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AN OVERVIEW ON LIPOSOMES AS A NOVEL DRUG DELIVERY SYSTEM

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ABSTRACT
The Liposomes are the sphere-shaped vesicles consisting of single or more bilayers of phospholipids. These can deliver both hydrophobic & hydrophilic drugs for the antibacterial, cancer, immunomodulation, antifungal, ophthalmic, diagnostics, enzymes, vaccines & genetic elements. The liposomes are characterized with respect to the physical, chemical & biological parameters. The multilamellar vesicle, the small unilamellar vesicle, and the large unilamellar vesicle are the major types of liposomes. Liposomes are biocompatible & biodegradable in nature. In this review article the mechanism, structural components, different methods of preparation, evaluation and applications of liposomes are explained.

Keywords: Liposomes, Structural components, Methods of preparation.

INTRODUCTION

The liposomes are concentric vesicles which are spherical in shape and it is derived from 2 Greek words lipos means fat & soma means body. In 1961 the Liposome were first made by Bangham et al, it was an accidental discovery in which he scattered in water the phosphatidyl choline molecule, during this he found that a molecule was forming the closed bilayer structure having an aqueous phase which were entrapped by the bilayer of lipid. The Liposome are very useful because they act as the carrier for the variety of drugs having the potential therapeutic action or other properties. The liposomes are the colloidal carriers having the size range of 0.01-5.0µm in diameter. The drug encapsulated by the liposome achieve the therapeutic level for longer duration as the drug must first be released from the liposome before the metabolism & excretion. These are small artificial vesicles which are spherical in shape which will be created from the cholesterol & natural phospholipids which are non-toxic. due to their size & hydrophilic & hydrophobic character [besides biocompatibility] the liposomes are the promising systems for the delivery of drug. To entrap drugs of both the aqueous & the lipid phase, is the unique ability of the liposomes & it makes them attractive drug delivery systems for the hydrophilic & hydrophobic drugs. The Liposomes are the novel drug delivery system that aims to deliver the drug directly to the site of action. To accommodate both hydrophilic and lipophilic compounds to protect the drug from degradation and release the active ingredients in a controlled manner, they have potential. It is found that glycerol is the backbone of the molecule that's why the phospholipid containing glycerol were found to be the essential component of the liposomal formulation & it represents 505 weights of lipid. [1-7]

THE STRUCTURAL COMPONENTS ARE

1] The Phospholipids:
The major structural components of liposome are the Phospholipids. The most common phospholipids used in liposomal preparation are the Phosphatidylcholine (PC). The Phosphatidyl-choline is the amphiphatic molecule which consist of:
a) The bridge of glycerol
b) The hydrophobic acyl hydrocarbon chains pair.
c) The polar head group which is hydrophilic, phosphocholine
The chemical structure of the naturally occurring Phosphatidylcholine has the glycerol moiety which is attached to 2 acyl chains which may be unsaturated or saturated. The stability of the liposome membrane depends on the packing of the hydrocarbon chains of a lipid molecules. The nature of the fatty acid in the lipid molecule, such as the number of double bonds in the chain is responsible for the bilayer properties such as the phase behavior & elasticity. The phospholipids are very abundant in nature & which contains the choline is used for the liposome's preparation. [8-10]
The examples of phospholipids are:
a) The Phosphatidyl ethanolamine[Cephalin]PE
b) The Phosphatidyl Glycerol [PG]
c) The Phosphatidyl serine [PS]
d) The Phosphatidyl choline [Lecithin] PC
2] Cholesterol

Another important structural component of liposome is the cholesterol. It is the commonly used sterol. The addition of the sterols modulates the function of the rigidity & stability. By itself It does not form the bilayer structure. In a very high concentration up to 1:1 or 2:1 molar ratio of cholesterol to phosphatidyl choline, it gets incorporated into phospholipids. The presence of the cholesterol in the lipid bilayer enhances the stability & form the highly ordered & rigid membrane structure. The cholesterol reduces the permeability of the water-soluble molecules & improves the fluidity & the stability of the biological membrane. By cholesterol the interaction & destabilization of the liposomes was prevented. [11-13]

CLASSIFICATION OF LIPOSOMES [14]

The Liposomes are classified on the basis of:

1. Structure.
2. Method of preparation.
3. Composition and application.
4. Conventional liposome.
5. Specialty liposome.

1. Classification Based on Structure

table-1. vesicle types with their size and number of lipid layers

Sr. No	Vesicle Type	Abbreviation	No Of Lipid Bilayer	Diameter Size
01	Unilamellar vesicle	UV	01	All size range
02	Small Unilamellar vesicle	SUV	01	20-100 nm
03	Medium Unilamellar vesicle	MUV	01	More than 100 nm
04	Large Unilamellar vesicle	LUV	01	More than 100 nm
05	Giant Unilamellar vesicle	GUV	01	More than 1 micro meter
06	Oligolamellar vesicle	OLV	Approx 5	0.1- 1 micro meter
07	Multilamellar vesicle	MLV	5-25	More than 0.5
08	Multi vesicular vesicle	MV	Multi compartmental structure	More than 1 micro meter

2. Based on Method of Preparation

table-2. different preparation methods and the vesicles formed by these methods

Sr. No	Method Of Preparation	Type Of Vesicle
01	Single or oligo lamellar vesicle made by reverse phase evaporation method	REV
02	Multi lamellar vesicle made by reverse phase evaporation method	MLV-REV
03	Frozen and thawed multi lamellar vesicle	FATMLV
04	Stable pluri lamellar vesicle	SPLV
05	Dehydration- Rehydration method	DR V
06	Vesicle prepared by extrusion technique	VET

3. Based on Composition and Application

table-3. different liposome with their compositions

Sr. No	Type Of Liposome Abbreviation Composition	Composition	Abbreviation
01	Fusogenic liposome	Reconstituted sendai virus envelops	RSVE
02	Conventional liposome	Neutral or negatively charge phospholipids and cholesterol	CL
03	Cationic liposome	Cationic lipid with DOPE	-
04	PH sensitive liposomes	Phospholipids such as PER or DOPE with either CHEMS or OA	-
05	Immuno liposome	CL or LCL with attached monoclonal antibody or recognition sequences	IL
06	Long circulatory liposome	Neutral high temp, cholesterol, and 510% PEG, DSP	LCL

4. Based Upon Conventional Liposome

table-4. based upon conventional liposome

Sr. No	Conventional Liposome
01	Synthetic identical, chain phospholipids
02	Stabilize natural lecithin (PC) mixtures
03	Glycolipids containing liposome

5. Based Upon Speciality Liposome

table-5. based upon speciality liposome

Sr. No	Speciality Liposomes
01	Antibody directed liposome.
02	Bipolar fatty acid
03	Lipoprotein coated liposome.
04	Methyl/ Methylene x- linked liposome.
05	Multiple encapsulated liposomes.
06	Carbohydrate coated liposome.

MECHANISM OF LIPOSOMAL FORMATION

The liposome encapsulates the region of the aqueous solution inside the hydrophobic membrane the dissolved hydrophilic solutes cannot readily pass through the lipids. The chemicals that are hydrophobic can be dissolved into the membrane & in this way liposome can carry both the hydrophilic molecules & hydrophobic molecules. With other bilayers such as the cell membrane, the lipid bilayer can fuse thus tranferring the contents of liposome to transfer the molecules to sites of action. Accordingly by making the liposomes in the solution of deoxyribonucleic acid or the drugs [which would normally be inadequate to diffuse through the membrane] they can be [indiscriminately] delivered past the bilayer of lipid. The liposomes are formed open hydration of lipid molecules normally the lipids are hydrated from the dry state [Thick or Thin film of lipid, spray dried powder] & stacks of the crystalline bilayers become fluid & swell myelin-long thin cylinders grow & upon agitation detach self-close in to the large multilameller liposomes because this eliminates the unfavourable interactions at the edges. When the large particles are formed, they could be either broken by the mechanical treatment in to a smaller bilayered fragments which close into the smaller liposomes. The size of the liposomes is very difficult to calculate in the budding off mechanism in the self-closing mechanism of bilayer depends on the size of the liposomes, the bending elasticity of a bilayer & the edge interactions of the fragments that are open. [15-16]

METHODS OF LIPOSOMES PREPARATIONS

Method:

On the following parameters the correct choice of liposome preparation method depends:

a] The nature of the medium in which a lipid vesicles are dispersed.

b] The physicochemical characteristics of a material to be entrapped & those of the liposomal ingredients.

c] The additional processes involved during the application/ delivery of the vesicles.

d] The possibility of large-scale production of safe and efficient liposomal products & batch-to-batch reproducibility. [17-18]

e] The optimum size, polydispersity & shelf-life of the vesicles for the intended application.

f] The entrapped substance's effective concentration & its potential toxicity.

