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# A REVIEW ON "DEVELOPMENT AND EVALUATION OF LIPOSOMES

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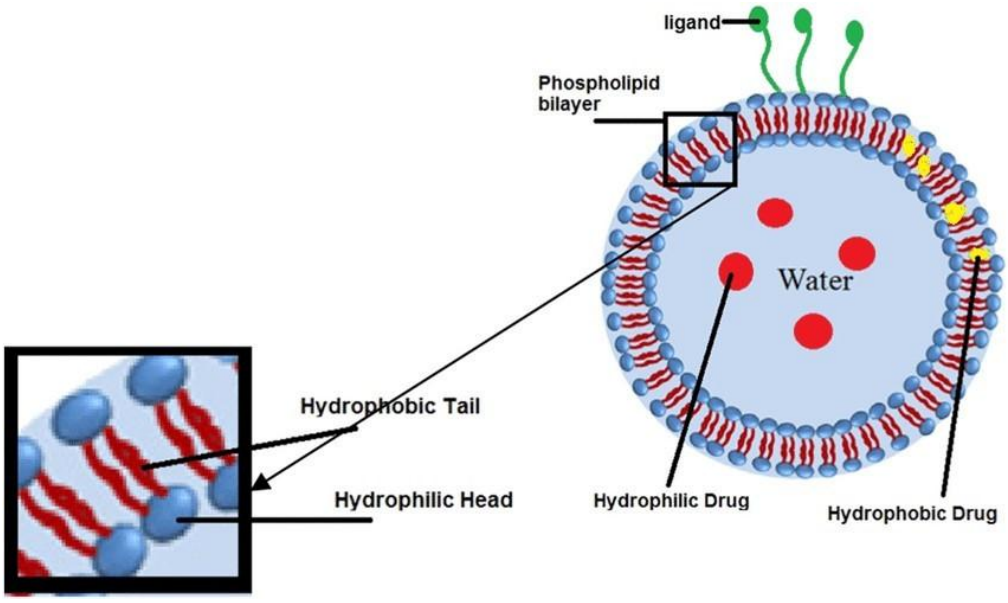
## ABSTRACT

Liposomes are simple microscopic vesicles in which an aqueous volume is entirely enclosed by a membrane compose of a lipid moiety and are well known to alter the bio-distribution of entrapped substances by protecting the enclosed materials. They are widely used as vehicles or carriers to target the specific molecule to a specific organ especially in condition of bacterial infections. Development of system which controls the release and enhances the bioavailability of chemical agents. This study aimed at developing and optimizing liposomal formulation for maximum therapeutic efficacy with minimal side effects by lipid film hydration technique using various ratios of soya lecithin and cholesterol.

**KEYWORDS:** Cholesterol and Soya lecithin, Phosphate buffer, Stearic acid acetone ethanol.

## INTRODUCTION

Liposomes, sphere-shaped vesicles consisting of one or more phospholipid bilayers, were first described in the mid-60s. Today, they are a very useful reproduction, reagent, and tool in various scientific disciplines, including mathematics and theoretical physics, biophysics, chemistry, colloid science, biochemistry, and biology. Since then, liposomes have made their way to the market. Among several talented new drug delivery systems, liposomes characterize an advanced technology to deliver active molecules to the site of action, and at present, several formulations are in clinical use. Research on liposome technology has progressed from conventional vesicles to 'second- generation liposomes', in which long-circulating liposomes are obtained by modulating the lipid composition, size, and charge of the vesicle.




Liposomes are small artificial vesicles of spherical shape that can be created from cholesterol and natural non-toxic phospholipids. Due to their size and hydrophobic and hydrophilic characters (besides biocompatibility), liposomes are promising systems for drug delivery. Liposome properties differ considerably with lipid composition, surface charge, size, and the method of preparation Furthermore, the choice of bilayer components determines the 'rigidity' or 'fluidity' and the charge of the bilayer. For instance, unsaturated phosphatidylcholine species from natural sources (egg or soybean phosphatidylcholine) give much more permeable and less stable bilayers, whereas the saturated phospholipids with long acyl chains (for example, dipalmitoylphos phatidylcholine) form a rigid, rather impermeable bilayer structure.

