EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 11
Issue: 3
302-310
Year: 2024

TUMOUR TARGETING VIA LIPOSOMAL DRUG DELIVERY SYSYTEMS FOR CANCER THERAPEUTICS

Putta Rajesh Kumar[1]*, Meena N.[1], Poojitha P.[1], Sowjanya P.[1], Shivaleela U.[1] and JVC Sharma[2]

[1]Department of Pharmaceutics, Joginpally BR Pharmacy College, Hyderabad - 500075, Telangana, India.
[2]Department of Pharmacognosy, Joginpally BR Pharmacy College, Hyderabad - 500075, Telangana, India.

***Corresponding Author: Dr. Putta Rajesh Kumar**

Department of Pharmaceutics, Joginpally BR Pharmacy College, Hyderabad - 500075, Telangana, India.

Article Received on 27/01/2024 **Article Revised on 17/02/2024** **Article Accepted on 07/03/2024**

ABSTRACT

Liposomes are spherical vesicles characterised by bilayer of lipids with an internal aqueous cavity. Liposome structural components are phospholipids or synthetic amphiphiles incorporated with sterols, such as cholesterol, to influence membrane permeability. Liposomes are the first nano drug delivery systems that have been successfully translated into real-time clinical applications. These closed bilayer phospholipid vesicles have witnessed many technical advances in recent years since their first development in 1965. Delivery of therapeutics by liposomes alters their biodistribution profile, which further enhances the therapeutic index of various drugs. Thin-film hydration is the most widely used preparation method for liposomes, in which lipid components with or without a drug are dissolved in an organic solvent. The solvent will be evaporated by rotary evaporation followed by rehydration of the film in an aqueous solvent. The other methods include reverse-phase evaporation, freeze-drying and ethanol injection. Techniques like membrane extrusion, sonication, homogenization and/or freeze-thawing are being employed to control the size and size distribution. Liposomes can be formulated and processed to differ in size, composition, charge and lamellarity. Extensive research is being carried out using these nano drug delivery systems in diverseareas including the delivery of anti-cancer, anti-fungal, anti-inflammatory drugs and therapeutic genes. The significant contribution of liposomes as drug delivery systems in the healthcare sector is known mainly by many clinical products, e.g., Doxil®, Ambisome®, DepoDur™, etc. Due to extensive developments in liposome technology, a number of liposome-based drug formulations are available for human use and many products are under different clinical trials. Encapsulation of drugs in liposomes enhanced the therapeutic indices of various agents, via improving their pharmacokinetics and pharmacodynamics.

KEYWORDS: Liposomes, Reverse-phase evaporation, Freeze-drying, Ethanol injection, Cancer therapeutics.

Liposomes concept

Paul Ehrlich in 1906 initiated the era of development for targeted delivery when he envisaged a drug delivery mechanism that would target drug directly to diseased cells, what he called as magic bullets**.** In comparison to conventional drugs, the primary purpose of a targeted drug delivery is to efficiently distribute a drug directly to the site of action to achieve better efficacy and to reduce adverse effects. Liposomes are bilayered vesicles that are concentric in shape, having a diameter of 0.01–5.0 μm as it may be made from cholesterols, glycolipids, nonharmful surfactants, long chains of fatty acids, sphingolipids and even membrane protein.[1]

Liposomes are vesicular structures that develop when phospholipids are spread in water and have an inner aqueous phase enclosed by phospholipid bilayer membranes. Liposomes consist of biocompatible and biodegradable materials capable of encapsulating both hydrophobic and hydrophilic molecules on one platform. The ongoing researches focus more on the growth of long circulating stealth liposomes and multi-functional liposomes with advanced *in vitro* properties.

The first closed bilayer phospholipid systems, called liposomes, were described in 1965 and soon were proposed as drug delivery systems. The pioneering work of countless liposome researchers over almost 5 decades led to the development of important technical advances such as remote drug loading, extrusion for homogeneous size, long-circulating (PEGylated) liposomes, triggered release liposomes, liposomes containing nucleic acid polymers, ligand-targeted liposomes and liposomes containing combinations of drugs. These advances have led to numerous clinical trials in such diverse areas as the delivery of anti-cancer, anti-fungal and antibiotic drugs,

the delivery of gene medicines, and the delivery of anesthetics and anti-inflammatory drugs. A number of liposomes (lipidic nanoparticles) are on the market, and many more are in the pipeline. Lipidic nanoparticles are the first nanomedicine delivery system to make the transition from concept to clinical application, and they are now an established technology platform with considerable clinical acceptance. We can look forward to many more clinical products in the future.[2]

Definition

Liposomes are colloidal, vesicular structures composed of one or more lipid bilayers surrounding and equal numbers of aqueous compartments". The sphere like shell encapsulated a liquid interior which contain substances such as peptides and proteins, hormones, enzymes, antibiotics, anti fungal and anti cancer agents. A free drug injected in blood stream typically achieves therapeutic level for short duration due to metabolism and excretion. Drug encapsulated by liposomes achieve therapeutic level for long duration as drug must first be release before metabolism and excretion.

