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Approaches for improvement of vesicular system-Pro-vesicular drug delivery

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ABSTRACT

Drug delivery systems using colloidal particulate carriers such as liposomes and niosomes have distinct advantages over conventional dosage forms. This class of drug carrier systems will likely play an increasingly important role in drug delivery. However, there remain significant problems like instability in the general application of liposomes and niosomes for drug delivery. Pro vesicular drug delivery developed to overcome the stability problems associated with vesicular drug delivery systems composed of water soluble porous powder as a carrier drug is dissolved in an organic solvent to produce free-flowing granular product. To overcome the limitations (especially chemical and physical stability) of vesicular drug delivery systems like liposomes, niosomes, trans-ferosomes, and pharmacosomes, the pro-vesicular approach was introduced. The present article gives a brief overview of introduction on proliposomes, Pro-niosomes, Dry granular liposomes, mixed micellar proliposomes, Pro-transferosomes and its applications.

Keywords*:* Proliposomes, Pro-niosomes, Pro-transferosomes, Dry granular liposomes.

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INTRODUCTION

Drug delivery systems using colloidal particulate carriers such as liposomes and niosomes have distinct advantages over conventional dosage forms. This class of drug carrier systems will likely play an increasingly important role in drug delivery. However, there remain significant problems like instability in the general application of liposomes and niosomes for drug delivery. The pro-vesicular concept has evolved to resolve the stability issues pertaining to the conventional vesicular systems i.e. liposomes and niosomes. Pro-vesicular systems are composed of water soluble porous powder as a carrier upon which one may load phospholipids/non-ionic surfactants and drugs dissolved in organic solvent. The resultant dry free-flowing granular product could be hydrated immediately before use and can avoid many of the problems associated with aqueous vesicular dispersions. The new emerging concept has demonstrated the potential of proliposomes/pro-niosomes in improving the oral bioavailability and permeation of drugs across the stratum corneum. Based on the investigations it is clear that pro-vesicular systems appear to be an alternate drug carrier for various routes of drug administration.

Approaches for improvement of vesicular system

Pro-vesicular drug delivery

Pro vesicular drug delivery developed to overcome the stability problems associated with vesicular drug delivery systems composed of water soluble porous powder as a carrier drug is dissolved in an organic solvent to produce free-flowing granular product. It can avoid many of the problems associated with aqueous vesicular dispersions [1].

To overcome the limitations (especially chemical and physical stability) of vesicular drug delivery systems like liposomes, niosomes, trans-ferosomes, and pharmacosomes, the pro-vesicular approach was introduced.

This includes-

A. Proliposomes
B. Pro-niosomes
C. Dry granular liposomes
D. Mixed micellar proliposomes
E. Pro-transferosomes

PROLIPOSOMES

Proliposomes (PLs) discovered by Payne et.al. In 1986, Proliposomes offer an elegant alternative to conventional liposomal formulations and defined as dry, free-flowing particles that

immediately form a liposomal suspension when they come in contact with water. They prepared by a penetrating solution of drugs and phospholipids in volatile organic solvents into the micro-porous matrix of water-soluble carrier particles, followed by evaporation of the organic solvents. In order to improve a stability of liposomes, the concept of proliposomes proposed [2]. Because of the solid properties, the stability problems of liposome can resolve without influencing their intrinsic characteristic [3-5].

Strategies for the preparation of proliposomes

Proliposomes composed of drug, phospholipid and a water-soluble porous powder and can be stored sterilized in a dried state [6, 7]. Moreover, by controlling the size of the porous powder in proliposomes, relatively narrow range of reconstituted liposome size can obtain [8] In preparation of proliposomes lipids, organic solvents like chloroform and porous powder like sorbitol used.Their free-flowing particulate properties permit fabrication of proliposomes into solid dosage forms, such as tablets and capsules, which then converted to liposomes on contact with water or biological fluids [9]. Proliposomal tablets and capsules represent the first example in which liposomes fabricated into solid-type dosage forms.

Comparisons between liposomes and proliposomes

Liposomes-Unilamellar or multilamellar spheroid structures composed of lipid molecules, often phospholipids. They show controlled release and increased solubility but have a tendency to aggregate or fuse, susceptible to hydrolysis or oxidation. Proliposomes-an alternative forms to conventional liposomal formulation Composed of water soluble porous powder as a carrier, phospholipids and drugs dissolved in organic solvent. Lipid and drug are coated onto a soluble carrier to form free-flowing granular material [10] Show controlled release, better stability, ease of handling and increased solubility. Comparison between liposomes and proliposomes shown in Table-**1**.

Advantages of proliposomes [11]

- Targeting of anti-cancer drugs to tumor sites.
- Targeting of drugs to non-RE tissues, which has not been possible with conventional liposomes.
- Proliposomes can use for controlling release within the vasculature by manipulating the phospholipid composition of bi-layers.
- For the diseases of vascular origin, proliposomes provide the best therapeutic effect over conventional drug.

Review of literature

•Keon Hyoung song et al. formulated Proliposomes contain salmon calcitonin by penetrating a methanol-chloroform solution of sCT and phosphotidylcholine into micro-porous sorbitol particles, which followed by vacuum evaporation of solvent by which free-flowing of liposomes obtained. These on contact with water certain amount of sCT entrapped by liposomes. Permeability of sCT across Caco-2 cell mono layers increased by incorporating sCT into proliposomes. They had suggested that the pharmacokinetics of sCT can modify if it's administered through proliposomes. The development of various dosage forms of sCT, especially solid dosage forms feasible with proliposomes [12].

• Byung Hwa Jung et al. formulated Proliposomes containing nicotine base (NB–proliposomes) or nicotine hydrogen tartarate salt (NS–proliposomes) and a mixture of powdered nicotine hydrogen tartarate salt and sorbitol (1:9 mixture, MP) administered intranasally to rats at a nicotine dose of 1 mg/ kg and the results Prolonged delivery of nicotine was found to achievable by the intranasal administration of NB– proliposomes, NS–proliposomes and NS–sorbitol mixed powders [13].

• Praveen S et al. developed Proliposomal formulations to enhance the oral bio-availability of exemestane by improving solubility, dissolution and/or intestinal permeability. Proliposomal powder formulations prepared to use different ratios of drug (exemestane), distearoyl–phosphatidylcholine (DSPC), cholesterol and dimyristoyl–phosphatidylglycerol (DMPG) by the solvent evaporation method. It is evident from this that Proliposomes provided enhanced exemestane dissolution due to incorporation into the phospholipid bi-layers and change in the physical state from crystalline to amorphous which confirmed by DSC studies [14].

• Hongtao Xu et al. formulated free-flowing proliposomes which contained vinpocetine prepared successfully to increase the oral bio-availability of vinpocetine, in this study the proliposomes prepared by a novel method which reported for the first time and the formulation optimized using the centre composite design (CCD)., The preparation method of VP proliposomes powders was more efficient and less toxic. The optimized vinpocetine proliposomes did improve the oral bio-availability of vinpocetine in New Zealand rabbits and offer a new approach to enhance the gastrointestinal absorption of poorly water-soluble drugs [15].

• Bo Young Hwang et al. formulated proliposomes as a sustained trans-dermal dosage examined. Proliposomes containing varying amounts of nicotine prepared by a standard

method using sorbitol and lecithin. The porous structure of sorbitol in the proliposomes maintained, indicating that most lecithin and nicotine deposited within their porous matrix of the sorbitol particles. As a consequence, the flow properties of the proliposomal particles were comparable to that of original sorbitol particles. Evidently, that sustained delivery of nicotine drug across the skin appears to achievable through topical application of nicotine-loaded proliposomes under occlusive conditions [16].

- Zeljka Pavelic et al. developed a liposomal drug carrier system, able to provide sustained and controlled release of appropriate drug for local vaginal therapy. To optimize the preparation of liposomes with regard to size and entrapment efficiency, liposomes a containing calcein prepared by five methods and is evident that Proliposome and polyol dilution methods would be the right choice of preparation methods for preparing liposomes due to their high trapping efficiency of model substance. Both methods are simple, reproducible and suitable for mass production of liposomes, stable in conditions chosen to mimic the human vaginal environment (a buffer pH 4.5). Incorporation of those liposomes in Carbopol gels further improved their stability and confirmed the applicability of liposome gels as a novel vaginal delivery system [17].
- Byung Nak Ahn et al. formulated free-flowing proliposomes containing propranolol hydrochloride (pH) evaluated for their potential as a nasal drug delivery system of propranolol to sustain the plasma concentration of the drug. In vitro release of pH was definitely retarded by an incorporating pH in the proliposomes, when compared with pH-loaded sorbitol;the results indicate that the proliposome system can be a potential candidate for the sustained delivery system of propranolol through nasal mucosa [18].
- Deepali D Deshmukh et al. formulated Cormolyn Beads for oral drug delivery formulated. Phospholipid (distearyl phosphatidyl choline) a cholesterol surfactant (Tween80/sodium holate) systems spray-coated on beads containing cromolyn sodium and the dosage forms characterized for vesicle formation and encapsulation efficiency. Spontaneous formation of vesicles upon dilution of beads observed. Enhancement in cromolyn transport was higher with phospholipids-surfactant proliposomal formulations compared to surfactant-free lipid formulations or pure surfactant solutions, the most significant enhancement being with formulations with low surfactant Concentration. Results suggested that a phospholipids-surfactant proliposomal bead offers a good potential for improved oral delivery of Cromolyn [19].

