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Review

Nano-niosomes as nanoscale drug delivery systems: An illustrated review

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ABSTRACT

The field of nanochemistry research has shown a great progress in the developing of novel nanocarriers as potential drug delivery systems. Niosome is a class of molecular cluster formed by self-association of non-ionic surfactants in an aqueous phase. The unique structure of niosome presents an effective novel drug delivery system (NDDS) with ability of loading both hydrophilic and lipophilic drugs. Numerous research articles have been published in scientific journals, reporting valuable results of individual case studies in this context. However, surveying and discussing the recent, rapidly growing reported studies along with their theoretical principals is required for the fully understanding and exploring the great potential of this approach. To this aim, we have provided an illustrated and comprehensive study from the view of a supramolecular chemist, interested in the synthesizing and studying chemical aggregates on the nanoscale for the development of nanotechnological clusters including niosomes. First, a connectional review of the molecular structure and physicochemical properties of niosome forming non-ionic surfactants and additive agents have been discussed. Second, a systematic survey of niosome preparation and loading methods, administration routes, characterization of niosomes, their toxicity studies and mechanism of drug release; used in recent articles have been performed.

Contents

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1. Introduction

Rapid progress in the application of nanotechnology for therapy and diagnosis has made a new field called “nanomedicine” and related subfields such as “pharmaceutical nanocarriers”. Nanoscale aggregates called nanocarriers are available in various classes including: nanoparticles made of metals, polymers, hydrogel, ceramic; lipid based carriers such as liposomes and niosomes [1,2]; nanoburrs [1] ,etc.

This review introduces nanometer scale materials named nanoniosomes, being part of nanochemistry, as well as nanomedicine fields. The field of nanochemistry closely related to nanotechnology, an extensive area of intense current endeavor that relies on physics, engineering and biotechnology, as well as chemistry. Here, we give a brief introduction to nanochemistry from the view of a supramolecular chemist interested in synthesizing and studying chemical aggregates on the nanoscale for the development of nanotechnological clusters called niosomes.

Nanomaterials having at least one dimension in the range between about 0.1 to 100 nm, exhibit novel physico-chemical proprieties that differ from the bulk material resulting in the novel characteristics. Nanotechnological materials have been traditionally prepared by the top-down approach which involves the break-down of materials using techniques developed by solid state physicists. However, an alternative approach called “bottom-up” forms the foundations of nanochemistry and enables the synthesis of nanostructures and nanomaterials through the utilization of supramolecular and biomimetic materials. Both approaches have interfaces with biology and biomimetic chemistry, engendering the field of nanobiology [3].

Reducing the size into nanoscale in drug carriers offer many advantages such as: improving pharmacokinetics and biodistribution of therapeutic agents due to higher ratio of surface area to volume; diminishing toxicity by their preferential accumulation at the target site, facilitating intracellular delivery and prolonging their retention time either inside the cell which improves therapeutic potential of drugs or in blood circulation [2,3].

In the bottom up approach, nanomaterials are built up of smaller building blocks, typically with the use of self-assembly methods for the formation of highly ordered two- and three-dimensional nanoscale structures. In this method sub-nanometer scale molecules spontaneously generate nanoscale aggregates such as a micelle or bilayer of surfactants according to their intrinsic molecular properties or as a result of the influence of a template, such as a molecule, or other self-assembled structures. Balance between supramolecular interactions and external forces govern the linking and orientation of potential building block molecules for nanoscale architectures. Molecules are more convenient building blocks than atoms, since molecules are less reactive than free atoms.

Drug delivery systems (DDS) play very important role in drug development. Vesicular drug delivery systems with a bilayer membrane and a hollow space have received a great attention as potential drug delivery systems. Vesicular systems can have high entrapping, low to high storage time, receptive surface for treating various targeting agents, capability of being synthesized by smart component for targeting certain environment, delivery of hydrophobic and hydrophilic drugs. One class of vesicular nanocarriers is non-ionic surfactant based vesicle called “Niosome” (Fig. 1).

This review attempts to provide a comprehensive explanation to the following aspects: first, an overview of issues related to non-ionic surfactants based nanocarriers (vesicles) by explaining 1) chemical components of niosomes and their formulations (non-ionic surfactants; common additives and properties); 2) methods of their synthesis; 3) niosomal drugs applications along with their advantages; 4) routes of administration for targeted, controlled and sustained niosomes; 5) toxicity and route of excretion of niosomes. Second, a collection of the findings of very recent investigations (the past two years) on the encapsulation ability of drugs in vesicles; Third, illustration the routes of administration in schematics in the form that had not been presented in the previously published literatures. Finally, this article tries to help developing niosomes as a DDS.

2. Niosomes

Niosomes are vesicles composed mainly of hydrated non-ionic surfactants in addition to, in many cases, cholesterol (CHOL) or its derivatives. The unique structures of niosomes make it capable of encapsulating both hydrophilic and lipophilic substances. This can be achieved by entrapping hydrophilic in vesicular aqueous core or adsorbed on the bilayer surfaces while the lipophilic substances are encapsulated by their partitioning into the lipophilic domain of the bilayers. Thin lipid films or lipid cakes are hydrated and stacks of liquid crystalline bilayers become fluid, swell and form liposome. Agitation makes the hydrated lipid sheets detach and self-associate to form vesicles, preventing interaction of water with the hydrocarbon core of the bilayer at the edges [2]. Niosome production was first started from cosmetic industry and then potential applications of niosome in drug delivery were explored [2].

Niosomes have been one of the illustrious vesicles into all vesicular systems, being the focus of a great attention as potential drug delivery

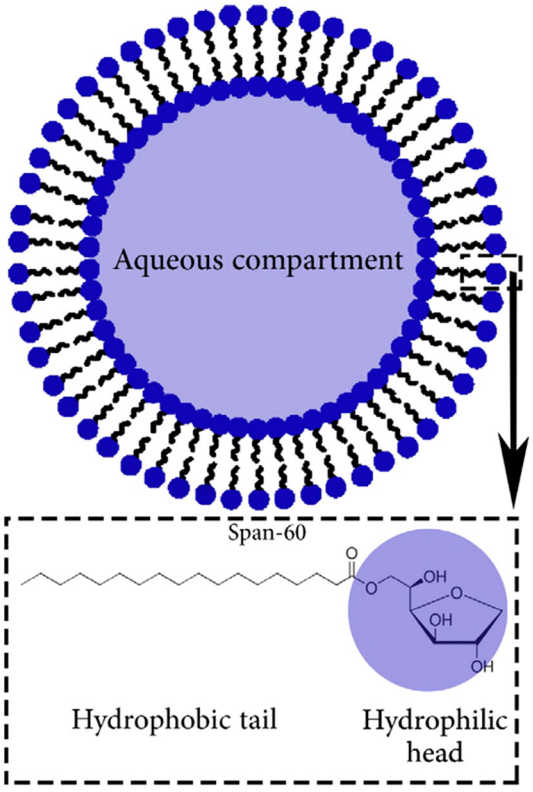

Fig. 1. Schematic representation of niosome prepared by sorbitan monostearate (Span-60) (redrawn from Ref. [35]).

systems for different routes of administration, in recent years. This is due to the fact that niosomes do not have the many disadvantages that others have and are a very useful drug delivery system with numerous applications; Niosomes have the ability of entrapping various types of drugs, genes, proteins and vaccines.

3. Formulation aspects

Formulation aspects are the most important independent parameter that can affect the characteristics of niosomes. Most commonly, niosomes are prepared by convenient accessible raw materials. Non-ionic surfactants are the basic components of niosomes.

3.1. Non-ionic surfactants

Surfactants form a unique class of chemical compounds. Surfactants are amphiphilic molecules with two distinct regions that have very different solubilities, a hydrophilic (water-soluble) end and a lipophilic (organic-soluble) end that is highly hydrophobic, for example, phospholipids (phosphatidyl choline) which are the foundation of biological cell membranes. The lipophilic region is chains made up of alkanes, fluorocarbons, aromatic or other non-polar groups. The head group involves highly solvated hydrophilic functionalities, such as sulfonates, carboxylates, phosphonates and ammonium derivatives. Surfactants can be classified to anionic, cationic, amphoteric and non-ionic; according to their hydrophilic functionality head group; being sulfonate, quaternary ammonium salts, zwitterionic butanes and fatty acids; respectively [3].

