Stealth Liposomes:A Novel Approach of Targeted Drug Delivery In Cancer Therapy

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Abstract: - Conventional liposomes are artificially prepared vesicles made of a lipid bilayer that can encapsulate drugs and used as a drug carrier system for the treatment of cancer and other diseases. Encapsulation of a drug in liposomes prevents its early degradation and alters the biodistribution profile in the body. This enables higher concentrations of the drug in the desired tumor site, leading to improved effectiveness, and reduced toxicity in the vital organs like heart, kidney etc. Conventional liposomes are eliminated rapidly from the biological fluids (e.g., blood), and therefore lack the stability to establish long circulation times required to realize the full efficacy of the drug. These conventional liposomes are detected by the body first line defense mechanism and phagocytes the liposomes which in turn leads to decrease the liposome concentration and effectiveness of drug. This problem leads in development of long circulatory liposomes which is far better than conventional liposomes in stability, efficacy and accuracy in treatment of cancer tissue. These Stealth liposomes recently used in treatment of Kaposi's sarcoma, e.g. DOXIL is marketed FDA Approved drug of Sequus Pharmaceuticals pvt Ltd.[19]

Keywords: - Stealth liposome, Long circulatory liposomes, Polyethylene glycol, PEGylation, Reticoendothelial system, mononuclear phagocytic system etc.

Introduction: - The drug-delivery market is changing drastically due to the introduction of new techniques and routes of drug delivery. The widely preferred drug carriers include soluble polymers, micro particles made of insoluble or biodegradable natural and synthetic polymers, microcapsules, cells, cell ghosts, lipoproteins, liposomes, micelles, drug-loaded biodegradable microspheres and drug polymer conjugates. In recent years, liposomes have seen phenomenal progress in drug-delivery systems and have received increased attention from the pharmaceutical community. Liposomes are artificially prepared vesicles made of a lipid bilayer that can encapsulate drugs and used as a drug carrier system for the treatment of cancer and other diseases. Encapsulation of a drug in liposomes prevents its early degradation and alters the biodistribution profile in the body. This enables higher concentrations of the drug in the desired tumor site, leading to improved effectiveness, while a lower concentration reaches into the vital organs, such as the heart and kidney, thus reducing the toxicity of the drug.

These conventional liposomes in treatment of cancer and cancer tissue encountered a serious problem of stability and inactivity of liposomes to deliver the effective concentration at the site of tumor tissue. These conventional liposomes faces bodies first line defense mechanism i.e. Reticular endoplasmic system (RES) and various phagocytes causing early metabolism of liposome by the process of phagocytosis and in turn leads to the decreased in concentration of the liposome. In conventional liposomes the outer layer is made up of Lipid bilayer which biocompatible and biodegradable by the enzymes and lipases causing the degradation of the lipid bilayer, by this the encapsulated drug matrix is exposed to the blood and enzymes, and also these drug complexes are unable to penetrate the tumor cell membranes and target the tumor cells. To avoid this problem a special technique has to modified or new design is essential to overcome this critical situation.

Conventional liposomes are good

Conventional liposomes**,** which are microscopic, manmade cells used as sustained-action delivery vehicles for a wide variety of drugs, vaccines, enzymes, nonenzyme proteins, and genetic material The molecular "payload" is encapsulated inside the liposomes, which eventually break down through natural processes and spill their contents into the bloodstream or into tissues to which they have migrated by diffusion through the walls of capillaries. Liposomes are a safe and effective way to introduce agents into our system. The problem with conventional liposomes, though, is that they're seen as alien invaders by phagocytes. Phagocytes are cell-eating cells whose mission is to devour anything that doesn't belong in our bloodstream. And when liposomes are devoured, so are their therapeutic payloads, which may thus go to waste. Another problem with conventional liposome is that they escape back into the bloodstream through leaky blood walls. Along with these problems there are other problems with conventional liposome delivery systems which are as follows:-

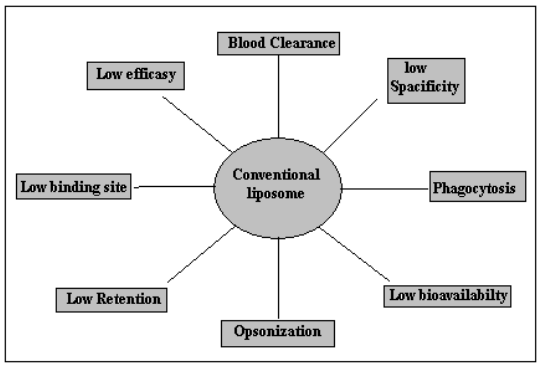


Fig. 1 Conventional liposome problems

To overcome this new modified liposomes are evolved.

