

## **EMERGING TRENDS OF LIPOSOMES USED AS NOVEL DRUG DELIVERY SYSTEM**

**Debjit Bhowmik\*, R.Margret chandira, Chiranjib.B.,B.jayakar**  
**Vinayaka missions college of pharmacy**  
**Vinayaka mission University, Salem, Tamilnadu, India**

**Email-debjit\_cr@yahoo.com**  
**Margret.jeni@yahoo.com**

### **ABSTRACT**

Drug delivery systems (DDS) have become important tools for the specific delivery of a large number of drug molecules. Since their discovery in the 1960s liposomes were recognized as models to study biological membranes and as versatile DDS of both hydrophilic and lipophilic molecules. Liposomes can be used as carriers. Liposomes--nanosized unilamellar phospholipid bilayer vesicles--undoubtedly represent the most extensively studied and advanced drug delivery vehicles. After a long period of research and development efforts, liposome-formulated drugs have now entered the clinics to treat cancer and systemic or local fungal infections, mainly because they are biologically inert and biocompatible and practically do not cause unwanted toxic or antigenic reactions. A novel, upcoming and promising therapy approach for the treatment of solid tumors is the depletion of macrophages, particularly tumor cells for both water and lipid soluble agents. Substances entrapped in liposomes include enzymes, glycolipids, immunoglobulin, monoclonal antibodies, drug-like antitumor, antiarthritic and antiparasitic, antigens, antifungal agents, antibiotics and chromosomes. Liposomes are artificial phospholipid bilayer vesicles. Liposomes are chiefly composed of natural or synthetic phospholipids especially lecithins hence can be metabolized in vivo and are generally non-toxic and non-antigenic. Entrapment of drug leads to different pharmacokinetics hence can result in better therapeutic index and enhanced cellular uptake. Thus the ADME of the agent can be altered by manipulating the physicochemical properties of liposomes, instead of the drug.

## INTRODUCTION

A liposome is a tiny bubble (vesicle), made out of the same material as a cell membrane. Liposomes can be filled with drugs, and used to deliver drugs for cancer and other diseases. Membranes are usually made of phospholipids, which are molecules that have a head group and a tail group. The head is attracted to water, and the tail, which is made of a long hydrocarbon chain, is repelled by water. In nature, phospholipids are found in stable membranes composed of two layers (a bilayer). In the presence of water, the heads are attracted to water and line up to form a surface facing the water. The tails are repelled by water, and line up to form a surface away from the water. In a cell, one layer of heads faces outside of the cell, attracted to the water in the environment. Another layer of heads faces inside the cell, attracted by the water inside the cell. The hydrocarbon tails of one layer face the hydrocarbon tails of the other layer, and the combined structure forms a bilayer. When membrane phospholipids are disrupted, they can reassemble themselves into tiny spheres, smaller than a normal cell, either as bilayers or monolayers. The bilayer structures are liposomes. The monolayer structures are called micelles. Liposomes are composite structures made of phospholipids and may contain small amounts of other molecules. Though liposome's can vary in size from low micrometer range to tens of micrometers, unilamellar liposomes, as pictured here, are typically in the lower size range with various targeting ligands attached to their surface allowing for their surface-attachment and accumulation in pathological areas for treatment of disease. Substances associated macrophages with bisphosphonate-containing liposomes. In the advent of the use of genetic material as therapeutic molecules the development of delivery systems to target such novel drug molecules to cells or to target organs becomes increasingly important. Liposomes, in particular lipid-DNA complexes termed lipoplexes, compete successfully with viral gene transfection systems in this field of application. Future DDS will mostly be based on protein, peptide and DNA therapeutics and their next generation analogs and derivatives. Due to their versatility and vast body of known properties liposome-based formulations will continue to occupy a leading role among the large selection of emerging DDS. Many sub-unit vaccines are successful in preventing the occurrence of disease, but their use is largely restrained due to low immunogenicity. Novel carrier-based vaccine could serve as a vaccine adjuvant to overcome low immunogenicity of sub-unit vaccines. The use of liposomes as a delivery system for antigen is well recognized but they are unstable and release of antigen from them cannot be controlled over a prolonged period of time. To overcome the limitation of liposomes, this study has developed gel core liposomes in which a core of polymer was incorporated inside the liposomal vesicles, which serve the function of skeleton and provide mechanical strength to vesicles. In the present investigation BSA(bovine serum albumin)-loaded gel core liposomes were prepared by reverse phase evaporation method and characterized for vesicles size, shape, entrapment efficiency, in vitro release and stability studies. The in vivo studies to evaluate antigen presenting potential of the gel-core liposomes was performed in Balb/c mice by measuring the immune response elicited by intramuscular administration of BSA-loaded gel core liposomes and compared with intramuscularly administered BSA-loaded conventional

