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Proniosomes – A Promising Drug Carriers

Sudhamani.T*, Priyadarisini.N, Radhakrishnan.M

Department of Pharmaceutics,

The Erode College of Pharmacy, Veppampalayam, Erode, 638112, Erode, INDIA

Corres.author: tsm_pd@yahoo.com

Abstract: Nanotechnology is an advancing technology expected to bring revolutionary changes in the field of life sciences including drug delivery, diagnostics, nutraceuticals and biomedical for implants and prosthesis. The advance in nanotechnology helps in preparing newer formulations. One of the advancement in nanotechnology is the preparation of proniosomes - derived niosomes. Proniosomes are solid colloidal particles which may be hydrated immediately before use to yield aqueous niosome dispersions similar to those produced by more cumbersome conventional methods. These 'proniosomes' minimize problems of niosome physical stability such as aggregation, fusion and leaking, and provide additional convenience in transportation, distribution, storage, and dosing. This article describes the preparation of dispersions of proniosome-derived niosomes; In addition proniosome-derived niosomes are described in terms of their morphology, particle size, particle size distribution, and drug release. In all parameters proniosome-derived niosomes are as good as or better than conventional niosomes.

Keywords: Niosomes, Proniosomes, Stability, Drug release.

Introduction

Colloidal particulate carriers such as liposomes [3] or niosomes [25] have been widely employed in drug delivery systems and producing them from proniosomes provides them a distinctive advantage. These carriers can act as drug reservoirs and the rate of drug release can be controlled by modification of their composition. These lipid vesicles can carry both hydrophilic drugs (by encapsulation) and hydrophobic drugs (in lipid domain). Due of their capability to carry a variety of drugs, these lipid vesicles have been extensively used in various drug delivery systems [32] like drug targeting [33] controlled release [34] and permeation enhancement of drugs [47] But there remains certain draw backs to be addressed and can be avoided if they are prepared in dry form. Proniosomes, prepared in dry form and hydrated by agitation in hot water to form niosomes provide an alternative with prospective for drug delivery via the transdermal route [6, 15, 42]

Niosomes:

Niosomes are non-ionic surfactant vesicles that can entrap a solute in a manner analogous to liposomes. They are osmotically active, and are stable on their own, while also increasing the stability of the entrapped drugs [1, 2]. Handling and storage of surfactants require no special conditions. Niosomes possess an infrastructure consisting of hydrophilic and hydrophobic moieties together, and as a result, can accommodate drug molecules with a wide range of solubilities [3]. Although niosomes as drug carriers have shown advantages such as being cheap and chemically stable, they are associated with problems related to physical stability such as fusion, aggregation, sedimentation and leakage on storage[4]. All methods traditionally used for preparation of niosomes are time consuming and many involve specialized equipments. Most of these methods allow only for a predetermined lot size so material is often wasted if smaller quantities are required for particular dose application [5] .The size of niosomes are microscopic and lies in nanometric scale. The particle size ranges from 10nm-100nm.

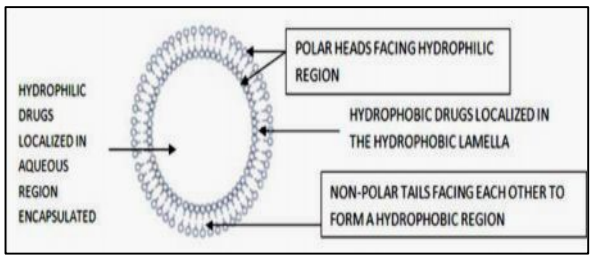

Fig 1: Representation of Niosomes.

Disadvantages of Niosomes:

1. Physical instability
2. Aggregation
3. Fusion
4. Leaking of entrapped drug
5. Hydrolysis of encapsulated drugs which limiting the shelf life of the dispersion

To overcome these Disadvantages, proniosomes are prepared and reconstituted into niosomes.

Proniosomes:

Hu and Rhodes et al [7] reported that Proniosomes are dry formulations of surfactant-coated carrier, which can be measured out as needed and rehydrated by brief agitation in hot water. These "proniosomes" minimize problems of niosomes physical stability such as aggregation, fusion and leaking and provided additional convenience in transportation, distribution, storage and dosing.

Proniosome-derived niosomes are superior to conventional niosomes in convenience of storage, transport and dosing. Stability of dry proniosomes is expected to be more stable than a pre-manufactured niosomal formulation. In release studies proniosomes appear to be equivalent to conventional niosomes. Size distributions of proniosome-derived niosomes are somewhat better that those of conventional niosomes so the release performance in more critical cases turns out to be superior [6-9].

