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Reviewon - "Liposomes as A Drug Delivery System"

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ABSTRACT
Liposomes are becoming increasingly popular as drug carriers because of their versatility. Among the controlled drug delivery systems employed in cancer therapy, these are the most extensively studied carriers. Drugs and other pharmaceuticals encapsulated in liposomes have higher efficacy than conventional formulations due to their effective protection from external conditions and prolonged and site-specific administration. The pharmacodynamics and pharmacokinetics features of the liposomal delivery method are adjusted, resulting in a higher therapeutic index with lower toxicity. Their manufacture is done in a variety of ways, ranging from lab size to industrial trial scale. Liposomes are also divided into categories based on their composition, preparation methods, size, and application. Before being tested in vivo, the liposomal formulations are evaluated in vitro for a variety of properties. In this study, we'll go over the different types of liposomes, their compositions, properties, different methods of preparation, and significant assessment metrics, as well as their applications in drug delivery research. We feel that this succinct study will be useful in gaining a basic understanding of liposomal delivery systems as well as some current ideas.

Keywords: Liposomes, Classification, Drug delivery systems, Methods of preparation of liposomes.

I. INTRODUCTION[7, 16]

Liposomes bilayer vesicles with concentric lipid bilayers are composed primarily of natural or synthetic phospholipids. Liposomes are biocompatible and decomposing particles that can contain both hydrophilic and lipophilic compounds in one place. The word liposome comes from two Greek words: 'Lipos,' meaning fat, and 'Soma,' meaning body. Liposomes can be made in various sizes, unilamellar or multilamellar.. Drugs can be confined to liposomes in phospholipid bilayers, in a sealed water volume, or in a bier interface. Naturally, liposomes are biocompatible. This feature makes them one of the most attractive options for construction scientists. They can hold both hydrophilic and hydrophobic drugs in their internal water and membranes. The ineffective effect of the external environment is avoided when drugs are contained in liposomes. They also transport medicines to cells and individual cell compounds with a continuous release profile and site-specific delivery. It is possible to change the size, charge, and more features of liposomes by using different production techniques and adding additional ingredients to the lipid mixture before liposome synthesis.

The development of long-acting secret liposomes and functional liposomes with improved in vivo structures is now the focus of current research. Additionally, research trials are currently underway to develop custom-made liposomes targeting specific antigens or indicators for more accurate drug delivery.

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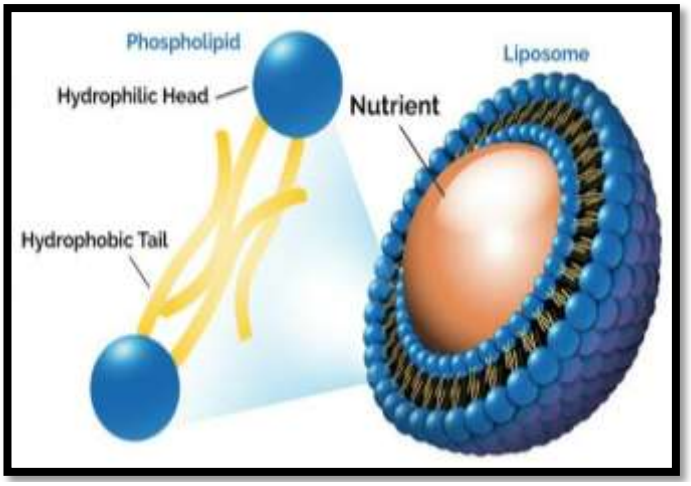

Figure 1Design of Liposomes

II. STRUCTURAL COMPONENTS[1-7]

1)Phospholipids

Glycerol-containing phospholipids are the most widely used ingredient in liposome formation and represent more than 50% of the lipid weight in the biological membrane. These are found in Phosphatidic acid.The glycerol moiety is the back bone of the molecule. In C, the OH group is esterified to phosphoric acid. OH at C_1& C_2 reinforced with long series. Fatty acids create a lipid environment. One of the remaining OH groups of phosphoric acid may remain esterified in a wide variety of organic alcohols including glycerol, choline, ethanolamine, serine and inositol. Thus the parent compound of the series is the phosphoric ester of glycerol**.**