- Anne Paavola et al. formulated liposomal solutions of ibuprofen-Na (20 mg: ml) prepared by high-pressure homogenization from egg phosphatidylcholine. The liposomal gel consisted of poloxamer 407 and the liposomal solution. No signs in the 1H-NMR spectroscopy of line broadening or chemical shifts observed. The liposomal formulations were reproducible and stable. The liposomal poloxamer gel represents a new formulation approach to increase the local epidural availability of ibuprofen. It appeared to a promising injectable controlled-release drug delivery system [20].
- Agarwal R et al. formulated Miconazole Nitrate loaded topical liposomes as miconozole nitrate is a widely used an anti-fungal agent, but its use in topical formulations is not efficacious because deep-seated fungal infections are difficult to treat with conventional formulations. So, entrapped drug in liposomes that can facilitate localized delivery of the drug and improved availability with a controlled release pattern which can advance treatment of deep fungal infections. The prepared products characterized for liposome specific properties such as microscopic appearance, size and degree of entrapment [21].

Proliposomes could be prepared by various methods including a crystal-film method [22], film-deposition on carrier's method [23], fluidized-bed method [24], powder bed grinding method [25], freezing and drying method [26] and spray drying method [27].

METHODS OF PREPARATION

Some of the normally used methods employed in preparation of PLs discussed below. They include

Film deposition on carrier method

This method involves a deposition of film about drugs and phospholipids onto a porous, water-soluble carrier material. As seen in Figure. **1**, solution of drug and phospholipid/s in a volatile organic solvent is an added drop wise via a feed tube onto a bed of carrier material held in a flask of a rotary flash evaporator under vacuum. At any given time, over-wetting of the matrix avoided and a subsequent aliquot of organic solution introduced only when a free-flowing powder matrix is obtained [28, 29]. The carriers chosen should have a high surface area and porosity so that the amount of carrier required can easily adjust to support the lipids. It also enables a high surfactant to a carrier mass ratio in the preparation of PLs. Further, being water-soluble they allow rapid formation of liposomal dispersion on hydration and by controlling the size of porous powder, relatively narrow range of reconstituted liposomes can obtain. Some of the carriers utilized include-maltodextrin, sorbitol, micro-crystalline cellulose, magnesium aluminium silicates, Mannitol,etc.

The manufacturing procedure, however, appears too tedious and not easy to control since the operation requires a discontinuous step of solvent addition and evaporation, which is time-consuming [30]. In order to solve this problem, the method modified wherein the carrier material dispersed in organic solution of the drug and phospholipid/s in a flask of the rotary evaporator, and subjected to vacuum evaporation. The suspension made the lipid distribution more uniform and efficient and the process is continuous and timesaving compared to the original method [31].

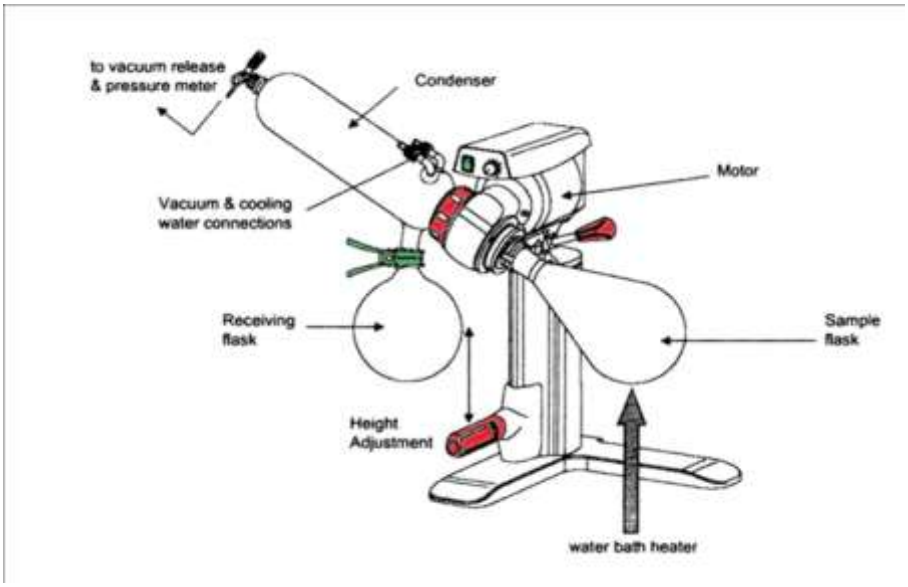

Figure.1. Apparatus for preparing PLs by Film Deposition on carrier method

Spray drying method

The unique feature of the spray drying process lies in its ability to involve particles formations and drying in a continuous single step, allowing better control of particle formation. Spray drying is not only limited to aqueous solutions, but can also be used for non-aqueous systems to prepare particles. This method is mainly used when particles of uniform size and shape required and can easily scale up its cost-effective and suitable for large-scale production of PLs [32, 33].

As seen in Figure. 2, the spray drying process involves four stages: atomization of the product into a spray nozzle, spray-air contact, drying of the spray droplets and collection of the solid product [34]. Initially liquid dispersions a containing pure lipid or lipids and carrier in organic solvent prepared and pumped into the drying chamber. The dispersions are atomized into the drying chamber using a spray nozzle and dried in a concurrent air flow, which is then collected in a reservoir.

Major concerns to spray drying are high working temperatures, shearing stresses and an absorption phenomenon that may lead to thermal and mechanical degradation of the active molecules. This can improve by optimizing the operating parameters such as drying air

temperature and liquid spraying rate. Stabilizing adjuvant such as disaccharide, cyclic oligosaccharides and polyols can also be used to protect the integrity of the active molecules and enhance the efficiency of hydration by increasing the surface area of lipids.

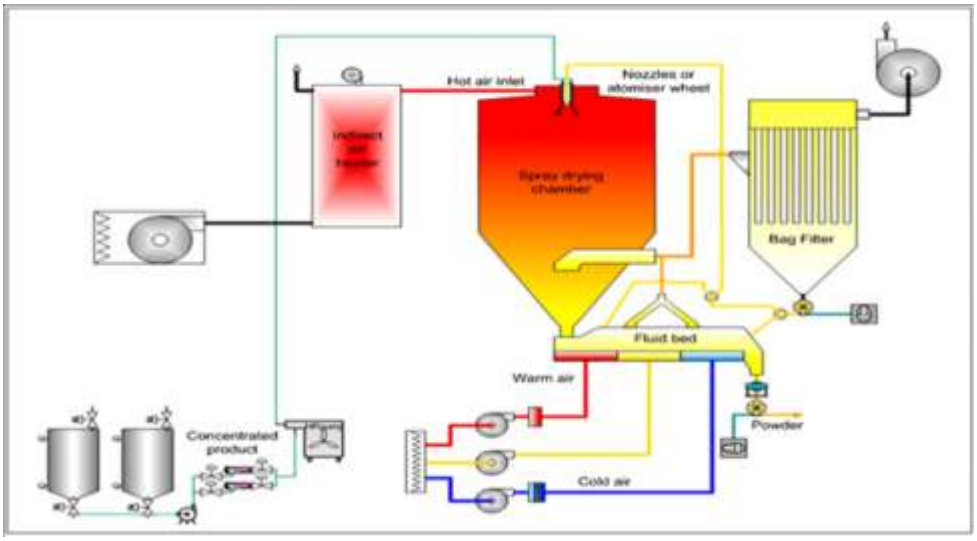

Figure.2. Apparatus for preparing PLs by Spray drying method

Fluidized bed method

This method can employ for the large-scale production of PLs and works on the principle of particle coating technology. The carrier material used here can vary from crystalline powder to nonpareil beads. When using beads as carrier material, initial seal coating applied to the beads to provide a smooth surface for further coating of phospholipids. These ensure formation of a thin uniform coating of phospholipid around the core and the formation of smaller sized liposomes upon hydration. The solution of the drug and phospholipid in organic solvent sprayed on to the carrier material through a nozzle. At the same time, the organic solvent removed by applying vacuum to the fluid bed. To remove the trace amount of residual solvent the finished lipid-coated powder/beads can dry an under vacuum overnight.

The method offers following advantages

A. It utilizes Film coating technology, which is well established and process able.
B. Various cores and coating materials are available or easy to prepare.
C. It is a cost-effective method to prepare liposomes for drug delivery [35, 36].

