Liposomes as Drug Delivery Systems in Dermal and Transdermal Drug Delivery

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Contents

2.1 Historical Perspectives

The term liposome is derived from two Greek words: "lipos" (fat), and "soma" (body). The term liposome was originally used in the literature before 1950 for colloidal aggregates of triglycerides which are very different from the lipid vesicles described in this review (Lasic 1996).

Liposomes were the first vesicular carrier studied for the delivery of drugs into the skin. The liposome story began with a paper in 1964, published in the *Journal of Molecular Biology*, in which Bangham and Horne from the Babraham Institute in Cambridge showed electron microscopic images of multilamellar phospholipid vesicles (Bangham and Horne 1964). The usage of the term liposomes for the description of lipid vesicles was proposed in 1968 (Sessa and Weissmann 1968; Kinsky et al. 1968). As lipid vesicles are often single-bilayer hollow spheres, which have nothing in common with "fat bodies," the use of the term liposome is in principle not

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appropriate (Lasic and Barenholz 1996a, b). Some authors use the term vesicle exclusively for a closed single or bilayer aggregate of amphiphiles (Kaler et al. 1992; Laughlin 1997).

Initially, liposomes were attractive as model systems for biological membranes, because of similarities in lipid composition and structure. Their usefulness as drug carriers was discovered by Sessa and Weissman (1970, 1997), who reported the encapsulation of lysozyme in multilamellar vesicles (MLV). Liposomes have been investigated extensively as a drug carrier system by various routes of administration and are accepted as potential carriers for a variety of drugs that include low molecular weight compounds, therapeutic proteins, diagnostic agents, and cosmetic actives (Egbaria and Weiner 1990; Du Plessis et al. 1994; Cevc 1996; Cevc and Vierl 2010; Kirjavainen et al. 1999; Walde and Ichikawa 2001; Blume et al. 2003; Choi and Maibach 2005; El Maghraby et al. 2008; Shailesh et al. 2009).

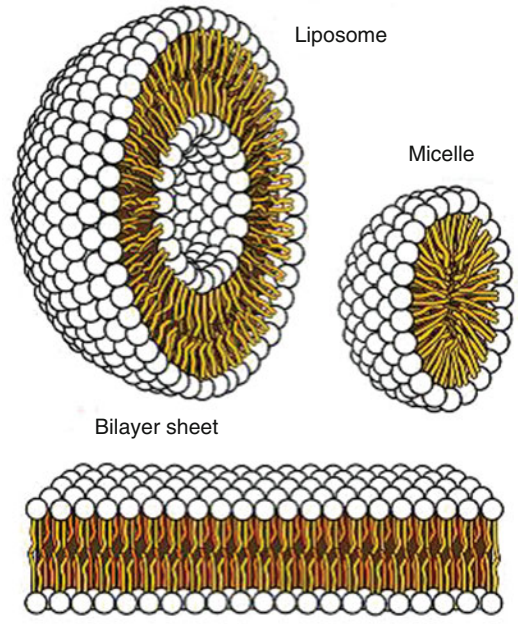

Fig. 2.1 Liposome structure (http://en.wikipedia.org/wiki/File:Liposome_cross_section.png)

2.2 Mechanism of Vesicle Formation

Liposomes are spherical vesicles, 0.05–5.0 μm in diameter, whose membranes consist of one (unilamellar) or more (oligolamellar, multilamellar) bilayers of phospholipids. The amphipathic nature of phospholipids and their analogues render them the ability to form closed concentric bilayers in the presence of water. When lipids are exposed to an aqueous environment, interactions between themselves (hydrophilic interactions between polar headgroups and Van der Waals interactions between hydrocarbon chains) and with water (hydrophilic interactions, hydrophobic effect) lead to spontaneous formation of closed bilayers. Spontaneous aggregation is not only determined by the hydrophobic contribution but is also related to the molecular parameters of the amphiphile (Lautenschläger 2006) (Fig. 2.1).

Liposomes may be characterized for their size, lamellarity, capture volume capacity, method of preparation, chemical integrity of the lipids, and application. Classification of liposomes based on structural parameters is represented in Fig. 2.2.

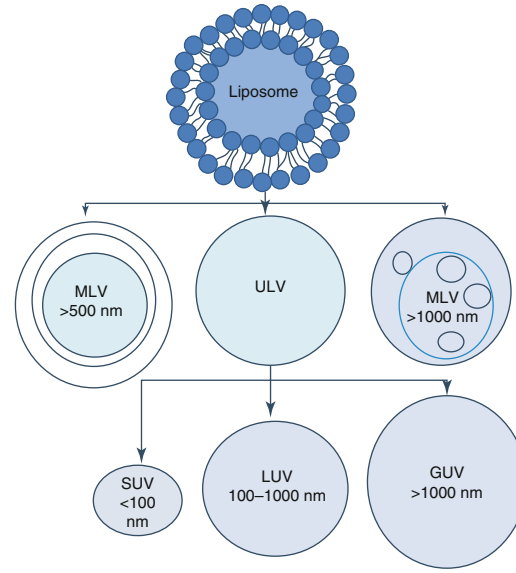

Fig. 2.2 Types of liposomes based on structure (Lautenschläger 2006)

2.3 Material for Vesicle Formation

Liposomes can be composed of naturally derived phospholipids with mixed lipids, hydrogenated

phospholipids, or of pure surfactants. Major structural components of conventional liposomes are phospholipids and cholesterol.

2.3.1 Phospholipids

Phospholipids, the major structural component of biological membranes, are amphiphilic with hydrophobic hydrocarbon tail and hydrophilic polar head (Fig. 2.3). The tails are repelled by water and line up to form a surface away from the water. The hydrocarbon tails of one layer face the hydrocarbon tails of the other layer, and the combined structures form a bilayer.

The glycerophospholipids are the most common used component of liposome formulations, derived from phosphatidic acid. Examples of natural phospholipids are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), and phosphatidylglycerol (PG).

The lipid composition determines the physical characteristics of the liposomes and the