Proniosomes are dry powder, which makes further processing and packaging possible. The powder form provides optimal flexibility, unit dosing, in which the proniosome powder is provided in capsule could be beneficial.

A proniosome formulation based on maltodextrin was recently developed that has potential applications in deliver of hydrophobic or amphiphilic drugs. The better of these formulations used a hollow particle with exceptionally high surface area. The principal advantage with this formulation was the amount of carrier required to support the surfactant could be easily adjusted and proniosomes with very high mass ratios of surfactant to carrier could be prepared. Because of the ease of production of proniosomes using the maltodextrin by slurry method, hydration of surfactant from proniosomes of a wide range of compositions can be studied [8-10].

Advantages of proniosomes over the niosomes [7-10]:

- Avoiding problem of physical stability like aggregation, fusion, leaking.
- Avoiding hydrolysis of encapsulated drugs which limiting the shelf life of the dispersion.

Methodology

Preparation of proniosomes:

The proniosomes can be prepared by

1. Spraying method.
2. Slurry method.

Spraying method [7]:

(Hu and Rhodes et al in 1999) prepared proniosomes by spraying the surfactant in organic solvent onto sorbitol powder and then evaporating the solvent. Because the sorbitol carrier is soluble in the organic solvent, it is necessary to repeat the process until the desired surfactant load has been achieved. The surfactant coating on the carrier comes out to be very thin and hydration of this coating allows multilamellar vesicles to form.

Slurry method [8-11]:

(Almira .I and Blazek - Walsh et al [8] in 2001) developed slurry method to produce proniosomes using maltodextrin as a carrier. The time required to produce proniosome by this is independent of the ration of surfactant solution to carrier material. In slurry method, the entire volume of surfactant solution is added to maltodextrin powder in a rotary evaporator and vacuum applied until the powder appears to be dry and free flowing.

Drug containing proniosome-derived niosomes can be prepared in manner analogous to that used for the conventional niosomes, by adding drug to the surfactant mixture prior to spraying the solution onto the carrier (sorbitol, maltodextrin) or by addition of drug to the aqueous solution used to dissolve hydrate the proniosomes.

Formation of Niosomes from Proniosomes [8 – 11]:

The niosomes can be prepared from the proniosomes by adding the aqueous phase with the drug to the proniosomes with brief agitation at a temperature greater than the mean transition phase temperature of the surfactant.

$$T > Tm$$

where,

T = Temperature
Tm = mean phase transition temperature

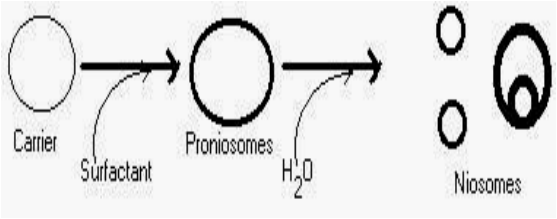

Fig 2: Formation of Niosomes from Proniosomes.

Blazek-Walsh A.I. et al has reported the formulation of niosomes from maltodextrin based proniosomes. This provides rapid reconstitution of niosomes with minimal residual carrier. Slurry of maltodextrin and surfactant was dried to form a free flowing powder, which could be rehydrated by addition of warm water [9].

Separation of Unentrapped Drug:

The removal of unentrapped solute from the vesicles can be accomplished by various techniques, which include: -

1. **Dialysis [11, 12, 15]:**

The aqueous niosomal dispersion is dialyzed in dialysis tubing against suitable dissolution medium at room temperature. The samples are withdrawn from the medium at suitable time intervals, centrifuged and analysed for drug content using suitable method (U.V. spectroscopy, HPLC etc).

2. **Gel Filtration [13, 14]:**

The unentrapped drug is removed by gel filtration of niosomal dispersion through a Sephadex-G-50 column and eluted with suitable mobile phase and analyzed with suitable analytical techniques.

3. **Centrifugation [6,7,11]:**

The proniosome derived niosomal suspension is centrifuged and the supernatant is separated. The pellet is washed and then resuspended to obtain a niosomal suspension free from unentrapped drug.

Characterization of proniosomes:

Measurement of Angle of repose [6 - 11]:

The angle of repose of dry proniosomes powder was measured by a funnel method (Lieberman et al 1990). The proniosomes powder was poured into a funnel which was fixed at a position so that the 13mm outlet orifice of the funnel is 5cm above a level black surface. The powder flows down from the funnel to form a cone on the surface and the angle of repose was then calculated by measuring the height of the cone and the diameter of its base.

