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PRONIOSOME: A NOVEL POLYMERIC VESICULAR DRUG CARRIER

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ABSTRACT

Vesicular system has large opportunities for the transdermal drug delivery system. Proniosomes are semisolid liquid crystalline product of nonionic surfactant. The surfactant which is used in the preparation of proniosomal gel is easily soluble in the minimal amount of acceptable solvent and also the least amount of aqueous phase. Proniosomal gel was prepared using different concentrations of non-ionic surfactants (span 20, 40, 60, 80 and tween 20, 40, 80) cholesterol and other solvents. Proniosomal gel was prepared by coacervation method. Proniosomes overcomes the problems related to niosomes. It has faster recovery , less cholinergic side effects on the salivery gland compared with that of oral TT. It has faster Vesicular system enhaneces the penetration of drug in the systemic circulation. this review mainly focus on the formulation , evaluation , entrapment effect , in vitro drug release , applications , advantages and disadvantages of proniosomal gel.

INTRODUCTION:

The main aim of novel vesicular drug delivery system is to deliver the drug in the body at specific site during the period of treatment. Drug encapsulation in the vesicles is one such system which helps to prolong drug duration in systemic circulation and decreases the toxicity [1]. Drug targeting means, the delivery of drug to a receptor or organs or any other specific site of body to which one wishes to deliver the entire body. [2].the targeted drug delivery system was first proposed by Paul Ehrlich in 1909.which delivered the drug directly to damaged cells only. number of carriers are needed to deliver the drug at specific sites like niosomes , liposomes , immunoglobulin , microsphers.[3]. To achieve the effective transdermal delivery, a carrier should avoid the major skin barrier called ‘stratum corneum’ which do not allow the permeation of pathogens and other molecules into systemic circulation. Several approaches have been used to improve the skin permeation of drugs like penetration enhancers, iontophoresis, microneedles and thermal ablation. But by using of these methods rely upon damaging the protective barrier function of the skin and it may cause irritation or other skin related problems. [4].pronisomes overcomes the issues such as trnesportatio, distribution, storage conditions. The main objective for the development of controlled release dosage forms is to prolong the extended duration of action

and thus gives assurance for higher patient compliance. [1].

TYPES: Targeted vesicles are classified based on their composition. [6]. Lipoidal biocarriers and Non-lipoidal biocarriers.

Proniosomes are dry formulation. The surfactants which are used in the preparation of pronisomes are easily soluble in water. They converted into niosomal dispersion immediately before use on agitation in hot aqueous media within minutes. Proniosomes are physically stable during the storage and transport.[6].

Structure of Proniosomes:

Proniosomes are transparent, translucent or semisolid gel structure because of having limited solvent and these are mixture of liquid crystals like lamellar, hexagonal, and cubic .Here lamellar phase shows sheets of surfactants arranged in bi layer, hexagonal phase shows cylindrical compact structure and these are arranged in hexagonal fashion whereas cubic phase consist of curved continuous lipid bi layer extending to three di mentions. While formulating this gel, in the beginning, less viscous composition is formed in some cases but addition of water leads to interaction between water and polar group of surfactant resulting swelling of bi layer. If amount of solvent is increased further, then a spherical structure is formed i.e., multi lamellar, multi-vesicular. This results complete hydration thereby formation of Niosomes. [7].

TYPES OF PRONIOSOMES:

According to the type of carrier and method of formulation of proniosomes they are of two types.[8].

Dry granular proniosomes:

1. Sorbitol based proniosomes
2. Maltodextrin based proniosomes

Sorbitol based proniosomes is a dry formulation that involves sorbitol as a carrier, which is further coated with non-ionic surfactant and is used as a noisome within minutes by the addition of hot water followed by agitation. Maltodextrin based proniosomes: these are formulations in which maltrodextrin, surfactant solution in the organic solvent are added together to form slurry. Later organic solvent is evaporated and to form dry powder.[1]

Liquid crystalline proniosomes:

This type of proniosomes is reservoirs for transdermal delivery of the drug. The transdermal patch involves an aluminium foil as a baking material along with a plastic sheet. Proniosomal gel is spread evenly on the circular plastic sheet followed by covering with a nylon mesh.[1]. Liquid crystalline proniosomes display a number of advantages:

1) Stability

2) High entrapment efficiency

3) As a penetration enhancer

4) Easy to scale up as no lengthy process is involved; moreover it avoids the use of pharmaceutically unacceptable additives.