In 1965, some researchers published the first description of swollen phospholipid systems. Within a few years, a variety of enclosed phospholipid bilayer structures consisting of single bilayers, initially 'bangosomes' and then 'liposomes', were described. The early pioneers such as Gregoriadis and Perrie have established the concept that liposomes can entrap drugs and can be used as drug delivery systems *In vivo* activity of liposome-entrapped drugs in animal models were first demonstrated by using the anti-cancer drug cytosine arabinoside to demonstrate significant increases in the survival times of mice bearing L1210 leukemia From then it became a popular 'model system' for testing the effects of a wide variety of liposome characteristics on therapeutic outcomes.

**Designing of liposomes is done to achieve the following optimized properties**

1. Drug loading and control of drug release rate
2. Overcoming the rapid clearance of liposomes
3. Intracellular delivery of drugs
4. Receptor-mediated endocytosis of ligand-targeted liposomes
5. Triggered release
6. Delivery of nucleic acids and DNA.

**Mechanism of liposome formation**

Liposomes are formed by phospholipids (amphiphilic molecules having a hydrophilic head and hydrophobic tail). The hydrophilic part is mainly phosphoric acid bound to a water soluble molecule whereas the hydrophobic part consists of two fatty acid chains with 10-24 carbon atoms and 0-6 double bonds in each chain. They form lamellar sheets when dispersed in aqueous medium by aligning themselves in such a way that the polar head group faces outwards the aqueous region while fatty acid groups face each other forming a spherical, vesicle like structures called as liposomes. The polar fraction remains in contact with the aqueous region along with the shielding of the non-polar part. When phospholipids are hydrated in water, along with the input of energy like sonication, shaking, heating, homogenization, etc. It is the hydrophilic/ hydrophobic interactions between lipid-lipid, lipid-water molecules that lead to the formation of bilayered vesicles in order to achieve a thermodynamic equilibrium in the aqueous phase. Phospholipids are the main components of the cell membrane hence they possess excellent biocompatibility with amphiphilic properties.

The amphiphilicity provide it the property of self assembly, emulsifying and wetting characteristics. When phospholipids introduced into aqueous milieu, they self assemble and it generates different structures with specific properties at different conditions. For example, phospholipids have a natural tendency to form liposomes, which can be employed as drug targeting molecules. They also have good emulsifying property to stabilize the emulsions. In addition of wetting property, this can be used in coating of drug to provide hydrophilicity to hydrophobic drugs .These three properties are employed in various drug designing.

Variation in aliphatic chains and alcohols lead to the occurrence of varieties of phospholipids. Additionally different sources of phospholipids enhance varieties of phospholipids.

**Classification of Liposome**

Liposomes can be classified on the basis of size and number of bilayers. They are classified as multilamellar vesicles (MLV), large unilamellar vesicles (LUV) and small unilamellar vesicles (SUV). Based on composition, they are classified as conventional liposomes (CL), pH- sensitive liposomes, cationic liposomes, long circulating liposomes (LCL) and immuno-liposomes. Based on the method of preparation, they are classified as reverse phase evaporation vesicles (REV), French press vesicles (FPV) and ether injection vesicles (EIV) as given in **Table 1**.

| S. No. | Classification based on structural features | Classification based on method of liposome preparation | Classification based on targeting concepts of liposomes |
|---|---|---|---|
| 1 | Multilamellar large vesicles | Single or oligolamellar vesicle made by reverse phase evaporation method. | PEGylated liposomes |
| 2 | Oligolamellar vesicles | Multilamellar vesicles made by reverse phase evaporation method. | Immunoliposomes |
| 3 | Unilamellar vesicles | Stable plurilamellar vesicles. | Cationic liposomes |
| 4 | Small unilamellar vesicles | Frozen and thawed MLV | Thermosensitive liposomes |
| 5 | Medium sized unilamellar vesicles | Vesicles prepared by extrusion method. | |
| 6 | Large unilamellar vesicles | Vesicles prepared by fusion | |
| 7 | Giant unilamellar vesicles | Vesicles prepared by French press | |
| 8 | Multivesicular vesicles | Dehydration- rehydration vesicles | |
| 9 | | Bubblesomes | |

