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Review Article

NIOSOMES AS AN APPROACH TO IMPROVE THE SOLUBILITY AND BIOAVAILABILITY OF BCS CLASS II DRUGS

GAURANG SAWANT[1*], GEETA BHAGWAT[1]

[1]Department of Pharmaceutics, H. K. College of Pharmacy, Oshiwara, Mumbai, India
*Email: sawant.gaurang10@gmail.com

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ABSTRACT

Based on their solubility and permeability, drugs are typically divided into four classes (Classes I–IV) according to the biopharmaceutics classification system (BCS). Of these classes, BCS class II drugs have high permeability and low solubility; not only do these characteristics constitute the rate-limiting step in the formulation of these drugs but the low solubility in water results in low bioavailability. Thus, methods for improving their solubility have been developed using lipid carriers such as liposomes, niosomes, and aquasomes; other approaches include self-micro-emulsifying drug delivery systems (SMEDDS) and self-nano-emulsifying drug delivery systems (SNEDDS). Currently, niosome-based drug delivery systems that utilize nonionic surfactants, drugs, and cholesterol in varying ratios are being widely used to deliver both hydrophilic and lipophilic drugs in addition to several other applications of niosomes.

Keywords: Biopharmaceutics, Solubility, Permeability, Lipid carriers, Liposomes, Niosomes

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INTRODUCTION

The biopharmaceutics classification system (BCS) is a useful mechanism used by researchers for obtaining biowaivers during *in vivo* bioequivalence studies and for decision making when determining the required solubility and permeability during drug discovery. This is because BCS is established upon a scientific blueprint highlighting the three rate-limiting steps critical in oral absorption: the liberation of the drug from its dosage form [1], prolongation of the dissolved state along with the whole gastrointestinal (GI) tract [2], and penetration of the drug molecules via the GI membrane into the blood [3]. Additionally, enterohepatic metabolism constitutes a fourth step that affects the systemic accessibility along with the release of metabolites into systemic blood circulation. The biopharmaceutical drug disposition classification system (BDDCS) proposed by Wu and Benet comprehensively describes the absorption operation by including the fourth rate-limiting step of first-pass metabolism. Niosomes are uncharged surfactant vesicles comprising microscopic lamellar structures built upon an amalgamation of uncharged surfactants such as the alkyl or dialkyl polyglycerol ether class and cholesterol formed via subsequent hydration in an aqueous buffer [2]. In niosomes, the vesicle-building amphiphiles are uncharged surfactants (e. g., Span 60) that are typically balanced by incorporating cholesterol and minuscule amounts [1.5 mg] of a negatively charged surfactant such as dicetyl phosphate [4]. Several methods exist for niosome fabrication such as ether injection [5, 6], the hand-shaking method [6], sonication [6], microfluidization [7], reverse phase evaporation [8], the bubble method [9], multiple membrane extrusion [7], and the proniosomal approach [10]. Characterization parameters include particle size, *in vitro* drug release, entrapment efficiency, and drug content together with some specific characteristics that depend on the formulation mechanism such as skin permeation etc. Herein, a literature survey was performed using accessible databases such as Google Scholar, PubMed, and Scopus to review research articles and thus compile a comprehensive yet concise introduction to the BCS and niosomes along with their applications.

Biopharmaceutics classification system

BCS is a scientific framework for classifying a drug based on its aqueous solubility and intestinal penetrability [11]. When used in conjunction with the *in vitro* dissolution properties of the concerned drug, BCS considers three important parameters: solubility, intestinal permeability, and dissolution rate. Together these parameters determine the essential factors of the speed and limit of oral drug absorption from immediate-release (IR) solid oral dosage forms [12, 13]. Based on the BCS framework, the drugs can be classified into four basic groups using the criteria of their solubility and permeability toward gastrointestinal tract (GIT) mucosa, as shown in fig. 1. The solubility categorization of a drug in the BCS is determined on the basis of the maximum dosage strength of the IR product. A drug is deemed highly soluble when its maximum dosage strength is soluble in a minimum of 250 ml of water-based media spanning a pH range of 1.0–7.5; otherwise, the drug is deemed a poorly soluble candidate. The volume approximation of 250 ml was established in the literature using traditional bioequivalence study methods [12, 13]. The permeability classification is directly based on a drug's intestinal absorption limit in humans or indirectly based on the calculations of mass transfer speed via the human intestinal membrane. A drug is deemed highly permeable when the intestinal absorption limit is ≥90%. Otherwise, the drug is deemed poorly permeable [12, 13]. An IR drug is categorized as a fast dissolution product when at least 85% of the stated amount of the drug dissolves in less than 30 min when utilizing the United States Pharmacopoeia (USP) Apparatus I set at 100 rotations per minute (rpm) or USP Apparatus II at 50 rpm comprising a minimum volume of 900 ml of each of the following media: 1) acidic media, such as 0.1 N hydrochloric acid or USP simulated gastric fluid with an absence of enzymes; 2) a pH 4.5 buffer, and 3) a pH 6.8 buffer or USP simulated intestinal fluid in the absence of enzymes. Otherwise, the drug is deemed a slow dissolution product.

