



**RECENT ADVANCES IN GLYCEROSOMES: FORMULATION STRATEGIES,
CHARACTERIZATION, SKIN PERMEATION MECHANISMS, AND THERAPEUTIC
APPLICATIONS**

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ABSTRACT

Topical drug delivery has emerged as an effective approach for the localized treatment of skin disorders by providing sustained drug release, improved therapeutic efficacy, and enhanced patient compliance while minimizing systemic adverse effects. However, the barrier function of the stratum corneum limits the penetration of many therapeutic agents, necessitating the development of advanced vesicular drug delivery systems. Among these, glycerosomes have gained considerable attention due to their superior flexibility, enhanced skin permeation, improved drug entrapment, and excellent stability compared to conventional liposomes. Glycerosomes are phospholipid-based nanovesicles containing a high concentration of glycerol, which increases membrane fluidity and facilitates deeper penetration into the skin. This review highlights the composition, characteristics, preparation methods, and evaluation parameters of glycerosomes, including thin-film hydration, reverse-phase evaporation, solvent injection, sonication, freeze-thaw, detergent removal, and other commonly employed techniques. The review also discusses physicochemical characterization, vesicle morphology, particle size, entrapment efficiency, deformability index, in vitro drug release, penetration studies, and stability evaluation. Furthermore, the therapeutic applications of glycerosomes in topical drug delivery are comprehensively summarized, including their use in skin cancer, inflammatory disorders, oxidative stress-related skin damage, rheumatoid arthritis, fungal infections, and wound healing. Recent research demonstrates that glycerosomes significantly enhance drug permeation, skin retention, controlled release, antioxidant activity, and therapeutic efficacy while reducing toxicity. Owing to these advantages, glycerosomes have emerged as promising nanocarriers for dermal and transdermal drug delivery. Continued research and clinical investigations are expected to expand their applications in the treatment of various dermatological disorders and improve the effectiveness of topical pharmaceutical formulations.

Keywords: Glycerosomes; Topical drug delivery; Transdermal drug delivery; Nanovesicles; Phospholipids; Glycerol; Skin permeation; Dermal delivery; Liposomes; Drug delivery systems; Controlled drug release; Skin disorders; Vesicular carriers; Entrapment efficiency; Nanotechnology.

INTRODUCTION

The skin acts as the main target as well as a principal barrier for dermal and transdermal drug delivery. It also represents an ideal route of drug administration in terms of accessibility and ease of application. Indeed, topical drug delivery has several advantages over other routes of administration including improved bioavailability for drugs that suffer the gastrointestinal environment and/or hepatic first effects. Moreover, topical application can give both a constant, continuous drug delivery and/or a targeting of the active with consequent fewer side effects and improved patient compliance. However, the barrier nature of the skin represents a significant obstacle for most drugs to be delivered into and through it. In order to overcome the barrier properties of the stratum corneum (SC) and to enhance drug transport across intact skin, several techniques have been developed. They include chemical methods based on the use of different penetration enhancers (Manconi *et al.*, 2009; Manconi *et al.*, 2011). As well as physical methods (iontophoresis, electroporation and low-frequency ultrasound) (Lavon and Kost, 2004). One of the most controversial methods is the use of vesicular formulations as drug skin delivery systems. Over the last two decades, the lack of ability of conventional liposomes to efficiently deliver drugs across the skin has led to intensive research in the field, with the introduction and development of new classes of lipid vesicles (Gupta *et al.*, 2012; Sinico and Fadda, 2009). Recently, it has become evident that vesicle composition plays a significant role in vesicles as skin delivery systems and many authors have reported that specially designed vesicular

carriers can be used to increase penetration and permeation through the SC for systemic delivery, but they can also be employed to localize the drug in the skin layers and deeper soft tissues (subcutaneous adipose and skeletal muscle tissue) for local treatment in the context of various diseases (Angelico *et al.*, 2013). Therefore, new, modified formulations have been tested and the so called deformable/elastic liposomes, niosomes as well as ethosomes were introduced. Recently, different authors have tested various penetration enhancers as “edge activator” in the formulation of new phospholipid vesicles such as propylene glycol-liposomes, invasomes, and PEVs (Penetration enhancer containing vesicles) (Dragicevic-Curic *et al.*, 2008; Manconi *et al.*, 2009).