**Advantages of liposome**

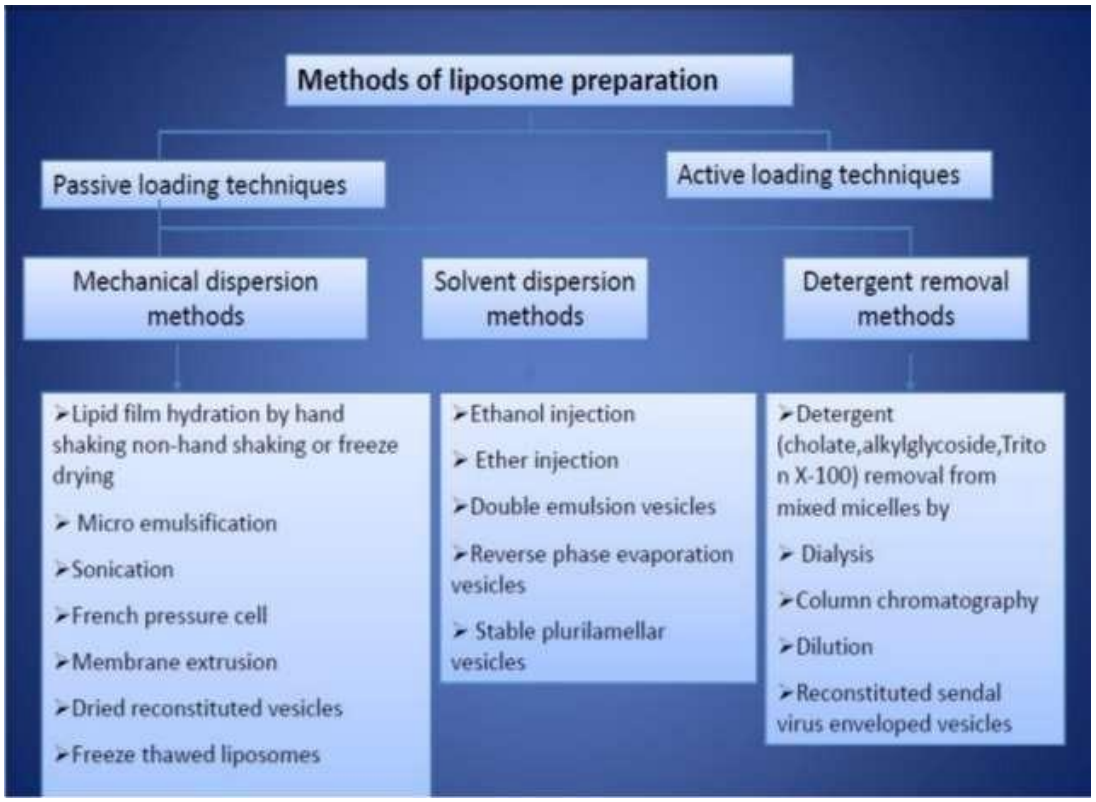
1. Liposomes increased efficacy and therapeutic index of drug (actinomycin-D
2. Liposome increased stability via encapsulation
3. Liposomes are non-toxic, flexible, biocompatible, completelybiodegradable, and non-immunogenic for systemic and non- systemic administrations
4. Liposomes help reduce the exposure of sensitive tissues to toxic drugs.
5. Flexibility to couple with site-specific ligands to achieve active targeting

**Disadvantages of liposome**

1. Short half-life
2. Low solubility
3. Sometimes phospholipid undergoes oxidation and hydrolysis-like reaction

4. Production cost is high.

**Methods of liposome preparation**




**General methods of preparation:** Method of liposome preparation and drug loading.

The following methods are used for the preparation of liposome

1. Passive loading techniques
2. Active loading technique

Passive loading techniques include three different methods

1. Mechanical dispersion method
2. Solvent dispersion method
3. Detergent removal method (removal of non-encapsulated material

All the methods of preparing the liposomes 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.

