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AN INTENSE REVIEW ON LIPOSOMAL DRUG DELIVERY SYSTEM

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ABSTRACT

Liposomes are artificially prepared spherical-shaped vesicles made of lipid bilayers. Liposomes have greater advantages than current drug delivery systems, such as site-targeting, modified release, prevention from clearance and drug degradation, increased therapeutic effects, and less toxic side effects. Liposomes have wide clinical applications as diagnostic, anticancer, topical cosmetic products, pulmonary, food applications, and so on. In biological, pharmacological, nutritional, and medical research, liposomes are being studied extensively as drug carriers for enhancing the distribution of bioactive agents and many different compounds. Most of those with clinical approval have sizes between 50 and 300 nm. This article represents liposomes as drug delivery, their composition, and mechanism, methods of preparation, characterization, and evaluation. Some advanced technology in liposomal drug delivery routes is inhalation and ocular.

Keywords: Liposomes, phospholipids, controlled delivery system, vesicles, encapsulation, active targeting

INTRODUCTION

The Greek terms Lipos-fat and Soma-body were combined to produce liposomes. The liposomes were discovered by Bangham and his teammates at the Babraham Institute in Cambridge, England, in the early 1960s. He discovered that the molecules were creating a closed bilayer structure with an aqueous phase encircled by a lipid bilayer after inadvertently scattering the phosphatidylcholine molecule in water. Since then, the majority of studies have focused on liposomes **[1]**. Both lipophilic

and hydrophilic substances can receive the drug from liposomes in an efficient manner **[2]**. The hydrophilic drug is kept in the liposome's core, while the hydrophobic drug is kept in the liposome's hydrophobic portion **[3]**. From extremely small (0.025 m) to big (2.5 m) vesicles, liposome size can vary. Liposomes have been utilized to improve the therapeutic index of a novel or current drugs by modifying drug absorption, lowering metabolism, extending biological half-life, and reducing toxicity **[4]**. The formation of liposomes is shown in **Figure 1**.

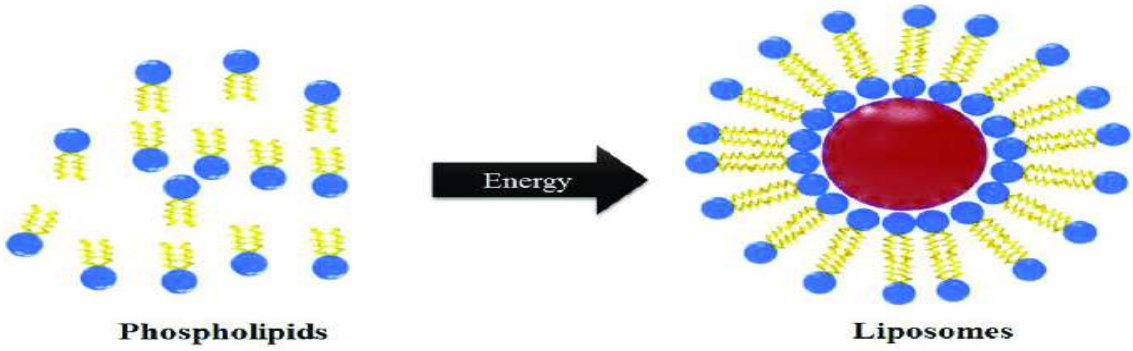

Figure 1: Liposomes formation

LIPOSOMES STRUCTURAL COMPONENTS: [5-7]

The main components of liposomes are as follows,

1. Phospholipid: [5-7]

Phospholipids are derived from phosphatidic acid. Glycerol moieties serve as the structural outlines for these molecules. The majority of phospholipids used in liposome formulation involve glycerol and represent more than half of the weight of lipids in biological membranes.

Saturated fatty acids are used to produce stable liposomes. Unsaturated fatty acids are rarely used. Several phospholipids include,

1) Phosphatidyl glycerol (PG),
2) Phosphatidyl serine (PS),
3) Phosphatidyl ethanolamine (Cephalin),
4) Phosphatidyl choline (Lecithin).

Phosphatidylcholine: Phosphatidylcholine (PC), an amphipathic molecule with a glycerol bridge, a hydrophilic polar head group, two hydrophobic acyl hydrocarbon chains, and phosphocholine, is the phospholipid that is used the most widely.

2. Cholesterol: [5-7]

Cholesterol is a fat, waxy-like component, which is an important substance in the human body. Cholesterol helps to produce hormones and vit-D. But cholesterol serves as a fluidity buffer and does not really produce a bilayer structure itself. Cholesterol modifies the movement of carbon molecules in the acyl chain by

interacting with phospholipid molecules and restricts the transformations of gauche from trans conformations.

3. **Sphingolipids: [8, 9]**

It is the support of sphingosine. Sphingolipids are vital components of both animal and plant cells. There are following three character-defining building blocks in sphingolipids.

a. A fatty acid mole.
b. A sphingosine mole.
c. A head group can range from very complex carbohydrates to basic alcohols like choline.

The most common Sphingolipid is Sphingomyelin and Glycosphingo lipids.

4. **Sterols: [8, 9]**

Liposomes frequently contain cholesterol and its derivatives for

a. A decrease in the bilayer's fluidity or microviscosity,
b. Maintain membrane stability in the presence of biological fluids like plasma which is used in the formulation of intravenous liposomes. Plasma proteins can also interact with liposomes without cholesterol through albumin, transferrin, and macroglobulin. Such proteins have the inclination to remove large amounts of phospholipids from liposomes, depleting the monolayer and causing the vesicles' physical instability.

5. **Synthetic phospholipids**: **[9, 10]**

Synthetic phospholipids are especially useful in membrane studies and in making lipoliposomes for drug delivery. Synthetic phospholipids can be classified into saturated and unsaturated synthetic phospholipids.

Example: For saturated synthetic phospholipids are:

a. DSPC- Disteroyl Phosphatidylcholine,
b. DPPE- Dipalmitoyl Phosphatidyl ethanolamine,
c. DPPC- Dipalmitoyl Phosphatidyl choline.

Examples of unsaturated phospholipids include:

a. DOPC- Dioleoyl Phosphatidylcholine,
b. DOPG- Dioleoyl Phosphatidyl glycerol.