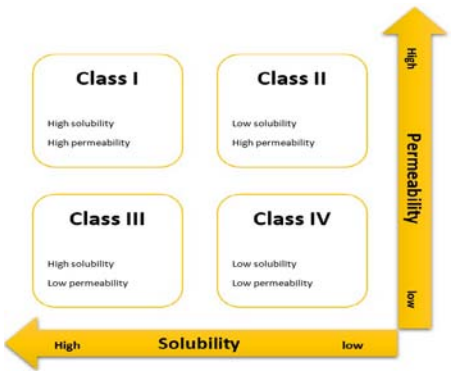

Fig. 1: Biopharmaceutics classification system†‡‡Adapted from commons. wikimedia.org, by MKD 2020 https://commons.wikimedia.org/wiki/File:Biopharmaceutics_Classification_System_(BCS).jpg. Copyright by, MKD 2020, ‡Copyright permission obtained under license CC BY 4.0
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Solubility and permeability measurement in the discovery/ development settings

Drug discovery begins with the recognition of a pharmacophore by scanning a library of recombinant chemical series through a biological method such as attachment to a receptor or enzyme blocking. The prototypical hit compounds are then directed toward high throughput screening (HTS) pharmaceutical profiling. Compounds with the required biological and pharmaceutical resources are subsequently examined *in vivo* for confirming the mechanism of action followed by lead optimization via chemical synthesis [14].

Solubility determination

HTS solubility examination typically begins with a dimethyl sulfoxide (DMSO) solution. This stock solution is incorporated into a pH 7 phosphate buffer with a volume of one microliter at all times until the compound precipitates out of the solution [15]. The light scattering phenomenon observed from the precipitated material is detected using an ultraviolet (UV) detector or directly via laser nephelometry [16]. Another method implements equilibration, followed by filtration of the DMSO-buffer suspension. Then, the filtrate is analyzed through a direct UV method. These automatic solubility procedures are currently being commercially used for HTS [17]. Precipitation of the compound from the DMSO stock solution is based on the rate and extent of nucleation occurring within the solution and hence demonstrates kinetic solubility. However, equilibrium solubility is mandatory by the Food and Drug Authority FDA for BCS classification. The shake flask method is a non-automatic method used to compute equilibrium solubility at various pH levels. The pH solubility profiling is usually performed only after the lead candidate has been selected. The kinetic solubilities are normally parallel but slightly higher than the equilibrium solubility.

Permeability determination

HTS permeability analysis has been used worldwide with 24 automated wells of the tissue-cultured human colon adenocarcinoma (Caco-2) cell system [18]. This system comprises a monolayer of cells that are allowed to develop onto a filter acting as support, which separates the drug donor and acceptor compartments. Permeability is examined by analyzing drug arrival in the acceptor compartment using the direct UV or liquid chromatography–mass spectrometry method. Although the Caco-2 cell system is an automated and well-established HTS method, it suffers from certain limitations. For compounds delivered through the passive transcellular course, the Caco-2 permeability is an appropriate procedure for forecasting human permeability. For compounds delivered via paracellular or transporter-governed processes or for highly insoluble compounds, the Caco-2 permeability tends toward miscalculation of human permeability because of three reasons: (1) excessive expression of p-glycoprotein (Pgp) efflux pumps, (2) lessening of the paracellular transport route owing to the absence of liquid pores, and (3) random attachment of insoluble compounds onto the filter support and plastic components thereby decreasing visible penetrability [19]. As a compound advances from discovery to the development stage, more tedious pressing procedures, e. g., the *in situ* rat gut perfusion method, are employed to rectify the false-negative conclusions produced by the Caco-2 method [20]. The compounds utilized in these tedious procedures are again characterized by low (BCS classes III and IV) to high permeability (BCS classes I and II) when entering the development stage. Moreover, the Caco-2 cells require 21 d to fully develop into a monolayer of integrity, whereas other cells such as the Madin–Darby canine kidney (MDCK) cell line require only 3–7 d; hence, the latter have been used more frequently to accelerate the analysis [21]. However, MDCK cells exhibit the same problems as the Caco-2 cells in terms of generating false-negative results and thus could represent low permeability. In addition, the MDCK cells present more issues than Caco-2 cells including less expression of various efflux pumps and failure to meet the criteria for screening of chemical series with identified efflux problems. Both the *in vitro* Caco-2/MDCK cell lines method and the *in situ* rat perfusion methods are trusted by the FDA in terms of BCS classification.