liposomes, alum adsorbed BSA and plain antigen. Results indicate that intramuscular immunization with gel core liposomes induces efficient systemic antibody responses against BSA as compared to other formulations. The gel core liposomal formulation provides good entrapment efficiency, enhanced in vitro stability, prolonged antigen release and effective immuno adjuvant property, justifying its potential for improved vaccine delivery.

### ***DISCOVERY OF LIPOSOMES***

Liposomes were first described by British haematologist Dr Alec D Bangham FRS in 1961 (published 1964), at the Babraham institute, Cambridge. They were discovered when Bangham and R. W. Horne were testing the institute's new electron microscope by adding negative stain to dry phospholipids. The resemblance to the [plasmalemma](#) was obvious, and the microscope pictures served as the first real evidence for the cell membrane being a bilayer lipid structure.

### **ADVANTAGES OF LIPOSOMES**

Liposomes offer several advantages in delivering genes to cells

- ❖ .Liposomes can complex both with negatively and positively charged molecules.
- ❖ Liposomes offer a degree of protection to the DNA from degradative processes.
- ❖ Liposomes can carry large pieces of DNA, potentially as large as a chromosome.
- ❖ Liposomes can be targeted to specific cells or tissues.

### **PROPERTIES OF LIPOSOMES**

- ❖ The system is composed of structures of bimolecular sheets intercalated by aqueous space.
- ❖ They are permeable to water.
- ❖ They are osmotically sensitive.
- ❖ Positively charged membranes are impermeable to cations and negative are relatively permeable to anions.

## ***APPLICATION OF LIPOSOMES***

Liposomes are used for drug delivery due to their unique properties. A liposome encapsulates a region on aqueous solution inside a [hydrophobic](#) membrane; dissolved [hydrophilic solutes](#) cannot readily pass through the lipids. Hydrophobic chemicals can be dissolved into the membrane, and in this way liposome can carry both hydrophobic molecules and hydrophilic molecules. To deliver the molecules to sites of action, the lipid bilayer can fuse with other bilayers such as the cell membrane, thus delivering the liposome contents. By making liposomes in a solution of DNA or [drugs](#) (which would normally be unable to [diffuse](#) through the membrane) they can be (indiscriminately) delivered past the lipid bilayer. There are three types of liposomes- MLV (multilamellar vesicles) SUV (Small Unilamellar Vesicles) & LUV (Large Unilamellar Vesicles). These are used to deliver different types of drugs. Liposomes are used as models for artificial cells. Liposomes can also be designed to deliver drugs in other ways. Liposomes that contain low (or high) pH can be constructed such that dissolved aqueous drugs will be [charged](#) in solution (i.e., the pH is outside the drug's pI range). As the pH naturally neutralizes within the liposome ([protons](#) can pass through some membranes), the drug will also be neutralized, allowing it to freely pass through a membrane. These liposomes work to deliver drug by [diffusion](#) rather than by direct cell fusion. Another strategy for liposome drug delivery is to target endocytosis events. Liposomes can be made in a particular size range that makes them viable targets for natural macrophage phagocytes. These liposomes may be digested while in the macrophage's phagosome, thus releasing its drug. Liposomes can also be decorated with opsonins and ligands to activate endocytosis in other cell types. The use of liposomes for transformation or transfection of DNA into a host cell is known as lipofection. In addition to gene and drug delivery applications, liposomes can be used as carriers for the delivery of dyes to textiles, pesticides to plants, enzymes and nutritional supplements to foods, and cosmetics to the skin. The use of liposomes in nano cosmetology also has many benefits, including improved penetration and diffusion of active ingredients, selective transport of active ingredients, longer release time, greater stability of active, reduction of unwanted side effects, and high biocompatibility.