6. **Polymeric material: [9]**

When exposed to UV, the diacetylene group in the hydrocarbon chain in the synthetic phopholipids polymerize. Lead to the development of polymerized liposomes with considerably increased penetrability barriers to drugs in water-soluble suspension. Eg. Lipids with conjugated dienes, methacrylate, and other substances are examples of various polymerizable lipids and also various polymerizable

surfactants are produced. Repulsive electrostatic forces dominate the stability of the repulsive interaction with macromolecules in polymers containing lipids. The coating of liposome surfaces with charge polymers can cause this repulsion. Higher solubility is achieved by water-compatible and non-ionic polymers such as polyvinyl alcohol, polyoxazolidine, and polyethylene oxide. However, the adsorption of such a copolymer, which combines hydrophobic portions with hydrophilic segments, causes liposome leak; hence, good outcomes can be obtained to phospholipids by covalently bonding polymers. Ex. PEG polymer and diacyl phosphatidyl ethanolamine are connected by a succinate or carbon bond.

Advantages of liposomes: [8, 9]

The several benefits of liposomes include,

1) Direct interaction of drug with cell.
2) Prevent oxidation of drugs.
3) enhanced stability through encapsulation
4) Improve protein stabilization.
5) Deliver sustained release.
6) Liposomes are biocompatible, completely biodegradable, non-toxic, and nonimmunogenic.
7) Alter pharmacokinetics and pharmacodynamics of drugs.

Some of the disadvantages of liposomes: [8, 9]

The several disadvantages of liposomes include

1) Leakage and fusion of encapsulated drug /molecules.
2) It has a short half-life.
3) Problem targeting various tissues due to their large size.
4) It has low solubility.
5) It has less stability.
6) High production cost.

LIPOSOMES CLASSIFICATION:

Liposomes are classified on the following basis,

- Specialty liposomes,
- Parameters based on structure,
- Conventional liposome,
- Method of preparation,
- Composition of the application.

Structural parameters [10, 11]

1) MLV [Multi lamellar vesicle (0.5 μm)],
2) MUV [Medium-sized unilamellar vesicle],
3) UV [Unilamellar vesicle (All size range)],
4) MV [Multivesicular vesicles (>1 mm)],
5) SUV [Small unilamellar vesicle (30-70 nm)],
6) OLV [Oligolamellar vesicle (0.1-1 μm)],

7) GUV [Giant unilamellar vesicle (> 1 μm)],
8) LUV [Large unilamellar vesicle (> 100 μm)].

Method of preparation [10, 11]

1) SPLV [Stable multilamellar vesicles],
2) REV [Reverse phase evaporation vesicles],
3) VET [Vesicle prepared by extraction method],
4) MLV-REV [Multi lamellar vesicle by REV],
5) FATMLV [Frozen and thawed MLV],
6) DRV [Dehydration- rehydration method]

Composition of the application [10, 11]

1) Long circulatory liposome,
2) Conventional liposome,
3) Cationic liposomes,
4) Fusogenic liposomes,
5) PH-sensitive liposomes,
6) Immuno liposomes.

Conventional Liposomes [12]

1) Synthetic identical, chain phospholipids,
2) Glycolipids containing liposomes,
3) Stabilize natural lecithin (PC) mixtures.

Speciality Liposome [12]

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

MECHANISM OF LIPOSOME FORMATION: [13]

The majority of the molecules in the liposome are amphiphilic phospholipids. Two fatty acid chains with 0–6 double bonds and 10–24 carbon atoms each make up the hydrophobic part [14], while phosphoric acid predominantly linked to a water-soluble molecule makes up the hydrophilic part. While the fatty acid groups of these phospholipids face one another and the polar head group of these phospholipids confronts the aqueous region, they arrange themselves to form liposomes, which are spherical/vesicle-like structures lamellar sheets are consequently produced. In addition, to protect the non-polar region the polar component maintains contact with the aqueous zone **[15]**.

To attain a thermodynamic equilibrium in the aqueous phase, phospholipids must be hydrated in water by applying energy such as shaking, sonication, homogenization, heating, etc. Hydrophilic/hydrophobic interactions between lipid-lipid and lipid-water molecules result in the formation of bilayer vesicles. Some of the following are reasons of bilayer formation:

Folding into vesicles can increase the stability of supramolecular self-assembled structures.

- Vesicles minimize the unfavorable interactions between the hydrophilic and hydrophobic phases.
- The formation of large bilayer vesicles promotes the reduction of significant free energy differences between the hydrophilic and hydrophobic environments.

LIPOSOMES - METHODS OF PREPARATION: [16]

All the techniques for producing liposomes in three fundamental steps:

A. Purifying the resultant liposome:

Differential centrifugation, density gradient centrifugation, and centrifugation are common types of centrifugations used to purify liposomes through molecular sieves (Torchilin and Weissing, 2002). Large liposomes can be easily separated from liposome mixtures using differential high-speed centrifugation.

B. Analysing the final product:

Drug development depends on assessing the liposome drug product's critical quality attributes (CQAs), which include physicochemical properties, composition, encapsulation effectiveness, and the release of drugs from the liposome formulation. It also depends on establishing the drug product specification.

C. Drying down lipids from an organic solvent:

The hydration of the lipid could anticipate the organic solvent's removal from the lipid solution depending on the particular formation process.

Drug loading and preparation of liposomes:

Liposomes can be prepared by following techniques:

A. Active loading technique.
B. Passive loading techniques.

There are three main passive loading techniques: **[17, 18]**

A. Method for detergent removal,
B. Mechanical dispersion method,
C. Solvent dispersion method.

PASSIVE LOADING TECHNIQUES:

Mechanical Dispersion Methods

1) Lipids film hydration by handshaking, non-hand shaking, and freeze-drying,
2) Membrane Extrusion method,
3) Freeze-thawed method,
4) Probe sonication,
5) Micro-emulsification liposomes,
6) Bath sonication,
7) Dried reconstituted vesicles,
8) Sonication of unicellular liposomes,
9) Calcium-induced fusion,
10) French Pressure Cell liposomes,

11) pH induced vesiculation.

Solvent Dispersion Methods

1) Stable multilamellar vesicles,
2) Ethanol injection,
3) Reverse phase evaporation vesicles,
4) Double emulsion vesicles,
5) Ether injection.

Detergent Removal Methods [17, 18]

1) Reconstituted Sendai virus enveloped vesicles,
2) Dilution,
3) Detergent.

