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NIOSOMES- A NOVEL TOOL FOR ANTI-AGEING COSMECEUTICALS

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ABSTRACT

Aging is a complex biological process that has not yet been completely elucidated. Human aging and longevity are affected by multiple reasons like genetic factors, environmental factors such as diseases, lifestyle, and social factors. Ageing gives rise to different age related diseases like cognitive impairment, diabetes mellitus, and frailty. Also people around the world have always been looking for better health and appearance. Therefore, the ideal anti-ageing formulation should be applicable for both maintaining the healthful appearance as a cosmetic product as well as preventing the development of degenerative diseases. With increasing advancement in the field of science and technology and also with increase in the information about products available through the use of social media, formulating new products and advertising them have become quite easy. Nowadays formulation containing nanotechnology combined with cosmeceuticals is growing on a larger scale. Thus, we can say that novel cosmetic delivery systems have enormous potential and are next generation carrier systems. Novel drug delivery systems like liposomes, were discovered in 1960's and since then they have been used effectively. However, due to their stability issues, new delivery systems like niosomes were discovered with improved stability. Niosomes are used in the field of cosmetics since the early 1970's. They are stable with good penetrating power and less irritating as compared to other colloidal carrier systems. This review article focuses on the role of niosomes as a carrier for anti-aging topical products.

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INTRODUCTION

With increasing average life span of people, the need for anti-ageing therapies is increasing since physical appearance is most essential in today's world. There are various technologies available for treatment of skin ageing such as facelifts, laser therapy, botox, microderma abrasion etc. These methods although quite famous are not used on a larger scale due to their invasive nature. Therefore, various novel non-invasive topical delivery systems are being developed. However, skin acts a major barrier for topical formulations, stratum corneum being the largest barrier. Hence, there is an urge to have a proper carrier to deliver the drugs through the skin which can be fulfilled using novel delivery systems[1-5]. The most widely used delivery systems are liposomes and they are being used in a variety of skin care rejuvenating products. Liposomes are capable of encapsulating various anti-ageing active ingredients and deliver them deep into the cells. The first liposomal anti-ageing cream to enter the market was "Capture" launched by Dior in 1986. But liposomes are associated with certain disadvantages so to overcome their drawbacks, surfactant based vesicles called niosomes were proposed [6]. Niosomes are preferred over liposomes because of several factors enlisted below:

- i. Liposomes require phospholipids which are highly susceptible to oxidative degradation making them unstable whereas niosomes do not require phospholipids.
- ii. In order to maintain the stability, liposomes and phospholipids have to be stored and handled in an inert nitrogen atmosphere.
- iii. Since phospholipids are natural in origin their purity is variable and require extensive purification.

All the above problems contribute to the high cost of liposomal formulations. Since niosomes do not have any of these problems they are cheaper and more stable as compared to liposomes. Moreover, niosomes behave in-vivo like liposomes, prolonging the circulation of entrapped drug. They can be used as a tool for targeted drug delivery to the desired site of action and provide controlled release.

MECHANISM OF SKIN AGEING

Aged skin has characteristics like loss of skin tone with resilience (loss of elasticity), increase in dryness & roughness, irregular pigmentation, accelerated skin ageing, wrinkles, sunburn, & various malignant skin cancers.

Ageing takes place because of two interactive and overlapping processes called primary and secondary ageing.

Primary aging, also known as "intrinsic senescence," is caused due to the progressive deterioration of the physical structure and biological functions of body which takes place with increasing age [7,8].

Secondary ageing is caused due to the degradation which takes place in body caused by diseases like diabetes, hypertension etc. Environmental factors as well as lifestyle effects like smoking tobacco also have an impact on body which in turn leads to ageing. Excessive exposure to sun leads to harmful impacts on skin [9-11]

Exact mechanisms for both these processes are not known, but the probable factors include the following:

1. Damage to protein and DNA which takes place due to oxidative stress along with improper repairment of DNA damage and genetic instability that takes place in mitochondria as well as in nuclear genomes [12-15].
2. Increased production of adipokine and cytokine that causes non-infectious chronic inflammation [16].
3. During fatty acid metabolism, excessive free fatty acids are released which undergoes alteration with subsequent tissue insulin resistance [17].
4. Interference in normal cellular functioning which takes place due to the accumulation of components like advanced glycation end products, proteins and amyloids [18-21].
5. Alterations in neuroendocrine systems along with activation of sympathetic nervous systems[22-24].
6. Deterioration of structure and function of cells of all tissues and organs with loss of post mitotic cells which leads to decrease in the number of neurons and mast cells [25].

Ageing is primarily found to be caused due to the free radicals present in the body which are known as reactive oxygen species (ROS).

Through different studies, various mechanisms have been found for skin ageing and age related skin changes which include oxidative stress theory of free radicals, mitochondrial dysfunction, telomere shortening and UV radiation. Also, there are various other mechanisms that are taken together or alone which may or may not accelerate the changes in skin.

Oxidative stress is considered to be a very important mechanism in the management of skin ageing. As per the free radical theory of ageing, molecular activity takes place due to the free radicals which are formed during the lifetime of a person. These free radicals are mainly the free oxygen radicals present in the body formed during aerobic metabolism. Thus in this situation ROS level rises and antioxidant activity declines.

Mitochondria also play an important role and if there is any accumulation of mutations in mitochondrial DNA, then there is an imbalance in the expression of antioxidant enzymes leading to over production of ROS.

Shortening of DNA telomerase is another cause for skin ageing. Additionally, there can be some premature ageing syndromes as well as nutritional factors responsible for ageing.