## 1. MECHANICAL DISPERSION METHOD

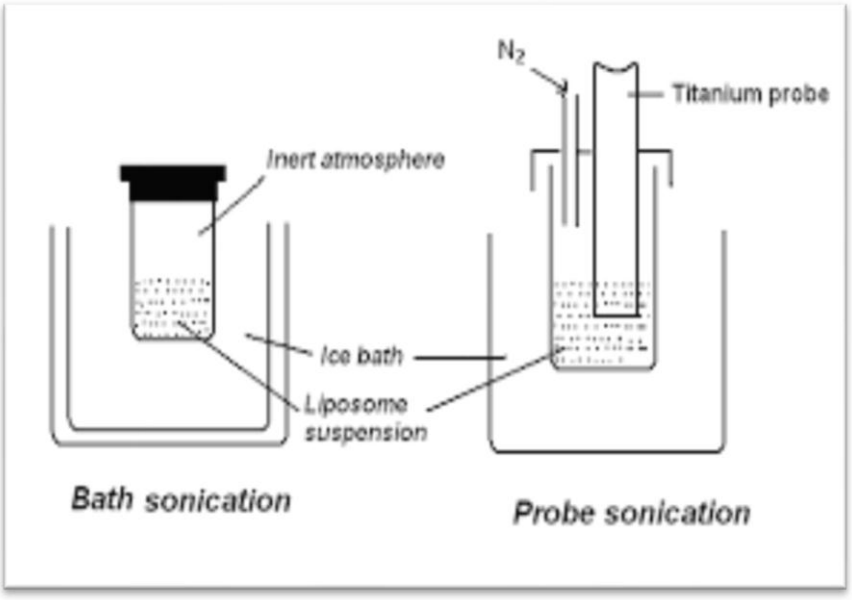
The following are types of mechanical dispersion methods:

- Sonication.
- French pressure cell: extrusion.
- Freeze-thawed liposomes.
- Lipid film hydration by hand shaking, non-hand. shaking or freeze drying.
- Micro-emulsification
- Membrane extrusion.
- Dried reconstituted

### A. Sonication

Sonication is perhaps the most extensively used method for the preparation of SUV. Here, MLVs are sonicated either with a bath type sonicator or a probe sonicator under a passive atmosphere. The main disadvantages of this method are very low internal volume/encapsulation efficacy, possible degradation of phospholipids and compounds to be encapsulated, elimination of large molecules, metal pollution from probe tip, and presence of MLV along with SUV:

There are two sonication technique:

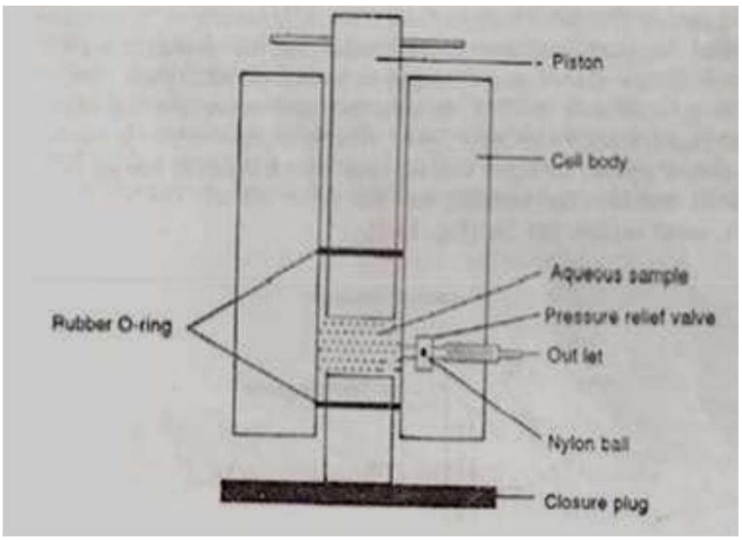



a. Probe sonication. The tip of a sonicator is directly engrossed into the liposome dispersion. The energy input into lipid dispersion is very high in this method. The coupling of energy at the tip results in local hotness; therefore, the vessel must be engrossed into a water/ice

bath. Throughout the sonication up to 1 h, more than 5% of the lipids can be de-esterified. Also, with the probe sonicator, titanium will slough off and pollute the solution.

b. Bath sonication. an inert The liposome dispersion in a cylinder is placed into a bath sonicator. Controlling the temperature of the lipid dispersion is usually easier in this method, in contrast to sonication by dispersal directly using the tip. The material being sonicated can be protected in a sterile vessel, dissimilar the probe units, or under atmosphere.

