NIOSOMES: A PROMISING NANOCARRIER FOR BCS CLASS IV DRUG DELIVERY

Sukanya Patil1* Jaya Agnihotri1 and Dr. Indira Parab1

1Department of Pharmaceutics, H. K. College of Pharmacy, Oshiwara, Mumbai, India.

*Corresponding Author: Sukanya Patil
Department of Pharmaceutics, H. K. College of Pharmacy, Oshiwara, Mumbai, India.

ABSTRACT
As per the biopharmaceutics classification system (BCS), all the drugs are categorized into four different classes following their solubility and permeability. Of these classes, BCS class IV drugs exhibit many problematic characteristics for effective oral and peroral drug delivery. Problems such as poor aqueous solubility and low intestinal permeability result in poor absorption and low bioavailability. Thus, the formulation and development of an efficacious system for the delivery of these class IV drugs is a tedious task for any formulator. To be effective clinically, drugs belonging to this class require the development of a proper system for both oral as well as peroral delivery. Thus, various techniques and strategies are applied for the delivery of BCS class IV drugs. Some of the techniques employed are lipid-based delivery systems by use of lipid carriers such as liposomes, niosomes, aquasomes, and ethosomes; polymer-based nanocarriers; nanocrystals and co-crystals; self-nano-emulsifying drug delivery system (SNEDDS) as well as self-micro-emulsifying drug delivery system (SMEDDS). Of these, the niosomal approach is widely used for the delivery of both hydrophilic as well as lipophilic drugs. By utilization of nonionic surfactants, cholesterol, and charge inducers in varying ratios.

KEYWORDS: Biopharmaceutics, Solubility, Permeability, BCS class IV drugs, Niosomes.

INTRODUCTION
Many factors such as physicochemical nature, molecular weight, size of the compounds, metabolic functions, physiological functions, surface and structure of the gut cells, etc. affect the absorption rate of the drug in the gastrointestinal tract (GIT) tract.1,2 Notwithstanding this complexity, Amidon et al. developed the Biopharmaceutics Classification System (BCS). Based on the Biopharmaceutics Classification System (BCS), drugs are classified into four groups depending on their solubility and permeability characters. Furthermore, when this classification system was deeply studied, it came into the light that formulation of the drug as well as their carrier system is equally responsible for determining the rate and extent of absorption in GIT, thereby increasing the bioavailability and therapeutic index of the drug. Many different approaches for improving drug delivery of these classes of drugs have been applied, also for enhancing solubility and permeability are constantly designed, especially for drugs belonging to classes II and IV.3,4 The approaches like the use of lipid carriers such as liposomes, niosomes, aquasomes, and ethosomes; polymer-based nanocarriers; nanocrystals and co-crystals; self-nano-emulsifying drug delivery systems (SNEDDS) and self-micro-emulsifying drug delivery systems (SMEDDS). Of these, the niosomal approach is widely used for the delivery of both hydrophilic as well as lipophilic drugs. Niosome is a non-ionic surfactant vesicle formed by hydration of nonionic surfactants, with or without the use of cholesterol/lipids. They are vesicular systems similar to liposomes and have been widely evaluated for controlled release and targeted delivery as well for the treatment of cancer, viral infections, and other microbial diseases. Niosomes can entrap both hydrophilic and lipophilic drugs and can also enhance the solubility of the drug. Encapsulation of drugs in vesicular systems like niosomes can be said to prolong the existence of the drug in the systemic circulation for a longer duration and thereby enhance the drug penetration into the target tissue, also reduce toxicity if selective uptake is achieved.5

