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REVIW ARTICLE

LIPOSOMES AS TARGETTED DRUG DELIVERY SYSTEMS PRESENT AND FUTURE PROSPECTIVES: A REVIEW

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ABSTRACT

Liposomes are the leading drug delivery systems have played a significant role in the formulation of potent drug to improve therapeutic effect. The mechanism giving rise to therapeutic advantages of liposomes such as the ability of long circulating liposomes to preferentially accumulate at disease sites such as tumours, site of infection, and site of inflammation. There are several new methods of liposomes preparation based on lipid drug interaction and liposomes disposition mechanism including the incubation of rapid clearance of liposomes by controlling particle size, and surface hydration. The liposomes are characterised with respect to physical, chemical and biological parameters. Present applications of liposomes are in field of immunology, dermatology, vaccine adjuvant, eye disorder, and brain targeting therapy. This review would be helpful to the researches working in the area of liposomal drug delivery and educates how this success is being built on to design, effective carriers for genetic drugs.
Key Words: Liposome, drug carrier, targeted site, phospholipids, characterisation.

INTRODUCTION

Liposomes were first described by British haematologist Dr Alec D Bangham FRS in 1961, observed from electron microscope by adding negative stain to dry phospholipids. The name liposome is derived from two Greek words: 'Lipos' meaning fat and 'Soma' meaning body1When phospholipids are dispersed in water, they spontaneously form closed structure with internal aqueous environment bounded by phospholipid bilayer membranes, this vesicular system is called as liposome.[2]

Liposomes are micro-particulate or colloidal carriers, usually 0.05-5.0 µm in diameter which form spontaneously when certain lipids are hydrated in aqueous media [3]Liposomes are composed of relatively bio-compatible and biodegradable material, and they consist of an aqueous volume entrapped by one or more bilayers of natural and /or synthetic lipids. Drugs widely varying lipophilicites can be encapsulated in liposomes, either in the phospholipid bilayer, in the entrapped aqueous volume or at the bilayer interface. Liposomes have been investigated as carriers of various pharmacologically active agents such as antineoplastic and anti-microbial drugs, chelating agents, steroids, vaccines and genetic material [4]

Due to recent developments in liposome technology more effective strategies are now available for controlling the stability and reactivity of liposomes after systemic administration [5] On the basis of the ability of liposomes to interact with cells or blood components, at least two types of liposome currently can be designed including: 1) non-interactive sterically stabilised (long circulating) liposomes (LCL) and 2) highly interactive cationic liposomes. Sterically stabilised liposomes can be formulated by incorporating hydrophilic long chain polymers in the bilayer which can form a coat on the liposome surface and repel opsonin penetration and adsorption.

ADVANTAGES

- Provide controlled drug delivery
- Biodegradable, biocompatible, flexible
- Can carry both water and lipid soluble drugs
- Drugs can be stabilized from oxidation
- Improve protein stabilization
- Provide sustained release
- Targeted drug delivery or site specific drug delivery
- Alter pharmacokinetics and pharmacodynamics of drug

CLASSIFICATION OF LIPOSOMES

On The Basis Of Composition

Liposomes are composed of natural or synthetic lipids (phospho- and sphingo-lipids,) and may also contain other bilayer constituents such as cholesterol and hydrophilic polymer conjugated lipids. The net physiochemical properties of lipids composing the liposomes, such as membrane fluidity, charge density, steric hindrance, and permeability, determine liposomes interactions with blood components and other tissues after systemic administration. The nature and extent of liposomes –cell interaction in turn determines the mode of intracellular delivery of drugs. Thus, the predominant mechanism behind intracellular delivery of drugs by liposomes may mainly depend on their composition.

Liposomes can be classified in terms of composition and mechanism of intracellular delivery into five types as:

1. Conventional liposomes
2. pH sensitive liposomes
3. Cationic liposomes
4. Immune liposomes
5. Long circulating liposomes

On The Basis of Size

The liposome size can vary from small (0.025µm) to large (2.5 µm) vesicles. Furthermore, liposomes may have single multiple bilayer membranes. The vesicle size is a critical parameter in determining circulation half-life of

liposomes; both size and number of bilayers influence the extent of drug encapsulation in the liposomes. On the basis of their size and number of bilayers, liposomes can be classified into one of the categories:

1. Multi lamellar vesicles (MLV)

2. Large uni lamellar vesicles (LUV)

3. Small uni lamellar vesicles (SUV)

METHODS OF LIPOSOMES PREPARATION

Liposomes of different sizes and characteristics usually require different methods of preparation. The most simple and the widely used method for preparation of MLV is the thin film hydration procedure in which an aqueous buffer at a temperature of lipids. The drug to be encapsulated is included either in the aqueous hydration buffer (for hydrophilic drugs) or in the lipid film (for lipophilic drugs). Thin –film hydration method produces a heterogeneous population of MLV (1-5 diameter) which can be sonicated or extruded through polycarbonate filters to produce small (up to 0.025 m) and more uniformly sized population of SUV .

