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REVIEW ARTICLE

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LIPID VESICLES: PHYSICAL PROPERTIES AND APPLICATION AS NANOCARRIERS IN DRUG DELIVERY SYSTEMS

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ABSTRACT

Lipid vesicles are nano spherical containers made from lipid molecules. The formation of vesicles is due to the nature of lipid molecules, which have hydrophilic and hydrophobic parts. When they are exposed to an aqueous environment, the hydrophobic parts will hide from the water, while hydrophilic part will face the water. As a consequence, they will form a bilayer, and eventually enclose to a vesicle. The varieties of lipid molecules available for vesicle compositions enrich them to have a wide range of physical properties, including the fluidity, permeability, and the strength. To be used as nanocarriers in drug delivery system, lipid vesicles should have ability to contain the drugs and deliver them directly to the targeted site in the body. Lipid vesicles have advantageous to be used as nanocarriers in drug delivery system since they are biocompatible, they can encapsulate both hydrophobic and hydrophilic drug, and they can be modified by combination with other materials such as polymers and ligands to face biology challenges in the body.

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INTRODUCTION

Lipid vesicle is a spherical nano container composed of lipid molecules which arrange themselves in the form of a bilayer, separating the inside aqueous materials to the outside aqueous environment. Lipid is an amphiphilic molecule, i.e., one part is hydrophilic and the other is hydrophobic. It is the nature of these amphiphilic molecules, which cause them to form of a bilayer, which spontaneously encloses to form a vesicle, when introduced to an aqueous environment (Figure 1). Lipid vesicles were first introduced in 1965 by Bangham and his colleagues, who described them as swollen phospholipid systems, which were used as model membrane systems [1]. In the following years after the introduction, the systems were developed and were known as bangasomes, and then liposomes. This is why lipid vesicles are also known as liposomes nowadays, and these two terms are used interchangeably.

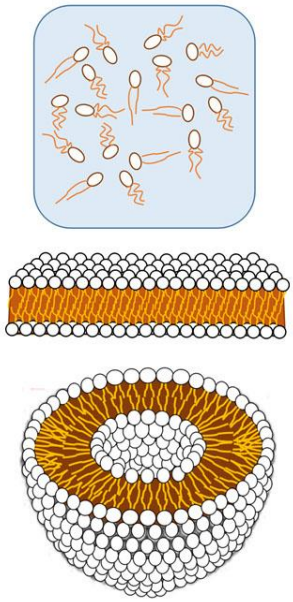

Figure 1. Diagram of lipid molecules in water, which spontaneously form a bilayer and enclose to a vesicle.

There are numbers of methods used to prepare nano size lipid vesicles. Two of the popular and widely used ones are: extrusion [2,3,4] and sonication [5,6]. In extrusion, lipid suspensions are repeatedly forced several times through the well defined size pores in a polycarbonate membrane filter, while in sonication, the suspensions were disrupted by sonic energy. Vesicles formed by extrusion have diameter varying from 50 nm to 200 nm, depending on the size of the pores and the pressured applied in extrusion process [4,7], and vesicles formed by sonication have diameter varying from 15 nm to 50 nm [8].

Since lipid vesicles can contain a material inside them and separating it from the outside environment, they can be used as nanocarriers. For examples, they have been used to contain many therapeutic agents, such as: drug molecules [9], gene therapy and bioactive agents [10]. The choice of lipid vesicles as nanocarriers in drug delivery system is an advantageous, since lipid molecules are biocompatible.

In drug delivery system using lipid vesicles as nanocarriers, the drug materials are loaded into the vesicles and are sent to the diseased tissue inside the body. Therefore to be used as nanocarriers, lipid vesicles should own chemical, biological, and physical properties in such a way that they are able to execute the job. These properties depend on the composition of lipids in the vesicles, other agents introduced, properties of drug materials, and medium surrounding the vesicles.