Concept of Stealth liposomes: - As stated by Yuanpeng Zhang, In World war II NAZI German's bombed the British command centers and they were helpless as their RADAR failed to detect the Nazi's bombers. These bombers were stealth bombers which were designed by Germans, were made up of special materials and unique design. As the allies failed to tackle the stealth bombers they were helpless and so the command centers were destroyed to bits…the same concept were used in long circulatory liposomes. We need such a newly designed drug delivery system which delivers the killer bombs deep inside tumor cells and destroy them, just like the stealth bombers did or was intended to. Camouflaging the liposomes so as to fool phagocytes into ignoring them thus became a key objective of pharmaceutical chemists, hence The result of their efforts was a process known as **PEGylation,** i.e. Covering outer side of liposome with polymer**,** To phagocytes, this molecular "cloak" of water of hydration makes the PEGylated liposomes look like little watery blobs rather than something edible, so they tend to leave them alone.

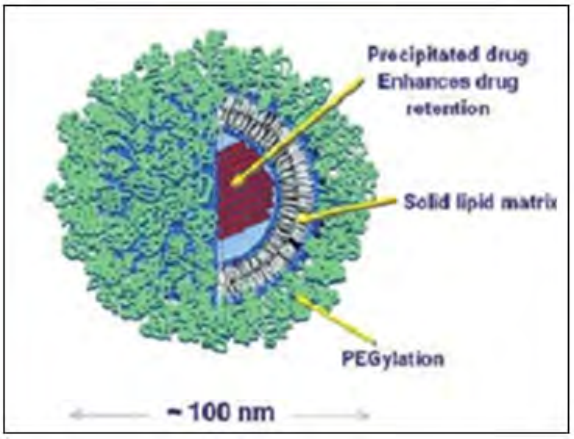
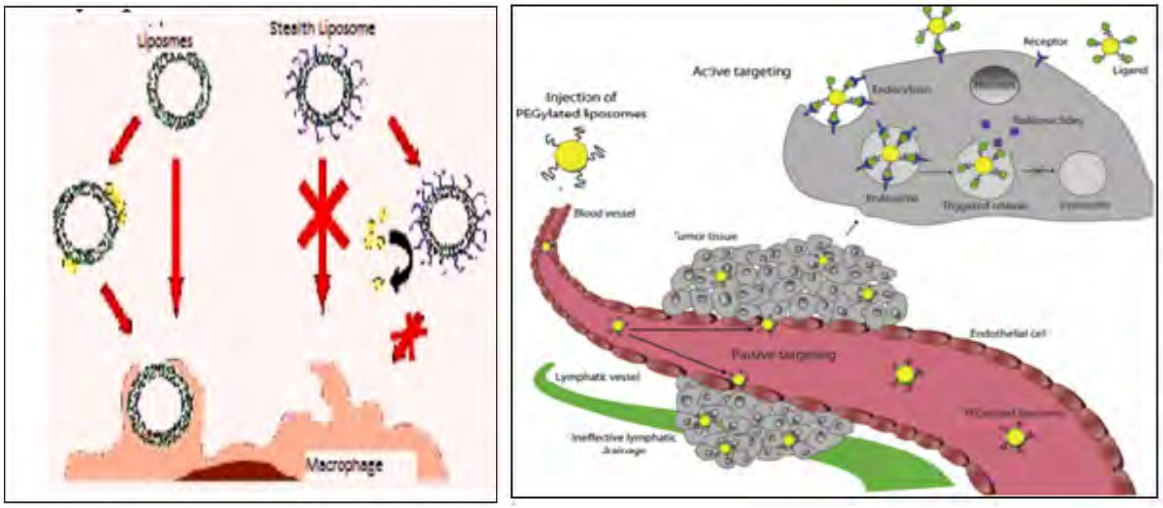


Fig.2 Stealth liposome showing PEGylated coat

These stealth liposomes are spherical vesicles with a membrane composed of phospholipids bilayer used to deliver drugs or genetic material into the systemic circulation. Moreover, these types of liposomes were composed of various polymers like Polyethylene glycol (PEG), Polyaniline (PA), polyacrylamide (PAA), Polyvinylpyrrolidine (PVPA) etc. These polymers were used to enclosed or to form the outer membrane of the liposomes [1].