Although thin –film hydration is a simple technique, one of the major advantages of this method is its relatively poor encapsulation efficiency (5-15 %) of hydrophilic drugs moreover, reduction of liposomes further decreases the amount of encapsulated drug. MLV with high entrapment efficiency (up to 40%) can be prepared by freeze – drying performed SUV dispersion in an aqueous solution of the drug to be encapsulated [6]. The encapsulated efficiency of MLV can also be increased by hydrating lipid in the presence of organic solvent. [7]

Several methods have been developed for the preparation of large unilamellar vesicles (LUV), including solvent (either or ethanol) injection, detergent dialysis, calcium induced fusion, and reverse – phase evaporation (REV) techniques. SUV can be prepared from MLV or LUV by sonication (using probe sonicator) or extrusion (passage through a small orifice under high pressure).

Inthemethodsdescribedabove,anamphiphilicionizabledrugwhichexhibitslipophilicandhydrophilicpropertiesdependingonthepHofthesolutionmaynotbeencapsulatedwithhighefficiencybecausethedrugmoleculescandiffuseinandoutofthelipid membrane.Thus, thedrugwouldbedifficulttoretaininsideliposomes.However,thesetypeofdrugscanbeencapsulatedintopreformedliposomeswithhighefficiency(up to90%)usingthe‘activeloading’technique.8Inthe'activeloading’method,thepHintheliposomeinteriorissuchthattheunionizeddrugwhichentersthelipsomeby passivediffusionisionizedinsidetheliposome,andionizeddrugmoleculesaccumulateintheliposomeinteriorin highconcentrationsdueto theirinabilitytodiffuseoutthroughthelipidbilayer.Forexample,doxorubicin andepirubicinmaybeentrappedinpreformedSUVwithhighefficacyusing‘activeloading’methods[9-14].