Senescence is yet another mechanism causing skin ageing and it is called as 'biological ageing'. It involves gradual deterioration of functional characteristics of many life forms and can cause both cellular as well as whole organism senescence. The study of biological aging is known as Biogerontology. However, it's not the primary cause of skin ageing and it depends on conditions like oxidative deterioration, genetic makeup and lifestyle.

CAUSES OF INTRINSIC AND EXTRINSIC AGEING

Ageing is influenced by various personal & environmental factors. Both cause progressive loss of structural integrity and physiological function. Damage due to light (photodamage) leads to an increase in the risk of cutaneous neoplasms.

Intrinsic Ageing:

It takes place due to the following factors:-

Ethnicity:

Effects of ethnicity on ageing is because of differences in pigmentation. More is the pigmentation, better is the protection obtained from cumulative effects of ageing.

Anatomical changes:

Differences in the thickness of skin in different areas of the body also alters ageing. Eyelids have skin thickness of <0.5 mm whereas the soles of feet have a thickness of 6mm. Thus, the skin of eyelids undergoes faster ageing than the skin of feet.

Hormonal changes:

As age changes, hormonal changes take place and this is another factor causing ageing.

Extrinsic Ageing:

It takes place due to the following factors:-

Lifestyle influence:

Lifestyle has a strong influence on skin ageing and include conditions like sleeping positions, diet, repetitive muscle movements, environmental pollution, exposure to harmful UV rays etc. Smoking causes elastosis and telangiectasia (red spots on skin). \ decreases the blood flow to skin and thus skin becomes deprived of oxygen and nutrients. There is less collagen and elastin fibres in dermis thus making skin slake, hard and less elastic. Nicotine in cigar causes constriction of vasculature which in turn leads to wrinkles. Thus, smoking causes an increase in dyslexia and roughness of skin.

Exposure to UV light (photoageing):

This type of skin ageing accounts for about 90% of the total skin ageing in people with less melanocytes.

Sunlight is composed of 3 different types of radiations namely UVC, UVB and UVA respectively.

UVC (100-290nm), these rays are largely blocked by ozone layer and hence have a little impact on skin. UVB (290-320 nm) rays penetrate into the epidermis of the skin and cause erythema from sunburn. UVA(320-400) rays however, requires 1000 fold high level to cause damage so earlier it was not considered as a factor for damaging skin but since it penetrates the dermis of skin it is now considered to cause photoageing.

UV radiations cause an indirect damage to the skin as they interfere with enzymes which are essential for DNA repair process, and they also interfere with components of the immune system (Example are T cells & Langerhans cells) that are involved in the removal of carcinogenic cells.

NIOSOMES

Niosomes were first discovered by Handjanivila et al. in 1979. Niosomes, also called as non-ionic surfactant vesicles are microscopic lamellar structures which are formed by the admixture of non-ionic surfactant and cholesterol [26]. They are formed by the self-assembly of amphiphilic molecules into closed bilayers[27]. Since they have an amphiphilic bilayer structure they can entrap both hydrophilic as well as hydrophobic drugs [28]. Appropriate mixtures of surfactants and charge inducing agents give thermodynamically stable vesicles. Other factors contributing to the formation of niosomes include HLB value of the amphiphilic molecule, aqueous interlayer, lipid chain-length, chain-packing and membrane asymmetry. The structure of niosome is represented below:

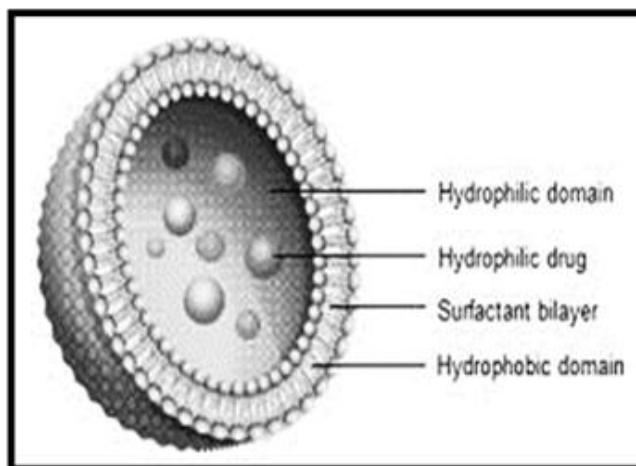


Figure 1: Structure of niosome [29].

They can be formulated as small unilamellar vesicles (SUV, size=0.025-0.05 μm), large unilamellar vesicles (LUV, size=>0.10 μm) and multilamellar vesicles (MLV, size=>0.05 μm).

These surfactant based vesicles provide targeted and controlled drug delivery and also enhance the permeation of drug through the skin thus providing efficient drug delivery. Niosomes are biodegradable, biocompatible, non-toxic, have low production cost, easy storage and handling [30].

Carlotta Marianecci et al. studied the anti-inflammatory activity of ammonium glycyrrhizinate niosomes in human and murine models. The vesicles had a particle size of $79.0 \pm 1.3 \text{ nm}$, zeta potential $79.0 \pm 1.3 \text{ mV}$ and entrapment efficiency 28.8 %. The *in vitro* evaluation of toxicity was evaluated on human keratinocyte NCTC2544 cells and a cellular mortality higher than 70% was observed. The *in vivo* evaluation of skin tolerability was done on human volunteers and the results showed that unloaded niosomes were not able to elicit the erythema induction with respect to the control. The study reported that niosomes showed no toxicity, good skin tolerability and were able to improve the drug anti-inflammatory activity in mice. Also, an improvement of the anti-inflammatory activity was observed on chemically induced skin erythema in humans [31].

