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Liposomes: Benchmark in the Era of Drug Carriers for Semisolid Based Topical Delivery System

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ABSTRACT

A liposome is a microscopic vesicle consisting of an aqueous core enclosed in one or more phospholipid layers, used to convey vaccines, drugs, enzymes, or other substances to target cells or organs. Liposomes are bilayered structures made of amphipathic (both hydrophobic and hydrophilic) phospholipids/cholesterol that spontaneously form closed structures when hydrated in aqueous solutions. Liposomes are acceptable and superior carriers having ability to encapsulate hydrophilic and lipophilic drugs and protect them from degradation. Topical liposomes have similarity to biological membranes they can store water-soluble and lipophilic substances in their different phases. Moreover, they are similar to the epidermis with respect to their lipid composition, which enables them to penetrate the epidermal barrier to a greater extent compared to other dosage forms. According to studies performed so far liposomes are biodegradable and non-toxic. The really new aspect with liposomes is that they are thought to act not only as drug transporters but also drug localizers. Thus vehicles can transport drugs to the wanted site of action within the skin by preventing systemic absorption and consecutively unwanted effects. The liposomal semisolid formulations could perform therapeutically better effects than the conventional formulations, as prolonged and controlled release topical dosage forms, which may lead to improved efficiency and better patient compliance. Such review giving an emphasis on topically applied liposomal formulations which encompasses methods of preparation, evaluation, mechanisms for enhancement in drug delivery through the skin and regulatory requirements necessitates as topical dosage form.

Keywords: Liposomes, Carriers, Topical, Evaluation

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INTRODUCTION

Topical Drug Delivery System:

For many years drug administration for the treatment of illness has been achieved via various routes namely oral, rectal, sublingual, parenteral, topical, inhalation, etc. But the topical delivery is very much popular for the application of many drugs to the skin for the treatment of cutaneous infection or manifestation with the intension of drugs pharmacological action or for other effects of drug to the surface of the skin or within the skin.

Topical drug delivery system has been a preferred route for cosmetic and drug delivery system. It covers the formulations which are to be applied to the body surface such as skin or mucous membranes of eyes, nose, throat, ear etc. Drugs are applied topically (to the skin) mainly for local action rather than systemic effect because the absorption is often poor and too erratic for the topical use for systemic therapy. There are many types of topical formulations available in the market like powders, lotions, creams, pastes, ointments, soaps, gels and sprays.[1-5]

Topical delivery includes two basic types of products –

1. External topical products which are spread, sprayed or dispersed on to the skin to cover affected area.
2. Internal topical products which are applied to mucous membrane orally, vaginally, nasally.

Advantages of Topical Drug Delivery System [6-9]:

- First pass metabolism is avoided.
- Medication can be easily terminated when needed.
- Ease of application and convenient.
- Patient compliance is improved (self medication is possible).
- It is a site specific drug delivery system.
- Physiological and pharmacological response is improved.
- Gastro intestinal incompatibility can be avoided by this route.

Disadvantages of Topical drug delivery system [10-12]:

- There are possibilities of skin irritation or contact dermatitis.
- Systemic absorption of drugs through skin is very erratic. Hence this drug delivery is useful for the drugs which require very small plasma concentration for action.
- Allergic reactions are also possible.
- For some drugs, permeability through skin is very poor. These drugs are poor candidates of topical drug delivery system.

- Denaturation of drugs can occur due to enzymes from epidermis.

Classification of Topical Drug delivery System based on physical state[13]:

1. Solid – Powder, Aerosol, Plaster
2. Liquid – Emulsion, lotion, Liniment, Solution, Suspension, Aerosol
3. Semi solids – Ointment, Cream, Paste, Gel, Jelly, Suppository

Amongst all these topical drug delivery system, cream is the popular dosage form in which the active substance must reach a skin compartment because skin itself the target. There are many active ingredients, which are very unstable, very reactive and easily effect when mixed together with other ingredients in the formulation. These active ingredients have been stabilized and thereby prevented from breaking down before being applied to and absorbed by the skin by use of technology such as embedding then in extremely small particles (liposomes). Present article reviews the information about liposomes as drug carrier for cream based topical applications and we are concerned with the modern technique of stabilization of drug for topical application. Stabilization of drug by hydrophilic lipids (liposomes) is useful method for protecting unstable drugs when used topically.

