QuSomes®

Novel Liposomes

Brian C. Keller

Handbook of Cosmetic Science and Technology, 2nd Edition
Edited by A. O. Barel, M. Paye and H. I. Maibach

Introduction

Liposomes are microscopic lipid vesicles. They are found naturally in human milk and are dynamic entities on many levels. Liposomes provide a valuable tool for dermatological formulation strategy because of there various proven benefits. Topically applied drug products using liposome technology were first commercialized in Switzerland when Janssen-Cilag launched Peveryl Lipogel in 1988.

A decade later the first topical liposome drug product introduced in the US was ELA-Max, developed and commercialized by Ferndale Laboratories, Inc. Other pharmaceutical companies have used liposome encapsulation in topical preparations for functional purposes including, improving percutaneous penetration, increasing therapeutic efficacy, and targeting specific sites in the skin.

Table 1 shows examples of several dermatological products that have become commercial successes.

Product Name	Drug	Use
Peveryl Lipogel	Econazole	Mycotic fungal infections
Hametum Crème	Hamamelis	Inflammation
Heparplus EmGel	Heparin	Anticoagulation
L.M.X.	Lidocaine	Dermal anesthesia
Miltrex	Miltefosine	Breast cancer tumors with cutaneous metastases

Table1

Since their introduction into cosmetics by Christian Dior in Capture® (1987), liposomes have become a staple for some and an enigma for others. Although barriers to use have been both spurious and real, ranging from cost, difficulty of formulation, scale-up problems, stability, and a general misunderstanding of the basis for which liposomes have the most value, they have made their way into numerous skin care products over the years and, by all counts, have passed the tests of time and utility.

Conventional liposomes, as we have seen in previous chapters, are made from purified phospholipids, mostly DPPC alone or DPPC plus cholesterol or another sterol. The ability of these amphiphilic lipids to orient themselves in the polar solvent in which they are suspended, usually water, into bilayer sheets sets the stage for liposome formation. The resultant colloidal aggregate, with the hydrophilic heads positioned outward and the hydrophobic tails inward, is the bilayer which can be assembled into vesicles, but only with the addition of outside energy. Fig.1

Just as it takes energy from your hands and fingers to physically bend a page of this book into a tube, energy is required to bend these bilayer sheets of phospholipids into vesicles. Contrary to popular belief these lipids do not form vesicles spontaneously and there is confusion between self-forming and spontaneous vesiculation. Phospholipids bilayers will self-close to form liposomes only when outside energy is added to the system. The source of external energy is process energy in the form of sonication, high-speed vortexing, high-pressure homogenization, and high shear fluid processing.

DPPC = dipalmitoylphosphatidylcholine

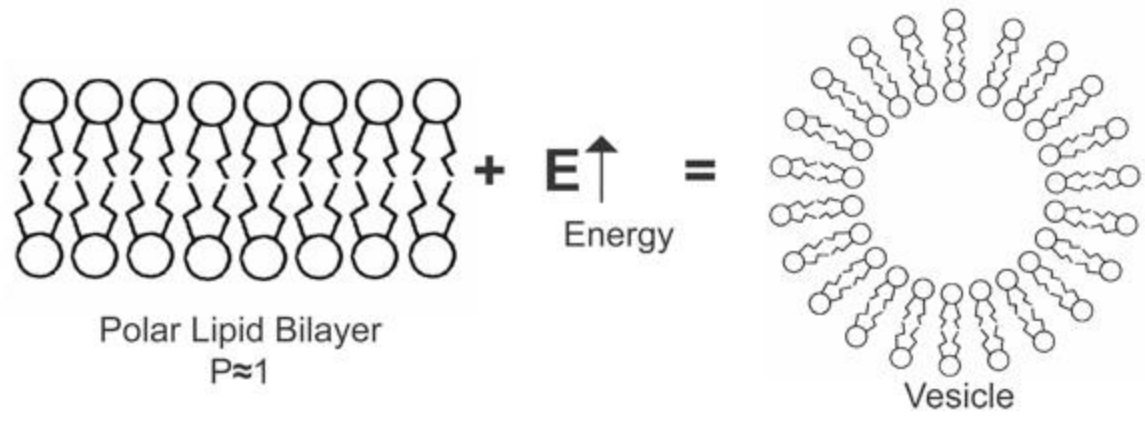


Figure1

Important derivatives of conventional liposomes are non-ionic surfactant liposomes and non-phospholipid vesicular systems, both are sometimes referred to as synthetic surfactant vesicles. These vesicles are

composed of single and double-chain synthetic surfactants with non-ionic polar heads. They form bilayer vesicles with the structure of the lipophilic tail controlling membrane properties.

