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A RECENT REVIEW ON MUCOADHESIVE LIPOSOME AS A NOVEL DRUG DELIVERY SYSTEM

S. Shravya Lakshmi*[1], S. Parthiban[1], G. P. Senthil kumar[1], T. Tamizh Mani[1]

[1]*Department of Pharmaceutics, Bharathi College of Pharmacy, Bharathinagar, Maddur, Mandya, Karnataka, India.

ABSTRACT

Liposomal mucoadhesive drug delivery system is developed in order to improve the bioavailability of poorly absorbed drugs. The bioavailability is improved by prolonging their gastric and intestinal residence time, by facilitating the intimate contact with the absorption membrane. Liposomes are artificially prepared vesicles and they are very important tools for improving delivery of drugs like antimicrobial, anticancer, antifungal drugs, peptide hormones, enzymes, vaccines and genetic materials. The term liposome means lipid body it has been derived on the bases of name of subcellular particles, ribosomes. Their size range is from 25-500nm. Liposomes are microscopic vesicles in which an aqueous volume is enclosed by a membrane composed of a lipid moiety, which alter the bio distribution of entrapped drug substances by protecting the enclosed materials. Gastro retentive dosage forms are having high potential for the usage as controlled drug delivery systems. A controlled release system designed to improve its residence time in stomach by making contact with the mucosa is achieved through formulating mucoadhesive liposomes.

KEYWORDS

Liposomes, Mucoadhesive drug delivery, Mechanism and Polymer of mucoadhesion, Method of preparation, Evaluation, Review of literature and Applications.

Author for Correspondence:

Shravya Lakshmi S,
Department of Pharmaceutics,
Bharathi College of Pharmacy,
Bharathinagar, Maddur, Mandya,
Karnataka, India.

Email: shravyals1993@gmail.com

INTRODUCTION

Liposomes are the vesicles made up of the same material as a cell membrane. Liposomes can incorporate with drugs, and used in the delivery of drugs for cancer and other diseases. The name liposome is derived from two Greek words: 'Lipos' meaning fat and 'Soma' meaning body[1]. "Liposomes are colloidal, vesicular structure composed of one or more lipid bilayers surrounding an equal numbers of aqueous compartments." The substances like peptides and protein, hormones, enzymes, antibiotics,

antifungal drugs, and anticancer agents are incorporate in a sphere shaped shell which is encapsulated a liquid interior[2]. The oral route remains to be the most convenient and comfortable way of drug administration, including peptide delivery. However, peptide drugs are readily degraded in presence of gastric acid and proteolytic enzymes in the gastrointestinal tract (GIT)[3]. Mucoadhesion delivery system is designed to prolong the residence time of the dosage form at the site of application or absorption. The delivery system is facilitating intimate contact of the dosage form with the underlying absorption surface to improve and enhance the bioavailability of drugs[4].

ADVANTAGES OF ORAL MUCOADHESIVE DRUG DELIVERY SYSTEMS[5]

- Prolongs the residence time of the dosage form at the site of application and improves the bioavailability.
- It provides rapid onset of action.
- Enormous blood supply and good blood flow rate increases rapid absorption.
- Drug degradation can be protected from gastric acidic environment in GIT.
- Patient compliance can be improved

DISADVANTAGES OF MUCOADHESIVE DRUG DELIVERY SYSTEMS[5]

- Prolonged contact of the drug possessing ulcerogenic property and leads to local ulcerous effect.
- The development of oral mucosal delivery is the lack of a good model for *in vitro* screening.
- Patient acceptability in terms to taste, irritancy and mouth feel are required to mask.

MECHANISM OF MUCOADHESIVE[6]

The mucoadhesion system is the attachment of the drug along with a suitable carrier to the mucous membrane. It is a complex phenomenon which involves wetting, adsorption and interpenetration of polymer.

Mechanism

- It forms Intimate contact between mucoadhesive system and mucous membrane and involve in wetting and swelling phenomenon.
- Penetration of the mucoadhesive system takes place into the surface of the mucous membrane due to interpenetration of polymer.

