International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 7 | Issue 4 | OCT-DEC| 2017 | 56-59
Review Article – Pharmaceutical Sciences| Open Access| UGC Approved | MCI Approved Journal

LIPOSOMES: AN ADVANCED TECHNOLOGY TO DELIVER ACTIVE DRUG MOLECULES

Deepak Singh* and Prashant Upadhyay
Department of Pharmaceutics, IFTM University Moradabad

***Corresponding Author Email:** deepakpharma88@rediffmail.com

ABSTRACT

Liposomes are spherical vesicles consisting of one or more phospholipid bilayers. Liposomes characterize an advanced technology to deliver active molecules to the site of action, and at present, several formulations are in clinical use. Liposomes can penetrate cell membrane easily and effectively deliver drug to such that a free drug could not penetrate.

KEY WORDS

Liposomes, Glycolipids, Drug formulations, Drug delivery systems

INTRODUCTION

Liposome was discovered about 40 years ago by Bangham and co-workers accidently when he dispersed Phosphatidyl choline molecules in water; it was forming a closed bilayer structure containing lipid bilayers [1].

The word liposome is derived from two Greek words: 'Lipos' meaning fat and 'Soma' meaning body. Structurally, liposomes are vesicles made up of lipid bilayer enclosing an aqueous volume. Liposomes may be used to deliver drug for treatment of cancer and other diseases [2].

Liposomes are vesicles of spherical shape prepared from cholesterol and natural nontoxic phospholipids. Choice of bilayer components determine the rigidity or fluidity and charge of the bilayer membrane (saturated phospholipids with long acyl chains for example, dipalmitoylphos phatidylcholine form a rigid, rather impermeable bilayer structure while unsaturated natural phosphatidylcholine species from natural sources give much more permeable and less stable bilayers) [3]. Vesicle size falls into nanometer to micrometer range: small unilamellar vesicles are 20–200 nm, unilamellar vesicles are 200 nm-1 µm, and giant unilamellar vesicles are larger than 1 µm [4].

History, of liposomes development can be divided into three ages: (1) Genesis (1968-1975)- physiochemical characterization of liposomes carried out in this period. (2) Middle age (1975-1985) - the period dealt with the discovery of various alternative method for the formulation of liposomes. (3) Modern era (1985 onwards) - Today, liposomes are used successfully in various scientific disciplines, including mathematics and theoretical physics, biophysics, chemistry, colloid science, biochemistry and biology [5]. The polar character of liposomes enables encapsulation of polar drug molecule, amphiphilic and lipophiilic molecules solubilized within the phospholipid bilayer [6]. Gas filled liposomes are also known [7]. Liosomes can act as a carrier for a variety of drug having therapeutic action. Liposomes gaining interest due to its contribution to varied areas like drug delivery, cosmetics and structure of biological membrane [8].

Types of Liposomes

The liposome size can vary from very small (0.025 µm) to large (2.5 µm) vesicles. Liposomes may have one or more bilayer membranes, size and number of bilayer membrane affect the amount of drug encapsulated in liposomes [9].

A) Classification Based on structural parameter [10]

i) Unilamellar Vesicle

- Small unilamellar vesicles (SUV)- size ranges from 20-40 nm.

- Large unilamellar vesicles (LUV)- size ranges from 40-80 nm.
- Medium unilamellar vesicles (MUV)-size ranges from 100- 1000 nm.

ii) **Oligolamellar Vesicles-** These are made up of 2-10 bilayers of lipid surrounding a large internal volume.

iii) **Multilamellar vesicles-**They have several bilayers. They differ according to way they prepared. The arrangements can be onion like arrangement of concentric spherical bilayers of luv/mlv enclosing a large no. of SUV.

B) Based on method of liposome preparation [10]

i) Single or oligolamellar vesicle prepared by reverse phase evaporation method

ii) Multilamellar vesicles made by reverse phase evaporation method

iii) Stable plurilamellar vesicles

iv) Dehydration rehydration method

v) Vesicles prepared by extrusion method

Methods of liposome preparation

General methods of preparation [6]

In this method of liposome formulation lipid is dissolved in organic solvent. Then the solvent is evaporated leaving a thin film of lipids on the wall of container after that an aqueous solution of drug is added. The mixture is agitated to produce multi lamellar vesicles and then sonicated to get SUV's.

Specific methods

These are classified as 3 types based on the modes of dispersion. They are

1. **Physical Dispersion methods**
2. **Solvent Dispersion methods**
3. **Detergent Solubilization methods**

1) Physical Dispersion Method

In this method the aqueous volumes enclosed within lipid membranes is about 5-10%, which is very small proportion of total volume used for preparation because large amount of water soluble drug is wasted during preparation, but lipid soluble drug can be encapsulated to high percentage. In these methods, MLVs are formed.