Super critical anti-solvent method

The Supercritical anti-solvent method utilizes Supercritical Carbon dioxide ($SCCO_2$) in preparation of PLs. $SCCO_2$ is a fluid state of carbon dioxide where it is held at or above its critical temperature and pressure. Anti-solvent technology is widely used in food industry and developed to prepare PLs because of its lower residual solvents, simpler steps and mild operation

temperatures. As shown in Figure. **3**, the apparatus used preparation of PLs includes three parts: a sample delivery unit, a precipitation unit and a separation unit. The sample delivery unit consists of two pumps: one for CO_2 and the other for solution. CO_2 is supplied from the CO_2 cylinder (1) which cooled down by a refrigerator (2) and introduced via a high pressure pump (3) to the buffer tank (4), in which it preheated. The drug solution introduced via HPLC pump (11). The solvent used for dissolving the drug should be completely miscible with CO_2. Opening the valves A and B allows the entry of the solution and CO_2 into the vessel through the nozzle (B). As seen in Figure. **3** solutions sprayed through the inner tubule whereas CO_2 is sprayed through the outer tubule of the nozzle. The precipitation unit consists of a vessel (9) heated by an air bath. The separation unit consists of a separator (13) and a wet gas meter (14). The organic solvent separated from $SCCO_2$ in the separator because of lower pressure whereas the volumetric flow rate of CO_2 is measured by the wet gas meter. After the temperature and pressure of the separating vessel reaches, the present value, valve A opened to allow entry to follow by opening of valve B allowing the entry of drug solution. $SCCO_2$ and solution mixed and diffused into one another rapidly as they are sprayed through the coaxial nozzle. This causes the solutes dissolved in an organic solvent to reach super saturation in a very short period because the solubility of solutes in the organic solvent decreases greatly. As a result, the PLs precipitated in the vessel. Once the solution is completely utilized, valves A and B closed while valve C opened in order to depressurize the vessel at the operating temperature. The samples are collected on the filter (8) at the bottom of the vessel. The pressure, temperature and flow rate of the drug solution need to optimize to obtain high drug loading in PL [37, 38].

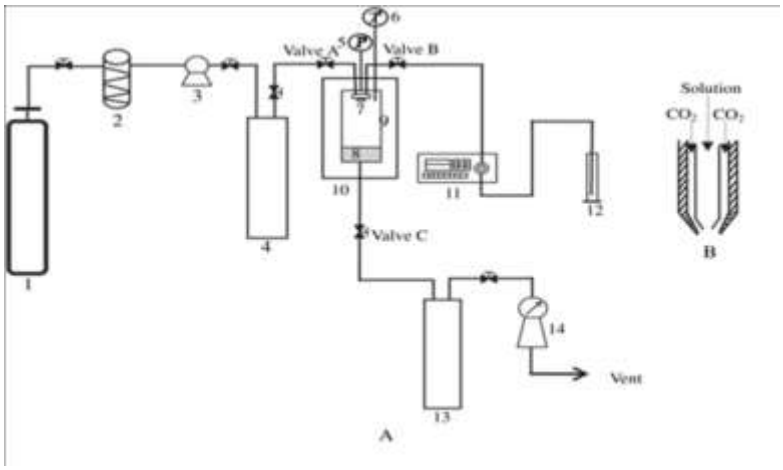

Figure.3. Apparatus for preparing PLs by Supercritical Anti Solvent Method.

Applications

PLs can be exploited for the following routes of administration-

Parenteral Delivery

For liposomes to be developed for parenteral application, their sterilization is mandatory. Routinely employed sterilization techniques in Pharmaceutical industry include- Steam sterilization, γ-irradiation, and Aseptic manufacturing and filtration sterilization. Terminal sterilization using steam at 121ºC may not be suitable for liposomal formulations, since high temperature may disrupt the liposome architecture due to hydrolysis of lipids, leading to physical destabilization. γ- Irradiation is also unsuitable for liposomal dispersions, since radiation causes hydrolysis and accelerates the peroxidation of unsaturated lipids. Aseptic manufacturing is not commonly used due to the expense and difficulty in validation. Filtration sterilization of the final product can be challenging due to the structural complexity of these vesicles and loss of lipids by their non-specific adsorb to filters [39, 40].

PLs are well suited for parenteral application of liposomes. The main advantage associated with PLs is that it allows sterilization without affecting the intrinsic characteristics. Besides, they can be stored as sterilized in dry state and can be hydrated prior to administration to form multilamellar liposomal suspension. In addition, several recent studies have reported that γ-irradiation sterilization is not as detrimental to liposomes as previously assumed, particularly when irradiated in the dry state. Since hydroxyl radicals (resulting from exposure of water to radiation), are a major source of the free radicals which cause the damage. Thus water content plays a key role in the stability of liposomes during this process. Being available in dry form, γ-irradiation may be used as a sterilization technique for PLs [41].

Oral Delivery

Oral drug delivery continues to be the preferred route of administration, but liposomes have limited success in delivering drugs through oral route [42]. This is due to the absence of a stable dosage form for oral delivery and erratic and unpredictable absorption profiles shown by liposomes. This is due to their inability to retain their integrity at the site of absorption. Being available as a free flowing powder, PL represents the first example of delivering liposomes into solid dosage form such as tablets or capsules. Further, liposomes are formed in contact with biological fluids at the site of absorption ensuring the retention of liposome integrity. PLs act as one of the most promising vehicles for enhancing the dissolution efficiency of poorly soluble drugs. It forms multi-lamellar vesicles on hydration which ensures higher incorporation of insoluble drugs due to increased hydrophobic volume within the liposomal lamellae. It also allows conversion of the drug from crystalline to amorphous form1. The larger particle size of multi lamellar liposomes formed on hydration ensures lymphatic uptake and improves the

bioavailability of drugs undergoing high first pass metabolism [43] Further, the phospholipid molecules which form the backbone of the bilayer structure help to enhance the solubility of drug molecule.

Zaleplon is a hypnotic drug indicated in insomnia and is a potent anticonvulsant. Due to its limited aqueous solubility and extensive first pass metabolism it shows poor bioavailability of 30%. Janga et al. Utilized PLs for oral delivery of Zaleplon and found 2-5 fold improvement in oral bioavailability in rats compared to pure drug. Vinpocetine is used in the prevention and treatment of ischemic stroke and other cerebrovascular diseases. Due to its poor aqueous solubility and high first pass metabolism, it has low oral bioavailability of 7% in humans. PLs utilized by Xu et.al improved the oral bioavailability of Vinpocetine by 3.5 fold in rabbits compared to the pure drug. Silymarin is widely used to maintain liver health and treat hepatic disorders but is slightly soluble in water and in oil and shows the poor permeation across the intestinal epithelial cells. PL utilized by Xiao et.al for Silymarin showed 3.4 fold increase in oral bioavailability in beagle dogs compared to the pure drug. PLs have also been successfully used for other poorly soluble drugs such as, Exemestane, Salamon Calcitonin, Glyburide [43], Halofantrine [44] and Progesterone [46]. PLs also have the ability to enhance the lipophilicity of highly hydrophilic drugs. This is due to the similarity between the liposomal bilayers and bio membranes. In addition, their relatively small size and bio adhesive nature help facilitate the absorption of poorly absorbed and poorly permeable drugs through endocytosis [46]. Cromolyn sodium is an anti-inflammatory drug used in the prophylactic treatment of bronchial asthma and allergic rhinitis. It is poorly absorbed from the gastrointestinal tract (bioavailability < 1%). Increasing the lipophilic character of Cromolyn could facilitate passive transport and, thereby, improves its absorption across the barrier membranes. PL utilized by Deshmukh et.al showed 4-7 fold increase in transepithelial flux compared to pure drug indicating enhanced lipophilicity of Cromolyn by liposome encapsulation. A patent titled “Enteric-coated proliposomal formulations for poorly water soluble drugs” is present relating to the oral delivery of PL. The advantage of this invention is that it provides a simple and inexpensive system to facilitate the oral administration of poorly water soluble drugs and enhancing their stability and bioavailability. Examples of drugs utilizing the invention are Halofantrine, Testosterone and Famotidine [47].

Pulmonary Delivery

Major advantage of liposomes as pulmonary drug delivery system is that they are prepared from phospholipids which are endogenous to lungs as component of lung surfactant. Drug encapsulation in liposomes provides modulated absorption, resulting in localized drug

action in the respiratory tract and prolonged drug presence in circulation and reduced systemic adverse effects [48.] Drug delivery to the pulmonary route is achieved by three types of devices namely-

Table 1: Comparison between liposomes and proliposomes

LIPOSOMES	PROLIPOSOMES
Unilamellar or multilamellar spheroid structures composed of lipid molecules, often phospholipids	An alternative formof conventional liposomal formulation
Advantages Controlled release and increased solubility	Composed of water soluble porous powder as a carrier, phospholipids and drugs dissolved in organic solvent
Disadvantages Tendency to aggregate or fuse susceptible to hydrolysis or oxidation	Lipid and drug are coated onto a soluble carrier to form a free-flowing granular material

Pressurized metered dose Inhalers (pMDI)

As the name suggests it consists of solution or suspension of drugs in liquefied propellants. Use of Hydrofluroalkanes as non-ozone depleting propellants over CFCs has the limitation for liposome delivery as they are poor solvents for phospholipids. PLs help overcome this limitation as they can be suspended in these propellants and serve as carrier for pulmonary delivery of liposomes through pMDI.