ADVANTAGES:

- Proniosomes do not required any special conditions of storage in case of niosomes and liposomes.
- proniosomes are physically stable compared to niosomes.
- Proniosomes are easily to handle, store and transport and distribution.
- They are easy to use as they can be hydrated just before use,
- Proniosomes are uniform in size.
- They overcomes the problems related to niosomes. [9]

DISADVANTAGES: Drug of large particle size not easy to absorb through the skin.

- Skin irritation or allergic reaction on contact dermatitis.
- Occurrence of bubble during formation of emulgel.[9].

Action of Proniosomes: Proniosomes show their action after converted to niosomes on hydration. The hydration may occur either by the skin or by the addition of aqueous solvents. Proniosomes can entrap both hydrophilic as well as lipophilic drugs.[10-13].

PREPARATION OF PRONIOSOMES:

proniosomes are prepared by three methods.

1. Slurry method.
2. Coacervation method.
3. Spray coated methods.

1. Slurry Method: slurry method is used to produce proniosomes. In this method maltodextrin is used as carrier. The time required to produce proniosomes. A 250 µmol stock solution of surfactant and membrane stabilizer was prepared in chloroform:methanol (2:1) solution. A definite volume of stock solution and drug dissolved in chloroform:methanol (2:1) the solution was added to a 100 ml round bottom flask containing the carrier marterial. Additional organic solvent solution added to form a slurry. if lower surfactant loading occurs. The flask was attached to a rotary flash evaporator, which evaporates solvent at 60 -70 rpm, a temperature was 45 ± 2°C, and a reduced pressure of 600 mmHg until the mass in the flask had become a dry, free flowing product. These materials were dried in a desiccator overnight at room temperature under vacuum. This dry preparation is referred to as "proniosomes" and was used for preparations and for further study on powder properties. These final products ,that is proniosomes were stored in a tightly closed container .[14] .

Advantages of slurry method:

a) Maltodextrin like polysaccharide which is easily soluble in water and it is used as carrier material in formulation; they were easily coated by simply adding surfactant in organic solvent to dry maltodextrin.

b) Due to uniform coating on the carrier it protect the active ingredient and the surfactants from hydrolysis and oxidation.

c) The higher surface area results in thinner surfactant coating which makes the rehydration process efficient.

Disadvantages of slurry method:

a) Method is time consuming and involves specialised equipment with vacuum and nitrogen gas.

b) The thin film approach allows only for a predetermined lot sizes so material often wasted, so small quantities and small dose batch can be tedious one.

2. Coacervation Phase Separation Method: This method is widely used to formulate Proniosomal gel. Precisely weighed amounts of surfactant, like span 60, lecithin (carrier), cholesterol, and drug was engaged in a clean and dry wide mouthed glass vial and alcohol 2.5 ml is added to it lipid and drug are taken in a clean and dry wide mouthed glass vial of 5.0 ml capacity and alcohol (0.5 ml) is added to it. After warming, all the ingredients are mixed well with a glass rod; the open end of the glass bottle is covered with a lid because of prevention the loss of solvent from it and warmed over water bath at 60-70°C for about 5 min until the surfactant mixture is dissolved completely. Then the aqueous phase (0.1% glycerol solution) is added and warmed on a water bath till a clear solution was formed which is then converted into Proniosomal gel on cooling.[15].

Advantages of this method :

a) Method is simple and without time consumable so it does required any specialized equipment.

b) Specially adopted for gel preparation

c) Small quantities or small dose formulation can be prepared on lab scale

3.Spray Coated Method: Proniosomes are generally formulated by spraying surfactant in organic solvent into sorbitol powder and evaporating the solvent. A 100 ml round bottom flask containing desired amount of carrier can be attached to rotary evaporator. The evaporator has to be evacuated and rotating flask can be rotated in a water bath under vacuum at 65-70°C for 15-20 min. This process is repeated until all of the surfactant solution has been applied. The evaporation should be continued until the powder becomes completely dry and to form multi cellular vesicles. [16, 17].