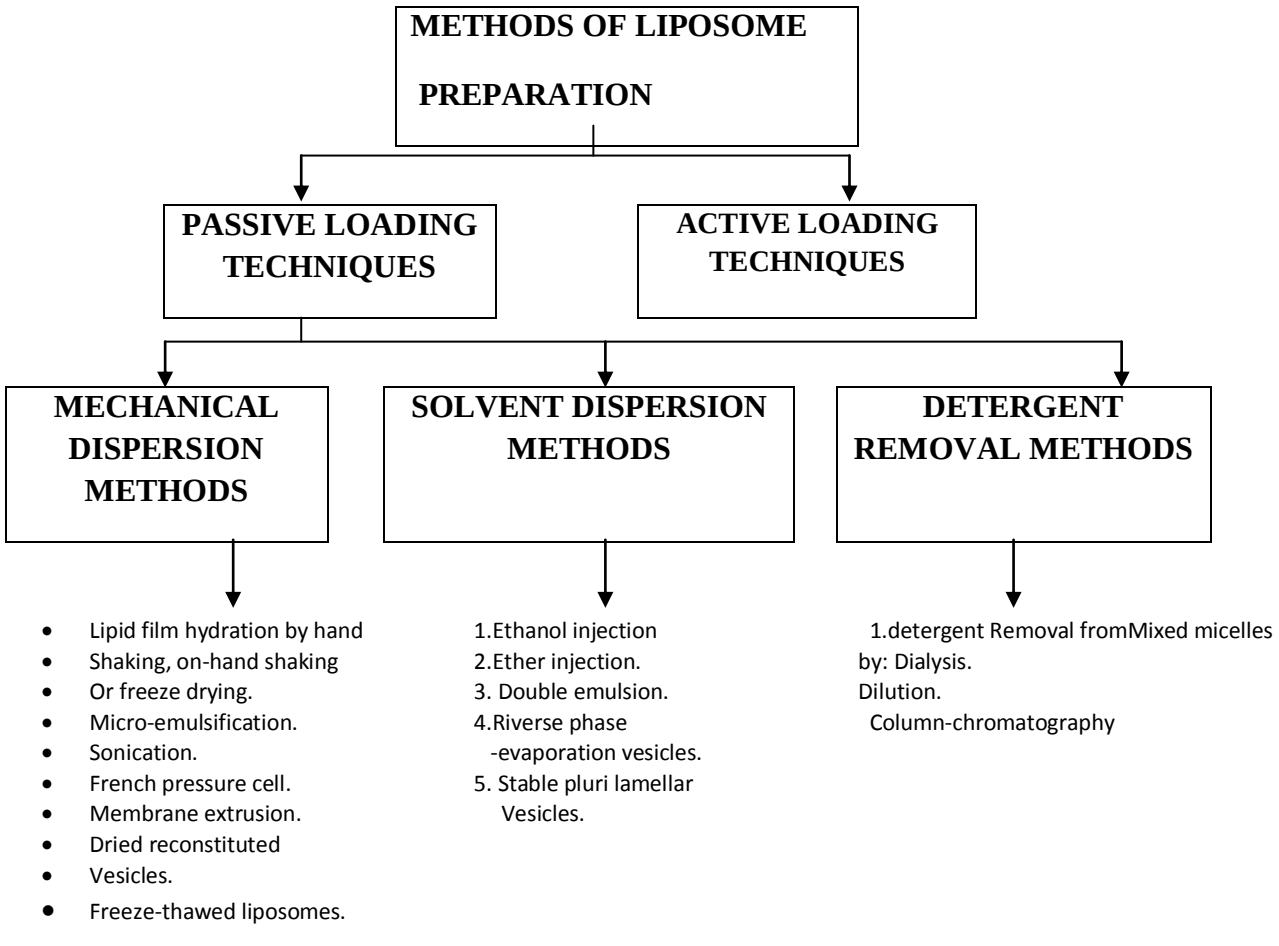

Figure 1: Shows Different schematic method of liposome preparations

I.MECHANICAL DISPERSION METHODS

1. Preparation of liposomes by lipid film hydration

Preparation of Lipid for Hydration

When preparing liposomes with mixed lipid composition, the lipids must first be dissolved and mixed in an organic solvent to assure a homogeneous mixture of lipids. Usually this process is carried out using chloroform or chloroform: methanol mixtures.

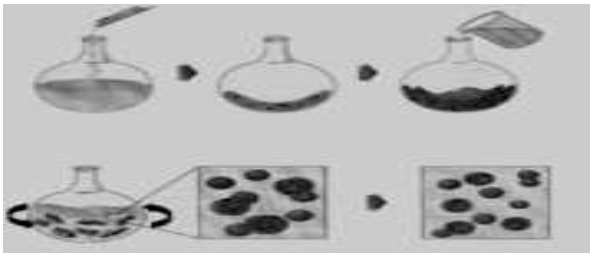

Figure 2: Liposomes prepared by thin layer evaporation technique.

The intent is to obtain a clear lipid solution for complete mixing of lipids. Typically lipid solutions are prepared at 10-20mg lipid/ml of organic solvent, although higher concentrations may be used if the lipid solubility and mixing are acceptable. Once the lipids are thoroughly mixed in the organic solvent, the solvent is removed to yield a lipid film. For small volumes of organic solvent (<1mL), the solvent may be evaporated using a dry nitrogen or argon stream in a fume hood. For larger volumes, the organic solvent should be removed by rotary evaporation yielding a thin lipid film on the sides of a round bottom flask. The lipid film is thoroughly dried to remove residual organic solvent by placing the vial or flask on a vacuum pump overnight. If the use of chloroform is objectionable, an alternative is to dissolve the lipid(s) in tertiary butanol or cyclohexane. The lipid solution is transferred to containers and frozen by placing the containers on a block of dry ice or swirling the container in a dry ice-acetone or alcohol (ethanol or methanol) bath. Care should be taken when using the bath procedure that the container can withstand sudden temperature changes without cracking. After freezing completely, the frozen lipid cake is placed on a vacuum pump and lyophilized until dry (1-3 days depending on volume). The thickness of the lipid cake should not be more than the diameter of the container being used for lyophilisation. Dry lipid films or cakes can be removed from the vacuum pump, the container should be closed tightly and taped, and stored frozen until ready to hydrate.[15]