Non-ionic surfactants are absolutely one of the best polymeric nanocarriers with a wide role in controlled, sustained, targeted and continuous drug delivery. Commonly, surfactants are classified according to their polar head group. A non-ionic surfactant has no charge groups in its head. The head of an ionic surfactant has a net charge and is called an anionic surfactant. Examples of such surfactants include: fatty acid salts ("soaps"), sulfates, ether sulfates and phosphate esters. If the head charge is positive, it is called a cationic surfactant. If a surfactant contains a head with two oppositely charged groups, it is termed as a zwitterionic (amphoteric) surfactant. Cationic surfactants are also frequently irritant and sometimes even toxic; therefore their application in drug delivery is more limited than the three other classes of surfactants. Examples of each category have been listed in Table 1 [31,32].

Non-ionic surfactants are a category of surfactants which have no charge groups in their hydrophilic heads. Therefore in solutions, non-ionic surfactants can form structures in which hydrophilic heads are opposite to aqueous solutions and hydrophilic tails are opposite to organic solutions. Because of this property of the non-ionic surfactants, niosomes are formed by the self-assembly of non-ionic surfactants in

Table 1
Surfactant classification (data has been collected from Refs. [3288]).

Surfactant class	Examples	Structures
Non-ionic	Polyoxyethylene alcohol	$C_nH_{2n+1}(OCH_2CH_2)_mOH$
	Polyoxyethylene glycol alkyl ethers (Brij)	$CH_3(CH_2)_{10-16}(O-C_2H_4)_{1-25}OH$
	Alkyl ethoxylate	$CH_3(CH_2)_{11}(OCH_2CH_2)_nOH$
	Alkyl phenol ethoxylate	$CH_3(CH_2)_8-C_6H_4-(OCH_2CH_2)_nOH$
	Fatty acid alkanolamides	$CH_3(CH_2)_{10}-OCN(CH_2CH_2OH)_2$
	Propylene oxide-modified polymethylsiloxane	$(CH_3)_3SiO((CH_3)_2SiO)_x(CH_3SiO)_ySi(CH_3)_3$
	(EO = ethyleneoxy, PO = propyleneoxy)	$C_3H_6O(EO)_m(PO)_nH$
Anionic	Stearate	$CH_3(CH_2)_{16}COO^-$
	Soap	$CH_3(CH_2)_{10}COO^-$
	Alkyl benzene sulfonate	$CH_3(CH_2)_9C(CH_3)C_6H_4SO_3^-$
	Alkyl sulfates	$CH_3(CH_2)_{11}OSO_3^-$
	Ether sulfates	$CH_3(CH_2)_{10}CH_2O(CH_2CH_2O)_4SO_3^-$
	Alkyl ether sulfate	$CH_3(CH_2)_{11}(C_2H_4O)OSO_3^-$
Cationic	Laurylamine	$CH_3(CH_2)_{11}NH_3^+$
	Trimethyl dodecylammonium	$C_{12}H_{25}N^+$
	Cetyl trimethylammonium	$CH_3(CH_2)_{15}N^+$
	Alkyl diamine salt	$CH_3(CH_2)_{12}(NH_2)^+(NH_3)^+$
	Benzylalkyldimethylamonium salts	$CH_3(CH_2)_{11}N^+(CH_3)_2CH_2(C_6H_5)$
	Alkyl quaternary ammonium salts	$CH_3(CH_2)_{15}N^+(CH_3)_3$
Zwitterionic	Dodecyl betaine	$C_{12}H_{25}N^+(CH_3)_2CH_2COO^-$
	Lauramidopropyl betaine	$C_{11}H_{23}CONH(CH_2)_3N^+(CH_3)_2CH_2COO^-$
	Cocoamido-2-hydroxypropyl sulfobetaine	$C_nH_{2n+1}CONH(CH_2)_3N^+(CH_3)_2CH_2CH(OH)CH_2SO^-{}_3$
	Alkyl imidazoline	$CH_3(CH_2)_8CONH(CH_2)_2NH^+(C_3H_6OH)C_2H_4COO^-$
	Alkylbetaines	$CH_3(CH_2)_{11}N^+(CH_3)_2CH_2COO^-$
	Sulfur-containing amphoterics	$CH_3(CH_2)_{15}N^+(CH_3)_2CH_2CH_2CH_2SO_3^-$

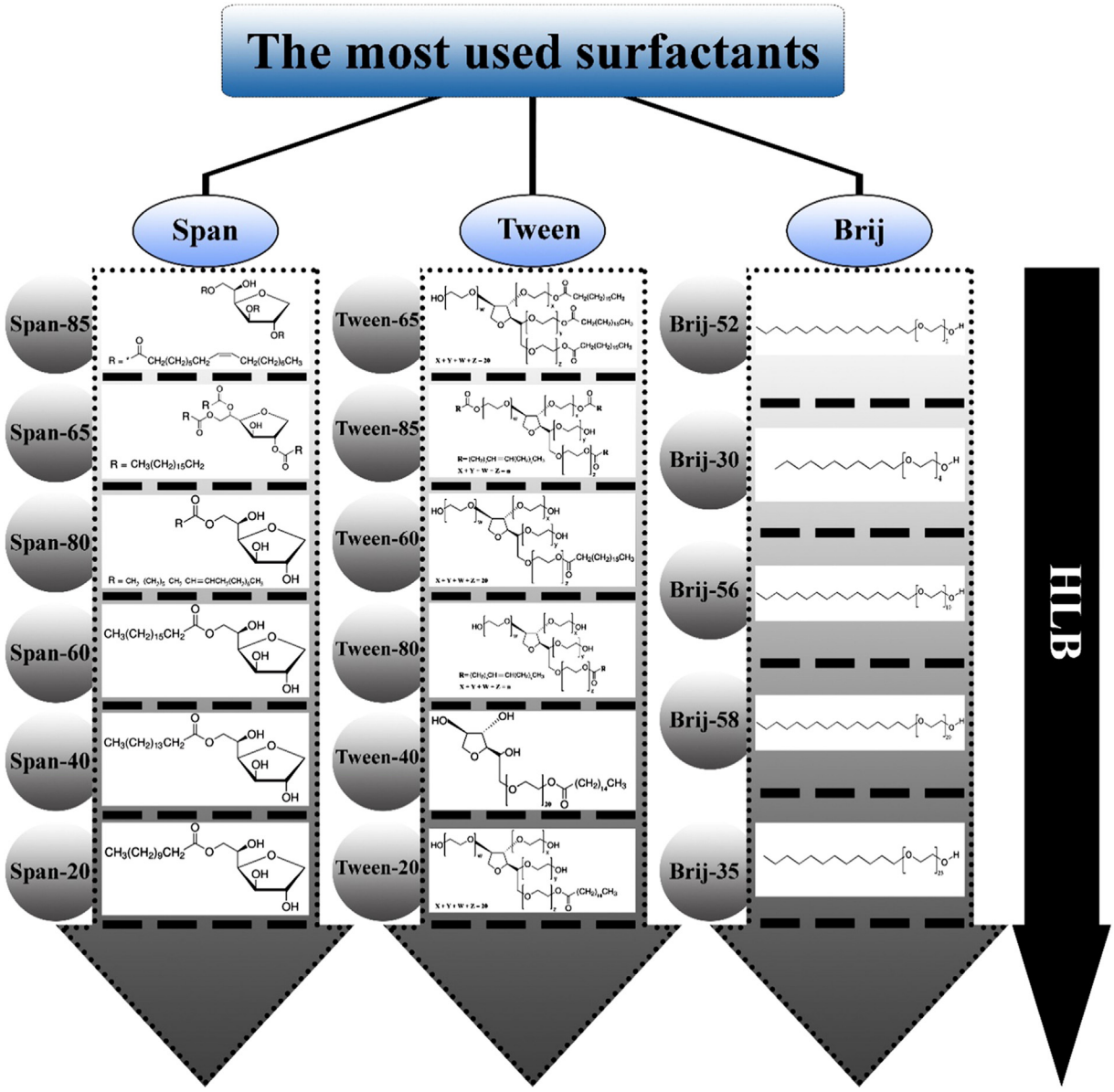

Fig. 2. The most used surfactants for niosome preparation.

aqueous dispersions. Non-ionic amphiphiles used in niosomes are classified in four categories: Alkyl esters, Alkyl amides, Alkyl ethers and esters of fatty acids [33]. The most used surfactants for niosome preparation have been illustrated in Fig. 2. Surfactant selection depends on the hydrophilic–lipophilic balance (HLB) and critical packing parameter (CPP) values which are explained below.