Fate of stealth liposome:-

Conventional liposomes are taken up by the reticular endoplasmic system (RES) and they are liable for degradation or inactivation by the phagocytosis. Conventional liposomes entering in the blood stream unable to targer the tumor site as they lack essential binding mechanism, by which they linked to tumor receptors and attack. This is the area of stealth liposomes show the concept of formulation. Stealth liposomes, due to presence of PEG derivatives on outer membranes provide stealth effect i.e. they are not detected by the phagocytes system. Eventually they will detect and eaten. But this process is too slow. And hence they provide long circulation time. Inability of detecting these liposomes by reticular endoplasmic system they resemble the stealth bombers, so they named after them. These stealth liposomes provide accurate, precise attack on the cancer cells and deliver the drug molecule at the site of action.



(a) (b)

Fig.3 (a) Fate of conventional stealth liposomes, (b) Fate of stealth liposome in vivo

Enhanced permeability and retention activity:-

Conventional liposomes on administration reach to the site of action through systemic circulation. Due to smaller size in µm they passively diffuse through the leaky walls of the blood vessels and reaches to the tumor site. Conventional liposome has permeability through cancer cells but has no such retention effect as there is no binding mechanism on outer surface. Due to this inability of binding to tumor site, conventional liposomes are proven inefficient in treating the tumor cells. On the other hand, Stealth liposome has smaller size up to ~50um and has PEGylated molecules attached to the outer side of the stealth liposome, providing better binding site to the cancer cell. PEGylation of stealth liposome mimic the host cell properties. And with the PEG it is to pass the leaky blood walls and reaches to cancer cell. This improves the retention activity of the drug molecules. Stealth liposomes provide targeted drug delivery and also provide bulk drug transfer at the tumor site.

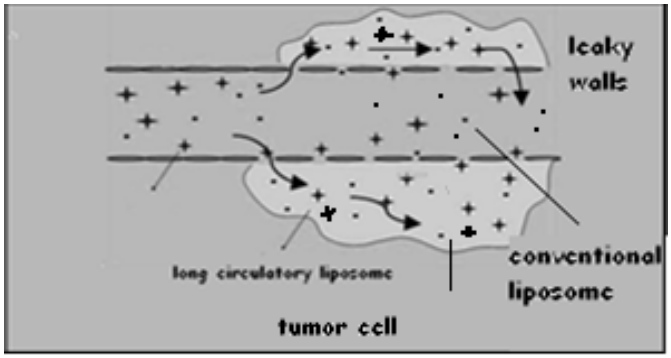


Fig 4. EPR effect of stealth liposome

Characteristic of stealth liposomes:-

1. Stealth liposome is composed of cholesterol and phospholipids such as phosphotidylcholine or diacetylphosphate, the composition and structure remains the same as in the host cell.
2. The phospholipids bilayer consists of hydrophilic head component and hydrophobic tail and PEG or other polymer as outer coat.
3. Stealth liposomes are stable in nature.
4. The size, shape of stealth liposome can be altered depending on drug and material used.
5. They can't be taken by endoplasmic reticular system and cause slow release of drug.
6. There size ranges from 50 to 5000nm.
7. The lipids most commonly used are phospholipids, spingolipids, glycolipids and sterols.
8. The stealth liposomes are colloidal and uniform in nature.

Polyethylene glycol (PEG) as polymer:-

Polyethylene Glycol is majorly and largely used in manufacturing of the Stealth liposome. Polyethylene glycol is hydrophilic in nature and at the final step of manufacturing of liposome polyethylene glycol is added. It directly adhered or covalently bonded to the outer surface of the liposome forming long circulating liposome or Stealth liposome. Polyethylene glycol posses following properties as ideal polymer:

It is biocompatible and biodegradable

The degradation product is nontoxic and does not produce inflammatory response.

Degradation time is within a reasonable period time as required for application.

It is completely or partially invisible to mononuclear phagocytic system (MPS).

It is permeable to lipid bilayer and blood brain barrier.

Other alternative polymer used:-

PolyAcrylamide (PAA)

Polyacrylamide is formed from the acryl amide subunits i.e. readily formed from polymerized acryl amide .it is highly hydrophilic and used as alternative to polyethylene glycol.