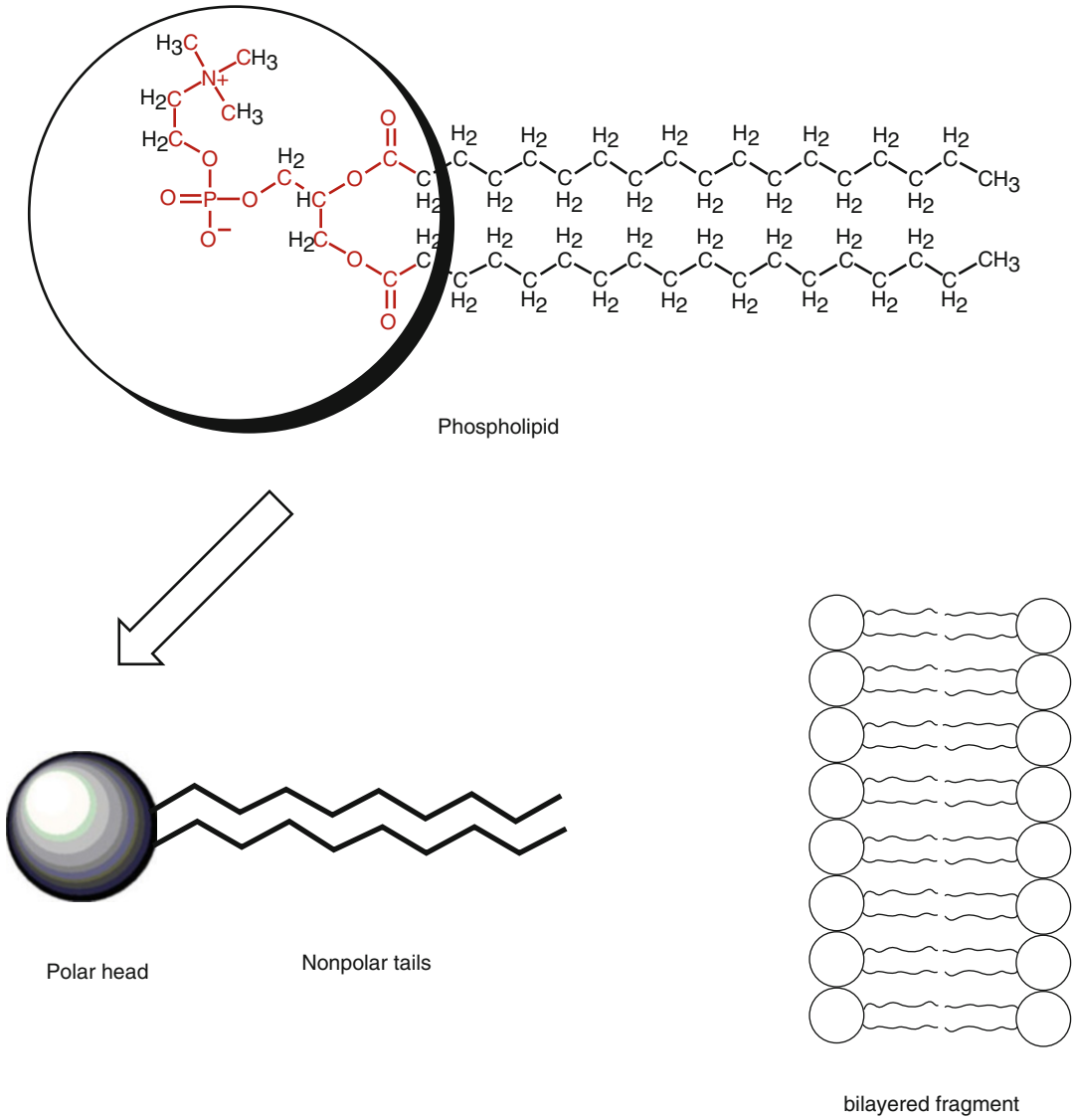

Fig. 2.3 Phospholipid structure

interaction of these carrier systems with the skin (Choi and Maibach 2005).

Natural phospholipids are obtained either from soy beans or eggs, which differ in composition of fatty acids and properties. Egg phospholipids have a higher content of saturated fatty acids (40 % of 16:0 and 18:0) in comparison to soy bean (80 % of 18:1 and 18:2). Soy phosphatidylcholine has a high content of unsaturated linoleic acid and a very low phase transition temperature of below 0 °C. This may be the reason behind its ability to fluidize the lipid bilayers of the horny layer in the skin. Because of its penetration capability, soy phosphatidylcholine delivers linoleic acid very effectively into the skin. By adhering very strongly to skin surface containing keratin, phosphatidylcholine shows moisturizing and softening effects. Liposomes composed of unsaturated fatty acids transported active agents better into the horny layer than liposomes composed of saturated fatty acids. The conditioning effect causes the horny layer to become a depot for these agents, and a more continuous permeation takes place outside the horny layer depot into the living part of the skin, over a longer period of time. This property makes this kind of liposomes very attractive for the application of substances influencing the regenerating ability of the living epidermis. In contrast, this kind of liposomes does not strengthen the natural barrier function of the skin with an exception of its indirect effect of supporting the formation of ceramide I. The liposomes based on natural phospholipids are not very stable, but the cost of purified lipids is very high.

The most common phospholipid used for the production of liposomes is PC. Phosphatidylcholine has a cylindrical molecule not soluble in water, which organizes itself into flat lamellae, i.e., bilayered structures, in order to minimize the unfavorable contact between the bulk aqueous phase and the long hydrocarbon fatty chains, which can further form liposomes. In contrast, other amphipathic molecules, such as detergents, have conical molecules and form micellar structures in water. Phosphatidylcholine membranes, like other lipid membranes, can exist in different phases, such as fluid liquid state and solid gel state, depending on the temperature. At phase transition temperature (Tm), lipid membranes pass from a tightly ordered gel state to a liquid crystal phase, where the freedom of movement of individual molecules is higher. Lipids with higher phase transition temperature have more stable molecules; however, they are at skin temperature in the gel state.

Instead of unsaturated PC, a fully hydrogenated PC should be selected for products with a longer shelf-life. Hydrogenated PC contains mainly stearic und palmitic acids and semisynthetic compounds, like dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylcholine (DSPC). Hydrogenated PC has high Tm in aqueous system of 50–60 °C, and it is insoluble in triglycerides, alcohols, and water. Such liposome preparations have better storage stability.

Recently, liposomes have been prepared using synthetic phospholipids. Commonly used synthetic phospholipids are dioleoylphosphatidylcholine (DOPC), distearoylphosphatidylcholine (DSPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylethanolamine (DOPE), and distearoylphosphatidylethanolamine (DSPE) (Lautenschläger 2006; Kulkarni 2005; Braun-Falco et al. 1998).

2.3.2 PEGylated Lipids

PEGylated lipids have a structure similar to phospholipids, but instead of the first acyl hydrocarbon chain, they have a polyethylene glycol (PEG) chain (Fig. 2.4). Glycerol provides the backbone, and the two hydrocarbon chains R1 and R2 can vary in length. The size of PEG chain can be from 8 to 45 subunits (*n*). The size of the PEG head relative to the length of the hydrophilic chain is the fundamental property that allows liposome formation (Lasic 1998).

This group of novel lipids has properties that allow formation of thermodynamically stable liposomes. PEGylated lipids prolong the plasma half-life of the encapsulated drugs. Coating of the liposomal surface with PEG would suppress the uptake of the drug by the reticuloendothelial system. PEG-12 glyceryl dioleate, PEG-12 glyceryl

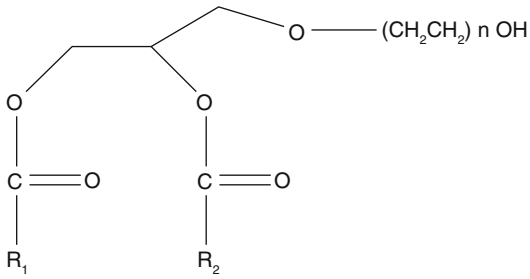

Fig. 2.4 Structure of PEGylated lipid

dimyristate, PEG-23 glyceryl palmitate, PEG-12 glyceryl distearate, and PEG-23 glyceryl distearate are suitable constituents of novel liposomes for cosmetic purposes (El Maghraby et al. 2004).

2.3.3 Polymeric Material

Synthetic phospholipids with diactylenic group in the hydrocarbon chain polymerize when exposed to UV light leading to the formation of polymerized liposomes. Polymerizable lipids contain conjugated diene or methacrylate (Regen et al. 1981).

2.3.4 Cholesterol

Cholesterol cannot form bilayer structures alone. However, it can be incorporated into phospholipid membranes in a very high concentration up to 1:1 or even 2:1 molar ratio (cholesterol to phosphatidylcholine). Cholesterol is included in liposomes for decreasing the fluidity of the bilayer, reducing the permeability of the membrane to water-soluble molecules, and stabilizing the membrane in the presence of biological fluids such as plasma (Praveen et al. 2009).

2.3.5 Other Substances

Varieties of other lipids or surfactants are used to form liposomes. Many single-chain surfactants or nonionic lipids can form liposomes, when mixed with cholesterol. A variety of polyglycerol and polyethoxylated mono and dialkyl amphiphiles are used mainly in cosmetic preparations. Single- and double-chain lipids having fluorocarbon chains can form very stable liposomes. Stearylamine and diacetyl phosphate are incorporated into liposomes to impart either a negative or a positive surface charge to these structures (Praveen et al. 2009).

2.4 Preparation of Liposomes

There are three groups of methods employed to produce liposomes (Fig. 2.5)

- Mechanical methods
- Methods based on replacement of organic solvent(s) by aqueous media
- Methods based on detergent removal

The choice of preparation method depends on the following parameters:

- The characteristics of major structural components of liposomes
- The characteristics, concentration, and toxicity of the material to be entrapped
- The nature of the medium in which the lipid vesicles are dispersed
- Additional processes involved during application/delivery of the vesicles
- Optimum size, polydispersity, and shelf-life of the vesicles for the intended application
- Reproducibility of the method and possibility of large-scale production

2.4.1 Mechanical Methods

The general steps of the production of liposomes are preparation of the lipids for hydration, hydration with agitation, and sizing to a homogeneous distribution of vesicles.

