Review Article

# Niosome: A Nano-carrier for Peptide and Protein Drug Delivery

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**Received:** 18-04-2023; **Revised:** 22-06-2023; **Accepted:** 30-06-2023; **Published on:** 15-07-2023.

**ABSTRACT**

Peptides and Proteins are the most essential component of biological cells. They are made up of different naturally occurring amino acids. A difference in peptides and proteins is in the number of amino acids. Peptides have lower than 20 amino acids, containing a molecular weight lower than 5000, and proteins have 50 or higher than 50 amino acids and contain a molecular weight higher than this value. Because of its high molecular weight, various problems arise with respect to the absorption of the drug. Recent advances in biotechnology evidence that proteins and peptides are the foundation of new modern drugs. However, the transportation of peptide and protein drugs in the body is restricted by their high molecular weight, which limits the permeation of tissue cell membranes. Furthermore, delivering the drugs selectively to target cells, tissues, or organ is the major challenge for the treatment of various human diseases, including cancer. Niosomes are nano-carrier of novel drug delivery systems containing non-ionic surfactants that entrap both lipophilic and hydrophilic drugs. Because of the small size, its advantage is an increase in the absorption of the drug. Niosomes in topical or transdermal formulations increase the penetration of the drug and increase stability of the drug.

**Keywords:** Niosome, Protein and Peptide, Topical or Transdermal, dermal delivery, Skin.

**QUICK RESPONSE CODE →**

**DOI:**
10.47583/ijpsrr.2023.v81i01.004

**DOI link:** http://dx.doi.org/10.47583/ijpsrr.2023.v81i01.004

## INTRODUCTION

Peptides and Proteins are the most essential components of biological cells. They present in functions such as hormones, enzymes, structural elements, and immunoglobulin. Naturally occurring amino acids combine with each other by peptide bonds and assemble polymers introduced as peptides and proteins[1]. Even though the difference between protein and peptide is in the number of amino acid present in it. Peptides have lower than 20 amino acids, containing a molecular weight lower than 5000, and proteins have 50 or higher than 50 amino acids and contain a molecular weight higher than this value. Maximum pharmaceutical peptides and proteins are absorbed through intramuscular, intravenous, or subcutaneous routes of administration, but these routes are all invasive. The more appropriate route for the absorption of peptides and protein is the oral route as compared to others but it has a low bioavailability. The reasons behind the low oral bioavailability of peptide and protein drugs are pre-systemic enzymatic degradation and poor permeation of the intestinal mucosa. The different problems related to the administration of peptide and protein drugs are required to be reduced by different pharmaceutical approaches[1].

Peptide and protein-based agents are typically administered by injection due to their poor absorption when administered through enteral routes administration of a drug. Regrettably, repeated administration of these drugs via multiple injections causes certain patient-related problems and it is difficult to mimic the normal physiological function through this mode of drug administration[1]. A requirement exists to non-invasively deliver these drugs using alternative ways such as dermal delivery.

The absorption of peptide and protein from the oral route is more convenient than other route but this route has some limitations for drugs that have short plasma half-life times or shows first-pass metabolism[2]. Topical or transdermal drug delivery systems have shown significant advantages in clinical practice for drug targeting to the active site in the body; this has reduced the systemic side effects. The administration of drugs through the skin is also performed to achieve controlled or prolonged drug delivery and this route can be explored as an alternative to the oral route.[2]

In the case of topical formulations drug products applied to the skin surface may penetrate to some extent into the skin layers, where their effects are expected for the treatment of skin disorders such as acne and cutaneous inflammatory diseases that include dermatitis, erythematous lupus, and psoriasis. Alternatively, transdermal formulations release drugs that permeate through the skin and enter the systemic circulation. Transdermal therapy must ensure that significant concentrations of the drug are absorbed to reach effective plasma concentrations[2].

Topical/TDDS showed promising results in comparison to oral and injectable administration because of the following advantages: It eliminates gastrointestinal interferences i.e first-pass metabolism of the drug, easy application, minimum fluctuating levels, achieves efficacy in a lower total dose, simple termination of medication if required, able to become more "site-specific" with, good adherence. Escaping of specific risks associated with oral or i.v intake, the utility of short half-life drugs, and patient compliance[3]. But skin acts as a principal barrier for topical/TDDS[3,4]. The stratum corneum plays a crucial role in barrier function because of the low diffusion rate of a drug across the stratum corneum i.e. only the lipophilic drug having a molecular weight (500 Daltons) can pass through it[3,5].