Advantages: It's a simple method suitable for hydrophobic drug without concerns of instability or susceptibility of active pharmaceutical ingredient to hydrolysis.

Disadvantages:

1. Method is time consuming and involves specialized equipment with vacuum and nitrogen gas.
2. The thin film approach allows only for a predetermined lot sizes so material often wasted so minute quantities or small dose batch can be tedious one.

Formulation of proniosomal gel: Proniosomes were formulated by coacervation-phase separation method with a slight modification. The drug (5 mg) and the blend of surfactant, soya lecithin and cholesterol were mixed with 125 µl absolute ethanol in a wide mouth glass tube and covered with lid to prevent loss of solvent. This mixture was heated on a water bath at 60–70 °C until it dissolved completely. To the resultant mixture, phosphate buffer (pH 7.4) was added and heated slightly on a water bath to obtain a clear solution which was allowed to cool at room temperature and then mixed with equal volume of 1% carbopol-934 to obtain clear proniosomal gel. These structures are liquid crystalline dense niosomes hybrids that can be converted into niosomes instantly upon hydration or used as such in the topical/transdermal applications.[18] Several grades of spans and tweens were used in the preparation to study their effect on formulation characteristics. For further optimisation of the preparations, different proniosomal formulations were prepared by different concentrations of surfactant, lecithin, and cholesterol.[19].

Evalutions of proniosomal gel: Proniosomes are characterized for vesicle size, size distribution, shape and surface morphological studies.

Measurement of Angle of Repose: The angle of repose of dry proniosomes powder was measured by a funnel method. The proniosomes powder was poured into a funnel and it 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.[20]. The cars compressibility index and haunsers ratio were calculated from the bulk and tapped density of the proniosom powder.[21].

Scanning Electron Microscopy: Particle size of proniosomes is very important evaluation. 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 aluminium stubs. The aluminium stub was placed in the vacuum chamber of a scanning electron. The samples were observed for morphological characterization using a gaseous secondary electron detector.[22].

Optical Microscopy: The niosomes were mounted on glass slides and viewed under a microscope with a magnification of

1200X for morphological observation after suitable dilution. The photomicrograph of the formulation also obtained from the microscope by using a digital SL camera.

Measurement of Vesicle Size : 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. The apparatus consists of a He-Ne laser beam of 632.8 nm focused with a minimum power of 5mW using a Fourier lens [R-5] to a point at the centre of multi element 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 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: The vesicles obtained after removal of un entrapped drug by dialysis is then re suspended in 30% v/v of PEG 200 and 1 ml of 0.1%v/v triton x-100 solution was added to solubilise vesicles, the resulted clear solution is then filtered and analysed for drug content [23]. The percentage of drug entrapped is calculated by using the following formula.

$$\%EE = \frac{\text{total amount of drug added} - \text{free drug in supernant}}{\text{total amount of drug added}} \times 100.$$

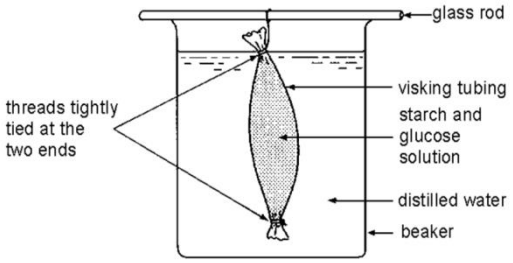
pH Measurement

The pH of the proniosomal gel were determined by a digital pH meter (Mettler Toledo, Greifensee, Switzerland). 1 g of gel was dissolved in 20 mL of distilled water and the electrode was then dipped into gel formulation and constant reading was noted. The measurements of pH of each formulation were replicated three times.

***In-vitro* methods for the assessment of Drug Release from Proniosomes:** In vitro drug release can be done by: Dialysis tubing, Reverse dialysis, Franz diffusion cell

Dialysis Tubing:

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.[24].