Thin film was the first method for liposome preparation. Lipids are dissolved in organic solvents (chloroform or mixtures with methanol), and the solvent is removed under a high vacuum with a rotor evaporator, forming a thin film on the walls of round-bottomed flask. The aqueous

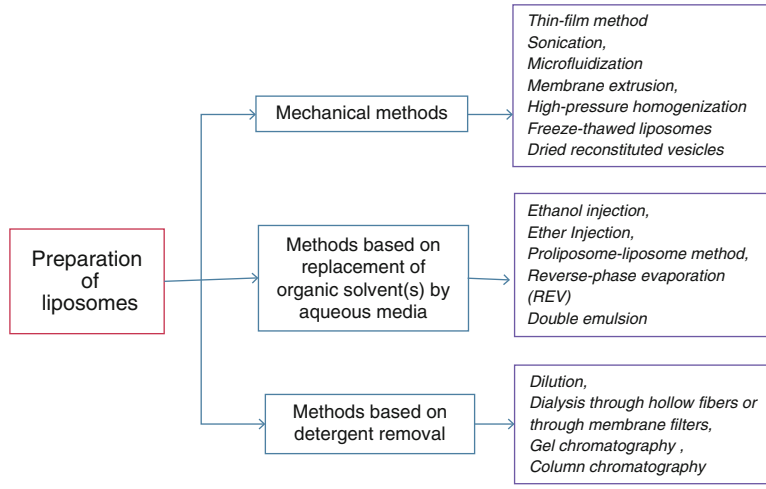

Fig. 2.5 Methods of preparation of liposomes

phase is prewarmed at temperatures above the Tm of the lipids used. After the addition of the aqueous phase, the thin film is detached from the flask walls by agitation and a highly heterogeneous population of MLVs is produced. Vesicle size is influenced by the lipid charge, nature of the aqueous phase, and power input of agitation. Since then, many different variations of this method have been developed. This method is easy to perform, and high encapsulation rates of lipid as well as aqueous soluble substances can be achieved because high lipid concentrations can be used. However, this method is not suitable for industrial production. Furthermore, the process becomes more time-consuming and cost intensive because additional processing is recommended for a defined liposome suspension (Braun-Falco et al. 1998).

The first published *homogenization technique* was sonication. A very high-energy input based on cavitation is applied to the liposomal dispersion either directly with a tip or indirectly in a bath sonicator. The position of the probe plays an important role on the ability to minimize vesicle size. Other methods are homogenization techniques, either by shear or pressure forces, such as microfluidization, high-pressure homogenization, and shear force-induced homogenization techniques. The most defined method for downsizing is the extrusion technique whereby liposomes are forced through filters with well-defined pores (Riaz 1996).

2.4.2 Methods Based on Replacement of Organic Solvent(s) by Aqueous Media

The liposome preparation methods described in this section have in common that organic solvents, either water miscible or immiscible, are replaced by an aqueous solution. This replacement is either performed by injection of the lipid carrying organic solution into the aqueous phase—the injection methods—or by stepwise addition of aqueous phase to the organic phase, in particular ethanol—the proliposome liposome method (Batzri and Korn 1973).

Ethanol injection method is a good alternative for the preparation of SUVs without sonication, based on injection of ethanolic solution of lipids into rapidly stirred aqueous buffer phase. By the immediate dilution of the ethanol in the aqueous phase, the lipid molecules precipitate and form bilayer planar fragments, which form liposomal systems, thereby encapsulating aqueous phase. The lipid concentration in ethanol is the only liposome formation influencing parameter. The method is very easy, but has an extra step to remove ethanol, and the concentration of vesicles produced and encapsulation of hydrophilic drugs is rather low (Kremer et al. 1977).

Ether injection method is a very similar method, and the only difference is that the lipid is injected slowly in the aqueous solution that is warm.

The proliposome-liposome method is based on the conversion of the initial proliposome preparation into a liposome dispersion by dilution with an aqueous phase (Jung et al. 2002). This method is suitable for the encapsulation of a wide range of drugs with varying solubility in water and alcohol and has extremely high encapsulation efficiencies.

In addition, the emulsification methods, namely, the *reverse-phase evaporation method (REV)* and the double emulsion technique, are based on the replacement of a water-immiscible solvent by an aqueous phase, thus forming liposomes with high encapsulation rates of hydrophilic as well as lipophilic substances (Szoka and Papahadjopoulos 1978).

2.4.3 Methods Based on Detergent Removal

In this group of methods, the phospholipids are brought into contact with the aqueous phase via detergents, which associate with phospholipid molecules and serve to screen the hydrophobic portion of the phospholipid molecule from water. In contrast to lipids, detergents are highly soluble in both aqueous and organic media. There is an equilibrium between the detergent molecules in the aqueous phase and the lipid environment of the micelles. Common used detergents are bile salts or alkylglycosides. The size, shape, and homogeneity of the resulting vesicles depend on the chemical nature of the lipids and detergent, their concentration, and the method and rate of detergent removal. Although liposomes are produced under mild conditions, this method suffers from low encapsulation efficiency of hydrophilic drugs (Schubert 2003).

Common procedures for detergent removal from the mixed micelles are dilution, gel chromatography (Schurtenberger et al. 1984), and dialysis through hollow fibers (Schurtenberger et al. 1976) or through membrane filters (Goldin 1979). Additionally, detergents can also be removed by adsorption to hydrophobic resins or cyclodextrins (Milsmann et al. 1978).

2.4.4 Large-Scale Industrial Production

In contrast to the many pessimistic forecasts during the 1980s, the reproducible preparation of large volumes of stable liposomes no longer presents a problem. They are prepared from well-characterized raw materials with established safety profiles. Furthermore, in most cases, shelf-life stability problems have been successfully solved as well. A characteristic liposome formulation consists of several different lipids that have to be mixed before hydration; normal practice is to lyophilize the lipid mixture from tert-butanol or to use a spray-dried lipid powder, or a thoroughly dried thin film. What is next performed? Adding organic solvents such as chloroform or methylene chloride to solubilize and mix lipids is not recommended (US regulations allow 50 ppm of chloroform and 500 ppm of methylene chloride in the formulation) (Lasic 1998).

Alternatively, lipid solutions in water-miscible (ethanol, propylene glycol) or water-immiscible (ether, freon) organic solvents can be injected into an aqueous phase followed by organic solvent removal (and possibly recycling) by evaporation, filtration, or dialysis. For cosmetic and some nutritional products, this may not be needed because the remaining solvent (propylene glycol or ethanol) may not be harmful and, additionally, prevents microbial growth. At higher lipid concentrations, all these hydration methods give rise to large multilamellar liposomes, which can be converted into smaller, unilamellar liposomes by extrusion and homogenization techniques (Riaz 1996).

A more suitable method for large-scale production of liposomes is "microfluidization." This process is similar to the "French Press" method. In this method, slurry-like concentrated lipid/water dispersions are introduced into the microfluidizer which then pumps it at a very high pressure (10,000–20,000 psi) through filters of 1–5 μm pore size. The fluid moving at a very high velocity is split into two streams by forcing them through two defined microchannels. The two streams are then made to collide together at right

angles at very high velocity. The fluid collected at the end is re-passed until a homogeneous dispersion is obtained. The microfluidization technique typically produces unilamellar liposomes of 50–500 nm diameter. The size of the liposomes can be roughly controlled by the processing pressure at which a microfluidizer is operated. Unfortunately, certain active ingredients, particularly proteins, may break down under the high processing pressure, which makes the microfluidization technique unsuitable for such actives (Vemuri et al. 1990).

In contrast to the microfluidizer, where the fluid stream is split and mixed by collision in a mixing chamber, homogenizers work on a different principle. In a homogenizer, the fluid beam is pressed with high pressure through an orifice, and this beam collides with a stainless steel wall. The liposome suspension is continuously pumped through the homogenizer system, where high pressures are generated to downsize lipid vesicles (Brandl et al. 1990).

The most prominent downsizing method is the extrusion. In this method, preformed vesicles are forced through defined membranes by a much lower pressure. Extrusion through polycarbonate filters was first published by Olson et al. in 1979. Depending on the apparatus and scale, the diameters of these membranes range from 25 to 142 mm (Mayer et al. 1986). As suggested for all downsizing methods, liposomes should be extruded above the Tm of the lipid composition. The main disadvantage of this method is the long-lasting preparation starting with preformed liposomes, eventually an additional freeze-thaw step, and finally the extrusion. In these procedures, high product losses may be generated, especially if clogging of the extrusion membranes occurs which may cause technical limitations with large-scale production (Table 2.1).

