



AN OVERVIEW ON ADVANCE VESICLES FORMULATION AS A DRUG CARRIER FOR N.D.D.S

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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. Provesicular systems, such as proniosomes which is one of the advancement in nanotechnology minimize problems of vesicular systems such as aggregation, fusion and leakage of drug and provide additional convenience in transportation, distribution, storage and dosing. Conventional vesicular systems such as liposomes and niosomes are particulate in nature and face stability related difficulty. This review article provides an insight about these approaches along with a novel vesicular approach known as proniosomes. Proniosomes is dry formulation using suitable carrier coated with non ionic surfactants and can be converted into niosomes immediately by hydration before administration. These proniosome-derived niosomes could equivalent or even superior than conventional niosomes. The drug carrier have been used in vesicle drug delivery system are Nanoparticles, Liposomes, Microspheres, Niosomes, Proniosomes, Ethosomes, Proliposomes.

KEYWORDS: Proniosomes, Niosomes, Vesicle drug delivery system, Convenience in transportation and Nanotechnology.

INTRODUCTION

The vital objective for the development of controlled release dosage forms is to prolong the duration of action, increased safety margin of high potency drugs due to better control of plasma levels, reduces fluctuations in plasma concentration, reduces serious side effects and gives assurance for higher patient compliance.^[1] Recently, different carrier systems and technologies have been extensively studied with aim of controlling the drug release and improving the efficacy and selectivity of formulation. Now-a-days, the vesicular systems like liposomes^[2] or niosomes^[3] have specific advantages while avoiding demerits associated with conventional dosage forms. To overcome the disadvantage of vesicular system, Proniosomes are designed. Niosomes are non-ionic surfactant vesicles obtained on hydration of synthetic non-ionic surfactant, with or without incorporation of cholesterol or other lipids.^[4]

These are vesicular systems similar to liposomes that can be used as carriers of amphiphilic and lipophilic drugs. One of the reasons for preparing niosomes is the assumed higher chemical stability of the surfactants than that of phospholipids, which are used in the preparation of liposomes. Due to the presence of ester bond,

phospholipids are easily hydrolyzed.^[5,6] Niosomes are promising vehicle for drug delivery and being non-ionic. It is less toxic and improves the therapeutic index of drug by restricting its action to target cells. Niosomes or non-ionic surfactants are microscopic lamellar structure formed of the alkyl or dialkyl polyglycerol ether hydration in aqueous media.^[6]

The advantages of Niosomes are.^[7, 8, 9]

1. They are osmotically stable. Drug molecules with a wide range of solubility can be accommodated in niosomes; they are able to entrap hydrophilic drug by partitioning of these molecules into their hydrophobic domain.
2. They can reduce drug toxicity because of their non-ionic nature.
3. Low cost of production as no special condition is required for handling and storage of niosomes. Non-ionic surfactants are biodegradable, biocompatible and non-immunogenic.
4. They can be tailored according to the desired situation by modifying their structural characteristics (composition, fluidity and size).

5. They can enhance performance of drug by improving availability and controlled delivery at a particular site.
6. Niosomal dispersion in an aqueous phase can be emulsified in a non-aqueous phase to regulate the delivery rate of drug and administer normal vesicle in external non-ionic phase.
7. Proniosomes are dry formulation of water-soluble carrier particles that are coated with surfactant and can be measured out as needed and dehydrated to form niosomal dispersion immediately before use on brief agitation in hot aqueous media within minutes. The resulting niosomes are very similar to conventional niosomes and more uniform in size.^[10]

Advantages of proniosomes over niosomes include

1. Avoiding problem of physical stability like aggregation, fusion, leaking.
2. Avoiding hydration of encapsulated drugs which is limiting the shelf-life of the dispersion.
3. Proniosomes are water soluble carrier particles that are coated with surfactant and can be hydrated to form niosomal dispersion immediately before use on brief agitation with hot aqueous medium. This has additional convenience of the transportation, distribution; storage and designing would be dry niosomes a promising industrial product.
4. Furthermore, unacceptable solvents are avoided in proniosomal formulations. The systems may be directly formulated into transdermal patches and doesn't require the dispersion of vesicles into polymeric matrix.
5. The storage makes proniosomes a versatile delivery system with potential for use with a wide range of active compounds.^[11,12]

Types of Proniosomes

Depending on the method of preparation, the proniosomes exists in two forms;

- A) Dry granular proniosome: According to the type of carrier these are again divided as a) Sorbitol based proniosomes
- B) Maltodextrin based proniosomes: Sorbitol based proniosomes is a dry formulation that involves sorbitol as a carrier. These are made by spraying surfactants mixture prepared in organic solvent on to the sorbitol powder and then evaporating the solvent. It is useful in case where the active ingredient is susceptible to hydrolysis.