3.1.1. Hydrophilic–lipophilic balance (HLB)

HLB is a dimensionless parameter for surfactants which is known as a time saving guide to surfactant selection. Also, the HLB value of a surfactant plays an important role in controlling drug entrapment efficiency [33]. Up to now, depending upon the administration of niosomes, a large number of non-ionic surfactants with various HLB values have been used such as polyglycerol alkylethers, glucosyl dialkyl ethers, crown ethers, polyoxyethylene ethers and esters such as series of Brijs, Spans and Tweens [23,34]. HLB range is from 0 to 20 for non-ionic surfactants; a low HLB (<9) refers to a lipophilic surfactant (oil soluble) and a high HLB (>11) to a hydrophilic (water soluble) surfactant. Surfactants with a HLB number between 3 and 8 are compatible with preparation bilayer surfaces and refer to water-in-oil (W/O) emulsifier. Also, oil-in-water (O/W) emulsifiers exhibit HLB values within the range of 8–18 [8].

3.1.2. Critical packing parameters (CPP)

In addition to the HLB number, for the prediction of surfactant vesicle forming ability, chemical structure and various other factors play an important role. CPP being another dimensionless scale of surfactants is defined as below [35].

$$CPP = v/l_c a_0. \quad (1)$$

Where v, lc and a_0 refer to: hydrophobic group volume, critical hydrophobic group length and the area of the hydrophilic head group; respectively (Fig. 3). The type of vesicle can be predicted by understanding the CPP value of the preparatory surfactant. CPP indicates the surfactant's ability to form spherical micelles (CPP < 1/3), non-spherical micelles (1/3 < CPP < 1/2), bilayer vesicles (1/2 < CPP < 1) or inverted micelles (CPP ≥ 1) [33,36,37].

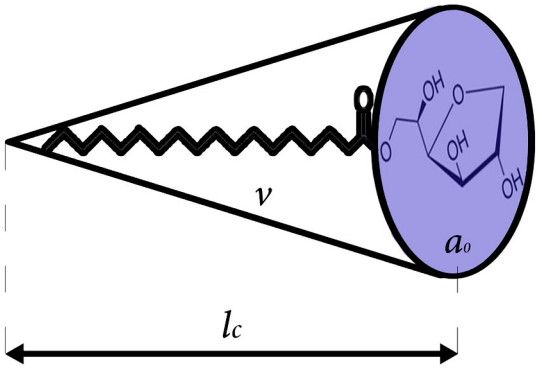

Fig. 3. Schematic structure of span-60 for detection parameters of CPP formulation (redrawn from Ref. [35]).

3.1.3. Gel liquid transition temperature (T_C)

One of the important parameters that has direct effect on the entrapment efficiency is the phase transition temperature (T_C) of the surfactant. Span-60, as an example of surfactants with high T_C, exhibits the highest entrapment efficiency [33,34].

3.2. Additive agents

In addition to the nature of the surfactant, the drug encapsulated and the method of niosome preparation; an additive agent can be an effective parameter on the self-assembly of surfactants into niosomes [35]. Up to now, various additives have been used for niosome membrane among which the most common and important of these agents is cholesterol.

Cholesterol content: The surfactant additive agent which has been widely used and seen in a large number of niosomal drug delivery studies is cholesterol. The position of cholesterol in bilayer of vesicle and its hydrogen bond with hydrophilic head of a surfactant (Span-60) has been shown in Fig. 4. In addition to surfactant properties, as discussed above, cholesterol content tends to affect the important vesicular properties such as entrapment efficiency, storage time, release and stability [23,34]. For surfactants with HLB > 6, cholesterol must be added in order to form a bilayer vesicle and for lower HLB values, cholesterol enhances stability of vesicles. Cholesterol provides greater stability to the surfactant bilayer by promoting the gel liquid transition temperature (T_C) of the vesicle [33].

Also, drug loading capacity can be altered by various content of cholesterol [23] and it is known that from a pharmaceutical viewpoint, drug entrapment efficiency plays an important role in niosomal formulations [20]. Therefore, cholesterol content should be optimized in terms of better properties. It has been shown [38] that cholesterol improves the stability of enoxacin with increasing cholesterol content, resulting in increases of entrapment efficiency [39,40]. Guinedi et al. in 2005 reported that cholesterol content tends to affect the rate of acetazolamide from niosomes prepared by Spans and cholesterol in different molar ratios [41]. It has also been seen that the addition of cholesterol enables more hydrophobic surfactants to form vesicles, suppressing the tendency of the surfactant to form aggregates [33].

Dicetyl phosphate (DCP) is another prevalent additive used for niosomes that have a charge inducer role and is usually used to impart a negative charge on the surface of niosomes to stabilize their bilayers or to achieve an electrophoretic mobility which is similar to that of erythrocytes as in the case of encapsulated hemoglobins. Thus however increasing the amount of DCP beyond the limit will prevent the formation of the Niosomes [4].

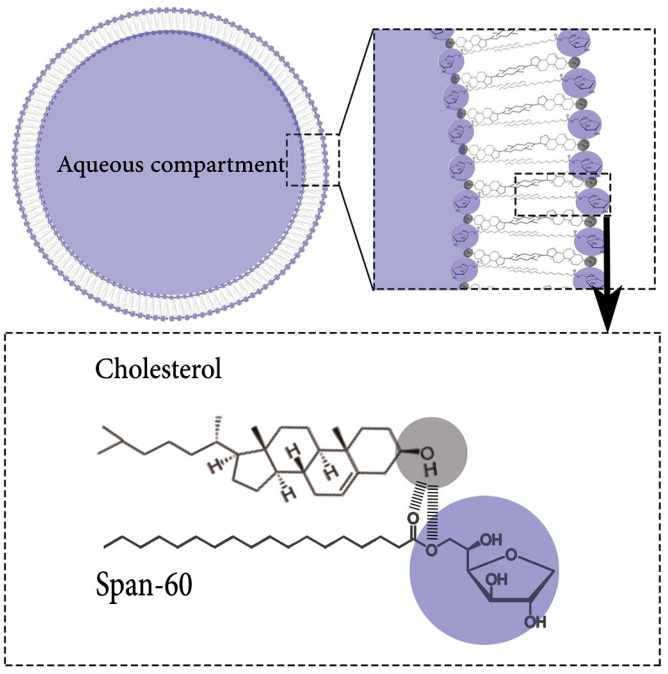

Fig. 4. Position of cholesterol in niosome bilayer, produced by surfactants and cholesterol (redrawn from Ref. [33,83]).

4. Advantages of niosomes

Niosomes offer numerous advantages as presented below:

(i) Niosomes are osmotically active, chemically stable and have long storage time compared to liposomes; (ii) their surface formation and modification are very easy because of the functional groups on their hydrophilic heads; (iii) they have high compatibility with biological systems and low toxicity because of their non-ionic nature; (iv) also, they are biodegradable and non-immunogenic; (v) they can entrap lipophilic drugs into vesicular bilayer membranes and hydrophilic drugs in aqueous compartments; (vi) they can improve the therapeutic performance of the drug molecules by protecting the drug from biological environment, resulting in better availability and controlled drug delivery by restricting the drug effects to target cells in targeted carriers and delaying clearance from the circulation in sustained drug delivery; (vii) access to raw materials is convenient; (viii) they exhibit a high patient compliance, because of the water-based suspension of niosomes;

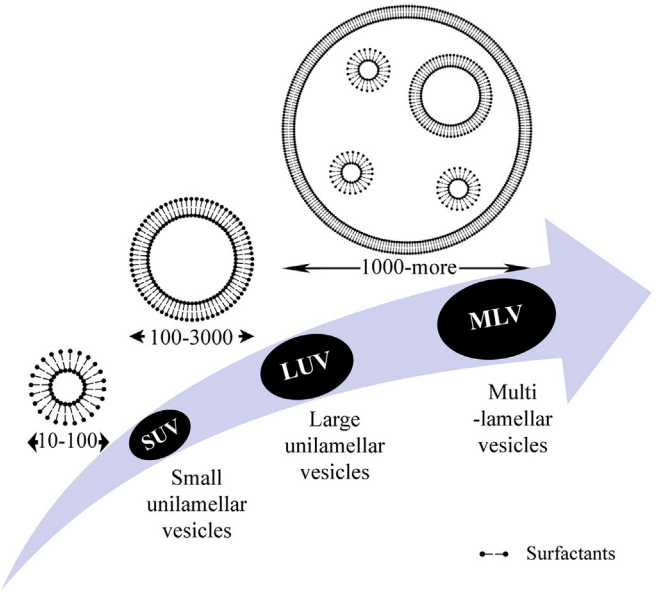

Fig. 5. Schematic structure of SUVs, LUVs and MLVs.

Table 2
Recent researches loading various drugs on to niosome.