Niosomes

Niosomes are microscopic layered structures of 10–1000-nm size, and their core is environmentally friendly and non-reactive toward the human immune system and biocompatible surfactants [22]. The niosomes are amphipathic, i.e., a water-soluble drug can be locked in their core cavity region and water-insoluble drugs in the non-polar region are present inside their bilayer; hence, both water-soluble and water-insoluble drugs can be added into niosomes as shown in fig. 2. Structurally, niosomes are similar to liposomes: they possess the same drug delivery potential and offer more chemical stability than liposomes at lower production costs. Both vesicles comprise a bilayer, which is composed of uncharged surfactants in the case of niosomes and of phospholipids in the case of liposomes.

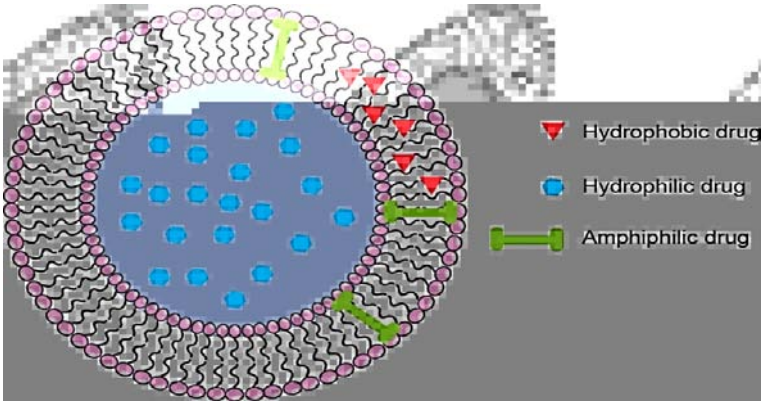

Fig. 2: Structure of niosome†‡,†Adapted from Reference [23], ‡Copyright License obtained under *CC BY license* (https://creativecommons.org/licenses/by/4.0/)

Preparation methods

Ether injection method

The primitive step in niosome formulation via the ether injection method involves surfactant dissolution in any volatile solvent such as diethyl ether, chloroform, or methanol. The solution is then incorporated into an aqueous drug solution via injection using a 14 Gauze needle maintained at 60 °C on a water bath or on a magnetic stirrer. Consequently, monolayered vesicles with sizes ranging from 50 to 1000 nm are produced through the volatile solvent's atomization [24].

Hand-shaking method

The hand-shaking method, also known as the thin-film hydration technique, involves the dissolution of the surfactant and cholesterol in a volatile organic solvent and subsequent transfer into a rotary

evaporator. Following evaporation, a thin layer of solid remains on the wall of the flask. This dried layer is then rehydrated using an aqueous phase of the drug of interest. Alternatively, this procedure can be performed at room temperature via light agitation [6, 25].

Sonication

Niosomes can be fabricated by sonicating an amalgamation of surfactant, cholesterol, and an aqueous phase containing the drug maintained at 60 °C for 3 min in a beaker placed in a probe or bath sonicator. The vesicles thus formed have less particle size exhibit size uniformity [6, 25].

Microfluidization

Microfluidization is another duplicable method that yields size uniformity via operating, i.e., two fluidized streams flowing forward and intersect with each other at ultrahigh speeds through an accurately defined microchannel [24, 25, 7].

Reverse-phase evaporation method

The reverse-phase evaporation method utilizes an amalgamation of surfactant and cholesterol in a 1:1 ratio in addition to ether and chloroform. An aqueous phase containing the target drug is incorporated into the concoction followed by sonication at a temperature of 4 °C–5 °C. Sonication is continued for about 5 min after incorporating about 10 ml of phosphate-buffered saline into the concoction. The organic solvent is atomized at 40 °C under low pressure, and the persisting suspension is thinned using phosphate-buffered saline. The amalgamation is heated at 60 °C for 10 min, and the ultimate product of niosomes is attained [24, 25, 8]. Fig. 3 shows a schematic of this method.

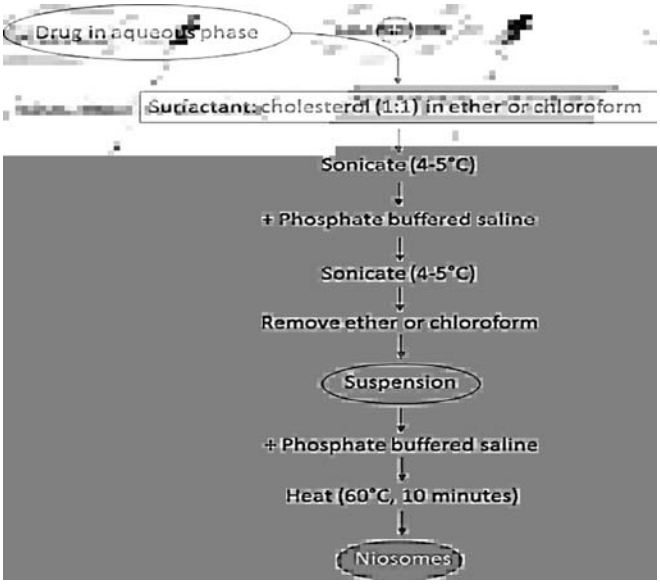

Fig. 3: Reverse-phase evaporation method for the preparation of niosomes†‡, †Adapted from Reference [26], ‡Copyright permission obtained under CC BY License (https://creativecommons.org/licenses/by-nc-nd/4.0/)