Examples of phospholipids are

- Phosphatidyl choline (Lecithin) - PC
- Phosphatidyl ethanolamine (cephalin) - PE Phosphatidyl serine (PS)
- Phosphatidyl inositol (PI)
- Phosphatidyl Glycerol (PG)
- For stable liposomes, saturated fatly acids are used.
- Unsaturated fatty acids are not used generally.

2) Sphingolipids

One of the most important components of sphingolipids is sphingosine. Both plant and animal cells produce sphingolipids. The most common sphingolipids are sphingomyelin and glycosphingolipids.which include saccharides containing one or more salicylic acid residues in their cooling group and as a result have one or more negatively charged pH neutral, are used as a minor component in liposome formation. Liposomes merge with them to provide coverage for top-down groups [7].

3) Sterols

Cholesterol & its derivatives are often included in liposomes for

- Decreasing the fluidity or microviscocity of the bilayer
- Reducing the permeability of the membrane to water
- Soluble molecules Stabilizing the membrane in the presence of biological fluids such as plasma.(this effect used in formulation of i.v. liposomes)

Cholesterol-free liposomes are known to interact rapidly with plasma proteins such as albumin, transferrin, and macroglobulin. These proteins tend to release more phospholipids from the liposomes, which by eliminating the external monolayer of vesicles leading to instability.

4) Synthetic phospholipids

Saturated phospholipids include the following

- Dipalmitoyl phosphatidyl choline (DPPC)
- Distearoyl phosphatidyl choline (DSPC)
- Dipalmitoyl phosphatidyl serine (DPPS)
- Dipalmitoyl phosphatidyl ethanolamine (DPPE)
- Dipalmitoyl phosphatidyl glycerol (DPPG)
- Dipalmitoyl phosphatidic acid (DPPA)
- Dioleoyl phosphatidyl choline (DOPC)
- Dioleoyl phosphatidyl glycerol (DOPG)

III. ADVANTAGES OF LIPOSOMES [7, 16]

- Liposomes are biocompatible, completely biodegradable, non-toxic and nonimmunogenic.

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- Suitable for delivery of hydrophobic, amphipathic and hydrophilic drugs.
- Protect the encapsulated drug from the external environment.
- Reduce exposure of sensitive tissues to toxic drugs.
- Provide sustained release.
- Nonionic.
- Targeted drug delivery or site specific drug de livery.
- Improve protein stabilization.
- Controlled hydration.
- Can be administered through various routes.
- Alter pharmacokinetics and pharmacodynamics of drugs

IV. DISADVANTAGES OF LIPOSOMES (7, 16)

- Production cost is high
- Leakage and fusion of encapsulated drug/molecules
- Short half-life
- Less stability.
- Quick uptake by cells of R.E.S.
- Phospholipids undergoes oxidation, hydrolysis.

V. MECHANISM OF FORMATION OF LIPOSOMES(15)

Liposomes are composed of phospholipids, which are the building blocks of the structure. Amphipathic molecules with a hydrophobic tail and a hydrophilic or polar head are the most common type. Phosphoric acid attached to a water-soluble molecule forms the polar limit of the molecule. In a wet environment, the binding molecule is oriented so that the polar part of the molecule stays in contact with the coil while protecting the non-cooled part. The latter (polar lipid) is commonly used to create a bilayer structure centered between amphiphiles used in the delivery of drugs, such as soap, detergent, and polar lipid. Phosphatidylcholine is the most common natural phospholipid.