2. Sizing of Lipid Suspension

Disruption of LMV suspensions using sonic energy (sonication) typically produces small, unilamellar vesicles (SUV) with diameters in the range of 15-50nm. The most common instrumentation for preparation of sonicated particles is bath and probe tip sonicators. Cup-horn sonicators, although less widely used, have successfully produced SUV. Probe tip sonicators deliver high energy input to the lipid suspension but suffer from overheating of the lipid suspension causing degradation. Sonication tips also tend to release titanium particles into the lipid suspension which must be removed by centrifugation prior to use. For these reasons, bath sonicators are the most widely used instrumentation for preparation of SUV. Sonication of an LMV dispersion is accomplished by placing a test tube containing the suspension in a bath sonicator (or placing the tip of the sonicator in the test tube) and sonicating for 5-10 minutes above the Tc of the lipid. The lipid suspension should begin to clarify to yield a slightly hazy transparent solution. The haze is due to light scattering induced by residual large particles remaining in the suspension. These particles can be removed by centrifugation to yield a clear suspension of SUV. Mean size and distribution is influenced by composition and concentration, temperature, sonication time and power, volume, and sonicator tuning. Since it is nearly impossible to reproduce the conditions of sonication, size variation between batches produced at different times is not uncommon. Also, due to the high degree of curvature of these membranes, SUV are inherently unstable and will spontaneously fuse to form larger vesicles when stored below their phase transition temperature

3. French Pressure Cell Method

The method involves the extrusion of MLV at 20,000 psi at 4°C through a small orifice. The method has several advantages over sonication method. The method is simple, rapid, and reproducible and involves gentle handling of unstable materials (Hamilton and uo, 1984). The resulting liposomes are somewhat larger than sonicated SUVs. The drawbacks of the method are that the temperature is difficult to achieve and the working volumes are relatively small (about 50 mL maximum). [16]

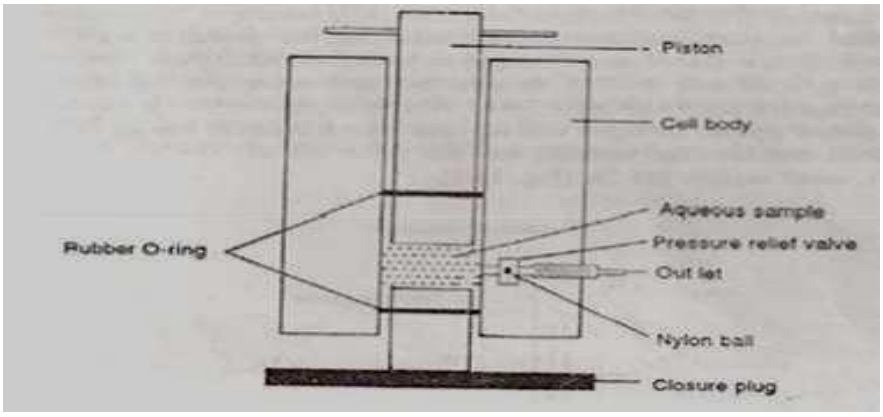

Figure 3: Liposomes prepared by French Pressure Cell Method

II. METHODS BASED ON REPLACEMENT OF ORGANIC SOLVENTS

In this method lipids are co-solvated in organic solution, which is then dispersed into aqueous phase containing material to be entrapped within the liposome. This method is of two types:

1. Reverse Phase Evaporation

The lipid mixture is added to a round bottom flask and the solvent is removed under reduced pressure by a rotary evaporator. The system is purged with nitrogen and lipids are re-dissolved in the organic phase which is the phase in which the reverse phase vesicle ill form. Diethyl ether and isopropyl ether are the usual solvents of choice. After the lipids are redissolved the emulsion are obtained and then the solvent is removed from an emulsion by evaporation to a semisolid gel under reduced pressure. Non encapsulated material is then removed. The resulting liposomes are called reverse phase evaporation vesicles (REV). This method is used for the preparation of large uni-lamellar and oligo-lamellar vesicles formulation and it has the ability to encapsulate large macromolecules with high efficiency. [17]

From observation of the phospatidylcholine cholesterol liposomes containing amphotericin B prepared by reverse phase evaporation method, the liposomes containing amphotericin B 2.0 mol % of total lipid demonstrated the highest percentage of drug entrapment, highest drug entrapment efficiency (approx. 95%) with particle size range of 1307- 1451 nm was obtained with the formulation containing 1:1 molar ratio of phosphatidycholine to cholesterol[18

2. Ether Vaporization Method

There are two methods according to the solvent used:

- Ethanol injection method.
- Ether injection method.

In ethanol injection method, the lipid is injected rapidly through a fine needle into an excess of saline or other aqueous medium. In ether injection method the lipid is injected very slowly trough a fine needle into an excess of saline or other aqueous medium.