FACTORS AFFECTING MUCOADHESION[7]

The mucoadhesion drug delivery system depends on the following factors:

- Physical Factors like pH, swelling of polymer, applied strength, contact time.
- Physiological Factors like presence of the enzyme mucin and disease condition.
- Polymer Based Factors like molecular weight, concentration, stereo chemistry, chain length and hydration factor of polymer.

ADVANTAGES OF LIPOSOMES[1,2]

- Liposomes are biocompatible, completely bio degradable, non-ionic and non-immunogenic.
- Suitable for delivery of hydrophobic, amphipathic and hydrophilic drugs.
- Increased efficacy and therapeutic index.
- Increased stability via encapsulation.
- Site avoidance effect.
- Reduce exposure of sensitive tissues to toxic drugs.

DISADVANTAGES OF LIPOSOMES[2]

- It leads to leakage and fusion of encapsulated drug molecules.
- Liposomes have short half-life.
- It is expensive.

TYPES OF LIPOSOMES[1,2,9]

Liposomes are classified on the bases of

BASED ON THE STRUCTURE PARAMETERS

Unilamellar

Vesicles SUV

Small unilamellar vesicles size ranges from 20-40nm

MUV

Medium unilamellar vesicles size range from 40-80nm

LUV

Large unilamellar vesicles size range from 100-1000nm

Oligolamellar Vesicles (OLV)

- Internal volume of lipid surrounding made up to 2-10 bilayers

Multilamellar Vesicles (MLV)

- They have several bilayers. The arrangements can be onion like arrangements of concentrate spherical bilayers of LUV/MLV enclosing a large number of SUV etc.

Based On the Method of Liposome Preparation

- **MLV-REV:** Multilamellar vesicles made by Reverse phase Evaporation method
- **SPLV:** Stable Plurilamellar Vesicles
- **REV:** Single or Oligolamellar vesicles made by Reverse phase Evaporation method
- **VET:** Vesicles prepared by extrusion technique
- **DRV:** Dehydration- rehydration method
- **FATMLV:** Frozen and Thawed MLV

BASED UPON COMPOSITION AND APPLICATION

Conventional Liposomes (CL)

Neutral or negatively charged phospholipid and cholesterol.

Fusogenic Liposome (RSVE)

Reconstituted Sendai virus envelopes.

Ph Sensitive Liposomes

Phospholipid such as PE or DOPE with either CHEMS or OA.

Cationic Liposomes

Cationic lipids with DOPE.

Long Circulatory Liposomes (LCL)

They have polyethylene glycol (PEG) derivatives attached to their surface to decrease their detection by phagocyte system.

Immuno- Liposomes

CL or LCL with attached monoclonal antibody or recognition sequence.

METHODS OF LIPID PREPARETION[1,2,12]

1. General method of preparation
2. Specific methods of preparation

General Method of Preparation

The lipid is melted in organic solvent. The solvent is dispersed and lives a film of lipids on the wall of the flask. An aqueous solution of drug is added. The mixture is stressed to create multi lamellar vesicle and sonicated to get SUVs. The mixture is sonicated and the solvent is dispersed to get LUVs. SUVs are found after extraction. The drug can be integrated into the aqueous solution in case of hydrophilic or organic solvent in case of hydrophobic.

Specific Methods of Preparation

There are 3 methods based on their dispersion.

1. Physical Dispersion methods
2. Solvent Dispersion methods
3. Detergent Solubilization methods

Physical Dispersion Methods

Methods the aqueous volume enclosed within lipid membrane is about 5-10% which is very small proportion of total volume used for preparation. So large amount of water soluble drugs is wasted during preparation. But lipid soluble drug can be encapsulated to high percentage. In these methods, MLVs are formed and further treatment is required for preparation of Unilamellar vesicles