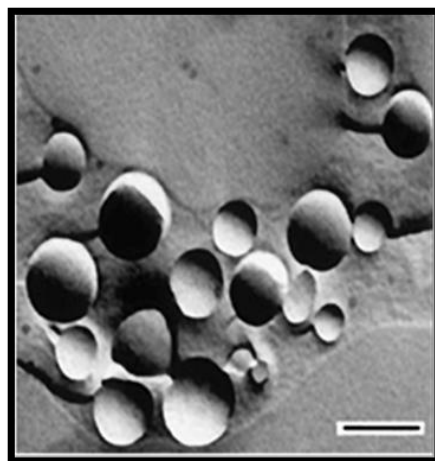


Figure 2: Transmission electron micrographs of drug-loaded vesicles after freeze-fracture. The bar corresponds to 100 nm [31].

Niosomes have been used for the dermatological purpose in cosmetic industry. The cosmetic brand that first developed and patented niosomes was L'Oréal. The first cosmetic product 'Niosome' was launched into the market by Lancome in 1987. Later the product 'Niosome Plus' an anti-ageing cream was developed. Niosomes offers several advantages in cosmetic and skin care products due to their ability to increase the stability of entrapped drugs with improved bioavailability of poorly absorbed ingredients thus enhancing skin penetration.

In a study conducted by Tavano et al. demonstrated that co-encapsulation of lipophilic anti-oxidants into niosomes improved percutaneous permeation. The anti-oxidants encapsulated include resveratrol/curcumin and alpha-tocopherol/curcumin. The *in-vitro* percutaneous permeation of the anti-oxidants was found to be controlled and improved as compared to the free solutions which were used as control. Also the combination of anti-oxidants gave a synergistic effect and promoted the ability to reduce free radicals [32].

MECHANISM OF TRANSDERMAL DRUG DELIVERY THROUGH NIOSOMES.

Transdermal delivery indicates the delivery of drugs through skin. Stratum corneum is the most impermeable barrier of the skin therefore for efficient drug delivery the formulation should be able to pass through this barrier.

- There is no single mechanism that can completely explain the ability of niosomes to increase drug transfer through the skin and several mechanisms have been proposed which are as follows: Niosomes diffuse through the layer of stratum corneum after which the lipophilic drug crosses the stratum corneum through fusion, aggregation and adhesion. Then the niosomes cause the cells of the stratum corneum to be loosened thereby increasing the permeation of drugs. This permeation is enhanced by non-ionic surfactant which leads to improved drug permeation through skin. Niosomes adsorb on to cell surface with little or no internalization of either aqueous or lipid components, it may take place because of two reasons, either as a result of attracting physical forces or as a result of binding by specific receptors to ligands on the vesicle membrane and then transfer of drug directly from vesicles to the skin.
- Niosomes may fuse with the cell membrane, which leads to complete mixing of the niosomal contents with the cytoplasm.
- Finally, by the process of endocytosis, niosomes may be engulfed by the cell thus releasing the entrapped drug into the medium [33].

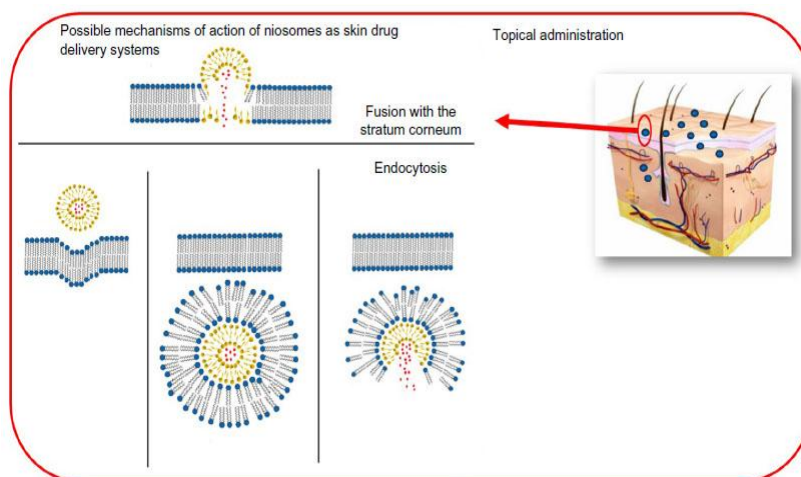


Figure 3: Mechanism of drug delivery into skin through niosome [33].

COMPONENTS REQUIRED FOR THE FORMATION OF NIOSOMES

The two major components used for the formation of niosomes are Non-ionic surfactant and Cholesterol.

Non-ionic surfactant

Non-ionic surfactants play a major role in niosomal formulations. The surfactant used must have a hydrophilic head and a hydrophobic tail. The hydrophobic moiety may consist of 1 or 2 alkyl or perfluoroalkyl groups or in certain cases a single stearyl group. The surfactants with alkyl chain length from C12-C18 are suitable for preparation of niosome. The most commonly used surfactants are given in Table 1:

Table 1: List of various types of non-ionic surfactants [26].

| Types of non-ionic surfactant | Examples |
|-------------------------------|--|
| Fatty alcohol | Cetyl alcohol, Stearyl alcohol, Cetostearyl alcohol, oleyl alcohol |
| Ethers | Brij, Lauryl glucoside, Decylglucoside, Octylglucoside, Triton X-100 |
| Esters | Spans, Polysorbates, Glyceryllaurate |
| Block co-polymers | Polaxomer |

A new class of surfactants called 'bola surfactants' have been synthesized. Bola forms amphiphiles and are composed of two identical Aza crown ether units, polar heads, linked to a long alkyl chain and represent a new class of non-ionic surfactants, which are able to assemble in colloidal structures if associated with cholesterol. The ether type surfactants with single chain alkyl as hydrophobic tail is more toxic than corresponding dialkylether chain. The ester type surfactants are chemically less stable than ether type surfactants and the former is less toxic than the latter due to ester-linked surfactant degraded by esterases to triglycerides and fatty acid in vivo[34].