BIOPHARMACEUTICS CLASSIFICATION SYSTEM (BCS)
The Biopharmaceutics Classification System (BCS) is a scientific framework designed for the classification of drugs as per their aqueous solubility and intestinal permeability. This classification system was put forward by Amidon et al.7 This particular concept published by BCS had finally led us to introduce the possibility of waiving in vivo bioequivalence (BE) studies in favor of comparative in vitro testing to conclude the BE of immediate-release (IR) oral products with systemic actions. The BCS has found international recognition in many fields such as industry, academic institutions, and public authorities.8 The principle of the BCS is that when two drug products yield the same concentration
profile along the gastrointestinal (GI) tract, they will result in the same plasma profile after oral administration.\(^9\) It is a drug-development tool that allows estimation of the contributions of three major factors, dissolution, solubility, and intestinal permeability that affect oral drug absorption from IR solid oral dosage forms.\(^8\) It was first introduced into the regulatory decision-making process in the guidance document on immediate-release solid oral dosage forms: Scale-up and post-approval changes.\(^10\) The drugs are divided into high/low-solubility and permeability classes. Currently, BCS guidelines are provided by USFDA,\(^11\) WHO,\(^12\) and EMEA.\(^13\)

According to BCS, drug substances or APIs are divided:\(^11\),\(^13\),\(^14\) as follows:

- **Class I**: High Solubility - High Permeability
- **Class II**: Low Solubility - High Permeability
- **Class III**: High Solubility - Low Permeability
- **Class IV**: Low Solubility - Low Permeability

In combination with the dissolution, the BCS also takes into account the three major factors governing BA, which are dissolution, solubility, and permeability. The BCS is per WHO guidelines as shown in Fig. 1. This classification is associated with the drug dissolution model and drug absorption model, which are identified as the key parameter which controls drug absorption as a set of dimensionless numbers.\(^14\)

**Absorption number**, \(A_n = \frac{\text{mean residence time}}{\text{mean absorption time}}\)

**Dissolution number**, \(D_n = \frac{\text{mean residence time}}{\text{mean dissolution time}}\)

**Fig.1**: Biopharmaceutics classification system.

**Table 1**: Examples of some drugs as per biopharmaceutical classification system.\(^3\),\(^17\)-\(^21\)

<table>
<thead>
<tr>
<th>CLASS I</th>
<th>CLASS II</th>
<th>CLASS III</th>
<th>CLASS IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroquine</td>
<td>Griseofulvin</td>
<td>Acyclovir</td>
<td>Indinavir</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>Danazol</td>
<td>Atenolol</td>
<td>Cyclosporin A</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>Glibenclamide</td>
<td>Captopril</td>
<td>Ellagic acid</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>Ketoconazole</td>
<td>Cimetidine</td>
<td>Furosemide</td>
</tr>
<tr>
<td>Propranolol</td>
<td>Nifedipine</td>
<td>Metformin</td>
<td>Ritonavir</td>
</tr>
<tr>
<td>Theophylline</td>
<td>Phenyltoin</td>
<td>Neomycin B</td>
<td>Saquinavir</td>
</tr>
<tr>
<td>Verapamil</td>
<td>Troglitazone</td>
<td>Ranitidine</td>
<td>Taxol</td>
</tr>
</tbody>
</table>

**SOLUBILITY DETERMINATION**

The solubility of any substance can be defined as the amount of substance that has passed through the solution when the equilibrium is reached between the solution and the transfer (a dissolved substance) at a given temperature and pressure.\(^14\) The substance of the drug or active ingredient of the drug (API) is considered to be most soluble when the maximum volume dissolves at 250 ml or below a liquid surface over a certain pH range.\(^11\)-\(^13\) A volume of 250 ml is taken from the normal volume of water used during oral administration of a volume form, which may be 1 glass or 8 ounces of water. This limit value is a representation (refill) of the small amount of water expected in the stomach during drug administration. The pH profile of the soluble substance is determined at 37 ± 1°C in a watery area with a pH range of 1.7-5.5 according to the guidelines of the United States Food and Drug Administration (USFDA),\(^11\) 1.2-6.8 according to the World Health Organization (WHO) guidelines\(^12\) and 1-8 as the European Medicines Academy\(^13\) (EMEA). A sufficient variety of pH conditions need to be tested as it should outline the pH-solubility profile. The number of pH conditions of a melting point depends on the ionization factors of the substance being tested. At least three repeated melting points in each pH condition must be made to predict
accurate melting. Common buffer solutions described in the pharmacopeia are considered suitable for use in melt studies. Methods other than the shake-flask method can also be used for justification to support the ability of such methods to predict the melting point of the substance being tested. If dehydration is seen as a function of buffer formation and/or pH, it should be considered. The saturation of the drug in selected baths or pH conditions should be determined using a melted solution that indicates the probability method of distinguishing between the components of the drug in its deteriorating products.