	Loaded drug	Highest entrap efficiency (EE %)	Title	Type of surfactant	Preparation method	Route of administration	Application	Biocompatibility aspects (in comparison to free drug)	Ref.
1	pCMSEGFP	–	A novel cationic niosome formulation for gene delivery to the retina	Combination of a cationic lipid, Tween-80 and squalene	REV	Ocular	Gene delivery to the retina	Decrease side effect with the aid of targeting	[31]
2	Antioxidants (gallic acid, ascorbic acid)	59.40 ± 1.43	Co-encapsulation of antioxidants into niosomal carriers: gastrointestinal release studies for nutraceutical applications	Tween-60	TFH	Oral	Nutraceutical applications, prevention of diseases caused by oxidative stress	Promote ability of reducing free radicals	[32]
3	Beclometasone dipropionate (BDP)	27.53 ± 3.01 36.37 ± 2.81	Air-jet and vibrating-mesh nebulization of niosomes generated using a particulate-based proniosome technology	Span-60	TFH PT	Pulmonary	Anti-asthma drug	Decrease side effect with the aid of fine particle fraction (FPF)	[33]
4	Methotrexate	94.8 ± 4.6	Alkyl glucopyranoside-based niosomes containing methotrexate for pharmaceutical applications: Evaluation of physico-chemical and biological properties	3 p-d-Glucopyranosides (octyl, decyl, dodecyl)	TFH	–	Anticancer drug	Decrease side effect with the aid of targeting	[34]
5	Morin hydrate (MH)	98.62 ± 0.01	Formulation, characterization and pharmacokinetics of Morin hydrate niosomes prepared from various nonionic surfactants	Span-60,80 Tween-60	HSM	Intra-venous	Antioxidant and anticancer drug	Decrease side effect, improve area under curve (AUC)	[20]
6	Diclofenac sodium (DCS)	48.37 ± 2.07 58.20 ± 1.75 50.14 ± 2.11	Preparation and *in-vitro* evaluation of diclofenac sodium niosomal formulations	Span-20,40,60,80,85 Tween-20,40,60,80	HSM EIM REV	–	Treatment of osteoarthritis, rheumatoid arthritis ankylosing spondylitis and antiinflammatory drug (NSAID)	Decrease immunogenicity and side effect of NSAID	[8]
7	Doxorubicin	38.73 ± 1.58	Transferrin-conjugated pluronic niosomes as a new drug delivery system for anticancer therapy	Pluronic L64	REV	–	Anticancer drug	Decrease side effect with the aid of targeting tumor cell lines	[35]
8	Curcumin	74.5 ± 3.2	Modulation of the photophysical; properties of curcumin in nonionic surfactant (Tween-20) forming micelles and niosomes: a comparative study of different microenvironments	Tween-20,80	Sonication method	–	Anticancer, antioxidant, antiinflammatory, antimicrobial, antiamyloid, anticystic fibrosis, anti-Alzheimer, and wound healing drug	Improve bioavailability and decrease side effect, increase drug concentration and decay time of curcumin	[14]
9	Hydroxychloroquine (HQ)	86.40	Hydroxychloroquine niosomes: a new trend in topical management of oral lichen planus	Tween-20	REV, sonication, HSM, EIM	Oral	Antimalarial drug and improve oral lichen planus	Improve topical treatment with less side effects	[36]
10	Ellagic acid (EA)	38.73 ± 1.58	Influence of chemical penetration enhancers on skin permeability of ellagic acid-loaded niosomes	Span-60 and Tween-60	REV	Transdermal	Antioxidant drug	Lower side effect with higher drug concentration in the dermis layer	[37]
11	Diallyl disulfide (DADS)	74.5 ± 3.2	Development, characterization and efficacy of niosomal diallyl disulfide in treatment of disseminated murine candidiasis	Span-20,40,60,80	Sonication method	Intraperitoneal	Anti-bacterial and anti-fungal drug	Decrease immunogenicity and lower erythrocyte lysis	[38]

(ix) unlike phospholipids, the handling of surfactants requires no special precautions and conditions; (x) they increase the oral bioavailability and skin penetration of drugs; (xi) the variable characteristics of the niosomes can be controlled. Characteristics; such as the type of the niosomes according to their size, entrapment efficiency and stability; can be controlled by the type of preparation method, of surfactant, cholesterol content, size, surface charge and suspension concentration; (xii) niosomes can enhance absorption of some drugs across cell membranes, to localize in targeted tissues and to elude the reticuloendothelial system; (xiii) also, they can regulate the drug delivery rate in the external non-aqueous phase by emulsifying an aqueous phase in a non-aqueous phase [40,42,43]. It should be noted that there is a physical instability during dispersion, may be equivalent to that of the liposome that could count as a drawback. During dispersion, both liposomes and niosomes are at risk of aggregation, fusion, drug leakage, or hydrolysis of encapsulated drugs [2].

5. Types of niosomes

According to niosome size, they can be divided into three categories. Small unilamellar vesicles (SUV) (10–100 nm), large unilamellar vesicles (LUV) (100–3000 nm) and multi-lamellar vesicles (MLV) where more than one bilayer is present [44] (Fig. 5). In addition to categorization based on size, many types of specialized niosomes are mentioned in the literature including proniosome, surfactant ethosomes, elastic niosomes, polyhedral niosomes, discomes (disk-shaped vesicle), aspasome (ascorbyl palmitate vesicle), surfactant ethosomes and so on [33,34]. Lipid vesicles exist in many classes. However, nano-liposome exclusively refers to nanoscale lipid vesicles which are known as small uni-lamellar vesicles (SUV) (10–100 nm). While most niosomes are in the nano or sub-micron (colloidal) size range, not many authors used the "nano-niosome" or "nanovesicle" term in their published articles which was due to introduction of new nanotechnology related phrases during the past few years [2,3].

Many research groups have performed studies on niosomes around the world, and the information about some recent studies has been shown in Table 2. Most niosomes are in the nano or sub-micron (colloidal) size range.

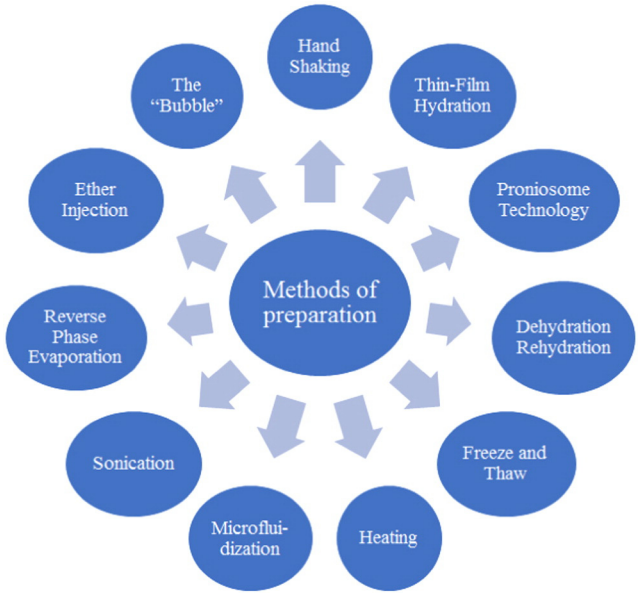

Fig. 6. Methods for niosome preparation.

6. Methods of preparation

Niosomes as drug delivery and gene therapy vectors are prepared by various methods, illustrated in Fig. 6. Also, for the sake of targeting, increasing circulation and target searching time, they could be coated by various types of agents such as polyethylene glycol (PEG) [24–26], hyaluronic acid (HA) [27–29], antibodies [30], etc. for specific applications. Surfactants with an additive component called cholesterol can form any types of non-ionic surfactant vesicles.

6.1. Thin-film hydration method (TFH)

Thin-film hydration method (TFH) is a simple preparation method and is widely used. In this method, the surfactants and some additives such as cholesterol are dissolved in an organic solvent in a round-bottomed flask. Then thin film is formed on the inside wall of the flask by removing the organic solvent using a rotary vacuum evaporator. An aqueous solution such as water or PBS (phosphate buffer saline) [containing drug] is added and the dry film is hydrated above the transition temperature (Tc) of the surfactant. MLVs were formed during the hydration [23,35]. The schematic of the protocol for niosome preparation through TFH has been illustrated in Fig. 7. TFH has been used for preparing niosomes entrapped in minoxidil [20], nimesulide [21], insulin [36], hydroxycampt-othecin [6], beclometasone dipropionate (Bdp) [13], glucocorticoid [14], salicylic acid and p-hydroxyl benzoic acid [45], methotrexate [46], doxorubicin [47], antioxidants [48], etc.