In a study conducted by Van Hal et al. also suggested the use of bola surfactant. It was reported that estradiol encapsulated in niosomes could be delivered through stratum corneum which is the most impermeable protective barrier using Bola surfactant for preparing niosomes. Bola surfactant has been proved to be highly effective in percutaneous drug delivery. Also studies have shown that they improve percutaneous passage of drugs through human stratum corneum and epidermis and are non-toxic[35-37]. (Show one study in details, do we have the chemical structure of bola)

Donatella Paolino et al. developed bola surfactant niosomes containing α, ω -hexadecyl-bis-(1-aza-18-crown-6) (Bola), Span 80® and cholesterol (2:5:2 molar ratio) for topical delivery of 5-fluorouracil (5-FU) to treat different forms of skin cancer. 5-FU-loaded bola-niosomes showed an improvement of the cytotoxic effect with respect to the free drug. They evaluated the percutaneous permeation of 5-FU loaded niosomes on human stratum corneum and epidermis membranes. They reported enhanced drug penetration of 8- and 4-folds with respect to an aqueous solution of drug and to a mixture of empty bola-niosomes in an aqueous solution of drug [38].

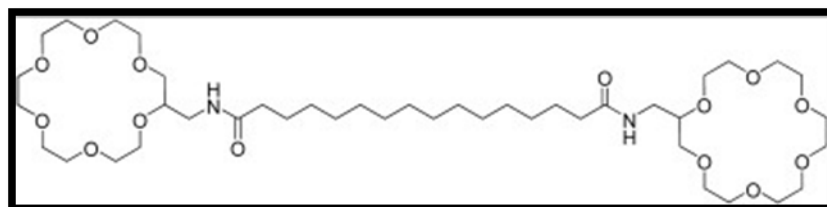


Figure 4: Chemical structure of α, ω -hexadecyl-bis-(1-aza-18-crown-6) (Bola) [38].

Maria Manconi et al. conducted a study in which a comparison was made between niosomes and liposomes both loaded with tretinoin and the commercial product of the drug RetinA® which was used as control. Tretinoin was incorporated into vesicles at saturated and unsaturated concentrations. Niosomes were prepared using two different commercial mixtures of Alkyl polyglycosides (APGs) that is ORAMIX CG110 and ORAMIX NS10 and with a polyoxyethylene lauryl ether (Br30) whereas the liposomes were prepared using soy phosphatidylcholine (P90). Thin film hydration method was adopted for the preparation of vesicles. It was observed that niosomes made from Br30 (HLB = 9.7) showed a smaller mean size than those prepared with ORAMIX NS10 (HLB = 11) whose niosomes in turn were smaller than vesicles made from ORAMIX CG110 (HLB = 16). The possible explanation for this behaviour was the higher hydrophilicity of the surfactant which led to increased water uptake and hence increased vesicle size and the amount of tretinoin which could be incorporated. Also drug saturated formulations gave high incorporation capacity, ranging from 94.96% (Br30/SA UVs) to 99% (ORAMIX CG110/SA UVs) while the liposomes showed the lowest incorporation efficiency 75%. The *in vitro* skin permeation studies was done on the skin of new born pig using Franz diffusion cells and the results showed that the permeation increased in the order of ORAMIX NS10 < Br 30 < P 90 < ORAMIX CG110 vesicles. Therefore the study concluded that niosomes are better vesicles for the transdermal delivery of tretinoin as compared to liposomes. Moreover saturated vesicles highly promoted drug accumulation in the pig skin, while the same did not occur when the vesicular bilayer is not saturated with the drug. Also this study proved that more hydrophilic surfactant such as ORAMIX CG110 (HLB = 16) are better candidates for niosome formation which greatly enhances the drug cutaneous retention [39].

Cholesterol

Cholesterol influences the physical properties and structure of niosomes because of its interaction with non-ionic surfactant. The incorporation of cholesterol into bilayer of niosome induces membrane stabilization and decreases the leakiness of membrane. Thus, it increases the entrapment efficiency of niosomes[35]. Many surfactants can form vesicles after the addition of cholesterol in concentrations of up to 30–50 mol%. Cholesterol being an amphiphilic molecule interacts with surfactants through hydrogen bonding between its hydroxyl group and surfactant's hydrocarbon chain which leads to an increase in the mechanical stiffness of the membranes and membrane cohesion. Cholesterol also has a condensing effect as it accommodates in the cavity which is formed by surfactant monomers. This is known as space filling function of cholesterol due to which the movement of carbons of hydrocarbon is restricted which leads to a decrease in the permeability of cholesterol containing membranes as compared to cholesterol free membranes. Also cholesterol increases the surface elasticity of membranes making them more rigid in nature. Aksornnarong Ritwiset et al. studied the molecular structure and dynamical properties of niosome bilayers with and without cholesterol incorporation. In this study, niosomes were investigated by using molecular dynamics simulations wherein the niosome bilayer was composed of sorbitan monostearate (Span60) with 0% and 50% cholesterol compositions. The simulations showed that niosome bilayer without cholesterol is in gel phase with a higher order structure whereas with cholesterol the bilayer has more fluidity with a less ordered structure. Also the niosome bilayer with 50% cholesterol inclusion showed an increase of area per lipid (~11%) and thickness (~39%). The study concluded that the addition of cholesterol to the bilayer enhances the lateral and transverse diffusion as well as an increase in the hydrogen bond number between Span60 and water. All these characteristics enhance the niosomal drug delivery system by imparting niosome bilayer the desired fluidity as well as stability[40].