PHYSICAL DISPERSION OR MECHANICAL DISPERSION METHOD

This method typically produces an enclosed aqueous volume of 5- 10%, which is a very tiny portion of the total volume used for swelling. As a result, numerous water-soluble substances are removed during swelling. Conversely, lipid-soluble compounds can entirely encapsulate themselves as long as their concentrations do not exceed those of the membrane's structural component. **[19]**

Lipid Film Hydration by handshaking: In this method, using a Flash rotatory evaporator, often known as "handshaking," releases the lipids from their organic solution as stacks of the film. The film formation and drying processes take place in the presence of reduced nitrogen, following the aqueous phase dispersion of the film stacks. The liquid hydrates to form multilamellar vesicles, which expand, peel away from the circular bottom flask's wall, and vesiculate. This is stored under the protection of a nitrogen umbrella store **[20]**. **Figure 2** shows the preparation of the liposomes by the handshaking method.

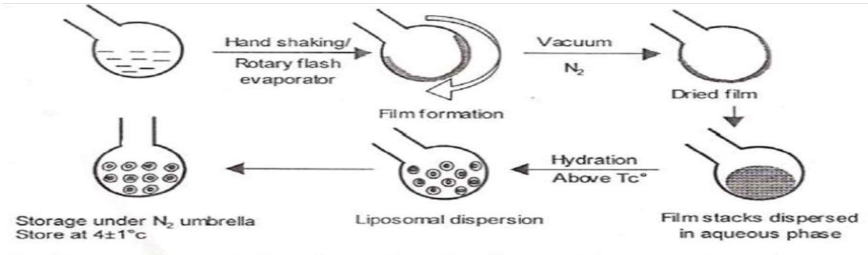

Figure 2: Handshaking Method

Non-hand shaking LUVs: These processes involve coating a conical flask with a solvent and lipid combination that is then permitted to vaporize naturally at room temperature without using nitrogen. The conical flask is hydrated with water-saturated nitrogen after the solution has dried, removing the opaqueness of the dried lipid coating, after hydration the lipid expands. The edge of the flask is progressively moved to one side, submerged in 10–20 ml of 0.2M sucrose in distilled

water, and then brought back to its original position. After being flushed with nitrogen and standing at room temperature for two hours, the flask is sealed. Following swelling, the suspension is centrifuged at 12,000 rpm for 10 minutes at room temperature. LUVs were then produced by adding iso-osmolar glucose solution to the remaining fluid. [20]

Freeze drying method: These techniques involve mixing and evaporation of lipids and solvents before allowing them to dry at room temperature. After that, add water-saturated nitrogen until the opacity is disappeared. To swell, mix 10–20ml of 0.2M sucrose solution with water. Then, after standing for two hours at 37ºC, centrifuged at room temperature for 10 minutes at 12000rpm with the residual fluid added to an iso-osmolar glucose solution to produce Large Unilamellar Vesicles **[22]**.

Sonication: This method is most frequently used to prepare SUVs. MLVs are sonicated using a passive atmosphere and by either a probe-type sonicator or a bath-type sonicator. This method's principal drawbacks include its extremely small internal volume, degradation of phospholipids, poor encapsulation efficiency, elimination of big molecules, metal pollution from the probe tip, and the presence of Multi Lamellar Vesicles along with Small Unilamellar Vesicles. There are two types of sonication, **Figure 3** shows the types of sonication methods **[23, 24]**.

a) **Probe sonication:** The liposome dispersion is promptly engulfed by the sonicator. This approach has a very large energy input into the lipid dispersion. The vessels must be submerged in water or an ice bath because the coupling of energy at the tip causes localized heat. More than 5% of the lipids can be deesterified for up to an hour during the sonication process.

b) **Bath sonication**: A bath sonicator is used to sonicate the liposome-containing cylinder at a set temperature. This method is frequently less complicated than sonication by dispersion utilising the tip. The chemical that is being sonicated can be kept safe in a sterile vessel, an inert atmosphere, or a unit with various probes.

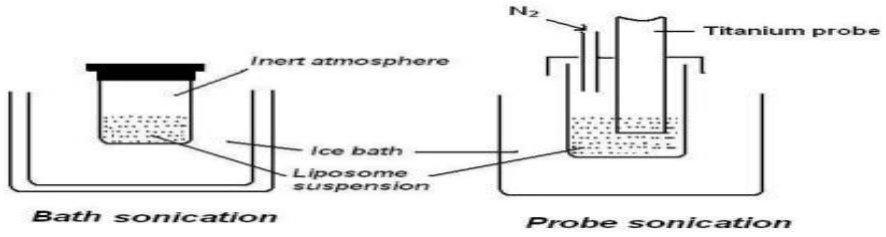

Figure 3: Bath and probe sonication method

Micro-Emulsification: Small MLVs are made from concentrated lipid dispersion using a "microfluidizer." The fluid is pumped through a 5mm orifice by a microfluidizer at 10,000 psi pressure. The fluid is then forced downward at predetermined microchannels, allowing two fluid channels to contact at right angles while moving quickly and transfer energy. Either large Multi Lamellar Vesicles or a slurry of a hydrated lipid in an organic medium can be used to deliver the lipids into the fluidizer. The fluid collected can be recycled through the pump and the interaction chamber, until vesicles of spherical dimensions are obtained. The size of the vesicles is reduced between 0.1 to 0.3µm after a single pass. **Figure 4** shows the preparation of liposomes using the micro emulsification method **[25]**.

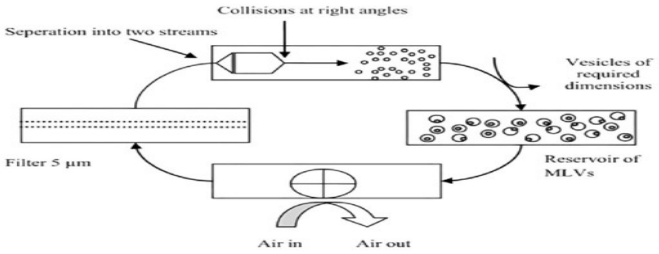

Figure 4: Micro-Emulsification preparation method

French pressure cell: This method comprises the extrusion of Multi Lamellar Vesicles through a small orifice **[23]**. Unlike the sonication method, this method has an essential feature is that the appearance of protein does not change significantly. Unstable methods should handle gently by using this technique. Compare to the sonication technique, this method has a number of advantages **[26]**. The final product liposomes are larger than sonicated Small Unilamellar Vesicles. Disadvantages of this method include the trouble in achieving the high temperature and achieving the working sample is relatively small (50ml at maximum). **Figure 5** shows the preparation of liposomes using the French Pressure cell method **[23, 27]**.