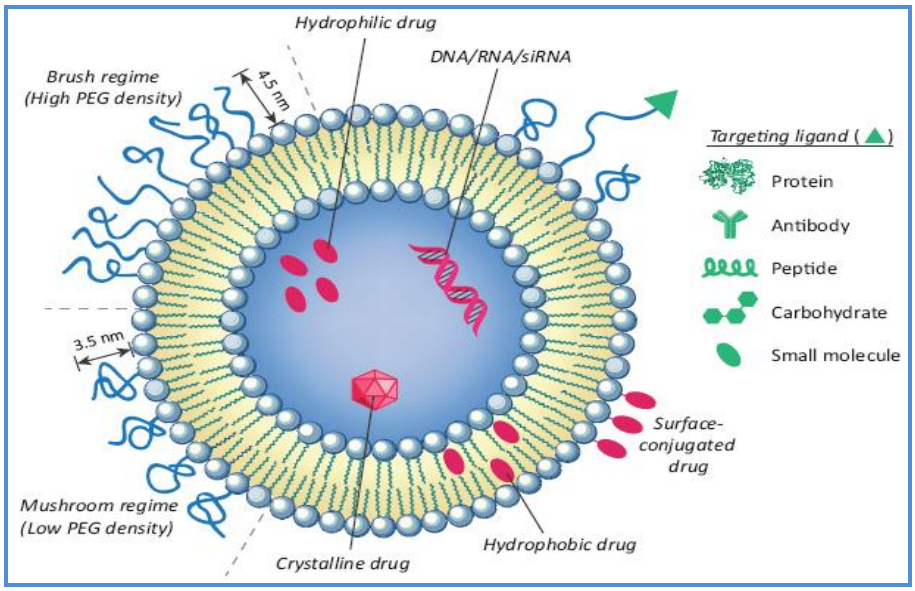

Fig. 1: Structural and Design considerations for liposomal drug delivery.

Liposomes composition

The major constituents of liposome includes

1. Phospholipids
2. Cholesterol

Phospholipids

The fundamental building blocks of bio membranes are phospholipids. Phosphatidylcholine (PC) is perhaps the most often utilised phospholipid in liposome composition. Phosphatidylcholines derived from natural origins or synthesized using semi-synthetic and synthetic techniques. In terms of bilayer sheet orientation in relation to micellar compositions, phosphatidylcholines vary significantly from other phospholipids. The preparation of liposomes is based on a number of natural and semi-synthetic phospholipids. The molecules of phosphatidylcholine are not miscible in water. They develop a planar bilayer configuration in aqueous environments to decrease undesirable interactions between the bulk aqueous environments as well as in nonesterified fatty acid. The sheets are then folded into enclosed capsules.[2]

Cholesterol

Typically, liposomes prepared by using only phospholipids are not sufficiently rigid primarily because of low phase transition temperature and/or unsaturation in the fatty alky chains creating defects in the cell membrane like packaging. During packaging those liposomes leak the encapsulated drug. One or two bilayer stabilizers are also used in most liposome formulation in order to avoid such leakage. The additives more widely used are cholesterol and alphatocopherol. Liposome encapsulation quality varies with variations in the composition of the phospholipid bilayer. Cholesterol is indeed an absolutely vital component of natural lipid bilayers and its presence in bilayer liposomes induces drastic alteration in their characteristics. Cholesterol by itself doesn't develop bilayer complexes, but can be integrated into high concentrations of phospholipid membranes. Rigidity is enhanced due to compact stacking of the bilayers, and permeability of water-soluble molecules is reduced. By reducing bilayer permeability, cholesterol enhances the durability of hydrophilic drugs. Cholesterol reduces the fluidity above the phase transition temperature (Tc) to make the bilayer more ordered. The tricyclic ring is wedged among the first few carbons of the fatty acyl chains, and the

hydroxyl group is exposed to the liquid phase, the cholesterol molecule fits in with the phospholipid molecules and orients itself among them. At very significant concentrations, cholesterol to phospholipids ratios of up to 1:1 or even 2:1 can be incorporated into the cellular membrane. Albumin, macroglobulin, and m-transferrin are blood proteins that interact more easily and frequently with cholesterol-free liposomes.[3]