In this review article, we explain the physical properties of lipid vesicles, showing that the vesicles can fulfil the criteria as nanocarriers. Since the physical properties of any material are determined by its composition, the review will start with description of various lipid molecules used to form lipid vesicles. Description of some physical properties relating to the job of nanocarriers is at the next section. These include: fluidity and permeability of the lipid bilayer, as well as the strength of the membrane. At the final section, we describe the relationship between the physical properties and the use of lipid vesicles as nanocarriers. This review is written with an emphasis on pedagogic purposes.

Phospholipids Molecular Structure

There are various lipid molecules, which can be found in plasma membranes [11]. However, since phospholipids are commonly used in vesicles formation [12], we mostly explain phospholipids in this article. In general, there are two parts of the lipid molecules: hydrophilic part, the head of the molecule, and hydrophobic part, the tails of the molecule. Phospholipids contain a phosphate group as the hydrophilic part, and two fatty acid chains as the hydrophobic part, where the two parts are connected by alcohol. The phospholipids, then, can be distinguished one from the others by the variation in headgroup, in the chains, and the alcohols. Based on the alcohol linking the headgroup and the chains, phospholipids can be classified as glycerolphospholipids and sphingomyelins. In glycerolphospholipids the alcohol is a glycerol, while in sphingomyelin, it is a sphingosine. For convenience,

we just discuss glycerolphospholipids in this review article, and just write it as phospholipids.

Based on the headgroup, phospholipids can be classified as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidic acid (PA), phosphatidylglycerol (PG), and cardiolipin (CL). In pH 7, PC and PE are electrically neutral, while others are negatively charged. The chains of the phospholipids differ in the number of carbons and number of carbon-carbon double bonds. Lipids with double bond/s are called unsaturated lipids, while the ones without double bond are called saturated lipids. The shortest chain for a saturated lipid is a 12 carbon chain lauric acid, followed by 14 carbon myristic acid, 16 carbons palmitic acid, 18 carbons stearic acid, 20 carbons arachidic acid, 22 carbons behenic acid, and 24 carbons lignoseric acid. Two of the popular examples of unsaturated chains are: palmitoleic acid with 16 carbons with 1 double bond, and oleic acid with 18 carbons with 1 double bond.

Figure 2 show diagram of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC), an example of unsaturated lipid, and 1,2-Dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC), an example of saturated lipid. POPC has 16 carbons (palmitic acid) and 18 carbons with one double bond (oleic acid), while DMPC has both 14 carbons (miristic acid). There is a kink at POPC chains due to the presence of double bond.

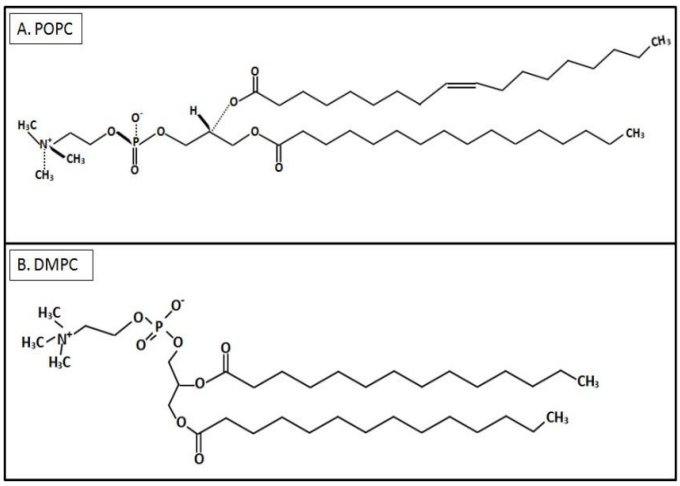

Figure 2. Molecular structure of POPC, an example of unsaturated lipids, and DMPC, an example of saturated lipids.

The composition of the headgroup and tailgroup of the phospholipid determines its molecule geometry: the composition of the headgroup determines the surface area a_0 occupied by the molecule, while that of the tails determine the volume v occupied by the molecule. Based on this geometry, the lipid molecules can be classified as truncated, cylindrical, and inverted cones as shown in Figure 3. It is this geometry of lipid molecules that determines what form of aggregation they assume, when exposed to an aqueous environmental. The formation is determined by packing factor p_f defined as [13]

$$p_f = \frac{v}{a_0 l_c},$$

where l_c is the maximum length the hydrocarbon chains can assume. Lipids with p_f less than 1/3 tend to aggregate to spherical micelles, while lipids with p_f between 1/3 and ½ tend to form cylindrical micelles. Lipids with p_f between ½ and 1 tend to form bilayer, while lipids with p_f greater than 1 tend to aggregate to inverted micelles.