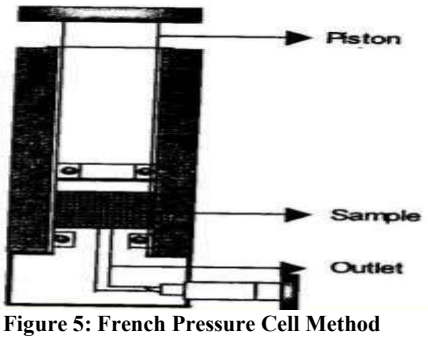

Figure 5: French Pressure Cell Method

Membrane extrusion: This method contains the phosphate lipid bilayer is broken and then resealed when the vesicles pass through a polycarbonate membrane. Compare to the French pressure cell, this method requires less pressure that is used to treat Multi Lamellar Vesicles and Large Unilamellar Vesicles. Finally, nucleation membranes and twisted membranes are formed. **Figure 6** shows the preparation of liposomes using the Membrane Extrusion method **[23, 27]**.

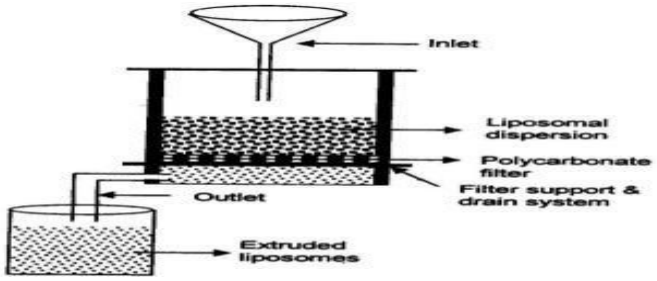

Figure 6: Membrane Extrusion Method

Dried Reconstituted Vesicles: This approach often yields unilamellar or oligolamellar liposomes with a diameter of 1m or less. A performed SUV dispersion is frozen and lyophilized in the first step of freeze drying. This results in an organized membrane structure that, when water is added can reseal, rehydrate, and fuse to produce vesicles with high capture efficiency. The dispersion of empty SUVs is combined with the water-soluble matrix to be trapped, and the two are then dried together. This approach has the advantages of high aqueous-soluble component entrapment and the utilisation of moderate conditions for bioactive synthesis and loading. The drawback of this approach is that it only functions on unilamellar vesicles, sometimes referred to as Small Unilamellar Vesicles. This technique is used to manufacture liposomes, as seen in **Figure 7 [28]**.

Freeze-Thaw Sonication: This technique is a development of dried reconstituted vesicles. Here, SUVs undergo a quick freezing process, a delayed thawing process that letting them stand at ambient temperature for 15 minutes, and then a sonication cycle that disperses aggregated components to Large Unilamellar Vesicles. The fusing of SUVs results in the production of unilamellar vesicles. 20–30% encapsulation efficiencies were attained. Sucrose, salt solutions with high ionic strengths, and the ineffective entrapment of divalent metal ions are disadvantages of the technique. **Figure 7** shows the liposomes preparation using dried reconstituted vesicles and freeze thawed methods **[28, 29]**.

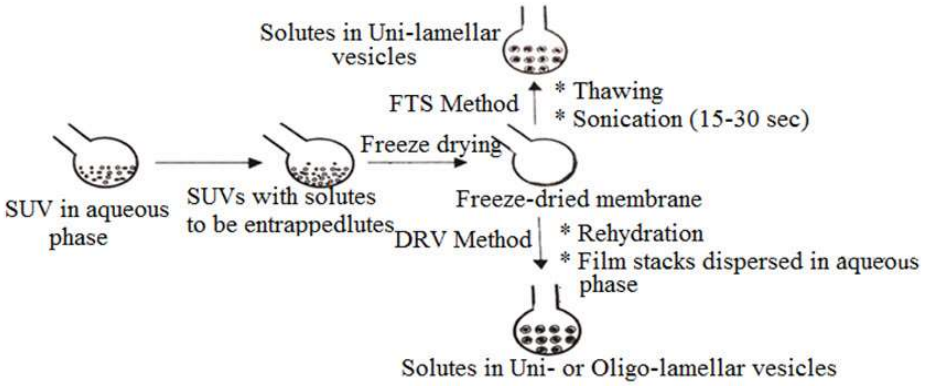

Figure 7: Dried Reconstituted Vesicles and Freeze-Thawed method

Calcium-induced fusion: This process dries out the lipid and suspends it in a sonication buffer (NaCl 0.385g, histidine 31.0mg, this base 24.2mg, water 100ml, pH 7.4). The lipid particles and large liposomes are subsequently separated using a 100,000rpm centrifuge. A precipitate of calcium solution is produced in an equimolar proportion. A spinner separates the precipitate after 60 minutes of incubation at 37°C. The pellet is reconstituted in buffered saline that also contains the substance to be entrapped, and it is then incubated for 10 minutes at 37°C. While mixing, the EDTA (170ml) is added to the buffer. The precipitation will shortly clear. Then, incubate for 15 minutes at room temperature after an additional 15 minutes of incubation at 37°C. Finally, removes the ca/EDTA complex by dialysis with buffer **[30, 31]**.