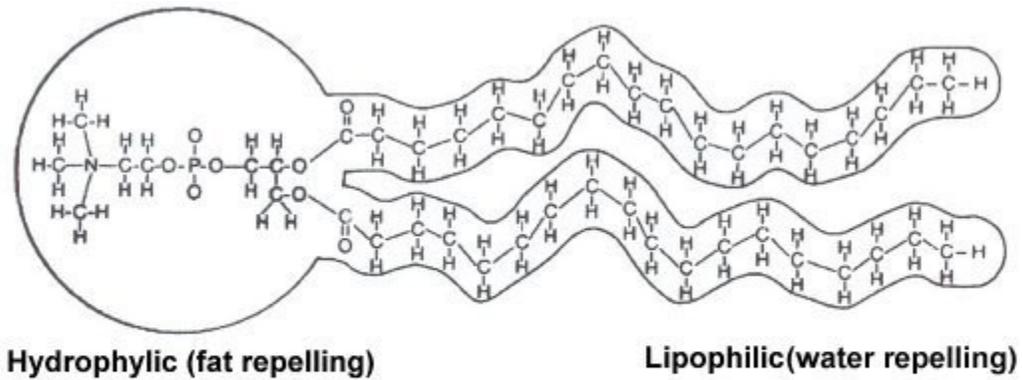
## **STRUCTURE OF LIPOSOMES**

Liposomes are spherical lipid bilayers from 50 nm to 1000 nm in diameter that serve as convenient delivery vehicles for biologically active compounds. The field of liposome research has expanded considerably over the last 30 years. It is now possible to engineer a wide range of liposomes varying in size, phospholipid composition and surface characteristics to suit the specific application for which they are intended. This paper gives an overview of the main advances in liposome research from a point of view of their applications in medicine. Aqueous contrast enhancing agents entrapped in liposomal carriers can be targeted to the liver and spleen and distinctions can be made between normal and tumorous tissue using computed tomography. Topical application of liposomes has great potential in dermatology. Liposomes have been used to deliver anticancer agents in order to reduce the toxic effects of the drugs when given alone or to increase the circulation time and effectiveness of the drugs. From the original concept of encapsulating hemoglobin in an inert shell, liposome-encapsulated hemoglobin (LEH) has evolved into a fluid proven to carry oxygen, capable of surviving for reasonable periods in the circulation and amenable to large-scale production. Liposomes may be used to target specific cells by attaching amino acid fragments such as antibodies or proteins or appropriate fragments that target specific receptor sites. Liposomal DNA delivery vectors and further enhancements in the forms of LPDI and LPDII are some of the safest and potentially most versatile transfer vectors used to date. DNA vaccination and improved efficiency of gene therapy are just a few of the upcoming applications of liposomes. liposome, microscopic, fluid-filled pouch whose walls are made of layers of phospholipids identical to the phospholipids that make up cell membranes. Liposomes are used to deliver certain vaccines, enzymes, or drugs (e.g., insulin and some cancer drugs) to the body. When used in the delivery of certain cancer drugs, liposomes help to shield healthy cells from the drugs' toxicity and prevent their concentration in vulnerable tissues (e.g., the kidneys, and liver), lessening or eliminating the common side effects of nausea, fatigue, and hair loss. Liposomes are especially effective in treating diseases that affect the phagocytes of the immune system because they tend to accumulate in the phagocytes, which recognize them as foreign invaders. They have also been used experimentally to carry normal genes into a cell in order to replace defective, disease-causing genes. Liposomes are sometimes used in cosmetics because of their moisturizing qualities.

Liposomes were first produced in England in 1961 by Alec D. Bangham, who was studying phospholipids and blood clotting. It was found that phospholipids combined with water immediately formed a sphere because one end of each molecule is water soluble, while the opposite end is water insoluble. Water-soluble medications added to the water were trapped inside the aggregation of the hydrophobic ends; fat-soluble medications were incorporated into the phospholipid layer.