The bubble method

Niosomes can also be fabricated in the absence of organic solvents through the bubble method, wherein a bubbling unit containing a round-bottomed flask with three necks is placed in a water bath; a water-cooled reflux condenser and thermometer are placed in the first and second necks, respectively, whereas nitrogen gas is introduced through the third neck. Surfactant and cholesterol amalgamated at 70 °C in a buffer are blended and bubbled at 70 °C by introducing nitrogen gas into the apparatus [24, 9]. Fig. 4 shows a schematic of this method.

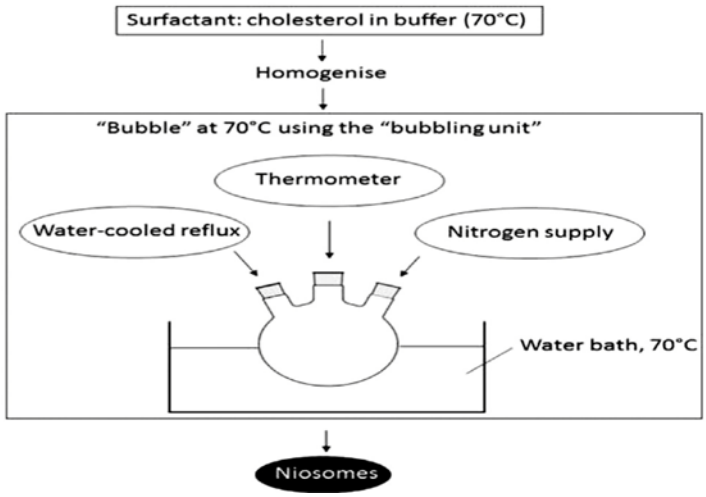

Fig. 4: The Bubble Method for Preparation of Niosomes†‡, †Adapted from Reference [26], ‡Copyright permission obtained under CC BY License (https://creativecommons.org/licenses/by-nc-nd/4.0/)

Multiple membrane extrusion method

In the multiple membrane extrusion method an amalgamation of surfactant, cholesterol, and dicetyl phosphate is dissolved in chloroform, and the resulting concoction is vaporized to form a thin film. This film is dampened with an aqueous drug solution, and the resulting suspension is extruded using polycarbonate membranes, which are inserted in series to create a maximum of 8 passages [24, 25, 27].

Proniosomal method

In this niosome fabrication method, a water-soluble transporter such as sorbitol is sprayed with a surfactant to form a dry formulation in which each water-soluble particle is laminated with a thin layer of dry surfactant. This formulation is labeled as a proniosome. The proniosome powder thus formed is subsequently loaded into a screw-capped vial, and blended with water or saline at 80 °C by vortexing. This is followed by stirring for about 2 min, thus producing the final niosomal suspension [28].

Niosome evaluation

Entrapment efficiency

For assessing a drug's entrapment efficiency within the niosomes, any of the following three methods can be used: centrifugation, gel filtration, or complete vesicle disruption using 50% n-propanol or 0.1% Triton X-100. This method estimates the quantity of the drug remaining locked inside the niosomes and examines the final solution through an assay method suitable for the target drug. In this method, entrapment efficiency (EF) can be defined as follows [29].

$$\text{Percentage drug entrapment (\%)} = \frac{[\text{Total Drug Content (mg)} - [\text{Free Drug Content (mg)}]}{\text{Total Drug Content (mg)}} \times 100$$

Drug content

The fabricated niosomal formulations are transferred into a test tube in which 10 ml of methanol is introduced to break down the niosomes followed by the destruction of the outer membrane, thereby liberating the entrapped drug. The quantity of the released drug can be estimated using a UV spectrophotometer at a specified wavelength, thus enabling the calculation of the total mass of drug present in the formulation [30, 31].

***In vitro* drug release**

The *in vitro* release rate can be determined using a dialysis tube. In this method, a dialysis sac is cleansed and immersed in distilled water. The niosome suspension is transferred using a pipette inside a bag composed of the dialysis tubing and fastened; then the bag is left in a 200 ml buffer solution placed in a 250 ml beaker and subjected to continuous vibration at 25 °C or 37 °C. The drug content in the resulting buffer solution is assessed at various time points via a suitable assay method [32].