Phosphocholine is amphipathic molecule with a glycerol bridge that connects two hydrophobic acyl chains with a hydrophilic polar head group. As a result of their amphiphilic character, phospholipids and their counterparts can form closed bilayers where water is present. However, in aqueous springs, these molecules can form various phases, some of which are stable and some that are always metastable. The most common building structure consists of lamellar, hexagonal or cubic sections called liposomes, hexasomes or cubosomes respectively.

VI. LIOSOMESASDRUGCARRIERS

Some special properties of liposomes include

Solubilisation- NLs may dissolve lipophilic drugs that would be difficult to administer intravenously (4).

Protection- Liposome-encapsulated drugs are inaccessible to metabolising enzymes;

Conversely, body components are not directly exposed to the full dose of the drug.

Amplification- Liposomes can be used as adjuvant invaccine formulations (10).

Internalisation- Liposomes are endocytosed or phagocytosed by cells, opening up opportunities to use 'liposome-dependent drugs'.

Duration of action- Liposomes can prolong drug actionby slowly releasing the drug in the body.

VII. CLASSIFICATION OF LIPOSOMES(17)

Liposomes can be classified on the basis of:

1. Method of preparation (Table 1).
2. Structure (Table 2).
3. Composition and application (Table 3).

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Table 1 Different preparation methods and vesicle form by these method

Vesicle Type	Method of Preparation
REVS	Single or oligo lamellar vesicle made by reverse phase evaporation method
MLV-REV	Multilamellar vesicle made by reverse phase evaporation method
VET	Vesicle prepared by extrusion technique
DRV	Dehydration- Rehydration method
SPLV	Stable plurilamellar vesicle
FATMLV	Frozen and thawed multi lamellar vesicle

Table 2 vesicles types with their size and number of lipid layer

Vesicle type	Abbreviation	No. of lipid bilayer	Diameter size
Small Unilamellar	SUV	1	20-100 nm
Unilamellar	UV	1	All size ranges
Medium Unilamellar	MUV	1	More than 100 nm
Large Unilamellar	LUV	1	More than 100 nm
Giant Unilamellar	GUV	1	More than 1 micrometer
Oligolamellar	OLV	5	0.1-1 micrometer
Multilamellar	MLV	5-25	More than 0.5 micrometer
Multi vesicular	MV	Multi compartmental structure	More than 1 micrometer

Table 3 different liposomes with their composition

Type oflipsome	Abbreviation	Composition
Conventional liposome	CL	Neutral of negatively charge phospholipids and cholesterol
Cationic liposome	-	Cationic lipid with DOPE
Long circulatory liposome	LCL	Neutral high temp, cholesterol and 5-10% PEG, DSP
Fusogenic liposome	RSVE	Reconstituted sendai virus enveops
pH sensitive Liposomes	-	Phospholipids such as PER or DOPE with either CHEMS or OA
Immune liposome	IL	CL or LCL with attached monoclonal antibody or recognition sequences

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VIII. METHODS OF LIPOSOME PREPARATION[17]

All the methods of preparing the liposomes basically involve four basic stages:

1. Drying down lipids from organic solvent.
2. Dispersing the lipid in aqueous media.
3. Purifying the resultant liposome.
4. Analyzing the final product.

Various methods of liposome preparation [16]

A. Passive Loading Technique

a. Mechanical Dispersion

I. Lipid film hydration (Hand shaking / non hand shaking)
II. Freeze drying.
III. Micro emulsification.
IV. Sonication
V. French pressure cell
VI. Membrane extrusion
VII. Dried reconstituted vesicles
VIII. Freeze-thawed liposomes

b. Solvent dispersion

I. Ethanol Injection
II. Ether injection
III. Double emulsion.
IV. Reverse phase evaporation

c. Detergent Removal.