**STRUCTURE OF SKIN**

Skin is a multilayered organ complex in both structure and function. Macroscopically, the outer epidermis, and the inner dermis are two distinct layers of the skin[2,6].

**Epidermis:**

Epidermis is the outermost layer of the skin, which is approximately 150 µm thick. Cells of lower layers of the skin move overhead throughout their life cycle and become flat dead cells of the corneum. The epidermis is a multilayered structure consisting of viable cells and dead keratinized cells.

The layers of the epidermis are[7]:

Stratum Corneum (Horny Layer)

Stratum Lucideum(Clear layer)

Stratum Granulosum (Granular Layer)

Stratum Spinosum (Prickly cell Layer)

Stratum Germinativum (Regenerative Layer)

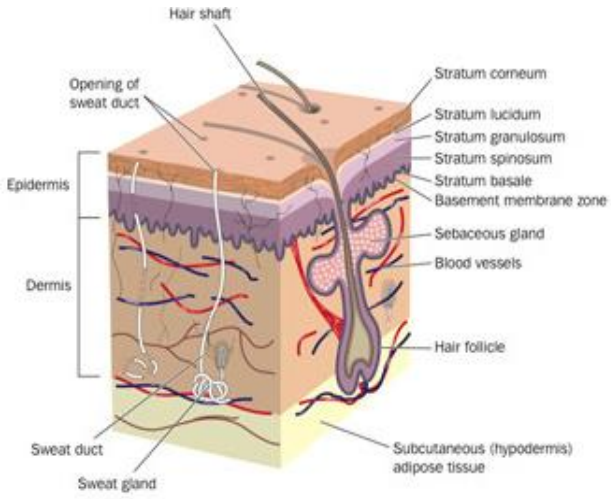


**Figure 1**: Structure of Skin

**1. Stratum corneum**:

This is the layer that interacts with the environment and it is also called the hornylayer. This layer contains 20 to 30 cell layers and is the uppermost layer, made up of dead keratinocytes, known as anucleate squamous cells. This layer varies in thickness, particularly in cellulose skin.

Keratinized cells are physiologically inactive and continuously shed with constant replacement from the underlying viable epidermal tissue. Within this layer, the dead keratinocytes secrete defensins which are part of our first immune defense. This layer has a water content of only 20% than to the normal physiological level of 70%, like in the physiologically active stratum germinativum (which is the regenerative layer of the epidermis).

Stratum corneum (10-15um in thickness) is the skin's primary defense layer against invasion. The major lipid contains within this layer are fatty acids, cholesterol, and ceramides. Their major structural components are aggregates of keratin filaments. All these are responsible for the tightness and impermeability characteristics of the skin.

**2. Stratum Lucidum:**

This layer is 2 to 3 cell layers, found in soles and palms comes in thicker skin, a thin translucent clear layer immediately above the granule layer containing eleidin. Eleidin is a product of the transformation of keratohyalin which acts as a limit to water and gives rise to its transparent or lucid properties.

**3. Stratum Granulosum:**

3-5 cell layers contain diamond-shaped cells with keratophyllin granules and lamellar granules. Keratophyllin granules contain keratin precursors that eventually aggregate, cross-link, and form bundles. The lamellar granules contain the glycolipids that get secreted to the surface of the cells and function as the glue, keeping the cells stuck together.

**4. Stratum Spinosum:**

8-10 cell layers, also known as the prickle cell layer contain irregular, polyhedral cells with cytoplasmic processes, sometimes called Spines. The cells of this layer are produced by morphological and histochemical alteration of the cells' basal layers as they moved upward. The cells flatten and their nuclei shrink. They are interconnected by fine prickles and form intercellular bridges- the desmosomes. These links maintain the integrity of the epidermis. Dendritic cells can be found in these layers.

**5. Stratum Germinativum:**

It is also called stratum basale. It is the deepest layer of the epidermis, separated by the basement membrane (basal lamina). It is attached to the basement membrane by hemidesmosomes.

Basal cells are nucleated and columnar. Cells of this layer have a high mitotic index and constantly renew the epidermis and this proliferation in healthy skin balances the loss of dead horny cells from the skin surface. These layers also contain melanocytes.

**Dermis:**

The human skin contains the dermis, approximately 2-3 mm thick, which forms the bulk of the skin. The dermis contains a network of blood vessels, lymph vessels, hair follicles,sweat glands & sebaceous gland -skin appendages.