Poly (2-methyl-2-oxozoline)

Poly(2-methyl-2-oxozoline), poly(2-alkyl-2-oxozoline), an important class of polymer used for extended or long circulation time and decreased uptake by mononuclear phagocyte system(MPS).[16]

Poly (Amino) Acid

Different synthetic poly(amino acid)s are currently being investigated as alternatives to PEG and are in different stages of development. Poly(glutamic acid) (PGA), which was first investigated by Li and Wallace, has already entered phase III clinical trial in the form of a 40 kDa PGA-paclitaxel conjugate.[21]

Poly (glycerol)

The close structural similarity of poly(glycerol) (PG) to PEG renders the polymer predetermined for biological applications. Indeed, linear as well as hyperbranched PG (HPG) with molar masses ranging from 150 Da to 540 kDa have already been used as hydrophilic shells for conjugates and liposomes, reverse micelles, and hydrogels.[21]

Poly (vinylpyrrolidone)

Poly (vinylpyrrolidone) (PVP) is commercially available, for example, under the brand name Kollidon from BASF. PLA-PVP micelles and microspheres as well as PVP-gelatin hydrogels and PVP have been studied for their formulation assistance**.**

Method of preparation of stealth liposomes:-

Stealth liposomes were prepared by same methods which are used to prepare the conventional liposomes. But in these methods one of the important steps to be included is PEGylation. These PEGylation is the method in which polymer is allowed to enclosed liposome.

Sonication method

These sonication methods were most largely used to prepare the small unilamellar vesicles.

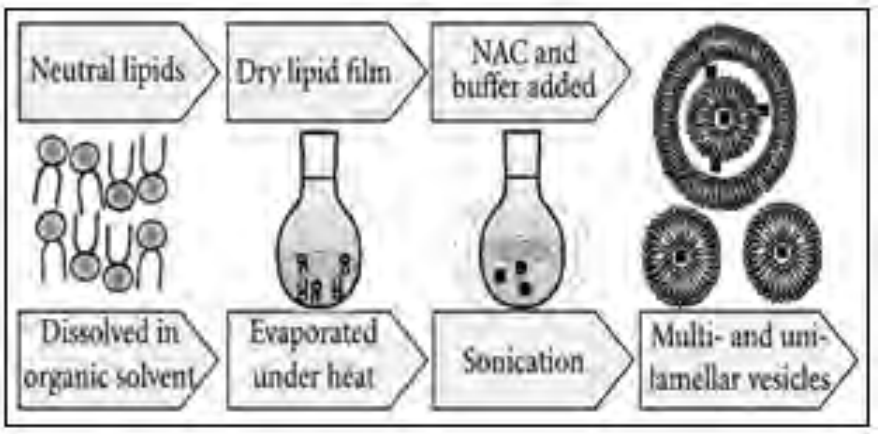


Fig.5 Sonication method

Probe Sonication

In this sonication method, a tip or probe is dipped in the liposome dispersion and high degree of energy is applied at the tip of the probe, such that dissipating of energy result in the overheating and the lipid bilayer fused with forming the liposome. After this step, the PEGylation is introduced in which the polymer derivative of the Poly Ethylene Glycol were incorporated and these polymer enclosed the liposome to produce the stealth liposome**.**

Bath sonication

This method is similar to the probe sonication, In this method instead of using probe for sonication, Bath sonicator is used. In Bath sonicator, the liposome dispersion in a tube is placed and while tube is sonicated. This resulted into the fusing the lipid bilayer with other membrane. After this, PEGylation is carried out by incorporating Polyethylene glycol in the liposomal dispersion. This leads to fusion of polyethylene glycol to the outer sheath of liposome providing PEGylated liposome**.**