The General Method of Preparation and Drug Loading

By using several procedures in which a water soluble [hydrophilic] materials are entangled by using the aqueous solution of these materials as hydrating fluid or by the addition of drug/drug solution at some stage during manufacturing of the liposomes, the liposomes are manufactured in majority. The lipophilic [lipid soluble] materials are solubilized in a organic solution of the constitutive lipid & then evaporated to the dry drug containing the lipid film followed by its hydration. These methods involve the loading of a entrapped agents before or during the procedure of manufacturing [Passive loading]. Hence after the formation of the intact vesicles [remote loading] certain type of compounds with ionizable groups & those which display both water & lipid solubility can be introduced into the liposomes.

THE MECHANICAL DISPERSION METHODS

The Preparation of liposomes by lipid film hydration:

Preparation of Lipid for Hydration:

The lipids must be first dissolved & mixed in a organic solvent to assure the homogeneous mixture of the lipids, when preparing liposomes with the mixed lipid composition generally this process is carried out using chloroform: methanol mixtures or chloroform. To obtain the clear lipid solution for the complete mixing of lipids is the intension behind doing this. The solutions of lipid are prepared generally at 10 to 20mg lipid/ml of the solvent that is organic however the higher concentrations may be used if the lipid solubility & mixing are acceptable. The solvent is removed to yield the lipid film once the lipids are thoroughly mixed in a organic solvent. The solvent may be evaporated using the dry nitrogen or the argon stream in the fume hood for small volumes of the organic solvent [<1mL]. The organic solvent should be removed by the rotary evaporation yielding the thin lipid film on the sides of the round bottom flask for the larger volumes. To remove the residual organic solvent the lipid film is thoroughly dried by placing a vial or the flask on the vacuum pump overnight. An alternative is to dissolve the lipid[s] in tertiary butanol or cyclohexane if the use of the chloroform is objectionable. The lipid solution is then transferred to the containers & frozen by placing the containers on the block of the dry ice or swirling the container in the dry ice-acetone or the alcohol [methanol or ethanol] bath. The care should be taken while using the bath procedure that the container may without cracking withstand sudden temperature changes. The frozen lipid cake is placed on the vacuum pump & lyophilized until dry [1-3 days depending on

volume] after freezing completely. The thickness of a lipid cake should not be more than the diameter of a container being used for the lyophilization. From the vacuum pump the dry lipid films or cakes can be removed the container should be closed tightly & taped & stored frozen until they are ready to hydrate. [19]

The Hydration of Lipid Film/Cake:

The hydration of a dry lipid film/cake is accomplished simply by adding the aqueous medium to a container of the dry lipid & agitating. The temperature of a hydrating medium should be above the gelliquid crystal transition temperature [Tm or Tc] of the lipid. The lipid suspension should be maintained above the Tc during the hydration period after addition of the hydrating medium. By transferring the lipid suspension to the round bottom flask & placing a flask on the rotary evaporation system without the vacuum, for high transition lipids this is easily accomplished. By spinning the round bottom flask in a warm water bath maintained at the temperature above the Tc of a lipid suspension allows the lipid to hydrate in its fluid phase with the adequate agitation. The hydration time may differ slightly among the lipid species & structure however the hydration time of one hour with vigorous stirring, shaking or mixing is recommended highly. It is also believed that by allowing the vesicle suspension to stand overnight [aging] prior to the downsizing makes the process of sizing easier & improves the homogeneity of a size distribution. For the high transition lipids, the aging is not recommended as the lipid hydrolysis increases with the elevated temperatures. By the application of the lipid vesicles the hydration medium is generally determined. The suitable hydration media include the buffer solutions, distilled water, non-electrolytes such as sugar solutions & saline. The 0.9% saline, 5% dextrose and 10% sucrose are the generally accepted solutions which meet these conditions. Some lipids form complexes unique to their structure during the hydration. When hydrated with low ionic strength solutions the highly charged lipids have been observed to form the viscous gel. By addition of salt or by downsizing the lipid suspension the problem can be alleviated. The poorly hydrating lipids such as the phosphatidylethanolamine have the tendency to self-aggregate upon the hydration. The lipid vesicles containing more than the 60 mol% of phosphatidylethanolamines form the particles having the small hydration layer surrounding the vesicle. There is no hydration repulsion to repel an approaching particle as the particles approach one another & the two membranes fall into an energy well where they adhere & form the aggregates. The aggregates settle out of the solution as large flocculates which will disperse on the agitation but reform upon sitting. A product of the hydration is the LMV [large multilamellar vesicle] analogous in structure to an onion with each lipid bilayer separated by the layer of water. A spacing between the lipid layers is dictated by the composition with the poly-hydrating layers being closer together than the highly charged layers which separates on electrostatic repulsion. Once the stable, hydrated large multilamellar vesicle suspension has been produced the particles can be downsized by the variety of techniques including the extrusion or sonication.