Maltodextrin based proniosomes prepared by slurry method. Maltodextrin is a polysaccharide easily soluble in water and it is used as carrier material in formulation. Since its morphology is preserved, hollow blown maltodextrin particles can be used for significant gain in surface area. The higher surface area results in thinner surfactant coating, which makes the rehydration process efficient. Time required for this process is independent of the ratio of surfactant solution.

C) Liquid crystalline proniosomes: When the surfactant molecule are kept in contact with water, there are three ways through which lipophilic chains of surfactant can be transformed into a disordered, liquid state called lyotropic liquid crystalline state. These three ways are

- Increasing temperature at kraft point (T_c),
- Addition of solvent which dissolve lipids,
- Use of both temperature and solvent.

The liquid crystalline proniosomes and proniosomal gel act as reservoir for transdermal delivery of drug.^[13,14]

1. Components of proniosomes: The essential components for the delivery system are as follows.

1.1. Surfactants

Surfactants are the surface active agent usually organic compounds that are amphiphilic in nature (having both hydrophobic and hydrophilic groups). Therefore, a surfactant molecule contains both a water insoluble (lipophilic) and a water soluble (hydrophilic) component. They have variety of functions including acting as solubilizers, wetting agents, emulsifiers and permeability enhancers.^[15] The most common non-ionic amphiphiles used for vesicle formation are alkyl ethers, alkyl esters, alkyl amides and esters of fatty acids.

1.2. Carrier material

The carrier when used in the proniosomes preparation permits the flexibility in the ratio of surfactant and other components that incorporated. In addition to this, it increases the surface area and hence efficient loading. The carriers should be safe and non-toxic, free flowing, poor solubility in the loaded mixture solution and good water solubility for ease of hydration.^[16,17] Commonly used carriers are listed below (**Table 1**).

Table 1: Carriers used for the preparation of Proniosomes.

Sr. No.	Carrier materials investigated
1	Maltodextrin
2	Sorbitol
3	Mannitol
4	Spray dried lactose
5	Glucose monohydrate
6	Lactose monohydrate
7	Sucrose stearate

1.3. Membrane stabilizer

Cholesterol and lecithin are mainly used as membrane stabilizer. Steroids are important components of cell membrane and their presence in membrane and their presence in membrane brings about significance changes with regard to bilayer stability, fluidity and permeability. Cholesterol is a naturally occurring steroid used as membrane additive. It prevents aggregation by the inclusion of molecules that stabilize the system against the formation of aggregate by repulsive steric or electrostatic effects. It leads transition from the gel state to liquid phase in niosome system.

Phosphatidylcholine is a major component of lecithin. It has low solubility in water and can form liposomes, bilayer sheets, micelles or lamellar structures depending on hydration and temperature. Depending upon the source from which they are obtained they are as named as egg lecithin and soya lecithin. It acts as stabilising as well as penetration enhancer. It is found those vesicles composed of soya lecithin are of larger size than vesicle composed of egg lecithin probably due to the difference in the intrinsic composition.^[18,19]

1.4. Solvent and Aqueous phase

Alcohol used in Proniosome has a great effect on vesicle size and drug permeation rate. Vesicles formed from different alcohols are of different size and they follow the order: Ethanol > Propanol > Butanol > Isopropanol. Ethanol has greater solubility in water hence leads to formation of highest size of vesicles instead of isopropanol which forms smallest size of vesicle due to branched chain present. Phosphate buffer pH 7.4, 0.1% glycerol, hot water is used as aqueous phase in preparation of proniosomes.^[19]

1.5. Drug

The drug selection criteria could be based on the following assumptions.^[18]

1. Low aqueous solubility of drugs.
2. High dosage frequency of drugs.
3. Short half life.
4. Controlled drug delivery suitable drugs.
5. Higher adverse drug reaction drugs.

2. FORMULATION CONSIDERATIONS

It is necessary to understand the role of basic formulation aspects of proniosomes before preparation which includes selection of surfactant, cholesterol concentration, the hydration medium, nature of encapsulated drug.

2.1. Selection of surfactant

Surfactants can improve the solubility of some poorly soluble drugs. Selection of surfactants should be done on the basis of HLB value which is a good indicator of the vesicle forming ability of any surfactant.

The formation of bilayer vesicles instead of micelles not only depends upon the HLB values of the surfactant but

also on the chemical structure of component and the critical packing parameter. The HLB value of a surfactant plays a key role in controlling drug entrapment of the vesicle it forms.

The critical packing parameter (CPP) is a geometric expression relating to hydrocarbon chain length (l) and volume (v) and the interfacial area occupied by the head group. $CPP = v / lc \times a$ v = hydrophobic group volume lc = critical hydrophobic group length a = area of hydrophilic head group A CPP below 0.5 and 1 indicates that the surfactant is likely to form vesicles.

A CPP of below 0.5 which indicates a large contribution from the hydrophilic head group area is said to give spherical micelles and a CPP of above 1 indicates a large contribution from hydrophobic group volume should produce inverted micelles.