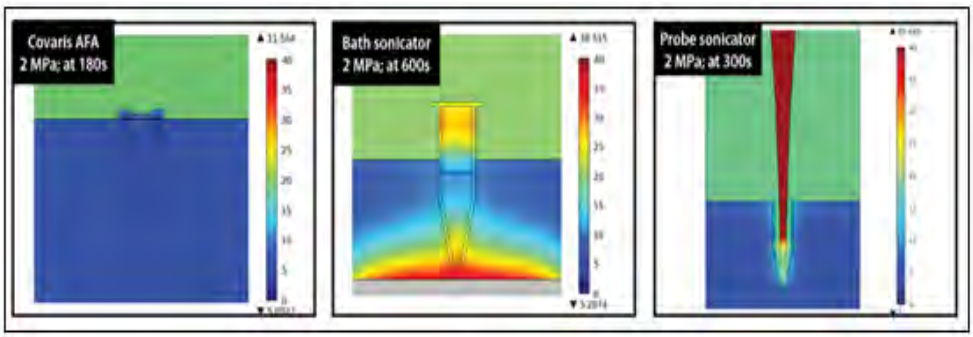


Fig.6 Bath sonication

Hand Shaken Method

In this hand shaken method, Mechanical agitation created by vortexing, shaking, swirling or pippeting which causes break in the lipid tubules and reseal the exposed hydrophobic edges resulting in the formation of liposome. By this method Multilameller liposome's can be prepared**.**

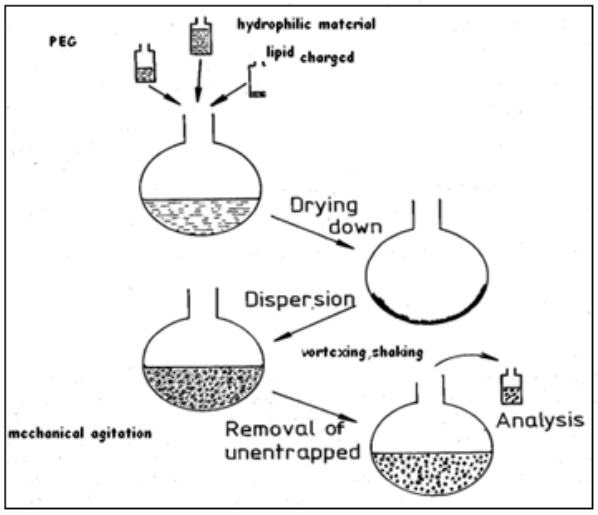


Fig.7 Hand Shaken method

Reverse-phase evaporation

First water in oil emulsion is formed by brief sonication of a two phase system containing phospholipids in organic solvent (diethyl ether or isopropyl ether or mixture of isopropyl ether and chloroform) and aqueous buffer. The organic solvents are removed under reduced pressure, resulting in the formation of a viscous gel. The liposomes are formed when residual solvent is removed by continued rotary evaporation under reduced pressure. With this method high encapsulation efficiency up to 65% can be obtained in a medium of low ionic strength. The main disadvantage of the method is the exposure of the materials to be encapsulated to organic solvents and to brief periods of sonication. These conditions may possibly result in the denaturation of some proteins or breakage of DNA strands (Szoka and Papahadjopoulos, 1978). We get a heterogeneous sized dispersion of vesicles by this method. Modified Reverse Phase Evaporation Method was presented by Handa et al. (1987) and the main advantage of the method is that the liposomes had high encapsulation efficiency (about 80%).

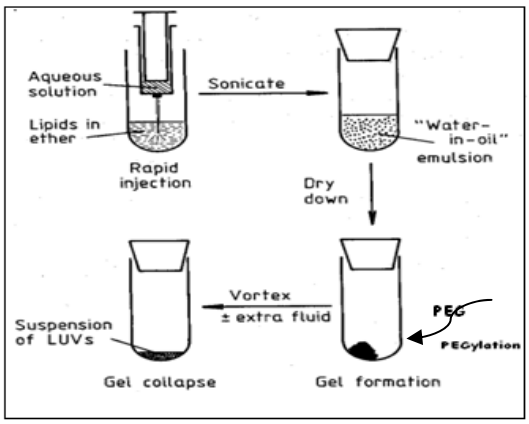


Fig. 8 Reverse phase evaporation

Liposome Extrusion techniques: -

Extrusion under nitrogen through polycarboriate filters LUV can be prepared by passing MLV under nitrogen through polycarbonate membrane filters (Jousma et al., 1987). The vesicles produced by this method have narrow size distribution. The extrusion is done under moderate pressures (100-250 psi). A special filter holder is required. Such devices are available commercially under the trade names such as LUVET and EXTRUDER and are equipped with a recirculation mechanism that permits multiple extrusions with little difficulty. Riaz and Weiner (1994) prepared liposomes by these technique Small quantities of liposome preparations (about 10 mL) can be easily prepared by the help of a commercial extruder. MLVs were passed through (Extruder Lipex Membrane Inc., Vancouver, Canada) ten times through a stalk of two 100 nm polycarbonate filters (Nudeopore Pleasanton, CA, USA) employing nitrogen pressures upto 250 psi. Freeze fracture electron microscopy and p31-FT NMR revealed that the liposomes were unilamellar. Photon Correlation Spectroscopy revealed that the size range was 99-135nm [2].