Hand Shaken Method

This is very simple and commonly used method. The lipid mixture is dissolved in chloroform and methanol mixture (2:1 ratio). Then the mixture is transferred in to a 250ml round bottomed flask. The flask is attached to rotary evaporator connected with vacuum pump and rotated at 60 rpm. The organic solvent is allowed to evaporate at room temperature. The rotation is continued for another 15 min and a dried residue is formed at the wall of the flask. The evaporator is detached from vacuum pump and

nitrogen is introduced in to it. The flask is then removed from evaporator and fixed on to lypholizer to remove residual solvent. Then the flask is again flushed with nitrogen and 5ml of phosphate buffer. The flask is attached to evaporator and rotated at 60 rpm for 30 minutes to remove the lipid from the wall of the flask. A milky white suspension is formed. The suspension is allowed to stand for 2 hours in order to complete swelling process to give MLVs.

Freeze Drying

Another method of dispersing the lipid in a finally divided from prior to addition of aqueous media is to freeze dry the lipid dissolve in a suitable organic solvent. The solvent usually used is tertiary butanol. All the above methods produce MLVs. these are too large or too heterogeneous. In order to modify the prepared MLVs are further processed using the following procedure.

PROCESSING OF LIPID HYDRATED BY PHYCICAL MEANS

Micro-Emulsification of Liposomes

Equipment called micro fluidizer is used to prepare small vesicles form concentrated lipid suspension. The lipids can be introduced in to the fluidizer as a suspension of large MLVs. This equipment pumps the fluid at very high pressure through 5 micrometer screen. Then the suspension is forced through long micro channels, which direct two streams of fluids collide together at right angles at very high velocity. The fluid collected is recycled through the pump and interaction chamber until vesicles of spherical dimensions are obtained.

Sonication

This is most widely used method for the preparation of small Unilamellar vesicles. There are two techniques.

Probe Sonication

The tip of sonicator is directly immersed into the liposome dispersion. The dissipation of energy at the tip due to local overheating and hence the vessel must be immersed into an ice bath. About 5% of the lipids can be desterify in one hour of sonication.

Bath Sonicator

The tube of liposome dispersion is placed into a bath sonicator. The temperature of the lipid dispersion can be easily controlled. Material being sonicated can be kept in a sterile container under an inert atmosphere. The lipid bilayer of the liposomes can fuse with other bilayers.

Membrane Extrusion Liposomes

In this method the size is reduced by passing those through a membrane filter of defind pore size. There are two types of membrane filter. The tortuous path type and the nucleation track type. The former is used for sterile filtration. In this random path arise between the criss cross fibers. The average diameter of these fibers is controlled by the density of fibers in the matrix. Liposomes that are larger than the channel diameter get struck when one tries to pass them through such membrane. The nucleation track type composed of thin continuous sheet of polycarbonate. They will offer less resistance to passage of liposomes as these consist of straight sided pore holes of exact diameter bored from one side to another. This method can be used to process both LUVs and MLVs.

Freez and Thaw Sonication

This is a method in which rupture and refusing of SUVs are done during which the solute equilibrates between the inside and outside. This process increases the entrapment volume and entrapment efficiency. This method will result in the formation of vesicles with in vesicled and vesicle between lamellae. This method can increase the entrapment volume up to 30%.

SOLVENT DISPERSION METHODS Ethanol Injection Method

A lipid solution of ethanol is rapidly injected to a vast excess of buffer. The MLVs are immediately formed. The disadvantages of the method are that the liposomes are heterogeneous (30-110 nm), and very dilute. The removal of ethanol is very difficult. Because it forms azeotrope with water and biologically active macromolecules become inactivation in the presence of ethanol.

Ether Injection Method

The lipid solution is dissolved in diethyl ether or ether-methanol mixture and slowly injected to an aqueous solution of the material to be encapsulated at 55-65°C or under reduced pressure. The ether solvent is subsequently removed under vacuum leads to the formation of liposomes. The main drawbacks of the method are the liposomes are heterogeneous (70-190 nm).