**Hypodermis:**

Beneath the dermis is the hypodermis, which is primarily composed of fibroblasts and adipocytes subcutaneous fatty tissues. Bulbs of hair project into these fatty tissues. The hypodermis binds the skin to the underlying structures, inaddition to serving as a thermo regulator and a cushion to internal organs against trauma.

The skin is interspersed with hair follicles and associated sebaceous glands and sweat glands. Collectively these are referred to as skin appendages. On an average of 10-70 hair follicles and 200-500 sweat ducts per square centimeter are present on the skin surface. These skin appendages occupy only 0.1% of the total human skin surface.

Despite major research & development efforts in the TDDS system and the advantages of these routes, low stratum corneum permeability limits the usefulness of these delivery systems. Many techniques have been aimed to disrupt or weaken the highly organized intercellular lipids in an attempt to enhance drug transport across the intact skin or to increase the driving force permeation of drugs across this skin barrier.

**The main approaches for Transdermal delivery are:**

**Physical methods**:

- Ultrasound
- Iontophoresis
- Sonophoresis
- Magnetophoresis
- Microneedle
- Electroporation
- Needle-free injection
- Photomechanical waves
- Laser-assisted

**Chemical methods**:

- Prodrug
- Chemical agent
- Ion pairs
- Supersaturated solutions
- Complexes
- Eutectic systems

**VESICLES AND PARTICLES**

- Ethosome
- Niosome
- Transferosome
- Microemulsion
- Lipid nanoparticle
- Liposome

As there are various drawbacks associated with physical & chemical methods & the specific requirements need for the use of such techniques, nowadays priority is given to the vesicles and particles by many researchers[8]. Researchers have understood vesicle structures for use in better drug delivery within their cavities that would allow for tagging the vesicle for cell specificity. One of the major advances in vesicle research was the finding of vesicle derivatives, known as Niosomes.

**Niosome:**

Niosomes are amphiphilic vesicles made up of a bilayer of non-ionic based surfactant hence named niosome. These are non-ionic amphiphilic because they can entrap both hydrophilic and lipophilic drugs[9]. It contains a non-ionic surfactant such as esters or polyoxyethylene alkyl ethers and they are produced through self-clustering of non-ionic surfactants in an aqueous medium. A closed bilayer structure is produced by the application of heat or physical agitation. L'Oréal developed and patented niosomes in the 1970s and 1980s but Lancome's first product which contains niosome was introduced by Loreal in 1987 (US Patent 4830857, 1989)[10,11].

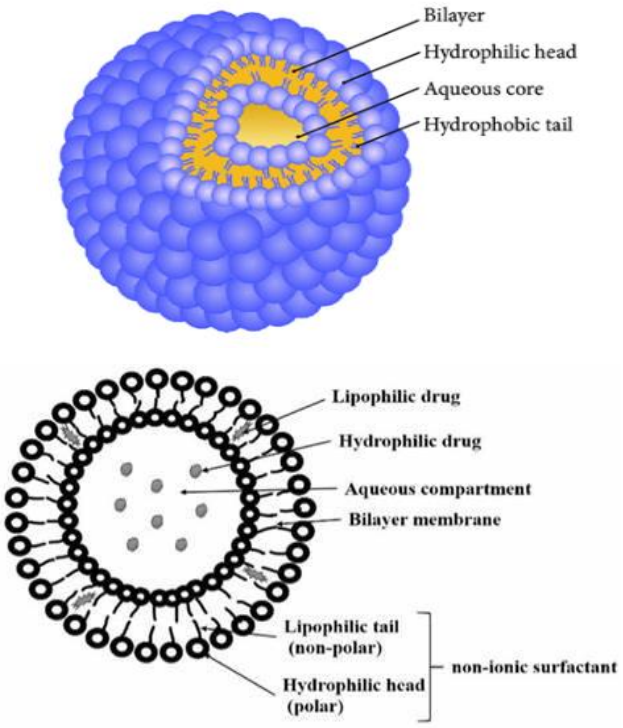


**Figure 2**: Structure of Niosome

Niosomes are structurally similar to liposomes[4]. Niosomes are favored over liposomes because

1) Surfactant present in a niosome is chemically stable over phospholipid present in a liposome.

2) Niosome is more economical than liposome.