PERMEABILITY DETERMINATION

The most commonly used methods for determining permeability include the following:

a. Pharmacokinetic studies in human subjects that include large-scale studies and comprehensive studies of bioavailability (BA) or gastrointestinal tract.

b. In vivo or in-situ perfusion in a suitable animal model.

c. In vitro penetration methods using excised intestinal tissue.

d. Elite epithelial cell monolayers e.g., Caco-2 cells or TC-7 cells.

In studies of large balance, non-labeled, stable isotopes or radio-based drug substances are used to determine the level of drug absorption. In complete BA studies, the oral BA is determined and compared with the BA implanted as a reference. Intestinal models and in vitro methods are being developed in portable drugs. Another interesting feature of intestinal tissue models is the use of in vitro systems depending on the human adenocarcinoma cell line Caco-2. These cells serve as an example of the small intestine tissue. Separated cells exhibit normal microvilli of the mucosa of the small intestine and essential proteins of the brush-border enzyme membrane. They also form ducts filled with normal fluid-filled epithelium. Recent research into Caco-2 cell lines has shown their ability to transport ions, sugars, and peptides. These structures have established the Caco-2 cell line as a reliable in vitro model of the small intestine.

Grès MC, Julian B, Bourrié M, Meunier V, Roques C, Berger M, et al studied the correlation between oral drug absorption in humans, and apparent drug permeability in TC-7 cells, a human epithelial intestinal cell line also compared with the parental Caco-2 cell line.

BCS CLASS IV DRUGS

The drugs with poor aqueous solubility and with low membrane permeability belong to BCS class IV, for example, indinavir, amphotericin B (AmB), furosemide (FUR), acetazolamide, etc. Usually, techniques used for BCS class II drugs do little to improve the absorption of class IV drugs due to the limited membrane permeability. As a result, the best solution to improve the bioavailability of class IV drugs is to go back to the lead optimization phase of drug discovery and modify their structures to obtain the appropriate physicochemical properties. However, the discovery of a novel therapeutic agent is a challenging, time-consuming as well as costly process. It takes the US $800–1200 million and 10–15 years to develop a new chemical entity. Also, only a very few of so many compounds after being tested reach the market. Hence, sending a drug molecule back to the lead optimization phase is not a feasible option because of the constraints associated with time, cost, labor, and resources. As a result, the proper formulation is of key importance to establish a successful product for the administration of BCS class IV drugs. Fig. 2 gives an overview of the different examples of BCS IV drugs and the hurdles posed by them during the formulation of successful oral and peroral drug delivery. This article highlights the use of niosomes to deliver these drugs more effectively.

Niosomes are bilayer lipidic systems consisting of nonionic surfactants, cholesterol, and charged molecules profoundly. Nonionic surfactants are mainly advantageous due to their ability to increase the solubility of poorly water-soluble drugs and thereby providing enhanced bioavailability.

![Fig. 2. BCS Class IV drugs- the hurdles posed by them in their delivery via oral and peroral route.](image-url)
Al-Zahraa and Bazigha optimized mucoadhesive coated niosomes as a sustained oral delivery system of famotidine.\cite{81} Helon, Liangxin, Yongxi, Zefan, Lutin, Junfeng, Jianfang and Kail, improved bioavailability and antitumor effect of docetaxel by TPGS modified proniosomes: in vitro and in vivo evaluations.\cite{82} Amal, Sanaa, Mohamed, Gamal Maghraby studied colloidal carriers for extended absorption window of furosemide \cite{83} Deepika, Alka and Indu developed niosomal formulations of acetazolamide that showed a comparable physiological effect (33% reduction of IOP in REV1bio and 37% reduction in dorzolamide) with a duration of up to 6 h (the duration being 3 h for dorzolamide). Results of the study indicated that it is possible to develop a safe (as indicated by corneal toxicity studies) and physiologically active topical niosomal formulation of acetazolamide relative in efficiency to the newer local carbonic anhydrase inhibitor, dorzolamide. The developed formulations could form a cost effective treatment plan, which was especially important in the treatment of glaucoma, a chronic ailment affecting middle-aged to old patients.\cite{84} Ashraf, Javad, Fateme, Hossein, Mojtaba, Seyed Ahmad and Somayeh formulated paclitaxel and curcumin coadministration in novel cationic PEGylated niosomal formulations which exhibited enhanced synergistic antitumor efficacy.\cite{85}

