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VESOSOMAL DRUG DELIVERY IN LIPOSOMES: A REVIEW

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ABSTRACT

Liposomal drug delivery can be put forth as the cynosure for the release kinetics of lipophillic drugs that require compartmental models in its therapeutics and triggers. The localization of the drug at the site of action, rate of achieving the therapeutic index and Circulation lifetime are the key parameters for a liposome. Lately, their arises a need for a multi-compartment structure consisting of drug-loaded liposomes encapsulated within another bilayer, is a promising drug carrier with better retention and stability. A vesosome contemplates a large lipid bilayer enclosing many smaller liposomes, serving as a support for the customization of separate environments for multiple therapeutics and release triggers, highlighting the vesosomes potential as a single site, single dose, and multiple component drug treatment. The permeation rate, membrane charge, specific recognition particles, steric stabilizers, membrane rigidity, and phase transition temperatures all play a role in optimization of vesicles for particular delivery applications. The vesosomes are optimized on the basis of phase behavior exhibited by homogeneous lipid mixtures. A variety of microscopy techniques, including freeze-fracture and cryo-transmission electron microscopy as well as fluorescence and confocal microscopy are used for characterization of these lipophillic, eukaryotic cell like species. The present paper tries to tap another vesicular drug delivery comport such that release and bioavailability of liposomes is taken to another level.

Keywords: Liposomal Drug Delivery, Vesosomes, Drug-Loaded Liposomes Encapsulated within another Bilayer, Eukaryotic Cell like Species

INTRODUCTION

A Vesosome is a more or less heterogeneous, aggregated, a large lipid bi-layer enclosing multiple, smaller liposomes that offers a second barrier of protection for interior components and can also serve as the anchor for active targeting components. Expecting a dosage form to be as versatile or as variegated in diffusion facets as those of the human conundrum is asking too much, but vesosomes perhaps strike an optimal balance that internal permits customization of separate environments for multiple therapeutics and release triggers. [1], [2]

An ideal dosage form epitomizes and comprehends:

1. The ability to localize at the site of disease.
2. Deliver contents at a rate appropriate for maximum therapeutic benefit and retain its contents over this timeframe.
3. Loading capacity and circulation lifetime may be more or less desirable, based on the type of treatment.

Unilamellar vesicles or "liposomes" are commonly used as simple cell models and as drug delivery vehicles. Encapsulating unilamellar liposomes within a second bilayer to form multi-compartment "vesosomes" extends contents retention by 2 orders of magnitude by preventing enzymes and/or proteins from reaching the interior bilayers. The multi-compartment structure of the vesosome can also allow for independent optimization of the interior compartments and exterior bilayer; however, just the bilayer-within-a-bilayer structure of the vesosome is

sufficient to increase drug retention from minutes to hours. [3], [4]

Liposomes are spherical assemblies of lipids separating an inside aqueous compartment from the outside aqueous environment, with a finite solute permeability through their lipid bilayer membrane. A major limitation of unilamellar liposomes in these applications has been premature contents release in physiological environments. This premature release is likely due to enzyme degradation or protein insertion into the liposome membrane, which significantly increases the bilayer permeability.

a) They have shown great potential in drug delivery applications, where they act as circulating reservoirs of drugs, at higher concentrations than the toxic dose of the free drug, thereby reducing toxic side effects. [5]
b) They also can be decorated with a variety of agents, for example to increase biocompatibility, to specifically target a diseased site, or to promote adhesion / fusion. Liposomes for drug delivery can be made to remain in the bloodstream for as much as 24 hours, allowing them to accumulate in growing, leaky tumors: this process is known as passive targeting. [6],[7]
c) However the downfall and maybe one reason only a handful of liposomal formulations are approved for cancer therapy, is that only a fraction of the encapsulated drug actually reaches the diseased site. [8]

This is mainly due to the numerous interactions of the liposome membrane with blood, either with enzymes degrading the carrier shell, or proteins inserting into the bilayers and making the liposomes leaky, hereby losing their contents rapidly. The drug just does not circulate as long as the carrier. [9], [10]

1. The different membranes allow a distribution of the tasks an efficient drug delivery vehicle has to achieve. For this purpose, we need to controllably form large open bilayers, and to close them at will: this is achieved by exploiting the unique phase behavior of various symmetrical saturated phospholipids in the presence of ethanol.
2. The latter induces interdigitation of the bilayer in its gel phase and subsequent fusion of small liposomes to form extended open bilayer sheets.
3. Removal of the excess ethanol until heated above the lipid chain melting transition temperature, which induces closure of the bilayers around whatever is in the liposomal suspension.