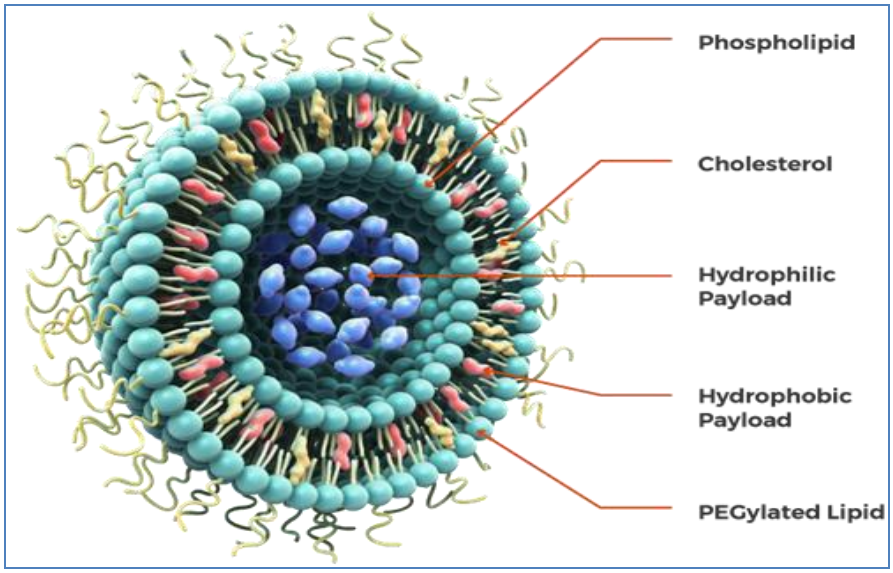

Fig. 2: Composition of liposomes.

Classification

The liposomes are classified based on

1. Structure
2. Preparation process
3. Conventional Liposomes
4. Constituent and its application
5. Liposome specialty.[4]

1. Based on structure

Diameter Size and Number of lipid layers of different vesicles			
Vesicle type	**Abbreviation**	**Diameter size**	**No. of lipid layer**
Unilamellar	UV	All size ranges	One
Small unilamellar	SUV	20-100nm	One
Medium unilamellar	MUV	More than 100nm	One
Large unilamellar	LUV	More than 100nm	One
Gaint unilamellar	GUV	More than 100mm	One
Oligolamellar	OLV	0.1-1.0mm	Approx 0.5
Multilamellar	MLV	More than 0.5mm	5-25
Multi vesicular	MV	More than 1.0mm	Multi compartmental

2. Based on method of preparation.

Different methods of preparation and the vesicles developed by those methods	
Prepation method	**Vesicle type**
Lamellar vesicle of a single or oligo formed by reverse phase evaporation	REV
Multi lamellar vesicles formed by reverse phase evaportion	MLV-REV
Stable pluri lamellar vesicles	SPLV
Frozen and thawed multi lamellar vesicle	FATMLV
Vesicle prepared by extrusion technique	VET
Dehydration –Rehydration method	DRV

3. Based upon conventional liposomes

1. Normalize mixtures of natural lecithine
2. Glycolipid –loaded liposome
4. Synthetic phospholipid with the same chain as natural phospholipids

5. Based on Composition and Application.

Different liposomes with their composition		
Liposomes	**Abbr'n**	**Composition**
Conventional	CL	Neutral or negitively charged phospolipid and cholestrol
Fusogenic	RSVE	Reconstituted sandal virus envelop
pH sensitive	-	Phospolipid such as DOPE or PER with either OA or CHEMS
Cationic	-	Cationic lipid with DOPE
Long circulatory	LCL	**N**eutral high temp, cholesterol, and 5-10% PEG, DSP
Immuno	IL	CL or LCL with monoclonal antibody linked or sequences of recognition

6. Based upon speciality liposomes

1. Carbohydrate coated
2. Bipolar fatty acid
3. Lipoprotein coated
4. Methyl / methylene x- linked
5. Multiple encapsulated
6. Antibody directed