Liposomes:

Liposomes are hollow spherical artificially prepared microscopic vesicles made up of lipid bilayer. In these liposomes, aqueous fluid is entirely enclosed by lipoid membrane. Many drug molecules are either be encapsulated in an aqueous phase or entrapped into the lipid bilayer. The outer wall of liposomes is made up of lipids in which lipophillic drugs are entrapped and the interior is filled with water in which various hydrophilic drugs can be dissolved.

Phospholipids are the main building blocks of liposomes. Each molecule has three major parts, one head and two tails. The head is made from three components: choline, phosphate and glycerol. The head is hydrophilic in nature. Tails are made of chain of fatty acids. This chain is hydrophobic in nature. When lipids are dispersed in aqueous solution, hydrophilic heads align themselves side by side with their tails behind. As these tails are hydrophobic in nature, another lipid layer lines up tail to tail in presence of aqueous medium. This natural alignment forms two rows of tightly bound lipids called as lipid bilayer. Thus the formation of liposomes is a self assembly process as shown in Figure 1. They are ampiphilic in nature in which drugs can be encapsulated (hydrophilic drugs) or dissolved in the membrane (hydrophobic drugs) of liposomes.[14-18]

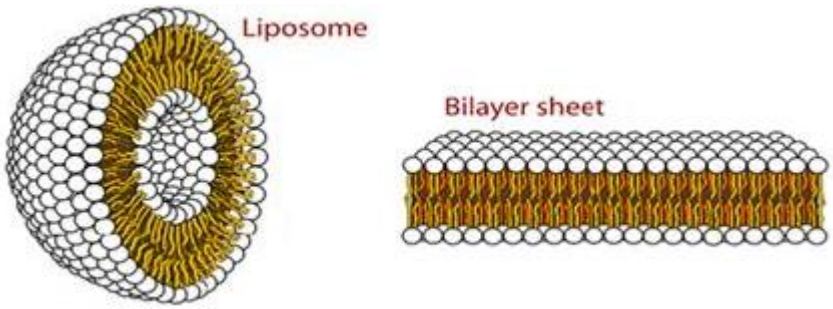

Figure 1: Self assembly and bilayer sheet of liposomes.

Selection of lipids:

There are number of components of liposomes, however phospholipids and cholesterol are the main components. Phospholipids are the main structural components of biological membrane. Glycerolipids can also be used (e.g.- glyceryl monomyristate). [27-28]

Following factors must be considered during selection of lipids:

1. **Chain of lipids** :

Lipids containing unsaturated fatty acids undergo oxidation. Hence unsaturated fatty acids should be replaced by saturated fatty acids. Lipid peroxidation is the process in which oxygen redical reacts with unsaturated fatty acid in the lipids. In this reaction the oxygen radical removes the hydrogen from fatty acids. This causes formation of the carbon centered radical within fatty acids. It then reacts with oxygen to produce peroxy radical, which can again react with other fatty acids. Therefore lipids containing unsaturated fatty acids like oleic acid are not preferable (example of unsaturated lipid: glyceryl monooleate).

2. Charge on lipids :

Anionic lipids have been incorporated into liposomes to increase the trapped volume and improve the loading of various molecules and drugs.

Liposomes with neutral charge containing lipids are the stable one. The presence of divalent metal ions such as Ca^{2+} and Mg^{2+} causes the aggregation of liposomes (those with negative charge). Therefore, aqueous buffer used for the preparation of liposomes should be free of divalent metal ions.

3. Lipid composition :

Lipid composition should be kept constant. Alterations in lipid composition can affect the size distributions achievable for certain liposomal drug systems Consequently, a comprehensive profile of the effects of liposome characteristics on the therapeutic activity of liposomal drug cannot be achieved.