In trying to improve the design of this type of drug delivery vehicle, we developed a method to efficiently encapsulate drug loaded liposomes within another bilayer – potentially of a different composition – to form a structure that we call Vesosome.

Hence, these multi-compartment vesosomes offer a versatile means for designing delivery systems because they can distribute often incompatible attributes among the various membranes. Interior vesicle sizes range from 20-500 nm and vesosome sizes range from about 0.1 micron to greater than 1.0 micron. [11]

Formulation, Designing and Evaluation of Vesosomes: The Eukaryotic Cell Mimic

Encapsulating vesicles or other chemically or biologically sensitive materials within a bilayer is complicated by the need to protect the interior contents while the exterior membrane is forming, which eliminates most conventional vesicle-forming processes. [12]

a) Steps in Vesosomal Production[13-15]

Many saturated phospholipids including phosphatidylcholines, phosphatidylglycerols, and phosphatidylserines form an Lβ' phase in water at temperatures below the main or chain-melting phase transition temperature, Tm.

1. In the Lβ' phase, the crystalline hydrocarbon chains of the lipids are tilted relative to the normal to the bilayer. The crystalline chains have a smaller projected area per molecule than the hydrated polar groups of the lipids at the bilayer-water interface; hence the chains must tilt to maintain close packing.
2. Above Tm, the chains melt and take up more area due to the greater conformational disorder of the chains on average, the chains are normal to the bilayer in the liquid crystalline Lα phase.
3. Adding ethanol or other alcohols, glycerol, or propylene glycol to aqueous dispersions of dipalmitoylphosphatidylcholine (DPPC) or other Lβ' phase dispersions of saturated phospholipids held below Tm leads to intercalation of these molecules in the lipid polar groups, causing the polar groups to swell and take up even more interfacial area.

4. These polar molecules effectively displace water molecules in the polar lipid heads, but do not penetrate deeply into the bilayer.
5. At some point, increasing the tilt of the hydrocarbon chains is insufficient to allow for efficient hydrocarbon packing while matching the increased interfacial area of the swollen polar groups. Instead of tilting, the hydrocarbon chains of the lipids interdigitate between each other, decreasing the thickness of the bilayer.
6. The amount of ethanol necessary to drive interdigitation is a function of both bilayer curvature and bilayer composition.
7. Interdigitation causes the bilayer to become much more rigid as the two monolayers in the bilayer can no longer glide over each other while bending. The bending rigidity of the bilayer is sufficiently high that the bilayers can no longer form stable, small vesicles; any such structures fuse to form bilayer sheets.
8. The energy of the exposed bilayer edges of the sheets is no longer sufficient to drive the rigid sheets into closed liposome structures as the energy costs of bending the interdigitated bilayers is too great.

b) LOADING OF THE DOSAGE FORM

Efficient loading of weakly acid or basic drugs has also been well established for conventional unilamellar liposomes using imposed pH gradients. In the case of any drug that is loaded in the vesicle, additional ions are added to further stabilize the drug (by precipitation or Complexation) within the liposome. [16]

Loading is based on the observation that the neutral form of drug is often several orders of magnitude more permeable through a lipid membrane than the charged form of the drug. A pH gradient is constructed such that the interior of the liposome is at a lower pH (for weakly basic drugs), usually by incorporating sodium citrate or ammonium sulfate in the liposome interior, followed by neutralizing the exterior solution. In the limit that the charged form of the drug is essentially impermeable, the encapsulated drug concentration inside the liposome, relative to the external drug concentration, is proportional to the ratio of the proton concentration inside and outside the liposome. [17], [18] To make the interior vesicles to be loaded into the vesosomes, lipid mixtures (DPPC, DPPC plus cholesterol, distearoyl phospha tidylcholine (DSPC) plus cholesterol, DSPC plus stearylamine and cholesterol, DPPC plus DPPG, egg phosphatidylcholines) were evaporated to dryness in glass ampoules to form thin films of lipids. [19]
These lipid films were hydrated with buffer at temperatures above the Tm of the lipids (45° C for DPPC, 65° C for DSPC). The hydrated lipids were then put through a series of 8 freeze cycles followed by a series of 8-10 high pressure (Approximately 400 psi dry nitrogen) extrusion cycles at 60° C through a Nucleopore filter of pore size 0.05 µm. Vesicles for aggregation were prepared as above, but with a 0.16 mol% biotin-X conjugated to dipalmitoylphosphatidylethanolamine. A stock solution of avidin was prepared in the same buffer at a concentration of 1 mg/ml (1.7 x 10-5 M/l). Aggregation was induced b adding an aliquot of a vesicle stock solution to sufficient avidin solution to form mixtures of the appropriate vesicle concentration at a ratio of receptor to biotin-X DPPE of 1:8. [20]