Hand Shaken Method:

This method is widely used for liposome's formulation. In this method the lipid mixture and charged components are dissolved in chloroform and methanol mixture (2:1 ratio) in a 250 ml round bottomed flask. This flask is attached to rotary evaporator connected with vacuum pump and rotated at 60 rpm. The organic solvents are evaporated at about 30^0c and a dry residue is formed at the walls of the flask. After that nitrogen is introduced into it. The flask is then fixed onto lyophilized to remove residual solvent. After that the flask is again flushed with nitrogen and 5 ml of phosphate buffer is added into it. Then flask is attached to evaporator again and rotated at about 60 rpm speed for 30 minutes or until all lipid has been removed from the wall of the flask. A milky white suspension is formed finally which is allowed to stand for 2 hours in order to complete swelling process to give MLVs

Non-Shaking Method:

This method is similar to shaking method except that care is taken in swelling procedure. In this method the solution of lipid in chloroform and methanol mixture is spread over the flat bottom of the conical flask. After that solution is evaporated at room temperature by flow of nitrogen through the flask. Then water saturated nitrogen is passed through the flask until the opacity of the dried film disappears. Lipid is swelled by addition of bulk liquid. Then10 to 20 ml of 0.2M sucrose in distilled water is introduced in to the flask and. The solution is allowed to run gently over the lipid layer on the bottom of the flask. The flask is flushed with nitrogen and allowed to stand for 2 hours at 37degrees for swelling. After that the vesicles are mixed to yield a milky suspension which is centrifuged at 1200 rpm for 10 minutes. The layer of MLVs floating on the surface is removed. From the remaining fluid, LUVs are produced.

Freeze Drying:

Freeze drying is another method of dispersing the lipid in a finally divided form prior to addition of aqueous media. Usually tertiary butanol is used as a solvent. All the above methods produce MLVs. In order to modify the size, the prepared MLVs are further processed using the following procedures.

Processing of lipids hydrated by physical means

1) Micro emulsification of liposomes:

In this method micro fluidizer is used to prepare small vesicles from concentrated lipid suspension. A suspension of large MLV's can be introduced in to the fluidizer. This equipment pumps the fluid at very high pressure through 5 micrometer screens. After that it is forced through long micro channels, which direct two streams of fluids collide together at right angles at very high velocity. The fluid collected can be recycled until vesicles of spherical dimensions are obtained.

Sonication:

Sonication is for the preparation of SUV. Here, MLVs are sonicated either with a bath type sonicator or a probe sonicator under a passive atmosphere. The main disadvantages of this method are very low internal volume/encapsulation efficacy, possible degradation of phospholipids and compounds to be encapsulated, elimination of large molecules, metal pollution from probe tip, and presence of MLV along with SUV. [9]

By this method the size of the vesicles can be reduced. This can be achieved by ultrasonic irradiation. There are two methods of sonication:

A) Using bath sonicator

B) Using probe sonicator

The bath sonicator is used for large volume of dilute lipids while the probe sonicator is used for suspensions which require high energy in small volume. (e.g.: high concentration of lipids or viscous aqueous phase). The disadvantage of probe sonicator is contamination of preparation with metal from tip of probe. By this sonication method small unilamellar vesicles are formed and they are purified by ultra-centrifugation.

2) Solvent Dispersion Methods

In these methods lipids are first dissolved in an organic solution and then brought into contact with aqueous phase containing materials to be entrapped within liposome.

Ethanol Injection Method

This is simple method. In this method an ethanol solution of the lipids is directly Injected rapidly to an excess of saline or another aqueous medium through a fine needle. The ethanol is diluted in water and phospholipids molecules are dispersed evenly through the medium. This procedure yields a high proportion of SUVs (about 25nm diameter). [The disadvantages of the method are that the population is heterogeneous (30 to 110 nm), liposomes are very dilute, the removal all ethanol is difficult because it forms into azeotrope with water, and the probability of the various biologically active macromolecules to inactivate in the presence of even low amounts of ethanol is high. [9]

Ether injection:

This method involves injecting the immiscible organic solution very slowly into an aqueous phase through a narrow needle at temperature of vaporizing of organic solvent. The advantage of this method is very less risk of oxidative degradation. [The disadvantage of this method is that population is heterogenous, long time is required for the process and careful control is needed for introduction of lipid solution. [9]

3) Detergent Solubilization Technique

In this method the phospholipids and aqueous phase are brought into close contact via detergents, which associate with phospholipids molecules. As a result of this association micelles are formed. They are composed of several hundreds of components. The concentration of detergent in water at which micelles start to form is called CMC. As the detergent molecule is dissolved in water at concentrations higher than the CMC, micelle form in large amounts. As the concentration of detergent added is increased more amount of detergent is incorporated into the bilayer, until a point is reached where conversion from lamellar form to spherical micellar form take place. On increasing the detergent concentration, the micelles are reduced in size. [9]

