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GLYCEROSOMES AS EMERGING GLYCEROL-ENRICHED VESICULAR CARRIERS FOR ENHANCED DRUG DELIVERY: A COMPREHENSIVE REVIEW

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ABSTRACT

Conventional topical and transdermal dosage forms often suffer from poor skin penetration, low bioavailability, frequent dosing, and inadequate patient compliance. These limitations have driven the development of novel drug delivery systems designed to enhance therapeutic efficacy and safety. Vesicular carriers have gained significant attention; however, issues such as instability and limited drug permeation remain challenges in many systems. Glycerosomes, an advanced lipid vesicular system enriched with glycerol, have emerged as a promising alternative due to their superior flexibility, enhanced hydration effect, and improved skin permeation. This review provides a comprehensive overview of glycerosomes, including their composition, physicochemical characteristics, advantages, and limitations. The mechanism of glycerosome-mediated skin penetration is discussed with emphasis on vesicle deformability and lipid–skin interactions. Additionally, various methods of preparation and key evaluation parameters such as vesicle size, entrapment efficiency, and *in vitro* release are highlighted. Glycerosomes act as promising vesicular carriers for enhancing drug penetration through the skin. Their flexible structure and glycerol content improve drug retention and permeation, making them suitable for effective topical and transdermal delivery systems.

KEYWORDS: *Glycerosomes, Novel drug delivery systems, Transdermal drug delivery, Skin penetration enhancement.*

INTRODUCTION

In the past, treating acute and chronic diseases mainly relied on conventional dosage forms. Although these forms are widely used, they have several limitations. Conventional drug delivery systems often distribute drugs non-specifically, leading to systemic side effects. They also lack control over drug release, which results in frequent dosing, higher doses, and fluctuating drug levels in the body. Another major drawback is poor bioavailability, especially for drugs that have low solubility or stability.

To address these issues, the pharmaceutical industry has shifted its focus to developing novel drug delivery systems (NDDS). These systems aim to boost drug bioavailability, control the rate and site of drug release, and cut down dosing frequency and side effects. Novel drug delivery systems improve patient compliance, treatment effectiveness, and safety by delivering the drug in a controlled and sustained way. Thus, NDDS offer a better approach to enhance the performance of existing drugs when compared to conventional dosage forms.[1]

Among the various novel vesicular drug delivery systems created to tackle the limitations of conventional dosage forms, glycerosomes have gained significant attention. They enhance drug penetration, improve bioavailability, provide controlled drug release, and reduce systemic side effects, particularly in topical and transdermal drug delivery.[2]

GLYCEROSOMES

Glycerosomes are bilayer vesicles used for delivering medications through the skin. They enable for transdermal medication administration. These vesicles differ from conventional liposomes in their bilayer

fluidity. Glycerosomes are prepared using phospholipids and glycerol in concentrations of 10, 20, and 30% v/v, and an increase in glycerol concentration results in significantly improved physical stability.[3] Their name comes from their high glycerol content.[4] These vesicles effectively deliver active ingredients to the skin. Glycerosomes are more. stable and have greater fluidity than liposomes, making them mainly used for topical drug delivery.[5] Glycerol improves the deformability of liposomal bilayers, which helps with skin penetration.[6]

Glycerol acts as a penetration enhancer and edge activator. Recently, these glycerosomes have shown potential in various therapeutic areas, including skin disorders as well as inflammatory and infectious diseases. Their capability to encapsulate both hydrophilic and hydrophobic drugs makes glycerosomes a promising method for drug delivery.[7,8]

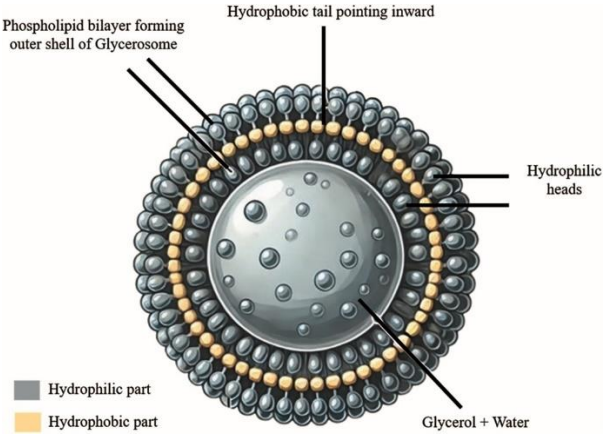

Fig. 1: Structure of Glycerosome.