c) ENCAPSULATION[21-23]

Encapsulation was typically carried out by adding the free vesicles or vesicle aggregates to the pelleted sheets after removing the residual ethanol. The mixture was briefly vortexed and then allowed to heat in a 46° C water bath while being gently stirred. The interdigitated sheets\ spontaneously closed to form vesosomes, encapsulating the contents of the solution. Separation of vesosomes from excess vesicles was carried out by low speed centrifugation in a desktop centrifuge (~ 2000 rpm). Samples were prepared by freeze-fracture replication for transmission electron microscopy (TEM) according to standard procedures. The interdigitated phase is sufficiently robust that small concentrations of a variety of polymers, ligands, etc. can be incorporated in the encapsulating membrane without disrupting the encapsulation process, thereby making stearic stabilization or specific recognition simple to incorporate.

d) VESOSOME STABILITY [24]

The stability of the vesosome structure against fusion of the interior vesicles is important to retain any advantages inherent to the vesosome. Freeze-fracture shows no apparent difference in vesosomes after month of storage at 4° C. The interior vesicles were well distributed inside the vesosome and there was no indication of the interior vesicles leaking out of or fusing with the encapsulating membrane. To quantify the stability of the interior vesicles against fusion, a pyrene-DPPE fluorescence assay was used. At sufficient pyrene-DPPE concentration in a bilayer (> 2 mole%), pyrene-DPPE excited state dimmers (excimers), which have an emission at 475 nm for an excitation at 340 nm, are formed. The concentration

of pyrene-DPPE in the bilayer is diluted by fusion with other bilayers with no pyrene-DPPE, the emission maxima moves to that of the pyrene-DPPE monomer, at about 377 nm.

e) IDEAL CHARACTERISTICS OF A VESOSOME[26-28]

1. The vesosome retains all of the essential features of conventional unilamellar liposomes including ease of manufacture, the possibility of extended circulation times and passive targeting to tumors or inflammation sites due to small (<250 nm) size and steric stabilization with PEG-lipids, and the ability to efficiently load weakly basic drugs with pH gradients. [29]
2. The vesosome also provides several important advantages over unilamellar liposomes. The interior bilayers can be of different composition from each other and from the exterior membrane. [30]
3. The interior vesicles bilayers can incorporate charged lipids or be decorated with other lipids that might lead to rapid aggregation if directly exposed to serum. The vesosome may contain different drugs to be delivered simultaneously in well-defined ratios. [31]
4. The vesosome can provide a significantly different release profile that can minimize spikes in drug release in comparison to unilamellar liposomes. [32]
5. Perhaps most important, the nested structure provides a significant barrier to degradation by lipolytic enzymes and other components of serum that might lead to premature release *in vivo*. [33]
6. The different membranes allow a distribution of the tasks an efficient drug delivery vehicle has to achieve. For this purpose, we need to controllably form large open bilayers, and to close them at will: this is achieved by exploiting the unique phase behavior of various symmetrical saturated phospholipids in the presence of ethanol. [34]

APPLICATIONS AND RECENT ADVANCES

Vesosomes being a complex structure have an infantile role to play in drug release and kinetics, but with the use of modern equipments and technology these are identified as possible solutions to various drug-release obstacles and hurdles in their way. Certain examples on which the vesosomes have being applied include:

DNA ENCAPSULATION[35-36]: DNA contained within or condensed by cationic liposomes penetrates anionic cell membranes relatively easily in culture. The transfection efficiency of cationic lipoplexes is greater than DNA delivered by other lipids but considerably lower than DNA delivered in a viral envelope. Additionally, poor reproducibility of transfection may be caused by variability of the structure and size of the liposome-DNA complexes. *In vivo*, the charged complexes are toxic and have immunoadjuvant activity prior to their rapid clearance from circulation by nonspecific interactions with blood proteins.