Scanning electron microscopy [8-11]:

Particle size of proniosomes is very important characteristic. The surface morphology (roundness, smoothness, and formation of aggregates) and the size distribution of proniosomes were studied by Scanning Electron Microscopy (SEM).Proniosomes were sprinkled on to the double- sided tape that was affixed on aluminum stubs. The aluminum stub was placed in the vacuum chamber of a scanning electron microscope (XL 30 ESEM with EDAX, Philips, Netherlands). The samples were observed for morphological characterization using a gaseous secondary electron detector (working pressure: 0.8 torr, acceleration voltage: 30.00 KV) XL 30, (Philips, Netherlands).

Optical Microscopy [9, 11, 15]:

The niosomes were mounted on glass slides and viewed under a microscope (Medilux-207RII, Kyowa-Getner, Ambala, India) with a magnification of 1200X for morphological observation after suitable dilution. The photomicrograph of the preparation also obtained from the microscope by using a digital SLR camera

Measurement of vesicle size [11, 15]:

The vesicle dispersions were diluted about 100 times in the same medium used for their preparation. Vesicle size was measured on a particle size analyzer (Laser diffraction particle size analyzer, Sympatec, Germany). The apparatus consists of a He-Ne laser beam of 632.8 nm focused with a minimum power of 5 mW using a Fourier lens [R-5] to a point at the center of multielement detector and a small volume sample holding cell (Su cell). The sample was stirred using a stirrer before determining the vesicle size. Hu C. and Rhodes [7] in 1999 reported that the average particle size of proniosomes derived niosomes is approximately

6µm while that of conventional niosomes is about 14µm.

Entrapment efficiency [7, 19, 42]:

Entrapment efficiency of the niosomal dispersion in can be done by separating the unentrapped drug by dialysis [11, 12, 15], centrifugation [6,7,11] or gel filtration [13, 14] as described above and the drug remained entrapped in niosomes is determined by complete vesicle disruption using 50% n-propanol or 0.1% Triton X-100 and analyzing the resultant solution by appropriate assay method for the drug. Where,

$$\text{Percent Entrapment} = \frac{\text{total drug} - \text{diffused drug}}{\text{total drug}} \times 100$$

In-vitro methods for the assessment of drug release from proniosomes

In vitro drug release can be done by *(Chen DB et al., 2001)*

- Dialysis tubing
- Reverse dialysis
- Franz diffusion cell

Dialysis tubing:

Muller et al [35] in 2002 studied in vitro drug release could be achieved by using dialysis tubing. The proniosomes is placed in prewashed dialysis tubing which can be hermetically sealed. The dialysis sac is then dialyzed against a suitable dissolution medium at room temperature; the samples are withdrawn from the medium at suitable intervals, centrifuged and analysed for drug content using suitable method (U.V. spectroscopy, HPLC etc). The maintenance of sink condition is essential.

Reverse dialysis [35]:

In this technique a number of small dialysis as containing 1ml of dissolution medium are placed in proniosomes. The proniosomes are then displaced into the dissolution medium. The direct dilution of the proniosomes is possible with this method; however the rapid release cannot be quantified using this method.

Franz diffusion cell [32, 36]:

The in vitro diffusion studies can be performed by using Franz diffusion cell. Proniosomes is placed in the donor chamber of a Franz diffusion cell fitted with a cellophane membrane. The proniosomes is then dialyzed against a suitable dissolution medium at room temperature; the samples are withdrawn from the medium at suitable intervals, and analysed for drug content using suitable method (U.V spectroscopy, HPLC, etc) .the maintenance of sink condition is essential.

Drug Release Kinetic Data Analysis [37]:

The release data obtained from various formulations were studied further for their fitness of data in different kinetic models like Zero order, Higuchi's and peppa's.

In order to understand the kinetic and mechanism of drug release, the result of *in-vitro* drug release study of Niosome were fitted with various kinetic equation like zero order (**Equation 1**) as cumulative % release vs. time, higuchi's model (**Equation 2**) as cumulative % drug release vs. square root of time. r^2 and k values were calculated for the linear curve obtained by regression analysis of the above plots

$$C = k_0 t \qquad \text{.....(1)}$$

Where k_0 is the zero order rate constant expressed in units of concentration / time and t is time in hours.

$$Q = k_H t^{1/2} \qquad \text{.....(2)}$$

Where k_H is higuchi's square root of time kinetic drug release constant

To understand the release mechanism ***in-vitro*** data was analyzed by peppa's model **(Equation 3)** as log cumulative % drug release vs. log time and the exponent n was calculated through the slope of the straight line.