Non-ionic surfactant are the most common type of surfactant used in preparing the vesicles due to the superiority over other counterparts having good stability, compatibility and toxicity aspects. It was found that the HLB value in between 4 and 8 was found to be compatible with vesicle formation. The entrapment efficiency of bilayered vesicle also depends upon the phase transition temperature (T_c) of the surfactant.^[15,13]

2.2. Cholesterol concentration

Cholesterol is an essential structural component of cell membrane and is required to establish proper membrane permeability and fluidity. It imparts rigidity to vesicles, which is very important under severe stress conditions. Cholesterol increases or decreases the percentage encapsulation efficiency depending on either the type of the surfactant or its concentration within the formulae. Cholesterol along with the addition of surfactant forms homogenous niosome dispersion rather than only a surfactant which forms a gel. Cholesterol is thus usually included in a 1:1 molar ratio in most formulations as it is known to abolish the gel to liquid phase transition of niosome systems resulting in niosomes that are less leaky. The amount of cholesterol to be added depends on the HLB value of the surfactants. As the HLB value increases above 10 it is necessary to increase the minimum amount of cholesterol to be added in order to compensate for the larger head groups. It was found that above a certain level of cholesterol, entrapment efficiency decreased possibly due to a decrease in volume diameter.^[19,20]

2.3. Hydration medium

Phosphate buffer having various pH are most widely used hydration medium for preparation of proniosome derived niosomes. The solubility of drug being encapsulated determines the actual pH of hydration medium. The temperature of hydration also plays an important role in governing the self assembly of non-ionic surfactant into vesicles and affects their shape and size. In case of proniosomal gel preparation, the

hydrating temperature used to make niosomes should usually be above the gel to liquid phase transition temperature of the system.^[15,20,21] The proniosome derived niosomes are very similar to conventional niosomes and more uniform in size. (Fig. 1).

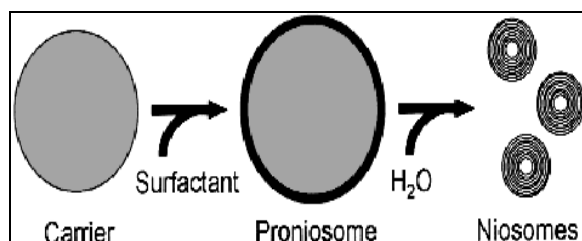


Figure 1: Formation of Niosome from Proniosome.

2.4. Nature of encapsulated drug

The main factor in the consideration is the influence of an amphiphilic drug on vesicle formation. When drug was encapsulated in niosomes, aggregation occurred and was overcome by the addition of a steric stabilizer. When more drug is added the increase in its encapsulation could be the result of saturation of the medium. This suggests that the solubility of certain poorly soluble drugs can be increased by formulation in niosomes but only up to a certain limit above which drug precipitation will occur. Increase in drug concentration showed an increase in both percentage encapsulation efficiency and the amount of drug encapsulated per mole total lipids upon hydration and formation of niosomes.^[15,20]

3. METHOD OF PREPARATION

Proniosome preparation mainly comprised of non-ionic surfactants, coating carriers and membrane stabilizers. The formulation may be prepared by following methods.

3.1. Slurry method

Proniosomes can be prepared by addition of the carrier and the entire surfactant solution in a round bottomed flask which is fitted to rotary flash evaporator and vacuum was applied to form a dry and free flowing powder. Finally, the formulation should be stored in tightly closed container under refrigeration in light. The time required for proniosome production is independent of the ratio of surfactant solution to carrier material and appears to be stable. This method is advantageous because due to uniform coating on carrier it protects the active ingredients and surfactants from hydrolysis and oxidation. Along with that the higher surface area results in a thinner surfactant coating, which makes the rehydration process more efficient.^[13,22]

3.2. Slow spray coating method

This method involves preparation of proniosomes by spraying surfactant in organic solvent onto the carrier and then evaporating the solvent. It is necessary to repeat the process until the desired surfactant loading has been achieved, because the carrier is soluble in the organic solvent. The surfactant coating on the carrier is very thin and hydration of this coating allows multilamellar vesicles to form as the carrier dissolves. The resulting

niosomes have uniform size distribution similar to those produced by conventional methods. The main advantage of this method is to provide a means to formulate hydrophobic drugs in a lipid suspension with or without problem with instability of the suspension or susceptibility of active ingredient to hydrolysis. This method was reported to be tedious since the sorbitol carrier is soluble in the solvent used to deposit the surfactant. It is also found to interfere with the encapsulation of certain drugs.^[13,22]

3.3. Co-acervation phase separation method

Proniosomal gels can be prepared by this method which comprises of surfactant, lipid and drug in a wide mouthed glass vial along with small amount of alcohol in it. The mixture is warmed over water bath at 60-70°C for 5min until the surfactant mixture is dissolved completely. Then the aqueous phase is added to the above vial and warmed still a clear solution is formed which is then converted into proniosomal gel on cooling.^[13,22] After hydration of proniosomes they are converted to uniformly sized niosomes.