Reverse Phase Evaporation Method

Water in oil emulsion is formed by simple sonication of a two phase system containing phospholipids in organic solvent and aqueous buffer. The organic solvents are removed under reduced pressure and this produce formation of a viscous gel. The liposomes are formed due to evaporation of solvent by rotary evaporation under reduced pressure. In this method 65% of encapsulation can be obtained in a medium of low ionic strength, 0.01M NaCl. Small and large macromolecules can be encapsulated using this method.

Detergent Solubilization Methods

In this method the phospholipids are brought in to close contact with the aqueous phase via detergents, which associate with phospholipids molecules. The structures formed as a result of this association are known as micelles. They are composed of several hundreds of component molecule. The concentration of detergent in water at which micelles start to form is called CMC. Below CMC the detergent molecule exist in free solution. As the detergent molecule is dissolved in water at concentration higher than the CMC, micelle form in large amounts. As the concentration of detergent added is increased more amount of detergent is incorporated in to the bilayer, until a point is reached where conversion from lamellar form to spherical micellar form take place. As detergent concentration is further increased, the micelles are reduced in size.

PREPARATION OF COATING OF LIPOSOMES[3,13]

Chitosan coating

Chitosan solutions 0.1% to 0.6% (w/v) were prepared in 0.1% (v/v) glacial acetic acid. A volume of 2.0 mL of chitosan solution was added drop-wise to the 2.0 mL of liposomes under magnetic stirring at room temperature for 1 hour, followed by incubation in refrigerator overnight.

Carbopol coating

Carbomers 974P was dissolved in PBS 7.4. A volume of 2.0 mL of Carbopol solution was added drop-wise to the 2.0 mL of liposomes under magnetic stirring at room temperature for 1 hour, followed by incubation in refrigerator overnight.

Eudragit coating

Eudragit S100 and Eudragit L100 were used. Eudragit were dissolved in acetone containing 3.0% of water and drop-wise added to liposomes and left overnight in refrigerator to stabilize before characterization.

USES OF LIPOSOMES[9]

- It is used as Chelation therapy for treatment of heavy metal poisoning
- It is used as Enzyme replacement
- It is used as Diagnostic imaging of tumors
- It is used as Cosmetics

APPLICATION OF LIPOSOMES[2]

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

TARGETING OF LIPOSOMES[2,10]

Passive Targeting

It refers to the accumulation of drug system at a specific site such as anti-cancerous drug attributed to physicochemical or pharmacological factors of the disease. Hence, in case of cancer treatment the size and surface properties of drug delivery nano-

particles must be controlled to avoid uptake by the reticulo-endothelial system (RES). Drug release or drug actions are limited to selective sites within the body such as a tumor. Other examples include targeting of antimalarial drugs for treatment of leishmiansis, brucellosis, and candiadsis.

Active Targeting

Active targeting means a specific ligand- receptor type interaction for intracellular localization. This occurs only after blood circulation and extravasations. It is further classified into three different levels of targeting; which are

1. First order targeting refers to distribution of the drug carrier systems to the capillary bed of a predetermined target site.

Ex: compartmental targeting in lymphatics, peritoneal cavity, plural cavity, cerebral ventricles.

2. Second order targeting refers to selective delivery of drugs to specific cells such as tumour cells

Ex: selective drug delivery to kupffer cells in the liver.

3. Third order targeting refers to drug delivery specifically to the intracellular site of targeted cells Ex: receptor based ligand mediated entry of a drug complex into a cell by endocytosis.

EVALUATION PARAMETER FOR MUCOADHESIVE LIPOSOME[3,2,11]

Review of Literature

Khameneh B., *et al* studied the properties of nanoliposomal formulation, they prepared Anionic liposomes by dehydration-rehydration method with an average size of 100 nm and coated with 0.01% (w/v) solution of trimethylchitosan with 50±10% of quaternization. They reported trimethyl chitosan-coated nanoliposomes have several positive potentials including good mucoadhesive properties, preserved integrity of loaded antigen and presence of TMC as a mucoadhesive polymer with innate immune adjuvant potential which make them suitable for efficient delivery system. Drug delivery via mucosal routes has been confirmed to be effective in including strong immune responses[14].