Niosomes are nano-carriers with a size range of 10-1000nm. Niosomes are classified based on

1) the nature of lamellarity

2) on the size of vesicles

**Table 1:** Classification of niosomes

| Based on the nature of lamellarity | Based on size |
|---|---|
| Multi-lamellar vesicles size -1µm to 5µm | Small Niosomes (100-200 nm) |
| Large Unilamellar vesicles Size-0.1µm-1 µm | Large Niosomes (800-900 nm) |
| Small Uni-lamellar vesicles Size 25nm-500 nm | Big Niosomes (2-4 µm in size) |

**I) Based on the nature of lamellarity of vesicles:**

1) Multil-amellar vesicle (MLV): (1-5µm in size)[12]

In this, the aqueous core is enclosed by no of the bilayer. The size of the vesicle is 0.5 to 10µm in diameter. This is the most widely used niosome and they are more suitable for a lipophilic drug.

2) Large unilamellar vesicle (LUV): (0.1-1µm in size)

This type of niosome contains a high amount of aqueous or lipid source so a high amount of bio-active material entrapped it.

3) Small unilamellar vesicle (SUV): (25-500nm in size)

This vesicle is mostly prepared from multilamellar vesicle by French press extrusion electrostatic stabilization is the inclusion of diacetyl phosphate in 5(6)-carboxyfluorescein (CF ) load span 60 based niosome.

**METHOD OF PREPARATION**

**Preparation of multilamellar vesicles:**

**1. Handshaking method:**

This method is also called as thin film hydration method. In this method, cholesterol, and surfactants dissolve in an organic solvent such as chloroform, methanol, or diethyl ether in a rotary shaker evaporator until a thin film of solid mixture is formed on the wall of a round bottom flask. This dried layer is hydrated with an aqueous phase containing the drug at normal temperature.

**2. Trans-membrane pH gradient method:**

In this method, cholesterol and surfactant are dissolved in chloroform. After that, the solvent is evaporated under reduced pressure to form a thin film on a round bottom flask. This dry film is then hydrated with 300mM citric acid( ph 4.0) by vortex mixing. The vesicles are frozen and thawed three times and then sonicated. After that, an aqueous phase containing 10mg/ml drug is added and vortexed. The ph of the sample is then raised to 7.0-7.2 with 1M disodium phosphate. After that, the mixture is heated at 60 degree for 10 min to produce desired multilamellar vesicle.

**Preparation of large unilamellar vesicles:**

**1. Reverse phase evaporation technique (REV):**

Surfactant and cholesterol are dissolved in a mixture of ether and chloroform. After that an aqueous solution containing the drug is added to this and the resulting two phases are sonicated at 4-5°C. The clear gel formed after the addition of a small amount of phosphate-buffered saline is further sonicated. The organic phase is removed at 40°C under low pressure. The formed viscous niosome suspension is diluted with phosphate-buffered saline and heated in a water bath at 60°C for 10 min to form niosomes.

**2. Ether injection method:**

This method is based on slow injection of ingredients to form niosome. Cholesterol and surfactant are dissolved in the volatile organic solvent. The aqueous phase containing the drug is placed on a magnetic stirrer at 60°C. The above solution is incorporated in an aqueous phase containing the drug dropwise by injection with the 14 gauge needle at the rate of approximate The probable reason behind the formation of large unilamellar vesicles is that the slow vaporization of solvent results in an ether gradient extending towards the interface of the aqueous-nonaqueous interface. The disadvantages of this method are that a small amount of ether is frequently present in the vesicle suspension and is difficult to remove.

**Miscellaneous:**

**1. Sonication method:**

A mixture of surfactant and cholesterol and the aqueous phase containing the drug are added together in a scintillation vial. Then the mixture is homogenized using a sonic probe at 60 c for 3 min. The vesicles are uniform and small in size.

**2. Microfluidization method:**

Two fluidized streams move forward through the precisely defined microchannel and interact at ultra-high velocities within the interaction chamber. Here, a common gateway is arranged such that the energy supplied to the system remains within the area of niosomes formation. The result is a smaller size, greater uniformity, and better reproducibility.

**3. Multiple membrane extrusion methods:**

A cholesterol, surfactant, and diacetyl phosphate are mixed in chloroform and are made into a thin film by evaporation. This film is hydrated with an aqueous phase

containing drug and the resultant suspension is extruded through polycarbonate membranes, which are placed in a series for up to eight passages. This is a good method for controlling niosome size.