French and Japanese inventions employing these types of technologies surfaced in the patent literature as early as 1980 and were incorporated into cosmetic products by the end of the decade. Niosomes were introduced into Lancôme and other L'Oreal brands in the late 1980's, and others followed.

Because topically applied products containing cosmetically active substances have evolved into "cosmeceuticals" or "cosmedics" a shift in formulation goals from an emphasis on organoleptics to skillfully coupling organoleptics and therapeutics, has become the priority. It is well known that working with phospholipids requires a great deal of processing and formulation skill to produce a finished product that is pleasing and elegant, yet maintains liposome integrity and ingredient encapsulation.

Colloidal Organization

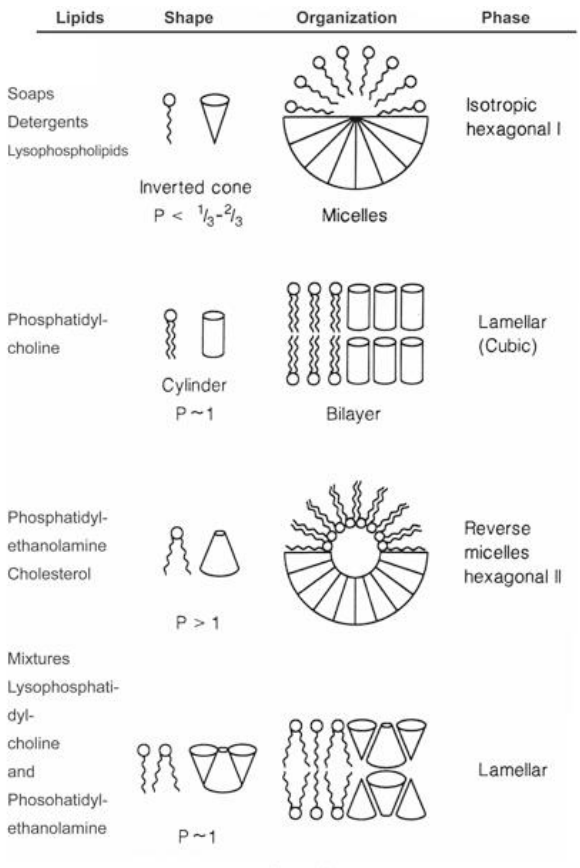


Figure2

A fundamental property of lipids is their geometric shape. This spatial configuration is determined by the packing parameter, P, of the lipid. Packing parameters are relative measure of a given lipid, determined by the size of the polar head and the length of the lipid hydrocarbon chain.

Although empirical, determining the packing parameter, P, gives us a very useful guide to the aggregate shape of amphiphilic lipids.

P is defined as: $P = v / al$

where v is the volume of the molecule, a is its polar head group area and l is the length of the hydrocarbon chain.

For single chain lipids, detergents, and surfactants the polar head is proportionately larger than the non-polar tail and $P<0.75$ so they organize into micelles. When $P\sim1$ the lipid is best suited for forming liposomes and when $P>1$, polar heads are smaller relative to the non-polar chain and inverse micelles form. Some interesting lipids used in cosmetic formulating, their packing parameter range, and their shapes and ultimate organization are shown in Fig. 2.

※（micelle）: The molecules of lipophilic (affinity for oils) and (affinity for water) substances that when in water, the hydrophilic substance will gather inside into a spherical. For example, the phospholipid bilayer membrane is also a micelle.

A Model of Geometric Packing of various amphiphilic lipids into colloidal aggregates.
(Adapted from D.D. Lasic, Liposomes; From Physics to Applications, Elsevier, 1993 pp 51)
A Model of Geometric Packing of various amphiphilic lipids into colloidal aggregates.

To form a bilayer, lipid head groups and hydrocarbon chains must organize themselves so that the radius of curvature results in a vesicle. If the hydrocarbon chains are too small relative to the head group, the radius of curvature will be too large and micelles will form. If the hydrocarbon chains are too large relative to the head groups, the radius of curvature will be of the opposite sign and an inverse micelle will form.