**B. French Pressure Cell: Extrusion**




French pressure cell involves the extrusion of MLV through a small orifice]. An important feature of the French press vesicle method is that the proteins do not seem to be significantly pretentious during the procedure as they are in sonication. An interesting comment is that French press vesicle appears to recall entrapped solutes significantly longer than SUVs do, produced by sonication or detergent removal. The method involves gentle handling of unstable materials. The method has several advantages over sonication method. The resulting liposomes are rather larger than sonicated SUVs. The drawbacks of the method are that the high temperature is difficult to attain, and the working volumes are comparatively small (about 50 mL as the maximum.

**C. Freeze-thawed liposome**

Liposomes are rapidly frozen and thawed slowly. The short-lived sonication disperses aggregated materials to LUV. The creation of unilamellar vesicles is as a result of the fusion of SUV throughout the processes of freezing and thawing [This type of synthesis is strongly

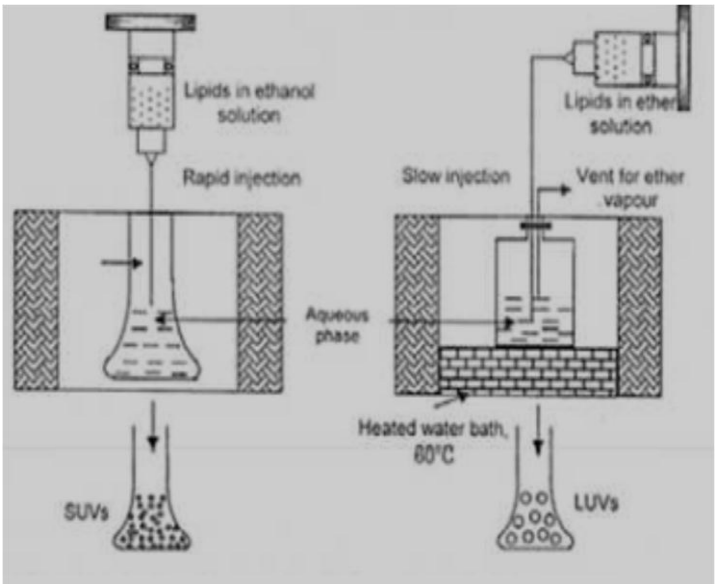
inhibited by increasing the phospholipid concentration and by increasing the ionic strength of the medium. The encapsulation efficacies from 20% to 30% were obtained.

## 2. SOLVENT DISPERSION METHOD

### A. Ether injection (solvent vaporization)

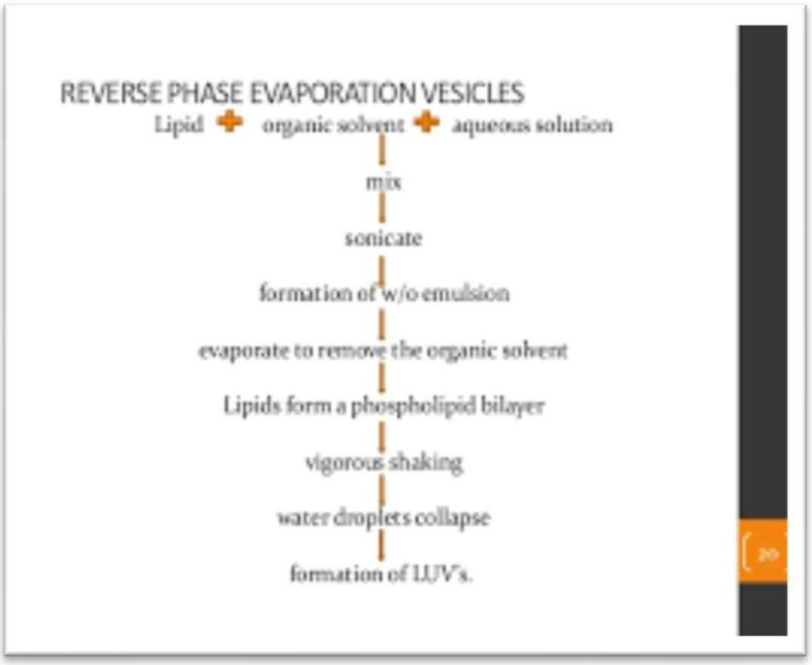
A solution of lipids dissolved in diethyl ether or ether-methanol mixture is gradually injected to an aqueous solution of the material to be encapsulated at 55°C to 65°C or under reduced pressure. The consequent removal of ether under vacuum leads to the creation of liposomes. The main disadvantages of the technique are that the population is heterogeneous (70 to 200 nm) and the exposure of compounds to be encapsulated to organic solvents at high temperature.