The Sizing of Lipid Suspension:

Sonication:

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

The French Pressure Cell Method:

This method involves the extrusion of the MLV at 20,000 psi at 4°C through the small orifice. This French pressure cell method has various advantages as compared to the sonication method. The method involves gentle handling of unstable materials (Hamilton and Guo, 1984) & is reproducible, simple & rapid. As compared to the sonicated SUVs the resulting liposomes are a bit larger. The disadvantage of the method is that the temperature is difficult to achieve & the working volumes are small relatively [about 50 mL maximum]. [20]

THE SOLVENT DISPERSION METHODS

The Ether Injection Method:

The solution of the lipids dissolved in the ether/methanol or diethyl ether mixture is injected slowly to the aqueous solution of a material to be encapsulated at the 55 to 65°C or under the reduced pressure. The subsequent removal of the ether under vacuum leads to a formation of the liposomes. The main disadvantage of this method are population is heterogeneous [70 to 190 nm] & the exposure of the compounds to be encapsulated to the organic solvents or the high temperature. [21] The ethanol injection method the lipid solution of the ethanol is rapidly injected to the vast excess of the buffer. Immediately the MLVs formed. The disadvantage of the method is that the population is heterogeneous [30 to 110 nm] the liposomes are very dilute it is difficult to remove all th ethanol because it forms the azeotrope with the water & the possibility of the various biologically active macromolecules to inactivation in the presence of even low amounts of the ethanol.

The Reverse Phase Evaporation Method:

The First water in oil [W/O] emulsion is formed by the brief sonication of the two-phase system containing the phospholipids in the organic solvent [mixture of isopropyl ether & chloroform or diethylether or isopropylether] & the aqueous buffer. The organic solvents are removed under the reduced pressure which resulted in the formation of the viscous gel. By continued rotary evaporation under reduced pressure the liposomes are formed when the residual solvent is removed. By this method the high encapsulation efficiency up to the 65 percent can be obtained in the medium of the low ionic strength, for eg: 0.01M NaCl. To

encapsulate small & large macromolecules the method has been used. The main drawback of the method is that the exposure of the materials to be encapsulated to the organic solvents & too brief periods of the sonication. [22]

THE DETERGENT REMOVAL METHOD:

At their critical micelles concentrations, the detergents have been used to solubilize the lipids. The micelles become progressively richer in phospholipid & finally combine to form the LUVs, as the detergent is removed. By dialysis the detergents can be removed. The merits of the detergent dialysis method are the excellent reproducibility & production of the liposome populations which are homogenous in the size. The main disadvantage of the method is the retention of the traces of the detergent[s] within a liposome. The commercial device called as LIPOPREP [Diachema AG, Switzerland] which is the version of the dialysis system is available for removal of the detergents.

For the removal of detergents, the other techniques have been used are:

[i] by binding of the octyl glucoside [a detergent] to Amberlite XAD-2 beads.

[ii] by using the gel chromatography involving the column of the Sephadex G25 [23]

[iii] by adsorption or binding of theTriton X-100 [a detergent] to Bio-Beads SM-2 [24-25]

CHARACTERIZATION OF LIPOSOMES:

For a specified purpose the liposomal formulation & the processing are characterized to ensure their predictable in vitro & in vivo performance. For the purpose of evaluation, the characterization parameters could be classified into the 3 broad categories, which include the physical, chemical and biological parameters.

a] The physical characterization evaluates various parameters which includes the shape, size, drug release profile, surface features & lamellarity phase-behaviour.

b] The chemical characterization includes those studies that establish the potency & purity of the different lipophillic constituents.

c] In establishing the safety & suitability of formulation for therapeutic application the biological characterization parameters are helpful.

1 Some of parameters are:

The vesicle shape & lamellarity vesicle shape can be assessed by using the electron microscopic techniques. The lamellarity of the vesicles i.e., the number of the bilayers presents in the liposomes is determined by using & P31 Nuclear Magnetic Resonance Analysis & Freeze Fracture Electron Microscopy.