COMPOSITION

Structure of glycerol

Glycerol is a thick liquid that functions as an alcohol. It contains three hydroxyl groups, which give it hydrophilic characteristics. As a component of triglycerides, glycerol can be found in both animal fats and vegetable oils. It can be produced during the soap-making process or generated as a by-product in biodiesel production. In pharmaceuticals, it serves various roles such as a lubricant, humectant, edge activator, and emulsifying agent.[9]

Phospholipids

Phospholipids: Both natural and synthetic phospholipids can be used to create glycerosomes, similar to traditional liposomes.[4] There is a wide variety of phospholipids due to variations in their hydrophilic head groups, hydrophobic tails. In addition to being amphiphilic, they are also compatible with nearly all ingredients. When placed in water, phospholipids organize into various shapes. The process of self-assembly in a hydration medium is influenced by the specific characteristics of the phospholipids. Various types of phospholipids are categorized based on differences in their backbone structures and the types of alcohol moieties they contain.[10]

TYPES OF PHOSPHOLIPIDS

Glycerophospholipids

Glycerophospholipids come from eukaryotes. Glycerol is the main part of these lipids. Changes in the hydrophilic head group lead to the formation of cardiolipin, phosphatidylcholine, phosphatidylserine, and others. Changes in the acyl chains result in dipalmitoyl phosphatidylcholine and dimyristoyl phosphatidylcholine.

Sphingomyelins

These phospholipids come from animal cell walls. They differ from glycerophospholipids because they have a sphingosine backbone, while glycerophospholipids have a glycerol backbone. They not only have different chemical structures but also differ in the number of groups in their acyl chains. Sphingophospholipids are asymmetric, whereas glycerophospholipids are symmetric. Naturally occurring sphingomyelins have over 20 acyl groups, while paraffin residues have fewer groups. This is why they are called asymmetric. In phosphatidylcholine, which is a glycerophospholipid, the chain lengths are equal, making them symmetric molecules.

Properties of phospholipids to form glycerosomes

Phospholipids need to be able to create vesicles that can encapsulate pharmaceutical components while remaining safe for use. These phospholipids should lead to the formation of vesicles that are biodegradable. Additionally, the phospholipids must produce vesicles that can work well with other ingredients in the formulation.[10]

Cholesterol

The cell membrane of animals predominantly consists of cholesterol, which is known to influence various membrane characteristics. The rigidity, thickness, stability, and fluidity of cell membranes are affected by cholesterol in various ways.[11] In glycerosomes, cholesterol is added to improve stability. Due to its hydrophobic properties, cholesterol primarily interacts with the inner cavity of liposomes, contributing to their stability.[12] It has been observed that the maximum amount of cholesterol that can be incorporated into vesicular systems is 50 mol percent. The optimal lipid to cholesterol ratio for creating effective liposomes has been noted to be 2:1. However, the explanation for this specific ratio remains unclear.[13-16]

The impact of adding cholesterol to vesicular structures has been extensively researched, leading scientists to determine that cholesterol has several functions.[13] It renders the membrane resistant to water and electrolytes, making it essential for liposome development.[17] It enhances the organization and packing effectiveness of lipid bilayers.[18] It facilitates the disaggregation of lipid

vesicles.[19] Additionally, it increases the stiffness of lipid bilayers by altering the fluidity of the vesicles.[20]

Nature of drugs encapsulated

Both hydrophilic and lipophilic pharmaceuticals can be encapsulated in glycerosomes.[4,21,22] Hydrophilic medications are contained in the aqueous core of these vesicular structures, while lipophilic compounds are housed within the phospholipid tails. The thin film hydration method is commonly employed for encapsulating hydrophilic substances. Although this technique is advantageous for the incorporation of water-loving drugs, it results in reduced encapsulation efficiency.[22] Drugs with intermediate log P values are positioned between the water-soluble and lipid-soluble regions.[23] These vesicular systems facilitate targeted drug delivery without leading to the degradation of the active compound.[24]

MECHANISM OF SKIN PERMEATION

Glycerosomes, a novel type of nanovesicle that includes glycerol in their composition, offer distinct benefits for drug delivery via multiple routes. These benefits encompass improved drug stability, solubility, and permeation, all of which are essential for enhancing therapeutic results. In the context of dermal and transdermal drug delivery, glycerosomes greatly improve the ability of drugs to penetrate the skin. Glycerol, an important ingredient, serves as a humectant, boosting the hydration of the stratum corneum. This increased hydration makes the skin more pliable and lessens its barrier properties, thus aiding drug penetration.[4,25] Moreover, the addition of glycerol to the lipid bilayer of glycerosomes enhances membrane fluidity. This increased fluidity encourages the merging of glycerosomes with the lipid matrix of the stratum corneum, enabling deeper drug absorption. In addition, glycerosomes have the capability to merge with the skin's lipid layers, releasing the drug straight into the deeper layers of the epidermis and dermis.[26,27]