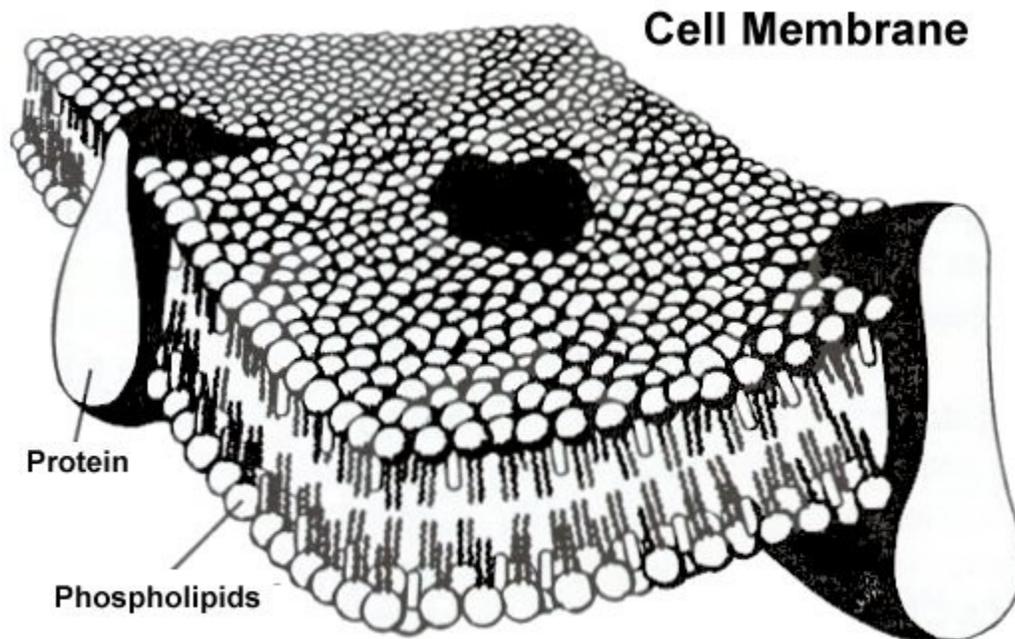
In some cases liposomes attach to cellular membranes and appear to fuse with them, releasing their contents into the cell. Sometimes they are taken up by the cell, and their phospholipids are incorporated into the cell membrane while the drug trapped inside is released. In the case of phagocytic cells, the liposomes are taken up, the phospholipid walls are acted upon by organelles called lysosomes, and the medication is released. Liposomal delivery systems are still largely experimental; the precise mechanisms of their action in the body are under study, as are ways in which to target them to specific diseased tissues.

The most important and interesting attribute of phospholipids is their ability to form special molecular structures called liposomes. The molecular shape of a phospholipid consists of a water-loving head and two oil-loving tails.



**Shape of phospholipid molecule**

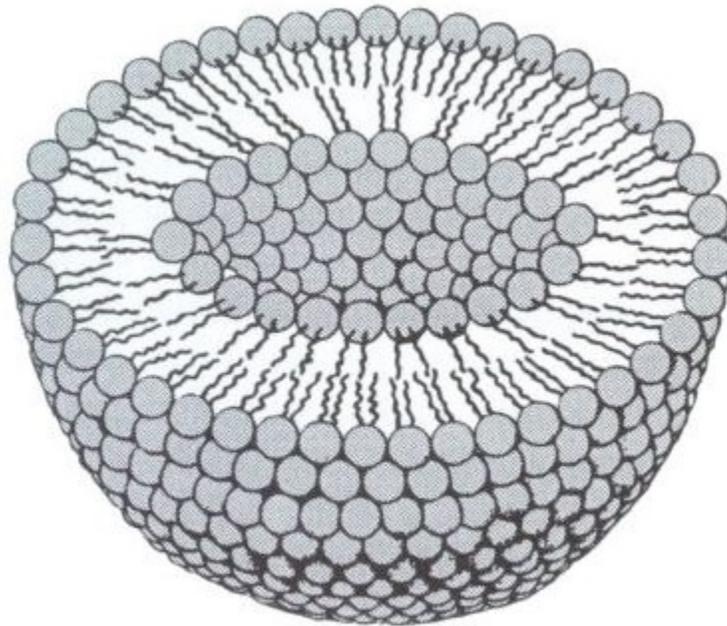
When placing a large number of these molecules into a limited space they will arrange themselves spontaneously to match their heads together and also their tails.