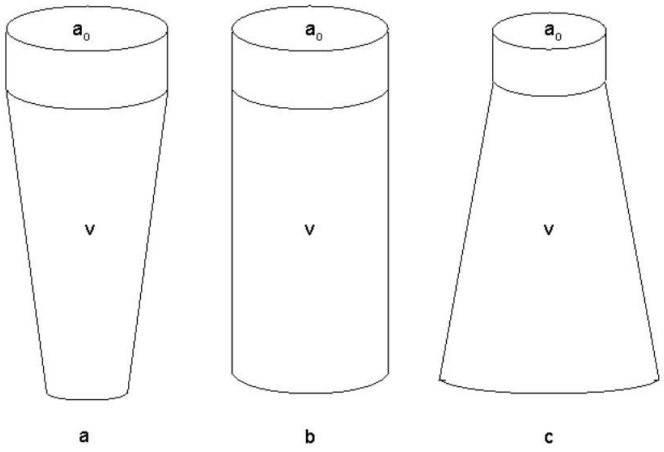

Figure 3. Geometry of lipid molecules: a. Truncated cone, b. Cylinder, and c. Inverted cone.

Fluidity of the Bilayer Membrane

Bilayer membrane can assume gel or liquid phases, depending on the temperature and the molecule characteristics. At a relatively high temperature, the bilayer membrane is in liquid phase, while at a relatively low temperature it is in gel phase. In liquid phase, the lipid molecules move much more freely than they are in gel phase. The temperature at which the membrane change from gel phase to liquid phase is called phase transition temperature T_m

The physical behaviour of the lipid molecules in bilayer membrane is determined by Van der Waals interaction of the chains of adjacent lipid molecules. For relatively long tailed-lipids, there is more interaction among the chains of adjacent lipids, strengthening Van der Waals interaction, thus lowering the mobility of the molecules: The T_m increases with increasing the chain length. For example, it has been reported that T_m of lipid

increase with increasing the chain length in phospholipids (14).

The van der Waals interaction is also affected by the degree of unsaturation in the chains. The double bond in the chain causes a kink, which prevents chains from adjacent molecules to interact, thus weakening the van der Waals interaction [13]. As a result, the increase in the double bonds in lipid molecules increases the mobility of the molecules in the membrane: The T_m decreases with increasing the degree of unsaturation. It has been reported that T_m for phospholipids decreases when the number of double bonds increases [15].

Permeability of Lipid Bilayer

Lipid vesicles are materials imitate plasma membrane, where the bilayer acts as protector of inside cell from its outside environment. The bilayer does the job by selecting certain materials, which can enter and exit the membrane. In lipid vesicles, lipid bilayer contains materials inside. Whether this material can permeate the bilayer to exit or the material from outside can permeate to enter the membrane depends on the properties of both the materials and the bilayer. For a molecule to cross the bilayer, it must overcome possible interfacial resistance or free energy barrier of the bilayer. The chains of the lipid is hydrophobic, thus hydrophobic molecule pays less energy to cross the barrier than hydrophilic molecule: ions and large molecules are difficult to across the bilayer. Then, the molecule must diffuse across the bilayer, before it exits the membrane to the outside, where there must be any possible interfacial resistance or free energy barrier to overcome too.

Experiments have shown that the lipid membrane permeability of water increases with increasing the degree of unsaturation of lipids [16]. It has been found that in the vicinity of gel-liquid phase transition temperature, the lipid membranes are permeable to water [17] and ions [18, 19]. The presence of cholesterol is believed to decrease the permeability of lipid membrane [20].

In terms of the materials inside vesicles, for example drug materials, it has been found that lipid membrane of vesicle has a relatively low permeability to hydrophilic drug, but high permeability to hydrophobic drug [21].

