmaceutics, 2023; 15(2): 581.
36. Johl S, Bhattacharyya S. Novel Deformable Vesicle for the Transdermal Delivery of Terbinafine Hydrochloride-Formulation and Cytotoxic Evaluation. Ind. J. Pharm. Edu. Res, 2024; 58(1): 1-0.
37. Rani D, Sharma V, Singh P, Singh R. Glycerosomes: A novel vesicular drug delivery system. Research Journal of Pharmacy and Technology, 2022; 15(2): 921-6.
38. Rani D, Singh C, Kumar A, Sharma VK. Formulation development and in-vitro evaluation of minoxidil bearing glycerosomes. Am. J. Biomed. Res, 2016; 4(2): 27-37.

39. Maan S, Khar RK, Mazumder R, Yadav N, Khan UA. Fabrication and Evaluation of Controlled Release Transdermal Drug Delivery System of Carvedilol using Design Expert® Software for the Management of Hypertension. Journal of Young Pharmacists, 2022; 14(3): 295-301.
40. Naguib MJ, Salah S, Halim SA, Badr-Eldin SM. Investigating the potential of utilizing glycerosomes as a novel vesicular platform for enhancing intranasal delivery of lacidipine. International Journal of Pharmaceutics, 2020; 582: 119-302.
41. Melis V, Manca ML, Bullita E, Tamburini E, Castangia I, Cardia MC *et al*. Inhalable polymer-glycerosomes as safe and effective carriers for rifampicin delivery to the lungs. Colloids and surfaces B: Biointerfaces, 2016; 143: 301-8.
42. Magdy M, Elmowafy E, El-Assal MI, Ishak RA. Engineered triamcinolone acetonide loaded glycerosomes as a novel ear delivery system for the treatment of otitis media. International Journal of Pharmaceutics, 2022; 628: 122-276.
43. Zaki RM, Alfadhel MM, Alossaimi MA, Elsawaf LA, Devanathadesikan Seshadri V, Almurshedi AS *et al*. Central Composite Optimization of Glycerosomes for the Enhanced Oral Bioavailability and Brain Delivery of Quetiapine Fumarate. Pharmaceuticals, 2022; 15(8): 940-58.