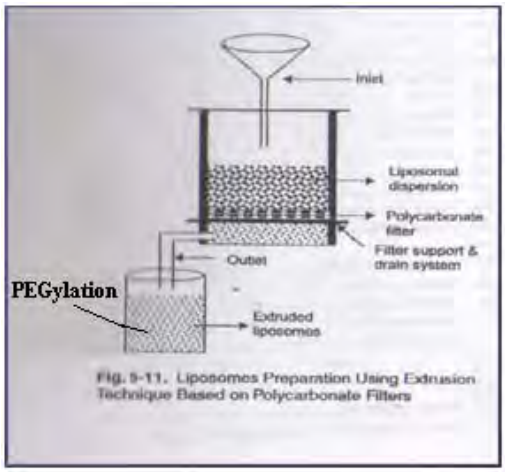


Fig. 9 Extrusion techniques

Detergent deletion method

Phospholipids are brought into intimate contact with the aqueous phase via intermediary of detergents, which associates with phospholipids molecules and serves to screen the hydrophobic portions of molecules from water. The structure formed as a result of this association are known as micelles, and composed of several hundred components molecules.[2] There size and shape depends on chemical nature of detergents, the concentration and other lipid involved. Detergent deletion method is A pilot plant under the trade name of LIPOPREP® II-CIS is available from Diachema, AG, Switzerland. The production capacity at higher lipid concentration (80 mg/ml) is 30 ml liposomes/minute. But when lipid concentration is 10-20 mg/ml 100 mg/ml then up to many litres of liposomes can be produced. In USA, LIPOPREP® is marketed by Dianorm-Geraete (Maierhofer, 1985) [1].

Freeze-Dried Rehydration method

Freeze-dried rehydration method involves the formation of liposome for preformed vesicles. The freeze drying of liposome vesicle brings the lipid bilayer and material to be entrapped into close contact. This procedure is to prepare the liposomal peptide antigen because of its high entrapment efficiency. In this method liposomal dispersion is allowed to freeze-dry below -16°C and rehydrated with the solvent for rehydration [2].

Micro-fluidization method:-

Micro-fluidization method involves passing of dispersion through Micro-fluidizer (Microlluidics Corporation, Newton, MA, USA) at 40 psi and this cause the annealing and resealing of the lipid bilayer with material to be entrapped and this liposome were then treated with the Polyethylene glycol polymer to enclose and to prepare long circulating liposomes. This technique is generally useful for production of large scale manufacture of liposomes [2].

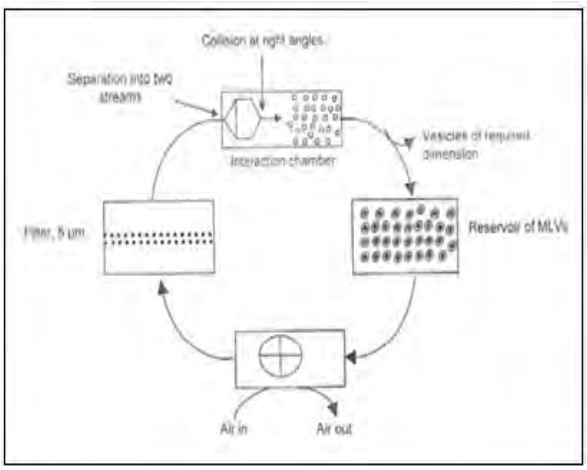


Fig.10 Micro-fluidization

Solvent injection method

(a)**Ether injection method**

A solution of lipids dissolved in diethyl ether or ether/methanol mixture is slowly injected to an aqueous solution of the material to be encapsulated at 55-65°C or under reduced pressure. The subsequent removal of ether under vacuum leads to the formation of liposomes. The main drawbacks of the method are that the population is heterogeneous (70-190 nm) and the exposure of compounds to be encapsulated to organic solvents or high temperature (Dcamcr and Bangham, 1976; Schieren et al., 1978).