**4. Niosome preparation using polyoxyethylene alkyl ether:**

The number and size of the vesicle bilayer consisting of cholesterol and polyoxyethylene alkyl ether can be changed using an alternative method. An increase in temperature above 60°C converts small unilamellar vesicles into large multilamellar vesicles (>1 µm), while vigorous shaking at room temperature shows the opposite effect, ie, the change of multilamellar vesicles into unilamellar vesicles. The transformation from unilamellar to multilamellar vesicles at higher temperatures might be the characteristic of polyoxyethylene alkyl ether (ester) surfactant since it is known that polyethylene glycol (PEG) and water remix at higher temperatures due to breakdown of hydrogen bonds between water and PEG moieties. Generally, the free drug is removed from the encapsulated drug by gel permeation chromatography dialysis method or centrifugation method [11,9].

## CHARACTERIZATION OF NIOSOME

**1. Size**

The vesicle shape of the niosome is expected to be spherical, and their mean diameter can be characterized by the laser light scattering method. The other method of mean diameter determination is by using electron microscopy, ultracentrifugation, molecular sieve chromatography, optical microscopy, photon correlation microscopy, and freeze-fracture electron microscopy.

**2. Bilayer formation**

The bilayer formation of vesicles from non-ionic surfactants is characterized by an X-cross formation under light polarisation microscopy.

**3. Number of lamellae**

Small-angle X-ray scattering, electron microscopy, and nuclear magnetic resonance (NMR) spectroscopy method is used to determine the number of lamellar in vesicles.

***4. Membrane rigidity***

Membrane rigidity can be determined by using the mobility of the fluorescence probe as a function of temperature.

***5. Entrapment efficiency***

The entrapment efficiency is calculated to determine the quantity of entrapped drugs in the niosome. For that free drug is separated from the niosome by lysis of the vesicles by centrifugation, sonication, or other methods. The free drug concentration is determine by UV- spectroscopy. It can be calculated by ;

Entrapment efficiency (EF) = (Amount entrapped / total amount) × 100

**Table 2:** Previous Published Patent on Niosomes:

| Patent publication number | Inventor | Date of publication | Title | Reference |
|---|---|---|---|---|
| RU2541156C1 | Bazikov Igor' Aleksandrovich (RU), et al | 10-2-2015 | Transdermal anthelmintic agent of silicon niosomes with albendazole | (13) |
| US2005/0239747 A1 | Chih-Chiyang Yang, Yuan-Chih le and Chao-Cheng Liu | 27-10-2005 | Composition and methods of enhancement of transdermal delivery of steroidal compounds and preparation methods | (14) |
| US4830857A | Rose M. Handjani, Alain Ribier, Guy Vanlerberghe, Arlette Zabotto, Jacqueline Griat | 16-05-1989 | Cosmetic and pharmaceutical compositions containing niosomes and a water-soluble polyamide, and a process for preparing these compositions | (15) |

## CONCLUSION

The niosomal drug delivery system is one of the best examples of great perception in drug delivery technologies and nanotechnology. It is most suitable for peptide and protein dermal drug delivery as it decreases the problems associated with the absorption of peptide and protein drugs. Proteins and peptides have many problems associated with the absorption of a drug because of their high molecular weight structure. Niosome nanocarriers avoid the problem that arises with peptide and protein drug absorption and increases drug bioavailability.

It is apparent that the niosome comes out to be a well-adopted drug delivery system compared to other dosage forms as the niosome is very much stable in structure and economics. There are adequate scopes to encapsulate toxic anti-cancer drugs, anti-AIDS drugs, anti-infective drugs, anti-viral drugs, anti-inflammatory drugs, etc. in niosomes and to use them as drug carriers to achieve better bioavailability and targeting properties and for reducing the toxicity and side effects of the drugs. Niosomes are more stable nanocarriers because of the components used in them.

Topical/transdermal dermal drug delivery is more advantageous compared to other routes of administration because it avoids first-pass metabolism, drug toxicity, patient in compliance, etc. Protein and peptide drugs encapsulate in niosomes containing formulations given through topical/transdermal drug delivery are the best examples of great evaluation in drug delivery technologies and nanotechnology. It targets the specific site of action and avoids patient incompliance. Thus, these areas require further advancement and research so as to come out with valuable and commercially available niosomal formulations. The concept of incorporating the drug into niosomes for better targeting and bioavailability of a drug at proper tissue destinations is widely accepted by researchers and academicians.

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**Source of Support:** The author(s) received no financial support for the research, authorship, and/or publication of this article.

**Conflict of Interest:** The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.