Niosomes are one of the most promising nano-drug carriers with a bilayer structure formed by the combination of cholesterol and nonionic surfactants in the liquid phase. Niosomes are biodegradable, biocompatible, and nonimmunogenic. They are said to have a long shelf life, exhibit high structural stability, and facilitate the delivery of drugs to the target area in a continuous and/or controlled manner.\cite{24} In more recent times, the potential for niosomes as a drug carrier has been extensively tested.\cite{25,27} Different types of nonionic surfactants have been reported to form niosomes and provide binding to a large class of drugs with a wide range of solubility.\cite{28-30} The composition, number of lamellae, size, and extra charge of the niosome can vary and can be fully utilized to improve the functioning of the niosomes as a drug transport system.

### Advantages

Niosomes combine several advantages concerning other nanocarriers:\cite{31}

- The surfactants used to repair niosomes are perishable, biocompatible, biodegradable, and not immunogenic.
- The use of unacceptable solvents is eliminated for routine as well as large-scale production of niosomes.
- The handling and storage of niosomes do not require any special conditions due to the chemical stability of their structural composition.
- The physicochemical properties of niosomes, such as their fluidity, size, and shape, can be easily controlled by varying their composition and/or production method.
- Niosomes can encapsulate a large amount of material in a small vesicular volume.
- The structure of niosome vesicles protect drug components from heterogeneous factors which are valid both inside and outside the body, hence niosomes can be utilized for the delivery of sensitive and labile drugs.
- The therapeutic performance of drug molecules is also enhanced using niosomal drug delivery by delaying drug clearance rate from the circulation and restricting drug effects to the target site.
- Different routes, such as oral, parenteral, and topical, using different dosage forms such as powders, suspensions, and semisolids, are explored for niosomal drug delivery; improving the oral bioavailability of drugs with low solubility and also enhancing the permeability of drugs via the skin when applied topically.
- The aqueous vehicle-based suspension formulation provides better patient compliance in comparison with oily vehicle base; in addition, niosomal dispersion, being aqueous, can be emulsified in a nonaqueous phase for controlling the drug release rate.
- Better patient adherence and satisfaction and also better effectiveness than conventional oily formulations are achieved by niosomal drug delivery.

### Disadvantages

- Due to the nature of the dispersal of niosomes, there may be a possibility of disposal, aggregation, compaction, or leakage of the captive drug during storage.\cite{32}
- Some formulation techniques are time-consuming and require special equipment.
- During the water infusion process, there may be a possibility of incomplete hydration of the surfactants.\cite{33}
- Niosomes toxicity: Niosome toxins are related to their components, that is, nonionic surfactants that are more biocompatible and less toxic than their anionic, amphoteric, and cationic counterparts. These structures are greatly reduced, where similar surfactants are in the form of vesicular systems. There is little published research on the toxicity of niosomes and the types of surfactants included.\cite{34} Hofland et al\cite{34} examined the toxicity of surfactants used in the production of niosomal in human keratinocytes and showed that ester types of surfactants are less toxic than ether species due to the degradation of enzymatic bonds in esters.
Hemolytic tests are widely used to predict non-ionic surfactant toxicity and to vesicular systems taken from them.\(^{[35]}\) Recently, it has been revised that the ability of niosomes to disrupt erythrocytes depends on the length of the alkyl chain present in the surfactant and the size of the colloidal aggregates in solution. Most likely, a short carbon chain binds better to the erythrocyte membrane, destroying its cellular structure; niosomes have a high degree of complex communication with the biological membrane, leading to the formation of hemolysis.\(^{[36]}\) Niosomes formulated with ballots showed enhanced safety and tolerance data in both in vitro in human keratinocytes and in vivo in human volunteers, who did not develop skin erythema when treated with a drug-free bola form niosome formulation.\(^{[37]}\)