(b) **Ethanol Injection Method**

A lipid solution of ethanol is rapidly injected to a vast excess of buffer. The liposomes are immediately formed. The drawbacks of the method are that the liposomes were heterogeneous (30-110 nm), liposomes are very dilute, it is difficult to remove all ethanol because it forms azeotrope with water and the possibility of various biologically active macromolecules to inactivation in the presence of even low amounts of ethanol (Batzri and Korn, 1973) [2].

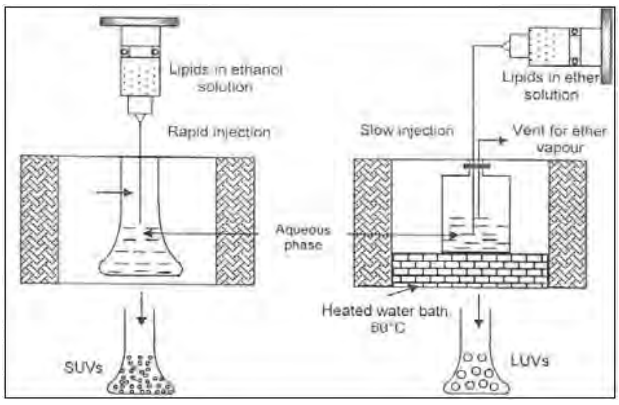


Fig.11 Ethanol/ether injection method

Evaluation of stealth liposomes:-

Zeta potential determination:-the zeta potential was evaluated by determination of electric mobility at the 90°C. The measurement was performed in triplicate using 3000 HS zetasizer equipment for this the sample were diluted before determination[12].

Size Distribution:- The uniform size of the liposome(50 to 5000nm) were determined by the photon correlation microscopy which ensures the uniformity in size of the liposomes[12].

Liposome stability: - The stability of the liposome was determined at various PH and temperature. For the evaluation, these liposomes were diluted at 10 fold in 0.9% NaCl solution at pH 7.4 and incubated at 37°C. Drug release from the liposome were separated and determined by ultracentrifugation at 150000g 10°C for 60 min [10].

Drug release determination:- Drug release determination is determined by dialysis method. The stealth liposome drug release is determined in comparison with conventional liposomes. These liposomes were taken in 2ml volumes and mixed with 2ml blank marine plasma and mixtures were placed in dialysis tubing's. These mixtures were placed in 50ml HBS solution. At 0, 5, 15, 30, 1hr, 4hr, 6hr, 10hr, 24hr and 48hr, aliquots of samples were carefully withdrawn and replaced with same quantity of HBS. These concentration of drug released were measured by flurospectrometry method and HPLC method. The release rate can be determined by the formula:

Rate of release = (W_n/W) X 100%, where W_n is release amt of drug, W is gross amt of drug present in liposomes.

Polydisperity index: - polydisperity index of liposome dispersion were determined light scattering using Malvern. The lipid content of the liposome dispersion were accessed phospholipids quantification according to rouscert radio activity of liposomes dispersion was assayed by ultima gold liquid scintillation counter [17].

Drug Quantification: - Drug concentrations of the leptosomic dispersion were quantified by specrtosopically. The drug concentration was confirmed by HPLC[1].

Advantages of Stealth liposome:-

- Increased bioavailability
- Stealth liposome provides long, slow release.
- Toxicity and side effects are minimized.
- PEGylated liposomes can offer some targeted delivery
- Passive targeting occurs for tumors and inflamed tissues.

Application of stealth liposome in clinical pharmacy:-

Stealth liposome in cancer therapy:-

Stealth liposome has advantage as they have long circulation time and targeted delivery, due to this reason stealth liposome are used in cancer liposome [44].PEGylated liposomes were first used and only Stealth liposome formulation available in the market for treatment of Kaposi's sarcoma and ovarian cancer. DOXIL® is brand name of Doxirubin by Seques pharma Ltd .[8,13]

Stealth Liposome in Vaccines:- Various vaccines are developed in the liposomal formulation for treatment of various diseases. Success of various liposomal based vaccines has been demonstrated in clinical trials and further human trials are also in progress. WHO commercial vaccines based on virosome technology are currently on the market. Epaxal®(Berna Biotech Ltd, Bern, Switzerland), a hepatitis A vaccine, has inactivated hepatitis A virus particles adsorbed on the surface of the immunopotentiating reconstituted influenza virosomes (IRIV) and Liposome-encapsulated malaria vaccine are effectively developed [22].