A new approach to increase liposome properties as dermal and transdermal drug delivery systems by modifying liposomal bilayer fluidity is represented by glycosomes, which are obtained from different phospholipids and high concentrations of glycerol (10–30%, v/v), a harmless and fully accepted compound for topical administration. Glycosomes are versatile vesicular carriers that might contain one or more of the additives commonly used in the composition of conventional liposomes such as cholesterol. Moreover, they can be obtained by any of the different techniques commonly used for the preparation of conventional liposomes (Zaru *et al.*, 2013).

Glycosomes

The concept of glycosomes was introduced by Manca *et al.* for the delivery of diclofenac to skin. Glycosomes are bilayer vesicles used for dermal and transdermal drug delivery. These vesicles differ from

conventional liposomes in bilayer fluidity, formed by the addition of phospholipids and varying concentrations of glycerol (10-30 % v/v) (Manca *et al.*, 2013). These are so named, as they contain high amount of glycerol (Manconi *et al.*, 2011). These vesicles deliver the active ingredients to skin with high efficiency (Ashtiani *et al.*, 2016). Glycosomes are found to be more stable and possess greater fluidity than liposomes and hence are predominantly used as topical drug delivery systems. Glycerol ameliorates the deformability index of liposomal bilayers, thus enhancing skin penetration (Zhang *et al.*, 2017).

Glycosomes 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 glycosomes, 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 (Gregoriadis, 1976; Jabin *et al.*, 2018; Kant *et al.*, 2013). Liposomes may encapsulate both polar and non-polar substances because of their amphiphilic nature (Sharma and Sharma, 1997). 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 (Dua *et al.*, 2012). 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. Vesicular systems with enhanced properties are required. Glycosomal carriers are currently the most fascinating research topic for scientists (Rani *et al.*, 2022).

Glycosomes 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. Glycosomes are a promising medication delivery vehicle since they can encapsulate both hydrophilic and hydrophobic medicines. The majority of the glycosome 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 (Singh *et al.*, 2023).

Method of preparation of glycosomes

The following is a description of the many preparation techniques of glycosomes.

Thin film hydration technique

This is the simplest method for making glycosomes that Bangham *et al.* have provided. 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 (Rani *et al.*, 2022; Kaddah *et al.*, 2018).

Reverse Phase Evaporation

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 re-dissolved 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 evaporate 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 is 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 glycrosomes by eliminating any remaining solvent. This technique can encapsulate big macromolecules with high encapsulation efficiency and is used to generate huge uni- and multi-lamellar vesicles.

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 glycrosomal formulation and homogeneous size distribution provided by this approach are advantages (Zhu *et al.*, 2022).

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 glycrosomal 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 glycrosomal dispersion into the sonicator and sonicating the suspension for five to ten minutes over the lipid's critical solution temperature (T_c). When using this technology instead of a probe sonicator, temperature control is simple.

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.

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.

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.

Detergent Removal Method

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. Decyl maltoside was found to be the best detergent for preparing vesicles using the detergent removal method because of how quickly it forms vesicles (Manca *et al.*, 2013; Gupta *et al.*, 2020).

French Pressure Cell Method

This method prepares multilamellar vesicles (MLVs) to flow through a small aperture at 20000 pressure and 4°C. 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.

Evaluation of glycosomes

Various parameters of glycosomes were evaluated

- a) Particle size analysis
- b) Development of vesicles
- c) Determination of deformation index
- d) Calculating entrapment efficiency (%)
- e) Determination of Penetration
- f) Measuring drug release *In vitro*
- g) Evaluation of fluidity
- h) Determination of Stability

Glycosomes for the treatment of topical disease

Role of Glycosomes in Skin Cancer Therapy

Shadab et al 2021, was developed, optimized and evaluate Plumbagin (PLM)-loaded glycosome (GM) gel and compare with conventional liposome (CL) for therapeutic efficacy against skin cancer. PLM is a phytochemical which has shown cytotoxicity against of cancer cells both *in vitro* and *in vivo*. However, the clinical application of PLM has been hindered due to poor aqueous solubility and low bioavailability. The GM formulations were optimized by employing design expert software by 3-level 3-factor design. The prepared GMs were characterized *in vitro* for vesicle size, size distribution, zeta potential, vesicle deformability, drug release, skin permeation, retention, texture, antioxidant and cytotoxicity activities. The optimized formulation showed a vesicle size of 119.20 ± 15.67 nm with a polydispersity index (PDI) of 0.145 ± 0.02 , the zeta potential of -27 ± 5.12 mV and entrapment efficiency of $76.42 \pm 9.98\%$. The optimized PLM-loaded GM formulation was transformed into a pre-formed gel which was prepared using Carbopol 934 polymer. The drug diffusion