Other additives

Other membrane additives like charge inducers are added into bilayer of niosomes to stabilize the formulation. Stearyl amine and cetyl pyridinium chloride are examples of positively charged molecules while dicetyl phosphate and phosphatidic acid are examples of negatively charged molecules both of which are commonly used for preventing flocculation, aggregation and fusion of niosomes because of electric repulsion. Usually they are added in concentration of 2.5 to 5 mol% since a higher concentration could inhibit the formation of niosomes.

Aranya Manosroi et al. studied niosomes prepared with hydrated mixture of various non-ionic surfactants and cholesterol. Span 60 (sorbitan monostearate), Brij 72 (polyoxyethylene 2 stearylether), Tween 61 (polyoxyethylene sorbitan monostearate), Glyceryl monostearate, Brij 30 (polyoxyethylene 4 laurylether), Diglyceryl monolaurate and Tetraglyceryl monolaurate were used as different non-ionic surfactants. The study reported that alkyl chain length of non-ionic surfactants and amount of cholesterol used to prepare vesicles affect the entrapment efficiencies of the vesicles and microviscosities of the vesicular membrane. The entrapment efficiencies of the vesicles obtained from stearyl (C18) chain surfactants (Brij 72, glyceryl monostearate, Span 60, Tween 61) were higher than surfactants with lauryl (C12) chain (Brij 30, tetraglyceryl monolaurate) because surfactants with longer alkyl chains generally give larger vesicles. Niosome prepared with Tween 61 in combination with cholesterol at 1:1 molar ratio was found to have the highest entrapment efficiency of water soluble substances because it had a larger hydrophilic head group and long alkyl side chain[41].

TYPES OF NIOSOMES

The different types of niosomes are proniosomes, aspasomes, deformable niosomes, vesicles in water and oil systems(v/w/o).

Pro-niosomes

Pro-niosome are dry granular product which get converted to niosomal suspension after subsequent hydration. They are more stable as compared to niosomes. Pro-niosomes are prepared using a mixture of a carrier and surfactant which upon hydration with excess aqueous phase gives a stable dispersion of niosomes.

Mahmoud Mokhtar et al. prepared flurbiprofen pro-niosomes using span 20, span 40, span 60, and span 80 without and with cholesterol. The effect of different processing and formulation variables such as surfactant chain length, total lipid concentration, negatively or positively charging lipids, and the pH of the dispersion medium on flurbiprofen entrapment efficiency (EE%) was studied. EE% was found to increase in the following order Span 60 (C18)>Span 40 (C16)>Span 20 (C12)>Span 80 (C18). when the hydrating medium was adjusted to pH 5.5 a maximum loading efficiency was 94.61% was obtained. It was found that increasing the amount of lipid increased the EE%. But the incorporation of either dicetyl phosphate which produces negative charge or stearyl amine which produces positive charge decreased the EE% of flurbiprofen into niosomes. *In-vitro* release data showed a biphasic release pattern from niosomes made of span 40 and span 60. Finally, upon hydrating the proniosomal formulation, niosomal vesicles were formed immediately. Thus, the study proved the potential of proniosomes as stable precursors of niosomes [42].

Aspasomes

These vesicles are formed from a mixture of ascorbyl palmitate, cholesterol and highly charged lipid. In order to form niosomes, aspasomes are first hydrated and then sonicated. Aspasomes enhance the transdermal permeation of drugs. They have been found to possess intrinsic anti-oxidant property which could be used to reduce the disorders caused by reactive oxygen species.

D. Gopinath et al. proved the potential of ascorbyl palmitate (ASP) in forming aspasomes to encapsulate azidothymidine in presence of cholesterol (CHOL) and dicetyl phosphate (DCP). A composition of ASP(45):CHOL(45):DCP(10) (mol%) gave the highest drug entrapment of $30.58 \pm 2.72\%$. The anti-oxidant activity of aspasome was found to be better than that of ascorbic acid solution. Also, the in-vitro transdermal permeation study of aspasomal azidothymidine conducted on excised rat skin using Franz diffusion cell showed much higher permeation than ascorbyl palmitate aqueous dispersion containing azidothymidine solution. This study thus indicates a promising future for aspasome as a transdermal drug delivery system [43].

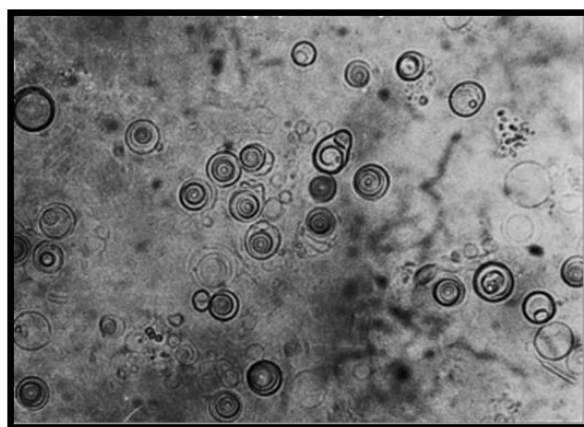


Figure 5: Unsonicated dispersion of aspasome under 450× magnification [43].

Deformable niosomes

These vesicles are also called as elastic niosomes as they are flexible in nature. They are composed of surfactants, ethanol and water. Due to their flexibility they can pass through the pores in the stratum corneum which is less than one tenth of these vesicles thus increasing the penetration efficiency and making them superior to conventional niosomes.