The Strength of the Vesicle Bilayer

The mechanical strength or just strength of the vesicle bilayer is commonly measured by its lysis tension, a minimum value of applied tensions on the bilayer at which the bilayer ruptures.

Lipid-lipid and lipid-water interactions set a bilayer with an area per molecules *a*, when lipid molecules are exposed to an aqueous environment. The hydrophobic interaction of lipid-water results in a compressive force, which tends to decrease *a*. The compressive force can be written as γa, where γ is the surface tension of lipid-water interface. This force is balanced by a steric repulsive force, due to the lipid-lipid interaction (between the tails) tending to increase *a*. This steric repulsive force is expressed in the form of C/a, where *C* is a positive constant. Energy per molecules *E* of a bilayer is a combination of these two forces [13] and can be written as

$$E = \gamma a + \frac{C}{a}.$$

Energy *E* has its minimum value when *a* is at its equilibrium value a_0. This can be determined by letting

$$\frac{dE}{da} = 0$$

We can determine *C* and work out to find

$$E = 2\gamma a_0 + \frac{\gamma}{a}(a - a_0)^2.$$

When tension or compression is applied on the bilayer at equilibrium, the area per molecule a_0 will change. The fractional change of a membrane area, $\Delta a/a_0$, can be written in terms of expansion (or compressibility) modulus K_a, and surface tension γ as

$$\frac{\Delta a}{a_0} = \frac{\gamma}{K_a}.$$

If the tension applied is larger than that of what the bilayer can withstand to keep the lipid molecules together, the vesicles will rupture. The minimum tension required to rupture the vesicles is called lysis tension γ_{lysis}. Vesicle bilayer ruptures when the fractional change of a membrane area is in the range of 2 to 5% [22]

Many groups have measured the lysis tension of lipid vesicles with various compositions of lipid materials. The lysis tension of SOPC and DAPC vesicles was determined to be 5.7 mN/m and be 2.3 mN/m, respectively [23]. The values increased with concentration of cholesterol reaching 30.9 mN/m at 89 mol % cholesterol in SOPC, and up to 3.4 mN/m at 80 mol % cholesterol in DAPC. The lysis tension of POPC vesicles also increases from 7.9 mN/m up to 11.9 mN/m with addition of 30 mol % cholesterol [24]. The measurements of lysis tension for vesicles made from unsaturated lipids show it decreases with increasing number of double bonds [16].

Lipid Vesicles as Nanocarriers in Drug Delivery Systems

For vesicles to execute the job as nanocarriers in drug delivery systems, they are required to have some abilities both as the drug container to encapsulate many varieties of drugs and as carriers to deliver the drug to the targeted site, where vesicles need to overcome biological barriers in the body. The advantages of using vesicles as nanocarriers are that the lipids are biocompatible [10], there are various kinds of lipids [11], which can be chosen based on the requirements, and they can be manipulated with other molecules [25] for specific requirements.

Lipid vesicles can handle both hydrophobic and hydrophilic drugs: hydrophobic drug can be inserted in the bilayer exposed to lipid tailgroups, while the hydrophobic drug can be trapped in the aqueous centre of the vesicle exposed to lipid headgroups [26]. To function well as nanocarriers, lipid vesicles should be non-permeable to prevent drug leakage. It has been shown that, membrane of vesicle has a relatively low permeability to hydrophilic drug, but high permeability to hydrophobic drug [21].

The body immune systems: the innate immune system and the adaptive immune systems, are the main barriers encountered by the vesicles. The innate immune system includes opsonization and reticuloendothelial system (RES) [27]. Opsonization is a process in which vesicles are marked by plasma proteins such as complement proteins, immunoglobulin, and fibronectin for phagocytosis. Phagocytosis occurs both in the blood and in organs with RES such as the liver, kidney, lungs, spleen, lymph nodes and bone marrow [28]. The presence of the proteins on vesicles is a signal for phagocytic cells to clear the vesicles. The opsonins themselves can also destroy the vesicles. Both opsonization and RES are responsible for vesicle clearance from blood circulation, thus shortening vesicle circulation time. The vesicles that have evaded opsonization and RES will encounter the adaptive immune systems of the body which involve antibody formation.