Particle size and morphology

The most commonly used methods for determining particle size and the morphology of niosomes are dynamic light scattering (DLS) [33], scanning electron microscopy (SEM) [34], transmission electron microscopy (TEM) [35], freeze-fracture replication-electron microscopy (FF-TEM) [36], and cryotransmission electron microscopy (cryo-TEM) [37]. DLS simultaneously provides detailed information on particle size and critical information on the solution's homogeneity. A single sharp peak in the DLS result indicates the presence of a single population of scatters. The polydispersity index (PI) is a useful parameter in this regard. If PI<0.3, then homogeneous population exists in the colloidal system and if PI>0.3 then the system is not homogeneous [33]. The abovementioned microscopic methods are commonly used to evaluate niosome morphology.

Niosome applications

Sustained release

A constant concentration of drugs possessing a narrow therapeutic index and low water solubility can be maintained in the blood plasma via niosomal encapsulation, thus achieving sustained release action. Azmin *et al.* [38] Proposed using part of the liver as a storage site for the absorption of methotrexate after niosome administration by the liver cells, thereby realizing sustained drug release.

Localized drug action

Niosomal dosage is an attractive method to realize localized drug action at the administration site because of the size of niosomes and their lesser penetrability compared with liposomes epithelium and connective tissue. This improves the drug's effectiveness and reduces its systemic toxic effects. For example, antimonials entrapped within niosomes are absorbed by mononuclear cells resulting in localized drug action, potency enhancement, and dual reduction in dosage as well as toxicity [9, 39].

Cancer

Common limitations of cancer chemotherapy manifest as side effects and less therapeutic efficacy. Doxorubicin, a broad-spectrum anthracycline used for anticancer activity, has demonstrated a dose-dependent irreversible cardiotoxic effect [4]. However, when this drug was administered in the form of niosomes to mice possessing an S-180 tumor, the lives of the mice were prolonged and the multiplication of sarcoma was reduced. This can be attributed to the high effectiveness of niosomes in drug entrapment, resulting in sustained blood circulation and changes in drug metabolism [4, 40]. Another popular anticancer drug, daunorubicin hydrochloride, demonstrated increased anticancer activity in its niosomal entrapped form when compared against the effectiveness of the drug alone. The niosomal formulation constructively shattered Dalton's ascitic lymphoma cells in a short duration. In the case of bleomycin, a potent anticancer drug, compared with its free drug form, the form of the drug entrapped within niosomes comprising 47.5% cholesterol accumulated in high levels at the tumor position [41]. Methotrexate is a well-proven toxic synthetic antineoplastic drug used in chemotherapy, either alone or in conjunction with other medications, to cure different types of cancers. Extensive research has demonstrated that intravenous administration of methotrexate entrapped in niosomes to S-180 tumor-possessing mice causes total retrogradation of the tumor, relaxed drug clearance, and an elevated plasma level of methotrexate [42]. Improved drug penetration was observed when 5-fluorouracil was developed in bola-surfactant niosomes to cure skin cancer [43]. In addition to the fact that boosted antitumor activity is observed, in some situations, drug entrapment in niosomal vesicles reduces the cytotoxicity toward normal cells, as reported in a study on the design of niosomes containing vincristine. Frequent side effects of the drug, such as neurological toxicity, diarrhea, and alopecia, were reduced, whereas antitumor activity was increased in a mouse model of S-180 sarcoma after niosome-entrapped vincristine was administered [44]. Tocotrienol was the foremost drug to be reported for its anticancer activity in the initial years of the 1990s, and was subsequently entrapped in niosomes by Fu *et al.* [45]. Applying the film hydration method doubled (at minimum) the cytotoxic effect of niosomal tocotrienol in shattering breast cancer cells with the cells' drug absorption improving 2.5-fold. The antitumor activity of the preparation was also observed in female BALB/c nude mice carrying breast cancer cells [46]. Curcumin is well known to demonstrate many therapeutic uses, including anticancer properties [47]. A new niosome system comprising Span 80, Tween 80, and poloxamer 188 was demonstrated to have excellent encapsulating efficiency (92.3%) with respect to curcumin. When niosomal curcumin was incorporated into ovarian cancer A2780 cells, it increased cytotoxic and apoptotic activities as compared with freely used curcumin. This can be attributed to the properly controlled liberation of curcumin from niosomes [48]. Sharma *et al.* [49] Fabricated niosomes using Tween 80 and cholesterol through a film hydration method. Two anticancer drugs, curcumin and doxorubicin hydrochloride, were entrapped within the formulated niosomes; while curcumin was found to be accumulated in the shell, doxorubicin hydrochloride was found to be accumulated in the inner aqueous core of the niosomes. Higher cytotoxicity toward cervical cancer cells (HeLa cells) was observed for the double-drug-encapsulated niosomes. Artemisinin, which is extracted from the Chinese herb *Artemisia annua*, is frequently used in the treatment of fevers and chills [50]. The herb