Aranya Manosroi et al. compared the penetration of papain from different gel formulations that is gel containing papain loaded in elastic niosomes (GEN), gel containing papain loaded in non-elastic niosomes (GNN), gel containing papain loaded in PLGA nanospheres (GPN) and gel containing free papain in phosphate buffer pH 7.0 (GS). The vesicular sizes of all niosomes and nanospheres in the gel formulations were in the range of 220.7–520.2 nm. Gel containing papain loaded elastic niosomes exhibited the accumulate amounts and fluxes of 0.226 mg/cm² and 0.029 mg/cm²/h in the whole rat skin and 0.220 mg/cm² and 0.037 mg/cm²/h in the receiving solution, which were higher than that found in GNN, GPN and GS respectively. The calculated primary irritation index (PII) values of all gel formulations were in the range of 0.00–0.44 indicating of no irritation, except GS that containing free papain (PII = 0.78, slight irritation). Gel containing papain loaded in elastic niosomes (GEN) exhibited higher reduction of hypertrophic scars of the induced scar on rabbits' ears and after histological examination it was found that the numbers of collagen fibres and the height of the scars treated with GEN were significantly decreased compared with the control group. Thus, this study concluded that papain loaded elastic niosomes gave superior skin permeation enhancement as well as reduction of hypertrophic scars therefore it can be developed as an efficient topical product for scar treatment[44].

Vesicles in water and oil system (v/w/o)

An aqueous suspension of niosomes is emulsified into the oily phase at 60°C to form vesicles in water in oil emulsion(v/w/o). Cooling this emulsion to room temperature gives vesicles in water in oil gel(v/w/o) that can entrap hydrophilic drugs which are susceptible to enzymatic degradation and also provide a controlled released pattern of drug delivery.

Toshimitsu Yoshioka et al. formulated vesicles in water and oil system (v/w/o) and investigated the *in vitro* release of 5(6)-carboxyfluorescein (CF). Different non-ionic surfactants that is sorbitan monoesters, Span 20, 40, 60 and 80 were dispersed in water droplets which were then dispersed in any one of the oils (octane, hexadecane and isopropyl myristate) leading to the formation of v/w/o emulsion. The study reported that the hydrophobicity of the Span had a significant influence on the release rate of CF. The Span 60 formulation had the slowest release rate because it has the highest phase transition temperature. When isopropyl myristate was used as the oil phase, the release rate constant was greater and almost same in all systems whereas in Span 40 and Span 60 systems, the release rate constants of v/w/o emulsions prepared with hexadecane were the almost same. but those prepared with octane varied with the surfactant. Also it was found that with increasing temperature the release rates of Span 40 and Span 80 systems increased but Span 60 system was unaffected because it caused gelation of the oil phase at 25°C. The results of the study thus conclude that the appropriate choice of surfactants, oil and temperature can be used to regulate the delivery rate of drugs by this system[45].

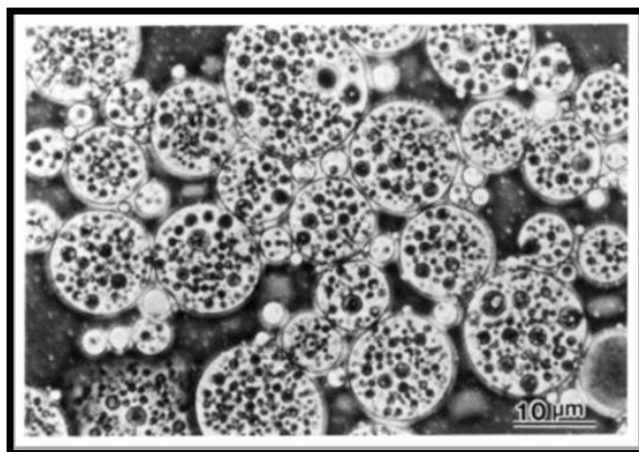


Figure 6: Photomicrograph of a v/w/o emulsion formulated with large niosomes prepared with Span 80 and 5% of Span 80 in the oil phase, octane [45].

METHODS FOR PREPARATION OF NIOSOMES

The method of preparation can also affect the niosomal properties. There are various methods used for preparation of niosomes such as ether injection, thin film hydration, reverse phase evaporation, sonication, bubble method, micro fluidisation, multiple membrane extrusion and transmembrane pH gradient technique.

Ether injection method

In this method cholesterol and non-ionic surfactant are dissolved in diethyl ether. This mixture is then injected through a 14 gauge needle into an aqueous solution of drug maintained at 60°C. The ether solution is evaporated to room temperature giving single layered vesicles having particle size in the range of 50-1000nm. The advantage of this method includes control of size, which can be obtained by controlling the size of the needle whereas limited solubility of materials in ether and difficulty to remove ether from the final formulation are the disadvantages of this method.

Thin film hydration method

In this method the vesicle forming agents, that is cholesterol and surfactant are dissolved in volatile organic solvent such as methanol, diethyl ether or chloroform in a round bottom flask. The solvent is then evaporated using rotary evaporator which leaves a thin film of solid mixture on the wall of the flask. This dried film of surfactant is hydrated using an aqueous solution of drug with gentle agitation giving milky niosomal dispersion. This is one of the most common methods forming multi lamellar niosomes.

Maria Letizia Manca et al. prepared novel diolein-niosomes for cutaneous delivery of tretinoin using thin film hydration technique. The vesicle forming agents used were Phospholipon 50 (120 mg/ml) or Plurol Oleique (100 mg/ml), Labrasol (100 mg/ml) and TRA (3.0 mg/ml). They were dissolved in chloroform and the solvent was evaporated by rotoevaporating under vacuum. This led to the formation of thin lipid film which was hydrated using phosphate buffer pH 7, resulting in the formation of MLV dispersion. The diolein niosomes had a particle size of 156 ± 4 nm, zeta potential of -48 ± 1 mV and incorporation efficiency of $15 \pm 9\%$. The results of *in vitro* studies showed that labrasol improves cutaneous drug deposition when used in combination with diolein. Finally the study reported that labrasol can be used along with non-ionic surfactants to obtain niosomes that has good potentiality in cutaneous drug delivery[46].