Channarong *et al*., developed chitosan-coated and polyplex-loaded liposomes containing DNA vaccine for Payer's patch targeting. Plain liposomes carrying plasmid pRc/CMV-HBs were prepared by the reverse-phase evaporation method. Chitosan coating was carried out by incubation of the liposomal suspensions with chitosan solution. Their results showed that zeta potentials of plain liposomes were strongly affected by the pH of the medium. Chitosan-coated polyplex-loaded liposomes demonstrated a higher potential to deliver the DNA to the distal intestine than the chitosan-coated liposomes due to the increase in permanent positive surface charges and the decreased enzymatic degradation[15].

Wu ZH *et al.,* evaluated the hypoglycemic efficacy of insulin liposomes coated by chitosan with different molecular weights and concentrations after oral administration in mice. Insulin-liposomes were prepared by reversed-phase evaporation. Chitosan coating was carried out by incubation of the liposomal suspensions with the chitosan solution. They concluded Chitosan-coated liposomes could reduce tryptic digestion on insulin, and enhanced enteral absorption of insulin. In all the insulin liposomes, the insulin liposomes coated by 0.2% chitosan showed a better hypoglycemic efficacy as compared with the other liposomes coated by chitosan[16].

Hirofumi *et al.,* evaluated the circulating properties of liposomes coated with modified polyvinyl alcohol-R having different molecular weights (6000, 9000 and 20000). Polymer coated liposomes were prepared by just mixing the resultant liposomal suspension and a polymer solution. The effects of polymer coating were evaluated by measuring the circulation time of the injected liposomes after i.v. administration in rats and the dispersing property of the liposomes in a biological condition. They reported prolonged circulation time of polyvinyl alcohol-R (molecular weight: 20000) coated liposomes). The circulation of the polyvinyl alcohol-R coated liposomes was prolonged with increasing the molecular weight of polyvinyl alcohol-R. The aggregation and/or fusion of the liposomes in the presence of the liposomes in the presence of serum *in vitro* was also depressed more

by coating the liposomes with polyvinyl alcohol-R having higher molecular weight[17.]

Jung I W*et al.,* They developed chitosan-coated mucoadhesive liposomes containing risedronate to improve intestinal drug absorption. Liposomes containing risedronate were prepared with 1, 2-distearoryl-sn-glycero-3-phosphocholine and distearoryl-sn-glycero-3-[phospho-rac-(1-glycerol)] using the freeze-drying method, with subsequent coating of the anionic surfaces of the liposomes with chitosan. Chitosan-coated liposomes also showed strong mucoadhesive properties, implying potential electrostatic interaction with the mucous layer in the gastrointestinal tract. Compared with the untreated drug, chitosan-coated liposomes significantly enhanced the cellular uptake of risedronate, resulting in an approximately 2.1 - 2.6-fold increase in Caco-2 cells[18].

Silvia F. Pantze *et al.,* Liposomes can be used as oral dosage form to improve the bioavailability of both hydrophilic drugs, such as peptides and proteins and lipophilic drugs. But liposome dispersions are not very stable under the harsh conditions of the GI-tract. Also a controlled release of embedded compounds is not possible. The lipid bilayer of standard egg-PC/cholesterol liposomes were stabilized by gelatin. The gelatin also works as a thickening agent by forming a matrix in which liposomes are embedded. Liposomes were prepared by dual asymmetric centrifugation with the addition of gelatin[19].