Size of the vesicles is an important parameter to face biological barrier in the body. It is one of the factors that determine the fate of the vesicles in blood circulation, since it affects the efficacy of vesicle opsonisation by serum proteins [29]. It has been shown also that opsonic activity decreases with decreasing size of vesicles [30]. The uptake of the vesicles by the RES organs is also size dependent [31]. It has been found that large vesicles are cleared from blood circulation rapidly than small neutral or positively charged vesicles [32]. In addition, electrically charged lipids can promote the interaction with opsonin biomolecules [30]. On the other hand, introducing cholesterol into the bilayer decreases interaction of vesicles with other biomolecules, as well as increases vesicle stability [27]. Vesicle size around 100 nm with cholesterol introduced is believed to increase the circulation time [33].

Other efforts to maintain the stability of the vesicles and prolong their circulation time in overcoming biological barriers are by introducing certain polymers and ligands on vesicle surface (Figure 4). The addition of the polyethylene glycol (PEG), a hydrophilic polymer on the vesicle surface, improves the ability of the vesicle to evade opsonization and clearance by the RES, thus improving the half-life of the vesicles [34].

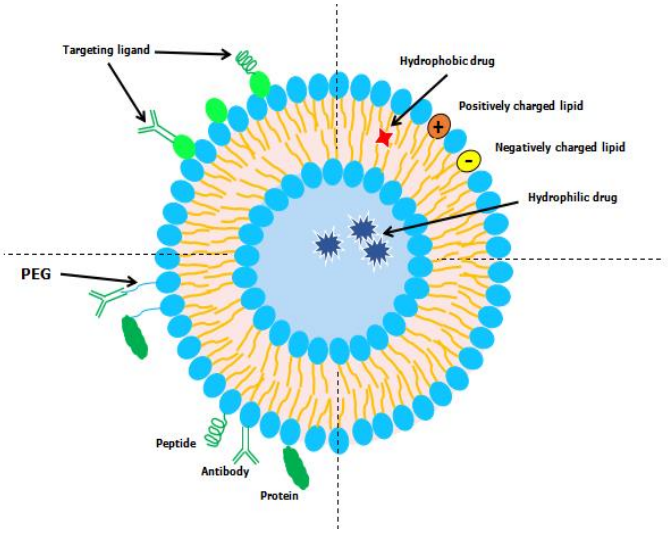

Figure 4. Diagram of a vesicle with hydrophilic and hydrophobic drugs, and certain polymers and ligands, which are introduced to vesicle surface in order to maintain the stability and prolong the circulation time.

The addition of ligands such as such as antibodies, proteins and carbohydrates on the vesicle surface enhances the chance of the vesicles to reach their targeted tissues. The specific ligands can promote the vesicle fusion with targeted cells by endocytosis resulting in vesicle-content delivery. Vesicle coated by monoclonal antibody has been used to target tumor-cell specific antigens and shown more effective results than free drug [35]. Vesicle coated by small protein has been used as a vehicle for breast cancer treatment [36]. The limitation of the vesicles coated by antibody to deliver the drug is caused by the possibility of the antibody to induce body immune response. Therefore the

combination of both antibody and PEG grafted on the vesicle surface has been used to get both low RES uptake and a long half-life [37].

CONCLUSION

Lipid vesicles own the physical properties that can make them appropriate to act as nanocarriers in drug delivery system. In this system, drugs are loaded into the vesicles, and are delivered to the targeted site in the body. Lipid vesicles can be made from many varieties of lipids, which can be chosen to optimise their physical properties as nanocarriers, including the fluidity, permeability, and strength. The materials are biocompatible, thus it is non-toxic. When introduced to blood circulation, lipid vesicles need to overcome biological barrier, especially body immune system, including the innate and the adaptive immune systems. Lipid vesicles have potential ability to face these systems, by introducing polymers and ligands, to prolong their blood circulation time and to precisely reach the targeted site.