4. CHARACTERIZATION OF PRONIOSOMES

Proniosomes are characterized for vesicle size, size distribution, shape and surface morphological studies.^[22,28]

4.1. Particle size analysis and Surface Morphology

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).

4.2. Microscopy

The vesicle formation by the particular procedure was confirmed by optical microscopy in 400x resolution. The niosome suspension placed over a glass slide and fixed over by drying at room temperature, the dry thin film of niosome suspension observed for the formation of vesicles. The photomicrograph of the preparation also obtained from the microscope by using a digital SLR camera.

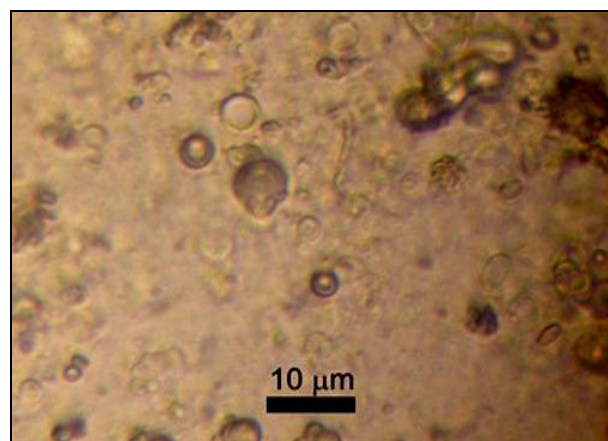


Figure 2: Microscopic View of Niosomes.

4.3. Measurement of angle of repose

The angle of repose of dry proniosomes powder and maltodextrin powder was measured by a funnel method. The maltodextrin powder or 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.

4.4. Separation of Free Un-entrapped Drug

The encapsulation efficiency of proniosomes is determined after separation of the un-entrapped drug using these techniques:

- A. Dialysis
- B. Gel filtration
- C. Centrifugation

A. Dialysis: The aqueous niosomal dispersion is dialysed tubing against suitable dissolution medium at room temperature. Then samples are withdrawn from the medium at suitable time interval centrifuged and analysed for drug content using UV spectroscopy.

B. Gel Filtration: The free drug is removed by gel filtration of niosomal dispersion through a sephadex G50 column and separated with suitable mobile phase and analysed with suitable analytical techniques.

C. Centrifugation: The niosomal suspension is centrifuged and the surfactant is separated. The pellet is washed and then re suspended to obtain a niosomal suspension free from un-entrapped drug.

4.5. Entrapment efficiency

Niosome entrapped drug was estimated by dialysis method. The calculated amount of prepared niosomes was placed in the dialysis bag 50 (pre-soaked for 24 hrs). Free drug was dialyzed for 30 minutes each time in 100 ml of phosphate buffer saline pH 6.8. The dialysis of free drug completed after 12-15 changes, when no drug was detectable in the recipient solution. The dialyzed drug was determined by finding out the concentration of bulk of solution by UV spectrophotometer at 275 nm. The samples from the bulk of solution diluted appropriately before going for absorbance measurement. The free drug in the bulk of solution gives us the total amount of un-entrapped drug. Encapsulation efficiency is expressed as the percent of drug trapped.

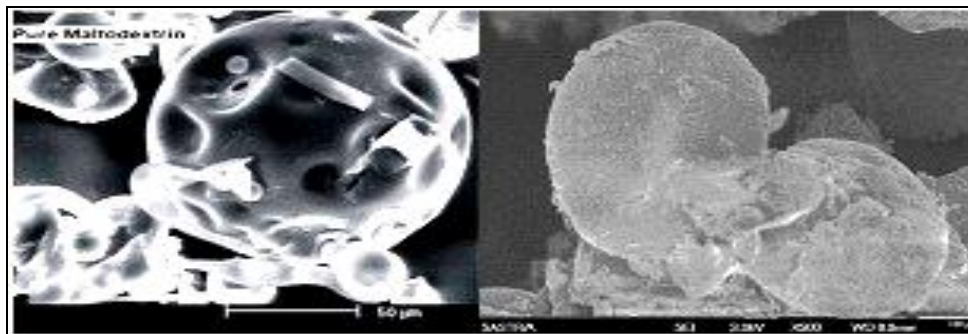


Figure 3: Scanning Electron Microphotograph of pure maltodextrin at higher magnification.

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 is added to solubilise vesicles the resulted clear solution is then filtered and analysed for drug content. The percentage of drug entrapped is calculated by using the following formula. $EE\% = \frac{\text{total drug} - \text{diffused drug}}{\text{total drug}} \times 100$.