Structural components of liposomes:

- The main components of liposomes are phospholipids and cholesterol.
- Phospholipids are the building blocks of the liposomes. The most commonly used phospholipids is phosphatidylcholine (PC). Phosphatidylcholine is an amphipathic molecule which consists of a hydrophilic polar head group phosphocholine, glycerol bridge, a pair of hydrophobic acyl hydrocarbon chains. They are water insoluble. But they align themselves into bilayer sheet. This reduces instability problem which exist when the molecule exist alone. They orient in such a way that fatty acid chains orient each other and polar head face an aqueous phase. Some other phospholipids that can be used in the formation of liposomes are phosphatidylethanoamine, phosphatidylserine, DOPC, DSPC,DOPE, DSPE.
- Second important component is cholesterol. It does not take part in formation of bilayer structure but it acts as fluidity buffer. Below the phase transition temperature of lipids, cholesterol makes the membrane slightly more permeable and less ordered. But above the phase transition temperature, cholesterol makes the lipid membrane more stable. Cholesterol inserts into the membrane with its aliphatic chain align parallel to the acyl chain and its hydroxyl group oriented towards the aqueous surface.

Classification of liposomes:

Based on structural parameters :

- MLV : Multilamellar vesicles (>0.5µm)
- OLV : Oligolamellar vesicles (0.1-1µm)
- MVV : Multi vesicular vesicles
- UV : Unilamellar vesicles – SUV : Small unilamellar vesicles (20-100µm)

MUV : Medium unilamellar vesicle

LUV : Large unilamellar vesicles (>100nm)

GUV : Giant unilamellar vesicles (>1µm)

Based on method of preparation:

- REV SUVs/OLVs – made by reverse phase evaporation method
- MLV-REV : MLVs made by reverse phase evaporation method
- SPLV :stable plurilamellar vesicle
- DRV : made by dehydrated rehydrated method
- VET : vesicles prepared by extrusion technique
- FATMLVs : frozen and thawed MLVs.

Based on composition and applications :

- CL : conventional liposomes
- Fugogenic liposomes
- Ph sensitive liposomes
- Cationic liposomes
- LCL : Long circulatory (stealth) liposomes
- Immuno-liposomes

Need of liposomal drug delivery system [19-21]**:**

- Lipid stabilization is the process in which many drugs are stabilized by forming hydrogen bonds with liposomes. Many drugs are very unstable when added in the emulsion. These drugs can undergo decomposition in presence of light, heat, oxidizing agent, reducing agent and enzymes. Prepared liposomal dispersion can show many fold increase in drug deposition compared to control (plain drug dispersion).
- Many drugs like curcumin are poorly soluble in aqueous medium. Liposomal formulation can overcome the limitation of these being so poorly soluble in aqueous medium.
- Drugs like Tacrolimus is found to be effective in treating atopic dermatitis with main side effects like burning sensation of the skin. The use of liposomes as drug carriers seems to have a promise for improved therapeutic prospects with a reduction of side effects.
- Lipid vehicles are designed to enhance drug delivery through the skin. Hence liposome formulation is needed to improve the ability of the delivery system to overcome the barrier posed by the stratum corneum.

Advantages of liposomal drug delivery system [22-24]**:**

Many drugs are stabilized by formulating them in liposome as drug carrier for drug delivery systems.

- Reduced Toxicity, Entrapped Drugs.
- Liposomes are made up of naturally occurring substances, hence they have a distinct advantages of being both nontoxic and biodegradable. Biologically active materials can be protected from their degradation when encapsulated by liposomes. Drugs entrapped in the liposomes are not activated under physiological conditions and do not cause unfavourable side effects. The entrapement of a drug in liposomes can facilitate localized delivery of drugs and improves availability by means of controlled release pattern.
- Sustained Delivery Systems.

- As systems for the delivery of drugs, liposomal dispersions may be beneficial through several mechanisms. They can solubilize water-insoluble drugs (such as Minoxidil or amphotericin B), provide site avoidance and reduced toxicity through minimized uptake in sensitive tissues.
- Liposomes hold drugs intact and deliver them to the targeted site.
- They also enhance drug penetration into the skins deepest layer.
- In addition to being good carrier of other ingredients, liposomes are advantageous in and of themselves because they can reduce skins dryness (emollient properties).
- They stabilize the drug hydrolysis and oxidation.
- Liposomal emulsions are low immunogenic.