Table No.1: Classification of Mucoahesive Polymer[1,7,8]

S.No	Synthetic polymers	Natural polymers
1	Hydroxy propyl methyl cellulose (HPMC)	Chitosan
2	Poly(acrylic acid) polymers (carbomers, polycarbophil)	Sodium alginate
3	Poly vinyl pyrrolidone (PVP)	Pectin
4	Poly vinyl alcohol (PVA)	Locust bean gum
5	Poly hydroxyethyl methylacrylate	Guar gum
6	Poly ethylene oxid	Xanthan gum
7	Sodium carboxy methyl cellulose (Na CMC)	Karaya gum
8	Hydroxyl ethyl cellulose (HEC)	Gelatin
9	Hydroxy propyl cellulose (HPC)	Tragacanth
10	Ethyl cellulose (EC)	Soluble starch
11	Methyl cellulose (MC)	Lecithin

Table No.2: Evaluation Parameters

S.No	Parameter	Instrument/apparatus
1	Surface morphology	SEM/TEM
2	Vesicle size analysis	optical microscope
3	Drug content	U.V spectrophotometer
4	Entrapment efficiency	Dialysis tube Centrifugation Gel chromatography
5	Zeta potential	Zetasizer Nano Z (Malvern Instruments).
6	*In vitro* diffusion study	Diffusion cell
7	*In vitro* wash - off test for mucoadhesive testing	USP disintegrations apparatus
8	Determination of mucoadhesive strength	HPLC
9	Stability study	ICH guidelines