**COMPONENTS OF NIOSOME**

The two main components used in the preparation of niosome are present in lipid compounds (cholesterol or L-α-soya phosphatidylcholine) and nonionic surfactants. Lipid compounds are used to provide a stable environment, proper structure, shape, and adaptation to niosomes.\(^{[38]}\) The surfactants play a key role in the development of niosomes. Many nonionic compatible surfactants are used for the design of niosome, spans (spans 60, 40, 20, 85, and 80), tweens (tweens 20, 40, 60, and 80), and Brij (30, 35, 52, 58, 72, and 76).\(^{[39-41]}\) Vesicles or niosomes based on Nonionic surfactants are competent drug carriers that require a bilayer structure made primarily of a combination of nonionic surfactant and lipid (cholesterol or L-α-soya phosphatidylcholine) incorporated into the aqueous phase.

- **Nonionic Surfactant**

  Niosomes are multilamellar vesicles prepared from nonionic synthetic surfactants. The nonionic surfactant has a hydrophilic head and a hydrophobic tail that affects the efficiency of the drug. As the HLB value of the surfactant increases, therefore, the alkyl chain increases, hence, the size of the niosomes increases. Therefore, the HLB ratio 14–17 is not suitable for the formation of niosomes.\(^{[42,23]}\) In addition to the amount of surfactant, the structure of the surfactant has played a major role in stabilizing and synthesizing the vesicle of niosomes by repulsion of steric or electrostatic forces.\(^{[43]}\) The effect of the surfactant structure on the formation of niosomes explains the critical packing parameter (CPP) specifying the following equation:\(^{[44]}\)

\[
CPP = \frac{V}{Ic} \times Ao.
\]

CPP is an important packing parameter, V is the hydrophobic group volume, Ic length is the important hydrophobic group, and Ao is the hydrophilic group area. The type of micellar structure was predicted by the value of the critical packaging parameter as assumed:

- If CPP <1/2 formation of spherical micelles
- If 1/2 < CPP <1 formation of bilayer micelles
- If CPP >1 formation of inverted micelles

Several types of surfactants are used in the preparation of niosomes such as alkyl ethers and alkyl glyceryl ethers, sorbitan fatty acid esters, polyoxyethylene fatty acid ester, and block copolymer (Pluronic L64 and Pluronic p105). To achieve these properties, some input power, for example, mechanical (regenerative or sonicates) or heat is required.

- **Cholesterol**

  In the structures of niosomes, cholesterol is an amphiphilic compound that can interact with surfactants to form hydrogen bonding between the hydroxyl groups of cholesterol and the hydrophilic head of the surfactant. This results in the development of mechanical stiffness for the vesicles and the interaction of the membrane and the leakage of the membrane and ultimately increases the efficiency of the entry of niosomes. The amount of cholesterol in niosomes influences the properties of niosomes and materials and affects the efficiency, structure, and release of payload. According to a previous study, it was revealed that the use of cholesterol in the preparation of niosomes and their amounts needed to be adjusted depending on the physical and chemical properties of surfactants and the type of drug in the future. The interaction of cholesterol with surfactant in the bilayer of niosomes is due to hydrogen bonding (Fig. 4).\(^{[46]}\)
Cholesterol can also be derived from natural sources such as ergosterol derived from yeast. This can be also used as a substitute for cholesterol and study their effect on the final niosomes formed. Barani, Hadi, Zaboli, Mirzaei, Torkzadeh-Mahani, Pardakhty and Karam studied in silico and in vitro model of magnetic niosomes for gene delivery and the effect of ergosterol and cholesterol on niosomes.\(^{[66]}\)