### B. Ethanol injection




A lipid solution of ethanol is rapidly injected to a huge excess of buffer. The MLVs are at once formed. The disadvantages of the method are that the population is heterogeneous (30 to 110 nm), liposomes are very dilute, the removal all ethanol is difficult because it forms into celecoxib with water, and the probability of the various biologically active macromolecules to inactivate in the presence of even low amounts of ethanol is high.

**C. Reverse phase evaporation method**




This method provided a progress in liposome technology, since it allowed for the first time the preparation of liposomes with a high aqueous space-to-lipid ratio and a capability to entrap a large percentage of the aqueous material presented. Reverse-phase evaporation is based on the creation of inverted micelles. These inverted micelles are shaped upon sonication of a mixture of a buffered aqueous phase, which contains the water-soluble molecules to be encapsulated into the liposomes and an organic phase in which the amphiphilic molecules are solubilized. The slow elimination of the organic solvent leads to the conversion of these inverted micelles into viscous state and gel form. At a critical point in this process, the gel state collapses, and some of the inverted micelles were disturbed. The excess of phospholipids in the environment donates to the formation of a complete bilayer around the residual micelles, which results in the creation of liposomes. Liposomes made by reverse phase evaporation method can be made from numerous lipid formulations and have aqueous volume-to-lipid ratios that are four times higher than hand-shaken liposomes or multilamellar liposomes.

**3. Detergent removal method (removal of non-encapsulated material)**

**A.** Detergent (cholate, alkyl glycoside, Triton X-100) removal of mixed micelles (absorption) Detergent absorption is attained by shaking mixed micelle solution with beaded organic polystyrene adsorbers such as XAD-2 beads (SERVA Electrophoresis GmbH, Heidelberg, Germany) and Bio-beads SM2 (Bio-RadLaboratories, Inc., Hercules,

USA). The great benefit of using detergent adsorbers is that they can eliminate detergents with a very low CMC, which are not entirely depleted.

**B. Gel-permeation chromatography**

In this method celecoxib can be used for gel filtration. The liposomes do not penetrate into the pores of the beads packed in a column. They percolate through the inter-bead spaces. At slow flow rates, the separation of liposomes from detergent monomers is very good. The swollen polysaccharide beads adsorb substantial amounts of amphiphilic lipids; therefore, pre-treatment is necessary. The pre-treatment is done by pre-saturation of the gel filtration column by lipids using empty liposome suspensions.

**3. 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 polydispersed micelles to vesicles occur.

**EVALUATION OF LIPOSOMES**

**1. Entrapment Efficiency**

The entrapped drug concentrations were determined after the lysis of prepared liposomes with absolute alcohol and sonicated for 10 min (Law and Shih 2001). The entrapment efficiency expressed as entrapment percentage was calculated through the following relationship.