SOLVENT DISPERSION TECHNIQUES:

Ether and Ethanol Injection: Multi Lamellar Vesicles are extruded as part of the procedure at a pressure of 20000 psi and a temperature of 4 °C. In contrast to sonication, Hamilton and Guo's approach had a number of benefits in 1984. The method delicately handles unstable materials and is quick and simple to replicate. The ethanol lipid solution is rapidly injected into a large volume of buffer, and the MLVs form quickly. The technique has certain drawbacks, such as a population with a diverse size range (30–110 nm), highly diluted liposomes, and difficulties completely removing ethanol since it forms an azeotrope with water. With the presence of even low amounts of ethanol, the potential for a number of biologically active macromolecules to become inactive (Batzri and Korn, 1973). **Figure 8** shows the preparation of liposomes using ethanol and ether injection methods. **[32]**

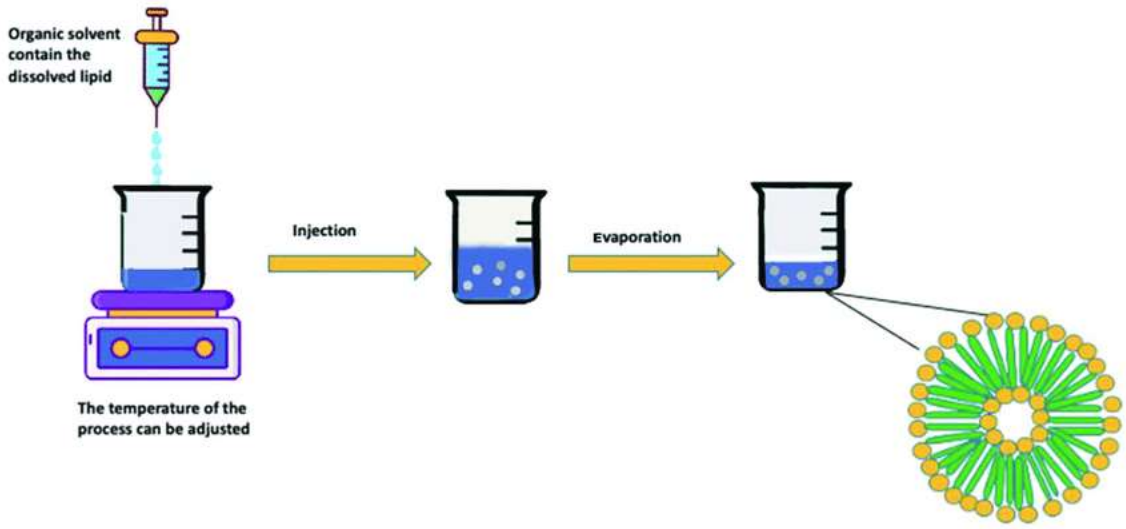

Figure 8: Preparation of liposomes using ethanol and ether injection methods

Double Emulsion Vesicles: The external portion of the liposome membrane is produced during a second-phase phase transition by emulsifying an organic solution with water. Multi-compartment vesicles can be produced by adding an organic solvent, which already contains water droplets to an excess aqueous media and then mechanically dispersing it. The resulting ordered dispersion is referred to as a W/O/W system. Multi-compartment vesicles can be formed by adding an excessive amount of aqueous medium to an organic solution that already contains water droplets and then mechanically dispersing it. The resulting ordered dispersion is referred to as a Water in Oil in Water system. During this stage, the phospholipid monolayers that surround each water compartment are in close opposition to each another. The next stage is to use a mechanical vortex mixer to vigorously shake the water droplets, causing a collapse to a specific proportion. The outer leaflet of the bilayer of the big unilamellar liposomes is formed by joining the lipid monolayer that enveloped the collapsed vesicle to the nearby intact vesicle. The resultant vesicles are unilamellar, 0.5 m in diameter, and 50% encapsulated **[33]**.

Reverse phase evaporation vesicles: This process produces the same amount of lipid without dispersion as earlier reports, but drying is followed by an extended bath sonication with a nitrogen stream. This time, the aqueous solvent and solute are divided among the many bilayers in each multilamellar vesicle and brought into equilibrium. The interior structure indicates a 30% average entrapment rate **[25, 28]**.

DETERGENT REMOVAL METHODS: [25, 33]

When non-ionic detergent is removed from detergent/phospholipid combinations employing the right adsorbents for the detergent, huge unilamellar vesicles may develop. In this procedure, detergent serves as an intermediate to link phospholipids with water-derived phospholipid molucules and bring them into touch with the aqueous phase. Micelles, which can consist of several hundred different component molecules, are the structural units that are created as a result of this interaction. Their shape and size are dictated by the detergent's chemical composition, concentration, and any other lipids that may be present. The crucial micelle concentration is the quantity of detergent in water at which micelles start to form (CMC). Sulphonic and phenolic compounds are a few of examples of substances that are used to eliminate detergents. **[34]**

ACTIVE LOADING TECHNIQUE:

Active loading is often referred to as remote loading. Because only a certain number of lipids may be provided systemically, it is essential that liposomes are efficiently loaded with active therapeutic components in order to transport the medicine in clinically efficacious amounts. The principle of the remote loading method is to move molecules from the bulk solution into the generated liposomes notwithstanding their concentration gradient. The medication may be loaded actively or passively (the drug is added when the liposomes are made) (i.e, after liposome formation). Depending on how well the drug dissolves in the liposome membrane, 100% trapping effectiveness may be reached. Liposomes must be able to absorb an aqueous buffer containing a dissolved medication during vesicle formation in order to passively encapsulate water-soluble medications **[43]**. The efficiency of drug trapping is frequently limited to 30% due to the medication's solubility and the amount of trapped drug specified in the liposomes. However, by using pH gradients, water-soluble medications with protonizable amine activities can be actively restricted [44], with trapping effectiveness that can approach 100% **[37]**. The following benefits of active loading methods over passive encapsulation methods: **[28]**

1) Less leakage of the substances that are encapsulated.
2) High capacity and encapsulation efficiency.

CHARACTERIZATION AND EVALUATION OF LIPOSOME

i) PHYSICAL CHARACTERISTICS

1) Vesicle shape and lamellarity:

The shape of the vesicle may be assessed using a variety of electron microscopy techniques. Using 31P NMR

analyses and freeze-fracture electron microscopy, it is possible to determine how many bilayers are present in liposomes or how lamellarly vesicles are composed **[24]**.

Freeze Fracture and Freeze-Etch Electron Microscopy: Evaluation of the liposomes' shape, lamellarity, and surface morphology can be done using freeze-fracture electron microscopy. In this method, the vesicles are positioned at random within the frozen state, and the fracture plan passes through them. The distance between the vesicle center and the plane of fracture affects the observed distribution profile. **[24, 28]**

Nuclear Magnetic Resonance: This method uses 31P NMR to track the strength of the signal of phospholipid phosphorus. A unilamellar vesicle is indicated by a 50% decrease in NMR signal strength, whereas a subsequent reduction implies a multilamellar vesicular preparation **[24, 28]**.

Scanning Electron Microscopy: Because of the distortion problem that occurs during sample preparation, this technique is less often used **[28]**.