also has antitumor properties [51]. However, the use of artemisinin is limited because of its low solubility in water and oil coupled with poor bioavailability. Moreover, it possesses a short half-life in humans [52]. To enhance the effectiveness of artemisinin, Dwivedi *et al.* [53] entrapped the compound within nanovesicular niosomes. They demonstrated that the entrapped artemisinin showed cytotoxicity toward melanoma cells with little to no toxicity toward normal skin cells, hinting at artemisinin's potential therapeutic use in melanoma treatment. Tamoxifen citrate is recognized as a hormone antagonist administered to breast cancer patients having estrogen receptor-positive tumors [54].

However, conditions such as localization, effectiveness, providing sustained drug release, and reducing side effects of drugs are significant issues encountered in this type of cancer treatment. Nevertheless, Shaker *et al.* [55] encapsulated the drug into niosomes and assessed its cellular uptake in addition to its cytotoxicity and *in vivo* effectiveness. Niosomal tamoxifen demonstrated increased cellular uptake coupled with higher cytotoxicity against the MCF-7 breast cancer cell line and showed increased regression in *in vivo* tumor volume. Mitoxantrone has been utilized in chemotherapy for various cancers such as leukemia, lymphoma, breast and prostate cancers, and multiple sclerosis. However, the administration of this drug is commonly followed by serious systemic toxicity, mainly cardiotoxicity. Tila *et al.* [56] prepared pH-sensitive, polymer-modified, and plasma-stable niosomes to deliver this drug. The cytotoxicity of mitoxantrone niosomes was examined against human ovarian cancer (OVCAR-3), human breast cancer (MCF-7), as well as human umbilical vein endothelial cell lines. Mitoxantrone entrapped in the pH-sensitive niosomes demonstrated increased cytotoxicity when compared against traditional niosomes on the cancer cells, but showed lesser cytotoxic activity when used on the endothelial cell line. These results demonstrate niosomal formulations to be promising carriers in minimizing the side effects of mitoxantrone. Cisplatin, a commonly used anticancer drug, acts by starting apoptosis and necrosis of the cells.

However, the use of cisplatin is accompanied by various harmful side effects, mainly nephrotoxicity and neurotoxicity [57]. Furthermore, the emergence of drug resistance toward cisplatin in patients is a significant issue in the cancer therapy of patients. Niosomal cisplatin manufactured via reverse-phase evaporation exhibited a 1.5-fold boost in cytotoxic activity against BT-20 breast cancer cells when compared against the unencapsulated drug [58]. Antineoplastic effects of the niosomal formulations mentioned above are summarized in table 1.

Table 1: Antineoplastic effects exhibited by various niosomal formulations

Drug	Action shown	References
Doxorubicin	Reduced proliferation of sarcoma cells	[4, 40]
Daunorubicin Hydrochloride	Shattered Dalton's ascetic lymphoma cells	[41]
Bleomycin	Collected in high levels at the tumor site	[41]
Methotrexate	Improved antitumor activity against sarcoma	[42]
5-Flourouracil	Improved drug penetration in skin cancer treatment	[43]
Vincristine	Improved antitumor activity against sarcoma	[44]
Tocotrienol	Improved cytotoxicity toward breast cancer cells	[46]
Curcumin	Improved cytotoxic and apoptotic effects toward ovarian cancer cells	[48]
Curcumin and Doxorubicin Hydrochloride	Improved cytotoxicity toward cervical cancer cells	[49]
Arteminisin	Cytotoxicity toward melanoma cells	[53]
Tamoxifen citrate	High cytotoxicity against breast cancer cell line	[55]
Mitoxantrone	High cytotoxicity against human ovarian cancer and breast cancer cell lines	[56]
Cisplatin	Improved cytotoxicity toward breast cancer cells	[58]

Ophthalmic drug delivery

Bioadhesive-coated niosomal formulation of acetazolamide fabricated from Span 60, cholesterol stearylamine, or dicetyl phosphate shows more affinity toward decreasing the intraocular pressure when compared against marketed formulation (Dorzolamide); in contrast, the chitosan smeared niosomal formulation timolol maleate (0.25%) demonstrates more activity in decreasing the intraocular pressure than marketed formulation with the reduced possibility of cardiovascular side effects [59].

Delivery of peptide drugs

Yoshida *et al.* [60] probed into the oral delivery of 9-desglycinamide, 8-arginine vasopressin as a model drug using niosome and by employing an *in vitro* intestinal loop model and enhanced the stability of peptide.