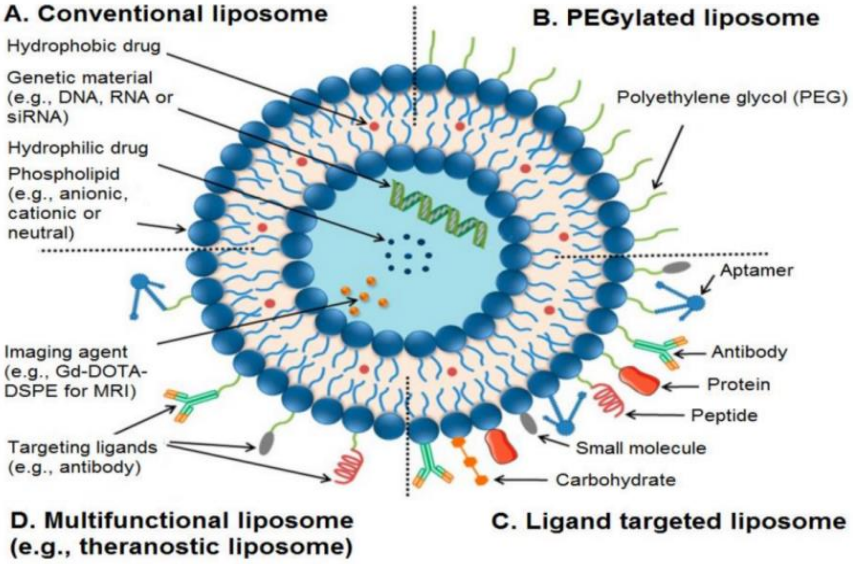

Fig. 3: Different types of Liposomes.

Advantages of liposomes

1. Liposomes increased efficacy and therapeutic index of drug.
2. Liposomes increased stability by encapsulation & passive targeting to tumor tissue.
3. Flexibility to couple with site-specific ligand to achive active targeting.
4. Improved pharmacokinetics (reduced elimination, increased circulation life time).
5. Liposomes reduce the toxicity of the encapsulated tablets.

Disadvantages of liposomes

1. Low solubility in water.
2. Production cost is high.
3. Short half-life.
4. Sometimes phospholipids undergo oxidation and hydrolysis like reaction.
5. Leakage and fusion of encapsulated drug /molecule.[5]

Liposome formulation methods

Parameters that should be considered during the method selection:

1) Physicochemical characteristics of the material to be entrapped
2) The nature of the medium in which the liposomes are dispersed
3) The effective concentration of the encapsulated material and its potential toxicity
4) Additional processes involved during application (delivery of the liposomes)
5) Optimum size, polydispersity and shelf-life of the liposomes
6) Batch-to-batch reproducibility and possibility of large-scale production.

Liposome size is a crucial parameter in determining the circulation half-life of liposomes in drug delivery. According to the desired formulation, different liposome preparation methods can be employed. The main difference in these methods is their approach to overcome the low solubility of lipids in water. Accordingly, these methods can be classified as mechanical agitation, solvent evaporation, solvent injection, and detergent solubilization. In all the above mentioned methods, drug loading is passive.[6]

Mechanical agitation

In this method, lipids are directly solubilized in water upon application of high mechanical agitation, through the use of probe sonication. It is one of the simplest methods of liposome Preparation but suffers from less yield value.

Solvent evaporation

In general this method consists of four major steps; first is the solubilization of the lipid (and a hydrophobic compound) in an organic solvent; second is solvent evaporation; third is hydration with a buffer (and the hydrophilic compound) and if need the fourth often involves obtaining unilamellar liposomes from the obtained multilamellar ones. lipid soluble drug can be encapsulated with 100% efficiency.

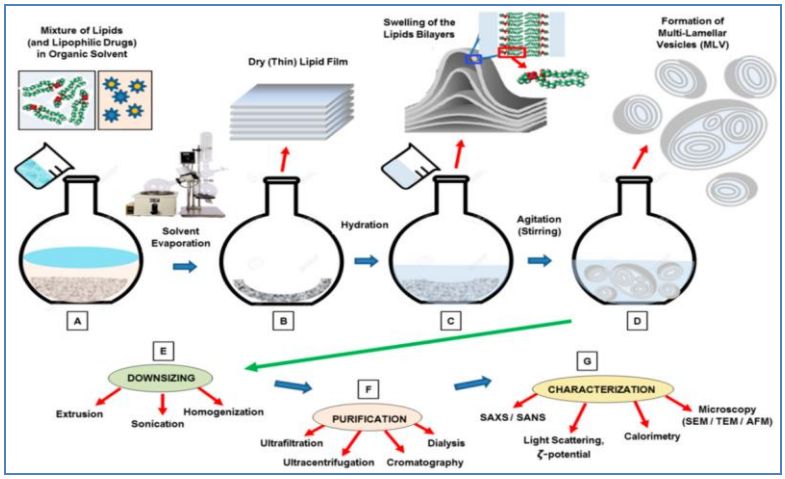

Fig. 4: Schematic representation of the main stages of solvent evaporation.