$$M_t / M_\infty = bt^n \qquad \text{.....(3)}$$

Where M_t is amount of drug release at time t, M_∞ is the overall amount of the drug, b is constant, and n is the release exponent indicative of the drug release mechanism. If the exponent n = 0.5 or near, then the drug release mechanism is Fickian diffusion, and if n have value near 1.0 then it is non-Fickian diffusion

Osmotic shock [3, 5]:

The change in the vesicle size can be determined by osmotic studies. Niosomal formulations are incubated with hypotonic, isotonic, hypertonic solutions for 3 hours. Then the changes in the size of vesicles in the formulations are viewed under optical microscopy.

Stability studies [15]:

To determine the stability of proniosomes, the optimized batch was stored in airtight sealed vials at different temperatures. Surface characteristics and percentage drug retained in proniosomes and

proniosomes derived niosomes were selected as parameters for evaluation of the stability, since instability of the formulation would reflect in drug leakage and a decrease. in the percentage drug retained. the proniosomes were sample at regular intervals of time (0,1,2,and 3months),observed for color change, surface characteristics and tested for the percentage drug retained after being hydrated to form niosomes and analysed by suitable analytical methods(UV spectroscopy, HPLC methods etc)

Zeta potential analysis [38]:

Zeta potential analysis is done for determining the colloidal properties of the prepared formulations. The suitably diluted proniosome derived niosome dispersion was determined using zeta potential analyzer based on electrophoretic light scattering and laser Doppler velocimetry method (Zetaplus™, Brookhaven Instrument Corporation, New York, USA). The temperature was set at 25°C. Charge on vesicles and their mean zeta potential values with standard deviation of 5 measurements were obtained directly from the measurement.

Niosomes as drug carriers:

Several studies have described the properties of niosomes as drug carriers. Niosomes behave similarly to liposomes *in vivo* by prolonging circulation time of the encapsulated drug and altering chemical distribution within the body [1, 16, 17]. However, niosomes have advantages over liposomes as drug carriers, including greater chemical stability, lower cost, easier storage and handling, and are less likely than liposomes to become toxic[39]. Niosomal encapsulation reduces toxicity of drugs in many different applications and therapies. Niosomal drug delivery has been studied using various methods of administration[9], including intramuscular[40], intravenous [41] peroral [30] and transdermal [8, 10,11,13,15] Nebulized surfactants entrapping all-*trans*-retinoic acid (ATRA) were delivered as an inhaled aerosol reducing the drug toxicity and altering the pharmacokinetics. In addition, as drug delivery vesicles, niosomes have been shown to enhance absorption of some drugs across cell membranes to localize in targeted organs [41] and tissues [1, 16] and to elude the reticulo-endothelial system (RES) [43] .Cellular uptake of niosomes can be via endocytosis [1]; however they have been shown to bind and fuse with cell plasma membranes via cellular receptors when vesicle surface charge is sufficiently negative [18].

Current trends in niosomes:

Use of niosomes in cosmetics was first done by **L'Oreal** (1975) as they offered the following advantages :[31]

- The vesicle suspension being water based offers greater patient compliance over oil based systems, since the structure of the niosome offers place to accommodate hydrophilic, lipophilic as well as ampiphilic drug moieties, they can be used for a variety of drugs.
- The characteristics such as size, lamellarity etc. of the vesicle can be varied depending on the requirement.
- The vesicles can act as a depot to release the drug slowly and offer a controlled release.

Marketed Products:

Lancôme has come out with a variety of anti-ageing products which are based on niosome formulations. L'Oreal is also conducting research on anti-ageing cosmetic products. Niosomal Preparation in the Market is – **Lancôme** (www.lancome.com)

Applications of Niosomes:

The application of niosomal technology is widely varied and can be used to treat a number of diseases. The following are the few uses of niosomes which are either proven or under research

Drug Targeting:

One of the most useful aspects of niosomes is their ability to target drugs. Niosomes can be used to target drugs to the reticulo-endothelial system. The reticulo-endothelial system [43] (RES) preferentially takes up niosome vesicles. The uptake of niosomes is controlled by circulating serum factors called opsonins. These opsonins mark the niosome for clearance. Such localization of drugs is utilized to treat tumors in animals known to metastasize to the liver and spleen [5]. This localization of drugs can also be used for treating parasitic infections of the liver. Niosomes can also be utilized for targeting drugs to organs other than the RES. A carrier system (such as antibodies) can be attached to niosomes (as immunoglobulin bind readily to the lipid surface of the niosome) to target them to specific organs[44]. Many cells also possess the intrinsic ability recognize and bind specific carbohydrate determinants, and this can be exploited by niosomes to direct carrier system to particular cells.