Structure of Liposomes

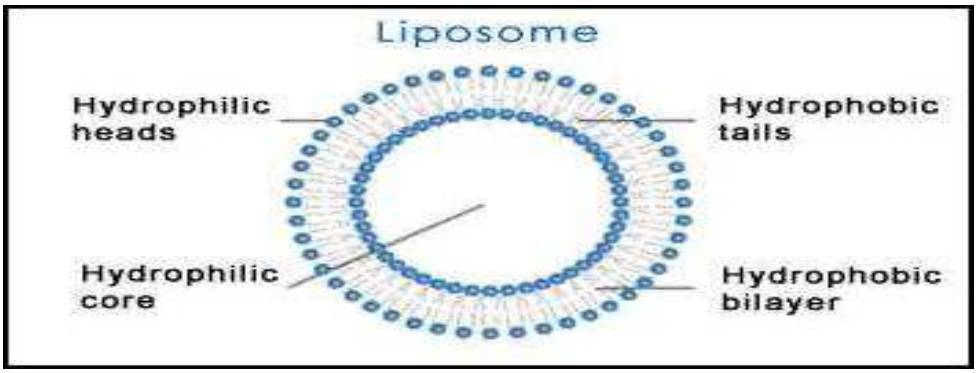

Figure No.1: Structure of liposomes

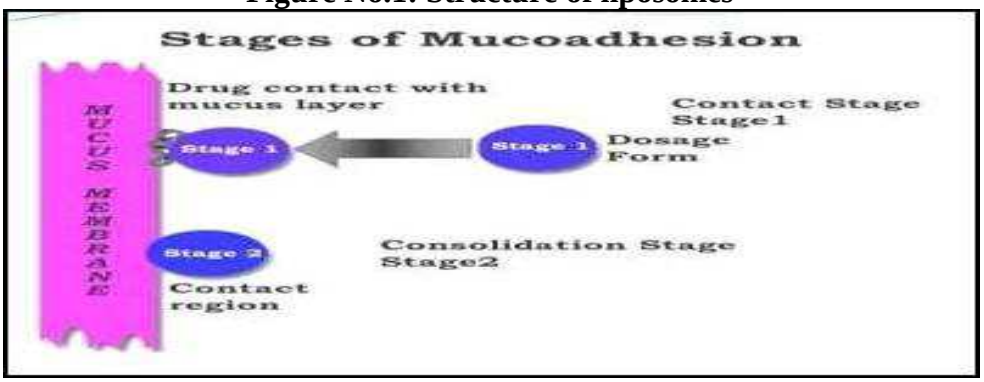

Figure No.2: Mechanism of mucoadhesive

Class of liposomes

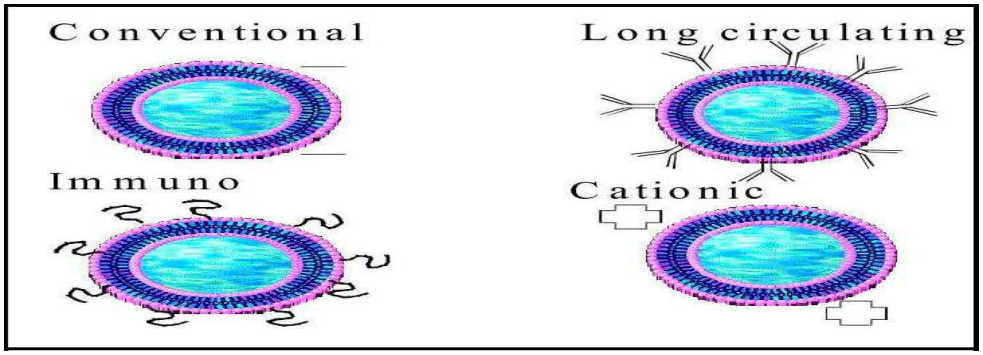

Figure No.3: Class of liposomes

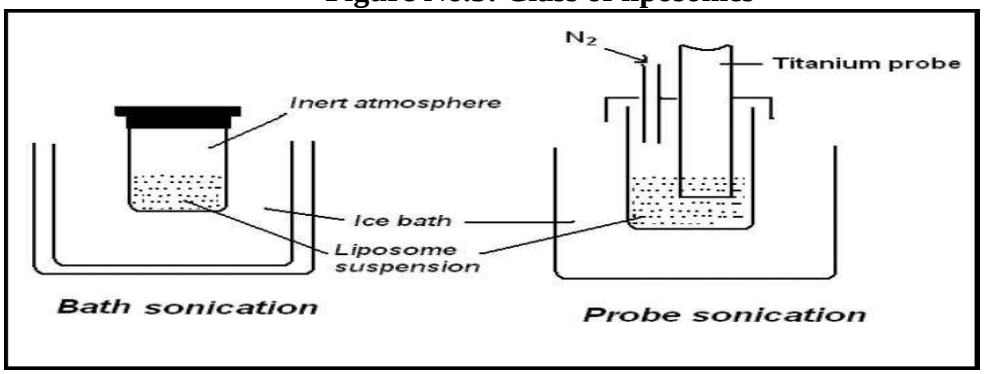

Figure No.4: Sonication apparatus

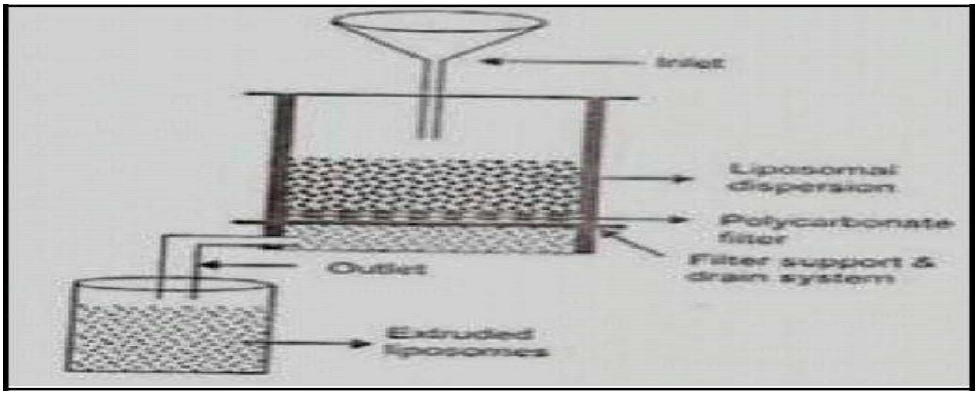

Figure No.5: liposomes prepared by Membrane extrusion method

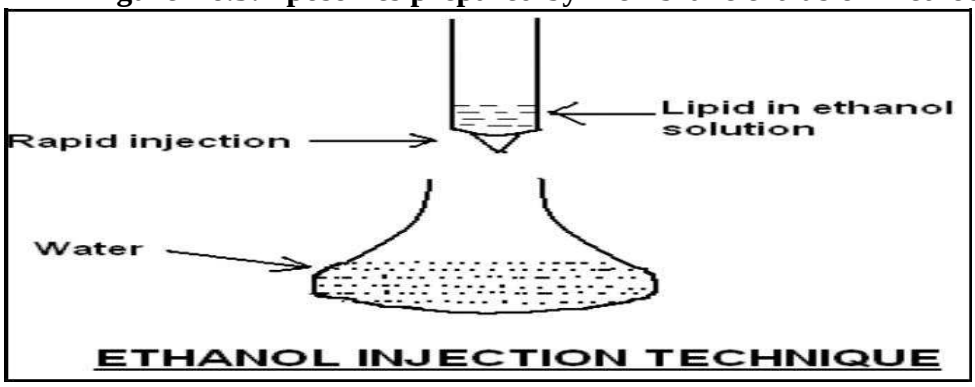

Figure No.6: Ethanol injection method

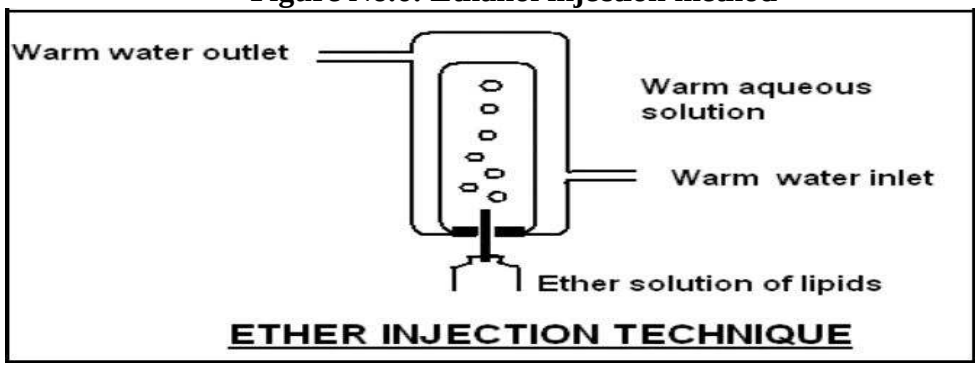

Figure No.7: Ether injection method

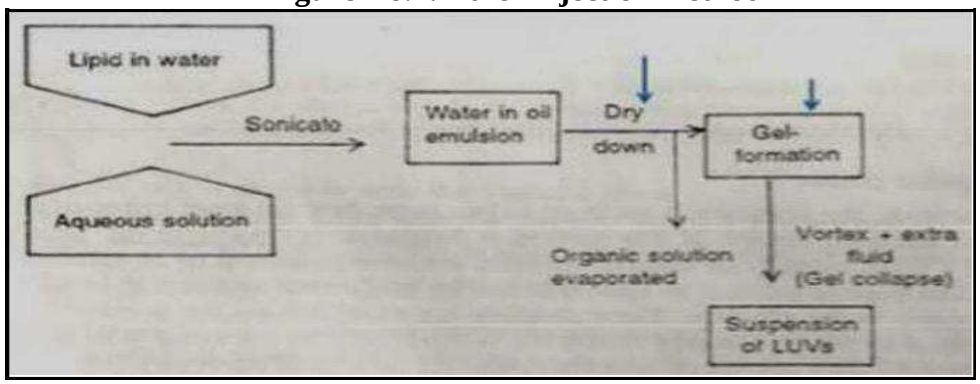

Figure No.8: Reverse Phase Evaporation Method

CONCLUSION

Liposomes have been realized as extremely useful carrier system for targeted drug delivery. The flexibility of their behavior can be exploited for the drug delivery through any route of administration and for any drug material irrespective of their solubility properties. The use of liposome in the delivery of drugs and genes are promising and is sure to undergo further development in future. Mucoadhesive drug delivery systems, are gaining popularity day by day in the global pharma industry and a burning area of further research and development. Extensive research efforts are having very significant advances in understanding the various aspects of mucoadhesion. The research on mucoadhesives is still in beginning stage, and further advances need to be made for the successful translation of the concept into practical application in controlled drug delivery system (CDDS).

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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