6.2. Hand shaking method (HSM)

One method for synthesis MLVs is hand shaking method (HSM) which is similar to TFH method and sometimes both HSM and TFH methods have been put in one category [40]. In this method, the surfactants and some additives such as cholesterol are dissolved in an organic solvent in a round-bottom flask. The organic solvent was removed using a rotary evaporator to form a thin film on the inside wall of the flask. The completely dried film was directly hydrated with aqueous solution [containing drug] for about 1 h with gentle mechanical shaking to form niosomal dispersion with a milky appearance. HSM has been used for preparation of niosome entrapped morin hydrate (MH) [4], diclofenac sodium (DCS) [8], luteinizing hormone releasing Hormone (LHRH) [49], adriamycin [50], flurbiprofe [51], etc.

6.3. The "bubble" method

The "bubble" method is a niosome preparation technique without the use of organic solvents. In this method, surfactants, additives and PBS (pH 7.4) were transferred into a glass reactor with three necks. The reactor is positioned in a water bath to control the temperature. A thermometer is positioned in the first neck, nitrogen is supplied through the second neck and water-cooled reflux in the third neck. Niosome components are dispersed at 70 °C and the dispersion is mixed for 15 s with high shear homogenizer and immediately followed by the bubbling of nitrogen gas at 70 °C [40].

6.4. Ether injection method (EIM)

In ether injection method (EIM), the surfactants with additives are dissolved in an organic solution such as diethyl ether and injected slowly through a needle in an aqueous solution [containing drug] maintained at constant temperature (about 60 °C). The organic solvent was evaporated using a rotary evaporator [8]. During ether vaporization surfactants are leaded to the formation of single layered vesicles. SUVs and LUVs produced by solvent injection technique, relatively have high entrapped aqueous volume. Depending upon the conditions, the diameter of the final vesicle ranges from 50 to 1000 nm [23,35,40]. Schematic of protocol for niosome preparation through EIM has been illustrated in

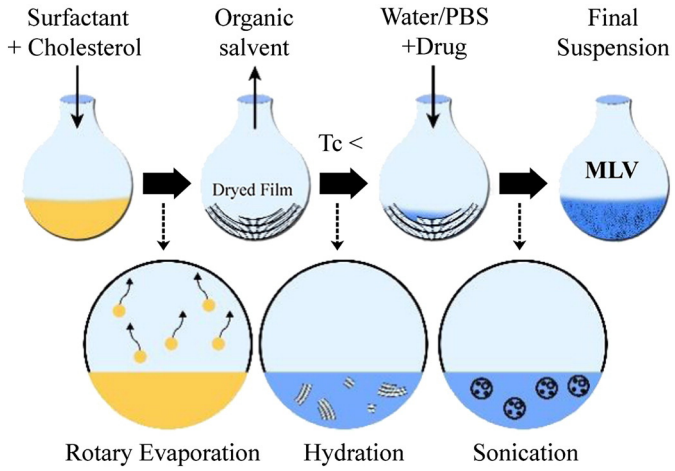

Fig. 7. Protocol for niosome preparation through thin-film hydration method.

Fig. 8. EIM has been used for the preparation of niosome entrapped gadobenate [52], diclofenac sodium (DCS) [8], fluconazole [53], rifampicin [54], adriamycin [50], etc.

6.5. Reverse phase evaporation method (REV)

LUVs are prepared via reverse phase evaporation method where niosomal ingredients, surfactants and additives are dissolved in an organic solvent. The aqueous phase [containing drug] is added to the organic phase and the mixture is sonicated in order to form an emulsion, followed by the slow removal of the organic phase using a rotary vacuum evaporator at about 40–60 °C. The evaporation is continued until the hydration is completed. LUVs are formed during the evaporation of organic solvent [8,11,55]. Schematic of protocol for niosome preparation through REV has been shown in Fig. 9.

Additionally, several methods such as reverse phase evaporation and ether or ethanol injection methods require vigorous conditions such as organic solvents, sonication and high temperatures for long periods of time [11,23,35,56,57]. REV method has been used for the preparation of niosomes entrapped in anti-HBsAg [7], naltrexone (NTX) [10], ellagic acid (EA) [19], diclofenac sodium (DCS) [8], acetazolamide (ACZ) [41], etc. Bendas, E. R. et al. investigated 4 preparation methods containing REV, Sonication, HSM and EIM for hydroxychloroquine niosomes and reported that with REV method, the highest entrapment efficiency has been achieved [58].

If the structure of the used drug has been deformed and lost its usability by being in temperatures greater than 50 °C or in organic solvents, Direct Entrapment method cannot be used (indicated in Section 7.1) in REV and EIM.

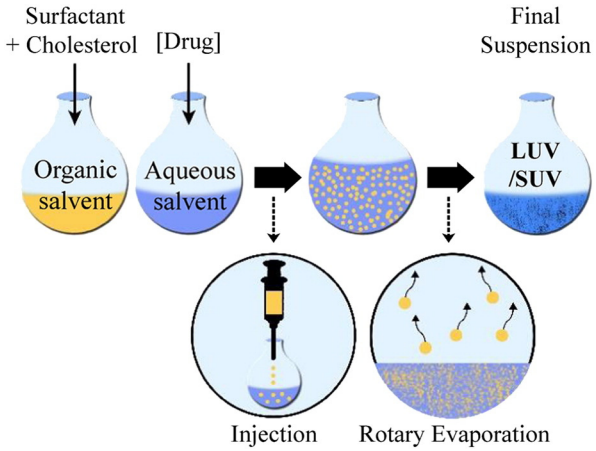

Fig. 8. Protocol for niosome preparation through ether injection method.

6.6. Sonication method

In this method, the solution of a drug, in buffer, is added to the surfactant/cholesterol mixture. The mixture is probe sonicated at 60 °C via a sonicator with a titanium probe for 3 min to yield niosomes [40]. Sonication method has been used for preparation diallyl disulfide (DADS) loaded niosomes [59].

6.7. Microfluidization method

Greater uniformity, smaller size, unilamellar vesicles and better reproducibility of niosomes could be achieved by using micro fluidization technique. In this method, the submerged jet principle in which two fluidized streams interact at ultra high velocities, in precisely defined micro channels within the interaction chamber is used. The impingement of a thin liquid sheet along a common front is arranged in such a way that the energy supplied to the system remains within the area of niosome formation [40].

6.8. Heating method (HM)

Surfactants and some additives such as cholesterol were separately hydrated in PBS (pH = 7.4) for one hour under nitrogen atmosphere at room temperature. Then, after about 15–20 min, the solution is heated (about 120 °C) on a hot-plate stirrer to dissolve cholesterol. The temperature is brought down to 60 °C and the other components, surfactants and other additives, are then added to the buffer in which cholesterol is dissolved while stirring continues for another 15 min. Niosomes obtained at this stage are left at room temperature for 30 min and then kept at 4–5 °C under nitrogen atmosphere until use [60–64]. The schematic of protocol for niosome preparation through HM has been shown in Fig. 10.

6.9. Freeze and thaw method (FAT)

This method can generate frozen and thawed multilamellar vesicles (FAT-MLVs). Niosomal suspensions, prepared using TFH method, were frozen in liquid nitrogen for 1 min and thawed in a water bath at 60 °C for another 1 min [11].

6.10. Dehydration rehydration method (DRM)

The dehydration rehydration technique was first described by Kirby and Gregoriadis in 1984 [35]. The dehydration rehydration vesicles (DRVs), prepared by TFH, are frozen in liquid nitrogen and then followed by freeze-drying overnight. Niosome powders are hydrated with PBS (pH = 7.4) at 60 °C [65].

6.11. Proniosome technology (PT)

The use of Proniosome technology for niosome preparation was started about two decades ago. Proniosome is a novel drug carrier preparation method and it has been used as stable precursors for the immediate preparation of niosomal carrier systems [56]. PT has been used for the preparation of niosomes entrapped vinpocetine [17], valsartan [9], 17β-estradiol [22], tenoxicam [16], etc.

Generally, SUVs are prepared by converting of MLV dispersions into SUVs by either sonication (a bath or a probe sonicator) or by high pressure homogenization (a microfluidizer) or by extrusion under high pressure (using French pressure cell). During application of energy the MLV structure is broken down and SUVs with a high radius of curvature are formed [4,23,35,66].

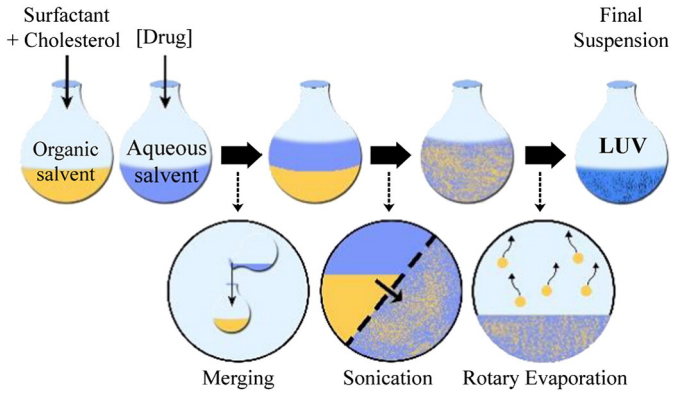

Fig. 9. Protocol for niosome preparation through reverse phase evaporation method.