Anti-neoplastic Treatment [16, 17]:

Most antineoplastic drugs cause severe side effects. Niosomes can alter the metabolism; prolong circulation and half life of the drug, thus decreasing the side effects of the drugs. Niosomal entrapment of Doxorubicin and Methotrexate [39, 18, 19] (in two separate studies) showed beneficial effects over the

unentrapped drugs, such as decreased rate of proliferation of the tumor and higher plasma levels accompanied by slower elimination [20].

Leishmaniasis [21]:

Leishmaniasis is a disease in which a parasite of the genus Leishmania invades the cells of the liver and spleen. Commonly prescribed drugs for the treatment are derivatives of antimony (antimonials), which in higher concentrations can cause cardiac, liver and kidney damage. Use of niosomes in tests conducted showed that it was possible to administer higher levels of the drug without the triggering of the side effects, and thus allowed greater efficacy in treatment.

Delivery of Peptide Drugs [22]:

Oral peptide drug delivery has long been faced with a challenge of bypassing the enzymes which would breakdown the peptide. Use of niosomes to successfully protect the peptides from gastrointestinal peptide breakdown is being investigated[(23)] .In an invitro study conducted by *Yoshida et al,* oral delivery of a vasopressin derivative entrapped in niosomes showed that entrapment of the drug significantly increased the stability of the peptide.

Uses in Studying Immune Response [45]:

(Brewer and Alexander in 1992) studied niosomes are used in studying immune response due to their immunological selectivity, low toxicity and greater stability. Niosomes are being used to study the nature of the immune response provoked by antigens.

Niosomes as Carriers for Haemoglobin[46]:

(Moser P. and Marchand Arvier M. in 1989) reported that niosomes can be used as carriers for haemoglobin within the blood. The niosomal vesicle is permeable to oxygen and hence can act as a carrier for haemoglobin in anaemic patients.

Transdermal Drug Delivery Systems Utilizing Niosomes [24]:

One of the most useful aspects of niosomes is that they greatly enhance the uptake of drugs through the skin. Transdermal drug delivery utilizing niosomal technology is widely used in cosmetics; In fact, it was one of the first uses of the niosomes [25]. Topical use of niosome entrapped antibiotics to treat acne is done. The penetration of the drugs through the skin is greatly increased as compared to un-entrapped drug [(26)]. Recently, transdermal vaccines utilizing niosomal technology is also being researched. A study conducted by P. N. Gupta et al has shown that niosomes (along with liposomes and transfersomes) can be utilized for topical immunization using tetanus toxoid. However, the current technology in niosomes allows only a weak immune response, and thus more research needs to be done in this field.

Other Applications:

a) Sustained Release:

Azmin *et al* suggested the role of liver as a depot for methotrexate after niosomes are taken up by the liver cells. Sustained release action of niosomes can be applied to drugs with low therapeutic index and low water solubility since those could be maintained in the circulation via niosomal encapsulation [27].

b) Localized Drug Action:

Drug delivery through niosomes is one of the approaches to achieve localized drug action, since their size and low penetrability through epithelium and connective tissue keeps the drug localized at the site of administration. Localized drug action results in enhancement of efficacy of potency of the drug and at the same time reduces its systemic toxic effects e.g. Antimonials encapsulated within niosomes are taken up by mononuclear cells resulting in localization of drug, increase in potency and hence decrease both in dose and toxicity. The evolution of niosomal drug delivery technology is still at an infancy stage, but this type of drug delivery system has shown promise in cancer chemotherapy and anti-leishmanial therapy[12, 18].

Conclusion

From the above article it is concluded that the concept of incorporating the drug into niosomes for a better targeting of the drug at appropriate tissue destination is widely accepted by researchers and academicians. Proniosomes derived niosomes represent a promising drug delivery module. They represent a structure similar to liposome and hence they can represent alternative vesicular systems with respect to liposomes, due to the niosome ability to encapsulate different type of drugs within their multienvironmental structure. Proniosomes based niosomes are thoughts to be better candidates drug delivery as compared to liposomes due to various factors like cost, stability etc. Various types of drug deliveries can be possible using proniosomes based niosomes like targeting, ophthalmic [(28, 29)] topical [6,11,24,25], parentral [40, 41], peroral vaccine [30] etc. More researches have to be made in this field to come out with all the potential in this novel drug delivery system.

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