I. Detergent removal from mixed micelles vesicles by- Dialysis Dilution.

B. Active Loading Technique:

a. Proliposome lyophilization

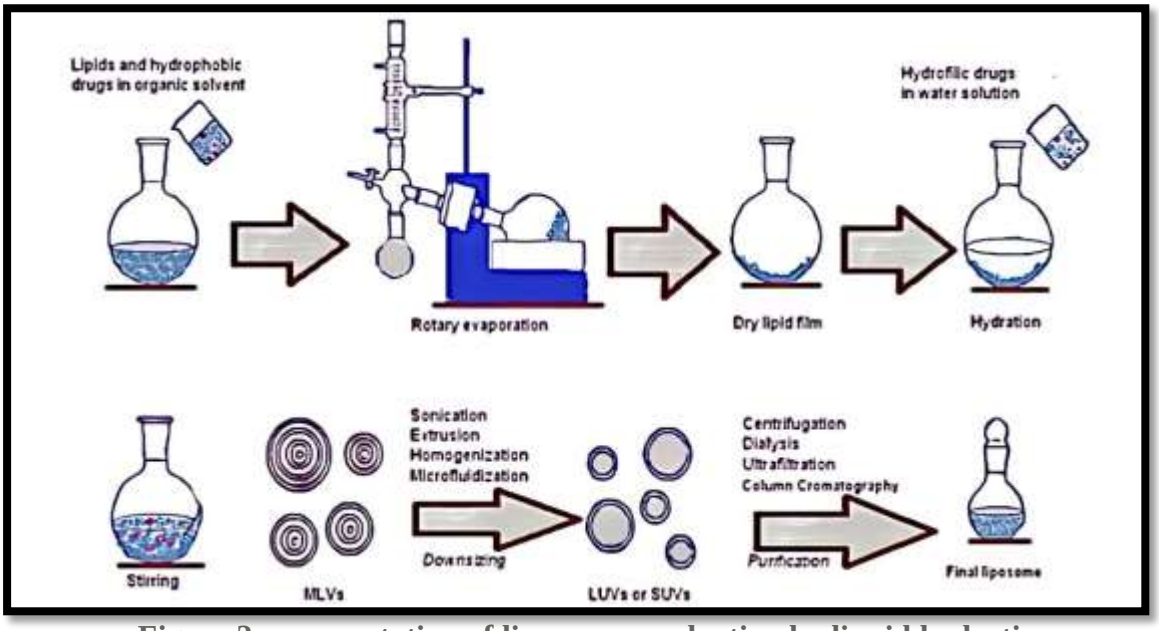

Figure 2 representation of liposome production by liquid hydration

8.1. Mechanical dispersion method[1-18]

1. Sonication: The sonication process is widely used in SUV preparation. In the case of inactivity, MLVS is integrated with a bath type sonicator or probe sonicator. The main obstacles to this method are its low encapsulation efficiency and phospholipid degradation and chemical degradation.

There are two sonication techniques

a) Bath Sonication: Modern bath sonicators have replaced most of the probe sonicators. In this hour, more than 5% of lipids can be removed esterified. And, in this way, titanium may slow down and contaminate the solution. [2]

method of controlling the temperature of the lipid dispersion is usually simple. Sonicated materials can be protected from sterile vessel or from under an inert atmosphere.

b)Probe Sonication: The tip of the sonicator is directly focused on the dispersion of the liposome. In the case of probo sonication the power input of lipid dispersion is very high. The convergence of energy eventually results in room temperature. Therefore, the vessel should be immersed in ice or a water bath. Sonication takes up to 1