**Human cell membrane formed by phospholipids**

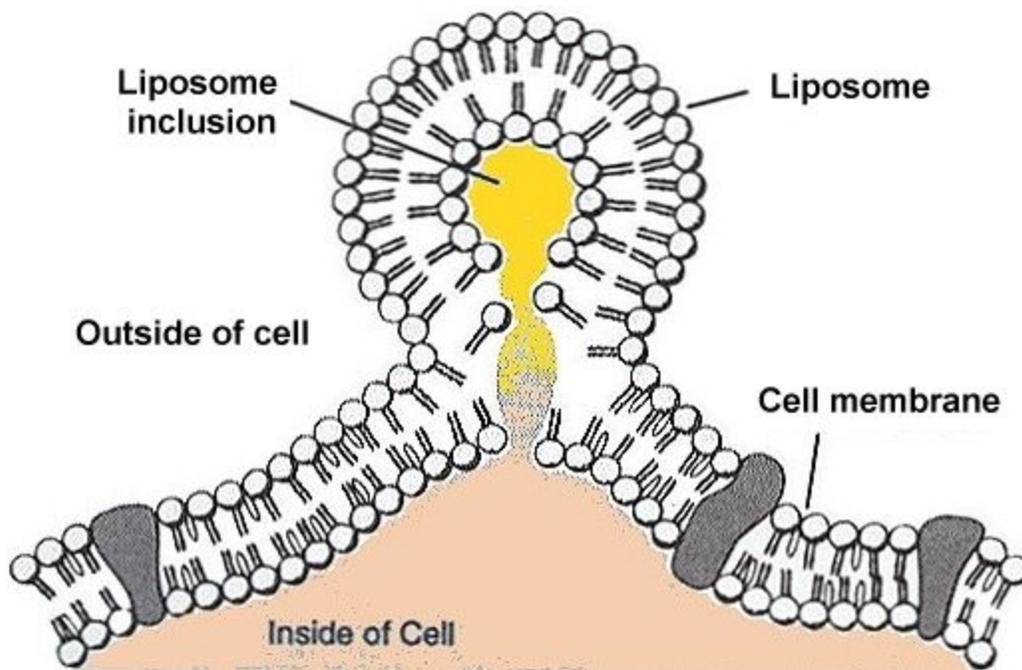
The figure above shows the arrangement of a section of a living cell membrane which consists largely of phospholipids. As you can see, the phospholipid molecules have arranged themselves to form a membrane so that oil droplets cannot penetrate the cell membrane because they would be repelled by the wall of hydrophilic heads. In like manner, no water can penetrate the membrane because the lipophilic tails inside will not allow passage. The only access through the membrane is by special protein molecules that are programmed to let only certain chemicals pass in and out of the cell.

Plant phospholipids are all very similar in structure and composition. Under certain physical conditions they will spontaneously form microscopic spheres whose walls are very similar in construction to the actual cell membrane shown above.



**Liposome structure formed by phospholipids**

The size of these spheres is very small, in the order of a nanometer. As illustrated, the spheres are hollow inside and enclose some of the liquid material in which they were formed (inclusion). Because of the small size of the phospholipid molecule and microspheres, they can pass through the epidermis and act as a carrier for the enclosed substances. It is postulated that when they reach the outside of a living cell membrane in the dermis they may become accepted as part of the membrane, being of the same composition. This process is as shown.



### Acceptance of liposome into cell

Thus, they are able to carry with them any enclosed substances into the dermis and to the individual cells.

The ability of phospholipids to act as the carrier mechanism for delivering active ingredients directly to the cell level has extensive implications for cosmetics. By themselves they are absolutely non-toxic and cause no skin irritations, not even around the eyes. Their danger lies in their ability to carry toxic or contaminated substances into the cells. The development of liposome technology offers the potential for many beneficial cosmetic products. However, the cosmetic developer has to deal very carefully with the selection of raw materials and the question of the biological fate of the preparation. The microspheres themselves are constantly undergoing changes due to thermal activity during preparation and storage. As a result, each ingredient of the preparation can end up inside the microspheres over time.