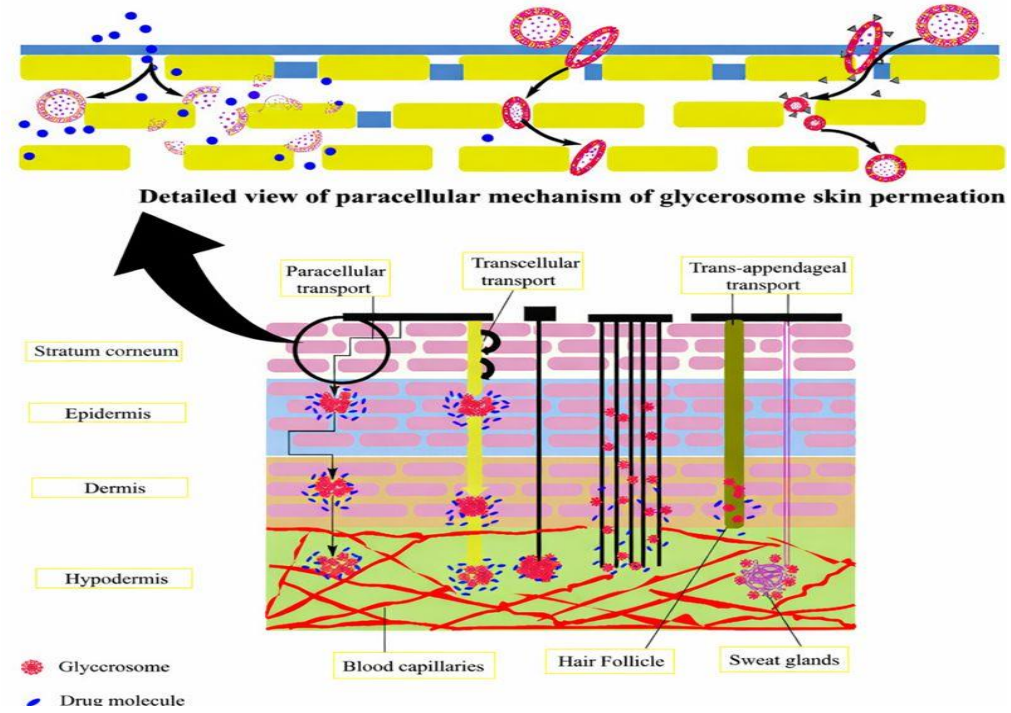

Fig. 2: Skin Permeation Mechanism of Glycerosome.

ADVANTAGES OF GLYCEROSOMES

- They provide a safe and non-toxic method for topical drug delivery.
- The creation of glycerosomes does not require specific transition temperatures.
- They can form at room temperatures (25 or 30°C), unlike conventional liposomes.
- Glycerosomes act as edge activators and penetration enhancers, facilitating drug absorption into the stratum corneum and deeper skin layers.[28]
- Due to the viscous nature of glycerol, glycerosomes distribute evenly across the skin, preventing leakage of the active pharmaceutical ingredient, which is a limitation of traditional liposomes.[29]
- Additionally, glycerosomes improve the plasticity of the skin layers, reducing barriers to transdermal drug delivery and increasing the moisture content of the stratum corneum.[30]
- Glycerosomes can change the configuration of hydrophilic phospholipid chains and influence how other vesicles in the system interact with each other. This is possible because glycerosomes have the capacity to modify the dielectric constant of the system.[31] These vesicles are unique in that they can

serve both as elastic materials and as enhancers of penetration.[30,32]

DISADVANTAGES OF GLYCEROSOMES

- Glycerol increases particle size and slows down drug release in vesicles.[6]
- The thickness of glycerosomes may extend the time it takes for vesicles to move from the formulation to the skin's surface, but it also improves stability.[31]