Disadvantages of liposomes [34]:

- Production cost is high
- Leakage and fusion of encapsulated drug / molecules.
- Sometimes phospholipid undergoes oxidation and hydrolysis like reaction
- Short half-life
- Low solubility
- Lesser stability

Mechanism of action of stabilization of drugs by liposomes [4, 25, 26, 32]**:**

Liposomes are used for drug delivery due to their unique properties. They can stabilize the drugs by three mechanisms. Interior part of liposomes is hydrophilic in nature. Hence they can encapsulate a region of aqueous solution inside the hydrophobic membrane. Hydrophobic drugs can be stabilized by dissolving into the membrane.

In this way liposomes can carry both hydrophobic and hydrophilic drug molecules. To deliver the molecules to the site of action, the lipid bilayer can fuse with other bilayer such as cell membrane, thus delivering the liposomal contents.

Some drugs (like hydrogen peroxide) can be stabilized by the formation of hydrogen bonding with hydrophilic lipids. The outermost surface of the hydrophilic lipids has hydrogen atoms. Therefore surface should be the perfect surface for creating hydrogen bonds. Hydrogen atoms on the surface act as hydrogen donor. Drugs with deficiency of hydrogen atoms act as hydrogen atom acceptor. Therefore, it is likely that the molecules of the drug in the lipid will show the preference of adhering to the surface of the hydrophilic crystals of liposomes. This adhering is responsible for very good stabilization of the drug.

Liposomes both penetrate the stratum corneum to some extent and then interact with the skin lipids to release the drug or their components alone enter the stratum corneum. General mechanism of liposome causes enhancement in drug delivery system via skin is as shown in Figure 2.