Dry Powder Inhalers (DPIs)

These disperse the drug into the patient's airstream as a fine powder during inhalation. Delivering liposomes through DPI have many advantages such as controlled delivery, increased potency, and reduced toxicity, uniform deposition of drugs locally, patient compliance, stability and high dose carrying capacity. Being available as dry powder form, PLs are the best alternative for delivering liposomes through DPIs [49]. Chougule [49] et.al developed spray dried liposome encapsulated Dapsone DPI for prolonged drug retention in lungs to prevent Pneumocystis carinii pneumonia. Prolonged drug release of up to 16 h was observed in vitro.

Nebulizers

Nebulization offers the simplest means, for delivering liposomes to the human respiratory tract but it is concerned with liposome leakage and drug stability. Use of dry powder formulations has been suggested to overcome these issues. Lyophilization and jet milling may be used to obtain dry powder but tend to have deleterious effect on liposomes due to the stresses involved in these processes. Thus, PLs serve as a stable alternative for delivering liposomes through nebulization. Besides, the ready formation of an isotonic liposome formulation in situ from PLs seems to offer advantages over other formulation approaches [48].

iv. Transdermal delivery

Phospholipids, being the major component of liposomal system, can easily get integrated with the skin lipids and maintain the desired hydration conditions to improve drug permeation. When PLs are applied to mucosal membrane, they are expected to form liposomes on contact with mucosal fluids whereby the resulting liposomes act as sustained release dosage form for loaded drugs. Liposomes formed on hydration have the ability to modulate diffusion across the skin. They do so by fusing with the skin surface and establishing concentration gradient of the intercalated drug across the skin. Thus they enhance skin permeation. Also, the vesicle intercalation into the intracellular lipid layers of the skin results in fluidization and disorganization of the regular skin structure, obviating the barrier function of the stratum corneum [50]

Exemestane, a novel steroidal aromatase in activator has limited bioavailability of 42% due to poor solubility and extensive first-pass metabolism. Jukanti [49] et.al utilized PL system for transdermal delivery of Exemestane and found a 2.4 fold increase in bioavailability from PL gel compared to oral suspension. PLs have also been developed for sustained delivery of Nicotine and Aceclofenac [51] trans-dermally.

v. Mucosal delivery

PLs form vesicular structures (liposomes) in vivo, triggered by the aqueous environment found on the mucosal surfaces. Phospholipids present in them have natural affinity for biological membranes. Besides they are generally nontoxic and non-irritant. The presence of drug as molecular dispersion in the bilayers offers improved drug activity.

Further, the difficulties associated with liposomal preparations such as stability and loading are circumvented because the PLs convert to vesicular structures in vivo, i.e., on the mucosa. Liposomes formed on hydration with the mucosal fluid, get deposited on the mucosa as drug reservoirs thereby increasing the drug retention capacity. The significantly higher mucosal retention of the liposomes, results in higher partitioning of the drug into the mucosa. This is responsible for prolonged and enhanced drug activity. This led to the utilization of PLs for vaginal and nasal drug delivery [52.]

Vaginal delivery systems are frequently required to treat local fungal infections. The poor aqueous solubility of antifungal and steroid compounds in conventional formulations limits their presence as molecular dispersion and consequently affects the drug concentration at active sites. The association of these lipophilic agents with the phospholipid molecules of PLs make them excellent carriers to molecularly disperse the drug [53]. Clotrimazole is widely and effectively used

for the treatment of vulvovaginal candidiasis but has low aqueous solubility. Commercially available conventional Clotrimazole vaginal delivery systems, such as creams, foams, and gels, are considered to reside the drug for a relatively short period of time at the targeted site. Ning [55] et.al developed a PL formulation of Clotrimazole and compared the fungicidal efficacy with the standard ointment in rats. The results indicated that Clotrimazole containing vaginal PL prolonged drug release and increased the drug retention into the mucosa. This resulted in higher antifungal efficacy compared to the standard ointment and in addition it did not affect the morphology of vaginal tissues confirming the non-toxic and non-irritant nature of the carrier.

Nasal mucoadhesive delivery has been used to improve local and systemic delivery of therapeutic compounds [55] It is a promising alternative for systemic administration of drugs that are poorly absorbed via the oral route [56]. Limitations associated with this route are mucociliary clearance which limits the residence time of drug in the nasal cavity and lack of sustained release of drugs with short half-life [55, 56]. Proliposomal delivery helps to overcome these limitations. Liposomes formed on hydration decrease the mucociliary clearance of drugs due to their surface viscosity and provide intimate and prolonged contact between the drug and mucus membrane. Hydration process of PL plays a role in sustaining the plasma concentration of drugs with short half-life in systemic circulation [57, 58].

Propranolol is a β-blocker which shows rapid absorption when administered intranasal as an aqueous solution. Due to this, it is eliminated very rapidly from the systemic circulation needing frequent dosing. Ahn [58] et.al utilized PL for nasal delivery of Propranolol. Sustained plasma concentration of Propranolol was obtained due to the slow hydration process of PL in nasal cavity. It was given by the Mean hydration time (MHT) of PLs which was defined as the difference of Mean Residence time between liposomes and PLs. It was found to be 80.4 minutes which confirmed longer residence time of PL in nasal cavity.

A. **PRO-NIOSOMES:**

Hu and Rhodes et al. reported that Pro-niosomes 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 for few minutes [59].The resulting niosomes are very similar to conventional niosomes and more uniform in size [60].These "pro-niosomes" [60, 61] minimize problems of niosomes physical stability such as aggregation, fusion and leaking and provided additional convenience in transportation, distribution, storage and dosing. Pro-niosomes- derived niosomes are superior to conventional niosomes in convenience of storage, transport and dosing. Stability of dry pro-niosomes is

expected to be more stable than a pre-manufactured niosomal formulation. In release studies pro-niosomes appear to be equivalent to conventional niosomes. Size distributions of pro-niosome-derived niosomes are somewhat better that those of conventional niosomes so the release performance in more critical cases turns out to be superior. Pro-niosomes are dry powder, which makes further processing and packaging possible. The powder form provides optimal flexibility, unit dosing, in which the pro-niosomes powder is provided in capsule could be beneficial. A pro-niosome 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 pro-niosomes with very high mass ratios of surfactant to carrier could be prepared. Because of the ease of production of pro-niosomes using the maltodextrin by slurry method, hydration of surfactant from pro-niosomes of a wide range of compositions can be studied.

Comparison between niosomes and pro-niosomes

Niosomes-are non-ionic surfactant based multilamellar or unilamellar vesicles, aqueous solution of solute is entirely enclosed by a membrane of surfactant macro-molecules as bilayers. They are cheap and chemically stable but posses' problems related to physical stability such as fusion, aggregation, sedimentation and leakage on storage. Pro-niosomes-approach minimizes the problems associated with niosomes as it is a dry and free flowing product which is more stable during sterilization and storage. Ease of transfer, distribution, measuring and storage make it a versatile delivery system. Pro-niosomes are water-soluble carrier particles that are coated with surfactant.

Advantages of pro-niosomes over the niosomes

Avoiding problem of physical stability like aggregation, fusion, leaking.

Avoiding hydrolysis of encapsulated drugs which limiting the shelf life of the dispersion.

Strategies for preparation of pro-niosomes

Pro-niosomes are product of non-ionic surfactants easily prepared by dissolving the surfactant in a minimal amount of an acceptable solvent and least amount of water. Typically, pro-niosomes may contain various non-ionic surfactants like span 20, 40, 60, 80 and 85, Tween 20, 40, 80; lecithin, alcohol (ethanol, methanol, isopropyl alcohol) and chloroform. Chemical structure of surfactants influences drug entrapment efficiency. Increasing the alkyl chain length leads to higher entrapment efficiency [62]. It had also been reported that spans provides highest entrapment for the drug [63]. Drug can be entrapped into pro-niosomes composed of tweens;

however the encapsulation efficiency was relatively low as compared to those composed of spans [64]. Most of surfactants used to make non-ionic surfactant vesicles have a low aqueous solubility. However, freely soluble non-ionic surfactants such as tween can form the micelles on hydration in presence of cholesterol [65.] Cholesterol concentration into pro-niosomal formulations could affect vesicle stability and permeability [66-67]. In addition, non-ionic surfactant and cholesterol can be combined with lecithin in these preparations. Formulations containing lecithin increase the entrapment efficiency of drugs compared to formulation containing cholesterol only [68.] However, the incorporation of lecithin into formulation requires special treatment during preparation and storage, which makes the product less stable and highly expensive. As stated earlier, pro-niosomes require minimal amount of acceptable solvent like ethanol, methanol, isopropyl alcohol and chloroform for dissolving surfactants. Various examples of different component of pro-niosomes are enlisted in Table **2** along with their use.