Table 2.1 Methods of liposome preparation and the resulting product

Method	Type of vesicles, size, nm	Encapsulation volume µl/µmol lipid	Encapsulation efficiency % active encapsulated (water soluble)
Mechanical methods			
Vortex or hand shaking of phospholipid dispersions	MLV 100–1000 nm		
French Press or microfluidization	LUV 100–1000 nm	2–7	Up to 50
French Press or microfluidization and harvesting by ultracentrifugation	SUV	0.2–0.5	Up to 15
High-pressure homogenization	Mainly SUV	0.2–0.5	Up to 15
Sonication followed by ultracentrifugation	SUV 20–100 nm	0.2–0.5	Up to 15
Extrusion through polycarbonate filters at low or medium pressure	OLV, LUV		
Dehydration-rehydration : Liposomes are stored freeze dried, rehydrated when required	MLV 100–1000 nm	2.5–6	Up to 60
Methods based on replacement of organic solvent(s) by aqueous media			
Removal of organic solvent(s)	MLV, OLV, SUV		
Hydration of dry lipid film followed by 3–10 freeze thaw (thawing to>Tm) cycles	MLV 100–1000 nm	2.5–6	Up to 60
Ethanol injection followed by extrusion	LUV, 100–1000 nm	2–7	Up to 50
Ether infusion (solvent vaporization)	LUV, OLV, MLV		
Reverse-phase evaporation	GUV, 1000 nm	As high as 720	Up to 60
Methods based on detergent removal			
Gel exclusion chromatography	SUV		
"Slow" dialysis	LUV, OLV, MLV		
Fast dilution	LUV, OLV		

Partly from Lasic and Barenholz (1996a)

2.5 Formulating with Liposomes

In formulating pharmaceutical or cosmetic products with liposomes, procedures and raw materials must be considered carefully to avoid adverse effects on liposome stability. In general, liposomes should be added to a formulation below 40 °C using low shear mixing. The addition of liposomes should also be the last step in the formulation's manufacturing process. Ethyl alcohol concentration should be kept below 5 %, solvents should be kept below 10 %, and high levels of salts (<0.5 %) should be avoided. Surfactants in general should also be avoided, but low levels (up to 1 %) of nonionic surfactants of high HLB values are usually well tolerated. Acceptable preservatives for liposome formulations include phenoxyethanol (and) methylparaben (and) ethylparaben (and) butylparaben (and) propylparaben (and) isobutylparaben. The recommended storage temperature of most liposome formulations is 25 °C (Braun-Falco et al. 1991).

2.6 Types of Liposomes

Specification of liposomes based on the structure of the vesicle and application includes many types of liposomes like conventional liposomes and novel liposomes [elastic liposomes, long-circulating liposomes, coated (lipoprotein-, carbohydrate-, PEG-coated), liposomes or sterically stabilized (Stealth®, manufacturer, country) liposomes, ethosomes, niosomes, Transfersomes® (manufacturer, country), invasomes, proliposomes, proniosomes, pharmacosomes, vesosomes, Marinosomes® (manufacturer, country), Rovisomes® (manufacturer, country), etc. (Table 2.2). Some of these liposomes are used for parenteral drug administration, but for dermal and transdermal application, conventional and flexible liposomes are still used.

Conventional liposomes can be defined as liposomes that are typically composed of only phospholipids (neutral and/or negatively charged) and cholesterol. Most early studies on liposomes as a drug carrier system employed this type of liposomes. They can vary widely in their physicochemical properties, such as size, lipid composition, surface charge and number, and fluidity of the phospholipid bilayers. Important derivatives of conventional liposomes are nonionic surfactant-based liposomes called niosomes (Choi and Maibach 2005).

Flexible (elastic) liposomes are similar to conventional liposomes but with the incorporation of an edge activator in the lipid bilayer structure to provide elasticity. Elastic liposomes are applied nonoccluded to the skin and have been shown to permeate through the SC lipid lamellar regions as a result of the hydration or osmotic force in the skin. They have been investigated as drug carriers for a range of small molecules, peptides, proteins, and vaccines.

Some of flexible liposomes are small, unilamellar vesicles (80–250 nm) prepared of soy PC (>80 %) having a high content of linoleic acid. They are used for topical application and provide the skin with essential polyunsaturated fatty acids, which support the formation of ceramide 1 and with choline which is a part of the natural moisturizing factor (NMF). In a clinical study it was proven that these liposomes have cosmetic properties, i.e., improve skin appearance, like inducing wrinkle reduction and an increase in skin smoothness. Additionally, these liposomes exert pharmaceutical effects, like decreasing of efflorescence in the acne treatment (Blume and Teichmüller 1997; Ghyczy et al. 1996). Recent approach in modeling transdermal drug delivery through the skin is the development of two ultraflexible vesicular carriers —ethosomes and Transfersomes®.

Sterically stabilized liposomes (long-circulating liposomes, Stealth® liposomes) are composed of hydrophilic polymer polyethylene glycol (PEG), attached covalently to the outer surface. PEG coating inhibits protein adsorption and opsonization of liposomes, thereby avoiding or retarding liposome recognition by the reticuloendothelial system (Lasic et al. 1991).

Table 2.2 defines the terms that are most commonly found in literature and relate them to different types of liposomes.

Table 2.2 Classification of commonly known lipid vesicles according to their structures and/or preparation

Identification	Definition
Archaeosomes	Archaeosomes are vesicles consisting of archaebacterial lipids which are chemically distinct from eukaryotic and prokaryotic species. They are less sensitive to oxidative stress, high temperature, and alkaline pH (Krishnan et al. 2000; Conlan et al. 2001)
Cationic liposomes	Composed of cationic lipids. Fuse with cell or endosome membranes; suitable for delivery of negatively charged macromolecules (DNA, RNA); ease of formation, structurally unstable; toxic at high dose, mainly restricted to local administration
Cochleates	Cochleates are derived from liposomes which are suspended in an aqueous two-phase polymer solution, allowing the logic partitioning of polar molecule-based structures by phase separation. The liposome containing two-phase polymer solution treated with positively charged molecules such as Ca2+ or Zn2+ form sacochleate precipitate of a particle dimension less than 1 μm (Gould-Fogerite et al. 1998)
Conventional liposomes	Composed of neutral or negatively charged phospholipids and cholesterol. Rapid and saturable uptake by reticuloendothelial system; short circulation half-life, dose-dependent pharmacokinetics
Dendrosomes	Nontoxic, neutral, biodegradable, covalent or self-assembled, hyperbranched, dendritic, spheroidal nanoparticles which are easy to prepare, inexpensive, highly stable (Sarbolouki et al. 2000)
Dried reconstituted vesicles (DRV)	By this preparation technique, small, "empty" unilamellar vesicles, containing different lipids or mixtures of them, are prepared. After mixing those SUVs with the solubilized drug, dehydration is performed. By addition of water, rehydration leads to the formation of large quantities of rather in homogeneous, multilamellar vesicles which need further processing (Gregoriadis et al. 1987)
Ethosomes	Ethosomes are composed of high amounts of ethanol (45 % v/v) and a low lecithin (2 % w/v) concentration, which provide an ethosome suspension with mean size of approximately 100 nm. Ethosomal systems are much more efficient at delivering to the skin, in terms of quantity and depth, than either conventional liposomes. Ethosomal systems composed of soy phosphatidylcholine and about 30 % of ethanol were shown to contain multilamellar vesicles by electron microscopy (Touitou et al. 2000; Godin and Touitou 2003)
Immunoliposomes	Conventional or stealth liposomes with attached antibody or recognition sequence
Immunosomes	Immunosomes are prepared by the anchorage of glycoprotein molecules to preformed liposomes. Under the electron microscope, immunosomes look like homogenous spherical vesicles (50–60 nm) evenly covered with spikes. Immunosomes have structural and immunogen characteristics closer to those of purified and inactivated viruses than any other forms of glycoprotein lipids association (Perrin et al. 1985)
Immune stimulating complex (ISCOM)	ISCOMs are spherical, micellar assemblies of about 40 nm. They are made of the saponin mixture Quil A, cholesterol, and phospholipids. They contain amphiphilic antigens like membrane proteins. ISCOMs already have a built-in adjuvant, quillaja saponin, which is a structural part of the vehicle (Kersten and Crommelin 2003)
Invasomes	Liposomes containing besides PC also ethanol and terpenes (Dragicevic-Curic et al 2008)
Lipoplexes	Liposomes, comprising cationic and neutral lipids, which are able to form complexes with negatively charged pDNA. They are efficient carriers for cell transfection but have certain drawbacks due to their toxicity. These toxic effects may result from either cationic lipids or nucleic acids (Khalil et al. 2006; Audouy and Hoekstra 2001)
Long-circulating liposomes	See stealth liposomes. Composed of neutral, high transition temperature lipid, cholesterol, and 5–10 % of PEG-DSPE. Hydrophilic surface coating, low opsonization, and thus low rate of uptake by RES long-circulating half-life (40 h); dose-independent pharmacokinetics (Lasic et al. 1991; Woodle and Lasic 1992; Moghimi and Szebeni 2003)
LUVETs	LUVETs are large unilamellar vesicles prepared by extrusion technique, mainly performed with high-pressure systems (Mayer et al. 1986)
Magnetic liposomes	Composed of PC, cholesterol, and small amount of a linear chain aldehyde and colloidal particles of magnetic iron oxide. Can be made use by an external vibrating magnetic field on their deliberate, on-site, rapture, and immediate release of their components