SERUM RELEASE [37-39]: The Vesosomal concept of release helps us understand that the increase in permeability due to pore and defects will be greatest for the charged molecules that have the lowest solubility in the hydrophobic interior of the bilayer.

Uncharged molecules may permeate via the solubility mechanism, while charged molecules may switch from solubility to pore transport depending on the density of pores relative to the bilayer dielectric properties. From the "solubility" model it is difficult to reconcile the enormous change in the release of carboxyfluorescein, vincristine, etc. from liposome carriers when serum is used instead of buffer as the external medium. No major differences are expected on changing isotonic saline for serum.

DRUG DELIVERY [41-44]: Various models of drug release for Ciprofloxacin, Vincristine, CF-Dyes (Carboxyfluorescein) have shown:

Nano-Carriers and Drug Targetting [43-44]: The minimum size for a nanocarrier is dictated by the kidney filtration cut-off size and is around 5 nm. However, the EPR effect varies from tumor to tumor, especially in solid tumors. Due to the poor perfusion out of the leaky blood vessels of the growing tumor, drugs penetrate only a few cell diameters into the extra vascular tumor tissue, resulting in a heterogeneous distribution of therapeutic agents. Controlling the rate of drug release from these nanocarrier formulations like the vesosomes may also provide improved penetration in the face of high interstitial pressure and convective flow Dysfunctional lymphatic drainage causes.

DISCUSSIONS

An optimal drug delivery vehicle should circulate in the body long enough to reach the site of illness or disease and also localize itself at the desired site to consequently deliver its contents at a rate appropriate for maximum therapeutic benefit. It should also possess a large drug loading capacity and retain its contents over the course of treatment. While liposomal systems have experienced success with extending circulation, content retention and controlled release remain problematic.

1. The vesosome - a large lipid bilayer enclosing many smaller liposomes - is the most suitable candidate for addressing these issues. The external lipid bilayer offers a second barrier of protection for interior components and also serves as the anchor for active targeting components.
2. Furthermore, internal compartmentalization permits customization of separate environments for multiple therapeutics and release triggers, highlighting the vesosomes potential as a single site, single dose, and multiple component drug treatment.
3. To assess the viability of the vesosome as a drug carrier, its in vivo lifetime and bio distribution was examined in live animals. Our work examines how these properties are affected by lipid composition and the addition of other functional components, including ones for controlled release and active targeting.
4. The vesosome preparations take advantage of the interdigitated phase of saturated lipids, which causes lipid bilayers to form flat, open sheets at low temperature, that close to form large unilamellar vesicles at higher temperatures. During this closure, the interdigitated sheets encapsulate other lipid vesicles or colloidal particles to form and thereby become the outer membrane of the vesosome.
5. The vesosome structure can take full advantage of the 40 years of progress in liposome development including steric stabilization, pH loading of drugs, and intrinsic biocompatibility. However, the multiple compartments of the vesosome give better protection to the interior contents in serum, leading to extended release of model compounds in comparison to unilamellar liposomes.
6. Vesicles of lipid bilayers are useful structures for drug delivery. The permeation rate, membrane charge, specific recognition particles, steric stabilizers, membrane rigidity, and phase transition temperatures all play a role in optimization of vesicles for particular delivery applications.