7. Loading methods

The encapsulation processes of drugs are called loading methods. Drugs can be entrapped based on their physical trapping, covalent and hydrogen bonds, adsorption, ionic interaction between drugs and vesicles, etc. but often in drug delivery systems, more than one of these parameters play role in loading process. Generally, niosome drug-loading processes contain two types of methods (Fig. 11).

7.1. Direct entrapment (passive loading)

Direct entrapment is the simplest loading method and is widely used where lipophilic drugs are dissolved in organic solvent and hydrophilic drugs are dissolved in aqueous phase and a percent of drugs are loaded among the preparation process [35] and unloaded drugs can be subtracted from suspension by dialysis, centrifugation, gel chromatography or filtration [40].

7.2. Remote loading (active loading)

This method enhances the efficiency drug loading by aid of pH and ion. Differential of these parameters is the real cause for some material transition across the niosome membrane [34,67].

7.3. Remote loading by using trans membrane pH gradient (in acidic range)

Basic drug in an unionized state passes the membrane barrier of the niosome, if the pH is higher in the outside of niosome vesicles. The basic drug becomes ionized and precipitate, due to the lower pH inside the niosome. Thus, it becomes unable to leave the vesicle, after encapsulation.

This method can experimentally be accomplished by hydration the thin film of surfactant and cholesterol with citric acid (pH 4.0) by vortex mixing. Therefore, the MLVs are frozen and thawed. Drugs are added into aqueous solution and suspension is vortexed. The pH is then raised to 7.0–7.2 and is later heated at 60 °C for 10 min to give niosomes [34,41].

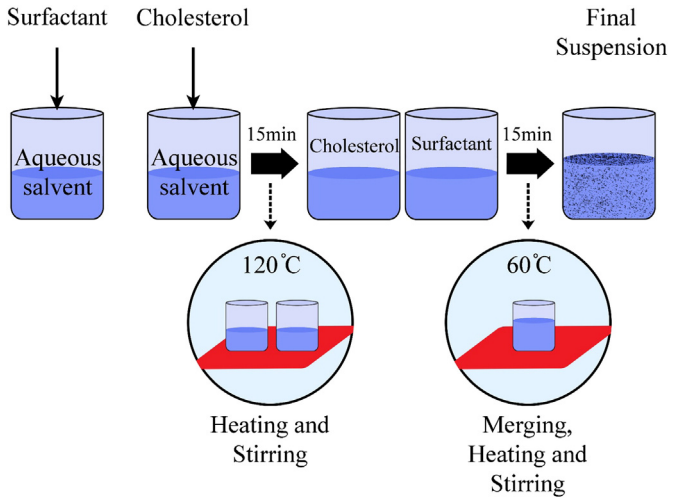

Fig. 10. Protocol for niosome preparation through heating method.

7.4. Remote loading by using trans membrane ion gradient

An alternative remote loading method has also been developed for DOX using ammonium sulfate [68]. In this trapping method, there is a trans membrane ion gradient where the concentration of *ammonium sulfate* ($(NH_4)_2SO_4$) *in vesicle is more than this concentration in medium and causes the reaction below* (Fig. 12). However, these remote loading procedures have not been used to increase the niosome encapsulation of acidic drugs [35].

8. Characterization

Physicochemical characterization and analyses of niosomes contain vesicle size, morphology, size distribution, charge and zeta potential, entrapment efficiency, curve of drug release, lamellarity, rigidity, stability, viscosity, conductivity and homogeneity.

8.1. Average vesicle size, morphology and size distribution

Niosomes are assumed to be spherical in shape and their size can be determined using several techniques such as light microscopy and coulter counter (for particles with diameter over 1 μm), photon correlation spectroscopy, electron microscopic analysis (scanning electron microscopy (SEM), transmission electron microscopy (TEM), freeze fracture replication-electron microscopy (FF-TEM)), light scattering techniques (spectrometer — dynamic light scattering (DLS) instrument), zetasizer and mastersizer. Also, niosomes size distribution and polydispersity index (PdI) can be measured by using dynamic light scattering particle size analyzer. Vesicle size can range from around 20 nm to around 50 μm [23,69].

8.2. Charge of vesicle and zeta potential

The zeta potential of vesicles can play an important role in the behavior of niosomes. In general, charged niosomes are more stable against aggregation and fusion than uncharged vesicles [40]. Also, negative zeta potential values ranging between −41.7 and −58.4 mV are sufficiently high for electrostatic stabilization and both surfactant type or encapsulation efficiencies might affect the zeta potential values [70]. Surface zeta potential can be determined using zetasizer, mastersizer, microelectrophoresis, pH-sensitive fluorophores, high performance capillary electrophoresis and DLS instruments [23,24].

8.3. Stability study

Storage feasibility of niosomal drug is investigated in stability study. Stability of vesicles is an important factor in successful development of a dosage form [40]. Stability of niosome is influenced by the entrapped drug, its concentration, type of surfactant and cholesterol content [34]. Stability studies are carried out to investigate the drug leaching from niosomes during storage and while in the general circulation. Using conditions that simulate both situations, this leaching can be evaluated by determining mean vesicle size, size distribution and entrapment efficiency over several month periods [19,41,71].

The stabilization strategies must be optimized depending on the agent to be entrapped to provide chemical stability of both the surfactant and drug components [71].

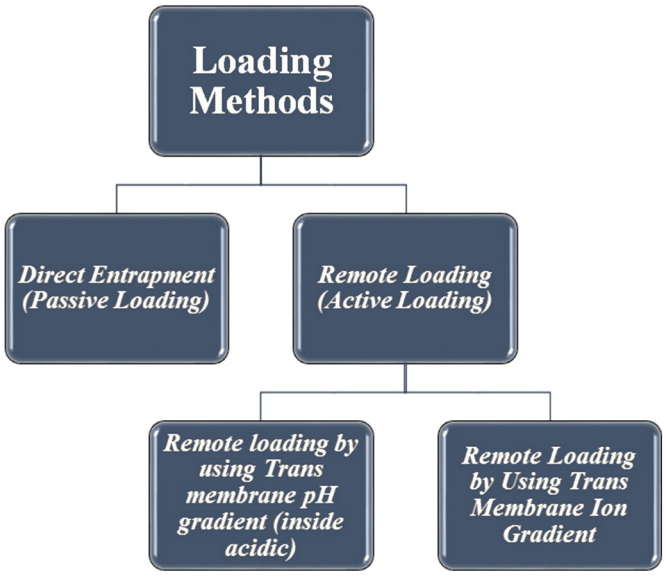

Fig. 11. Loading methods.

8.4. Entrapment efficiency

For the use of therapeutic vesicles in pharmaceutical application, the most important parameter of niosome is entrapment efficiency (EE%) [20]. After preparation of niosomes suspension, unencapsulated drug (free drug) can be subtracted from the total amount of drug by dialysis, centrifugation, gel chromatography or filtration [40].

For the, encapsulation of the drug in niosomes, some parameters must be determined. First, the amount of total drug (loaded and free drug) (μg/ml) in constant amount of suspension is determined by a spectrophotometer instrument, high-performance liquid chromatography (HPLC), enzyme-linked immunosorbent assay (Elisa) and soon. The concentration of drug in μg/ml is found using a standard curve of absorbance values. After one or more separation step/s (depending on the separation methods), the total concentration of free drugs can be obtained. Entrapment efficiency of niosome can be obtained now by using Eq. (2).

For expressed *EE%* by using Eq. (3), the concentration of loaded drug is needed. This concentration is obtained by determining drug concentration in complete destructed vesicles mixture (adding 50% propane or 0.1% Triton X-100 to niosomal suspension and about 1 h incubation) [72].

$$EE = (WT - WF)/WT \times 100\% \quad (2)$$

Or

$$EE = WL/WT \times 100\% \quad (3)$$

Where *WT* is total amount of drug in suspension, *WF* is the amount of free drug in suspension (unloaded) and *WL* is the total amount of loaded drug in vesicles. Eq. (2) is usually used for EE % measurement with the aid of dialysis and centrifugation methods, which can indicate the concentration of free drug. Dialysis method separates free drug, using dialysis membrane bags with certain molecular weight cutoff and then centrifugation with a rotation speed of about 12,000–15,000 rpm, duration about 5–60 min (related to MW of components) and at a temperature of usually 4 °C, leading to the formation of two sections, niosomal pellets and supernatant containing free drug.