Diffraction and Scattering Techniques: Laser Light Scattering Techniques: Quasi-elastic light scattering techniques, which involve lasers, are helpful for examining homogeneous colloids. A unimodal system with a diameter of less than 1 micrometer (<1 μm) is applied by this technique **[24, 28]**.

2. DISTRIBUTION AND VESICLE SIZE

Microscopic Techniques:

Optical Microscopy: Vesicular dispersion is diluted, moist mounted on a hemocytometer, and phase contrast microscopy photographs is taken. The negatives are then projected using a photographic enlarger x1250 on a piece of calibrated paper. Then measure the diameters of about 500 vesicles. Bright field, phase contrast, and fluorescent microscopes are used in this microscopic technique. It is helpful in determining the size of big vesicles greater than one micrometer (>1μm) **[24, 28, 38]**.

Negative Stain Transmission Electron Microscopy: With this technique, a thin coating of an electron-dense heavy metal stain is applied over the liposomes. The relatively electron transparent liposomes can be observed as bright spots on a dark backdrop when utilising negative stain transmission electron microscopy **[24, 28, 38]**.

Cryo Transmission Electron Microscopy Technique: The samples are freeze-fractured using this technique, then visualized. Transmission electron microscopy is used. In a specially designed environment chamber, thin sample films are produced at a controlled temperature of 25 degrees Celsius and humidity levels. The films are then transported to TEM analysis

after being quickly frozen in liquid ethanol **[24, 28, 38]**.

Hydrodynamic Techniques:

Field Flow Fractionation Techniques (FFF): Sedimentation FFF and flow FFF measure various vesicle characteristics due to fundamentally different driving forces **[24, 28]**.

Gel Permeation: Estimating the presence of different-sized particles in the dispersion could be done by using the preparations' capacity to separate different components. Additionally, it is used to separate different heterodispersed liposomal preparations **[28]**.

3) Surface Charge: The charge is typically determined using the two techniques of Zeta potential measurement and free flow electrophoresis **[4]**. The Helmholtz-Smolochowski equation for the mobility of the liposomal dispersion in a suitable buffer may be used to determine the surface charge **[28]**.

4) Encapsulation Efficiency: The percentage of drug entrapment in the aqueous phase is often represented sas % entrapment/mg lipid. The protamine aggregation method and the microcolumn centrifugation method are the two methods used to measure the effectiveness of encapsulation **[28, 38]**.

$$\%Entrapment\ efficiency = \frac{Entrapped\ Drug\ (mg)}{Total\ Drug\ addded\ (mg)} \times 100$$

5) Phase Response and Transitional Behavior: Liposomal membrane properties like permeability, fusion, aggregation and protein binding are determined by the phase behavior of the membrane. Using freeze-fracture electron microscopy, the phase change has been assessed. By analyzing data from a differential scanning calorimeter, they are more thorough. Furthermore, it is crucial to define these phase behavior while creating liposomes because different lipids have distinct phase transition temperatures and because polymer PEG grafting prevents macrophage absorption and, thus, long circulation **[28, 38]**.

7) Drug Release: Before using in vivo investigations, which are expensive and time-consuming, liposome-based formulations can benefit from using *in vitro* assays to estimate the pharmacokinetics and bioavailability of the medication **[28, 38]**.

II) CHEMICAL CHARACTERISTICS

1) Phospholipid Identification and Assay [28]

The concentration of phospholipids in the liposomal formulation can be estimated using the Bartlett assay, Stewart assay, and thin-layer chromatography.

The sample's phospholipid phosphorus is first hydrolyzed to produce inorganic phosphates before being used in the Bartlett assay. Ammonium molybdate is added, and this is changed into

phosphomolybdate acid. Amino naphthylsulphonic acid quantitatively reduces phosphor molybdic acid to a blue-colored product. Using spectrophotometry, the blue color's intensity is determined and compared with the phospholipid standard curve. The disadvantage of the method is measuring phospholipids because it is so sensitive to inorganic phosphates **[28]**.

In the Stewart test, phospholipids and ammonium ferrothiocynate are combined to produce a complex in an organic solution. First, the standard curve is prepared by mixing different amounts of phospholipid in chloroform with 0.1M ammonium ferrothiocynate solution. The samples are processed similarly, the solutions' optical densities are measured at 485 nm, and the absorbance of the samples is compared to a phospholipid reference curve. The benefit of this approach is that the assay is unaffected by the presence of inorganic phosphates. This method's drawback is that it cannot be used with samples that may contain mixers of unidentified phospholipids.

To evaluate the concentration and purity of lipids, thin layer chromatography is employed. In each elution, the material should only show up as a single spot if it is pure. In contrast to the pure material, which runs as a single, distinct spot, degraded phospholipids appear as long streaks with a tail that stretches back to the origin **[28]**.

2) Cholesterol Analysis [37]

- Cholesterol concentrations can be measured using the ferric perchlorate method or cholesterol oxidase test and gas-liquid chromatography techniques **[28]**.
- Using a flexible fused silica capillary column, cholesterol is qualitatively analyzed.
- The absorbance of the complex created when iron interacts with an ethyl acetate, ferric perchlorate, and sulfuric acid solution at 610 nm may be used to calculate the quantity of cholesterol.

STABILITY OF LIPOSOMES [37]

During preparation and storage, a stable dosage form maintains the active ingredient's physical and chemical stability. As a result, a stability test is necessary to guarantee the pharmaceutical product's physical and chemical stability while it is being kept.

Physical Stability: Liposomes are affected physically by a variety of things, such as fusion, aggregation, shape, and size. Leakage of drug ingredients is the main problem that occurs. The shape and size distribution plays an important role in determining stability. The physical stability of the phospholipids can be maintained by avoiding excessive unsaturation. Without

freezing or exposure to light, they must be stored at 4°C **[28, 38-42]**.

Chemical Stability: A drug's stability can be hydrolyzed and altered by phospholipids, which are unsaturated fatty acids. The oxidative degradation of liposomes can be stopped by using antioxidants like butylated hydroxy anisole **[28, 38-42]**.

APPLICATIONS OF LIPOSOMES: [42-52]

Liposome usage for therapeutic and diagnostic purposes can be recognised in pharmacology and medicine. Contains several markers or drugs along with instructions on how to utilise them in fundamental research on cell contact, pattern recognition, and the mechanism of action of certain drugs. Pharmaceuticals, including anticancer medicines, encapsulated in liposomes demonstrated decreased toxicities while retaining higher efficacy, according to a number of pre-clinical and clinical studies.