Solvent injection

In this type of preparation methods, lipids are first dissolved in an organic solvent and then brought into contact with the aqueous phase containing the materials to be encapsulated within the liposome. The lipids align themselves into a monolayer at the interface between the organic and aqueous phase which is an important step to form the bilayer of the liposome. There are three categories in solvent dispersion method including; (i) a miscible organic solvent with the aqueous phase, (ii) an immiscible organic solvent with the aqueous phase that is used in excess, and (iii) an immiscible organic solvent used in excess with the aqueous phase.

Ethanol injection method

In this method an ethanol solution of lipids is injected rapidly into an excess saline or other aqueous medium by a fine needle. The injection force is usually sufficient to achieve complete mixing, so that ethanol is diluted in water, and lipids are dispersed evenly throughout the medium. This method yields a high proportion of SUVs.

Ether injection method

This method involves injecting the immiscible organic solution very slowly into an aqueous phase through a narrow needle at a temperature that the organic solvent is removed by vaporization during the process. In this method, large vesicles are formed which might be due to the slow vaporization of solvent giving rise to ether: water gradient extending on both sides of the interfacial lipid monolayer, resulting in the eventual formation of a bilayer sheet which folds itself to form a sealed vesicle.

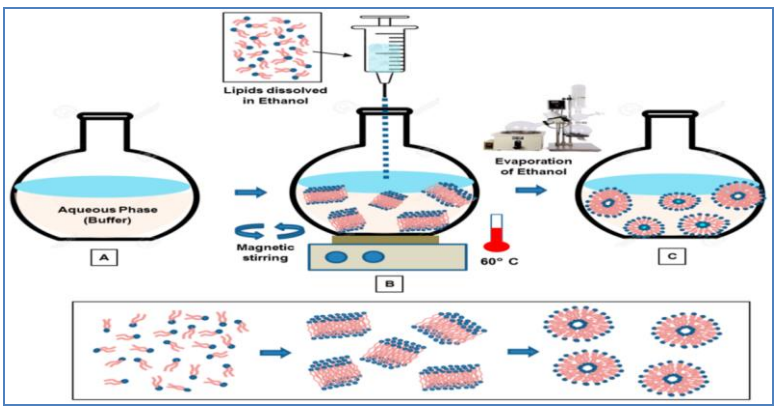

Fig. 5: Schematic representation of the main stages of the ethanol injection.

Surfactant (Detergent) solubilization method

In this method, the phospholipids are brought into contact with the aqueous phase via the intermediary of surfactants. Phospholipid molecules associate with surfactants and form mixed micelles. The basic feature of this method is the removal of the surfactant from preformed mixed micelles containing phospholipids, whereupon unilamellar liposomes form spontaneously.

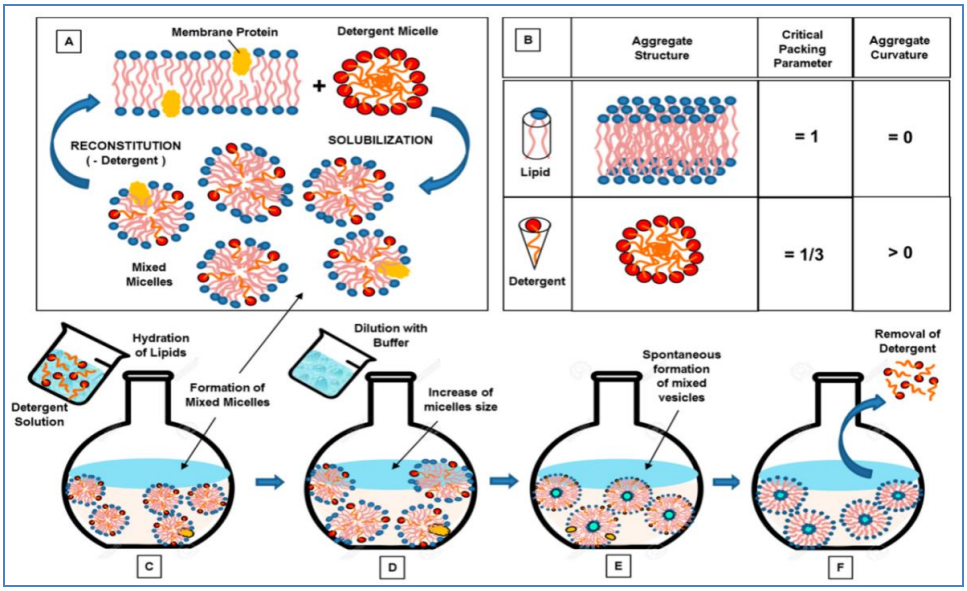

Fig. 6: Surfactant solubilization method schematic representation.