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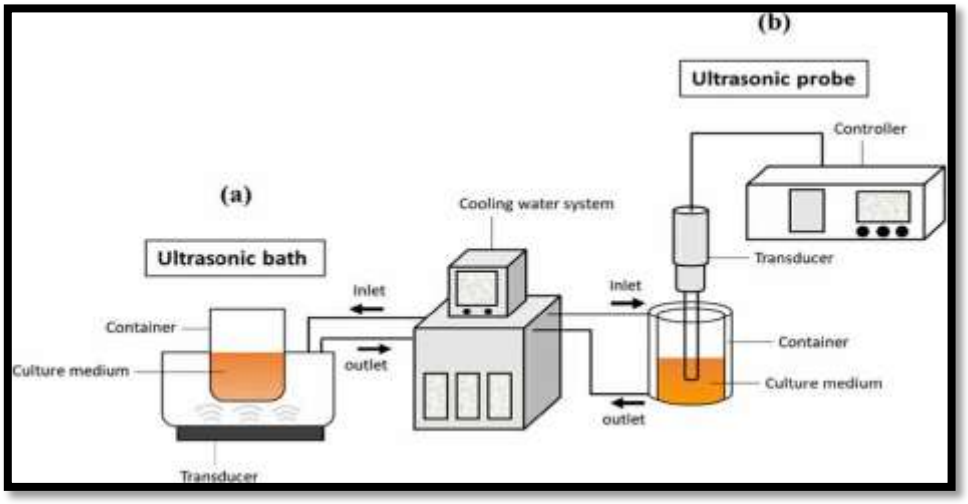

Figure 3 Sonication apparatus

2. French pressure cell:MLV extraction through a small hole is performed in a French compression cell, which involves careful handling of unstable substances. This method will give uni- or oligo-lamellar liposomes of medium size (30-80nm), theseliposomes are stable compared to sonicatedliposomes. This method has the advantage of producing liposomes larger than sonicated SUVs. However, maintaining a high temperature is a challenge, and operating costs are very modest.[(5)]

3. Freeze-thawed liposomes: SUVs are frozen quickly too melted slightly. Temporary sonication disperses set the integrated equipment to LUV. Creation of unilamellar vesicles are the result of SUV integration in all freezing and thawing processes. This type of synthesis is severely inhibited by increasing the concentration of phospholipid andto increase the ionic strength of the medium. The effectiveness of encapsulation was found to be 20% to 30%.[(16-17)]

8.2. Solvent dispersion method

1. Ethanol injection:

The ethanol lipid solution is rapidly injected into a large buffer volume leading to the formation of a larger size of MLVs. The disadvantage of the method is that the population varies (30 to 110 nm). Liposomes are highly depleted, the release of all ethanol is difficult and the potential for various active macromolecules is ineffective if there are low ethanol levels. [(6)].

2. Ether injection:

A solution of lipids dissolved in ether methanol mixture or diethyl ether. And the mixture is gradually injected to an aqueous solution of the material to be encapsulated at 55°C to 65°C or under reduced pressure[(16)] Removal of ether from the mixture under vacuum leads to the creation of liposomes. The main drawback of the technique is that the exposure of com pounds to be encapsulated to organic solvents at high temperature and population is heterogeneous (70 to 200 nm).

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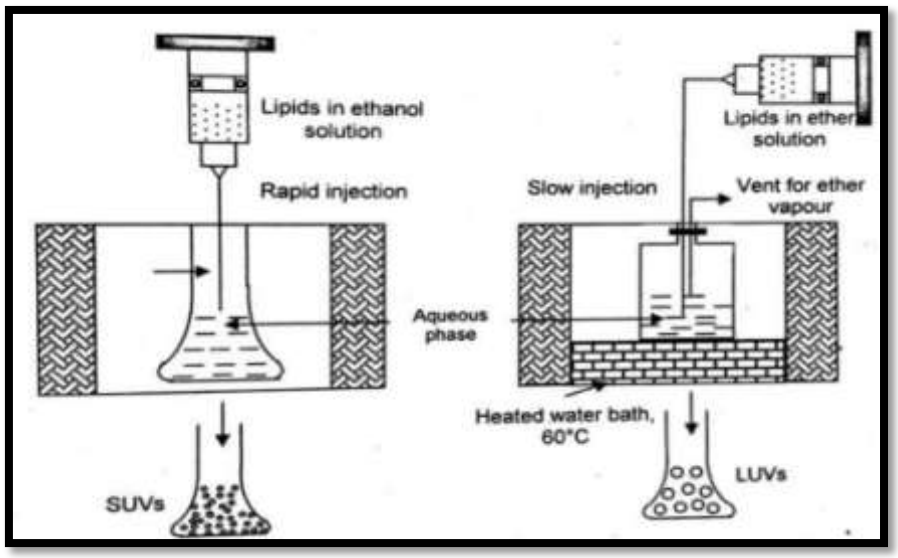