Table 2.2 (continued)

Identification	Definition
Marinosomes	Liposomes based on a natural marine lipid extract containing a high polyunsaturated fatty acid (PUFA) ratio (Moussaoui et al. 2002)
PLARosomes	Liposomes with phospholipid alkylresorcinol (PLAR), which improves the stability and the entrapment of the liposomal drug formulation (Gómez-Hens and Fernández-Romero 2006)
pH-sensitive liposomes	Four basic classes of pH-sensitive liposomes have been described The first class combines polymorphic lipids, such as unsaturated phosphatidylethanolamines, with mild acidic amphiphiles that act as stabilizers at neutral pH; suitable for intracellular delivery of weak base and macromolecules The second class includes liposomes composed of lipid derivatives resulting in increased permeability to encapsulated solutes A third class utilizes pH-sensitive peptides or reconstituted fusion proteins to destabilize membranes at low pH The fourth class uses pH-titratable polymers to destabilize membranes following change of the polymer conformation at low pH (Drummond et al. 2000)
Polymerized liposomes	Polymerized phosphatidylcholine vesicles (35–140 nm) have been synthesized from lipids bearing one or two methacrylate groups per monomer. Compared to nonpolymeric analogues, these vesicles exhibited improved stability and controllable time-release properties (Regen et al. 1981)
Proliposomes	Proliposomes are defined as dry, free-flowing particles that immediately form a liposomal dispersion on contact with water
Proteasomes	Liposomal vesicles of bacterial origin, with a protein to lipid ratio higher than that achieved with purified protein incorporated in liposomes. Vesicles were solubilized, followed by ammonium sulfate precipitation and dialysis against detergent buffer. Proteins and peptides are noncovalently complexed to the membrane, making them highly immunogenic (Lowell et al. 1988)
Reverse-phase evaporation vesicles (REV)	Vesicles are formed by evaporation of oil in water emulsions resulting in large unilamellar liposomes (Szoka and Papahadjopoulos 1987)
Stealth liposomes	Liposomes sterically stabilized by covalent attachment of hydrophilic polymers, mainly polyethyleneglycols (PEGylated liposomes) to the bilayer, which show prolonged circulation half-lives and can evade interception by the immune system. PEG coating inhibits protein adsorption and opsonization of liposomes, thereby avoiding or retarding liposome recognition by the reticuloendothelial system (RES). These PEG-coated liposomes are also referred to as sterically stabilized or stealth liposomes. The PEG-stabilizing effect results from local surface concentration of highly hydrated groups that sterically inhibit both hydrophobic and electrostatic interactions of a variety of blood components at the liposome surface (Allen et al. 1991; Klibanov et al. 1990)
Temperature-sensitive liposomes	Such liposomes have been prepared using lipids which undergo a gel-to-liquid crystalline phase transition a few degrees above physiological temperature. The most suitable lipid is dipalmitoylphosphatidyl choline. Temperature-sensitive liposomes are considered to be a promising tool to achieve site-specific delivery of drugs They release the entrapped content at the target cell surface upon a brief heating to the phase transition temperature of the liposome membrane (Kono 2001; Needham and Dewhirst 2001)
Transfersomes	Transfersomes are elastic, very deformable vesicles which consist of PC in combination with an edge active surfactant like sodium cholate, which softens the membrane of the vesicle and makes the bilayer much more flexible. The strong hydrophilicity and the vesicle's extreme ability to deform enable the transfersome to temporarily open the pores through which water normally evaporates between the cells. Such newly activated intercellular passages can accommodate sufficiently deformable vesicles maintaining their integrity but changing their shape to fit the channel. Along these said pathways in the horny layer, transfersomes reach regions of high water content in the deeper skin layers (Paul and Cevc 1995). Transfersome is a term registered as a trademark by IDEA AG, Germany
Virosomes	Liposomal formulations with viral envelope proteins anchored in their lipid membrane (Glück 1999)

Partly from Wagner and Vorauer-Uhl (2011)

2.7 Methods for Characterization, Quality Control, and Quantification of Percutaneous Penetration of Liposomes

The use of liposomes requires the control of several features, such a size distribution, composition, encapsulation efficiency (EE), and chemical stability. The tests, including lipid integrity, particle size, morphology of liposomes, phase transition temperature, and drug release rates, are proposed for the stability study of liposomes.

Chemical analysis of liposomes sheds light on the purity of lipids and can quantitatively reveal the absence or presence of degradation products formed by lipid hydrolysis or peroxidation. The hydrolysis of lipids leads to the formation of lysolipids. As the concentration of lysolipids increases in a given solution of liposomes, the bilayer structure of the liposome is eventually disintegrated and the undesirable formation of micelles takes place. The hydrolysis of lipids depends on the pH of the media employed. Reported data indicate that, at pH 6.5, lipid hydrolysis is minimal (Lasic and Templeton 1996).

Lipids with unsaturated acyl chains tend to oxidize due to the presence of oxidizing agents present in the media. This process results in fragmented acyl chains which contribute to the destabilization of the bilayer structure of the liposomes. It is, therefore, very important to provide an optimum pH range as well as the presence of antioxidants in liposome products in order to reduce hydrolysis and oxidation. Ultraviolet (UV) spectra of an ethanolic solution of a lipid can reveal if the lipid is oxidized. An absorbance at 230 nm will suggest the presence of conjugated dienes, while absorbance between 270 and 280 nm will indicate the presence of conjugated trienes, and this suggests that degradation of the lipid has occurred (Mhashilkar et al. 2001). Other commonly used methods of lipid analysis include thin-layer chromatography (TLC) (Oberholzer et al. 1995), high-performance liquid chromatography (HPLC) (Arts et al. 1997), gas chromatography (Müller et al. 2004), nuclear magnetic resonance (NMR) spectroscopy, and differential scanning calorimetry (DSC).

The size of liposomes is commonly characterized by two different methods: light scattering and transmission electron microscopy (TEM). Light scattering, including low-angle light scattering and photon correlation spectroscopy (PCS), is commonly applied for generating specifications of liposomes in the cosmetic and pharmaceutical industries. For SUV or LUV types of liposomes, the PCS method is preferred over the low-angle light scattering method. Further, TEM (negative staining method and cryo-TEM) images allow accurate measurement of the liposome size, while the freeze fracture TEM sheds light on lamellarity and surface morphology of liposomes.

Lipid thermal behavior is studied using a differential scanning calorimeter.

A new method to determine the integrity and intactness of liposomes in final formulations is electron spin resonance (ESR) spectroscopy. First of all, liposomes are labeled with an ESR-active probe (chemically similar to PC), and afterward ESR spectra of formulations are taken. By computer simulation the degree of stability or degradation can be calculated. An excellent stability could be detected in aqueous systems. In w/o emulsions the stability of liposomes varied between 50 and 100 % (over a time period of 8 weeks) depending on the emulsifiers used. The stability less than 50 % was found in a w/o formulation, and a total breakdown could be observed in shampoos (Magdassi 1997).

Electron paramagnetic resonance (EPR) has been used to investigate the influence of liposome size on the transport of substances and the interactions of liposomes with the skin (Šentjurc et al. 1999, 2004).

Most of the methods described to determine the percentage of drug carried by liposomes (i.e., encapsulation efficiency, EE) involve removal of the non-encapsulated drug using size exclusion chromatography (SEC) (Kaiser et al. 2003; Junping et al 2000), centrifugation (Moribe et al. 1998), dialysis, and filtration (Wu et al. 2004). Proton NMR spectroscopy is an alternative technique that allows the determination of liposomal

EE without physical separation of entrapped and non-entrapped drug (Zhang et al. 2004).