New Spontaneous, Thermodynamically Stable Liposomes (STS)

Most, if not all, known liposome suspensions are not thermodynamically stable. Instead, the liposomes are kinetically trapped into higher energy states by the energy used in their formation. Again, energy may

be provided as heat, sonication extrusion or homogenization. Since every high-energy state tries to lower its free energy, known liposomes formulations experience problems with aggregation, fusion, sedimentation and leakage of liposome associate material. However, when well prepared the liposomes and final product can remain stable for years provide they are stored properly and not able to chemically degrade. A thermodynamically stable liposome suspension, which could avoid some of these difficult to overcome problems, is desirable.

Notwithstanding their success, and the progress that has been made in expanding the use of conventional liposomes there are various disadvantages that prompted the search for novel liposome-forming lipids.

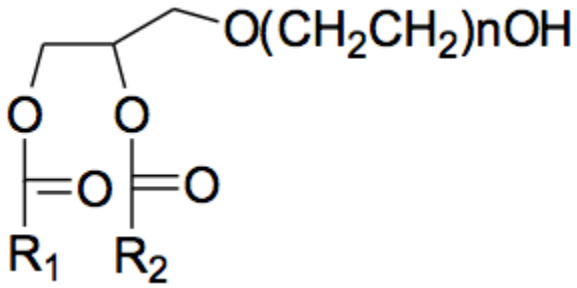


Figure3

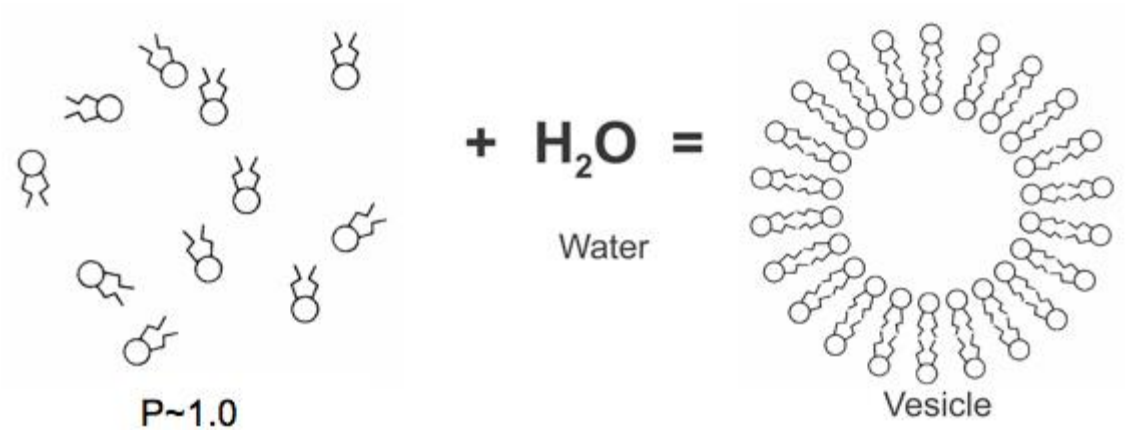
A group of novel lipids, which have fundamental properties that allow thermodynamically stable liposomes to form easily and cost effectively is presented in this chapter. These lipids have the general structure in Fig. 3.

This structure has similarities to phospholipids but the fundamental differences are essential to the formation of thermodynamically stable liposomes. The essential hydrophilic head group is a PEG chain, which can range in size anywhere from 8-45 subunits, or 300 Daltons to 5000 Daltons, n=8-45. PEGylated lipids have been incorporated into the membranes of some liposome compositions (i.e.. Stealth®) made from phospholipid liposomes which have a phosphate head group. Glycerol, a three-carbon chain provides the backbone and the two hydrocarbon chains R1 and R2 can vary in length, C= 8-25. The size of the PEG head relative to the length of the to the hydrophilic chain is the fundamental property that allows liposome formation when added to water with the addition of little or no energy. When mixed with water the liposomes form spontaneously and remain in the lowest free energy state.

※ PEG = polyethylene glycol: nonionic surface-active agent
※ Dalton = Dalton: a unit of measurement for the mass of atoms and molecules. 1Dalton = 1/1000nm

By using these lipid molecules little or no energy is needed when mixing the lipid and an aqueous solution to form liposomes. When mixed with water the lipid molecules disperse and self assemble into vesicles as the system settles into its natural low free energy state. The resultant suspension is a thermodynamically stable system containing multilamellar lipid vesicles.