4.6. In-vitro Drug Release from Proniosomal Vesicles:

In- vitro drug release and skin permeation studies for proniosome can be determined by different techniques like

- A. Franz diffusion cell
- B. Dialysis tubing
- C. Reverse dialysis

A. Franz Diffusion Cell: This franz diffusion cell has a donor chamber fitted with a cellophane membrane. The Proniosomes are placed in it and dialysed against a suitable dissolution medium at room

temperature. The drug content is analysed using suitable method (UV spectroscopy, HPLC) maintenance of sink conditions is essential.

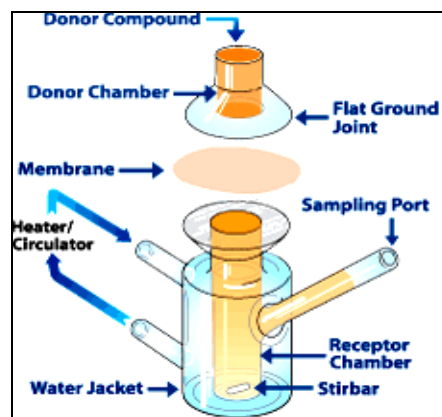


Figure 4: Franz Diffusion cell.

B. Dialysis Tubing

This apparatus has prewashed dialysis tubing which can be hermetically sealed. The Proniosomes are placed in it and then dialysed against a suitable dissolution medium at a room temperature. *In vitro* release pattern of niosomal suspension was carried out in dialysis bag method. 10 mg equivalent of drug niosomal suspension was taken in dialysis bag (Hi media) and the bag was placed in a beaker containing 75 ml of 0.1 N HCl. The beaker was placed over magnetic stirrer having stirring speed of 100 rpm and the temperature was maintained at

37±10°C. 5 ml samples were withdrawn periodically and were replaced by fresh buffer. After two hours, 25 ml of 0.2 M tribasic sodium phosphate was added to change the pH of test medium to 6.8 and the test was continued for a further 22 hours. The sink condition was maintained throughout the experiment. The withdrawn samples were appropriately diluted and analyzed for drug content using U.V. spectrophotometer keeping phosphate buffer pH 6.8 as blank. All the determinations were made in triplicate.

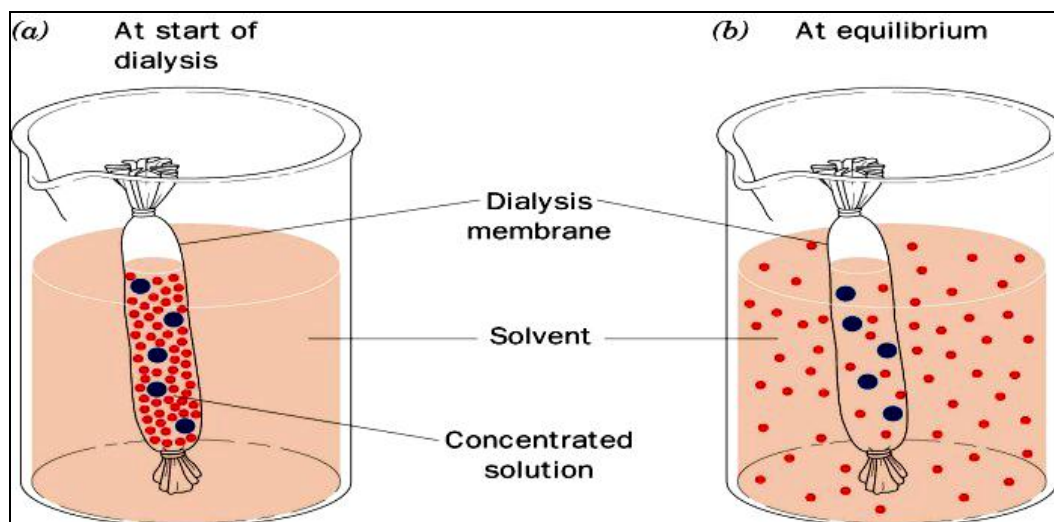


Figure 5: Dialysis bag method Reverse Dialysis.

In this apparatus a number of small dialysis tubes containing 1 ml of dissolution medium are placed. Proniosomes is then displaced into the dissolution medium. The direct dilution of the proniosomes is possible with this method. But the rapid release cannot be quantified using this method.

C. Drug Release Kinetic Data Analysis

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 peppas's.

4.7. Stability Studies on Proniosomes

Physical stability study was carried out to investigate the degradation of drug from proniosome during storage. Stability studies carried out by storing the prepared proniosomes at various temperature conditions like refrigeration on (2-8°C), room temperature (25°C±0.5°C) and elevated temperature (45°C±0.5°C) from a period of one month to 3 months. Drug content and variation in the average vesicle diameter are periodically monitored. ICH guidelines suggests stability studies for dry proniosomes powder meant for reconstitution should be studied for accelerated stability at 75% relative humidity as per international climatic zones and climatic conditions.