Sizing of liposomes

Size characteristics of liposome have a major effect on the application they can be used. Physical integrity and stability of lipid bilayers structure influence the therapeutic applications of liposome. Therefore particle size of the liposome must be considered for the liposome production procedure and it must be predictable and reproducible with particle size distribution within a certain size range. Sequential extrusion and gel chromatography are common methods of sizing of liposomes.

Sterilization of liposomes

There are several sterilization methods; such as filtration, gamma irradiation, final steam sterilization, ethylene oxide sterilization, and ultraviolet sterilization. Filtration is the most suitable sterilization technique for the thermolabile liposomes since it does not include any form of heat that can result in the degradation of liposomes.

Evaluation and Characterization of liposomes

Liposome should be characterized for visual appearance, turbidity, size distribution, lamellarity, concentration, composition, presence of degradation products, and stability. The behaviour of liposomes in both physical and biological system is governed by these factors; therefore liposomes are characterized for physical attributes and chemical compositions.[7]

Biological characterization

Sterility - Aerobic/anaerobic culture, Pyrogenicity - Temperature (Rabbit) response, Animal toxicity - Monitoring survival of animals (rats)

Chemical characterization

Phospholipids concentration - HPLC/Barrlet assay, Cholesterol concentration - HPLC / cholesterol oxide assay.

Physical characterization

Vesicle shape, and surface morphology – SEM / TEM

1. Visual appearance

Based on the particle size and composition the appearance of the liposomal suspension may be varying from translucent to milky. The samples are homogeneous if the turbidity has a bluish shade; the presence of a nonliposomal dispersion is by flat, grey colour and is most likely a disperse inverse hexagonal phase or dispersed micro crystallites. An optical microscope can detect liposome of size greater than 0.3 μm as well as contamination with larger particles.

2. Determination of liposomal size

Size Distribution was usually measured by dynamic light scattering. Liposomes with relatively homogeneous size distribution are reliable for this method. Gel exclusion chromatography is a simple method, in which a truly hydrodynamic radius can be detected. Sephacryl-S100 can separate liposome in size range of 30-300nm. Sepharose -4B and -2B columns can separate SUV from micelles.

3. Determination of lamellarity

The lamellarity of liposomes can be measured by electron microscopy or spectroscopic techniques. The NMR spectrum of liposome is recorded most frequently with and without the addition of a paramagnetic agent

that shifts or bleaches the signal of the observed nuclei on the outer surface of liposome.

4. Entrapped volume

The entrapped volume of liposome (in µL/ mg phospholipids) can often be deduced from measurements of the total quantity of solute entrapped inside liposome assuring that the concentration of solute in the aqueous medium inside liposomes is the same after separation from unentrapped material. For example, in two phase method of preparation, water can be lost from internal compartment during the drying down step to remove organic solvent.

5. Surface charge

Liposomes are usually prepared using charge imparting / constituting lipids and hence it is imparting to study the charge on the vesicle surface. The two methods used in general to assess the charge are free flow electrophoresis and zeta potential measurement.

Stability of liposomes

Liposome should be physically, chemically, and biologically stable. Physical stability indicates the ratio of lipid to therapeutic agent and steadiness of the size. The chemical stability may be affected by oxidative and hydrolytic degradation pathways. Oxidation of phospholipids in liposomes mainly takes place in unsaturated fatty acyl chain-carrying phospholipids. These chains are oxidised in the absence of particular oxidants. Reduction of oxidation can be achieved by storage at low temperatures and protection from light and oxygen.

Stabilization of liposomes

Usually liposomes may create problem in stability during the storage period. In general certain parameters should be considered to achieve successful formulation of stable liposomal drug product: Processing with fresh, purified lipids and solvents. Avoid high temperature and excessive shearing stress. Maintain low oxygen potential, use of antioxidant or metal chelators. Formulate at neutral pH. Use of lyo-protectant when freeze drying.

The liposomal stability of the drug molecules regulates their therapeutic function. Size, size distribution, composition and drug retention are correlated with the physical integrity of liposomes, whereas Chemical equilibrium struggles with phospholipid oxidation and hydrolysis, as well as drug degradation. A safe liposomal medicinal product has a qualified physical, chemical and microbial stability that guarantees the integrity of the products during its storage time. Therefore, a given stability study protocol emphasizes its significance in deciding the products physical and chemical integrity having a well-established methods for development, characterization, effectiveness and stability testing.