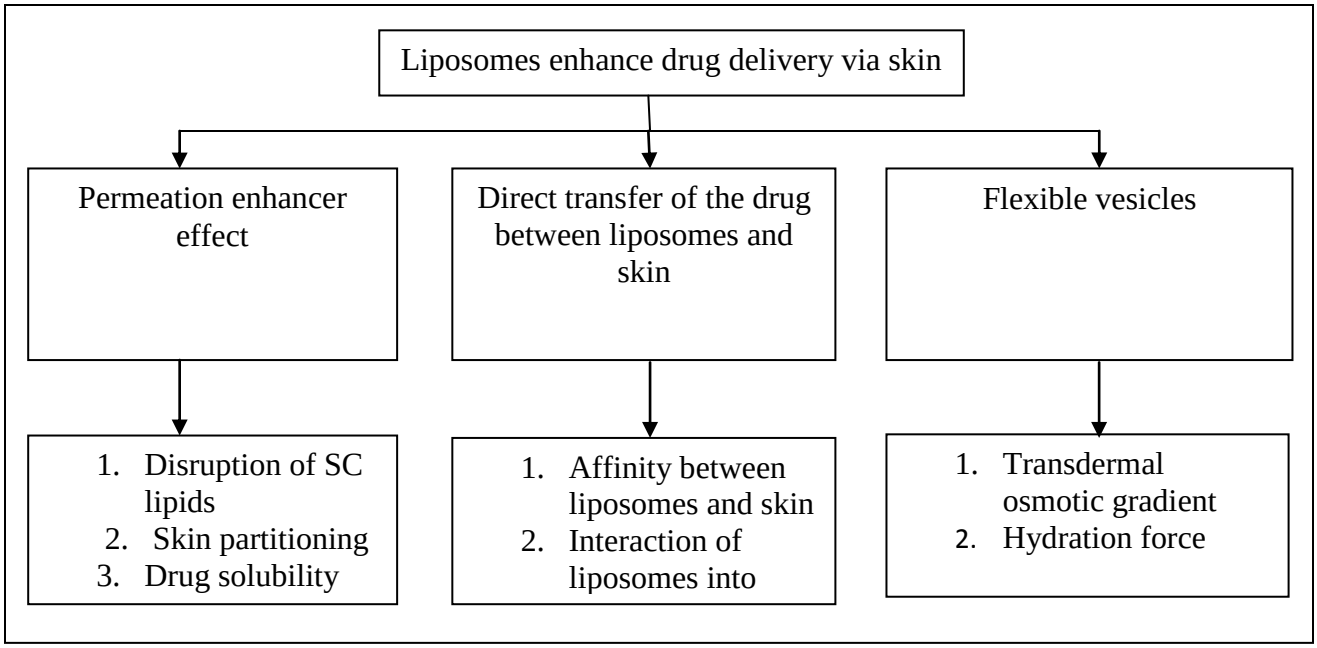

Figure 2: General mechanism of enhancement of drug delivery through skin by liposomes.

Most widely used method of preparation of liposomal topical based formulation [34]:

There are many methods used in the preparation of liposomes but only two methods are widely used in topical cream based liposomal formulations.

1. Lipid Hydration method :

Lipid hydration method is widely used method for the preparation of MLV.

It is divided into two types – Hand shaken MLVs and Non shaking vesicles.

Hand shaken MLVs:

This is the simplest and most widely used method.

It involves dissolution of lipids in suitable solvent. It consists of drying a solution of lipids and formation of thin film at the bottom of round bottom flask. This film is dried by evaporation of solvent in rotary evaporator or by hand shaking. This film is further dried by lyophilization to remove residual moisture. The hydration is done at a temperature above the gel-liquid crystalline transition temperature (Tc) of the lipid or above the (Tc) of the highest melting component in the lipid mixture. The casted film is then dispersed in the aqueous medium. Upon hydration, lipids swell and detached from the wall of the conical flask to form MLVs. The drug compounds which

is to be encapsulated is added either in the aqueous buffer or in the organic solvent containing lipids depending upon their solubilities.

Disadvantages :

Low internal volume

Low encapsulation efficiency and the size distribution is heterogeneous

Non shaking Vesicles:

MLVs with high encapsulation efficiency are normally prepared by hydrating the lipids in the presence of an immiscible organic solvent (diethyl ether, petroleum ether). These mixture is then emulsified by vigorous vortexing or sonication. The organic solvent is removed by passing a stream of nitrogen gas over the mixture. MLVs are formed in the aqueous phase after the removal of organic solvent.

Disadvantage :

This method is the exposure of the materials to be encapsulated to organic solvent and to sonication.

2. Ethanol evaporation method :

Solvent injection method is suitable for preparing SUV or LUV.

The lipids and lipid soluble component (drug) are first dissolve in organic solvents (ethanol). A lipid solution of ethanol is rapidly injected to a vast excess of buffer. The MLVs are immediately formed with continuous stirring. The solvent was evaporated by heating so as to obtain drug loaded liposomes.

Disadvantages :

The population is heterogeneous (30-110 nm),

Liposomes are very dilute,

It is difficult to remove all ethanol because it forms azeotrope with water and the possibility of various biologically active macromolecules to inactivation in the presence of even low amounts of ethanol.

Characterization of liposome [17, 18]**:**

Behavior of liposomes depends on their physical size, membrane permeability, percent of entrapped solute, chemical composition, quantity and purity of starting material. Hence liposomes are evaluated for their physical attributes and chemical composition. Evaluation of liposomes consists of particle size, zeta potential, entrapment efficiency, in vitro drug release, in vitro skin permeation study, drug deposition in skin and rheological study etc.

Physical properties:

1. Shape, size and its distribution:

Microscopic methods:

Electron microscopy - Most precise method to determine size of the liposomes is electron microscopy. Electron microscopy allows observing each individual liposome and helps in finding exact information about liposome profile. Freeze etch electron microscopy (for measurement of small vesicles) and freeze fracture electron microscopy (for measurement of large size vesicle diameter) can also be used to study structure and size of vesicles in liposomes. But drawback of freeze fracture electron microscopy is, it determines the size distribution and mean vesicle size.