- **Charge inducing molecule**

  Some charged molecules are added to niosomes to enhance the stability of the niosomes by electrostatic repulsion that avoids vesicle aggregation. The negatively charged molecules used in the processes of niosomes are dicetyl phosphate (DCP) and phosphatidic acid. Stearyl amine (STR) and stearyl pyridinium chloride are well-known positively charged molecules used in the formation of niosomes. 2.5–5 molar% coagulation of charged molecules is acceptable as a high concentration can prevent the formation of niosomes.\(^{[66]}\)

The concentration and type of charge inducers also affect the final stability of niosomal formulation. Varaporn et. al. Studied the effect of charged and non-ionic membrane additives on physicochemical properties and stability of niosomes.\(^{[83]}\)

**STRUCTURE OF NIOSOME**

The niosome structures are made up of a mixture of surfactant and cholesterol followed by hydration in water. The bilayer in niosomes is formulated as a nonionic surfactant with its hydrophilic ends exposed on the outside and inside of the vesicle, while the hydrophobic chains are exposed within the bilayer. As shown in Fig. 5, due to the high surface tension between the water and the hydrophobic tail, the monomer units combine to form vesicles, forming closed bilayer structures. To achieve these properties, a certain contribution power, for example, mechanical (regenerative or sonicates) or heat, is essential. Thus, the vesicle captures hydrophilic compounds within the space around the vesicle, and hydrophobic compounds are bound within the bilayer itself, while amphiphilic compounds interact with lipophilicity-based compounds in the space between the hydrophilic core and the lipophilic tail (Fig. 5).

![Fig. 5: Schematic representation of a niosomes as drug-delivery system.\(^{[47]}\)](image)

**PREPARATION METHODS OF NIOSOME**

A common method for preparing niosomes is by the hydration of nonionic surfactants using a hydration medium. However, they are prepared by several techniques, such as transmembrane pH gradient method, lipid layer hydration, reverse phase evaporation, EER injection, nitrogen depletion, sonicatation, enzymatic method, single-process pass, and micro fluidization which are described herein detail.

- **Transmembrane pH Gradient Method**

  The surfactant and cholesterol are ready with chloroform or any other organic solvent and evaporated under reduced pressure and N2 flow was exposed to produce a thin lipid film on the wall of the lower end. The available lipid film is coated with an acidic compound (usually citric acid). The resulting correction (multilamellar vesicles) is expressed in the freeze-thaw cycles.\(^{[48-50]}\) The pH of the sample is then increased to 7.2 (Fig. 6). Bhaskaran and Lakshmi\(^{[51]}\) reported that niosomes that could be formed by this process have high entrapment efficiency up to 87.5%.


**Lipid Layer Hydration**
As shown in Fig. 7, cholesterol and non-ionic surfactant are dissolved in organic solvent and then evaporated under reduced pressure to form a thin lipid film on the wall of the RBF. The obtained film is soaked in water with an aqueous solution and heated at a slightly higher temperature than the transition phase of surfactants under moderate shaking conditions. Several variables were confirmed that included the difficulty for each group, the evaporation angle, the rotational speed of the vacuum rotary evaporator, and the hydration process. The latter variables were developed by different solvents (water, phosphate buffer (PB), and PB / tree) as well as the hydration temperature below and above the temperature of the gel transition. Sathali and Rajalakshmi prepared terbinafine niosomes by this method and resolved this process, in which sonication resulted in the formation of small unilamellar niosomes (EE = 85%).

**Reversed-Phase Evaporation**
The surfactants are dissolved in a mixture of ether and chloroform or any other organic solvents and added to the aqueous phase where the drug is emulsified to obtain a w/o emulsion. The resulting mixture is homogenized, and then the organic phase is evaporated. Firstly, the lipid or surfactant forms a gel and then hydrates to form stable uniform spherical vesicles.