Liposomal technologies in tumor targeting for anticancer therapy

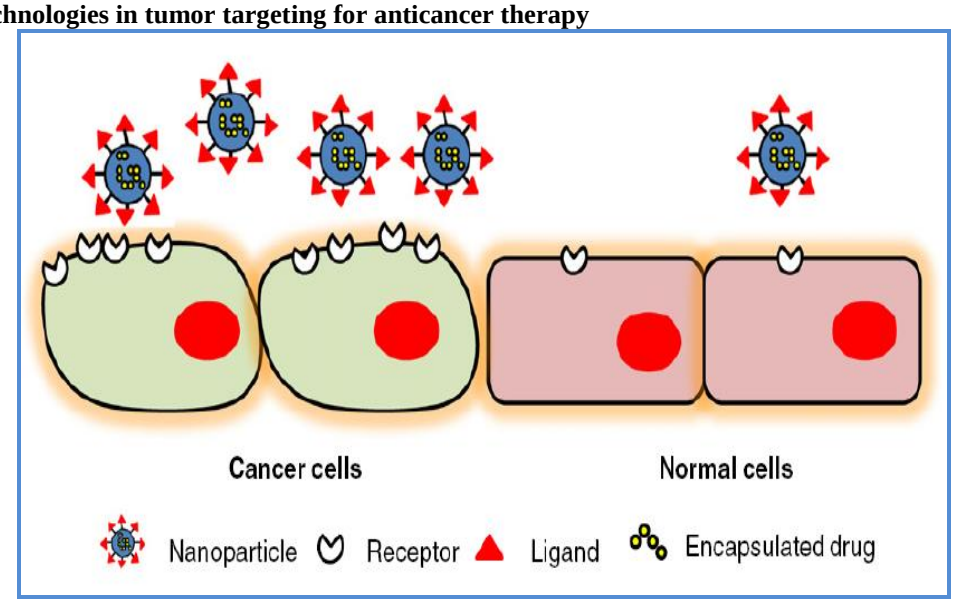

Fig. 7: Liposomal active targeting of cancer drugs via receptors to tumors

Clinical applications of liposomes

Liposomes have demonstrated one of the most established nanoplatforms with several FDA-approved formulations for cancer treatment, and had the greatest impact on oncology to date, because of their size, biocompatibility, biodegradability, hydrophobic and hydrophilic character, low toxicity and immunogenicity a wide range of drugs, including anti-cancer and antimicrobial agents, peptide hormones, enzymes, other proteins, vaccines and genetic materials, are feasible for encapsulation in aqueous or lipid phases of liposomes which showed enhanced therapeutic activity.

Based on these liposome properties, several modes of drug delivery can be listed: the major ones are enhanced drug solubilization (e.g. amphotericin B, minoxidil), protection of sensitive drug molecules (e.g. cytosine arabinose, DNA, RNA, antisense olgionucleotides,

ribozymes), enhanced intracellular uptake (all agents, including antineoplastic agents, antibiotics and antivirals) and altered pharmacokinetics and biodistribution of the encapsulated drug. Long circulating PEG coated liposomes (stealth liposomes), are now being investigated in detail and are widely used in vitro and in vivo studies due their flexibility and also they found their place in the clinical applications.[8]

Liposomes have great pharmaceutical applications in oral and transdermal drug delivery systems. Reduction in toxic effect and enhancement of effectiveness of drugs

1. Enhancement of solubilisation (Amphotericin-B, Paclitaxel)
2. Protection of sensitive drugs (Cytosine arabinosa, DNA, RNA, Ribozymes)
3. Enhancement of intracellular uptake (Anticancer, antiviral and antimicrobial drugs)
4. Alteration in pharmacokinetics and bio-distribution (prolonged or SR drugs with short circulatory half life)

Liposomes for brain targeting

The biocompatible and biodegradable character of liposomes makes its used in brain drug delivery system. Liposomes with a small diameter (100 nm) and large diameter undergo free diffusion through the BBB. However small unilamellar vesicles (SUVs) coupled to brain drug transport vectors may be transported by receptor mediated or absorptive mediated transcytosis through the BBB. Cationic liposomes undergo absorptive mediated endocytosis into cells whereas the same undergoing absorptive mediated transcytosis through the BBB has not yet been determined. Liposomes coated with mannose reach brain and assist transport of loaded drug through BBB. The neutropeptides, leuenkephaline and mefenkephalin normally do not cross BBB when given systemically. The anti depressant amitriptylline normally penetrate BBB, due to versatility of this method.[9]