fluxes of CL gel and GM-loaded gel were 23.31 ± 6.0 and 79.43 ± 12.43 $\mu\text{g}/\text{cm}^2/\text{h}$, respectively. The result of texture analysis revealed the adequate hardness, cohesiveness, consistency, and viscosity of the developed GM-loaded gel compared to CL gel. The confocal images showed that glycosomal gel has deeper skin layer penetration as compared to the control solution. GM-loaded gel treated rat skin showed significantly ($p < 0.05$) higher drug accumulation in the dermis, higher cytotoxicity and higher antioxidant activity as compared to CL gel and PLM suspension. Thus, findings revealed that novel GM-loaded gel could be potential carriers for therapeutic intervention in skin cancer (Shadab et al., 2021).

For dermal and epidermal delivery of Diclofenac as analgesic

Manca et al., 2013, have prepared glycosomes by hydrating dipalmitoylglycerol phosphatidylcholine-cholesterol films with glycerol aqueous solutions (10–30%, v/v). Glycosomes, vesicles composed of phospholipids, glycerol, and water, as novel vesicular carriers for (trans) dermal drug delivery. The model drug was diclofenac sodium salt and conventional liposomes were used as control. Prepared formulations were characterized in terms of size distribution, morphology, zeta potential, and vesicle deformability. Glycosomes and liposomes were oligo/multilamellar vesicles, spherical in shape with a mean diameter ranging between 81 and 97 nm and a fairly narrow distribution (P.I. = 0.14–0.19), negative zeta potential values (from -35 to -48) and drug loading capacity between 64 and 73%. Deformability index of both conventional

liposomes and glycrosomes showed that glycerol is able to act as edge activator for dipalmitoylglycerol phosphatidylcholine bilayers when used in concentration higher than 10%. DSC studies suggested that glycrosomes are in a more fluid state than conventional liposomes. *In vitro* transdermal delivery experiments showed an improved skin deposition and permeation of diclofenac when 20 and 30% glycrosomes were used. MTT test demonstrated that glycrosomes were able to reduce the *in vitro* drug toxicity versus keratinocytes (Manca et al., 2013).

Protection of skin Oxidative stress

Manca et al 2014, have incorporated quercetin in glycrosomes, new phospholipid-glycerol vesicles, and evaluated their protective effect against oxidative stress skin damages. In particular, the concentration-dependent effect of glycerol (from 10 to 50%) on vesicle suitability as cutaneous carriers of quercetin was carefully assessed. All vesicles were unilamellar and small in size (~80–110 nm), as confirmed by cryo-TEM observation, with a drug incorporation efficiency ranging between 81 and 91%. SAXS studies, performed to investigate the bilayer arrangement, indicated a strong, dose-dependent interaction of glycerol with the polar portions of the phospholipid molecules, while quercetin did not significantly change the bilayer packing. *In vitro* studies on newborn pig skin underlined the concentration-dependent ability of glycrosomes to promote quercetin accumulation in the different layers, also confirmed by confocal microscopic observation of skin treated with fluorescent vesicles. Quercetin incorporated into

liposomal and glycrosomal nanoformulations showed a strong ability to scavenge free radicals (DPPH test) and protect human keratinocytes *in vitro* against hydrogen peroxide damage. Moreover, quercetin-loaded vesicles were avidly taken up by keratinocytes *in vitro*. Results indicate 40 and 50% glycrosomes as promising nanosystems for the improvement of cutaneous quercetin delivery and keratinocyte protection against oxidative stress damage (Manca et al., 2014).

Glycrosome for rheumatoid arthritis treatment

Zhang et al 2017 have reported novel glycrosome carrier containing essential oils for topical administration of paeoniflorin (PF) to enhance its transdermal drug delivery and improve drug absorption in the synovium. The formulation of glycrosomes 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. The *in vitro* transdermal flux of PF loaded in the STO-glycrosomes was 1.4-fold, 1.6-fold, and 1.7-fold higher than those of glycrosomes, liposomes, and tinctures, respectively. *In vivo* studies showed that the use of STO-glycrosomes was associated with a 3.1-fold greater accumulation of PF in the synovium than that of common glycrosomes. This finding was confirmed by *in vivo* imaging studies, which found that the fluorescence intensity of Cy5.5-loaded STO-glycrosomes in mice knee joints was 1.8-fold higher than that of the common glycrosomes 5 h after administration. The glycrosomes mediated by STO exhibited considerable skin

permeability as well as improved drug absorption in the synovium, indicating that STO-glycrosomes may be a potential PF transdermal delivery vehicle for the treatment

of rheumatoid arthritis caused by synovium lesions (Zhang *et al.*, 2017).