METHODS OF PREPARATION OF GLYCEROSOMES

1. Thin Film Hydration Method/ Lipid Hydration Method

The phospholipid dissolves in an organic solvent and dries to form a thin film. Next, this thin film is hydrated by mixing it with an aqueous phase, which is a mixture of water and glycerol. The prepared dispersion is then sonicated using a high-intensity ultrasonic sonicator. This method produces a formulation with higher encapsulation efficiency and improved physical characteristics, such as a spherical shape and smooth texture, compared to other methods.[28,33]

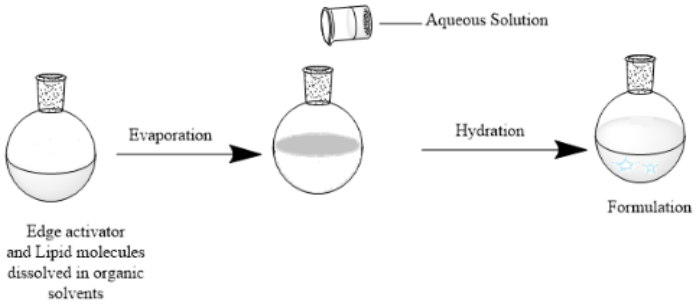

Fig. 3: Thin film hydration method.

2. Reverse-phase Evaporation Method

In this method, a specific amount of cholesterol and phospholipid is dissolved in a mixture of methanol and chloroform (3:1, v/v). Meanwhile, the drug is dissolved in a mix of glycerol and water. Both phases, the aqueous and organic, are combined in a ratio of 1:3 to create a uniform emulsion using a bath sonicator. A rotary evaporator recovers the organic solvent. This method has a unique advantage for trapping valuable hydrophilic substances like drugs, proteins, and nucleic acids.[28,33]

3. Solvent spherule method

Phospholipids are mixed with an organic solvent and then dissolved in water using the solvent spherule process. Spherules of lipids with organic solvents (o/w emulsion) are created by vortexing or stirring this mixture for one hour under low vacuum. In a water bath, the spherules undergo controlled evaporation to remove the organic solvents and produce MLVs.[28,33]

4. Solvent injections method, ether injection method

This technique involves dissolving lipids in a diethyl ether and ether methanol mixture. Next, the solution is injected into a heated aqueous solution containing the substance that needs to be encapsulated. It is essential to keep the heated aqueous phase above the boiling point of ether. During encapsulation, the temperature is typically maintained between 55 and 65°. The injection should happen slowly rather than quickly. Ether evaporates when it comes into contact with the heated aqueous phase, leading to the formation of unilamellar vesicles. This method has drawbacks, including the limited yield of heterogeneous liposomes and the exposure of the chemicals being encapsulated to organic solvents and high temperatures.[28,33]

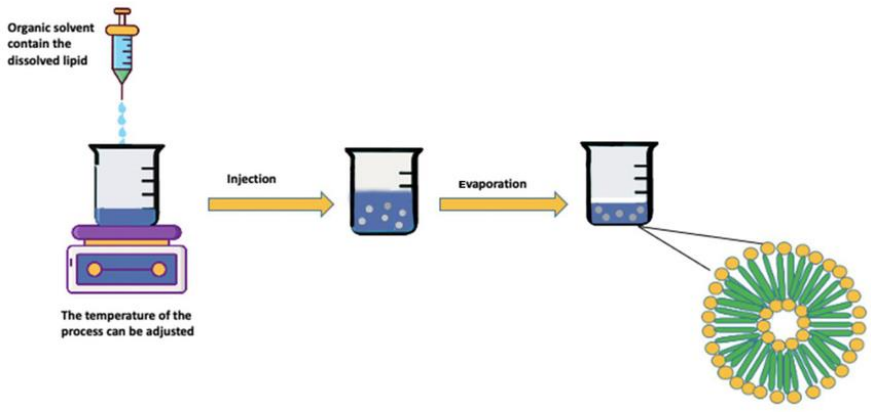

Fig. 4: Ether injection method.

5. Ethanol injection method

Batzri and Korn first explained the ethanol injection technique in 1976. This method involves dissolving lipid in ethanol and pushing it through a small opening, which may be a syringe, into a large amount of water. When injecting an ethanolic lipid solution into water, the speed should ensure the two mix completely. For phospholipids to disperse in water and for ethanol to dilute right away in the hydration medium, they must combine well. The main advantage of this technique is that by injecting a lipid solution dissolved in ether into water, tiny liposomes smaller than 100 nm can form without needing sonication or extrusion. It also produces diluted and uniform liposomes. However, the limitation caused by lipid solubility in ethanol restricts the amount of ethanol that can mix with water, which consequently limits the lipid that can dissolve in ethanol. This is one drawback of the ethanol injection method. Although dialysis can remove it, ethanol remains in the liposomes.[28,33]