2 The vesicle size & size distribution:

For determination of the size & size distribution the various techniques are described in the literature. These include the Fluorescent Microscopy, Light Microscopy, Laser light scattering Photon correlation Spectroscopy, Electron Microscopy [Transmission Electron Microscopy], Field Flow fractionation, Gel Exclusion & Gel Permeation. Electron Microscopy is the most precise method of determine size of liposome. It is very time consuming & require the equipments that may not always be immediately to hand. In contrast the laser light scattering method is very simple & is rapid to perform but having drawback of measuring an average property of the bulk of liposomes. The other more recently developed microscopic technique known as the atomic force microscopy has been utilized to study the size, morphology & stability of the liposome. [23] The most of the methods used in the size, shape & distribution analysis can be grouped into the various categories namely the diffraction, microscopic, hydrodynamic techniques & scattering.

3 The Microscopic Techniques

Optical Microscopy

This method includes the use of the Bright- Field, Fluorescent Microscope & Phase-Contrast Microscope and is useful in evaluating the vesicle size of the large vesicles.

Cryo-TEM [Cryo-Transmission Electron Microscopy Techniques]

To elucidate the surface morphology and size of vesicles this technique has been used.

Diffraction & Scattering Techniques

The Laser Light Scattering Photon correlation spectroscopy [PCS]

Due to the Brownian motion of particles in solution/suspension it is the analysis of time dependence of the intensity fluctuation in scattered laser light. As the particles those are small diffuse more quickly than those particles which are large in size the rate of fluctuation of the intensity of the scattered light varies accordingly. Hence the translational diffusion coefficient [D] can be measured which in turn can be used to determine a mean hydrodynamic radius [Rh] of the particles by using the Stoke-Einstein equation. One can measure particles in range of about 3nm by using this technique.

4 The Hydrodynamic Techniques

The hydrodynamic techniques includes the gel permeation & ultracentrifuge. The exclusion chromatography on the large pure gels was introduced to separate the SUVs from the radial MLVs. However, the large vesicles of 13µmdiameter usually fail to enter into the gel & are retained on the top of the column. The thin layer chromatography system using agarose beads has been introduced as the fast technique, convenient for obtaining a rough estimation of the size distribution of the liposome preparation. However it was not reported if this procedure was sensitive to the physical blockage of the pores of an agarose gel as is the more conventional column chromatography.

5 Encapsulation Efficiency

To Determine the rate & amount of entrapment of the water-soluble agents in the aqueous compartment of the liposome. [26]

Advantages Of Liposome

1] The liposomes hold the normally immiscible materials together in the microsphere with controllable release of the encapsulated ingredients.

2] Liposomes deliver drug to the site of action.

3] Liposomes carry both hydrophilic & hydrophobic drugs.

4] Liposomes are biodegradable.

5] Liposomes are nontoxic.

6] They are controlled drug delivery system.

7] They Prevent Oxidation [15,27]

Disadvantages Of Liposomes

1] Liposomes have Low solubility.

2] They have short half life
3] Their Production cost is high [28].
4] The leakage and fusion of encapsulated drug may occur.
5] The oxidation of phospholipids may occur [29].
6] They are less stable.

Marketed Formulations of Liposomes

The first liposome DDS approved for human use by the US FDA was Doxil (PEGylated liposome-encapsulate doxorubicin) in the year 1995. There was list of marketed formulations of liposomes. [30]

table-6. marketed formulations of liposomes

Sr. No	Drug	Product Name	Company
01	Amphotericin B	Abelcet	The Liposome company N.J.
02	Doxorubicin	Doxil	Sequus pharmaceuticals Inc.CA
03	Amphotericin B	Ambisome	NeXstar pharmaceuticals Inc.CO
04	Amphotericin B	Amphocil	Sequus pharmaceuticals Inc.CA
05	Amikacin	Mikasome	NeXstar pharmaceuticals Inc.CO
06	Daunorubicin	DaunoXome	NeXstar pharmaceuticals Inc.CO
07	Lidocaine	ELA max	Biozone Labs, CA, USA
08	Doxorubicin	DC99	Liposome CO., NJ, USA
09	Hepatitis A vaccine	Epaxel	Swiss Serum Institue, Switzerland

Stabilization of The Liposome

The stability of the liposome should meet the same standards as that of conventional pharmaceutical formulation. The stability of any pharmaceutical product is the capabilities of a delivery system in a prescribed formulation to remain within the defined or the pre-established limits for the predetermined period of the time. The chemical stability involves the prevention of both the oxidation of the unsaturated sites in the lipid chain & hydrolysis of the ester bonds in the phospholipid's bilayers. The chemical instability leads to the physical instability or the leakage of the encapsulated drug from the bilayers & fusion & finally the aggregation of vesicles. [31]