**Ether Injection method**
A mixture of a non-ionic surfactant, cholesterol, charged molecule, and the drug is dissolved in diethyl ether and over gauze, is then injected slowly into an aqueous phase. The ether solution is evaporated by a rotating evaporator above the boiling point of the organic solvent. Large unilamellar vesicles, after evaporation of the organic solvent and re-exposed to reduce the size and to provide single-layered vesicles.
Fig. 9: Schematic diagram of the preparation of niosomes via ether injection method.

- **Bubbling of Nitrogen**
  This method is a new process for the development of a single-step process for niosomes formulation without the use of any natural solvents. By using this method, buffer, cholesterol, and surfactant are distributed together (pH 7.4) at 70°C conditions. It is presumed by a round-bottomed flask with three necks. The first two necks are immersed in cool reflux with water to control the temperature. Due to a homogenized (cholesterol and surfactant) sample, nitrogen gas was transferred to the third neck. Thus, large unilamellar vesicles are produced. An undisturbed stream of nitrogen gas bubbles is formed and is introduced by dispersion to form small unilamellar vesicles (Fig. 10).[57]

![Diagram of ether injection method](image)

Fig. 10: Schematic small unilamellar vesicles (niosomes) formation by bubbling of nitrogen method.

- **Sonication**
  In a sonication-mediated process, niosomes were modified by Baillie et al.[58] The cholesterol compound of the surfactant is still distributed in the water phase containing the drug in flax. The mixture is subjected to probe sonication or a sonicator bath for 3 minutes at 60°C until multilamellar vesicles are formed (Fig. 11).[59]

![Diagram of sonication method](image)

Fig. 11: Schematic small unilamellar vesicles (niosomes) formation by sonication method.
• **Enzymatic Method**
In this process, niosomes are produced by the enzymatic route from a mixed micellar solution. Ester bond is broken down by esterases that cause the breakdown of products such as cholesterol and polyoxyethylene, which are mixed with dicetyl phosphate and other lipids that produce multilamellar niosomes. The different non-ionic surfactants which are used in this method are polyoxyethylene cholesterol sebacate diacetate[61] and polyoxyethylene stearyl derivatives.[60]

• **Single-Pass Method**
It is a patented method that involves a continuous process that leads to the removal of a solution or suspension of lipids that concludes a device with holes and later through a nozzle. It combines high-pressure extrusion with homogenization to provide niosomes with a small size distribution of a range of 50–500 nm.[62]

• **Micro fluidization**
Micro fluidization was the current strategy to provide unilamellar vesicles with limited circulation. Based on the underwater jet system, in this strategy, two water streams are connected at high speeds, with negative signals of small-scale channels inside the interaction chamber. The insertion of a thin sheet of liquid near the normal front was resolved in such a way that the energy brought into the system remained in place for the formation of niosomes. The result was a more pronounced consistency, a reduced size, and better reproducibility of the structure and shape of niosomes.

**CHARACTERIZATION OF NIOSOMES**

**Size and Viscule Charge**
The size and charge of the vesicles played a major role in their durability, encapsulation, steadiness, and coagulation. The size and charge can be determined by a powerful zeta analysis, in which the size of the vesicles was the result of a tossing force between the bilayers and the entrapped drug. The size of the vesicles can be enhanced by electron microscopy, molecular sieve chromatography, photon correlation, ultracentrifugation, and optical and freeze-fracture electron microscopy.[51]

**Encapsulation Efficiency**
Vesicles composed of suitable solvents such as 50% n-propanol or 0.1% triton X-100 and tested for appropriate analysis.[60] The percentage of encapsulation effectiveness (EE) is calculated according to the following equation:

\[
\text{Entrapment efficiency (\%)} = \frac{\text{Total entrapped drug} - \text{unentrapped drug}}{\text{Total entrapped drug}} \times 100
\]

**In Vitro Release Study**
In vitro release studies were performed on a regular release frequency that included the use of a dialysis tube. The vesicle suspension was incorporated into an open dialysis membrane and inserted into a receptor site consisting of a buffer solution with continuous movement at 25°C or 37°C. Trials are periodically collected and evaluated by authorized procedures.[48,60,63]