Figure 4.Ethanol And Ether Injection

8.3. Reverse phase evaporation method: This approach has provided advances in liposome technology. The evaporation of the regenerative phase is based on the creation of distorted micelles formed on the sonication of a water-soluble compound, consisting of water-soluble molecules to be covered in liposomes and the biological phase in which the amphiphilic molecules are dissolved. The dissolution of the living solvent leads to the conversion of these distorted micelles into a viscous and gel-like form. In a critical situation in this process, the gel state collapses, and some distorted micelles are disrupted and excess phospholipids in the environment contribute to the formation of a complete bilayer around the remaining micelles, leading to the formation of liposomes. The main disadvantage of the method is the contact of the material to be incorporated into the organic chemicals and the short periods of sonication that may result in the breakdown of DNA strands or the release of other proteins [(2)].

8.4. Detergent removal method (removal of non-encapsulated material):

1) Dialysis: The filters in their essential concentrate micellers (CMC) are used to dissolve lipid and when the filters are separated, the micelles become better at phospholipid and eventually combine to form LUVS. Dialysis was used to remove the cleaning products. A sales machine called Lipo-Prep (Dia-chema AG, Switzerland) is available to eliminate cleaning products.

2) Detergent removal by adsorbers: Purification absorption is achieved by stirring a micellent solution mixed with organic polystyrene beads. The advantage of using detergent adsorbers is that they can finish the filter with very low CMC, not completely eliminating.

Organic-beads adsorber - XAD-2 beads (SERVA Electrophoresis GmbH, Heidelberg, Germany).

Bio-beads adsorber SM2 (Bio-Rad Laboratories, Inc., Hercules, USA).

3) Gel-permeation chromatography: In this method, the detergent is depleted by size special chromatography. The liposomes do not penetrate into the pores of the beads packed in a column rather; they percolate through the inter-bead spaces at slow flow rates. The separation of liposomes from detergent monomers remains very good. The swollen polysaccharide beads adsorb substantial amounts of amphiphilic lipids; so, pretreatment is necessary. The pre-treatment is done by pre-saturation of the gel filtration column by lipids using empty liposome suspensions.

4) Dilution: Upon dilution of aqueous mixed micellar solution of detergent and phospholipids with buffer, the micellar size and the polydispersity increase fundamentally, and as the system is diluted beyond the mixed micellar phase boundary, a spontaneous transition from poly-dispersed micelles to vesicles occurs. Sephadex G-50, Sephadex G-1 00 (Sigma-Aldrich, MO, USA) and Sepharose 2B-6B can be used for gel filtration.

8.5. Freeze-protectant for liposomes (lyophilization)

Natural products are often destroyed due to oxidation and other chemical reactions. Ice drying has become a common process used in the

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production of many pharmaceutical products. There are many pharmaceutical products that need to be frozen in organic co-solvent systems. Freezing involves the removal of water from products in an ice pack under very low pressure. The process is often used to dry products that are thermo-labile and that will be destroyed by heat dissipation. The process has great potential as a way to solve long-term stabilization difficulties.