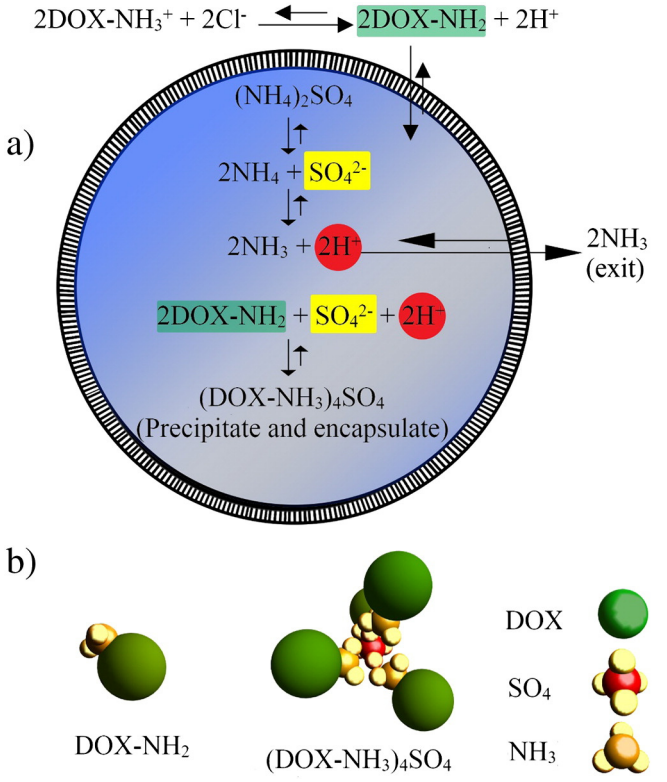

Fig. 12. Schematic illustration of the remote loading by using trans membrane ion gradient: a) Reactions that occur inside and outside of the vesicle to $(DOX\text{-}NH_3)_4SO_4$ precipitate and encapsulate. b) Spatial structure of $DOX\text{-}NH_2$ and $(DOX\text{-}NH_3)_4SO_4$, the increase in the spatial volume is the reason of the precipitation (redrawn from Ref. [67]).

8.5. In vitro and in vivo studies

Based on the route of administration, *in vitro* release can be determined by dialyzing the niosomal suspension against buffer at definite temperature and determining the content of dialysate [40]. Also, permeation of niosomes from certain biological barrier can be achieved using specific filters in the test cell culture systems. For example, about oral administration of insulin, Coca-2 permeability may be measured by calculating the concentration of permeated insulin in transwell polyester plate in various times and the opening of tight junctions reversibly in Caco-2 cell monolayers can be determined by measuring transepithelial electrical resistance (TEER) using epithelial volt-ohmmeter (EVOM) fitted with planar electrodes. The decreasing of TEER indicates the increasing paracellular permeability of Caco-2 cell monolayers [73–77].

In vivo studies of niosomal drug release depended on the route of administration investigated, the concentration, effect and presence time of drug in tissues such as liver, lung, spleen and bone marrow. Also, variation in the size of niosomes influences the their residence pattern in tissue, the retention of vesicle and the effect of phagocytic cells on carriers, while small sized vesicles can penetrate in liver sinusoidal epithelium, and have better access to spleen [40]. Entrapment efficiency of niosomes is directly influenced by vesicle size and for per administration, there is an optimum size, with an appropriate entrapment efficiency. Some characterizations techniques for analyzing niosomal formulation, identified in the published literature, have been listed in Table 3.

9. Modification in niosomes

Modification of vesicles can improve wettability and decrease drug side effects by creation targeted niosomes. Amount of applied modified agents can be determined using nuclear magnetic resonance (NMR), Fourier-transform infrared spectroscopy (FTIR) spectroscopy and so on.

Table 3
Characterizations of several niosomal drug.

	Loaded drug	Size			Polydispersity index (PdI)		Morphology	Zeta potential		Ref.
		Light microscope	Zetasizer	DLS	Zetasizer	DLS	TEM	ζ-potential analyzer	Zetasizer	
1	Flurbiprofen	*					*			[51]
2	pCMSEGFP			*		*	*		*	[31]
3	Antioxidants			*		*	*			[32]
4	Beclometasone dipropionate (BDP)		*		*		*		*	[33]
5	Methotrexate			*		*	*			[34]
6	Morin hydrate (MH)			*		*	*	*		[20]
7	Diclofenac sodium (DCS)	*					*			[8]
8	Doxorubicin			*		*	*			[35]
9	Curcumin			*		*	*			[14]
10	Hydroxychloroquine (HQ)	*								[36]
11	Ellagic acid (EA)		*		*					[37]
12	Diallyl disulfide (DADS)		*		*					[38]

	Loaded drug	EE %				Release	*In vitro*	*In vivo*	Clinical	Stability and rigidity	Toxicity	Ref.
		Dialysis	Centrifugation	Chromatography	Filtration	Sampling						
1	Flurbiprofen	*	*			*	*			*		[51]
2	pCMSEGFP					*	*	*			*	[31]
3	Antioxidants			*		*	*					[32]
4	Beclometasone Dipropionate (BDP)			*	*		*					[33]
5	Methotrexate	*		*		*	*				*	[34]
6	Morin hydrate (MH)		*	*		*	*	*		*		[20]
7	Diclofenac sodium (DCS)	*				*	*					[8]
8	Doxorubicin	*				*	*				*	[35]
9	Curcumin		*							*		[14]
10	Hydroxychloroquine (HQ)		*			*	*		*	*		[36]
11	Ellagic acid (EA)		*	*		*	*	*		*		[37]
12	Diallyl disulfide (DADS)			*		*	*	*			*	[38]

Asterisks indicate the characterizations of the drugs within the niosomes.

There are several types of modification methods for niosomes and one of the more important modifying agents is PEG (polyethylene glycol).

9.1. PEG (polyethylene glycol)

The modification of vesicles with PEG makes them able to circulate for longer periods of time in the blood stream, because of being hidden from the body's immune system. This provides enough time for the vesicles to search for their target, before being taken through the reticulo-endothelial-system (RES) [78]. High hydrophilicity of polyethylene glycol (PEG) causes the niosomes with PEG coating to have a layer of H_2O molecules around themselves and this layer prevents them from recognition and endocytosis by RES, and prolongs the circulation and target search time, which was a key factor for the successful application *in vivo* [79]. PEGylation is widely used for niosomal vesicles [6,24,80–82].

10. Administrations

Depending on the types of drugs, surfactants, diseases or locations of defects, various routes of administration exist for niosomal drugs which have been listed below:

Intravenous (IV): Intravenous administration of drugs can directly put drugs into the circulation system and drug loaded niosomes compared to free drugs can enhance stability of the drugs and prolong the circulation time. Loaded drug can be released into the bloodstream or into target tissue under certain condition or into the targeted cells.

Intramuscular (IM): After IM injection of the drugs, a gentle drug penetration from tissues to capillaries has been observed.

Transdermal: The specific characteristic of transdermal route is slow penetration of the drug through the skin [42].

Oral: The oral route is the most preferred route for delivering a therapeutically active substance. But acids and digestive enzymes in the stomach and small intestine can degrade some active substances [70]. However, niosomes have been reported as conceivable vesicles to deliver drug molecules to the desired mucous membrane or skin layers [58].

Ocular: Topical ocular drug delivery is one of the commonly used and preferred routes for treating conditions that affect the anterior segment of the eye. However, there are many anatomical and physiological barriers such as exclusive tight junctions of corneal epithelium and precorneal tear film that prevent absorption of the administered particles from residing on the eye surface for deeper sites. Therefore, the bioavailability of drugs administrated by ocular route from simple solutions is typically less than 5% and often less than 1% [11].

Subcutaneous (SC): After SC injection, drugs transit to capillaries and this route of administration is used for several drugs such as insulin, hydroxycamptothecin and so on [6,12]. However, IV, IM and SC injections are more invasive routes than others which generally are not an ideal method for the administration of drugs.

Pulmonary: Pulmonary administration, through inhalation of drugs, is one of appropriate routes used for glucocorticoids such as beclomethasone dipropionate (BDP) for patients with asthma [14]. Pulmonary delivery of BDP through polysorbate 20 niosomes offers the advantages of sustained delivery, an improved mucus permeation, targeted drug delivery and amplified therapeutic effect [23].