**Stability Study**
Major problems related to vesicle retention are deterioration due to light, aggregation, merging, and leakage of drugs. Ammar et al.[61] reported the stable formation of tenoxicam as this indicates the efficacy of high entrapment (> 60%) and retention (> 90%) over 30 days. After 30 days, the only stable formation was selected to last another 30 days. It was found that there was no significant change in vesicle size after 90 days when it was equal to those newly formed niosomes. However, the efficiency of the entrapment was reduced (10%) after the last.[65]

**APPLICATION OF NIOSOMAL CARRIER FOR CLASS IV DRUG DELIVERY**
An important requirement for transdermal delivery is that the drug carried by a vehicle should be able to reach the skin surface at an adequate rate and insufficient amounts. A wide range of applications and several mechanisms have been reported because of their ability to enhance the percutaneous drug delivery to the deeper layer of skin. In general, a permeant applied to the skin has three possible routes across the epidermis. The transcellular route, a lipid domain associated with the proteins inside cornocytes, the intercellular route, and the appendageal route, through hair follicles, via associated sebaceous glands, and sweat ducts. Based on the drug nature, the mechanism of drug transport may be varied.[66–68]

Mechanisms for transdermal enhancement of hydrophilic drugs include (i) increasing drug thermodynamic activity—the encapsulated drug vesicles get adsorbed and fused onto the surface of the skin. Then, a thermodynamic activity gradient is developed, which enhances the diffusion pressure for drug permeation at the surface that acts as a driving force for drug penetration across stratum corneum (sc).[69–71]; (ii) modification of surface electrical charge of ionic drugs; (iii) solubilizing of sebum by vesicles to facilitate follicular delivery; and (iv) pore pathway of large water-soluble molecules loaded in niosomes.

Mechanisms for transdermal enhancement of hydrophobic drugs include (i) disruption of the lipid bilayer of the stratum corneum (SC)—structural modification of stratum corneum, i.e., the densely packed lipid bilayer, helps to fill the extracellular spaces by a disruption to enhance the permeation rate; (ii) enhancement of transdermal permeation through nanosizing; (iii) changing drug partition into skin layers; (iv) hydrating skin and the dilation of the SC intercellular channels—niosomes cause an alteration in the barrier property of stratum corneum, which enhances sc hydration by reducing trans-epidermal water loss, leading to loosening the tightly packed structure and lyses of the membrane by lysozyme and releasing the entrapped drug into the system.[72–77]; and (v) changing
the permeation pathway of lipophilic permeants to follicular delivery.[78] The non-ionic surfactants play a crucial role as a penetration enhancer that enters into the intercellular lipids through endocytosis.[79,80] The possible mechanisms of action to enhance skin penetration are as shown in Fig. 12.

**Fig. 12:** Possible mechanisms of action of niosomes for penetration in topical and transdermal drug delivery.

In the above figure different mechanisms mentioned are:
A) Release of drug molecules by niosomes.
B) Niosomes adsorption and fusion with stratum corneum.
C) Penetration of niosomes through the intact sc.
D) Components of niosomes act as a penetration enhancer and increase absorption of the drug.
E) Penetration of niosomes through hair follicles or pilosebaceous units.[16]

**CONCLUSION**
Nonionic surfactant vesicles were introduced as an innovative and capable method for drug delivery. BCS forms a base upon which drugs are classified into respective classes according to their solubility in water and permeability through the GIT; thus, through BCS, the problems of drugs can be identified and potentially resolved. BCS employs various methods for determining solubility and permeability. Various drug delivery systems are available for BCS class IV drugs, of which niosomes are more economical and safer carriers than any other traditional drug delivery system available. This review forms an insightful reference base for the various administration and preparation methods along with evaluation parameters and applications of niosomes for the delivery of class IV drugs.

**CONFLICTS OF INTEREST**
The authors declare that they have no conflicts of interest.

**REFERENCES**

8. Helga M. The Biopharmaceutical Classification System (BCS) and its usage. Drugs Made in Germany, 2002; 45: 63-5.