4.8. Zeta potential analysis

Zeta potential analysis is done for determining the colloidal properties of the prepared formulations. The

suitably diluted proniosome derived niosome dispersion is determined using zeta potential analyzer based on electrophoretic light scattering and laser Doppler velocimetry method. The temperature is set at 25°C. Charge on vesicles and their mean zeta potential values with standard deviation of 5 measurements are obtained directly from the measurement.

5. CLINICAL APPLICATIONS

Approaches to stabilize niosomal drug delivery system without affecting its properties of merits have resulted in the development of the promising drug carrier, proniosomes. Proniosomes is dry formulation using suitable carrier coated with non ionic surfactants and can be converted into niosomes immediately before use by hydration.

These proniosome-derived niosomes are as good as or even better than conventional niosomes.^[29] The application of proniosomal technology is widely varied and can be used to treat a number of diseases. The following are the few uses of proniosomes which are either proven or under research.

5.1. Drug Targeting

One of the most useful aspects of proniosomes is their ability to target drugs. Proniosomes can be used to target drugs to the reticulo-endothelial system. The reticulo-endothelial system^[30] (RES) preferentially takes up

proniosome vesicles. The uptake of proniosomes is controlled by circulating serum factors called opsonins. These opsonins mark the proniosome for clearance. Such localization of drugs is utilized to treat tumors in animals known to metastasize to the liver and spleen. This localization of drugs can also be used for treating parasitic infections of the liver.

Proniosomes can also be utilized for targeting drugs to organs other than the RES. A carrier system (such as antibodies) can be attached to proniosomes (as immunoglobulin bind readily to the lipid surface of the niosome) to target them to specific organs.^[31] Many cells also possess the intrinsic ability recognize and bind specific carbohydrate determinants and this can be exploited by proniosomes to direct carrier system to particular Cells.^[32,33]

5.2. Anti-neoplastic Treatment

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^[34,35,36] (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.^[33] Podophyllotoxin-dipalmitoylphosphatidylcholine (PPT-DPPC) proliposomes (PPT-DPPC-PL) for improvement of the stability of PPT-DPPC liposome. Methods Freeze-drying method was used to prepare PPT-DPPC-PL and the particle morphology, size range, encapsulation efficiency and stability of PPT-DPPC liposome were investigated. Results After hydration of PPTDPPC- PL, PPT-DPPC liposome appeared multivesicular under electron microscope and the particles were distributed homogenously with an average particle size of 1.45 ± 0.38 μm . The encapsulation efficiency of PPT was 72.3% and alters storage at 4 to 40 for 6 months, the proliposome remained stable. Conclusion the prepared PPT-DPPC-PL particles by freeze-drying method are evenly distributed. The preparation method is relatively simple with higher embedding ratio and better stability.^[37]

5.3. Leishmaniasis

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 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.^[33,29,38]

5.4. Delivery of Peptide Drugs

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.^[39] 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.^[33]

5.5. 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.^[33]

5.6. Proniosomes as Carriers for Haemoglobin

Moser P. et al., 1989 reported that, the 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 anemic patients.^[33,40]

5.7. 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 various ratios of sorbitan fatty acid esters, cholesterol, lecithin prepared by coacervation-phase separation method. The formulated systems were characterized in vitro for size, vesicle count, drug entrapment, drug release profiles and vesicular stability at different storage conditions. Stability studies for proniosomal gel were carried out for 4 weeks.^[41,42] The current study was to investigate the feasibility of proniosomes as transdermal drug delivery system for losartan potassium. Different preparations of proniosomes were fabricated using different nonionic surfactants, such as Span 20, Span 40, Span 60, Span 80, Tween 20, Tween 40 and Tween 80. Different formulae were prepared and coded as PNG-1 (proniosomal gel-1) to PNG-7.

The best *in vitro* skin permeation profile was obtained with proniosomal formulation PNG-2 in 24 h. The permeability parameters such as flux, permeability coefficient and enhancement ratio were significant for PNG-2 compared with other formulations ($P < 0.05$). This optimized PNG-2 was fabricated in the form of transdermal patch using HPMC gel as a suitable base.^[43]

5.8. 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.^[44]

5.9. 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 niosome 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 antileishmanial therapy.^[33,45,46]

5.10. Antibacterial treapy

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. 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 phospholipid.^[47]

5.11. Hormonal Therapy

A proniosome based transdermal drug delivery system of levonorgestrel (LN) was developed and extensively characterized both *in vitro* and *in vivo*.