Several other routes of administration of niosomal drugs have been reported such as intraperitoneal route, brain and vaginal deliveries where niosomes enhanced brain uptake. Also, it has been reported that niosomes might be a good carrier for vaginal delivery of protein drugs [23]. Schematic illustration of the whole process of

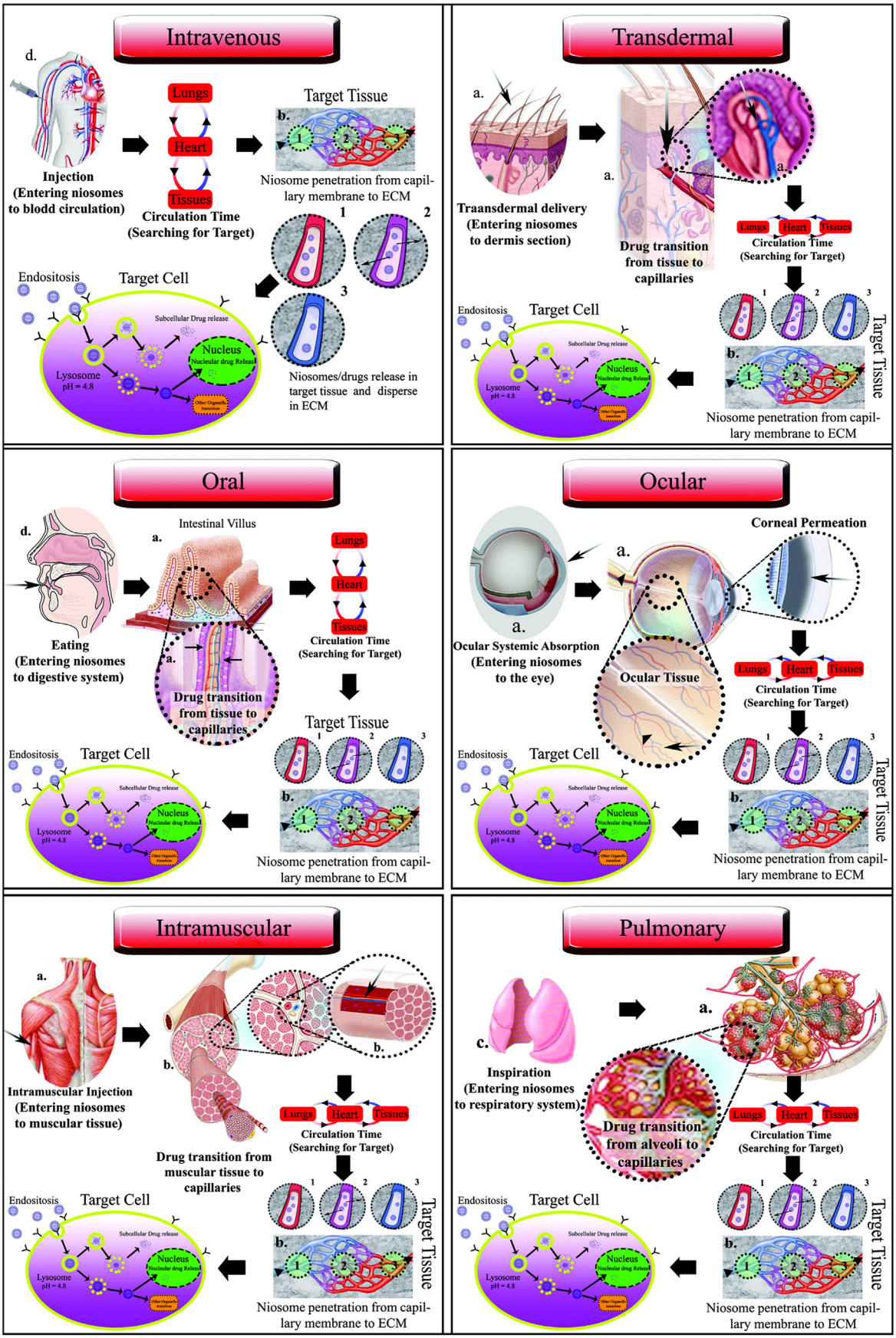

Fig. 13. Routes of administration. Schematic illustration of the whole process of intravenous, ocular, and transversal, oral, pulmonary and intramuscular drug delivery *in vivo*, involving stages of systemic penetration, circulation time, tissue and intracellular targeting. Drug releasing can be achieved in per step of process, depending on the route of administration and asked releasing site, size, charge, stability, sensitivity and niosome coating can be altered (panels a, b, c have been adapted from [84–86] and d [87]; respectively).

Table 4
Drugs administrated by several routes.

Route	Surfactant	Preparation method	Drug	Ref.
Intravenous	Span-60,80	HSM	Morin hydrate (MH)	[4]
	Tween-60			
	Span-60	FTH	Hydroxycamptothecin	[6]
Intramuscular	Span-85	REV	Anti-HBsAg	[7]
	polysorbate 20	HSM	Bovine serum albumin (BSA)	[90]
	Span-60		5,6-Carboxyfluorescein (CF)	
	Solulan C24	HSM	Luteinizing hormone releasing hormone (LHRH)	[49]
Transdermal	Brij-52,76	TFH	Minoxidil	[20]
	Span-20,40,60			
	Tween-20,60,80	TFH	Nimesulide	[21]
	Span-20,60,80,85			
	Some sugar ester, sucrose palmitate, sucrose myristate	PT	Vinpocetine	[17]
	Span-40,60,85	PT	17β-Estradiol	[22]
	Tween-20,60,80			
	Span-60,80	PT	Tenoxicam	[16]
	Tween-20,60,80			
	Span-60	REV	Ellagic acid (EA)	[19]
	Tween-60			
	Tween-61	FDEL method [91]	Gallidermin (Gdm)	[18]
Oral	Span-60	PT	Valsartan	[9]
	Brij-52,72,76, 92,97	TFH	Insulin	[36]
	Tween-60	TFH	Antioxidants (gallic acid, ascorbic acid)	[48]
	Tween-20	REV, Sonication, HSM, EIM	Hydroxychloroquine (HQ)	[58]
Ocular	Span-60	REV	Naltrexone (NTX)	[10,11]
	Combination of a cationic lipid, Tween-80 and squalene	REV	pCMSEGFP	[89]
	Span-40, 60	REV	Acetazolamide (ACZ)	[41]
Pulmonary	Polysorbate 20	TFH	Glucocorticoid	[14]
	Span-60	TFH	Beclometasone dipropionate (Bdp)	[13]
		PT		

administrations has been shown in Fig. 13. Also, drugs administrated by per routes have been shown in Table 4.

11. Application

Niosomes have been first used in cosmetic industry and then have come to the attention of the pharmaceutical companies. They have enormous potential for therapeutic applications, being the subject of an intensive research studies. Niosomes can encapsulate various drugs including doxorubicin, insulin, monoxide, ovalbumin, oligonucleotide, EGFP, hemagglutinin, DNA vaccine, α-interferon, bovine basic pancreatic inhibitor and many others [23]. These can have various applications such as antioxidant, anticancer, anti-inflammatory, anti-asthma, antimicrobial, anti-amyloid, anti-Alzheimer, anti-bacterial, etc. Some of these applications have been shown in Table 2. Also, niosomes have been applied to various delivery routes such as: intravenous [4–6], intramuscular [7], oral [5,8,9], ocular [10,11], subcutaneous [12], Pulmonary [13,14], intraperitoneal [15] and transdermal [16–22], as indicated in Table 4.

12. Biocompatibility aspects

Niosomes have attracted a great deal of attention in controlled drug delivery systems because of many advantages, such as biodegradability, non-immunogenicity nature, bioavailability and effective in the modulation of drug release properties [4,9]. Also, niosomes by their nonionic nature and admirable biodegradability have shown excellent biocompatibility and low toxicity. Therefore, niosomes having versatile biological activities such as low immunogenicity, have provided ample opportunities for further drug delivery developments. Biocompatibility aspects of niosomal formulation have been shown in Table 2.

13. Toxicity

It is thought that niosomes have low toxicity because of their non-ionic nature. In fact, non-ionic surfactants have more compatibility and low toxicity compared to their anionic, amphoteric or cationic counterparts [33]. However, segregation of non-ionic surfactants may cause toxicity. Also, when the role of niosome is targeting and reducing side effects of drug, another explanation for toxicity is the location and concentration of released drugIn addition, though, niosomes play the role of targeting, resulting in the reduction of the drug's side effects, the inappropriate location and high concentration of the released drug could still be toxic in some cases.

14. Conclusion

In the recent years, attentions have been attracted toward vesicular drug delivery systems such as liposomes and niosomes. It is obvious that niosome appears to be a well preferred drug delivery system over liposome. Niosomes present a convenient, prolonged, targeted and effective drug delivery system with the ability of loading both hydrophilic and lipophilic drugs. The potential of niosome can be enhanced by using novel preparations, loading and modification methods. Thus, these areas need further exploration and research so as to bring out commercially available niosomal preparations.

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