Hemoglobin carriers

Niosomes can be employed as hemoglobin carriers. The formulated niosomal suspension demonstrated a visible spectrum that can be superimposed over free hemoglobin. Vesicles are open for oxygen to travel inside the molecule and the hemoglobin dissociation curve can be altered similar to that of non-entrapped hemoglobin [61, 62].

Antiviral drug delivery

Furthermore, niosomes can deliver various antiviral drugs. Ruckmani and Sankar [63] prepared zidovudine encapsulated niosomes and assessed their entrapment efficiency and sustainability of drug release. The niosomes comprised Tween 80, Span 60, and cholesterol in various proportions. Niosomes comprising Tween 80 exhibited greater entrapment of zidovudine while the addition of dicetyl phosphate improved drug release for a longer duration. The drug outflow from Tween 80 containing formulations maintained at room temperature was substantial when compared to niosomes stored at 4 °C for 90 d. Regardless, a pharmacokinetic study conducted on rabbits also verified that Tween 80 formulations prepared with dicetyl phosphate can be removed from systemic blood circulation in less than five h [64].

Transdermal drug delivery

Transdermal drug delivery is the delivery of drugs via the skin. The advantage of this delivery route is that transdermally administered drugs fail to undergo the first-pass metabolism; however, the penetration of drugs occurs slowly through the skin and this limitation can be resolved using niosome preparations. The mechanism obeyed by the niosomes for transdermal drug delivery is as follows.

- Diffusion via the stratum corneum layer.
- Water concentration in the skin is critical to this mechanism.
- The lipophilic drugs transverse the stratum corneum through various mechanisms such as aggregation, fusion, and adhesion.
- Nonionic surfactants improve the permeation thereby improving drug permeation via the skin [65, 66].

Niosomes as drug carriers

Niosomes have also been employed as carriers for iobitridol, a diagnostic agent used in X-ray imaging. Topical niosomes can perform several duties such as solubilization of the lipid matrix and act as local storage to provide sustained release of dermally active compounds, penetration boosters, or modifiers of the rate-limiting

membrane barrier for the alteration of systemic drug absorption [67].