Stealth Liposome in Targeted delivery:-

Stealth liposome has property of long circulation and accuracy in drug delivery along with stealth effect; provide targeted drug delivery of drug molecule to the site of action with large amount of drug. The targeted delivery of Stealth liposome can be improved Ligand activity and degree of immune uptake provides "Stander killing effect", as drug molecule can diffuse through tumor cells[22, 34].

Stealth Liposome in Diagnostic Imaging: -

Stealth liposome can be used in diagnostic imaging by carrying different type of materials in bilayer for various diagnostic magnetic resonance imaging (MRI), Gamma scintillation, Computed tomography Imaging (CTG), and Sonography etc [15,19].

Future scope of the stealth liposomes:-

Active targeting of STEALTH liposomes to tumor cells has long been an area of intense investigation. To achieve active targeting, Ligand (such as antibodies or antibody fragments), which may bind selectively to cell surface receptors, are chemically tethered to the termini of the PEG chains extending from the liposome surface. In one formulation, a single-chain antibody fragment targeted against HER2, a growth factor receptor overexpressed in a wide range of human epithelial tumors, has been introduced onto the surface of Doxil[24, 25].In animal models, the anti-HER2 targeted Doxil exhibits superior antitumor activity relative to unencapsulated doxorubicin and Doxil[24,26].Other ligands being explored for targeting of STEALTH liposomes include vitamins, such as folate, and growth factors[28].

Ligand-bearing STEALTH liposomes may also modulate important biological receptor-binding events. For example, in inflammatory tissues, PEGylated liposomes with Sialyl Lewisx moieties on their surface effectively

inhibit the binding of circulating lymphocytes to selectins expressed on vascular endothelial cells.[7] In a feline myocardial reperfusion model, these liposomes significantly attenuate myocardial necrosis and preserve coronary endothelial functions after a myocardial ischaemia /reperfusion challenge [24].

STEALTH liposomes also offer a potential vehicle for the delivery of plasmid genes or catalytic polynucleotide to sites of disseminated disease. DNA constructs formulated with mPEG-containing liposomes appear to exhibit favorable pharmacokinetic and tumor distribution properties, in part by protecting these biopolymers against degradation from nuclease digestion. Unfortunately, the steric stabilization afforded by the mPEG a group appears to interfere with cellular uptake of the liposome-encapsulated DNA[7].Possible solutions to this classic drug delivery dilemma have recently been proposed. One such liposome system, termed PolyVERSE^{TM}, employs mPEG groups that may be shed from the liposome surface after extravasations and entry into tumors [28, 30].

Delivery of anti-inflammatory agents encapsulated in STEALTH liposomes to sites of inflammation is an exciting prospect. Clinical data indicate that STEALTH liposomes containing radioactive tracers passively target to sites of infection and inflammation in much the same way as Doxil targets to tumors[18, 19].

Some recent patented technologies to stealth liposomes: -

Table no. 1 some recent patent technologies of stealth liposomes

Company name	Brand name	Targeted disease	**Position**
Neo Pharm	LE-SN38 LEM LE-AON LEP	Various solid tumors Prostate cancer Various solid tumor Various solid tumor	Phase I/II Phase I/II Phase I/II Phase I/II
Celsion	ThermaDox	Prostate cancer	Phase I
Antigenics Inc	Aroplatin ATRA-IV	Colorectal cancer Acute Promyeolocytic Cancer	Phase II Phase II
Ghed science	AmoBisome DaunoXome	Kaposi's cancer	Marketed Marketed
ALZA	DOXIL	Ovarian Cancer	Marketed

Conclusion:-

Stealth liposomes are the new drug delivery systems which were recently developed to overcome the errors encountered in the conventional liposomes. The systems, due to their advantage of fooling the phagocytic system i.e. MPS, provide the accurate and efficient drug delivery at the site of action. Large of drugs were design in this system to administer the large amount of drugs at the site of treatment. By this stealth liposomes were used to treatment of various kinds of cancer and tumors. These stealth liposomes will again modified to improve the targeted drug delivery systems by modifying outer layer of liposome with antibodies. These provide the effective and selective treatment to selective diseases. Doxil® and Stealyth® is officially marketed preparation preparations based on the stealth technology and functioned effectively for Kaposi's sarcoma. These types of targeted drug delivery systems were now developed to improve the medication and pharmaceutical product to improve the dosage form.

Author's contribution: -

KDB is main author of this review article. KDB have undertaken all the study for this review and collect all the information related to work for this review. BGS guided for this article and MS More help in corresponding work.

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