Figure 4 depicts the formation of vesicles when a lipid is mixed with water at the melting point of the lipid.



＜Figure4＞

Working with these lipids in cosmetic formulations is uncomplicated and has made liposome preparations on both small and large-scale considerably less labor intensive. Because the melting temperatures of these lipids are low and, heating them to temperatures near 75oC-80oC is not denaturing, they display excellent versatility in a variety of dermatological formulations.

Lipid	**Melting Point**	**Spontaneous Liposomes at Melting temperature**
PEG-12 Glyceryl dioleate	Fluid @ 25oC	Yes
PEG-12 Glyceryl dimyristate	Fluid @25oC	Yes
PEG-23 Glyceryl palmitate	31.2oC	Yes

PEG-12 Glyceryl disterate	40.0oC	Yes
PEG-23 Glyceryl disterate	39.8oC	Yes

No organic solvents are necessary to dissolve the lipid prior to incorporating them into a formulation and simple mixing with conventional processing equipment is all that is required for liposome formation.

Table2 gives examples of the lipids that have been used in cosmetic formulations.

Table 2

The liposomes formed using these lipids have a size range of 750-1500Å with a mean bilayer thickness of around 40 Å. They appear uniform in size upon microscopic analysis and tend to be perfectly round as opposed to oval or anomalous shaped that some liposomes assume. The most likely explanation for this is the purity and uniformity of these synthetic lipids.

※ Å = Angstrom:A very small unit of size that represents the length of molecules and atoms. 1Å = 10-10m = 0.1nm

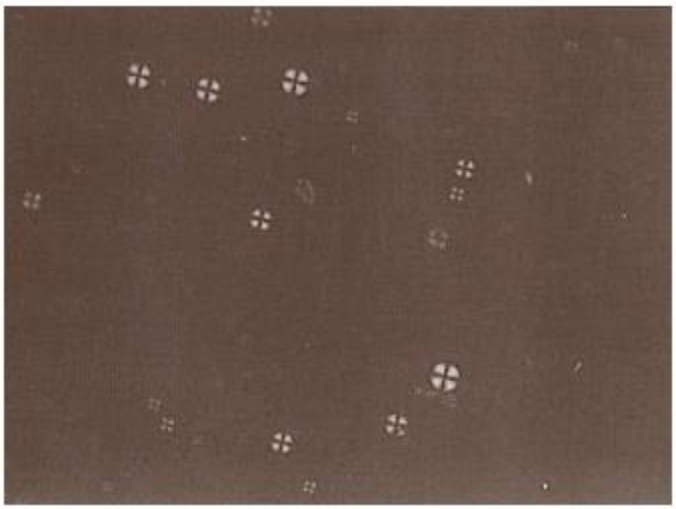


Figure5

The ideal way to view liposomes in a cosmetic laboratory and to validate the presence or absence of liposomes in a formulation is through an optical light microscope with polarized light. Figure 5 shows a

photograph of several multilamellar liposomes formed from PEG-12 GDO and cholesterol. These are the perfectly round, white objects with a demarcating cross over the center. They are robust and have been stable under stressors; 40oC and 50oC for 90 days and during freeze thaw cycling. In cosmetic formulations these liposomes are able to withstand higher ionic surfactant levels and a broader pH range, from 2.5 to 9.18 compared to conventional liposomes. Additionally PEG-12 GDO and PEG-12 GDM have been used as an ingredient solubilizer with great success, dissolving difficult ingredients including cholesterol.

Because of their thermodynamic stability they will not fuse, aggregate or destabilize trying to get to the lowest energy state.

Using these non-phospholipid lipids in a cosmetic preparation makes the product less susceptible to microbial over growth due to the absence of phosphate, an essential microbial nutrient. Contributing to their overall stability, the absence of this type of head group prevents oxidation of the amino group in the polar head of some phospholipids that causes a fishy smell.

Toxicity of phospholipids has been reviewed thoroughly and they are essentially non-toxic. An extensive test on the toxicity of the lipids in table 2 was recently conducted and concluded that they are nonirritating and non-toxic. It has also been observed them that these lipids are well tolerated with all skin phenotypes in many skin care products.