Niosomes in vaccine delivery

A] Protein subunit vaccines

The development of novel, safe, and efficacious vaccines, is a crucial aim for scientists worldwide. Subunit proteins or the deoxyribonucleic acid (DNA) of multiple organisms are less harmful than live organism-based vaccines, which may be relatively less clinically efficient. Adjuvants are being used in vaccines to improve the immunogenicity of the subunit vaccines via protection (i.e., preventing the destruction of the antigen *in vivo*) and upgraded targeting of these antigens to the desired antigen-presenting cells [68]. Brewer and Alexander [69] announced the first implementation of niosome antigen distribution for the immunization of Balb/c mice to develop resistance toward bovine serum albumin (BSA). They concluded that niosomes were possibly superior stimulators of the Th1 lymphocyte subset when compared against Freund's complete adjuvant, thus demonstrating very powerful stimulators of cellular immunity. Hassan *et al.* [70] announced enhanced immunogenicity with the herpes simplex virus 1 antigen encapsulated within l-mono palmitoyl glycerol (MP)/-CHOL/DCP niosomes in mice. In contrast, only partial protection was observed against homologous (type 2 herpes simplex virus HSV-2) infection induced in the mice by the HSV2 antigen encapsulated niosomes [71], demonstrating the significance of composition in addition to the preparation methods for niosomal adjuvant formulations. Yoshioka *et al.* [72] prepared Span/CHOL/DCP niosomes comprising tetanus toxoid (TT) emulsified in an external oil phase to prepare a vesicle-in-water-in-oil (v/w/o) formulation. Initial studies on the system adopting cottonseed oil as the external oil phase *in vivo*, demonstrated improved immunological activity when compared to free antigen or vesicles. Murdan [73] entrapped BSA or hemagglutinin (HA) in the v/w/o emulsion and demonstrated via immunogenicity studies that in addition to the water-in-oil (w/o) gel that served as a control, the v/w/o gel exhibited immunoadjuvant abilities that intensified the primary and secondary antibody titers (of total IgG, IgG1, IgG2a, and IgG2b) to the HA antigen. Chambers *et al.* [74] announced an exclusive subcutaneous dose of killed Mycobacterium Bovis BCG in Brij® 52-containing nanoniosomes (NovasomeTM), which shielded guinea pigs from deadly tuberculosis. Vangala *et al.* [75] amalgamated three dissimilar protein antigens entrapped within positively charged niosomes synthesized from MP/CHOL/α, α'-trehalose 6, 6'-dibehenate (TDB) or MP/CHOL/TDB/ dimethyldioctadecylammonium (DDA). Antigen entrapment within the niosomes enhanced the size of vesicles from sub microns to micrometers (1–2.7 μm), which can be attributed to the high molecular weight of antigens and their stronger hydrophobic nature that causes protein aggregation in the hydrophobic zones of the vesicle bilayers while feasibly establishing a level of vesicle fusion or manipulating the packing positioning of the surfactants. Their conclusions recommend that both DDA-and MP-based vesicular systems can boost the immunogenicity of the subunit vaccines, primarily with the subunit antigen Ag85B-ESAT-6 immunized against tuberculosis, wherein the requirement of a high cell-mediated Th1 immune response is critical. Vangala *et al.* [76] also announced DDA formulations containing TDB, which demonstrated markedly improved hepatitis B surface antigen-specific splenocyte multiplication and induced cytokine synthesis in conjunction with a strong T-cell-based response, thus outlining formulations that can be useful for the supplementary assessment of their clinical value. Ferro and Stimson [77] adopted gonadotrophin-releasing hormone (GnRH) analog, GnRH-glycs, and joined two dissimilar carrier molecules and entrapped them within niosomes vesicular (NSV)-based formulations to attain immune-neutralization of GnRH in male Sprague-Dawley rats. They concluded that NSVs can be adopted as a safe immune adjuvant. Further, an altered GnRH peptide (CHWSYGLRPG-NH2) was linked to tetanus toxoid (TT), which was designed with unlike adjuvants such as C18EO2/CHOL-/DCP niosomes [78]. When used with nanoniosomes, the sterilization effect demonstrated in the synthesis of IgG2b antibodies is not as promising as that attained with sustained-release poly-(lactideco-glycolide)/triacetin (PLGA) formulation. An encouraging immunization outcome was announced by Lezama-Davila [79] in C57BL/10 mice immunized with L. m. mexicana leishmanolysin (gp63). For the formulation of non-parenteral niosomal vaccines, Rentel *et al.* synthesized sucrose ester niosomes for the entrapment of ovalbumin and orally administered the vesicular formulations to Balb/c mice. Considerable enhancement in antibody titers was observed after oral vaccination with reduced hydrophilic vesicular formulation [80]. Chattaraj and Das [81] encapsulated hemagglutinin antigens from three divergent influenza A strains in Span 40 or 60 niosomes for nasal mucosal delivery. BSA-loaded niosomes fabricated from Span 60/Span 85/CHOL/stearylamine were smeared with an altered polysaccharide O-palmitoyl mannan (OPM) for making them selective toward Langerhans' cells, the critical antigen-presenting cells existing in substantial amounts below the stratum corneum. Analyzing serum IgG titer and its subclasses (IgG2a/IgG1 ratio) induced a significantly greater serum IgG titer upon topical application of mannosylated niosomes as compared to topically applied alum adsorbed BSA ($p<0.05$). The mannosylated niosomes were also administered orally for showing oral mucosal immunization against TT. OPM coating was performed to shield the antigen-entrapped vesicles from bile salt dissolution activity and enzymatic breakdown in the gastrointestinal tract in addition to boosting the attraction of the antigens toward the antigen-presenting cells of Payer's patches [82]. Furthermore, Gupta *et al.* reported that, after secondary immunization, topically administered TT co-transferosomes could induce an immune response (anti-TT-IgG) equal to that obtained after performing intramuscular alum-adsorbed TT-based immunization. The immunity response of Span 85/CHOL niosomes was found to be shaky than that of the transferases [83].

B] DNA vaccines

DNA is a nucleic acid (biomolecule) that contains the genetic code specifying the biological development of all cellular forms of life and is often referred to as the molecule of heredity as it is responsible for the genetic propagation of all traits [84]. The fundamental principle of DNA vaccination is to induce immunity by transfecting host cells with plasmid DNA encoding the required antigen, contrary to the typical approach of injecting an antigen in the form of a protein or peptide [85]. Dengue ribonucleic aid has been discovered to possess more than five stereotypes [86]. DNA encapsulation in niosomes can be attributed to the shielding of genetic material in the biological environment, enhancing significant humoral as well as cell-mediated immune responses against the encoded antigen in immunized mice [87]. Perrie *et al.* [88] announced the encapsulation of nucleoprotein demonstrating the plasmid of the H3N2 influenza virus in NSVs followed by subcutaneous injection of the formulations that improved the immunization of treated mice relative to naked DNA. Vyas *et al.* prepared Span 85/CHOL niosomes containing DNA encoding HBsAg, which enhanced the serum anti HBsAg titer and the cytokine levels (IL-2 and IFN-γ) upon topical application in Balb/c mice, demonstrating the practical usefulness of the topical vesicular vaccine drug delivery system [89].

CONCLUSION

BCS is the 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 potentially resolved. BCS employs various methods for determining solubility and permeability. Various drug delivery systems are available for BCS class II drugs, of which niosomes are more economical and safer carriers than liposomes. This review forms an insightful reference base for the various administration and preparation methods together with evaluation parameters and applications of niosomes in various fields of medicine.

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CONFLICT OF INTERESTS

None

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