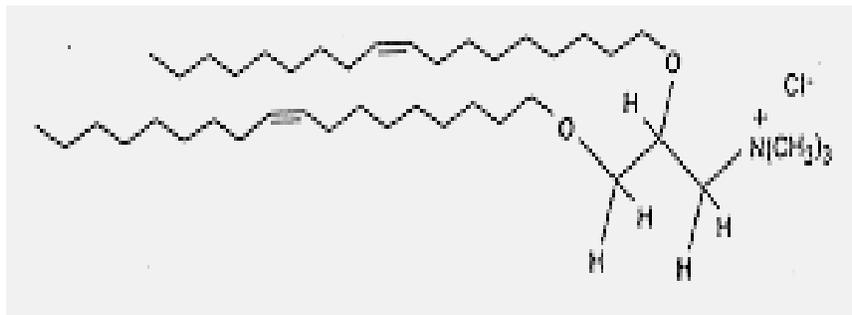
More than 80 percent of the cosmetic products on the market contain toxic substances that if used in liposome products will eventually become part of the inclusion inside liposomes that, in turn, will get inside your skin cells. Therefore, beware of products with liposomes that also contain substances causing adverse effects. As an example, preservatives fall into this hazardous category since they are all cellular toxins.

It has not been decided by the FDA whether liposome products with inclusions should be considered a medicine and put under the scrutiny of medical doctors with the advantage of documenting and tracking of potential long-term adverse effects. According to the

Cosmetic Handbook, published by the Food and Drug Administration, "Products that are cosmetics but are also intended to treat or prevent disease, or affect the structure or functions of the human body, are considered also drugs and must comply with both the drug and cosmetic provisions of the law."<sup>1</sup> Some combinations of liposomes and active substances certainly qualify for this category. The cosmetic industry has no intention of waiting for a decision before cashing in on the remarkable properties of liposomes.

## MECHANISM OF LIPOSOMES

Liposomes were first described in 1965 as a model of cellular membranes and quickly were applied to the delivery of substances to cells. Liposomes entrap DNA by one of two mechanisms which has resulted in their classification as either cationic liposomes or pH-sensitive liposomes. Cationic liposomes are positively charged liposomes which interact with the negatively charged DNA molecules to form a stable complex. Cationic liposomes consist of a positively charged lipid and a co-lipid. Commonly used co-lipids include dioleoyl phosphatidylethanolamine (DOPE) or dioleoyl phosphatidylcholine (DOPC). Co-lipids, also called helper lipids, are in most cases required for stabilization of liposome complex. A variety of positively charged lipid formulations are commercially available and many other are under development. One of the most frequently cited cationic lipids is lipofectin. Lipofectin is a commercially available cationic lipid first reported by Phil Felgner in 1987 to deliver genes to cells in culture. Lipofectin is a mixture of N-[1-(2, 3-dioleoyloxy) propyl]-N-N-N-trimethyl ammonia chloride (DOTMA) and DOPE. The structure of DOTMA is shown below.



DNA and lipofectin interact spontaneously to form complexes that have a 100% loading efficiency. In other words, the entire DNA is complexed with the lipofectin, provided enough lipofectin is available. It is assumed that the negative charge of the DNA molecule interacts with the positively charged groups of the DOTMA. The lipid: DNA ratio and overall lipid concentrations used in forming these complexes are extremely important for efficient gene transfer and vary with application. Lipofectin has been used to deliver linear DNA, plasmid DNA, and RNA to a variety of cells in culture. Shortly after its introduction,

it was shown that lipofectin could be used to deliver genes *in vivo*. Following intravenous administration of lipofectin-DNA complexes, both the lung and liver showed marked affinity for uptake of these complexes and transgene expression. Injection of these complexes into other tissues has had varying results and, for the most part, are much less efficient than lipofectin-mediated gene transfer into either the lung or the liver.

pH-sensitive, or negatively-charged liposomes, entrap DNA rather than complex with it. Since both the DNA and the lipid are similarly charged, repulsion rather than complex formation occurs. Yet, some DNA does manage to get entrapped within the aqueous interior of these liposomes. In some cases, these liposomes are destabilized by low pH and hence the term pH-sensitive. To date, cationic liposomes have been much more efficient at gene delivery both *in vivo* and *in vitro* than pH-sensitive liposomes. pH-sensitive liposomes have the potential to be much more efficient at *in vivo* DNA delivery than their cationic counterparts and should be able to do so with reduced toxicity and interference from serum protein.

## THE FUTURE OF LIPOSOMAL PREPARATIONS

Liposomal dispersions have proved not only to be innovative and effective cosmetic ingredients, but also to be a very convenient form to work with phosphatidylcholine. In dermatology, they will be used with success for preventing and treating several skin diseases. Complementary formulations are established where liposomal dispersions come up against limiting factors. Table 2 shows liposomal and complementary formulations in a direct comparison.