Light microscopy has been used to determine gross size distribution of large vesicles. If the bilayers of liposomes have fluorescent probes, fluorescent microscopy can be utilized. Resolution is one of the limitations of light microscopy.

Laser light scattering:

The principal of diffraction of light is used here. It tells that when a ray of light is incident on a particle it gets diffracted through certain angle. This causes light to change its path. Detectors with high precision then record the signal caused by scattering of light and compare them with the signal recorded when no molecule is present in the path. This technique is called dynamic light scattering (DLS). Small particle cause rapid fluctuation of light than larger particles. This fluctuation of signal is used for particle size determination. It is very simple and rapid to perform. But the major disadvantage is it does not allow viewing individual particle. It gives average count (average range -3nm to 3µm).

If approximate idea of size range of particle is required then chromatographic technique, gel extrusion method can be used.

2. Surface charge:

Zeta analyzer can be used to determine charge distribution on liposomes. Zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle. A value of 25 mV (positive or negative) can be taken as the arbitrary value that separates low-charged surfaces from highly-charged surfaces.

Free flow electrophoresis is also used to determine surface charge of liposomes (MLVs). This technique is based on the principal of separation of extruded vesicles on the basis of their surface charge by electrophoresis. Lipids are applied on the plate and electrophoresis is carried out for specified time. The plate is then dries and the phospholipids are then observed by using some reagent (like molybdenum blue reagent). Liposomes with diameter upto 0.2 µm can be migrated

on this support. This assay is very sensitive as only 2 mole % of charged lipids can be detected. This is mainly used to determine heterogenisity in liposome preparation.

3. Percent drug capture:

For determination of percent entrapment it is essential to remove unincorporated material by separation technique. In general two techniques are used –

1) Mini column centrifugation
2) Protamine aggregation.

In mini column centrifugation, dried gel column is used. Liposomal preparation is applied dropwise to the top of the gel column and column is then centrifuge at high speed to expel drug from liposomes. This drug is then removed for assay.

Before carrying out protamine aggregation, it should be confirmed that the solute material entrapped does not precipitate in presence of protamine after releasing from liposomes. In this method, protamine solution is added to liposomal preparation in centrifuge tube and spun for few minutes with saline. Supernatant is assayed for free, unentrapped drug. The suspended pellets are resuspended in buffer (triton X100) and the material is allowed to dissolve completely. This is then assayed for entrapped drug.

4. Lamellarity:

Lamellarity is the average number of bilayers present in liposomes. This can be found out by two techniques -1) Feeze electron microscopy 2) 31P – NMR. Freeze fracturing electron microscopy is very much popular method to study structural details of aqueous lipid preparation.

5. Percentage of drug release:

Percent drug release from liposomes can be calculated by diffusion cell (well calibrated).

Chemical characterization

1. Quantitative estimation of phospholipids

Most widely used method is indirect method. In this method phosphate content of the sample is measured. The phospholipids are measured using Bartlett assay and Stewart assay.

In Bartlett method Phosphorous in phospholipids is first hydrolyzed and then converted into phosphor-molybdic acid by ammonium molybdate which is then reduced to the blue colored compound amino-naphthyl-sulfonic acid. Intensity of blue colour is measured spectrophotometrically. This assay is more sensitive but non reproducible.

In Stewart assay method, in presence of organic solution phospholipids form a complex with ammonium ferrothyocynate. It is not applicable when unknown mixtures of phospholipids are

present. The advantage if this method is the presence of inorganic phosphate does not interfere with the assay as in the Bartlett assay method.

TLC method can be employed to find out concentration and purity of lipids.

2. Phospholipid oxidization and hydrolysis

Major product of phospholipid (lecithin) hydrolysis is lysolecithin by de-etherification. This lysolecithin is estimated quantitatively by HPLC.

Fatty acids in phospholipids gets oxidized by free radical mechanism. Many techniques like UV absorbance method, iodometric method, GLC method are used for estimation of oxidation of phospholipids at different stages.

3. Analysis of cholesterol

Cholesterol is analyzed quantitatively by capillary column of fused flexible silica. Purple complex by the reaction of cholesterol with iron from the reagent. Then absorbance is measured by UV.