Table 2; commonly used materials for pro-niosomes preparation

Class	Example	Uses
Surfactants	Span 20, 40, 60, 80,85, Tween 20, 40, 80	To increase drug flux across the skin.
Cholesterol	Cholesterol	To prevent leakage of drug formulation
Lecithin.	SoyaLecithin, Egg Lecithin	Penetration enhancer
Maltodextrin	Maltodextrin	Provides flexibility in surfactant and other component ratio.
Sorbitol	Sorbitol	Alters the drug distribution
Alcohol	Ethanol, methanol, Isopropyl alcohol	For providing the softness for vesicle membrane As a penetration enhancer

Suitability of Drug to the Pro-niosomes

Different categories of drugs selections for pro-niosomes formation based upon the below mentioned points;

- Low Aqueous solubility drugs
- High dosage frequency drugs
- Low half-life
- Controlled drug delivery suitable drugs
- Higher adverse drug reactions drugs

REVIEW OF LITERATURE:

- Almira et al [69] reported a novel method for rapid preparation of pro-niosomes with a wide range of surfactant loading. They developed slurry method to produce pro-niosomes using maltodextrin as the carrier. The time required to produce pro-niosomes by this simple method is independent of the ratio of surfactant solution to carrier material and appears to be scalable. The flexibility of the pro-niosome preparation method would allow for the optimization of drug encapsulation in the final formulation based on the type and amount of maltodextrin. This formulation of pro-niosomes is a practical and simple method of producing niosomes at the point of use for drug delivery.
- Tamizharasi et al [70] describes the preparation of indomethacin loaded maltodextrin based pro-niosome by slurry method with different surfactant to cholesterol ratio. Prepared pro-niosomes were optimized for highest percentage drug entrapment. They confirm all particles are uniform in size and shape through microscopy and entrapment efficiency was determined by separating the untrapped drug using dialysis. The in vitro release studies of drug from niosomes exhibited a prolonged release as studied over a period of 24 h. On the basis of in vitro characterization, the niosome showing maximum entrapment and suitable release rate were selected for in vivo performance evaluation. They conclude that the niosomal formulation could be a promising delivery system for indomethacin with improved bioavailability and prolonged drug release profile.
- Mahmoud et al [71] developed pro-niosomal gels or solutions of flurbiprofen based on span 20 ,span 40, span 60 and span 80 with and without cholesterol. Non-ionic surfactant vesicles (niosomes) formed immediately upon hydrating pro-niosomal formulae. They studied influence of different processing and formulation variables such as surfactant chain length, cholesterol content, drug concentration, total lipid concentration, negatively or positively charging lipids and pH of the dispersion medium on flurbiprofen percentage encapsulation efficiency and also, they studied release of the prepared niosomes in phosphate buffer (pH 7.4). Results indicated that the percentage encapsulation efficiency followed the trend Sp 60 >Sp 40 >Sp 20 >Sp 80. Cholesterol increased or decreased the percentage encapsulation efficiency depending on either the type of the surfactant or its concentration within the formulae. The maximum loading efficiency was 94.61% when the hydrating medium was adjusted to pH 5.5. Increasing total lipid or drug concentration also increased the percentage encapsulation efficiency of flurbiprofen into niosomes. However, incorporation of either dicetyl phosphate (DCP) which induces negative charge or stearyl amine (SA) which

induces positive charge decreased the percentage encapsulation efficiency of flurbiprofen into niosomal vesicles. Finally, they suggest in vitro release data for niosomes of Sp 40 and Sp 60 showed release profiles of flurbiprofen from niosomes of different cholesterol contents is an apparently biphasic release process. As a result, this study suggested the potential of pro-niosomes as stable precursors for the immediate preparation of niosomal carrier systems.

- Ajay et al [72] investigated the combined influence of 3 independent variables in the preparation of piroxicam pro-niosomes by the slurry method. They used a 3-factor, 3-level Box-Behnken design to derive a second order polynomial equation and construct contour plots to predict responses. The independent variables selected were molar ratio of Span 60: cholesterol (X1), surfactant loading (X2), and amount of drug (X3). They prepared fifteen batches by slurry method and evaluated for percentage drug entrapment (PDE) and vesicle size. The transformed values of the independent variables and the PDE (dependent variable) were subjected to multiple regressions to establish a full-model second-order polynomial equation. F was calculated to confirm the omission of insignificant terms from the full-model equation to derive a reduced model polynomial equation to predict the PDE of pro-niosome derived niosomes. Contour plots were constructed to show the effects of X1, X2 and X3 on the PDE. A model was validated for accurate prediction of the PDE by performing checkpoint analysis. The computer optimization process and contour plots predicted the levels of independent variables X1, X2, and X3 (0, -0.158 and –0.158 respectively), for maximized response of PDE with constraints on vesicle size. The Box-Behnken design demonstrated the role of the derived equation and contour plots in predicting the values of dependent variables for the preparation and optimization of piroxicam pro-niosomes.
- Ajay et al [73] characterize and optimize aceclofenac pro-niosomes using central composite design and carry out stability studies. They selected three independent variables molar ratio of drug to lipid (X1), surfactant loading (X2) and volume of hydration (X3). Based on central composite design, they prepared 16 batches of pro-niosomes by slurry method and evaluated for the percentage drug entrapment and mean volume diameter. The percentage drug entrapment and mean volume diameter (dependent variables) and the transformed values of independent variables were subjected to multiple regressions to establish a second order polynomial equation. Contour plots were constructed to further elucidate the relationship between the independent and dependent variables. The conformity of the

polynomial equations was checked by preparing three checkpoint batches. From the computer optimization process and contour plots, predicted levels of independent variables X1, X2, and X3(-0.77, -0.8 and 0 respectively), for an optimum response of percentage drug entrapment with constraints on mean volume diameter were determined. The optimized batch was subjected to stability studies. The polynomial equations and contour plots developed using central composite design suggested preparation of pro-niosomes with optimum responses.

- Chintankumar et al [74] prepared aceclofenac loaded maltodextrin based pro-niosome by slurry method with different surfactant to cholesterol ratio. The pro-niosome formulations were evaluated for FT-IR study, angle of repose and scanning electron microscopy. The niosomal suspensions were further evaluated for entrapment efficiency, in vitro release study, kinetic data analysis, stability study, in vivo anti-inflammatory study. The result from SEM analyses has showed smooth surface of pro-niosome. The formulation F4 which showed higher entrapment efficiency of 83.24 ± 1.34 and in-vitro releases of 97.122% at the end of 24hr was found to be best among the all 7 formulation. Release was best explained by the zero order kinetics. Kinetic analysis shows that the drug release follows super case II transport diffusion. Pro-niosome formulation has showed appropriate stability for 90 days by storing the formulation at refrigerator condition.
- Chandra et al [75] prepared piroxicam pro-niosomes by conventional technique and employing maltodextrin and sorbitol as base. The prepared lipid vesicles were evaluated for entrapment efficiency and vesicle size of niosomes formed. The morphology of the pro-niosomes was studied by scanning electron microscopy. The results reveals that span 60 based formulations produced vesicles of smallest size and higher entrapment efficiency while those of span 80 produced vesicles of least entrapment efficiency. Incorporation of lecithin further enhanced entrapment efficiency. Further they investigate permeation of piroxicam from pro-niosome based reservoir type transdermal gel formulation across excised rat abdominal skin Keshery Chein diffusion cell. There was considerable improvement in flux over the control gel formulation. Pro-niosomes were prepared Maximum flux achieved was 35.61g/cm2/h; an enhancement of 7.39 times was achieved for transdermal system based on pro-niosomal gel as compared to control gel. The in-vivo anti-inflammatory studies revealed that pro-niosome based transdermal drug delivery system of piroxicam were promising carriers for delivery of piroxicam. There was significant reduction in carrageenan induced rat paw inflammation compared to control.

- Ibrahim et al [76] prepared ketorolac pro-niosomes using spans and tweens for transdermal delivery. The encapsulation efficiency and size of niosomal vesicles formed by pro-niosome hydration were also characterized by specific high performance liquid chromatography method and scanning electron microscopy. Each of the prepared niosomes achieved about 99% drug encapsulation. Vesicle size was markedly dependent on the composition of the pro-niosomal formulations. Further they investigated permeation of a potent non-steroidal anti-inflammatory ketorolac; across excised rabbit skin from various pro-niosome gel formulations was investigated using Franz diffusion cells. Each of the prepared pro-niosomes significantly improved drug permeation and reduced the lag time ($P>0.05$). Pro-niosomes prepared with span 60 provided a higher ketorolac flux across the skin than did those prepared with Tween 20 (7- and 4-fold the control, respectively). A change in the cholesterol content did not affect the efficiency of the pro-niosomes, and the reduction in the lecithin content did not significantly decrease the flux ($P>0.05$).
- M.Intakhab et al. described the pharmacodynamic evaluation of pro-niosomal transdermal therapeutic gel containing celecoxib which minimizes the hepatic first pass metabolism by the development of low dose transdermal drug delivery system and produced 100% inhibition of paw oedema in rats.
- K.Raja. et al. prepared glipizide loaded maltodextrin based pro-niosome by slurry method with different surfactant–cholesterol ratio. Release was best explained by the zero order kinetics and showed that the drug release follows super-case II transport diffusion and showed appropriate stability for 90 days when compared with reconstituted niosome by storing the formulation at refrigeration condition.
- Ankur Gupta et al. developed pro-niosomal carrier system for captopril for the treatment of hypertension that is capable of efficiently delivering entrapped drug over an extended period of time, and the potential of pro-niosomes as a transdermal drug delivery system was investigated by encapsulating the drug in various formulations of pro-niosomal gel composed of various ratios of sorbitan fatty acid esters, cholesterol, lecithin prepared by coacervation phase separation method and characterized in vitro for size, vesicle count, drug entrapment, drug release profiles and vesicular stability at storage conditions and the studies showed prolonged release of entrapped captopril.
- Sabareesh et al. formulated and evaluated lisinopril dihydrate transdermal pro-niosomal gels using lecithin and cholesterol as encapsulating agents and observed that the formulation

showed good spreadability and viscosity and follows zero order drug release by diffusion mechanism and could be formulated for controlled release of lisinopril.