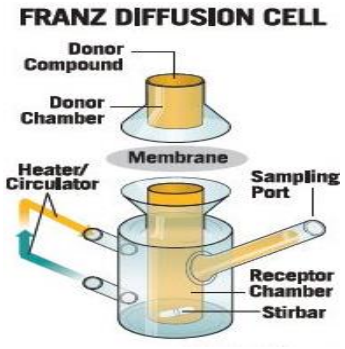
Apparatus to show dialysis

Reverse Dialysis:

In this technique, a number of small dialysis as containing 1ml of dissolution medium are placed in proniosomes. The proniosomes are then displaced in to 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:

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 were 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.



***Ex-vivo* permeation studies:**

Ex-vivo permeation study was carried out using male wistar rat skin . [26]. The rat skin was mounted between the donor and receptor compartment with the stratum corneum facing upper side on the diffusion cell. To maintain the sink conditions .30% (v/v) ethanol in phosphate buffer pH is 7.4 (basic) was taken in receptor compartment. The temperature was maintained at 37 ± 1 °C. The receptor compartment content was stirred with the help of magnetic beads. The samples were collected at different time intervals and were immediately replaced with the fresh media [26,27]. The samples were analysed for drug content using UV spectrophotometer. The cumulative amount of drug permeated (Q) at different time intervals and various parameters like steady state flux (Jss), permeability coeffcient (Kp) and enhancement ratio (ER) were calculated using following equations:[26] Steady sate flux(Jss) = $\frac{amount\ of\ drug\ permeated}{time\ X\ area\ of\ membrane.} = \frac{Q}{(t\ X\ a)}$

Permeability co efficient (Kp) = $\frac{flux}{initial\ concentration\ of\ drug\ in\ donar\ chameber\ .}$

Enhancement ratio(ER) = $\frac{(Jss)\ of\ proniosome}{(Jss\ )\ of\ control.}$

Applications of Proniosomes: 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:

Anti-Neoplastic Treatment : Most of these drugs cause severe side effects. Niosomes can alter the metabolism; prolong systemic circulation and half life of the drug, thus decreasing the side effects of the drugs

Leishmaniasis: Leishmaniasis is a disease in which a parasite of the genus Leishmania invades the cells of the liver and spleen. Use of proniosome 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.

Uses In Studying Immune Response: Proniosomes 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.

Proniosomes As Carriers For Haemoglobin: 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 hemoglobin in anaemic patients.[28.].

Proniosomes Used in Cardiac Disorders: Proniosomal carrier system for captopril for the treatment of hypertension that is capable of efficiently delivering entrapped drug over an extended period of time. The potential of proniosomes as a transdermal drug delivery system for captopril was investigated by encapsulating the drug in various formulations of proniosomal gel composed of different concentrations of sorbitan fatty acid esters, cholesterol and lecithin prepared by coacervation-phase separation method.

Antibacterial Therapy: Amphotericin-B proliposomes could be stored for 9 months without significant changes in distribution of vesicle size and for 6 months without loss of pharmacological activity.

Lipoidal biocarriers for site specific targeting.	
1.Liposomes.	2. Emulsomes
3. Enzymosomes	4. Ethosomes
5. Sphingosomes	6. Transferosomes
7. Pharmacosomes	8. Virosomes
Non- lipoidal biocarriers for site-specific targeting:	
1. Niosomes	2. Bilosomes
3. Aquasomes	