Table 1 and Table 2 indicates the characterization pattern and its quality control assay.[33]

Current Scenario:

Current scenario for some marketed liposomal cream based topical preparations as shown in Table 3. [29, 30]

Table 1 Physical Characterization of Liposomes

Characterization Pattern		Instrument for Analysis
1)	Vesicle shape and surface morphology	TEM and SEM
2)	Vesicle size and size distribution	Dynamic light scattering, TEM
3)	Surface charge	Free flow electrophoresis
4)	Electrical surface potential and pH	Zeta potential measurement and pH sensitive probe
5)	Lamellarity	P31-NMR
6)	Drug release	Diffusion cell, dialysis
7)	Encapsulation efficiency	Mini column centrifugation, Gel permeation

Table 2 Chemical Characterization of Liposomes

Characterization Pattern	Instrument for Analysis
1) Phospholipid concentration	HPLC, Bartlett assay
2) Cholesterol concentration	HPLC
3) Phospholipid oxidation	UV absorbance
4) Phospholipid hydrolysis	HPLC/TLC
5) pH	pH meter
6) Osmolarity	Osmometer

Table 3 Some Marketed Liposomal Cream Based Topical Preparations [32]

Trade Name	Company	Specifications
Lipo-Gest™ Natural Balancing Cream	Lippomix	Lipo-Gest™ Natural Balancing Cream leaves the skin feeling moist, soft and natural, Lipo-Gest™ is hypoallergenic, fragrance-free, paraben-free, contains no animal products, no mineral oil, no artificial colors, and no artificial or harmful preservatives.
OPTISOMETM	Lippomix	It is a topical anesthetic cream comprising OPTISOMETM encapsulated tetracaine (5%).
Natural Progesterone Liposomal Skin Cream - 3 oz.	Now	Liposomal Skin Cream Calming Lavender 20 mg of Natural Progesterone per Pump No Artificial Colors or Fragrances Paraben Free Natural Progesterone Cream from Wild Yam and balancing herbs to support hormone levels.
Crystacide cream	AFT Pharmaceutical Ltd	It contains lipid stabilised Hydrogen peroxide 1%w/w. It is used as anti infective for small cuts, burns.
LIPOSOME CFT™ A slimming liposome	Cosmetic Surgery Suppliers, Inc	Aminophylline is key ingredient in slimming cream. Unlike other creams that have a 2% concentration, Liposome CFT has a 2.5%, the maximum concentration that will not cause skin irratation. Other ingredients are Co-enzyme A (an important catalyst in penetrating the skin & fatty tissue.), Aloe (as opposed to water as in the other similar products), Gilycerin makes this slimming cream soothing to the skin, as well as moisturizing.
Dermal-K Clarifying cream	-	A Vitamin K Cream that promotes healing and reduces bruising this cream utilizes a unique all natural dermaceutical delivery system. It also helps to treat burns, surface ulcers, cuts, abrasions, and skin irratation.

Regulatory requirements [31]:

The European Agency of the Evaluation of Medical Products (EMA) and the FDA has implemented the subject of liposome into their guidelines in last decades. Currently, EMA has covered general aspects of liposomes in several guidelines such as “Note of Guidance on the Quality, Preclinical and Clinical Aspects of gene transfer medicinal,” and “Guideline on adjuvant in vaccines for human use”.

FDA has published a draft version in 2001 entitled “Liposome Drug Products: chemistry, manufacturing, and controls, human pharmacokinetics and bioavailability and labeling documentation.” This draft consists of recommendations explicitly for liposome drug products submitted in new drug applications (NDAs). In detail, recommendations concerning the submission of a new liposomal product are given regarding physiochemical properties, description of manufacturing process and process controls, and control of excipients and drug products. Control of excipients involves those parameters which are essential to define lipid components, including description, characterization, manufacture, and stability. Furthermore,