Characterization of Liposomes [10]

Liposome characterization should be performed immediately after preparation. Different type of chromatography can be used to separate bilayer components (TLC, GLC). One major problem is detection and quantitation as UV molar absorptivity of lipids is low and depends on the degree of saturation of the acyl chains. For those phospholipids with only saturated acyl chains, alternative detection systems not based on UV absorption are described i.e. systems based on differential refractometry, light scattering and flame ionization. The physical properties of liposomes have a direct impact on the behaviour of the liposomes with its content in vivo. size, number of lamellae, internal

morphology charge, bilayer fluidity are the factors that play a role in the in vivo disposition.

Techniques for physical characterization of liposomes

Parameter	Technique
Size	Electron microscopy
Number of lamellae	NMR, Electron microscopy
Bilayer Fluidity	Fluorescence
Charge	Microelctrophoresis

Applications [10]

- Gene therapy
- Liposomes as carriers for vaccines
- Liposomes as carrier of drug in oral treatment
- Liposomes for topical applications
- Liposomes for pulmonary delivery
- Against Leishmaniasis
- Lysosomal storage disease
- Cell biological application
- Metal storage disease
- Ophthalmic delivery of drugs.
- Liposome as drug/protein delivery vehicle.
- Liposome in antimicrobial, antifungal and antiviral therapy
- Vehicle for macromolecule as cytokines or genes.
- Liposomes in enzyme immobilization and bioreactor technology.

CONCLUSION

The use of liposomes in pharmaceuticals is very broad. Liposomes are proved as intracellular delivery systems for anti-sense molecules, ribosomes, proteins/peptides, and DNA. Liposomes alsopromote targeting of particular diseased cells within the disease site. Based on the pharmaceutical applications and available products liposome's have definitely established their position in novel delivery systems. The use of liposomes in the delivery of drugs and genes are promising and is sure to undergo further developments in future.

References

1. Thulasiramaraju T.V., Sudhakar Babu A.M., Arunachalam A., Pratap M., Srikanth S., and Sivaiah P. 2012. Liposomes: A Novel Drug Delivery System. International Journal of Biopharmaceutics, 3(1), 5-16.
2. Dual J.S., Rana A.C., Bhandari A.K. 2012. Liposome: Methods of Preparation and Applications. International Journal of Pharmaceutical Studies and Research, 3(2), 14-20.
3. Akbarzadeh Abolfazl, Rezaei-Sadabady Rogaie, Davaran Soodabeh, Woo Joo Sang, Zarghami Nosratollah, Hanifehpour Younes, Mohammad Samiei, Kouhi Mohammad and Nejati-Koshki Kazem. 2013. Liposome: Classification, Preparation, and Applications. Nanoscale Research Letters, 8(1), 102.
4. Deepthi V and Kavitha AN. 2014. Liposomal Drug Delivery System-A Revie Liposomal Drug Delivery System-A Review.Int. J. Drug Dev. & Res., 4(2), 47-56.
5. Rangasamy Manivannam and Parthiban Kugalur Ganesan. 2010. International Journal of Research in Ayurveda And Pharmacy, 1(2), 316-326.
6. Yadav A.V., Murthy M.S., Shete A.S. and Sfurti Sakhare. 2011. Stability Aspects of Liposomes. Indian Journal of Pharmaceutical Education And Research, 45(4),
7. Kant Sashi, Kumar Satinder, Bharat Prashar. 2012. A complete review on: liposomes- International research journal of pharmacy, 3(7), 10-16.
8. Sirisha1 V.N.L., Harika I. Bhavani, Sruthi B., Namrata M., Kirankumar P., Kiran Kumar Rao Y., Pranavi K., Sindhura K., Vamsi Krishna N., Uma Maheshwara Rao O. 2011. Liposomes – the potential drug carriers. IOSR Journal of Pharmacy, 2(5), 26-38.
9. Kulkarni Priyanka R, Yadav Jaydeep D, Vaidya Kumar. 2011. A Liposomes: A Novel Drug Delivery System. International Journal of Current Pharmaceutical Research, 3(2), *1*-9.
10. Marripati Saraswathi., Umasankar K., Jayachandra Reddy.2014. International Journal of Research in Pharmaceutical and Nano Sciences. 3(3), 2014, 159 - 169.
11. Kalepu Sandeep, Kumar Sunil, Betha Sudheer K T, Varma Mohan M. 2013. Liposomal drug delivery system - A Comprehensive Review. Int. J. Drug Dev. & Res. 5(4), 62-75.

***Corresponding Author:**
Deepak Singh
Email: deepakpharma88@rediffmail.com