To avoid physicochemical instability encountered in the liposome suspension such as the fusion, aggregation, hydrolysis &/or oxidation, Chen et al. introduced the pro-liposome concept of the liposome preparation. [32] To increase the liposomal stability the approaches that can be taken which include efficient formulation & lyophillization. The formulation involves the selection of a appropriate lipid composition, concentration of the bilayers, the aqueous phase ingredients such as the buffers, antioxidant, metal chelators & cryo protectants. To decrease the fusion the charge inducing lipid such as the phosphotidyl glycerol can be incorporated into the liposome bilayers, to decrease the permeability & leakage of the encapsulated drugs the cholesterol & sphingomyellin can be included in the formulation. The buffers at neutral pH can decrease the hydrolysis; the addition of the antioxidant such as sodium ascorbate can decrease the oxidation. The oxygen potential is kept to minimum during the processing by nitrogen purging solution. [33]

Generally, for the successful formulation of the stable liposomal drug product, following precautions are required:
1] The processing with fresh, purified lipids & solvents.
2] When freeze drying, Use of lyo-protectant.
3] Use of metal chelators or antioxidant.
4] Avoidance of high temperature & excessive shear forces
5] Formulating at neutral pH.
6] Maintenance of low oxygen potential [Nitrogen purging]

APPLICATIONS OF LIPOSOME

The current deepening & widening of interest in the liposomes in many scientific disciplines & their applications in diagnostics, cosmetics, medicine, immunology, cleansing, ecology & the food industry are promising novel breakthrough & products. Over last 30 years the field of the liposome research has expanded considerably. For a wide range of application, it is now possible to engineer a wide range of liposome of varying size, composition, phospholipids, cholesterol composition, surface morphology suitable. [34] In many ways Liposomes interact with the cells to cause the liposomal components to be associated with the target cells. The liposome carrier can be targeted to liver & spleen & with the help of tomography distinction can be made between the normal & tumors tissue. Liposome has the great application In case of the TDDS [transdermal drug delivery system]. The liposomal drug delivery system when used to target a tumor cell leads to the reduction in the toxic effect & increases the effectiveness of the drugs. By the attachment of the amino acid fragment such as the antibody or the protein or appropriate fragments that target the specific receptors cell the targeting of the liposome to the site of action takes place. The liposomal DNA delivery vectors & further enhancement in the form of LPDI -I & LPD-II are some of the safest & potential most versatile transfer vectors which are used to date. The improved capability of the gene therapy & deoxyribonucleic acid vaccination are some of the hardly recent application of the liposome.

Different modes of drug delivery applications have been purposed for the liposomal DDS, some of them are as below:
a. The altered pharmacokinetics & bio-distribution [sustained or prolonged released drugs with short circulatory half-life].
b. To Enhance drug solublisation [Minoxidil, Amphotericin-B, Cyclosporins & Paclitaxels]
c. To Enhance intracellular uptake [Antimicrobial, Anticancer & antiviral drugs]
d. The protection of sensitive drug molecules [DNA, Cytosine arabinosa, Anti-sense oligo-nucleotides, Ribozymes, RNA]

The recent applications of liposomal drug delivery system are as follows:

In Anticancer Therapy

Long term therapy of the anticancer drug leads to the several side effects which are toxic. For the targeting to the tumour cell the liposomal therapy have been revolutionized the world of cancer therapy with side effects which are least. The stable & small liposome are targeted passively to the different tumour because for longer time they can circulate & they can extra vasate in tissue

with the enhanced vascular permeability. The liposome macrophage uptake by liver & spleen hampered the development of the liposome as the drug delivery for over 20 years. The Doxil is the liposomal formulation of the doxorubicin, intravenous, chemotherapeutic agent. The Doxil is prepared by a new technology called stealth technology stealth liposome. These are the long circulatory liposome which are prepared by several means. The myocet & caelyx are the liposomal formulations of the doxorubicin. For the treatment of metastatic ovarian cancer, the caelyx is used but now it is used in advanced breast cancer. For metastatic breast cancer the myocet's approved. [35-39]

For Respiratory Drug Delivery System

In several types of the respiratory disorders the liposome is widely used. Over ordinary aerosol the liposomal aerosol has several advantages which are as follows:

1] Reduced toxicity.
2] Sustained release.
3] Improved stability in the large aqueous core.
4] Prevention of local irritation.