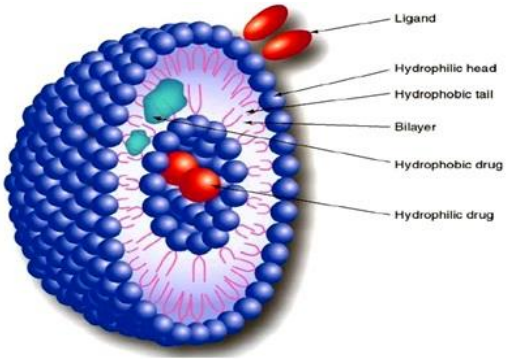


Fig 1: Structure of Proniosomes

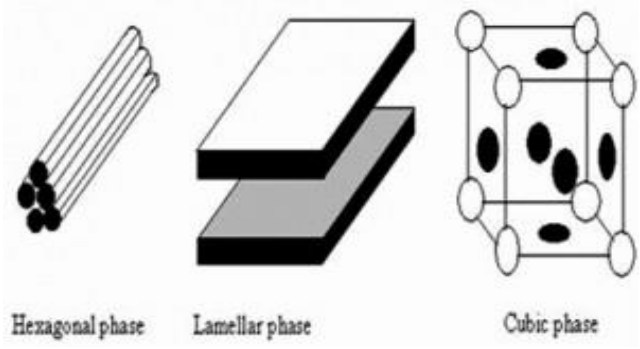


Fig 2: Schematic representation of various liquid crystalline phases

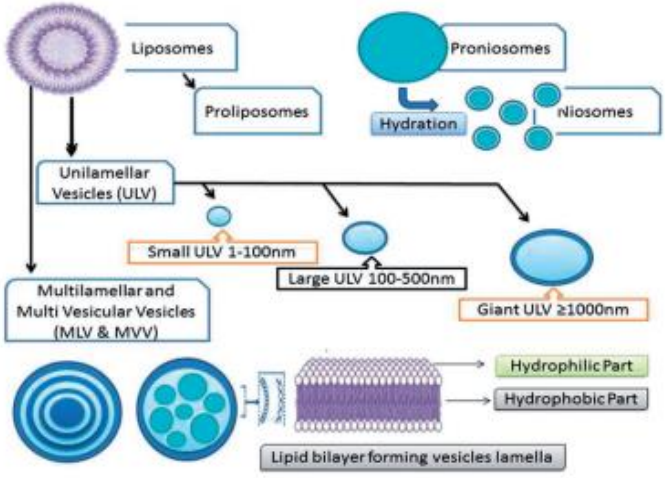


Fig 3: Schematic representation of niosomes and proniosomes

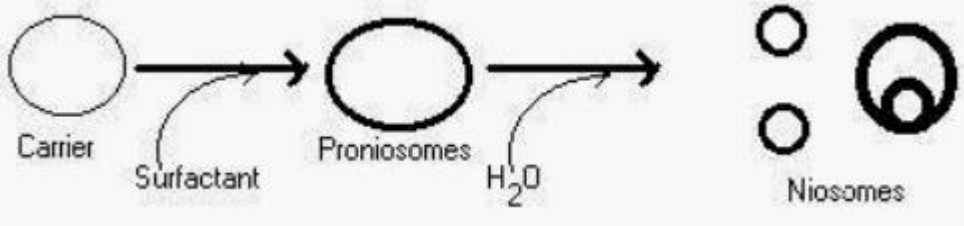
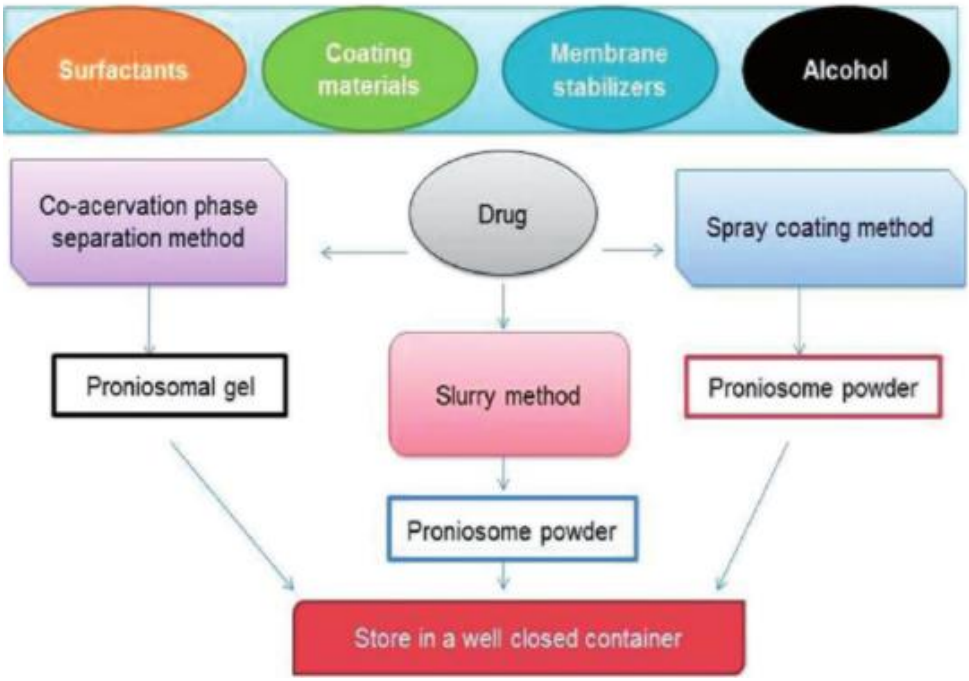