Utility

Cosmetic products incorporating conventional liposomes have become ubiquitous. At times, the practical significance of liposomes has been a secondary consideration. Taking precedence is the label claim and marketing propaganda that publicizes the presence of liposomes. The microscopic system of these vesicles with the entrapped freight in a cosmetically elegant vehicle has a consumer benefit far beyond the limits of cosmetic labeling, however, meaningful therapeutic amounts of liposomes relative to the ingredient concentrations need be present to achieve payback.

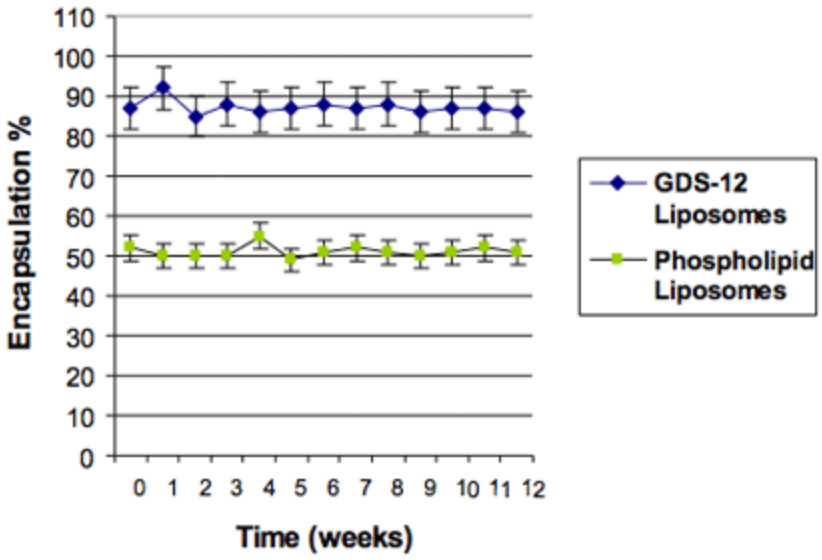
Encapsulation Efficiency

The tremendous advantages of liposomes in dermatological products are; improved ingredient solubilization, microencapsulation of the ingredient for improved ingredient stability in the cosmetic system, enhanced skin penetration resulting in longer residence time of the ingredient in the skin,

sustained release of the ingredient for prolonged effect, the beneficial effects of the lipid molecule itself by providing lipid material to the skin to improve barrier function and moisture.

In considering the constructs of a cosmetic formulation the art of commingling liquids, solids, oils, water, crystals, cellulosics, and pastes, among others, is required. As mentioned earlier in this chapter, although the therapeutic value of a particular product has become a focus, overlooking the effects of touch, feel and smell of a product would be a mistake. Therefore, great time and effort are expended on consideration of the concentrations of each ingredient.

The potency of cosmetically active ingredients is low and therefore the usage levels are in the 1-5% range, generally. In addition, most formulas contain more than one ingredient that has benefits to the skin. To achieve liposomal encapsulation of the proposed active ingredients the physico-chemical properties of the materials must be known. Lipophilic ingredients have a higher encapsulation rate and are entrapped in the acyl chains of the lipid whereas hydrophilic compounds reside in the water layer and have a lower degree of encapsulation.



<Figure6>

The type of lipid, the compounds being encapsulated, the charge of the lipid and the active, all contribute to the encapsulation efficiency of the active ingredients. A simple way to empirically begin formulation and potentially achieve maximum encapsulation is start with a lipid-to-active molar ratio of 2:1. For hydrophilic ingredients a higher relative amount may be necessary.

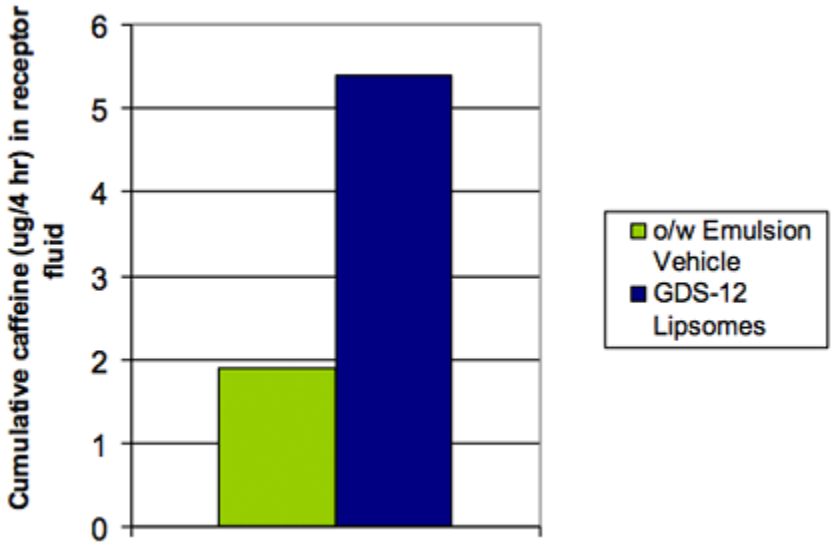
A comparative study of two liposomal formulations with equal molar concentrations of lipids found that novel PEG lipid that forms STS liposomes had greater encapsulation efficiency then the conventional phospholipid liposomes. (Fig. 6)