Table 4 list of clinically approved liposomal drugs

Name	Trade name	Company	Indication
Liposomal Amphotericin B	Abelcet	Enzon	Fungal infections
Liposomal Amphotericin B	Ambisome	Gilead Sciences	Fungal and protozoal Infections
Liposomal Daunorubicin	DaunoXome	Gilead Sciences	HIV-related Kaposi's Sarcoma
Vincristine	Onco TCS	--	Non-Hodgkin's lymphoma
Nystatin	Nyotran	--	Topical antifungal agent
Liposome-PEG Doxorubicin	Doxil/Caelyx	Ortho Biotech, Schering-Plough	HIV-related Kaposi's sarcoma, metastatic breast cancer, metastatic ovarian cancer
Liposomal Vaccine	Epaxal	Berna Biotech	Hepatitis A
Liposomal Vaccine	Inflexal V	Berna Biotech	Influenza

IX. EVALUATION OF LIPOSOMES[3,11-19]

1) Vesicle shape and lamellarity: The shape of thevesicles were studied by using electron microscope.

2) Particle size and distribution: The size analysed by an analyzer based on laser diffraction theory focused with minimum power of 5MW.

3) Entrapment Efficiency– It determines amount and rate of entrapment of water soluble agents in aqueous compartment of liposomes.

This can be calculated by a given formula

% Entrapment Efficiency = Entrapped Drug ×100/ Total Drug

4) Trapped Volume – It is an important parameterRelated to liposomes .It is aqueous entrapped volume Per quantity of lipids. This can vary from 0.5 L Microlitre/micromol.

5) In vitro drug release – This can be carried by using Franz Diffusion cell which has a diameter of 25 mm .Itcontains reservoir compartment of 22 ml which was filled with buffer which contains 20%v/v methanol to maintain sink condition.

6) Percentage yield of liposomes- The preparedliposomes were prepared and collected. Themeasured weight was divided by the total amount ofdrug and ingredients which were used for the preparation of liposomes.

X. TARGETING OF LIPOSOMES[19-23]

Two types of targeting

1) Passive targeting

As a practical diagnostic method, such liposomes are usually regulated to be rapidly excreted in the bloodstream and taken up by the RES in the liver. So macrophage energy can be used when liposomes are to be targeted to macrophages. This was demonstrated by the successful delivery of liposomal antimicrobial agents to macrophages.

2) Active targeting

A pre-requisite for targeting is the targeting agents are positioned on the liposomal surface such that the interaction with the target i.e., the receptor is tabulated such as a plug and socket device. The liposome physically prepared such that the lipophilic part of the connector is anchored into the membrane during the formation of the membrane. The hydrophilic part on the surface of the liposome, to which the targeting agent should be held in a stericaly correct position to bond to the receptor on the cell surface. The active targeting can be brought about the using.

i. Immune liposomes: These are conventional or stealth liposomes with attached Antibodies or other recognition sequence [e.g. Carbohydrate

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determinants like glycoprotein] the antibody bound, direct the liposome to specific antigenic receptors located on a particular cell. Glycoprotein or Glycolipid cell surface component that play a role in cell-cell recognition and adhesion

ii. Magnetic liposomes: Contain magnetic iron oxide. These liposomes can be directed by an external vibrating magnetic field in their delivery sites.

iii.Temperature or heat sensitive liposomes: Made in such a way that their transition temperature is just above body temperature. After reaching the site, externally heated the site to release the drug.

XI. APPLICATIONS[23]

- Cancer chemotherapy
- Gene therapy
- Liposomes as carriers for vaccines
- Liposomes as carrier of drug in oral treatment
- Liposomes for topical applications
- Liposomes for pulmonary delivery
- Against Leishmaniasis
- Lysosomal storage disease
- Cell biological application
- Metal storage disease
- Ophthalmic delivery of drugs

XII. CONCLUSION

Liposomes have been identified as the most useful transport systems for targeted drug delivery. The flexibility of their behavior can be used for the delivery of drugs by any means of handling any active substance of the drug regardless of its melting point. The use of liposomes in drug delivery and genetics is promising and sure to improve in the future.

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