The development of analytical methods to control the effectiveness of liposomal delivery systems (LDSs) runs parallel to the development of these LDSs (Koppenhagen et al. 1998). Although many methods have been described for the control of liposomal drug formulations, most of them measure only total drug concentration in the sample and do not distinguish between free and entrapped drug, which would be desirable to know to establish the real behavior of these formulations.

2.7.1 Methods for Separation Liposomal and Non-liposomal Drug Forms

Solid-phase extraction (SPE) is of great interest in the separation of liposomal and non-liposomal drug forms. Separation is based on the property of liposomes to cross reversed-phase C18 silica gel cartridges without being retained, while a non-liposomal drug is retained on the stationary phase (Deshpande et al. 2010).

Size exclusion chromatography (SEC) is a simple and powerful technique for the investigation of encapsulation, insertion/interaction of substances from small solutes (ions, surfactants, drugs, etc.) up to large molecules (proteins, peptides, and nucleic acids) in liposomes. The drug retention capacity of the liposomes is usually determined. SEC is widely used to narrow the size distribution. For example, Sepharose 2B, 4B Sephacryl S-1000, and high-performance exclusion gels of the TSK-PW series are suitable for separating small unilamellar vesicles from larger ones using SEC. The potentiality of SEC is strongly improved by using a HPLC system associated to gel columns with a size selectivity range allowing liposome characterization in addition to particle (Lesieur et al. 1993; Lundahl et al. 1999; Grabielle-Madelmont et al. 2003).

Thin-layer chromatography (TLC) is an old method for characterization and investigation of chemical stability of phospholipide.

High-performance liquid chromatography (HPLC) has been widely applied for the determination of drugs in liposome formulations (Chimanuka et al. 2002; Ferdous et al. 1997; Rossi et al 2004; Grohganz et al. 2004). A limitation of this method is that it involves the disruption of liposomes, measuring total drug in the sample and not the entrapped drug.

Capillary electrophoresis (CE) with chemiluminescence detection has been used for the characterization of liposomes in order to study different properties, such as homogeneity, trapped volume, stability, and permeability (Tsukagoshi et al. 1998; Griese et al. 2001).

Future innovations in analytical techniques will be oriented toward the development of new approaches to provide online and in situ information on the penetration of drugs into the skin.

There have been several methods reported in literature for the quantification of the percutaneous penetration enhancement. These include diffusion experiments (Du Plessis et al. 1994), visualization by electron microscopy (Hofland et al. 1995), and micro dialysis (Schnetz and Fartasch 2001). Micro dialysis and diffusion experiments provide information about the amount and the rate of drug penetration of the model compound, but do not give any information about the physiological effects of the model drug on cells and lipid organization. The visualization by electron microscopy provides detailed information about the structure of the cells and lipid organization in the skin, but does not provide information about the penetration pathways. Other techniques used are fluoromicrography (Yarosh et al. 1994) and confocal laser scanning microscopy (CLSM). Fluoromicrographs of the skin treated with fluorescently labeled liposomes demonstrated that the fluorescent marker remained in the SC or penetrated deeper in the epidermis mainly along the follicle. A disadvantage of fluoromicrography is that the tissue needs to be (cryo)fixed, which may change skin lipid organization or may result in redistribution of the marker (Shotton and White 1989). CLSM provides information about the localization and the permeation pathway of a fluorescent model compound in the tissue. The major advantage of CLSM is that the distribution of the fluorescent model compound in the sample can be visualized

without cryofixing or embedding the tissue. However, in the case of penetration studies with liposomes, CLSM does not provide information about the permeation of the entire liposome, but only about the penetration of the fluorescent label (Õan Kuijk-Meuwissen et al. 1998).

2.8 The Stability of Liposomes

The stability of liposomes involves the stability of the particles and all their components, including the encapsulated substance. Liposome stability can be subdivided into physical, chemical, and biological stabilities, which are all interrelated. Generally, the shelf-life of liposomes is determined by the physical and chemical stability. By optimizing the size distribution, pH, and ionic strength, as well as the addition of antioxidants and chelating agents, liquid liposome formulations can be stable (Mojović et al. 1996a, b; Šiler-Marinković et al. 1996).

2.8.1 Physical Stability

Physical instability of the liposomes is expressed as a fusion of vesicles, aggregation, and encapsulation capacity reduction. The consequence of fusion is the loss of encapsulated material. By proper selection of lipid components as well as storing conditions for liposomes, an optimum membrane permeability can be obtained. Risk of fusion can be reduced by applying negatively charged phospholipids, which increase the density of charge on the surface of the membrane of liposomes. Addition of cholesterol reduces the permeability of the membrane when it is in liquid-crystalline thermodynamic state. Some authors believe that cholesterol can autoxidize over time, leading to loss of encapsulated material (Šobić and Šiler Marinković 2002).

The high membrane permeability for water-soluble molecules is the cause of the sensitivity liposomes to the osmotic pressure. Larger liposomes (MLV and LUV) are more sensitive, and they can swell on the high osmotic pressure. At the critical value of the osmotic pressure, liposomes may rupture.

2.8.2 Chemical Stability

Chemical instability of the liposomes is reflected in the oxidation of unsaturated fatty acids present in the lipid bilayer, lipid hydrolysis, and degradation of the active components, which may lead to disruption of liposome membranes and leaking of encapsulated materials.

The oxidation rate depends on the nature of the phospholipids and the presence of antioxidants and prooxidants. Oxidation can be slowed down using hydrogenated phospholipids as starting material, avoiding contact with oxygen, reducing the exposure to light and heat (Šiler-Marinković and Mojović 1996).

Liposomes are susceptible to enzymatic and chemical hydrolysis, but it is not an important cause of instability. Enzymatic hydrolysis of lipids is catalyzed by lipolytic enzymes. Lipases of vegetable origin operate normally under acidic conditions, while animal lipases act in an alkaline environment. The rate of enzymatic hydrolysis was higher for lipids with unsaturated fatty acids. Chemical hydrolysis is, in contrast to the enzyme hydrolysis, less controlled. This process depends on pH, and it is slowest at pH 6.5 for most phospholipids. In order to stop the hydrolytic changes in the liposomes, dispersions can be prepared with Tris-buffered saline or phosphate-buffered saline of certain pH. Temperature affects the rate of hydrolysis which is much faster at temperatures higher than 25 °C. The reason may be the activation of lipases, which are present in the raw materials of natural origin.

2.8.3 Microbiological Stability

Liposomes are an excellent medium for bacterial growth. Liposomes used for cosmetic purposes are not sterilized, but adequate sterility can be achieved by the passage of liposomes through up to millipore filters (400 nm), and after that, dispersions are protected with preservatives. The preservatives may interact with double-layer membranes, which are manifested by changes in Tm. This phenomenon is most pronounced in propylparaben and must be taken into account in

the formulation of liposomal dermatics (Wallhaeusser 1995). Ethanol, as a possible preservative, has also limitations, because its addition increases the average diameter of liposomes. Addition of 10 % ethanol increases the average diameter of liposomes by about 20 %, while the addition of 30 % ethanol causes an increase in the mean diameter of about two times (Kirjavainen et al. 1997; Maitani et al. 2001).

2.9 Liposomes as Skin Drug Delivery Systems

Delivery, as we know, is the process of transporting the right chemical to the right location at a relevant concentration for a sufficient period of time. Many factors govern the delivery of active ingredients from topically applied formulations into the skin. These factors include type of formulation, the lipophilicity of the component, the size of the molecule, presence of penetration enhancers, and physical state of the stratum corneum (SC).

Many studies showed significantly higher absorption rates and greater pharmacological effects for drugs encapsulated in liposomes, as compared to conventional topical formulations (Michel et al. 1992; Mezei and Gulasekharam 1980; Cevc 1996; Rownicka-Zubik et al. 2012).