Table 1: Review of published studies on Glycrosomes for topical drug delivery

S. No.	Author (s) & Year	Title/Study	Major Findings
1	Gotpagar et al. (2025)	Glycrosomes in topical antifungal therapy	Reported that glycrosomes improve drug penetration, stability, fluidity, and entrapment efficiency, making them promising carriers for topical antifungal delivery.
2	Banode et al. (2025)	Review on glycrosomes	Highlighted the versatility of glycrosomes for topical, transdermal, pulmonary, oral, and ocular drug delivery due to enhanced membrane fluidity and penetration.
3	Abualsunun et al. (2025)	Quercetin-loaded hyaluronic acid modified glycrosomes	Developed Quercetin-loaded glycerohyalurosomes that significantly improved wound healing, skin penetration, and topical therapeutic efficacy.
4	Sadek et al. (2024)	Voriconazole augmented glycrosomes	Optimized glycrosomes exhibited high entrapment efficiency, enhanced skin permeation, prolonged drug release, improved antifungal activity, and good stability.
5	Anjali et al. (2024)	Novel glycerosomal drug delivery	Reviewed glycrosomes as advanced vesicular systems offering superior penetration, stability, entrapment efficiency, and fluidity compared with conventional liposomes.
6	Patil et al. (2024)	Atorvastatin-loaded glycrosomes	Developed optimized glycrosomes with sustained drug release, enhanced transdermal permeation, and improved

			skin retention.
7	Alam et al. (2023)	Rutin glycosomal Carbopol gel	Prepared optimized rutin-loaded glycosomes that showed prolonged drug release, enhanced skin penetration, and strong antioxidant activity for sunburn treatment.
8	Fatima et al. (2023)	Advances and patents on glycosomes	Reviewed recent developments and patents, emphasizing glycosomes as flexible vesicular carriers capable of encapsulating hydrophilic and lipophilic drugs.
9	Singh et al. (2023)	Glycosomes: Recent research and patents	Reported that glycosomes improve drug bioavailability, minimize adverse effects, and serve as efficient targeted drug delivery systems.
10	Deepika et al. (2022)	Overview of glycosomes	Summarized composition, preparation methods, characterization, advantages, and applications of glycosomes for topical and systemic drug delivery.
11	Abhishek et al. (2022)	Glycosomal nanovesicles	Described glycosomes as superior to liposomes in encapsulation efficiency, penetration, stability, and scalability with applications in wound healing, infections, and cosmetics.
12	Salem et al. (2022)	Dexamethasone glycosomal in situ gel	Formulated Box–Behnken optimized glycosomes with enhanced drug permeation and significantly improved bioavailability.
13	Priya et al. (2020)	Glycosomes as improved liposomes	Concluded that glycerol enhances liposomal stability, penetration, entrapment efficiency, and membrane fluidity, making glycosomes superior topical carriers.
14	Kai et al. (2017)	Essential oil-loaded	Developed glycosomes containing essential oils for improved transdermal

		glycosomes	delivery of paeoniflorin, showing significantly enhanced skin permeation and synovial accumulation.
15	Manca et al. (2013)	Development of glycosomes	First demonstrated glycosomes as effective vesicular carriers for transdermal delivery with improved deformability, drug loading, skin penetration, and reduced cytotoxicity compared with conventional liposomes.

CONCLUSION

Glycosomes are advanced phospholipid-based vesicular carriers that offer significant advantages for topical and transdermal drug delivery due to their enhanced flexibility, improved skin permeation, high drug entrapment efficiency, and excellent stability. Their ability to encapsulate both hydrophilic and lipophilic drugs makes them versatile drug delivery systems. Numerous studies have demonstrated their effectiveness in improving drug penetration, controlled release, and therapeutic outcomes in various dermatological disorders. Therefore, glycosomes represent a promising and innovative nanocarrier system with considerable potential for future topical pharmaceutical formulations and clinical applications.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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