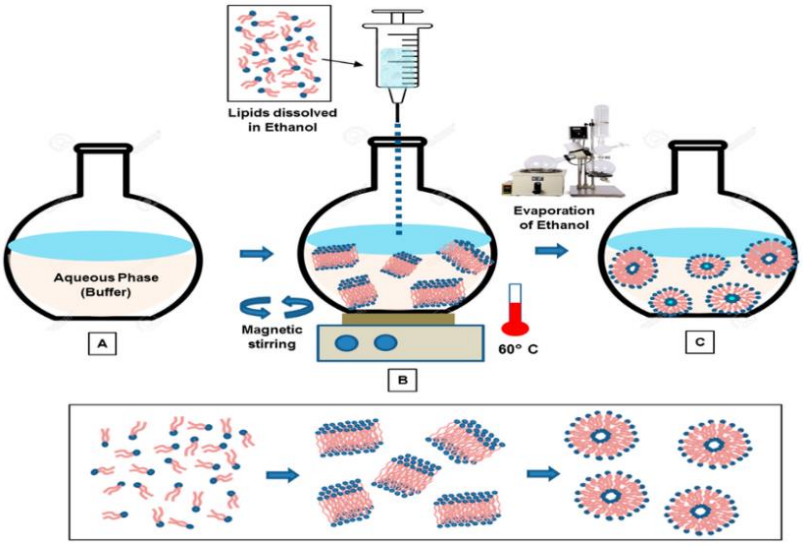

Fig. 5: Ethanol injection method.

6. Detergent removal method/detergent dialysis method

The detergent removal, or detergent dialysis, method is commonly used to encapsulate proteins and other biological molecules. In this process, phospholipids are first dissolved in detergents, and then the detergent is carefully removed to form vesicles. Researchers often use non-ionic, anionic, or cationic detergents with high critical micelle concentration (CMC). Gel chromatography or Lipoprep systems are typically used to remove the detergent. This approach helps create uniform vesicles that replicate efficiently. Alpes et al. studied alkyl maltosides as detergents in this method and found that the speed of detergent removal mainly depends on the dialysis membrane's permeability and the detergent's CMC. They also observed that the length of the alkyl chain affects the CMC, and identified decyl maltoside as a good choice because it is removed quickly and supports effective vesicle formation.[28,33]

7. Double emulsion evaporation

This technique creates a double emulsion of the type W1/O/W2. There are an inner and an exterior aqueous phase to it. Individual oil globules are scattered across the outer phase, while tiny droplets within each outer aqueous phase oil globule represent the inner aqueous phase. The following steps make up the double emulsion evaporation process The drug's aqueous phase is introduced to an organic solvent that contains lipids after being dissolved in water. This creates a water-in-oil emulsion, which is subsequently homogenized appropriately to create the primary emulsion (W1/O). Double emulsion is created when the primary emulsion is mixed with an outer aqueous phase that contains stabilizer.[28,33]

8. Calcium-induced fusion method

This process creates LUVs by fusing SUVs with calcium (Ca2+). Large planar lamellae are created during fusion, and these eventually develop into cochleate cylinders. To create LUVs, these are further combined with EDTA and undergo additional transformation. EDTA keeps the membrane fluid and aids in the restoration of negative charge.[28,33]

9. Microfluidization method

This technique uses a microfluidizer to produce liposomes with a high aqueous volume continuously and on a big scale. According to Mayhew et al., liposomes made with a microfluidizer had characteristics similar to those of tiny extruded multilamellar liposomes. Microfluidized liposomes had a more consistent size distribution and were smaller than traditional multilamellar liposomes. This procedure involves pumping an aqueous lipid suspension through filters, usually with pore sizes of 5 μm, at high pressure after it has been added to a reservoir. After entering an interaction chamber, the suspension is split into two

streams that encounter in microchannels at extremely high speeds. Following the production of liposomes as a result of this intensive contact, the outflow is gathered and examined.[28,33]

10. Freeze-thaw method

The freeze-thaw method utilizes the process of freezing and thawing. The freeze-thaw approach can be applied to phospholipids that are crude in nature or to mixes of charged phospholipids, that is, phospholipids that exhibit both positive and negative charge. Small unilamellar liposomes are quickly frozen and then thawed. After that, they are sonicated to produce LUVs. As a result of this process, bilayers of SUVs fuse together during freezing and thawing, forming LUVs. By using this technique, the synthesis of liposomes is decreased by higher liposome concentration or increased ionic strength.[28,33]