There are three different skin penetration routes for applied drugs. These include the intercellular route, the transcellular route, and follicular penetration. In the past, the intercellular route has been considered the predominant pathway of skin penetration. Hair follicles and sweat glands account for approximately only 1 % of the skin surface, and, therefore, the follicular route was not previously considered to represent a significant route of penetration. Surprisingly, however, recent in vitro and in vivo studies suggest that the follicular route is also important for skin penetration (Lademann et al. 2001). However, the mechanisms of action of liposomes in inserting active ingredients into the skin remain controversial. But, in the last few years, some authors highlighted important factors that influence the penetration of active ingredients encapsulated in liposomes. Several factors, such as total lipid concentrations and composition, lamellarity, charge of liposomal surface, and mode of application, have been proven to influence drug deposition into the skin layers (Cevc and Blume 1992; Weiner et al. 1989). The experimental design may have also some effects on the recorded action. Accordingly, it is not possible to describe a general mode or mechanism of action of liposomes (acting as skin drug delivery systems) with skin, and in each situation, a detailed description of the formulation and experimental design has to be considered (Tikshdeep et al. 2012).

Four alternative mechanisms have been suggested for liposomes acting as delivery systems (Fig. 2.6) (El Maghraby et al. 2005, 2006). The first mechanism (A) represents a free drug operation, whereby molecules are initially released from liposomes and they independently permeate through the skin. In this case, vesicles can be considered only as carriers that can control drug release. The second mechanism (B) suggested a possible penetration enhancement effect, because lecithin enchases transdermal delivery by lowering the permeability barrier of the skin. The third mechanism (C) is the adhesion of liposome lipids onto the skin surface, with possible fusion or mixing with the lipid matrix of the SC. It is supposed that once in contact with the skin, some budding of liposomal membrane might occur (Schaller and Korting 1996; Vrhovnik et al. 1998). This could cause a mixing of the liposome bilayers with intracellular lipids in the SC, which may change the hydration conditions and thereby the structure of lipid lamellae of the SC. This may enhance the permeation of the lipophilic drug into the SC and ease the diffusion of hydrophilic drugs into the interlamellar spaces. The concept of intact vesicular skin penetration (D) is not recent and was suggested in the first reports on liposomes as skin drug delivery systems. It was difficult to conceive that large lipid vesicles could penetrate the densely packed SC. Consequently, many research groups have tested this hypothesis, and electron micrography showed the presence of few intact liposomes in the dermis (Foldvari et al. 1990). Although the applied formulation included large multilamellar vesicles (LMLVs), most of the

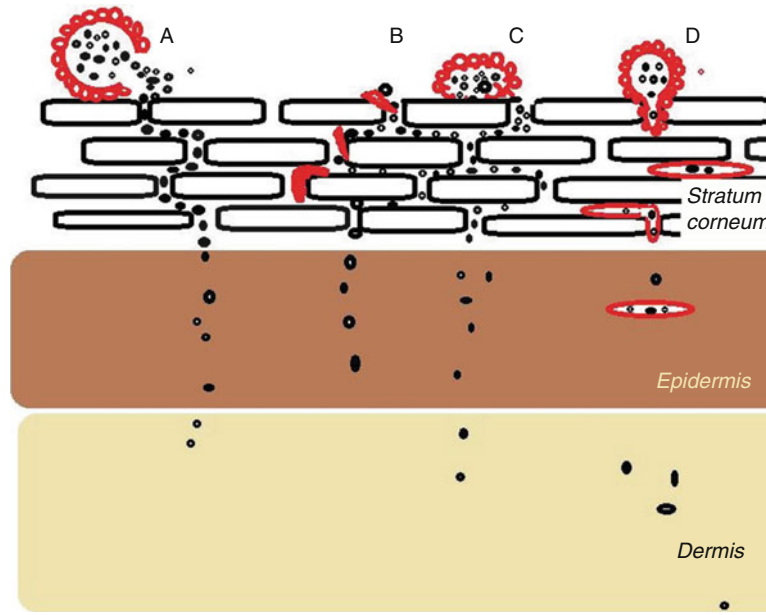

Fig. 2.6 Interaction of the liposomes and skin (El Maghraby et al. 2006)

liposomes detected in the dermis were unilamellar (300–500 nm) with some LMLVs. The authors proposed that liposomes could penetrate the epidermis carrying the drug into the skin and that the smaller nano-aggregates could have come from LMLVs that lost their external bilayers during penetration. The liposomes could be adsorbed intact on the skin surface before penetration, with a possibility that some vesicles might rupture. It may be possible that some vesicles, which are deformable enough, will pass the SC as intact structures (Cevc and Blume 1992; Cevc et al. 2002) or may accumulate in the channel-like regions in the SC (Honeywell-Nguyen et al. 2004) depending upon their compositions. It is reported by several authors that the high elasticity of vesicles could result in enhanced drug transport across the skin as compared to vesicles with rigid membranes. In any case, liquid-state, flexible liposomes showed greater skin penetration than those in a gel state, and small-sized and unilamellar vesicles seem to result in a higher degree of skin penetration (Blume et al. 2003; Fresta and Puglisi 1996; Verma et al. 2003; Honeywell-Nguyen et al. 2004).

The application conditions can also influence the penetration kinetics. The flexible vesicles work more efficiently under nonocclusive application. The driving force for the transport of vesicles into the skin is the trans epidermal osmotic gradient, and nonocclusive application is the key to create it (Cevc and Blume 1992). The driving force for the movement of flexible liposomes is generated by the hydration gradient across the skin, which builds up because of the different water content in the SC and stratum granulosum (SG), i.e., the content varies from 15 to 20 % in the SC to 70 % in the SG (Fresta and Puglisi 1996). When the flexible liposomes are applied onto the skin and allowed to dry, the vesicles as hydrophilic entities/structures are attracted by the moisture in the epidermis, and due to their flexibility, they penetrate the skin. After reaching in the local area, a liposome can interact with the skin cell by any of the following methods (Anwekar 2011):

- Endocytosis by phagocytic cells of the reticuloendothelial system (RES) such as macrophages and neutrophils
- Adsorption to the cell surface either by nonspecific weak hydrophobic or electrostatic forces or by specific interaction with cell surface components
- Fusion with the plasma cell membrane by insertion of lipid bilayer of liposome into the

plasma membrane with simultaneous release of liposomal contents into the cytoplasm
- Transfer of liposomal lipids to cellular or subcellular membrane or vice versa without any association of the liposome contents

It is often difficult to determine what mechanism is operative and more than one may operate at the same time.

To continue the action of drugs to a particular site in the body, the general approach is to deposit drug-bearing liposome directly into the site where therapy is desired. Since liposomes are large and do not easily cross epithelial or connective barriers, they are likely to remain at the site of local administration. The liposomes would then slowly released into the target site or perhaps create a local drug level higher than the systemic level. Alternatively the drug-loaded liposomes might interact directly with cells in the target site, without producing drug release. The goal of this approach is to maximize the amount of effective drug at the target site, while minimizing the drug levels at other sites and thus decreasing systemic toxicity (Tatsuhiro 2002).

2.10 Application of Liposomes as Dermal Delivery Systems

Liposomes are efficient delivery systems for drugs. They can act as drug carriers controlling release of the therapeutic agent. Alternatively they may provide a localized depot in the skin so reducing the amounts of drug permeating through the skin, thus minimizing systemic effects. They may also provide targeted delivery to skin appendages. Further, vesicles can enhance transdermal drug delivery, increasing systemic drug concentrations. In addition, the use of liposomes in nanocosmetology also has many benefits, including improved penetration and diffusion of active ingredients, transport of active ingredients, extended release time, greater stability of active ingredients, reduction of unwanted side effects, and high biocompatibility. Liposomal preparations reduce the skin roughness because of their interaction with the corneocytes and with the intercellular lipids resulting in skin softening and smoothening (Pierre and dos Santos Miranda Costa 2011).

Liposomes can be loaded with different hydrophilic, lipophilic, or amphiphilic *cosmetic actives*. Examples include vitamins, α-hydroxy acids, UV filters, ceramides, unsaturated fatty acids, growth factors, botanical extracts, and antioxidants (Blume 2008). In these cases, formation of gels containing the corresponding active-loaded liposomes is easily achieved. There is a considerable interest in the use of liposomes for products that retard premature aging of skin, or prevent photoaging. Antioxidants along with natural botanical extracts are commonly used in antiaging products, and encapsulation of vitamins in liposomes has been shown to enhance their stability. Encapsulation and release studies of personal care actives like α-hydroxy acids, β-hydroxy acids (salicylic acid) (Perugini et al. 2000), retinoids (Li and Lishko 2001), and ascorbic acid (Fočo et al. 2005) have been published in the past. The first liposomal cosmetic product to appear on the market was the antiaging cream "Capture" launched by Dior in 1986. Topical liposomal preparations containing encapsulated compounds have recently attained commercial value.