Table 1: Liposomes in the pharmaceutical industry

Liposome Effectiveness	Treated Disease States	Applications
Specific Targeting	Wide therapeutic	Cells bearing specific antigens
Site-Avoidance	Fungal infections	Amphotericin B – reduced Fungal infections, and nephrotoxicity.
Accumulation	Cardiovascular diseases	Prostaglandins
Sustained-Release	Cancer, biotherapeutics	Systemic antineoplastic drugs, hormones, Cancer.
Solubilization	Fungal infections	Amphotericin B, minoxidil
RES Targeting	Cancer, MAI, tropical	Immunomodulators, vaccines, MAI.

Liposomal applications in parasitic infections and diseases: Conventional liposomes are the best delivery systems for drugs that target these macrophages since they are broken down by phagocytic cells in the body after intravenous administration. Several fungi infections and parasite illnesses are examples of this "Trojan horse-like process."

Cancer Therapy: It is effective to entrap anticancer drugs in liposomes. This prolongs the circulation time and protects against metabolic degradation.

Liposomes in oral treatment

- Large MLVs could contain arthritis-related steroids.
- By giving liposome-encapsulated insulin orally to diabetic rats, blood glucose levels were altered.

Liposome for topical application: Topical liposomes can be successfully used to deliver drugs like triamcinolone, benzocaine, corticosteroids, etc.

Cosmetic Applications: The delivery of substances in cosmetics also makes use of the qualities of liposomes. Liposomes restore lipids, particularly linolenic acid, for the skin. The antiaging cream capture,

introduced by Christian Dior in 1986, was the first liposomal cosmetic item to come to the market. Hair loss has also been treated using liposomes. **Table 1** lists some of the liposomal cosmetic formulations that are currently available. **[44-47]**

Food application: Currently employed in the food business, biopolymer matrices made of synthetic materials, starch, alginates, gum, protein, dextrin, and sugar are the foundation for the majority of microencapsulation processes. However, liposomes have just started to gain more importance in food preparation **[46, 48]**.

Ophthalmic Disorders: Liposomes have been proven to be effective in treating a variety of eye conditions, including proliferative Vitro retinopathy, corneal transplant rejection, keratitis, uveitis, and endophthalmitis **[49]**.

Immunological adjuvants in vaccines: Liposomes are utilized in immune adjuvants, and immune diagnosis.

Pulmonary Application: Due to their ability to solubilize substances, they are effective tools for the pulmonary delivery of drugs **[48-50]**.

Site-specific targeting: Target cells are more precisely identified and bound by immunoliposomes **[51,52]**.

CONCLUSION:

Nowadays, one of the distinctive and most promising drug delivery systems is the liposome. This article shows the various broad range advantages of liposomes in pharmaceutical and pharmacological aspects and it is administered as parenteral, oral, and topical routes. The other applications include diagnostic, anticancer, topical cosmetic products, food products, and so on. The liposome is a nontoxic vehicle so, it can be used for insoluble drugs and improves the solubility of highly potent drugs. The liposome can cross the BBB easily and achieve good site specific action than any other dosage form. This article also fulfills the liposomes as drug delivery and explains their structural components, classifications, mechanisms, preparation methods. Various liposome based formulations have been currently undergoing clinical trials and are also available commercially.

REFERENCE:

[1] Bangham AD, and Horne RW. Negative staining of phospholipids and their structural modification by surface-active agents as observed in the electron microscope. J Mol Bio. 1964; 8(5): 660-668.

[2] Torchilin VP. Recent Advances with Liposomes as Pharmaceutical Carriers. Nat Rev, February. 2005; 4(2): 145-160.

[3] Mehran A, Naser K and Mohsen S. Application of Various Types of Liposomes in Drug Delivery Systems. Adv Pharm Bull. 2017; 7(1): 3-9.

[4] Sarika S Lokhande. Liposome drug delivery: an updated review. Int J Pharm Sci. 2018; 9(1): 88-202.

[5] Maheswaran A, Brindha P, Mullaicharam AR, Masilamani K. Liposomal Drug Delivery Systems-A Review. A Rev Int J Pharm Sci Rev Res. 2013; 55: 295-301.

[6] Roy AS, Das SU, Samanta AR. Design Formulation and Evaluation of Liposome Containing Isoniazid. Int J App Pharm. 2018; 10: 52-56.

[7] Li J, Wang X, Zhang T, Wang C, Huang Z, Luo X, et al. A Review on Phospholipids and Their Main Applications in Drug Delivery Systems. Asian J Pharm Sci. 2015; 10:81-98.

[8] Thulasiramaraju TV, Babu AS, Arunachalam A, Prathap M, Srikanth S, Sivaiah P. Liposome: A novel drug delivery system. Int J Biopharm. 2012; 3(1): 5-16.

[9] Shashi K, Satinder K, Bharat P. A complete review on; Liposome. Int Res J Pharm. 2012: 3(7): 10-6.

[10] Kant S, Kumar S, Prashar B. A Complete Review on Liposome. Int Res J Pharm. 2012; 3(7): 10-16.

[11] A Review on Liposome. Int J Adv Res Pharm Biosci. 2012; 2: 453-464.

[12] Hope MY, Bally MB, Mayer LD. Phase behavior of large unilamellar vesicles composed of synthetic phospholipids. Chem Phy Lip. 1986; 40: 89-107.

[13] Kalepu Sandeep, Sunil Kumar KT, Mohan Varma M, Sudheer Betha. Liposomal drug delivery system - A Comprehensive Review. Int J Drug Dev Res. October - December 2013; 5 (4): 62-75.

[14] Vyas SP, Khar RK. (Eds.). Targeted and controlled drug delivery: Novel carrier systems. CBS publishers. 2002; 173-248.

[15] Lasic DD. The mechanism of vesicle formation Biochem J. 1988; 256: 1-11.

[16] Abolfazl Akbarzadeh, Rogaie Rezaei Sadabady, Soodabeh Davaran, Sang Woo Joo, Nosratollah Zarghami, Younes Hanifehpour, et al. Liposome: classification, preparation, and applications. Nano Res L. 2013; 8: 102-108.

[17] Riaz M. Liposome preparation method. Pak J Pharm Sci. 1996; 9(1): 65-77.