- Litha Thomas et al. described the pro-vesicular niosomes gel as a novel absorption modulator. It was an insight into the exploitation of the various properties of drug to be encapsulated, preparation, mechanism of penetration and application in transdermal delivery. The factors affecting the entrapment and penetration of drug through the skin was also reviewed.
- Jalesh Varshosaz et al. reviewed sorbitan monopalmitate based pro-niosome for transdermal delivery of chlorpheniramine maleate. The system evaluated for the effect of composition of formulation, type of surfactants and alcohols on the drug loading, rate of hydrates, vesicle size. The results showed that lecithin produced more stable and larger vesicles with higher loading efficiency.
- Bhavana vora et al. described pro-niosome based transdermal delivery of levonorgestral for effective contraception and extensively characterized both in vitro and in vivo studies. The study revealed the pro-niosome structure was liquid crystalline compact and niosome hybrid could be converted to niosome upon hydration. The effect of composition of formulation, amount of drug, alcohols, type of spans and sonication time on transdermal permeation profile was observed. The study demonstrated the utility of pro-niosomal transdermal patch bearing levonorgestrel for effective contraception.
- V .Sankar et al. formulated and evaluated pro-niosome of hydrocortisone gel in comparison with a commercial cream. Hydrocortisone pro-niosome gel was prepared by coacervation phase separation method using different combination of non-ionic surfactants with cholesterol and lecithin. Pro-niosome formulation were characterized for vesicle size, entrapment efficiency and drug content uniformity and found excellent result in increasing permeation of hydrocortisone gel through the skin.
- Rishu Kakkar et al. prepared non-ionic surfactant vesicles of valsartan by coacervation phase separation method. The prepared systems were characterized for encapsulation efficiency, size, shape and in vitro drug release. Stability study was carried out to investigate the leaching of drug from the pro-niosomal system during storage. Results showed that valsartan in all the form was successfully entrapped and a substantial change in release rate and an alteration in the encapsulation efficiency of valsartan from pro-niosomes were observed upon varying the type of surfactant and cholesterol content. The

encapsulation efficiency of pro-niosomes prepared with span60 was superior to that prepared with span[40].

- Reena Thakur et al. developed a pro-niosomal gel of losartan potassium and studied its pharmacokinetic parameter. Pro-niosomal gel of Losartan potassium using different surfactant was formulated and evaluated. The optimized formulation used to evaluate the bioavailability of the formulated product with the marketed product and revealed that significant greater amount of drug reached the systemic circulation than the marketed formulation.
- J.-Y. Fang et al. examined the feasibility of pro-niosomes as a transdermal delivery system for estradiol. The study revealed that encapsulation efficiency of pro-niosomes was nearly 100% and the permeation of estradiol with span 40 and span 60 was higher through the skin. In addition to this, cholesterol in the vesicular bilayer did not significantly affect the entrapment efficiency and penetration. Hence, the study concluded that type and content of non-ionic surfactant is the important parameter that affects efficiency of transdermal estradiol delivery.
- A.Azeem et al. carried out the research with an aim to explore the mechanism of the penetration of drug through the skin. The study showed the stratum corneum is the rate limiting step and when the pro-niosomes were applied to the skin, it get hydrated and converts to niosomes. The hydrated niosomes will break the hydrogen bond network leading to high thermodynamic activity at the interface. This will increase the concentration gradient and hence increases the diffusion pressure for the driving of drug through the stratum corneum.
- Akhilesh et al. studied Gliclazide pro-niosome based niosomal drug delivery system using maltodextrin as carrier and to evaluate its performance. The pro-niosomes with various types and contents of non-ionic surfactant and cholesterol is evaluated in this study. In this study entrapment efficiency is found to be cholesterol: surfactant ratio dependent. The release rate also found to be dependent of cholesterol: surfactant ratio. For effective pro-niosomes, it is essential that the surfactant coating be smooth and uniform to allow rapid and consistent hydration. Angle of repose measurements indicated that the fluidity of pro-niosomes dry powder is equal to or better than that of maltodextrin powder, so further processing of pro-niosomes powder should be straightforward.

- Narayan dutt et al. reviewed approaches to stabilize niosomal drug delivery system. Pro-niosomes is a dry formulation using suitable carrier coated with non-ionic surfactants and can be converted into niosomes immediately before use by hydration. These pro-niosome-derived niosomes are as good as or even better than conventional niosomes. The focus of the study is to bring out different aspects related to pro-niosomes preparation, clinical and merits of proliposomes.
- Hengjiu et al. described procedure for producing a dry product which may be hydrated immediately before use to yield aqueous niosome dispersions similar to those produced by more cumbersome conventional methods. This report describes the preparation of dispersions of pro-niosome derived niosomes, comparison of these niosomes to conventional niosomes, and optimization of pro-niosome formulations. In addition, conventional and pro-niosome derived niosomes are compared in terms of their morphology, particle size, particle size distribution, and drug release performance in synthetic gastric or intestinal fluid. In all comparisons, pro-niosome derived niosomes are as good as or better than conventional niosomes. Further they suggest that these prepared pro-niosomes minimize problems of niosome physical stability such as aggregation, fusion and leaking and provide additional convenience in transportation, distribution, storage and dosing.

Factors affecting the formulation of pro-niosomes [77-79]

Various processing and formulation variables affect the pro-niosomes characteristics. They include surfactant chain length, cholesterol content, drug concentration, total lipid concentration, charge of lipids, pH of the dispersion medium and type of alcohol used in the preparation.

1. Surfactant chain length

Spans are commonly used in the preparation of pro-niosomes. All span types have the same head group and different alkyl chain. Increasing the alkyl chain length is leading to higher entrapment efficiency. The entrapment efficiency followed the trend Span60 (C18)>Span40 (C16)>Span20 (C12)>Span80 (C18). Span 60 and Span 80 have the same head groups but Span80 has an unsaturated alkyl chain. De Giere demonstrated that the introduction of double bonds into the paraffin chains causes a marked enhancement of the permeability of liposomes, possibly explaining the lower entrapment efficiency of the Span80 formulation.

2. Cholesterol content

Cholesterol increases or decreases the percentage encapsulation efficiency depending on either the type of the surfactant or its concentration within the formulae.

3. pH of the hydration medium

The percentage encapsulation efficiency of niosomes prepared by hydration of pro-niosomal gels of Span 60/cholesterol (9:1) was found to be greatly affected by the pH of the hydrating medium. For example, the fraction of flurbiprofen encapsulated was increased to about 1.5 times as the pH decreased from pH 8 to 5.5. The increase in the percentage encapsulation efficiency of flurbiprofen by decreasing the pH could be attributed to the presence of the ionisable carboxylic group in its chemical structure. Decreasing the pH could increase the proportions of the unionized species of flurbiprofen, which have higher partitioning to the bilayer lipid phase compared to the ionized species.

4. Total lipid concentration

The percentage encapsulation efficiency of flurbiprofen was increased as the lipid concentration was increased from 25 to 200mol/ml, respectively. The increase in percentage encapsulation efficiency of flurbiprofen as a function of total lipid concentration was linear. On the other hand, the amount of flurbioprofen entrapped was decreased on increasing the lipid concentration from 25 to 200mol/ml, respectively. This leads to the fact that the fraction of lipid taking part in encapsulation decreases as the concentration of lipid increases.

5. Drug concentration

Increasing flurbiprofen concentration from 25 to 75mg/mmol lipids in the Pro-niosomes prepared from Span 60/cholesterol (9:1) showed an increase in both percentage encapsulation efficiency and the amount of drug encapsulated per mol total lipids upon hydration and formation of niosomes.

6. Charge of the lipids

Incorporation of either dicetyl phosphate (DCP) which induces negative charge or stearylamine (SA) which induces positive charge decreased the percentage encapsulation efficiency of flurbiprofen into niosomal vesicles.