III. METHODS BASED ON SIZE TRANSFORMATION OR

FUSION OF PREFORMED VESICLE

1. The Dehydration- Rehydration Method

In this method the empty buffer containing SUVs and rehydrating it with the aqueous fluid containing the material to be entrapped after which they are dried. This leads to a dispersion of solid lipids in finely subdivided form. Freeze drying is often the method of choice. The vesicles are than rehydrated. Liposomes obtained by this method are usually oligolamellar vesicle. [19]

Liposome prepared by the conventional thin film hydration technique, [20] the result showed that the formation of bi-layered liposomes in the particle size range of 0.2-0.8276 occurred with a maximum entrapment efficiency of 42.6% and the liposomes stored at 4-5^{o}C showed maximum stability as compared to those stored at any other temperature.

CHARACTERIZATION OF LIPOSOMES

Evaluation could be classified into three broad categories which are physical, chemical and biological methods. The physical methods include various parameters, which are size, shape, surface features, lamellarity phase behaviours and drug release profile.

Structural integrity of liposomal phospholipids membrane by a new technique of gamma-ray perturbing Sular correlation (PAC) spectroscopy. In this In-label diethyenetriaminepentaaceticacid (DTPA) derivative dipalmitoylphosphatidyl ethanolamine (DPPE) lipid were incorporated in the SUVs. This helped in the continuous non- invasive monitoring of the microenvironment of the lipid bilayer.

Quasi elastic light scattering (QELS) or photon correlate spectroscopic (PCS) technique for determining distribution of vesicles prepared by freeze-thaw extrusion method.[22] The influence of filter pore size, extrusion press and lipid concentration on the size and size distribution extruded vesicles was studied. Chemicalcharacterization includes those studies which established the purity and potency of various liposomal constituents.

Biological characterization is helpful in establishing the safety and suitability of formulation for the *in vivo* use for therapeutic application [23].The characteristics of the carrier through appropriate choice of membrane components, size and charge determines the final behaviour of liposomes both *in vitro* and *in vivo* as well[24,25] Characterized vesicles are obtained by repetitive extrusion through polycarbonate membranes using freeze fracture electron microscopy, small angle X-ray scattering (SAXS), and encapsulated volume using 6-carboxy fluroescein (6-CF) and 31p-NMR as aqueous markers. [26]

CHARACTERIZATION OF LIPOSOMES WITH THEIR QUALITY CONTROL ASSAYS [27-45]

Table 1: Biological characterization of Liposomes

Characterization parameters	Instrument for analysis
Sterility	Aerobic/anaerobic culture
Pyrogenicity	Rabbit fever response
Animal toxicity	Monitoring survival rats

Table 2: Chemical characterization of Liposomes

Characterization parameter	Instrument for analysis
Phospholipids concentration	HPLC/Barrlet assay
Cholesterol concentration	HPLC / cholesterol oxide assay
Drug concentration	Assay method
Phospholipids per oxidation	UV observance
Phospholipids hydrolysis	HPLC/ TLC
Cholesterol auto-oxidation	HPLC/ TLC
Anti-oxidant degradation	HPLC/TLC
PH	PH meter
Osmolarity	Osmometer

Table 3.Physical Characterization of Liposomes

Characterization parameter	Instrument for analysis
Vesicle shape, and surface morphology	TEM and SEM
Vesicle size and size distribution	Dynamic light scattering ,TEM
Surface charge	Free flow electrophoresis
Electrical surface potential and surfacePH	Zeta potential measurement and PHsensitive probes
Lamellarity	P31NMR
Phase behaviour	DSC, freeze fracture electron microscopy
Percent capture	Mini column centrifugation, gel exclusion
Drug release	Diffuse cell/ dialysis

STABILIZATION OF LIPOSOME

The stability of liposome should meet the same standard as conventional pharmaceutical formulation. The stability of any pharmaceutical product is the capabilities of the delivery system in the prescribed formulation to remain within defined or pre established limits for predetermined period of time. Chemical stability involves prevention of both the hydrolysis of ester bonds in the phospholipids bilayers and the oxidation of unsaturated sites in the lipid chain. Chemical instability leads to physical instability or leakage of encapsulated drug from the bilayers and fusion and finally aggregation of vesicles.[46]

The pro-liposome concept of liposome preparation was introduced to avoid physicochemical instability encountered in liposome suspension such as aggregation, fusion, hydrolysis, and/or oxidation.[47]