The proniosomal structure was liquid crystalline compact niosomes hybrid which could be converted into niosomes upon hydration. The system was evaluated *in vitro* for drug loading, rate of hydration (spontaneity), vesicle size, polydispersity, entrapment efficiency and drug diffusion across rat skin. The effect of composition of formulation, amount of drug, type of Spans, alcohols and sonication time on transdermal permeation profile was observed. The stability studies were performed at 4°C and at room temperature. The biological assay for progestational activity included endometrial assay and inhibition with the formation of corpora lutea. The study demonstrated the utility of proniosomal transdermal patch bearing levonorgestrel for effective contraception.^[41]

5.12. Cosmetics/Cosmeceuticals

Proniosomal gels are generally present in transparent, translucent or white semisolid gel texture, which makes them physically stable during storage and transport. Due to the limited solvent system present, the proniosomes formed were the mixture of many phases of liquid crystal, viz. lamellar, hexagonal and cubic phase liquid crystals. Dissolution of most surfactants in water, leads to the formation of lyotropic liquid crystals rather than micellar solution. Lamellar phase shows sheets of surfactants arranged in bilayer form, whereas in hexagonal phase cylindrical units are packed in hexagonal fashion.

Cubic phase consists of curved bio-continuous lipid bilayer extending in three dimensions, separating two congruent networks of water channels. These liquid crystals present an attractive appearance because of their, transparency and high viscosity, although in the beginning of its formation, a short range of less viscous compositions (so called liquid/gel compositions) appear in some cases. Addition of water leads to interaction between water and polar groups of the surfactant results in swelling of bilayer. Proniosomes exists in two forms, i.e. semisolid liquid crystal gel and dry granular powder, depending on their method of preparation. Out of these two forms, the proniosome gel is mainly used for topical/transdermal applications. Nowadays consumers are replacing cosmetics frequently with cosmeceuticals. Cosmeceuticals are skin care medicines which combine cosmetics and medicines. Many times consumer claims that their cosmetics are not effective this is true because the availability of the cosmetic agent is must at the site of action. The skin is a complex organ and allows entry of only selective components. So the formulation of a cosmetic/Cosmeceuticals is very important in terms of delivering the active agent at the site of action. The new drug delivery systems are required to deliver the actives into the skin. Applying a cosmetic/Cosmeceuticals in a certain way may change its activity. For example, increased time of application usually leads to higher activity. Occlusion (covering the product with something, as plastic or a medical membrane/hydrogel) usually increases activity.

Proniosome gel can be used as an effective delivery systems for cosmetics and Cosmeceuticals due to their unique properties. For applying therapeutic and cosmetic agents onto or through skin requires a non toxic, dermatologically acceptable carrier, which not only control the release of the agent for prolong action but also enhances the penetration to the skin layer. Proniosome gel is promising delivery systems for delivering the actives through skin via different formulations of drugs and cosmetics. The delivery system not only enhances the delivery of active agent through skin but also controls the rate of release. A wide variety of active agents of different therapeutic functions were formulated into proniosomal gel delivery system. Proniosomal gel carrier system entraps not only hydrophilic but hydrophobic agents also. There is great scope for cosmetic agents to be incorporate in the proniosomal gel delivery system.

Nowadays a large number 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 harm to the skin. It is proved that proniosomes are as effective as niosomes and liposomes, but their preparation, 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, antiageing, cleansing, sunscreen particles, etc. The different liquid crystalline phases prepared by using various surfactants which appear in proniosomes gel also, are used for various cosmetic applications. Proniosomes gel system is a step forward to noisome, which can be utilized for various applications in delivery of actives at desired site. Proniosomes are easily hydrated using aqueous phase or by skin itself if used topically. Their incorporation into base gel/cream/ointment along with other ingredients leads to form a cosmetic preparation. These cosmetic formulations can be used for topical/transdermal applications for various functions. Proniosomes gel formulation shows advantages in controlled drug delivery improved bioavailability, reduced side effects and entrapment of both hydrophilic and hydrophobic drugs. Proniosomes gel has an affinity towards biological membranes which helps in enhancing the permeation of actives through skin. A brief compilation related to wide range of actives including therapeutic agents and cosmetic agents are which have been reported to be utilized for various applications.^[48,49]

5.13. NSAIDs Application

Ketorolac tromethamine (KT) is a nonsteroidal agent with potent analgesic and moderate anti-inflammatory activity. The drug is currently administered intramuscularly and orally in divided multiple doses for short-term management of postoperative pain (30 mg q.i.d. by IM injection and 10 mg q.i.d. as oral tablets). This frequent dosing, which results in unacceptable patient compliance, is required due to the short half-life of the drug (4– 6 h). Therefore, an alternative noninvasive mode of delivery of the drug is needed. Transdermal delivery certainly appears to be an attractive route of administration to maintain the drug blood levels of KT for an extended period of time.^[50]

Piroxicam, a non-steroidal anti-inflammatory drug (NSAID), are used in the treatment of dysmenorrheal various acute and chronic musculoskeletal disorders like rheumatoid arthritis, osteoarthritis etc. and also as potent analgesics (Andersson et al., 1998). However, the use of piroxicam has been associated with a number of gastrointestinal disorders (Schiantarelli and Cadel, 1981). Dermal delivery is an alternative route, but requires a formulation which ensures the deep skin penetration.^[51] Aceclofenac in the present study the slurry method was used for the preparation and optimization study of aceclofenac, as this method is simple and easy to scale up. Aceclofenac is a poorly water soluble, non-steroidal anti-inflammatory drug which acts specifically on inflammatory sites and thereby decreases the inflammation. It is highly effective as an anti-inflammatory drug for various inflammatory conditions like rheumatoid arthritis, osteoarthritis and ankylosing spondylitis.^[52,39] A new proniosomal delivery system for

a poorly watersoluble drug such as celecoxib was developed and subjected to in vitro and in vivo studies.