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REFERENCES

1. D.W. Deamer, From "banghasomes" to liposomes: a memoir of Alec Bangham, 1921–2010, ***FASEB. J*. 2010; 24:1308–1310**.
2. M.J. Hope, M.B. Bally, G. Webb, P.R. Cullis. Production of large unilamellar vesicles by a rapid extrusion procedure. Characterization of size distribution, trapped volume and ability to maintain a membrane potential, ***Biochimica et Biophysica Acta–Biomembranes* 1985; 812:55–65.**
3. B. J. Frisken, C. Asman, and P. J. Patty. Study of Vesicle Extrusion**.** ***Langmuir* 2000; 16:928-933.**
4. P.J. Patty and B.J. Frisken. The Pressure-Dependence of the Size of Extruded Vesicles, ***Biophys. J*. 2003; 85: 996-1004.**
5. N.J. Cho., L. Y. Hwang, J.J.R. Solandt, and C. W. Frank, Comparison of Extruded and Sonicated Vesicles for Planar Bilayer Self-Assembly, ***Material* 2013; 6: 3294-3308.**
6. M.M. Lapinski, A. Castro-Forero, A.J. Greiner, R.Y. Ofoli, and G.J. Blanchard, Comparison of Liposomes Formed by Sonication and Extrusion: Rotational and Translational Diffusion of an Embedded Chromophore**,** ***Langmuir* 2007; 23:11677–11683**
7. P.J. Patty, Using Vesicles to Study the Effect of Sterols on the Mechanical Strength of Lipid Membranes and the Protein-Lipid Membrane Interaction, **Ph.D. Thesis. Simon Fraser University, 2006. Canada**
8. G. Maulucci, M. De Spirito, G. Arcovito, F. Boffi, A.C. Castellano, and G. Brigantiy, Particle Size Distribution in DMPC Vesicles Solutions Undergoing Different Sonication Times, **Biophys. J. 2005; 88:3545-3550.**
9. G. Bozzuto, and A. Molinari, Liposomes as nanomedical devices, **Int.*J. Nanomedicine, 2015;* 10:975–999 .**
10. N. Monteiro, A. Martins. R.L. Reis, and N.M. Neves, Liposomes in tissue engineering and regenerative medicine, ***J.R.Soc.Interface,* 2014; 11:20140459.**
11. Robert B. Gennis, Biomembrane Molecular Structure and Function, **1989; Springer-Verlag.**
12. J. Li, X. Wang, T. Zhang, C. Wang, Z. Huang, X. Luo, Y Deng, A review on phospholipids and their main applications in drug delivery systems, ***Asian Journal of pharmaceutical sciences*, 2015; 10:81-98.**
13. J. Israelachvili, Intermolecular and Surface Forces, Third Edition. **2011; Academic Press, San Diego.**
14. D. Marsh, Structural and thermodynamic determinants of chain-melting transition temperatures for phospholipid and glycolipids membranes, ***Biochimica et Biophysica Acta*, 2010; 1798:40–51.**
15. G. Wang, S. Li, H. Lin, E. E. Brumbaugh, and C. Huang, Effects of Various Numbers and Positions of cis Double Bonds in the sn-2 Acyl Chain of Phosphatidylethanolamine on the Chain-melting Temperature, ***J. Biol. Chem.*, 1999; 274:12289–12299.**
16. K. Olbrich, W. Rawicz, D. Needham, and E. Evans, Water Permeability and Mechanical Strength of Polyunsaturated Lipid Bilayers, ***Biophys. J*. 2000; 79:321–327**
17. M. Jansen and A. Blume, A comparative study of diffusive and osmotic water permeation across bilayers composed of phospholipids with

different head groups and fatty acyl chains. ***Biophys. J.*, 1995; 68:997–1008**.