Approaches that can be taken to increase liposomal stability involve efficient formulation and lyophilisation. Formulation involves the selection of the appropriate lipid composition, concentration of bilayers, aqueous phase ingredients such as buffers, antioxidant, metal chelators and cryo protectants. Charge inducing lipid such as phosphotidyl glycerol can be incorporated into liposome bilayers to decrease fusion while cholesterol and sphingomyellin can be included in the formulation to decrease permeability and leakage of encapsulated drugs. Buffers at neutral pH can decrease hydrolysis; addition of antioxidant such as sodium ascorbate can decrease oxidation. Oxygen potential is kept to minimum during processing by nitrogen purging solution.[48]

In general successful formulation of stable liposomal drug product requires the following precautions:

1. Processing with fresh, purified lipids and solvents.

2. Avoidance of high temperature and excessive shear forces

3. Maintenance of low oxygen potential (Nitrogen purging)

4. Use of antioxidant or metal chelators

5. Formulating at neutral pH.

6. Use of lyo-protectant when freeze drying

APPLICATION OF LIPOSOMES

A. Liposome for Respiratory Drug Delivery System

Liposome is widely used in several types of respiratory disorders. Liposomal aerosol has several advantages over ordinary aerosol which are as follows [49]

1. Sustained release

2. Prevention of local irritation

3. Reduced toxicity and

4. Improved stability in the large aqueous core.

Several injectable liposome based product are now in the market including ambisome, fungisome and Mycoses. To be effective, liposomal drug delivery system for the lung is dependent on the following parameters:

1. Lipid composition

2. Size

3. Charge

4. Drug and Lipid ratio and

5. Method of delivery

Table 4: Liposomal Formulation for the Respiratory Disorder[50]

Active constituent	Effect
Insulin	Isoniazid and Rifampicin
Catalase	Conferred resistance to pulmonary oxygen toxicity
Super oxide dusmutase	Minimize toxicity to subsequent hyperoxia and improvedSurvival
Cyclosporins	Preferentially adsorbed by lung and shows sustainedRelease
Ricins vaccine	Improved safety profile for intra pulmonary vaccination
Interleukin-2	The lungs Facilitated bioactivity and reduce toxicity
Isoniazid and Rifampicin	Improved the effect of drugs for the tuberculosis

B. Liposomes for Brain Targeting drug delivery

The biocompatible and biodegradable behaviour of liposomes have recently led to their exploration as drug delivery system to brain. [51]Liposomes with a small diameter (100 nm) as well as large diameter undergo free diffusion through the Blood Brain Barrier (BBB). However it is possible that a small unilamellar vesicles (SUVS) coupled to brain drug transport vectors may be

transported through the BBB by receptor mediated or absorptive mediated transcytosis. Similarly, cationic liposomes which were developed recently showed these structures to undergo absorptive mediated endocytosis into cells. Whether cationic liposomes successfully undergo absorptive mediated transcytosis through the BBB has not yet been determined. The transport of substances through BBB by liposomes was extensively studied. The important finding issues from their studies are that the addition of the sulphatide (a sulphur ester of galactocerebroside) to liposome composition increases their several recent applications ability to cross BBB [52]. Liposomes coated with the mannose reach brain tissue and the mannose coat assist transport of loaded drug through BBB [53]. The neutropeptides, leu-enkephaline and mefenkephalinkyoforphin normally do not cross BBB when given systemically. The anti depressantamitriptylline normally penetrate the BBB, due to versatility of this method. Nanoparticles (NP) were fabricated with different stabilizers. It was found that amitriptyline level was significantly enhanced in brain when the substance was adsorbed onto the NP and coated or particle stabilized with polysorbate 85. [54]

C. Liposome in Tumour Therapy

The long term therapy of anticancer drug leads to several toxic side effect. The liposomal therapy for the targeting to the tumour cell have been revolutionized the world of cancer therapy with least side effect. It has been said that the small and stable liposome are passively targeted to different tumour because they can circulate for longer time and they can extra vasate in tissue with enhanced vascular permeability. [55, 56.]

NEW GENERATION LIPOSOMES

- Virosomes.
- Magnetic liposomes.
- ATP liposomes.
- Liposomes in photodynamic delivery.
- Cytoskeleton-specific immune-liposomes
- Liposomal haemoglobin.