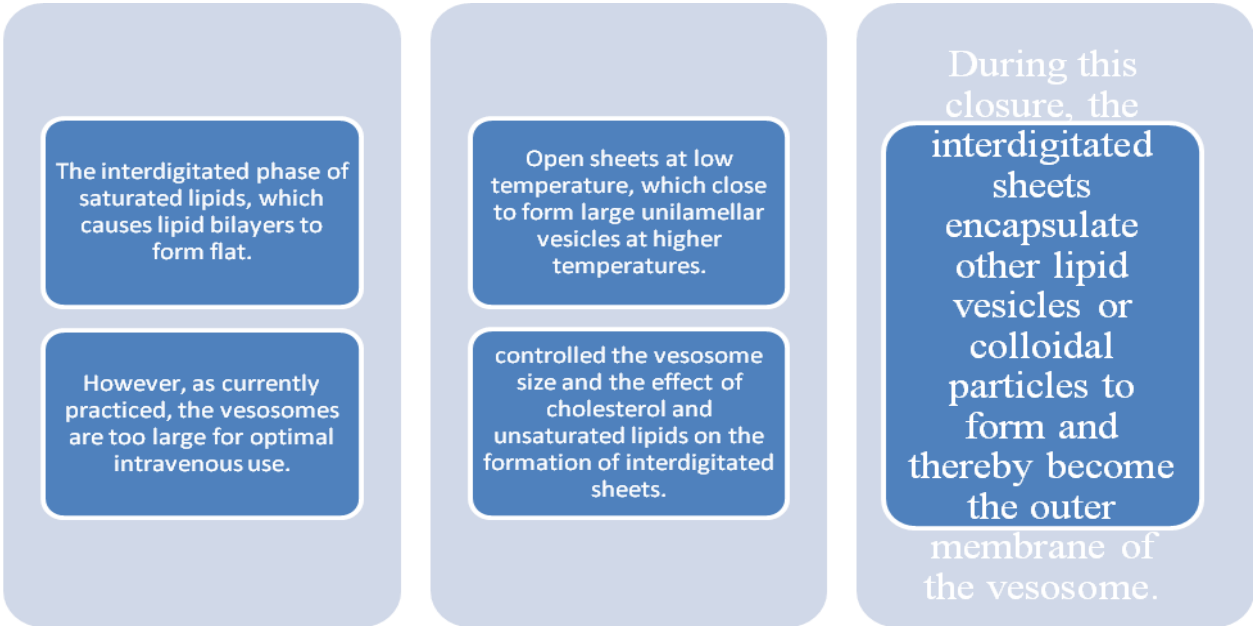

Figure 1: Comprehended advantages of Vesosomal drug delivery [9], [10]

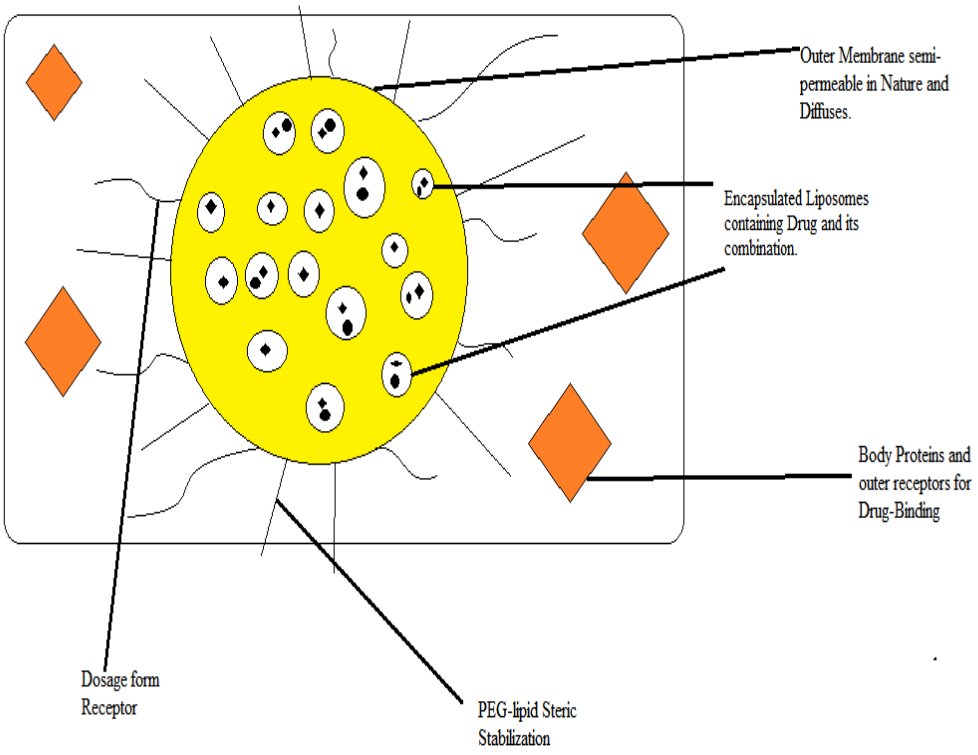

Figure 2: An ideal Vesosomal Liposome [1-10]