Reverse phase evaporation method

In a mixture of organic solvent (ether and chloroform), equal ratios (1:1) of cholesterol and surfactant is dissolved. To the above phase an aqueous phase containing the drug is added giving a mixture of two phases. This phase is sonicated at 4-5°C to form a clear gel which is further sonicated after the addition of phosphate buffer saline. The organic phase is removed using rotary vacuum evaporator at 40°C and at reduced pressure. This results in a viscous suspension of niosome which is diluted with phosphate buffer saline at 60°C for 10 minutes to yield niosomes.

Varaporn Junyaprasert et al. studied the influence of penetration enhancers such as Dimethyl sulfoxide (DMSO) or N-methyl-2-pyrrolidone (NMP) on skin penetration of ellagic acid loaded niosomes, using Reverse Phase Evaporation Method. Span 60, Tween 60 and cholesterol were used as vesicle forming agents, solulan C24 as steric stabilizer and polyethylene glycol 400 (PEG) as solubilizer. The EA-loaded niosomes had mean particles sizes in the range of 312-402 nm with PI values of lower than 0.4. The niosomes were observed under transmission electron microscope and optical microscopy which determined it to be spherical multilamellar vesicles. All niosomes were stable after 4 months storage at 4 degree. The results of *in vitro* evaluation of skin permeation through human epidermis was done, and the result showed that the skin enhancers affected the penetration of EA from the niosomes at 24 h. DMSO niosomes were found to have highest EA amount in epidermis; whereas the NMP niosomes had the highest EA amount in acceptor medium. The skin distribution by confocal laser scanning microscopy showed the high fluorescence intensity of the DMSO niosomes and NMP niosomes at a penetration depth of between 30-90 micrometer (the epidermis layer) and 90-120 micrometer (the dermis layer) under the skin, respectively. The study concluded that the DMSO niosomes are suitable for epidermis delivery of EA and the NMP niosomes can be used for dermis delivery of EA[47].

Sonication

An aqueous solution of drug in buffer is added to the mixture of surfactant and cholesterol. This mixture is added to a 10ml glass vial which is probe (titanium probe) sonicated for 3 minutes at 60°C to yield niosomes. This method is also used to form SUV's from MLV's.

Warintorn et al. evaluated transdermal absorption and physicochemical properties of gel containing niosomes loaded with *Volvarella Volvacea* extract. *V. volvaceais* a paddy straw mushroom and has been found to possess anti-oxidant properties and hence potent anti-ageing activity. *V. Volvacea* extract loaded niosomes were prepared by the method of sonication using 1:1 molar ratio of 20Mm of Tween 61 and cholesterol which were then incorporated into gel. A maximum loading of 2% (w/v) *V. Volvacea* extract was done into the niosomes which gave an average size of 254 ± 20.32 nm and zeta potential of -50.07 ± 3.11 mV showing good physical stability. The transdermal absorption of gel was studied on rat skin using Franz diffusion cell and the result showed that formulation retarded the cumulative amounts and fluxes of the total phenolic content in the extract in the first hour of skin permeation, while enhanced the skin permeation at the 6th hour. Thus, the study concluded that niosomes containing *V. volvacea* extract could be developed as an topical anti-ageing formulation giving superior occlusion effect beneficial for saturation in the skin [48].

Bubble method

This method has the advantage of preparing niosomes in one step without the use of organic solvent. In this method, all the vesicle forming components were dispersed in aqueous solution of drug and mixed for 15 sec using a high shear homogenizer. This homogenous dispersion was placed in a round bottom flask which had three necks. First neck was water cooled reflex. Second neck was thermometer to check the temperature and third neck was to provide the nitrogen supply. After homogenization the dispersion was immediately bubbled using a continuous stream of nitrogen gas bubbles giving niosomes, having mean particle size between 0.2 and 0.5 μ m.

Micro fluidization method

In this method two fluidized streams were made to interact at high velocities in micro channels within an interaction chamber. Due to the effect of high speed and energy small uniform uni-lamellar niosomes were formed. This was known as submerged jet principle which was found to have better reproducibility as compared to other methods.

Multiple membrane extrusion

A mixture of surfactant, cholesterol and di-acetyl phosphate was dissolved in chloroform after which the solvent was evaporated using rotary evaporator leaving a thin film. This film was then hydrated using an aqueous solution of drug. The suspension formed was then extruded through polycarbonate membrane which was placed in series for up to 8 passages giving niosomes.

Transmembrane pH gradient technique

In this technique the surfactant and cholesterol placed in a round bottom flask and dissolved using an organic solvent such as chloroform. The solvent evaporated under reduced pressure thereby leaving a thin film on the walls of the flask. This film was hydrated by vortex mixing using citric acid having pH 4.0 which leads to the formation of multi lamellar vesicles. These vesicles were then frozen and thawed 3 times after which they were subjected to sonication thus forming niosomal suspension to which an aqueous solution of drug was further added. The pH of the aqueous drug sample was then increased to 7-7.5 using a base, usually disodium hydrogen phosphate so that a pH gradient was created across the niosomal membrane. In order to obtain niosomes this mixture was heated for 10 minutes at 60°C. The neutral pH gave mixture of protonated (membrane impermeable) and un-protonated (membrane permeable) forms of drug of which the un-protonated form of the drug crossed the niosome membrane and became protonated after entering the acidic medium thus getting trapped into the vesicle. This diffusion across the bilayer continued until the interior and exterior concentrations of drug attained equilibrium[49].