CONCLUSION

In summary, this article reviewed the possible applications of liposomes and discussed in brief about some problems associated with formulation and development. Liposomes are prepared by various methods in which the most common methods applied for research purpose are film and dehydration rehydration methods. Stabilization of liposomes has been an area of concern for optimum shelf life of the liposomal formulation. Nowadays, stealth liposome (Pegylated liposome) is under development, with prolonged circulation and residence time in the body. The new developments in the liposome are the specific binding properties of a drug-carrying liposome to a target cell (tumour cell and specific molecules), stealth liposomes for targeting hydrophilic (water soluble) anticancer drugs like doxorubicin, Mitoxantrone which leads to decrease in side effects because the drug is mostly concentrated at the site of action. Other development is bisphosphonate-liposome mediated depletion of macrophages. Several commercial liposomes have already been discovered, registered and introduced with great success in pharmaceutical market. There is even greater promise in future for marketing of more sophisticated and highly stabilized liposomal formulations.

REFERENCES

1. Biology Pages, "Cell Membranes." Stryer S. Biochemistry, 1981, 213.
2. Grubber,S.M. Liposome Biophysics Therapeutics,Marcel Dekker: New York, 1987.
3 .Bangham, A.D.Horne, R.W Negative staining of phospholipids and their structural modification by surface- active agents as observed in the electronmicroscope. J. Mol. Biol. 8, 1964.660-668.
4. Gregoriadis G, Florence A.T., Liposomesin drug delivery: Clinical,diagnosticandophthalmicpotential.Drugs 45, 1993.15- 28
5. LasicD.D,PapahadjopoulosD Liposomes revisited Science267 1996. 1275-1276
6. Ohsawa T., Miura, H., Harada, K. A novel method for preparing liposome with a high capacity to encapsulate proteinous drugs: freeze-drying method. ChemPhaiin. Bull. 32, 1984 .2442 —2445
7. PapahadjopouD.Watkins, J.C.Phospholipids model membranes. Permeability properties of hydrated liquid crystals. Biochim.Biophys.Acta 1 35, 1967.639— 652
8. Clerc, S.Barenholz, Y.Loading of amphipathic weak acids into liposomes in response to transmembrane calcium acetate gradients. Biochim.Biophys.Acta 1240, 1995. 257—265
9.Mayer, L. D.Tai, L. C., Batty, M. B., Mitilenes, G.N.Gins- berg, R. S.Cullis,P. R., Characterization of liposomal systems containing doxorubicin entrapped in response to pH gradients. Biochim.Biophys.Actu 1025, 1990. 143— 15I
10. Mayhew, E. G.Lasic, D., Babbar, S., Martin, F.J.. Pharmacokinetics and antitumor activity of epirubicin encapsulated in long-circulating liposomes incorporating polyethylene glycol-derivative phospholipid Int.J Cancer 51, 1992.302 — 309.
11. Szoka, F Jr.Papahadjopoulos, D. Comparative properties und methods of preparation of lipid vesicles (liposomes). Ann. Rev. Biophys.Bioeng.9, 1980. 467-508
12. Deamer, D. and Uster, P., Liposome preparation methods and monitoring liposome fusion. In: Baserga, R., Croce, C. and Royeza, G. (Eds.), Introduction of Macromolecules into viable Mammalian Cells, Alan R. Liss, New York, 1980, pp. 205—220
13. Mayer, L. D.Bally, M. B., and Cull is, P. R. Uptake of Adriamycin into tune unilamellar vesicles in response to a pH gradient. Biochim.Biophys.Acta 857, 1986.123-126.
14. New, R.R.C., Preparation of liposomes. In: New, R.R.C. (Ed.), Liposomes: a practical approach, IRL Press, Oxford, 1990, pp. 33— 104
15. Danilo D. L. “Liposomes in Gene Delivery”. CRC press, 1997.
16. Riaz M.; “review: liposomes preparation methods,” Pak. J. Pharm. Sci.; 1996, 19, 65-77
17. Papahadjopoulos, D.Vali, W.J.Jacobson, K.;Poste,G.Biochim. Biophys.Acta, 1975, 394, 483.
18.Pleumchitt, R.; Narong, S.Korakot, C.Krisana, K. Drug Dev. Ind. Pharm.,2000, *29*(1), 31
19.Gregoriadis, G. Leathwood, P.D. Ryman, B.E. FEBSLett.1971, *19*, 95.
20. Bhalerao, S.S.; RajeHarshal, A. Drug. Dev. Ind. Pharm.,2003, *29*, 451.
21. Ma, W. Hwang, K.J.; Lee, V.H.L. Pharm. Res.1993, *10*(2), 252.
22. Kolchens, S.; Ram swami, V.; Birgenheier, J.Nett, L.O’Brien, D.F. Chem. Phys. Lipids,1993, *65*, 1.
23. Talsma, H.; Crommelin, D.J.A. Pharmaceut. Technol., 1992a, 16, 19
24 .Abra, R.M.; Hunt, C.A. Biochim.Biophys.Acta, 1981, 666, 493.
25. Jaroni, H.W.; Schubert, R.E.; Schmidt, K.H. Liposomes as Drug Carries,Georg thiemeVerlag: Stutgart, 1986.