18. V.F. Antonov, V. V. Petrov, A. A. Molnar, D. A. Predvoditelev, and A. S. Ivanov, The appearance of single-ion channels in unmodified lipid bilayer membranes at the phase transition temperature, ***Nature,* 1980; 283:585– 586.**
19. V.F. Antonov., A. A. Anosov, V. P. Norik, and E. Y. Smirnova, Soft perforation of planar bilayer lipid membranes of dipalmitoylphosphatidylcholine at the temperature of the phase transition from the liquid crystalline to gel state. ***Eur. Biophys. J*. 2005; 34:155–162.**
20. Sophie Raffy and Justin Teissie, Control of Lipid Membrane Stability by Cholesterol Content, ***Biophys. J.*, 1999; 76:2072–2080.**
21. T.M.Allen and P.R.Cullis, Liposomal drug delivery systems: from concept to clinical applications. ***Adv. Drug Deliv. Rev*., 2013;65:36–48.**
22. D. Boal, Mechanics of the Cell, 2003; ***Cambridge University Press.***
23. D. Needham and R.S. Nunn, Elastic deformation and failure of lipid bilayer membranes containing cholesterol, ***Biophys. J.*, 1990; 58: 997-1009.**
24. Y.W Hsueh, M.T. Chen, P.J. Patty, C. Code, J. Cheng, Barbara J. Frisken, M. Zuckermann, and J. Thewalt, Ergosterol in POPC Membranes: Physical Properties and Comparison with Structurally Similar Sterols, ***Biophys. J.*, 2007; 92:1606–1615**.
25. L. Sercombe, T.Veerati , F. Moheimani, S.Y.Wu, A. K. Sood, and S. Hua, Advances and Challenges of Liposome Assisted Drug Delivery**, *Frontiers in Pharmacology,* 2015; 6: Article 286.**
26. S. Hua and S.Y.Wu, The use of lipid-based nanocarriers for targeted pain therapies. ***Front.Pharmacol.* 2013; 4:143.**
27. M. Willis and E. Forssen, Ligand-targeted liposomes, ***Adv.Drug Deliv. Rev.* 1998; 29:249–271.**
28. J.H. Senior, (1987).Fate and behaviour of liposomes *invivo*: are view of controlling factors. ***Crit. Rev.Ther.DrugCarrierSyst.,* 1987; 3,123–193**.
29. P.R.Cullis, A. Chonn, and S.C. Semple, Interactions of liposomes and lipid-based carrier systems with blood proteins: relation to clearance behaviour *invivo*, ***Adv. Drug Deliv. Rev.* 1998; 32,3–17.**
30. S.S. Chrai, R. Murari, and I. Ahmad, Liposomes (a review) part two:drug delivery systems. ***Bio.Pharm.*, 2002;17,40–43.**
31. D. Liu, F. Liu, and Y.K. Song, Recognition and Clearance of Liposomes Containing Phosphatidiserine Are Mediated by Serum Opsonin, ***Biochim. Biophys. Acta,* 1995; 1235:140 –146.**
32. A.S. Ulrich, Biophysical aspects of using liposomes as delivery vehicles. ***Biosci. Rep.* 2002; 22:129–150.**
33. S. Geng, B. Yang, G. Wang, G. Qin, S. Wada, and J.Y. Wang, Two cholesterol derivative-based PEGylated liposomes as drug delivery system, study on pharmacokinetics and drug delivery to retina. ***Nanotechnology,* 2014; 25:275103.**
34. V.P. Torchilin, A.L. Klibanov, L. Huang, S. O'Donnell, N.D. Nossiff, and B.A. Khaw, Targeted accumulation of polyethylene glycolabilcoated immuno liposomes in infarctedrabbit myocardium. ***FASEBJ.* 1992;6:2716–2719.**
35. I. Ahmad, M. Longenecker, J. Samuel, and T.M. Allen, Antibody-Targeted Delivery of Doxorubicin Entrapped in Sterically Stabilized Liposomes Can Eradicate Lung Cancer in Mice, ***Cancer Res.* 1993; 53: 1484–1488.**
36. A. Puri, Kramer-Marek G, Campbell-Massa R, Yavlovich A, Tele SC, Lee SB, Clogston JD, Patri AK, Blumenthal R, Capala J. HER2-specific affibody-conjugated thermosensitive liposomes (Affisomes) for improved delivery of anticancer agents, *J. **Liposome Res*. 2008;18:293–307.**
37. K. Maruyama, PEG-immunoliposome. ***Biosci.Rep.* 2002; 22,251–266**.

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