CHARACTERIZATION OF NIOSOMES

Niosomes are characterized for their size, shape and morphology, bilayer of niosomes, number of lamellae, vesicle diameter and charge, encapsulation efficiency, in-vitro release kinetics etc.

Size, shape and morphology

Structure of niosomes is studied using Scanning electron microscopy (SEM)/Transmission electron microscopy (TEM). The size or diameter of niosomal vesicle can be determined by Photon correlation spectroscopy where it helps to determine the mean diameter of vesicles. Visualization can simply be done by freeze fracture microscopy. Laser beam spectroscopy is used to study the morphology as well as size distribution of niosomes. Electron microscopy can also be used to study morphology of niosomal vesicles. Molecular sieve chromatography is also being used to study mean diameter of vesicles.

Formation of bilayer and number of lamellae

Bilayer which is formed by the assembly of non-ionic surfactants is characterized using X-cross formation under light polarization microscopy. Number of lamellae can be determined using various techniques like electron microscopy, nuclear magnetic resonance (NMR), small angle x-ray scattering etc.

Vesicle charge

Vesicle charge plays an important role and zeta potential of individual niosome can help to estimate surface charge. Since, charged niosomes are more stable against fusion and aggregation they are preferred over uncharged ones. Charged vesicles are measured using micro electrophoresis. Recently various methods have been used for same, like pH sensitive fluorophores, dynamic light scattering etc [49].

Measurement of Vesicle size

In order to study vesicle size, particle size analyser is used. Vesicular dispersion is diluted 100 times using solvent used for its formulation. In this, the apparatus consists of Helium-Neon laser beam of 632.8 nm which is focused with a minimum power of 5 mW with a Fourier lens [R-5] toward the point at the centre of multielement detector and it has a small volume sample holding cell. The sample used to check the particle size is stirred first and then used. Average niosomal vesicle size ranges from 6µm to 14µm respectively depending on method from which they are derived [50].

Membrane rigidity and homogeneity

Membrane rigidity is determined by studying rigidity of fluorescence probe. It is studied as a function of temperature. Both biodistribution and biodegradation of niosomes depends on membrane rigidity. Homogeneity is identified in both cases namely in niosomes structure themselves and between niosomes in dispersions. Techniques used to identify are FT-IR, NMR, DSC etc.

Drug loading and encapsulation efficiency

Drug loading and encapsulation efficiency of niosomes is done using ultracentrifugation method in which from the aqueous suspension of niosomes supernatant layer is removed and the sediment is washed with suitable solvent so that any adsorbed drug is removed.

Niosomal recovery is calculated as:

$$\text{Niosomal recovery\%} = \frac{\text{Amount of niosome recovered}}{\text{Amount of polymer + drug + excipient}} \times 100$$

Drug loading is calculated as:

$$\text{Drug loading} = \frac{\text{Amount of drug in niosomes}}{\text{Amount of niosomes recovered}} \times 100$$

***In-vitro* drug release**

In order to study *in-vitro* drug release, dialysis bag is used. The suspension of niosomes is inserted into the dialysis bag with pipette and then sealed. This is placed in buffer solution of 200ml in 250 ml beaker and stirred magnetically. Temperature is maintained at 25-37°C and sample is withdrawn at regular intervals of time. This sample is then analysed at appropriate wavelength using UV or any other assay method.

Stability studies

In order to study the stability of niosomes they are sealed in airtight containers and kept at different temperatures. Drug retained in the niosomes is used as a parameter to carry out the stability studies and the drug retained is withdrawn after particular time intervals like (0,1,2,3 months) and their colour, drug retaining capacity etc. are determined. Further their stability is conducted using various analytical methods like UV, HPLC etc.

In a study conducted by Manosroi A et al. can help us understand about stability of niosomes. In this a gel was prepared containing niosomes loaded with a semi-purified fraction containing gallic acid from Terminalia chebula galls (Family Combretaceae) which was found to enhance longevity. The semi-purified fraction containing phenolic compounds was loaded in elastic and non-elastic niosomes and the prepared gel was evaluated using closed patch test for rabbit skin irritation and the skin anti-ageing effect was studied in human volunteers by measuring the skin elasticity and roughness. The results demonstrated that when elastic and non-elastic niosomal gel was applied the % parameter changes of skin elastic recovery and skin elastic extension were -21.25 and -22.63%, +28.73 and +32.57; respectively. Also these gels showed a significant decrease of the maximum and average roughness values with the parameter changes of -39.47 and -35.28%, -29.43 and -32.38; respectively. Thus, the loaded niosomes gave higher chemical stability of gallic acid and more *in-vivo* anti-ageing activity [51].

CONCLUSION

Niosomes are a promising dermal drug delivery system with remarkable advantages such as good permeation, cost effective, more stable, and relatively non-toxic. Thus, niosomes have proven to be a new generation drug delivery systems after liposomes. Niosomal formulations with proper preparation techniques along with selection of good non-ionic surfactant, cholesterol content and nature of incorporated drug can prove to be a potential consideration in the field of cosmetics which could help in the treatment of skin ageing and wrinkles. Overall, niosomes are an effective tool for drug delivery and have the potential to provide better treatment than the conventional drug delivery systems. However, the technology utilized in niosomes is still in its infancy and requires further research and exploration in order to bring out a commercially available niosomal anti-ageing product.

COMPETING INTERESTS:

The authors declare no conflict of interest.

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