Fig 4: Formation of Niosomes from Proniosomes.



S.no	Class	Examples	Use
1.	Surfactants	Span 20, 40,60,80,85 and tween 20, 40, 80.	To enhnces dry flux rate across the skin.
2.	cholesterol	cholesterol	To prevent leakage of drug formulation.
3.	lecithin	lecithin	Penetration enhancer.
4.	maltodextrin	maltodextrin	It gives the flexibility in surfactants and other component ratio.
5.	Sorbital.	Sorbital.	Alters the drug distribution.

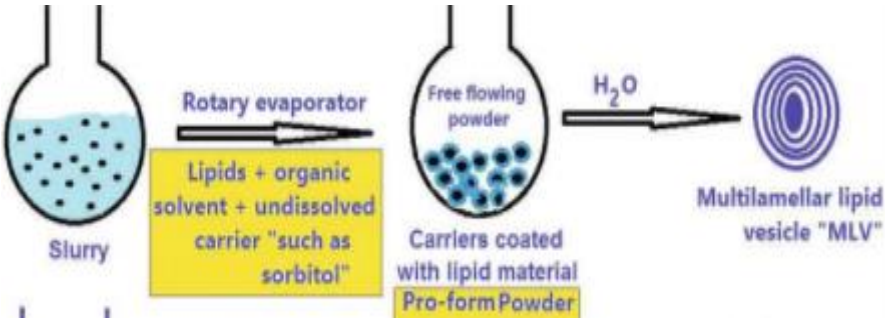


Fig 5: Poniosomes prepared by slurry method

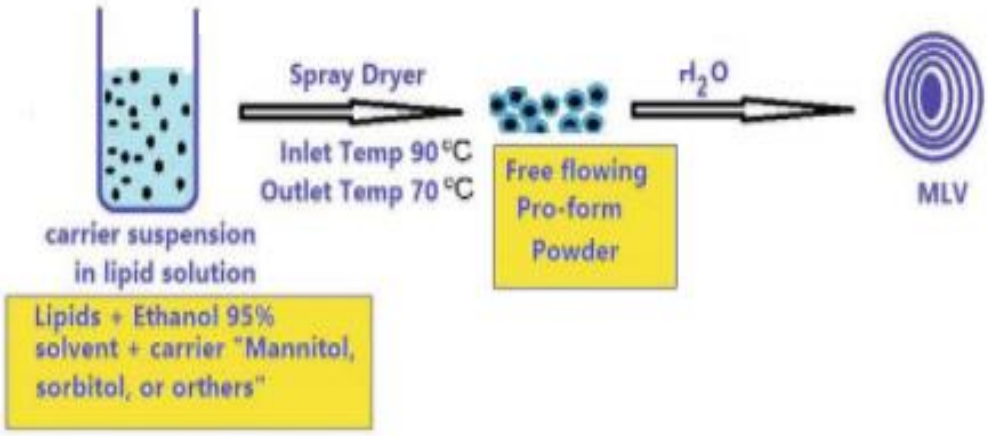


Fig 6: Proniosomes are formulted by Spray Coated Method

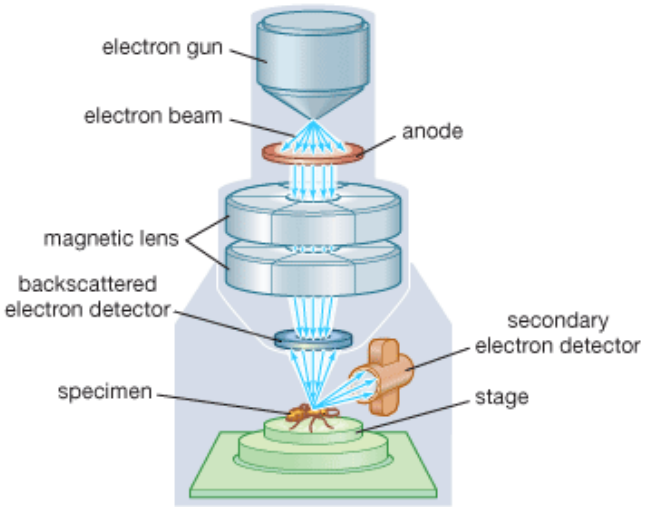


Fig 7: Particle size of proniosomes determination by Scanning Electron Microscopy (SEM)

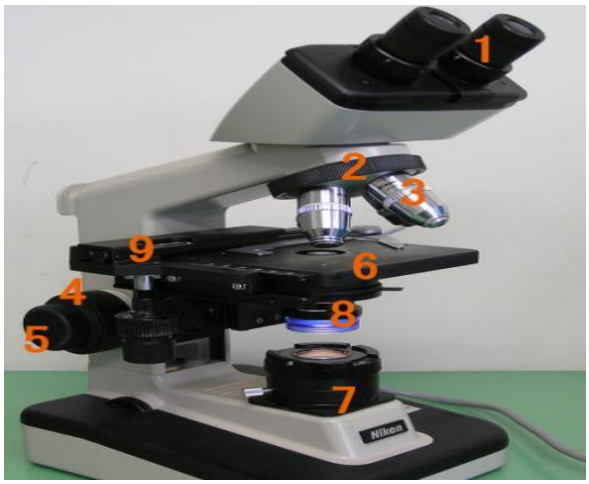


Fig 8: Optical Microscopy

Marketed products of proniosomal gel :

S.no	Drug	Category
1.	Ttenoxicum	Anti-inflamatory
2.	Ketorolac	NSAID
3.	Levontreloges	Contraceptive
4.	Guggul lipid	Herbal
5.	Carvedilol	Antihypertensive
6.	Tacrolimus	Immunosuppressive agent
7.	Valsartan	Antihypertensive
8.	Metformin	Anti diabetic
9.	Celecoxib	NSAID
10.	Neem seed oil	Therapeutic and cosmetic agent

Patents of proniosomes:

Patent publication Number.	INVENTORS.	TITLE.
US.4830857A	R.Handjain, A.Riber , G.Vanlerberghe , Z.zabotto , j.Griat	Cosmotic and pharmaceutical composition containing niosomes and a water soluble polymte, and a process for prepairing there compositions.