Glycolic acid, excellent as an exfoliative agent and moisturizer used in cosmetic products, has an irritant effect when applied on the skin. Loading glycolic acid into the liposomes showed the advantage of liposomes in reducing side effects (e.g., irritation) of glycolic acid. The results obtained showed that liposomes are suitable to modulate glycolic acid release and thereby its irritation potential and that the best condition to achieve this control is obtained by the liposomal systems in which glycolic acid/lipid molar ratio is 5:1. Further significant release control is obtained by addition of chitosan into the liposome (Perugini et al. 2000).

Retinoids, drugs useful in the treatments of acne, psoriasis, and many tumors, were recently shown to have reduced side effects when applied entrapped in liposomes. Decreased skin burning and increased drug stability after exposition to light was demonstrated by the use of this delivery

system (Li and Lishko 2001; Trapasso et al. 2009). Cationic liposomes consisting of double-chained cationic surfactants, PC, and retinoic acid were found to increase delivery of retinoic acid about twofold suggesting the potential of cationic liposomes' use for the intradermal delivery of lipophilic drugs like retinoic acid (Kitagawa and Kamasaki 2006).

Stability and low pH value of *ascorbic acid* are the difficulties for formulating effective cosmetic products containing these actives. In the study of Fočo et al. (2005), two types of multilamellar vesicles, one from non-hydrogenated and the other from hydrogenated soybean lecithin, containing cholesterol, were prepared for the encapsulation of sodium ascorbyl phosphate (SAP). It was shown that liposome carriers enabled high SAP penetration into SC. The difference in liposome composition did not have any significant effect on the penetration profile of SAP which was much more influenced by the concentration of the drug. At higher concentrations of SAP, the absolute penetrated amount of SAP was higher. As to the stability of SAP in liposome formulations, it was much more influenced by the storage temperature than by liposome composition.

Liposomes with encapsulated lipolytic actives are used for the treatment of *obesity*. Recently, Tholon et al. have prepared so-called slimming liposomes from soy phospholipids and extract of *Centella asiatica*, L-carnitine, and other actives. Their in vivo data indicate that a daily topical application of gel with 3 % slimming liposomes reduced thigh circumference by 10 mm in 28 days (for >20 % of the subjects) (Tholon et al. 2002).

The treatment of many dermatological diseases by topical application of liposomal formulations is expected to be more efficient, because a significant concentration of the drug is retained in the living epidermis and dermis. Triamcinolone, methotrexate, hydrocortisone, and diclofenac are some of the drugs encapsulated in the liposomes with promising results. Liposomes with methotrexate reduced the percutaneous drug absorption, while the drug retention in the skin was two- to threefold higher than when the free drug form was used (Betz et al. 2001; Schmid and Korting 1994; Yarosh 2001; Samad et al. 2007).

Liposomal preparations were found superior in the treatment of acne vulgaris compared with conventional preparations including alcoholic lotions (Meybeck 1992; Ghyczy et al. 1996). A double-blind clinical study was conducted to assess the safety and efficiency of liposome-encapsulated 1 % clindamycin solution versus 1 % clindamycin solution (Klimicin® T, Lek). On the basis of the clinical trial, it may be concluded that liposome-encapsulated 1 % clindamycin solution was therapeutically superior over conventional 1 % clindamycin solution in the treatment of acne vulgaris (Honzak and Sentjurc 2000).

The transdermal route for *local anesthetics* has advantages over injection pathway, such as improving patient compliance, providing continuous drug delivery, and avoiding side effects including hematoma and nerve damage. Poor permeability and slow penetration rate into the skin of existing topical anesthetics, however, have significantly limited their clinical applications. An alternative delivery strategy used to increase penetration of topical anesthetics is the incorporation of the drug into the liposomes (Eichenfield et al. 2002; Lener et al. 1997; Miler et al. 2004). The potential of liposome-encapsulated local anesthetics to provide topical anesthesia to intact skin was investigated. Using the pin-prick assay, prolonged anesthesia from a tetracaine or a lidocaine liposome formulation was shown, whereas a cream control formulation was ineffective. The improved anesthetic effect after vesicle delivery was probably due to improved skin accumulation (Gesztes and Mezei 1988). Vesicles provided even stronger and deeper anesthesia relative to a commercial eutectic mixture of local anesthetics EMLA (APP Pharmaceuticals LLC, Schaumburg, Il.) 2.5 % lidocaine and 2.5 % prilocaine in man (Fisher et al. 1998). Biozone Labs, Pittsburg, CA,USA, produces a liposomal formulation with lidocaine available at the market under the commercial name ELA-Max ™. ELA-Max is a registered trademark of Ferndale Laboratories, Inc.

Dithranol is one of the drugs used in the topical treatment of psoriasis, but it has irritating, burning, staining, and necrotizing effects on the skin. The entrapment of drugs in liposomes will

reduce the dose-dependent side effects, like irritation and staining. The entrapment efficiency of dithranol in the liposomes was optimized by altering the proportion of PC and cholesterol. The in vitro permeation study using mouse abdominal skin showed significantly enhanced permeation with vesicles as indicated by the flux of dithranol from liposomes (23.13 μg/cm^2/h) and niosomes (7.78 μg/cm^2/h) as compared with the cream base (4.10 μg/cm^2/h) (Agarwal et al. 2001).

The deposition of *hydrocortisone* into the human skin was significantly higher when liposomes were used compared with the ointment form. The blanching effect of hydrocortisone paralleled the deposition results, with the vesicles producing a greater effect than the ointment even when urea was incorporated into this base (Wohlrab et al. 1992).

Liposomal delivery of *local antibiotics* may be clinically useful in surgical wound prophylaxis, and the advantages include the achievement of adequate local antimicrobial concentration at the time of operation as well as increased efficacy of the antibiotic. The liposomal delivery of local antibiotics in this model of surgical wound infection reduced the number of organisms more effectively than the applied free drug. Treatment of contaminated surgical wounds is often complicated by the failure of local or systemic antibiotic treatment and prophylaxis. Locally administered liposome-encapsulated antimicrobials may offer advantages over free antibiotics, including an increase in efficacy, ease of administration, and safety. The local delivery of antibiotics by liposomes may also have advantages in other areas such as oncology, wound healing, immunology, and cellular biology. The therapeutic advantages, as well as the absorption and distribution of locally administered liposome-encapsulated antibiotics, were compared with those of locally applied unencapsulated antibiotics in a contaminated wound model. Liposomes can potentially alter toxicity and target drug delivery to specific sites (Šiler-Marinković et al. 1997; Honzak and Sentjurc 2000).

The successful targeting of actives via hair follicles using liposome delivery systems is an "open door" for cosmetic applications as well. Cotsarelis and his group at the University of Pennsylvania have investigated gene delivery to hair follicles using liposomes (Domashenko et al. 2000; Gupta et al. 2001). Hoffman has also reported on the liposomal targeting of hair follicles (Hoffman 1998). These studies have shown encouraging results and may become a practical way of treating hair disorders by means of DNA treatment. Introducing hair growth promoters or hair growth retardants through liposomal formulations may also be a practical approach.

Liposomes have also been used in the treatment of hair loss. *Minoxidil*, a vasodilator, is the active ingredient that claims to prevent hair loss. It is formulated in liposomes to improve the flux of contents through the skin (Bhushan 2008). Minoxidil sulfate in PG-coated liposomes is also marketed as Nanominox-MS (Sinere, USA). Liposomes with *progesterone*, on the other hand, have been used to reduce the rate of hair growth in idiopathic hirsutism (Ganesan et al. 1984).

A formulation with *melanin*-encapsulating liposomes in a spray form (Lipoxome®, Dalton Medicare B.V., the Netherlands) delivers melanin selectively to the hair follicle and the hair shaft to stain hair follicles of people with bold, white, or gray hair (Sand et al. 2007).

Conclusion

As a conclusion, liposomes offer great value in dermal/transdermal drug delivery, and recent advances of liposomes appear to have generated increased therapeutic potential. Alteration in their composition and structure results in vesicles with better properties. Flexible liposomes are one such advance with claims of enhanced transdermal drug delivery.

Liposomes continue to be an area of research to be further explored for a better understanding and characterization of the transport path and interaction with the skin. Most of the researchers agree that the use of liposomes has many benefits, including improved penetration of active ingredients, selective transport of active ingredients, longer release time, greater stability of active ingredients, reduction of unwanted side effects, and high biocompatibility.

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