In the market several injectable liposome-based products are now available including Myocet, ambisome, Fungisome. To be effective the liposomal drug delivery system for the lung is dependent on the following parameters:

a] Method of delivery
b] Lipid composition
c] Drug and Lipid ratio and
d] Charge
e] Size

The recent use of the liposome for the delivery of the DNA to the lung means that the greater understanding of their use in the macromolecular delivery via inhalational is now emerging. In the development of the liposome-based protein formulations, much of this new knowledge, which includes new lipids & the analytical techniques can be used. The dry form or the liquid is taken for the inhalation of the liposome & during nebulization the release of drug occurs. The drug powder liposome has been produced by spry drying or by milling. [40]

In Nucleic Acid Therapy

The recombinant DNA technologies & studies of the gene function & gene therapy all depends on the successful delivery of the nucleic acid into cells in vivo & in vitro. For the selective delivery of the gene to the malignant cells the non-viral vectors will be developed. The vector will exploit the increase requirements of the rapidly growing cells for the more nutrients by attaching the nutrients ligand onto the vector [liposome]. This vector additionally will have the passively charged lipid to enhance nucleic acid binding along with the novel pH sensitive surfactants. The role of the surfactants is to increase the amount of the nucleic acid escaping the endosome & correspondingly increase the transfection efficiency. In an animal model of cancer, the vector system will be evaluated first. The lipid-based gene delivery is the focus of the several specialized high technology companies of which Vical [San Diago, USA, CA], Genzyme [MA, Farmington, USA] & Megabios [USA Burhingam, CA] have products in the clinical trials. The preliminary studies will be carried out by using the marker gene [BETA GALCTOSIDASE] with later experiments using the gene encoding for the cytosine deaminase. The cytosine deaminase can catalyze the conversion of a innocuous agent 5- Flourocytosine to a anticancer agent 5- Fluorouracil. By the selective delivery of this gene only to the cancer cells, the therapeutic index of the 5-Flourouracil can then be increased. The some of the engineered liposomal & non liposomal versions like the pH sensitive anionic & cationic liposome, fusagenic liposome, pH sensitiveimmuno-liposome, lipofections, genosomes, & recently cochleats are being investigated as a major gene vectors. This agent will become activated at the endosomal pH has the membrane disrupting effects & will be inactivated before reaching the lysosome. The surfactant must enter the cell by endocytosis for this agent to work. The soft surfactant will be incorporated into the liposome that has shown to be entering the cell in this manner. The novelty of this delivery system stems from the SPS [soft pH sensitive surfactants]. For the cytosolic delivery of DNA, the pH sensitive liposomes have been reported as the plasmid expression vectors. For the intracellular trafficking of anti-sense oligo-nucleotides it is also effective carrier. [41-44]

In Opthalmic Disorders

For the ophthalmic drug delivery the liposomes have been investigated since it offers advantages as a carrier system. It is the biocompatible & biodegradable nanocarrier. It can enhance the permeation Of the poorly absorbed drug molecules by binding to the corneal surface & improving the residence time. It can encapsulate both the hydrophobic & hydrophilic drug molecules. In addition the liposomes can enhance therapeutic effect, improve the pharmacokinetic profile & reduce the toxicity associated with the higher dose. The liposomes have been widely investigated for the treatment of both anterior and posterior segment eye disorders, Owing to their versatile nature. The current approaches for the anterior. Segment of the drug delivery are focused on improving the corneal adhesion & permeation by incorporating the various bioadhesive & penetration enhancing polymers. Hence in the case of the posterior segment disorders the improvement of the intravitreal half-life & targeted drug delivery to the retina is necessary. In photodynamic therapy for the treatment of subfoveal choroidal neovascularization [CNV], pathological myopia, ocular histoplasmosis effectively, currently verteporfin is being used clinically. The verteporfin is the light-activated drug which is administered by the intravenous infusion. After the drug is injected in the photodynamic therapy the low-energy laser is applied to a retina through the contact lens in order to activate the verteporfin that results in the closure of a abnormal blood vessels. Unfortunately the photodynamic therapy usually does not permanently close the abnormal vessels & after several months the choroidal neovessels reappear. For the treatment of age-related macular degeneration another liposomal photosensitizing agent rostaporfin was evaluated. Now it is under phase three clinical trial. The rostaporfin requires less frequent administration as compared to the verteporfin. For the ophthalmic drug delivery the liposome technology has been explored. There are some issues to be addressed such as the formulation & storage of the liposomes is very difficult & they are known to cause the long-term side effects. The intravitreal administration of the liposomes has resulted in the vitreal condensation, the vitreal bodies in the lower part of the eye & retinal abnormalities. Therefore, while developing the liposomal formulation for the ophthalmic application all these factors should be taken into account. [45-49]

As Vaccine Adjuvant

The liposome has been firmly established as the immuno-adjuvant potentiating both the cell mediated & the non-cell mediated [humoral] immunity.