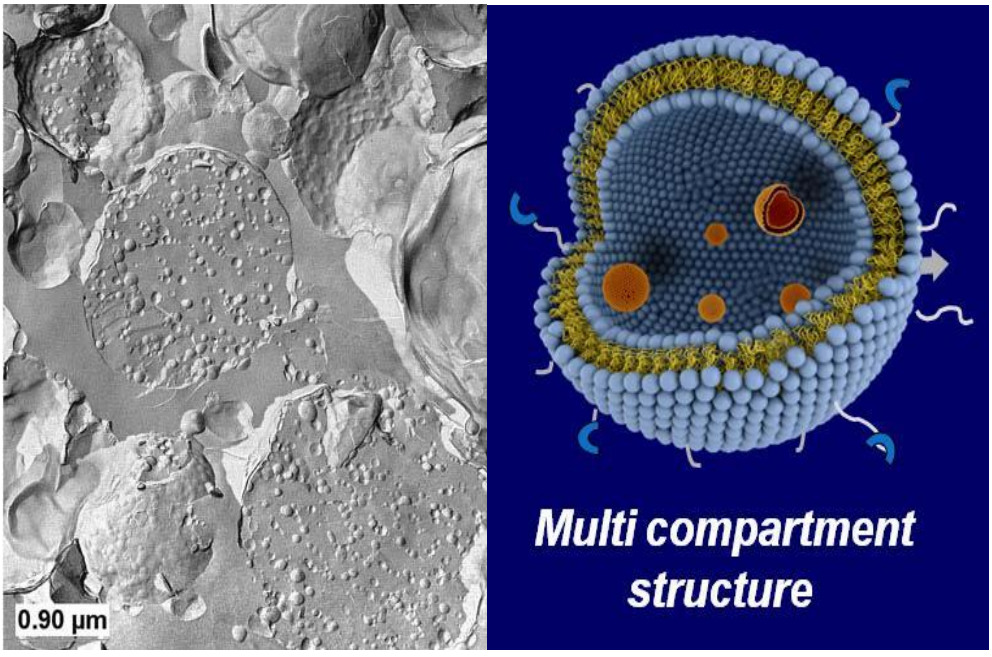

Figure 3: Internal and External membranes of vesicles [12]

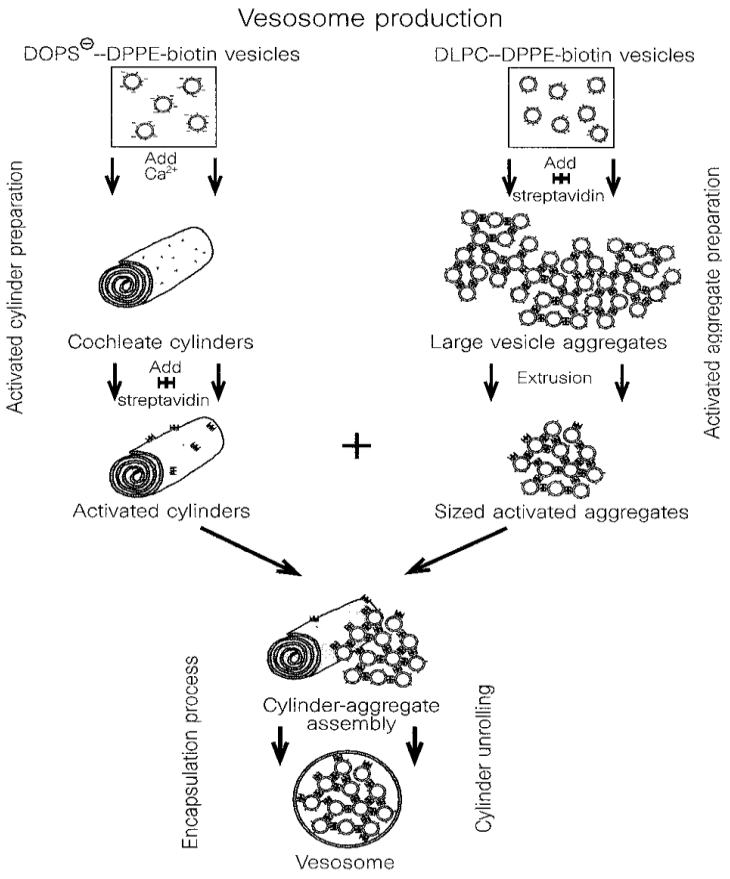

Figure 4: Vesosomes Production and activation [15]