aspects are addressed such as assaying encapsulated and nonencapsulated drug substance, lipid components, and degradation products, as well as *in vitro* tests for drug release from liposomes. The second part of this document is dealing with human pharmacokinetics and bioavailability. In particular, requirements concerning the quality and potency of bioanalytical methods are discussed. Therefore, the recommendations are focused on the validation of these methods and the capability to distinguish between encapsulated and nonencapsulated drug substances. Similar recommendations are given for *in vivo* integrity and stability considerations, respectively. For safety assessment, validated *in vitro* assays are recommended to simulate the liposomal release. In an additional chapter, studies for pharmacokinetics and bioavailability are recommended, such as mass balance studies and pharmacokinetic studies. Lastly, general recommendations concerning the labeling requirements are given. This draft guidance does not provide recommendations on clinical efficacy and safety studies, nonclinical pharmacology and/or toxicology studies, bioequivalence studies, liposomal formulations of vaccine adjuvant or biologics, and drug-lipid complexes. Unfortunately, during the intensive discussion process no conclusion regarding the appropriate approaches to access pharmacokinetics and bioavailability was achieved. Hence, this document has only draft status to this date.

In 2006, a reflection paper was published on nanotechnology-based medicinal products for human use reflecting the current thinking and the initiatives by EMA in relation of recent developments. As mentioned in this document, medicinal products containing nanoparticles, including liposomes, have already been authorized both in EU and US under the existing regulatory frameworks. Nevertheless, the European Commission has developed a number of initiatives with emphasis on safety and ethical considerations but also to evaluate the appropriateness of existing methodologies to assess the potential risks associated with nanotechnology. It is also mentioned that there is still insufficient knowledge and data concerning characterization, their detection and measurement, the persistence of liposomes in humans and the environment, and all aspects of toxicology related to these particles to allow satisfactory risk assessments. For dealing this issue, EMEA has created the Innovative Task Force for the coordination of scientific and regulatory competence. Because novel applications of liposomes and nanotechnology will span the regulatory boundaries between medicinal products and medical devices, the mechanism of action will be the key to decide whether a product should be regulated as a medical product or a medical device. Furthermore, evaluation of the quality, safety, efficacy, and risk management must be discussed in more detail.

Future aspects [31, 32]**:**

There are many liposomal topical products currently available in the market which are approved by regulatory different agencies. But there is still more insufficient information and data concerning to the characterization, many aspects of toxicology and risk management of new products. Hence evaluation of safety, efficacy, quality, risk management is more important for newly developed liposomal products and will require special consideration. EMEA will promote this process soon, either to develop specific guidelines or for the updating existing guidelines.

Stability of the liposomes is the main challenge for future inventions of topical liposomal formulation. Stability depends on the proper balance between maintenance of the drug within the vesicle and making it bioavailable to the affected site. Preparation of liposomes with optimum composition of lipids will be the big challenge during liposome preparation. Proper selection of lipids is very necessary as composition in bilayer controls permeability of the membrane and the nature of the drug. association with the membrane. Form of the drug substance and location of that drug within liposome lead to differences in drug leakage rate through the same membrane. Hence avoidance of drug leakage is the important challenge.

Successful stable liposomal topical formulation can be achieved in future by considering following points:

1. Processing with fresh, purified lipids and solvents
2. Avoidance of high temperature and excessive shear forces
3. Maintenance of low oxygen potential (Nitrogen purging)
4. Use of antioxidant or metal chelators
5. Formulating at neutral pH
6. Use of lyo-protectant when freeze drying

Many studies have proved the enhancing efficiency of liposomes on topical drug delivery. So future challenges of skin or topical application of liposomes is the first need to establish clinical data (in vivo studies). Even though the liposomal preparation is successfully prepared on laboratory scale, many problems like instability of liposomes can arise during mass production. These problems need to be overcome before the preparation is commercialized. Resolving these issues is one of the important challenges for future development of new formulations of liposomes.

CONCLUSION :

Numerous studies for the pharmaceutical application of liposomes have appeared during the past few decades. So liposomes are proved to be a versatile carrier systems for topical use. Liposomal

preparations are successfully used for delivering encapsulated drug to the targeted area. The new developments in the liposome are the specific binding properties of a drug-carrying liposome to a target cell.

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