APPLICATIONS OF PRO-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.Pro-niosomes can be used for many purposes in drug delivery. Ethosomes are mainly used as replacement of noisome. Mainly the transdermal route of drugdelivery is preferred. Pro-niosomes can be used for the transdermal delivery of hydrophilic and hydrophobic drugs through the skin. Table 3 shows drugs have been used with pro-niosomes carrier:

Table 3: Applications of Proniosomes as a Drug Carrier

S.No.	Drug	Nature of drug (Hydrophilic Or Lipophilic)	Category
1.	Ibuprofen [60]	Lipophilic	NSAIDS
2.	Aceclofenac [75]	Lipophilic	NSAIDS
3.	Haloperidol[68]	Hydrophilic	Antipsychotic effect
4.	Piroxicam[70]	Lipophilic	NSAIDS
5.	Indomethacin [80]	Lipophilic	NSAIDS
6.	Alprenolol Hydrochloride [61]	Lipophilic	Antihypertensive
7.	Captopril[81]	Hydrophilic	Antihypertensive
8.	Griseofulvin[82]	Lipophilic	Antifungal
9.	Flurbiprofen [66]	Lipophilic	NSAIDS
10.	Estradiol[64]	Lipophilic	For Symptomatic Treatment Of The Usual Symptoms Associated With Menopause
11.	Ketorolac [83]	Lipophilic	NSAIDS
12.	Losartan Potassium [84]	Hydrophilic	Antihypertensive
13.	Levonorgestrel [85]	Lipophilic	Anti-contraceptives
14.	Celecoxib[86]	Lipophilic	Cyclooxygenase inhibitor
15.	Cromolyn Sodium [87]	Hydrophilic	Antiasthmatic and Antiallergic
16.	ChlorpheniramineMaleate [88]	Hydrophilic	Antihistamine

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 (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. 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. 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

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 (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

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. In an in-vitro 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 (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 (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

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. 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. 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.

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. 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.

Pro-niosomes are promising drug carriers for the future with greater physical, chemical stability and potentially scalable for commercial viability. Pro-niosomal delivery system holds effective delivery for amphiphilic drugs. Due to advantages of nontoxicity & penetration enhancing effect of surfactants & effective modification of drug release, pro-niosomes has attracted a greater deal of attention for delivery of drugs through transdermal route. Pro-niosomes in dry form makes the possibility of convenient unit dosing as they further converted into beads, tablets, capsules.The finding of the studies on pro-niosomes opens the door for the future use of different carriers' materials with bio-Compatability and suitability for the preparation of pro-niosomes. The future experiments would explore the suitability of pro-niosomes with more drugs having defined drawbacks for improved & effective intended therapy.

Methods for preparing pro-niosomes

The pro-niosomes can be prepared by

i. Spraying method.
ii. Slurry method.
iii. Co-acervation phase separation method.
iv. Formation of Niosomes from Proniosomes

I. Spraying method

(Hu and Rhodes et al in 1999) prepared pro-niosomes. A100mlroundbottomflaskcontaining desiredamountofcarriercanbeattachedto rotary flashevaporator. A mixture of surfactants and cholesterol should be prepared and introduced in to round bottom flask on rotary evaporator by sequential spraying of aliquots on to carrier's surface. The evaporator has to be evacuated and rotating flask can be rotated in water bath under vacuum at 65-70^{O}C for 15–20 min. This process has to be repeated until all of the surfactant solution had been applied. The evaporation should be continued until the powder becomes completely dry [94].

II. Slurry method

(Almira. I and Blazek - Walsh et al 8 in 2001) developed slurry method to produce pro-niosomes using maltodextrin as a carrier. Pro-niosomes can be prepared from a stock solution of surfactants and cholesterol in suitable solvent. The required volume of surfactant and cholesterol stock solution per gram of carrier and drug should be dissolved in the solvent in 100 ml round bottom flask containing the carrier (maltodextrin or lecithin). Additional chloroform can be added to form the slurry in case of lower surfactant loading. The flask has to be attached to a rotary flash evaporator to evaporate solvent at 50-60 rpm at a temperature of 45±20 ^{o}C and a reduced pressure of 600mm Hg until the mass in the flask had become a dry, free flowing product. Finally, the formulation should be stored in tightly closed container under refrigeration in light [89-91]

III. Coacervation phase separation method

Accurately weighed or required amount of surfactant, carrier (lecithin), cholesterol and drug can be taken in a clean and dry wide mouthed glass vial (5 ml) and solvent should be added to it. All these ingredients have to be heated and after heating all the ingredients should be mixed with glass rod. To prevent the loss of solvent, the open end of the glass vial can be covered with a lid. It has to be warmed over water bath at 60-700oC for 5 minutes until the surfactant dissolved completely. The mixture should be allowed to cool down at room temperature till the dispersion gets converted to a proniosomal gel [92, 93].Figure.4 shows apparatuses for Coacervation phase separation method

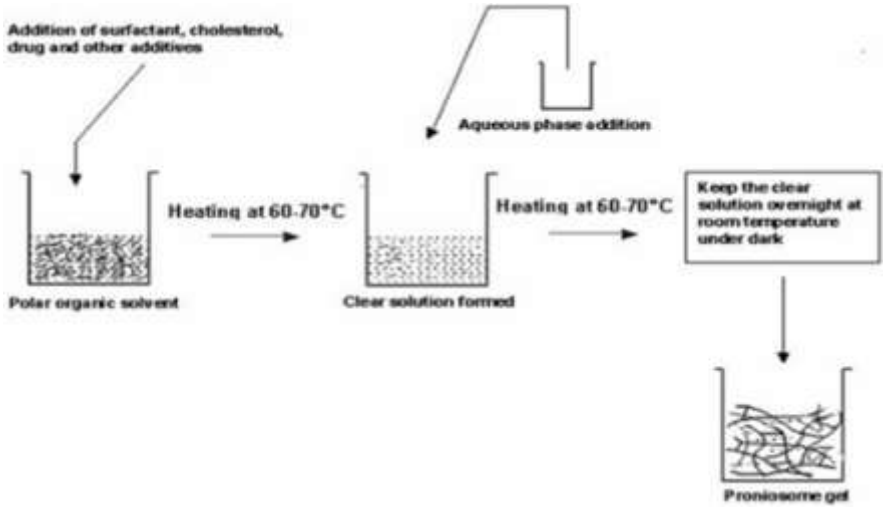

Figure 4: Coacervation phase separation method

IV. Formation of Niosomes from Proniosomes

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.Figure.**5** shows formation of Niosomes from Proniosomes.

T > Tm

Where, T = Temperature, Tm = mean phase transition temperature

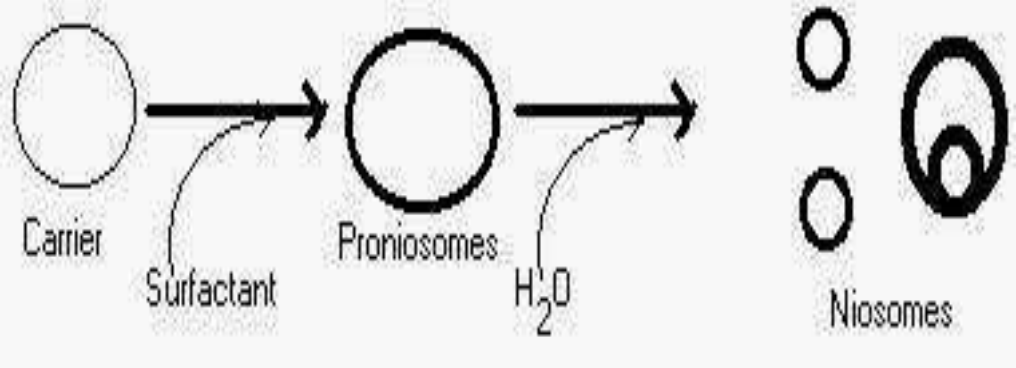

Figure.5: Formation of Niosomes from Proniosomes.

B. **DRYGRANULARLIPOSOMES**

Liposomes are composed of phospholipids that can either be unsaturated such as egg phosphotidyl choline, soya phosphotidyl choline, etc., or saturated such as hydrogenated soya phosphotidyl choline and cholesterol as bilayer component. Phospholipids aid in formation of bilayer structure and cholesterol retains the rigidity and intactness of the bilayer [95]. But it was observed that liposomal formulations were highly unstable and did not have a long shelf life [96]. The underlying reason was susceptibility to oxidation, hydrolysis and leakage of drug in aqueous formulations which is unacceptable in terms of product performance or safety.

Oxidation of the formulation occurs if unsaturated phospholipids are used. So, this could be overcome by using saturated phospholipids in the liposomal formulation [97]. The reason behind hydrolysis and leakage of drug from the formulation is the physical state of the formulation. Therefore a change in the physical state appears to be necessary for formulating a stable formulation to have a long shelf life; hence conversion of colloidal liposomal suspension into a dry powder has been attempted.