Proniosomes were prepared by sequential spraying method, which consisted of cholesterol, span 60 and dicetyl phosphate in a molar ratio of 1:1: 0.1, respectively. The average entrapment percent of celecoxib proniosome-derived niosomes was about 95%. The prepared proniosomes showed marked enhancement in the dissolution of celecoxib as compared to pure drug powder. The bioavailability of 200 mg single dose of both celecoxib proniosomal formulation and a conventional marketed celecoxib capsule was studied in human volunteers. The obtained results show that the proniosomal formulation significantly improved the extent of celecoxib absorption than conventional capsule. The mean relative bioavailability of the proniosomal formulation to the conventional capsule was 172.06 +/- 0.14%. The mean T (max) for celecoxib was prolonged when given as proniosomal capsule. There was no significant difference between the values of K (el) and t (1/2) for both celecoxib preparations. In conclusion, the proniosomal oral delivery system of celecoxib with improved bioavailability was established.

5.14. Antiallergic:

Investigate the preparation and safety of carboplatin proliposomes Methods: Carboplatin liposomes were prepared by film extrusion. Mannitol was incorporated into the liposome to prevent the liposome vesicles from aggregation. The carboplatin proliposomes were obtained by lyophilization. Allergic reactions to carboplatin proliposomes were evaluated in Guinea pigs. The vein irritation and hemolysis post the administration of liposomes in rabbits were characterized. Results: The inclusion efficiency and drug delivery ratio of the rehydrated carboplatin proliposomes were 72.0% and 24.0%, respectively. The particle size of the liposomes was well-distributed and in well-rounded shape, averaging 125.1 nm. No allergic reactions were found in Guinea pigs. None of hemolysis, hemagglutination and vein irritation was reported, either, after intravenous injections into rabbits. Conclusion: The highly efficient and safe carboplatin proliposomes were achieved.^[53]

5.15. Preparation of Vitamins

To prepare vitamin A proliposomes and to enhance stability of vitamin A. Methods: Freeze drying method was used to prepare vitamin A proliposomes. The particle morphology, the size range, encapsulation efficiency and stability of vitamin A liposomes were studied. Results: After vitamin A proliposomes was hydrated, vitamin A liposomes with unilamellar vesicle was formed. The particles were distributed homogenously. The average particle size was 0.615 µm. The encapsulation efficiency of vitamin A was 98.5%. The liposomes were stored at 40°C for 3 months and were stable. Conclusion: Vitamin A proliposomes exhibit greater stability.^[54]

5.16. Co-Enzyme preparation of Proliposome

To enhance the stability of coenzyme Q10 liposomes, freeze-drying method was used to prepare coenzyme Q10 proliposomes. Sucrose, trehalose, mannitol and lactose were selected as cryoprotectant. The particle morphology, the size range and encapsulation efficiency of coenzyme Q10 liposomes before freeze-drying and after hydrated were studied. The weight ratios of the optimized formulation were trehalose egg lecithin=41. After coenzyme Q10 proliposomes was hydrated, the average diameter of rehydration liposomes was 508nm and the encapsulation efficiency was about 75%. The analysis of FTIR spectrum showed that the hydrogen bond between trehalose and lipid head groups formed in the dry state.^[55]

6. CONCLUSION

Nanoparticulate systems have great potentials, being able to convert poorly soluble, poorly absorbed and labile biological active substance into promising deliverable drugs. Generally nanoparticle have relatively higher intracellular uptake compared to microparticles and available to a wide range of biological targets due to their small size and relative mobility.

The development of proniosome represents a significant advance over the conventional vesicular systems. This concept of incorporating the drug into proniosomes for a better targeting at appropriate tissue destination and for controlled delivery is widely accepted by researchers. As a drug delivery device, proniosomes are osmotically active and stable.

Proniosomes are thought to be better candidates of drug delivery as compared to liposomes and niosomes due to various factors like cost, stability etc. Compared to liposome or niosomes, proniosomes are very promising as drug carriers and compared to liposome and niosome suspension, proniosome represents a significant improvement by eliminating physical stability problems, such as aggregation or fusion of vesicles and leaking of entrapped drug's during long term storage. Proniosome are convenient to store, transport and for unit dosing since proniosome's have similar release characteristics as conventional niosomes, it may offer improved bioavailability of some drugs with poor solubility controlled release formulations or reduced adverse effects of some drugs. The slurry method was found to be simple and suitable for laboratory scale preparation of proniosomes.

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