EVALUATION PARAMETERS OF GLYCEROSOMES

1. Particle size analysis

A Zetasizer can examine the polydispersity index (PI) and particle size of glycerosome preparation. This method is called photon correlation spectroscopy or dynamic laser light scattering. For this, a Malvern Zetasizer is typically used.[33,28,3]

2. Vesicle formation

SEM (scanning electron microscopy), TEM (transmission electron microscopy), and cryo-TEM are used to confirm vesicle formation. The materials are first stained with 1% phosphotungstic acid in TEM before being examined with an electron microscope. Cryo-TEM works by coating the samples on a carbon rod and immersing it in ethane at its melting temperature. The TEM is then used for investigation**.**[33,28,3]

3. Determination of deformation index

This involves creating glycerosomal preparations that can pass through a membrane extruder with a specific pore size and using an extruder at a certain pressure. The membrane holes must be smaller than the average phospholipid vesicle size. Flexible vesicles that can easily flow through skin pores are necessary for penetration into the skin. Glycerol is thought to form these types of vesicles, so the deformation index is calculated to see if glycerol can create vesicles that can change shape.[33,28,3]

4. Determination of entrapment efficiency (%)

Entrapment efficiency (EE) of glycerosomes is commonly determined by the indirect centrifugation method. In this procedure, a known amount of drug-loaded glycerosomal suspension is centrifuged at high speed to separate the vesicles (pellet) from the free, unentrapped drug (supernatant). The concentration of free drug in the supernatant is measured using a UV-Visible spectrophotometer at the drug's λmax.[33,28,3]

The percentage entrapment efficiency is then calculated using the formula.

$$\%EE = (\text{Total Drug} - \text{Free Drug}) / \text{Total Drug} \times 100$$

5. Determination of penetration

This evaluation measures how well glycerosomes penetrate the epidermal layer. This can be done ex vivo and helps determine how well drugs pass through the epidermis. It is conducted using a Franz diffusion cell. Animal skin is positioned with the stratum corneum facing the donor side between the donor and receptor compartments. After applying the glycerosome preparations to the skin at specific intervals, the medium collected in the receptor compartment is removed and replaced with new medium. It is analyzed for drug content using an appropriate method like HPLC or UV. Once all samples have been delivered, the skin is removed from the Franz diffusion cell. The layers are sonicated after separating the epidermis from the dermis, and then the drug concentration is measured.[33,28,3]

6. Determination of drug release in vitro

In-vitro drug release using the Franz diffusion cell is a standard method to evaluate how a drug diffuses from a topical glycerosomal formulation. The apparatus consists of two compartments separated by a membrane (synthetic membrane or animal skin). The glycerosomal gel or dispersion is placed in the donor compartment, while the receptor compartment is filled with a suitable and maintained at 37 ± 0.5 °C with continuous stirring to simulate body conditions. At predetermined time intervals, samples are withdrawn from the receptor medium and replaced with fresh buffer to maintain sink conditions. The collected samples are analyzed using a UV-Visible spectrophotometer at the drug's λmax, and the cumulative percentage drug release is calculated and plotted against time to determine the release profile.[33,28,3]

7. Determination of fluidity

Lipid bilayer fluidity is assessed by differential scanning calorimetry (DSC) studies. This method helps determine the transition temperature of the phospholipids. Some reports indicate that the transition temperature shows how they interact with other substances. Adding glycerol changes the transition temperature, supporting the idea that glycerosomes are more fluid.[33,28,3]

8. Determination of stability

Using a Zetasizer to measure the zeta potential provides insight into the stability of the preparation and the charges present on the surface of glycerosomes due to the addition of charged species in the formulation. When the zeta potential is negative, the formulation is more stable.[33,28,3]

CONCLUSION

Glycerosomes have been discovered as incredibly valuable carrier system for regulated and targeted medication delivery. Vesicles' great flexibility and

deformability allow them to be used for drug delivery by any mode of administration and for any medication, regardless of its solubility. Glycerosomes can be manufactured by several methods in which the most popular approach employed for therapeutic is lipid thin film hydration process. Additionally, it has been demonstrated that this technology can triple a medication's effectiveness. The usage of glycerosomes in the delivery of bioactive are promising and are sure to undergo additional improvements in future.

CONFLICT OF INTEREST

Nil.

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Nil.

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