US.6051250	Ribier , A. Simonnet , Jean-thierry.	Process for the stabilization of vesicles of amphiphilic lipid and composition for topical application the said stabilized vesicles.
US 06576625B2	A.Singh, R. Jain.	Targeted vesicles constructions for cytoprotection and treatment of H.Pyroli infections.
US.06951655B2	Y.Cho.k.H.Lee.	Pro-micelle pharmaceutical compositions.
US.4912032.	Allan S. Hoffmar , Nobuo Monji.	Methods for selectively reacting ligands immobilized within a temperature-sensitive polymer gel

Even though physical stability of the preparation can be increased, a vacuum or nitrogen atmosphere is still required during preparation and storage to prevent oxidation of phospholipids.

Cosmetics Formulation: Now a day's large numbers of cosmetic preparations available in the market are utilizing niosomes and liposomes as a carrier for delivery of actives. Liposomes were prepared using unacceptable organic solvents, whose traces in the final preparation can cause damage to the skin. It is proved that proniosomes are as effective as noisome and liposomes, but their formulation, handling, storage and transportation make them superior over others. The therapeutic agents which can be utilized for incorporation into proniosomal carrier systems include, moisturizing, nutritional, anti wrinkle, anti-ageing, cleansing, sunscreen particles.

Other Applications:

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.

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. Antimonies 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.

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