※ Acyl chain = Tail portion of the lipophilic group of lipid molecules

※ Molarity = One way to display the concentration of a solution. It is expressed as the amount of a substance of solute per unit volume of a solution.

Skin Penetration

One of the chief reasons to use delivery vehicles, even in cosmetic products, is their ability to promote skin penetration. Delivering active ingredients into layers of the skin below the stratum corneum is desirable for many cosmetic purposes including hydration, improving barrier function, scavenging for oxy-radicals to prevent aging, fortifying the epidermis and dermis with nutrients and vitamins such as vitamin A and it's congegers, the tocopherols, and vitamin C, and the delivery of other dermally active ingredients for other purported benefits.



<Figure7>

Dermal delivery of liposome encapsulated biologically active ingredients for cosmetic and drug applications was well underway by the mid 1990's. The ability of vesicles to promote cutaneous permeation and sequester active ingredients in the skin is discussed in numerous studies. This has

become an important benefit of liposomal encapsulation; the use of liposomes not only accelerates the skin permeation of the active but also accelerates and possibly enhances the ‘therapeutic’ effect.

A skin penetration study conducted using STS liposome encapsulated caffeine on human cadaver skin and a continuous flow-through diffusion cell system gave interesting results.

Radio labeled caffeine encapsulated into a liposome made from PEG-12 GDS was compared to a non-encapsulated oil-water emulsion containing the same concentration of the [C14]-caffeine. After eight hours more caffeine penetrated the skin and diffused into the receptor fluid. (Figure 7)

Cosmetic Applications

Applications for conventional liposomes continue to show promising outcomes in therapeutic as well as pharmaceutical areas. Treatments for skin discoloration problems, which include lightening products as well as vitiligo therapy, have been encouraging. Stabilizing retinol with liposomes and quenching ingredients has potential to solve a use-limiting problem with a valuable cosmetic ingredient. Another more intrepid cosmeceutical use of liposomes has been in targeting hair follicles to deliver hair-growth stimulating molecules and potentially DNA. The ease of use, cost, stability and vast utility of STS liposomes has created another viable delivery candidate of these applications as well.

Basic research has established spontaneous, thermodynamically stable (STS) liposomes in cosmetic science as a viable delivery system. These lipids, principally PEG-12 GDS and PEG-12 GDO have been used employing this technology in products for therapeutic uses like acne, dry skin, inflammation, skin lightening, and photo-wrinkles. Laboratory testing has demonstrated the pharmaceutical capabilities of ingredient solubilization, ingredient stability and sequestering to be superior to currently available delivery vehicles. STS liposomes also offer options for the delivery of peptides and growth factors, hydrolyzed wheat proteins, glycolic acids, retinal, co-factors and co-enzymes.

Conclusion

Vesicular delivery systems in topical formulations have been available to cosmetic scientists since the late 1980’s when conventional phospholipid and, to a limited extent due to patent protection, non-phospholipid vesicles entered this field. During the past 20 years many success stories have unfolded with topically applied liposomes products. There is continued focus on the internal ‘operating systems’ of cosmetic formulations where liposomes are becoming more used and appreciated and consequently seem to be the system of choice.

The ideal cosmeceutical formulation should contain proper concentrations of solubilize “active” ingredients encapsulated in a vesicle and incorporated in a visually appealing cream, gel, lotion or serum that contains an engaging fragrance which effortlessly rubs into the skin. Laboratory formulation and large-scale manufacturing should not require unconventional processing or expensive equipment to make the resultant product commercial. A novel delivery system that has can help achieve this idyllic definition has been discussed. Presently it has over come the barriers of entry to liposome use, and adds another tool to the dermatological formulators toolbox.