Liposome in cancer therapy

All cancer drugs on long term usage produce stern toxic effects. The liposomal approach causes targeting of drug to tumour with lesser toxic effects. The small and stable liposome is passively targeted to different tumour because they can circulate for longer time. Nowadays many anti-cancer herbal drugs also formulated into liposomes to provide better targeting with enhanced bioavailability.[10]

New generation Liposomes and Their features

Type	Main constituent	Advantage
Liposomes	Phospholipids	
Archaesomes	One or more lipids containing diether linkages	High stability at several conditions
Niosomes	Non-ionic surfactant and cholesterol	Less prone to action of bile salts
Novasomes	Monoester of polyoxyethylene fatty acids, cholesterol and free fatty acids. Two to seven bilayer shells	High loading of drugs
Transfersomes	Lipid supramolecular aggregates	More flexible for better transdermal delivery
Ethosomes	Phospholipids and alcohol in relatively high concentration	More distruptive in the skin lipid bilayer organization hence better transdermal delivery
Virosomes	Lipids surface modified with fusogenic viral envelope proteins	Intracellular delivery of antigens, drugs and DNA
Cryptosomes	Phospholipids and polaxamers or PEG	More stable
Emulsomes	Internal solid fat core surrounded by phospholipid bilayer	Better for encapsulation of hydrophobic drugs
Vesesomes	Multilamellar liposomes	Multidrug formulations
Genosomes	Complex of cationic phospholipids and a functional gene or DNA	Suitable for gene delivery

CONCLUSION

Liposomes are one of the classical specific drug delivery system, which can be used for controlled and targeted action. These systems can be administered through oral, parenteral as well as topical route. This wide range of selection of route of administration makes its flexible in designing the drug delivery system. Also these systems provide as an effective carrier for cosmetic formulations also. The liposome systems have been explored in the clinic for applications as diverse as sites of infection and imaging, for vaccine, gene delivery and small molecular drugs, for treatment of infections and for cancer treatment, for lung disease and for skin conditions etc. Several liposomal formulations are already on the market, while quite a few are still in the pipeline for treatment of diseases. Conventional techniques for liposome preparation and size reduction remain popular as these are simple to implement and do not require sophisticated equipment. The major problem in the formulation of liposome is its stability problem. These problems can be overcome by employing modification in the preparation method and also by using some

specialized carriers. Nowadays liposomes are used as carrier for wide variety of drugs. However, not all laboratory scale techniques are easy to scale-up for industrial liposome production. Many conventional methods, for preparing small and large unilamellar vesicles, involve use of either water miscible/immiscible organic solvents or detergent molecules. The need for improvements in the design and stability of liposomal diagnostic and therapeutic systems will continue to motivate innovative and efficient routes to their production.

REFERENCES

1. Farooque F, Wasi M, Mughees MM, Liposomes as Drug Delivery System: An updated review, Journal of drug delivery and therapeutics, 2021; 11(5): 149-158.
2. Liu, P. Chen, G. Zhang, J. A Review of Liposomes as a Drug Delivery System: Current Status of Approved Products, Regulatory Environments and Future Perspectives. Molecules, 2022; 27: 1372.
3. Roger R.C. New Chapter 1 Introduction, Liposomes: A Practical Approach, Edited by R. R. C. New, IRL Press at Oxford University press, 1990.
4. Shashi K., Satinder K, Bharat P. A Complete Review on Liposomes. International Research Journal of Pharmacy, 2012; 3(7): 10-16.
5. Jain N. K. Controlled and Novel Drug Delivery. CBS Publisher, 304-326.
6. Dua J.S, Rana A.C, Bhandari A.K. Liposome: Methods of Preparation and Applications. International Journal of Pharmaceutical Studies and Research, 2012; 3(2): 14-20.
7. Lombardo, D. Kiselev, M.A. Methods of Liposomes Preparation: Formation and Control Factors of Versatile Nanocarriers for Biomedical and Nanomedicine Application. Pharmaceutics, 2022; 14: 543.
8. Upendra Be, Sindhu D, Nagavendra K, Wahid K. Liposomal formulations in clinical use: An updated review. Pharmaceutics, 2017; 9(12): 1-33.
9. Anatoly NL Tamer AE. Ananthsrinivas RC, Vladimir PT. Tumor-targeted liposomes: Doxorubicin-loaded long-circulating liposomes modified with anti-cancer antibody. Journal of Controlled Release, 2004; 100: 135–144.
10. Sercombe L, Veerati T, Moheimani F, Wu SY, Sood AK, Hua S. Advances and Challenges of Liposome Assisted Drug Delivery. Front. Pharmacol, 2015; 6: 286.