**Entrapment Efficiency Percentage = Total Entrapped drug/Total drug X 100.**

**2. *In Vitro* Difussion Studies**

The release of drug from all multilamellar liposomal formulations with different compositions was determined using the membrane diffusion technique. In brief, an accurately measured amount of Celecoxib solution or Celecoxib liposomal formulations, equivalent to 20 mg Celecoxib was suspended in 1 mL of phosphate buffered saline [pH 7.4] in a glass cylinder having a length of 20 cm and a diameter of 2.5 cm. This cylinder was fitted, before addition of liposomal suspension, with a presoaked membrane [Spectra/Pore membrane] and was placed in a beaker (200 mL) containing 50 mL phosphate buffered saline [pH 7.4]. The whole set was placed on a magnetic stirrer adjusted to a constant speed [150 rpm]. Samples were collected every 1 h over a period of 48 h and the same volume of fresh test solution was

replenished and the drug release weredetermined spectro-photometrically (Hathout et al 2007.

### 3. Particle Size Analysis and Zeta Potential

The mean particle size of the liposomal formulations were determined by light scattering based on laser diffraction using laser diffraction particle size analyzer.

### 4. Lyophilization

The drug was used in different concentrations was dissolved in suitable buffer, along with the liposomal formulations were frozen over night at-80 ℃ and then freeze dried for 24hrs under vaccum at -50 C, the resulting lyophilized cakes were rehydrated to its original volume at room temperature with PBS and subjected for further investigation.

### 5. Scanning Electron Microscopy Studies

Scanning electron microscopy has been extensively employed to study the morphology and surface topography of the prepared liposomes. The samples to be examined were mounted on the SEM sample stab using a double sided sticking tape. The samples mounted were coated with gold (200 A°) under reduced pressure (0.001torr) for 5 min. The gold-coated samples were observed under the SEM with suitable magnifications

### 6. Drug Release Kinetics

The data obtained from *In vitro* drug release studies were fitted into various kinetic models such as zero order, Higuchi's model and Peppas model to study the release kinetics. The stability studies were carried out for the most satisfactory formulation as per the ICH guidelines to estimate the stability of the prepared liposomal formulation. The formulation were sealed in aluminium package and kept in cooling chamber maintained at $4 \pm 2$ ℃, and at $25 \pm 2$ ℃, for 2 months.

## Applications of liposomes

### 1. Applications of liposomes in Basic sciences

A number of new theoretical concepts were developed to understand their conformational behaviour. On the other hand they can be used as a model in order to understand the topology, shape fluctuations, phase behaviour, permeability, fission and fusion of biological membranes.

**2. Applications of liposomes in medicine**

Sustained release system of systemically or locally administered drugs can be formulated in liposomes. Examples are doxorubicin, cytosine arabinose, cortisones, biological proteins or peptides such as vasopressin.

**3. Application of liposomes in cosmetics**

In addition to the drug application, liposome pastes are used as replacement for creams, gels, and ointments for cosmetic products. And also they are used for formulating various extracts, moisturizers, and complex products containing recombinant proteins for wound or sunburn healing.

**4. Application of liposomes in treatment of AIDS**

The liposomes also find its application informulating the drugs for the treatment of AIDS.

**5. Application of liposomes in agro-food industry**

Lipid molecules, from fats to polar lipids, are one of the fundamental ingredients in almost any food. For instance, lecithin and some other polar lipids are routinely extracted from nutrients, such as egg yolks or soya beans. Liposomes are also used in agro food industries.

**6. Liposomes in parasitic diseases and infections**

From the time when conventional liposomes are digested by phagocytic cells in the body after intravenous management, they are ideal vehicles for the targeting drug molecules into these macrophages. The best known instances of this ‘Trojan horse-like’ mechanism in several parasitic diseases which normally exist in the cell of MPS.

They comprise leishmaniasis and several fungal Infections.

**7. Marketed products of liposomes**

Many of the drugs like anticancer, antibacterial, antifungal drugs, enzymes, hormones, NSAIDs, have been marketed in liposomal formulations.

**CONCLUSION**

Among the various Novel Drug Delivery systems the Liposomal drug delivery system is one of the best advantageous carriers for the drug delivery because of its lipophillic moieties in the formulations.

The drug release pattern was in controlled manner. Prepared liposomes showed good stability profile without any much declination in their properties. Thus we conclude that in addition to other Novel Drug Delivery carriers like nanoparticles, microspheres & floating beads, liposomes are best system for the delivery of biphasic drugs.

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