By the following therapeutic points of view the liposome acts as the immuno-adjuvant:
a] Liposomes as a tool in immuno diagnostics.
b] Liposomes as an immunological (vaccine) adjuvant.
c] Liposomes as carrier of immuno modulation.
d] Liposomal vaccines.

By slowly releasing the encapsulated antigen on the intramuscular injection & also by passively accumulating within the regional lymph node the liposomal immuno-adjuvant act. By the targeting of liposome with the help of the phosphotidyl serine the accumulation of liposome to lymphoid is done. The liposomal vaccine can be prepared by inoculating the microbes, soluble antigen, cytokinesis of deoxyribonucleic acid with the liposome. The latter stimulating an immune response on expression of the antigenic protein. The antigens can be covalently coupled to liposomal membrane. The liposomes which are encapsulating antigen are second time encapsulated within the alginate lysine microcapsules to control the release of antigen & improve the antibodies responses. For about twelve months the liposomal vaccines can be stored at the refrigerated condition. [50-52]

For Brain Targeting

The biodegradable & biocompatible behaviour of the liposomes have recently led to their exploration as the drug delivery system to the brain. The liposomes with the small diameter [100 nm] as well as the large diameter undergo the free diffusion through the BBB [Blood Brain Barrier]. It is possible that the SUVS [small unilamellar vesicles] coupled to the brain drug transport vectors may be transported through a blood brain barrier by the receptor mediated or the absorptive mediated trans-cytosis. Similarly, the cationic liposomes which were recently developed showed these structures to undergo the absorptive mediated endocytosis into the cells. Whether the cationic liposomes successfully undergo the absorptive mediated transcytosis through a blood brain barrier has not yet been determined. The transport of the substances through the blood brain barrier by the liposomes was studied extensively. From their studies the important finding issues are that, the addition of the sulphatide [a sulphur ester of the galactocerebroside] to the liposome composition increases their several recent applications ability to cross the blood brain barrier. The liposomes coated with the mannose reach brain tissue and the mannose coat assist transport of loaded drug through blood brain barrier was reported by Wang et al. When given systemically the leu-enkephaline, neutropeptides, & mefenkephalin kyoforphin normally do not cross blood brain barrier. Due to the versatility of this method, the anti-depressant amitriptylline normally penetrate the blood brain barrier. With different stabilizers the nanoparticles were fabricated. It was found that level of amitriptylline was significantly enhanced in the brain when the substance was adsorbed onto the nanoparticles & coated or the particle are stabilized with the polysorbate 85. [53-56]

As Anti-Infective Agents

In the liver & spleen the Intracellular pathogen like bacterial, protozoal & fungal reside & the therapeutic agent may be targeted to these organs using liposome as vehicle system thus to remove these pathogen. The disease like candidiasis, leishmaniasis, histoplasmosis, aspergelosis, gerardiasis, erythrococosis, tuberculosis & malaria are targeted by the respective therapeutic agent by using the liposome as a carrier. [57-59]

CONCLUSION

For the targeted drug delivery the Liposomes have been realized as extremely useful carrier systems. Many factors contribute to the success of liposomes as drug delivery vehicles. The liposomes solubilise the lipophillic drug candidates that would otherwise be difficult to administer intravenously. The drug which is encapsulated is inaccessible to metabolizing the enzyme, conversely, body component such as the erythrocyte & tissue injection site are directly not exposed to the full dose of the drug. The liposomes can cross the BBB [Blood brain barrier] because of the phospholipids lipophillic nature, so even the drugs which are hydrophilic [which otherwise cannot easily cross the Blood brain barrier] might be formulated as liposomes. By slowly delivering the drug in the body the liposome can extend the action of drug. The targeting option change the distribution of the drug in the body. As an adjuvant they can be used in formulation of vaccine. The use of the liposomes in the delivery of drugs & genes are promising & is sure in future to undergo further developments.

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