Table PC-Containing Formulations

Parameter	Liposomes	Nanoparticles	DMS	Conventional emulsions
PC	++	+	Used as additive	(+)
PC hydrogenated	Rarely used	+	++	Rarely used
Lipophilic ingredients	Limited	+	+	+
Hydrophilic ingredients	+	+	+	+
Amphiphilic ingredients	Limited	Limited	+	+
Auxiliary compounds	As few as possible	As few as possible	Rarely used	++
Preparation (usual)	Usual and high pressure homogenizers	High pressure homogenizer	High pressure homogenizer	Phase conversion method
Physical stability	(+)	+	++	++
Chemical stability	Depending on pH	Depending on pH	Depending on pH	Depending on pH
Preservation	Glycols, ethanol	Glycols, ethanol	Glycols, ethanol	Conventional preservatives
Penetration	++	+	+	(+)
Skin protection	- (Unsaturated PC)	(+)	++	(+)
Convenient particle size	100-300 nm	50-200 nm	Not detectable	Usual droplets
Typical cosmetic applications	Antiaging, regeneration	Lotions for sensible skin	Skin protection, sun protection	Versatile
Prevention of skin diseases	++ (e.g., acne)	++ (e.g., encapsulated primrose oil: neurodermatitis)	++ (e.g., neurodermatitis, dehydrated skin)	(+)

*Abbreviations:* DMS, derma membrane structure; PC, phosphatidylcholine

Generally, members of the membrane family like liposomes, nanoparticles, and DMS are

more compatible with the skin structure than usually applied conventional emulsions. "Compatible" means that formulations do not disturb the integrity of the skin lipid bilayers and are not washed out when the skin is cleaned. In the sense of modern strategies of cosmetics, these formulations get by with a minimum of auxiliary compounds, which put only a strain on the skin. Moreover, compatibility means embedding lipids and hydrophilic agents in the horny layer and being in line with the natural situation. Remarkably, phosphatidylcholine need not be applied in high concentrations, because experience shows that formulations are stable at lower amounts. Also, there is a cumulative effect in the horny layer with repeated application of phosphatidylcholine. In many cases liposomes, nanoparticles and DMS are compatible with each other in a sense that they can be used as a modular system. So these formulations are believed to still have a great future in cosmetic science. How far new findings about the importance of the choline moiety of phosphatidylcholine will impact skincare research and development cannot be estimated today.

### **TARGETING CANCER**

Another interesting property of liposomes are their natural ability to target **cancer**. The endothelial wall of all healthy human **blood vessels** are encapsulated by endothelial cells that are bound together by **tight junctions**. These tight junctions stop any large particle in the **blood** from leaking out of the vessel. Tumor vessels do not contain the same level of seal between cells and are diagnostically leaky. This ability is known as the Enhanced Permeability and Retention effect. Liposomes of certain sizes, typically less than 400nm, can rapidly enter tumor sites from the blood, but are kept in the bloodstream by the endothelial wall in healthy tissue vasculature. Anti-cancer drugs such as **Doxorubicin** (Doxil), **Camptothecin** and **Daunorubicin** (Daunoxome) are currently being marketed in liposome delivery systems.

### **CONCLUSION**

The potential use of liposomes in man necessitates the production of sterile, pyrogen free preparations of liposomes which requires specific conditions for their preparation. For use as drug carriers, liposomes should be able to fuse with the arbitrary cells in a spontaneous and controllable manner. One major drawback of liposomal drug delivery system is poor encapsulation of certain drugs in which case the drug is derivised. Application of liposomes medicine include encapsulation of both Lipid and water soluble drugs. Apart from use as drug carrier perhaps the most promising immunological property of liposomes is their cation as adjuvants. Liposomes are less likely to produce undesirable effects as they may be formed from lipids occurring naturally in the body. In addition, liposomes overcome problems inherent with viral vectors - specifically concerns of immunogenicity and replication competent virus contamination. The ability to chemically synthesize a wide variety of liposomes has resulted in a highly adaptable and flexible system capable of gene delivery both in vitro and in vivo. As liposome technology is better understood, it should be possible to produce reagents with improved in vivo gene delivery into specific tissues. Studies involving protein-DNA-liposome complexes are already showing promise in the ability to target DNA delivery into specific cells. Current limitations regarding in vivo

application of liposomes revolve around the low transfection efficiencies and transient gene expression. Also, liposomes display a small degree of cellular toxicity and appear to be inhibited by serum components. The ability to overcome these problems should greatly facilitate their application to a variety of gene delivery mechanisms