[18] Himanshu A, Sitasharan P, Singhai AK. Liposomes as drug carriers. Int J Pharm L Sci. 2011; 2(7): 945-951.

[19] Rajan K Verma and Sanjay Garg. Current Status of Drug Delivery Technologies and Future Directions. Pharm Tech. 2001; 25 (2): 1-14.

[20] Riaz Mohammad. Review Liposomes Preparation Method. Pak J Pharm Sci. January 1996; 19(1): 65-77.

[21] Samad A, Sultana Y, Aqil M. Liposomal Drug Delivery Systems: An Update Review. Cur Drug Del. 2007; 4: 297-305.

[22] Akbarzadeh A, Sadabady RR, Davaran S, Joo WS, Zarghami N, Hanifehpour Y et al. Liposome: Classification, Preparation, and Applications. Nano Res Let. 2013; 8(102): 01-09.

[23] Riaz M. Liposome preparation method. Pak J Pharm Sci. 1996; 9(1): 65–77.

[24] Saraswathi Marripati, Umasankar K. Jayachandra Reddy P. A Review on Liposomes. Int J Res Pharm Nano Sci. 2014; 3(3): 159-169.

[25] Chandraprakash Dwivedi, Shekhar Verma. Review on Preparation and

Characterization of Liposomes with Application. J Sci Inno Res. March-April 2013; 2(2): 486-508.

[26] Mayer LD, MJ Hope, and PR Cullis. Vesicles of variable sizes produced by a rapid extrusion procedure. Biochim. Biophys. Acta. 1986; 858(1): 161-168.

[27] Himanshu A, Sitasharan P, Singhai AK. Liposomes as drug carriers. Int J Pharm L Sci. 2011; 2(7): 945–951.

[28] Vyas SP, Khar RK. Targeted and Controlled Drug Delivery, Novel Carrier Systems, 2004; 173-248.

[29] Xiaoling Li, Bhaskara R Jasti, Mcgram Hill. Chemical Engineering, Design of Controlled Release Drug Delivery System, 2004; 65: 367-378.

[30] Rajan K Verma and Sanjay Garg. Current Status of Drug Delivery Technologies and Future Directions. Pharm Tech. 2001; 25(2): 1-14.

[31] Dwivedi Chandraprakash, Verma. Review on Preparation and Characterization of Liposomes with Application, J Sci Inno Res. 2013; 2(2): 1-31.

[32] Dipak K Banerjee, Subroto Chatterjee. Preparation, Isolation, and Characterization of liposomes containing natural and synthetic lipids, Methods Mol Bio. 2002; 199: 03-16.

[33] Vyas SP and Khar RK. Target and Controlled Drug Delivery Novel carrier Systems. Reprinted In 2007; 177-242.

[34] Devender Sharma, Aashiya Aara E. Ali, Leena R, Trivedi. An Updated Review on: Liposomes as drug delivery system, PharmaTutor, 2018; 6(2): 50-62.

[35] Akbarzadeh A, Sadabady RR, Davaran S, Joo WS, Zarghami N, Hanifehpour Y, Samiei M. Liposome: Classification, Preparation, and Applications. Nano Res Let. 2013; 8(1): 102.

[36] Kersten GFA, Crommelin DJA. Liposomes and ISCOMS as vaccine formulations. Biochim. Biophys. Acta. 1995; 124(1); 117-138.

[37] Sharma A, Straubinger RM. Novel taxol formulations: preparation and characterization of taxol containing liposomes. Pharm Res. 1994; 11: 889-896.

[38] Kanth Shashi, Kumar Satinder, Prashar Bhara. A Complete Review on Liposomes. Int J Pharm. 2012; 7 (3): 10-16.

[39] Rabiya Ansari, Abdul Mannan. Liposomes as a novel drug delivery system. Int J Pharm Tech. 2017; 9(2): 29735-29758.

[40] Sanjay K Jain and NK Jain. Liposomes as Drug Carriers. A handbook of Controlled and Novel Drug Delivery. 304-348.

[41] Sercombe L, Veerati T, Moheimani F, Sherry YW, Sood AK, Hua1 S. Advances And Challenges Of Liposome Assisted Drug Delivery. Front Pharmacol. 2015; 6: 1-13.

[42] Giuseppina B, Agnese M. Liposomes as Nanomedical Devices. Int J Nano Med. 2015; 10: 975- 999.

[43] Saha S, Roy A, Bahadur S, Choudhury A. Fabrication and in-vitro evaluation of liposomal quercetin and its optimization. Res J Pharm Tech. 2018; 11(1): 61-64.

[44] Roy A, Saha S, Choudhury A, Bahadur S. Bioenhancement of curcumin by combined approaches of adjuvants and liposomal

fabrication. Asian J Pharm. 2016; 10(4): 688-692.

[45] Müller Goymann CC. Physicochemical characterization of colloidal drug delivery systems such as reverse micelles, vesicles, liquid crystals, and nanoparticles for topical administration. Eur J Pharm Biopharm. 2004; 58(2): 343-356.

[46] Lautenschläger H. Liposomes. Handbook of Cosmetic Science and Technology (AO Barel, M. Paye and HI Maibach). 2020; 155-163.

[47] Patravale VB, Mandawgade SD. Novel cosmetic delivery systems: an application update. Int J Cos Sci. 2008; 30(1): 19-33.

[48] Reza Mozafari M, Johnson C, Hatziantoniou S, Demetzos C. Nanoliposomes and their applications in food nanotechnology. J Lipo Res. 2008; 18(4): 309-317.

[49] Gabizon A. Liposomes as a drug delivery system in cancer chemotherapy. Hor Biochem Biophy. 1989; 9: 185-190.

[50] Ugochukwu AE, Nnedimkpa OJ, Rita NO. Preparation and characterization of Tolterodine tartrate proniosomes. Uni J Pharm Res. 2017; 2(2): 22-25.

[51] Li X, Chen D, Le C, Zhu C, Gan Y, Hovgaard L. Novel mucus penetrating liposomes as a potential oral drug delivery system: preparation, in vitro characterization, and enhanced cellular uptake. Int J Nano Med. 2011; 6(9): 3151-3162.

[52] Kant Shashi, Kumar Satinder, Prashar Bharat. A complete review on liposomes. Int Res J Pharm. 2012; 3(7): 10-16.