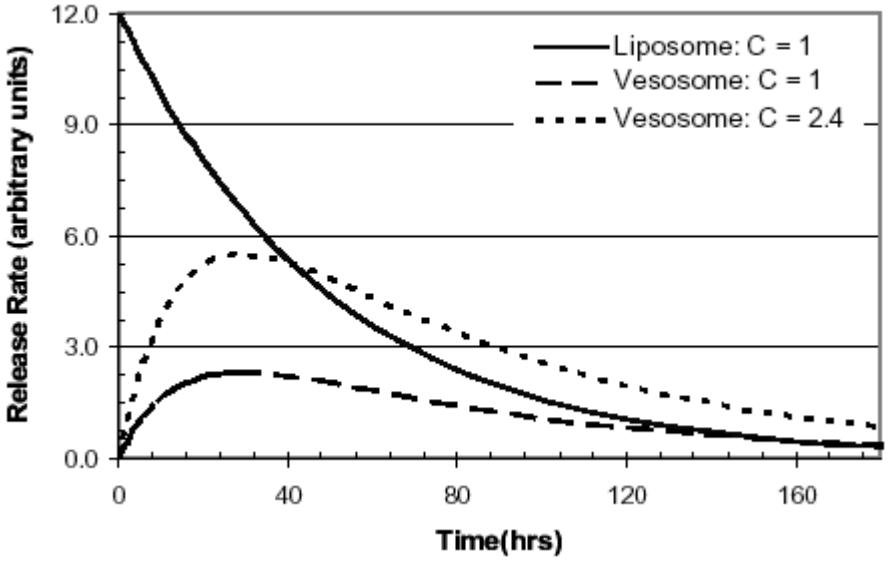

Figure 5: Predicted model of drug release for a vesosome [28]

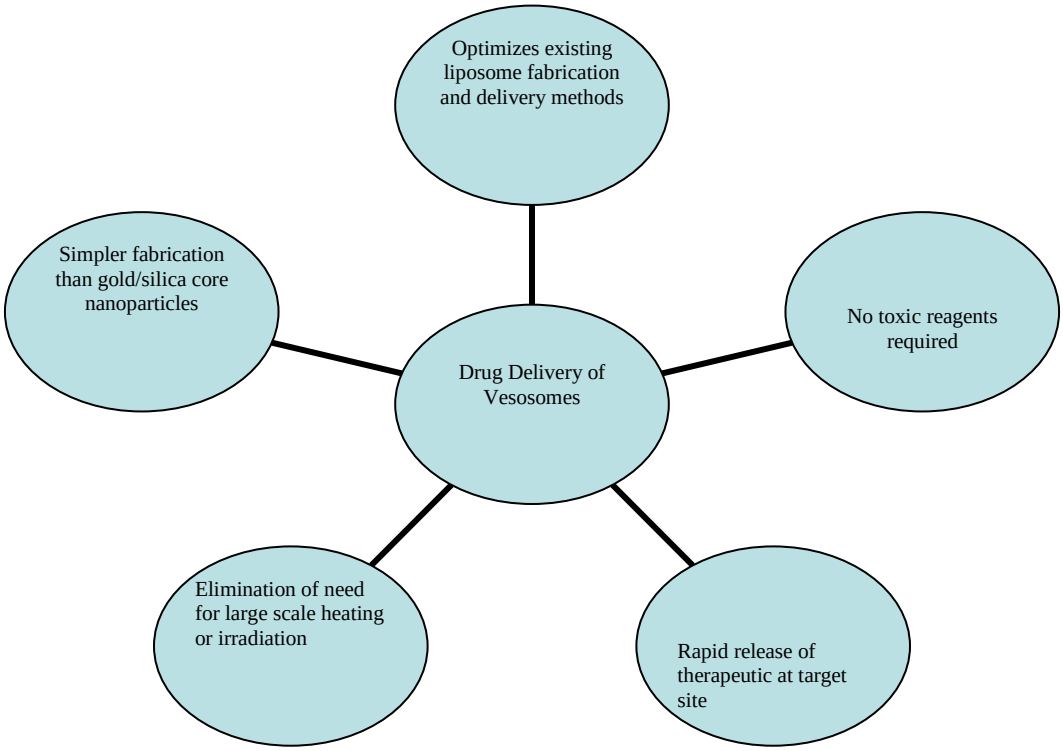

Fig 6: Aspects of Vesosomal drug delivery in tested drugs [41-42]

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