## REFERENCES

- 1) Lasic DD. Liposomes and niosomes. In: Rieger MM, Rhein LD, eds. *Surfactants in Cosmetics*. 2d ed. New York: Marcel Dekker, 1997:263-283.
- 2) Wendel A. Lecithins, phospholipids, liposomes in cosmetics, dermatology and in washing and cleansing preparations. Augsburg: Verlag fuer chemische Industrie, 1994.
- 3) Wendel A. Lecithins, phospholipids, liposomes in cosmetics, dermatology and in washing and cleansing preparations Part II. Augsburg: Verlag fuer chemische Industrie, 1997.
- 4) Braun-Falco O, Korting HC, Maibach HI, eds. *Liposome Dermatics*. Berlin: Springer-Verlag, 1992.
- 5) Ghyczy M, Nissen H-P, Biltz H. The treatment of acne vulgaris by phosphatidylcholine from soybeans, with a high content of linoleic acid. *J Appl Cosmetol* 1996; 14:137-145.
- 6) Lautenschlaeger H. Kuehlschmierstoffe und Hautschutz - neue Perspektiven. *Mineraloeltechnik* 1998 (5):1-16.
- 7) *Cosmetic Ingredient Review. Lecithin and Hydrogenated Lecithin*. Washington: The Cosmetic, Toiletry, and Fragrance Association, 1996.
- 8) Lautenschlaeger H. Liposomes in dermatological preparations Part II. *Cosmet Toilet* 1990; 105 (7):63-72.
- 9) Nippon Surfactant Kogyo KK, Japanese Patent 199104364104 (1992).
- 10) Lautenschlaeger, German Patent 4021082 (1990).
- 11) Kutz G. Galenische Charakterisierung ausgewaehlter Hautpflegeprodukte. *Pharmazeutische Zeitung* 1997; 142 (45):4015-4019.
- 12) Wallhaeusser KH. *Praxis der Sterilisation, Desinfektion - Konservierung*. 5th ed. Stuttgart: Georg Thieme Verlag, 1995:43, 394.
- 13) Roeding J. Properties and characterisation of pre-liposome systems. In Braun-Falco O, Korting HC, Maibach HI, eds. *Liposome Dermatics*. Berlin: Springer-Verlag, 1992:110-117.
- 14) Lautenschlaeger, German Patent 4021083 (1990).
- 15) Feingold KR. Permeability barrier homeostasis: Its biochemical basis and regulation. *Cosmet Toilet* 1997; 112 (7):49-59.
- 16) Blusztajn JK. Chorines, a vital amine. *Science* 1998; 281:794-795.
- 17) Torchilin VP. (2006) *Adv Drug Deliv Rev*. 2006 Dec 1;58(14):1532-55
- 18) Kimball's Biology Pages, "Cell Membranes."
- 19) Stryer S. (1981) *Biochemistry*, 213
- 20) Barani, H. & Montazer, M. (2008) A Review on Applications of Liposomes in Textile Processing. *Journal of Liposome Research*, 18 (3) 249-262

- 21) Meure, L.A., Knott, R., Foster, N.R., Dehghani, F. (2009) The Depressurization of an Expanded Solution into Aqueous Media for the Bulk Production of Liposomes. *Langmuir*, 25, 326-337
- 22) Gomez-Hens, A., Fernandez-Romero, J.M. (2006). Analytical methods for the control of liposomal delivery systems. *Trends Anal Chem* 25:167–178.
- 23) Mozafari, M.R., Johnson, C., Hatziantoniou, S. & Demetzos, C. (2008) Nanoliposomes and their applications in food nanotechnology. *Journal of Liposome Research*. 18 (4), 309-327.
- 24) Mozafari, M.R. & Mortazavi, S.M. (2005) *Nanoliposomes: From Fundamentals to Recent Developments*. Trafford Publishing Ltd, Oxford, UK.
- 25) Colas, J.C., Shi, W.L., Rao, V.S.N.M., Omri, A., Mozafari, M.R., Singh, H. (2007) Microscopical investigations of nisin-loaded nanoliposomes prepared by Mozafari method and their bacterial targeting. *Micron* 38:841–847.