This was accomplished by freeze drying or Lyophilization technique as it employs low temperature and hence there is no damage to the vesicle components [98]. Since Lyophilization involves several steps, each of the steps may damage the liposomal vesicles during freeze drying. For example, in the freezing step the total solute concentration increases as a function of temperature. As freezing process is continued, solute will be precipitated from the product

mixture when saturation solubility are reached and causes major pH shifts that would be damaging. As a result vesicle size will be increased and entrapped drug may be leaked [99]

This undesirable precipitation can be prevented by the addition of suitable excipients like lyoprotectants which usually are poly hydroxy compounds that do not readily crystallize from a frozen solution [100]. Literature reports the usage of various sugars such as glucose, sucrose, maltose, trehalose as protectants during dehydration/ rehydration of vesicles [101.]

Apart from the addition of lyoprotectant, a few studies have also reported that nature and composition of bilayer also influences the integrity of liposomal membrane. Hence, apart from cholesterol other bilayer components such as oleic acid, stearic acid, alpha tocopherol and MPEG were used for forming bilayer and their influence on liposomal size and vesicle integrity was measured upon freeze thawing and freeze drying.

Integrity of the bilayer is measured as a function of drug leakage. Lower the drug leakage, more integral is the membrane. For measuring the drug leakage doxorubicin hydrochloride an anti-cancer drug has been used as a marker which is estimated colorimetrically at 495 nm for sucrose and trehalose as lyoprotectants.

REVIEW OF LITERATURE

- T.D. Madden et al. studied the integrity of freeze dried liposomes prepared using EPC. Trehalose was used as disaccharide, glycerol as cryoprotectant and CF as the fluorescence indicator to estimate the vesicle integrity by measuring the leak out of CF on reconstitution. This study reported that presence of Trehalose on both inside and outside of liposome showed better integrity than that of liposomes which had Trehalose either inside or outside. It has further revealed that susceptibility to leakage induces by both dehydration and freezing is critically dependent upon vesicle size. With increase in concentration of glycerol upto 40%w/w, integrity of membrane was found to increase [102.]
- W. Zhang et al studied the bilayer permeability of freeze dried liposomal bilayers upon rehydration using DPPC and DPPG in the ratio 1:10. CF was used as fluorescence indicator and leak in studies were performed. They reported that presence of sucrose in and outside the vesicles caused temporary increase in the bilayer permeability of CF and this effect was seen upto 20 hours and later this effect levelled off. They also reported that amount of CF that leaked in, which is a direct measure of permeability increase with both size and lamellarity of the vesicles and decrease with number of bilayers [103.]
- Ewound C. A. van Winden et al. have done research on Effect of freezing rate on stability of

liposomes during freeze drying and rehydration. They subjected the prepared liposomal dispersions to MTDSC, and the dry cakes to SEM for texture of liposomes, FTIR to know the interaction between phospholipids head groups and lyoprotectant and MTDSC to know the thermal behaviour. Percentage encapsulation of CF of rehydrated liposome was analysed using Fluorimetry, vesicle size was measured using dynamic light scattering technique and bilayer transition with DSC. They reported that lyoprotectants added to liposome dispersions form glassy matrix during freezing. This protects fusion of vesicles and also acts by suppression of bilayer transition temperature [104].

- W. L. J. Hinrichs et al. made an attempt to prepare liposomes using oligosaccharides like dextran and inulin as lyoprotectants in place of disaccharides and study the aggregation in pegylated and non pegylated complexes. It was observed that both inulin and dextran prevent severe aggregation of non pegylated lipoplexes. In case of pegylated lipoplexes aggregation was prevented only with inulin whereas, dextran increased aggregation. The reason was attributed to compatibility of PEG with insulin [105]
- Christopher Womersley et al. studied inhibition of dehydration induced fusion between liposomal membranes by carbohydrates between palmitoy loleoyl phosphatidyl choline:phosphatidyl serine (85:15) LUV. He measured this fusion using the technique of fluorescence energy transfer (FET). Trehalose was most effective at inhibiting fusion (0.4 g/trehalose/g lipid showed 30% probe intermixing), followed by maltose (60% intermixing), fructose (60%), sucrose (70%), glucose (80%), cellobiose, glycerol, raffinose, and myo-inositol (90%). The relative abilities of these carbohydrates to inhibit fusion correlate directly with their abilities to interact with phospholipids and maintain bilayer fluidity [107].
- Lois. M. Crowe et al. had done research on preservation of freeze dried liposomes by trehalose. They studied ability of trehalose to stabilize freeze dried liposomes. Inhibition of fusion between liposomes during drying was assessed by freeze-fracture and resonance energy transfer between fluorescent probes incorporated into the bilayer. Results revealed that with trehalose both inside and outside the bilayer, almost 100% of trapped solute was retained in rehydrated vesicles. Further the study provided evidence stabilization of the dry liposomes requires depression of transition temperature and consequent maintenance of the constituent lipids in the dry liposomes in a liquid crystalline phase [108].
- Brigitte Stark et al. determined the impact of different cryoprotectants on physicochemical parameters of sterically stabilized PEGylated liposomes, They investigated particle stability

in terms of size, lamellarity and thickness of the lipid bilayer using photon correlation spectroscopy and small angle X-ray scattering and also evaluated the impact of cryoprotectants on the thermal lipid phase behavior of either frozen/thawn or lyophilized/rehydrated PEGylated liposome formulations by DSC. Optimal results for the preservation of the average size of the extruded unilamellar liposomes during freezing were achieved using a mixture of glycerol and carbohydrate concentrations of about 1% w/v, irrespective of the carbohydrate used. No significant changes in the bilayer organization was found and and the transition behavior of lipids was almost unaffected by freezing [109.]

- Samuni AM et al. studied liposomal lipid activated damage and hydrolysis on long term storage with liposomes made of EPC and cholesterol in the ratio 10:1 with 3.4% of acyl chains of EPC being poly unsaturated fatty acids. Chemical and physical changes were monitored at several time points to assess oxidative and hydrolytic damage and reported that liposomes with EPC and cholesterol are more stable because cholesterol decreases lipid bilayer hydration [110.]
- Gerd Bendas et al. prepared liposomes using SPC and tested for their activity as membrane-bound cryoprotectants in the freeze-drying process of large unilamellar vesicles (LUV). These glycosidic derivatives possess the same hydrophobic proportions but different head group sugars (galactose or cellobiose) and a number (0–3) of ethoxy spacer units between the chain and head group as modifications in the hydrophilic moieties. They measured CF retention, resonance energy transfer (RET), particle size distribution and analyzed the electron micrographs. Results revealed that galactosides cause increased 6-CF retention in the presence of free carbohydrates (glucose or sucrose) which could not be explained by a simple addition of cryoprotective effects of free and membrane bound sugars [111.]

C. MIXEDMICELLARPROLIPOSOMES

Mixed micelles contain bile salts, cholesterol, and phospholipids which upon dilution undergo micelles to vesicle transition of form liposomes.

D. PROTRANSFEROSOMES

Protransferosomes are proultraflexiblevesicles,whichcanbeconvertedintoultra-flexiblevesicles.

1. Characterization of provesicular system shown in table 4

- Morphology.
- Angle of repose.
- Size and size distribution.

- Rate of hydration.
- Entrapment efficiency.
- Degree of deformability and permeability measurement.
- In vitro release rate.
- In vivo fate and pharmacokinetic.

Table 4: characterization of provesicular system

Characterization Parameters	Analytical method / Instrumentation
Physical characterization	
Appearance	Pharmacopoeial protocols (visual inspection)
Vesicle shape and surface Morphology	Transmission electron microscopy, freeze fracture electron microscopy
Vesicle size distribution	
Micron range	Coulter counter, light microscopy, laser light scattering.
Electric surface potential surface pH	Membrane bound electrical field probes and pH and sensitive probes
Zeta potential	Electrophoretic mobility
Lamellarity	Freeze-fracture electron microscopy, PNMR
Phase behaviour	Differential scanning calorimetry (DSC)
Percentage of free drug	Gel exclusion, ion-exchange chromatography, minicolumn centrifugation, protamine aggregation, radiolabelling
Drug release	Diffusion cell/dialysis
Chemical characterization	
Phospholipids concentration	Lipid phosphorous content using Barlettassay/ Stewart assay, HPLC
Cholesterol concentration	Cholesterol oxidase assay assay and HPCL
Drug concentration	Appropriate methods given in the monograph for individual drug
Phospholipids peroxidation	UV absorbance, TBA (for endoperoxidase), iodometric (for hydroperoxidase) and GLC
Phospholipid hydrolysis	HPLC and TLC and fatty acid concentration
Cholesterol auto-oxidation	HPLC and TLC
Anti –oxidant degradation	HPLC and TLC
Ph	pH meter
Osmolarity	Osmometer
Biological characterization	
Sterility	Aerobic or anaerobic cultures Gel exclusion, ion-exchange chromatography
Pyrogenicity	Rabbit fever response test or Limulus Amebocyte Lysate (LAL) test
Animal toxicity	Monitoring survival rates, histology and pathology

CONCLUSION:

Compared to liposome or niosomes, proniosomes are very promising as drug carriers.

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. This formulation of proniosomes is a practical and simple method of producing niosomes at the point of use for drug delivery.

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