26. Jousma, H.Talsma, H.Spies, F. Int. J. Pharm., 1987, 35, 263.
27. Lasic, D.D. Liposome Biophysics to Application, Elsevier: New York, 1993.
28. Lasic, D.D.Frederik, P.M.; Stuart, M.C.A.Barenholz, Y.McIntosh, T.J. FEBS Letts, 1992, 312(2, 3), 255.
29. Lasic, D.D.Papahadjopoulos, D. Medical Applications of Liposome, Elsevier: New York, 1998.
30 Lasic, D.D.Ceh, D.d.; Stuart, M.C.A.; Guo, L.Frederik, P.M. Barenholtz, Y. Biochim. Biophys.Acta, 1995, 1239, 15.
31. Lasic, D.D. Liposome in Gene Therapy, CRC press: Boca Raton, FL, 1997. Lasic, D.D. Trends Biotechnology.,1998, *16*, 307
32. Lasic D.D. Biochim. J., 1988, 29, 35
33. Ostro, M. J. Liposome Biophysics to Therapeutics, Marcel Dekker: New York, 1987.
34. Mandal, T.K.; Downing, D. T. ActaDerm. Venereol., 1993, 73, 12
35. Vyas, S.P.; Katare, Y.K.; Mishra, V.; Sihorkar, V. Int. J. Pharm., 2000, 210, 1
36. New, R.C. Liposomes a Practical Approach, IRL Oxford University Press: Oxford, 1990
37. Kolchens, S.; Ram swami, V.; Birgenheier, J.; Nett, L.; O'Brein, D.F. Chem. Phys. Lipids, 1993, 65, 1.
38. Allen, Z.; Tong, X.; Mangeed, P.; Lan, M.; Sydney, U.; Shahid, A.; Imran,Int. J. Pharm., 2004, 270, 93
39. New, R.R.C. Liposome a Practical Approach, OIRL press: Oxford, London, 1989.
40. Wiener, N.; Lieb, L. Medical Applications of Liposome, Elsevier:Oxford, 1998
41. Weiner, N.; Martin, F.; Riaz, M. Drug Dev. Ind. Pharm., 1989, 15, 1523.
42.Weiner, N.; Williams, N.; Birch, G.; Ramachandran, C.; Shipman, J. R.; Flynn, G. Antimicrob. Agents Chemother., 1989, 33, 1217.
43. Chamman, D. Quart. Rev. Biophys., 1975, 8, 185
44. Jones, G. R.; Cosins A.R. Liposome A Practical Approach, New, R.R.C Ed, OIRLS press: Oxford, 1989.
45 Grit, M.; Zuidam, N.J.; Crommelin, D.J.A. Liposome Technology, CRC press: Boca Raton, FL, 1993.
46. Chen, C.M.; Alli, D. J. Pharm. Sci., 1987, 76(5), 419.
47. Uster, P.S.; Deamer, D.W. Arch. Biochim. Biophys., 1981, 209, 385.
48. Mayer, L.D.; Cullis, P.R.; Balley, M.B. Medical Application of Liposome, Elsevier science BV: New York, 1998.
49. Jaroni, H.W.; Schubert, R.E.; Schmidt K.H. Liposomes as Drug Carries, GeorgthiemeVerlag: Stutgart, 1986.
50. Gursoy, A.; Kut, E.; Ozkirimi, S. Int. J. Pharm., 2004, 271, 115
51. McCauley, J.A.; Flory's Book.Mc Comb, T.G. Biochim. Biophys.Acta, 1992, 30, 112.
52. Rose, J.K..; Buoncore, L.; Whitt, M.A. Biotechniques, 1991, 10, 520.
53. Wang, H.S.; Toy, P.C. Biochim. Biophys.Acta, 2002, 15, 25.
54. Schroeder, U.; Somerfield, P.; Ulrich, S.; Sabel, B.A. J. Pharm.Sci., 1998, 87, 1305
55. Gabizon, A. Cancer Res.,1992a, *52*, 891.
56. Lawrence, M.; Jennifer, R.M.; Marcel, B. J. Pharm. Sci.,1998, *88*(1), S96.

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