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Review Article

Liposomes for the Drug Delivery: A Review

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Abstract: Formulation of drugs in liposomes has provided an opportunity to enhance the therapeutic indices of various agents mainly through alteration in their bio distribution. Liposomes are a novel drug delivery system (NDDS), they are vesicular structures consisting of bilayer which form spontaneously when phospholipids are dispersed in water. They are microscopic vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid bilayers. The goal of any drug delivery system is spatial placement and temporal delivery of the medicament. Research works are going on to prepare an ideal drug delivery system which satisfies these needs. Liposomes are small vesicles (100 nm) composed various lipid molecules which build their membrane bilayers. These formulations are mainly composed of phosphatidylcholine and other constituents such as cholesterol and lipid-conjugated hydrophilic polymers. Liposomes are biodegradable and biocompatible in nature.

Keywords: Liposomes, NDDS, Vesicular structures, Phospholipids.

INTRODUCTION

Liposome are simple microscopic vesicles in which an aqueous volume is entirely enclosed by membrane composed of lipid molecules. Various amphipathic molecules have been used to form liposomes [1, 2]. Phospholipids are the major structural components of the biological membranes in the human body, where two types of phospholipids exist i.e. phosphor-diglycerides and sphingolipids, together with their corresponding hydrolysis products. The way they work and form membranes are elegant and miraculous. Each phospholipid molecule has three major parts, one head and two tails. The head is made from three molecularcomponents: choline, phosphate, and glycerol. The head is hydrophilic in other words, it is attracted to water. Each tail is a long, essential fatty acid chain. These fatty acids are hydrophobic that is, they repelled by water. The drug molecules can either be encapsulated in aqueous space or intercalated in to the lipid bilayers. The number of components of the liposomes is varied; however phospholipids and cholesterol are the main components. The most commonly used phospholipids include phosphatidyl choline [PC]. PC is an amphipathic molecule in which a glycerol bridge links a pair of hydrophobic acyl hydrocarbon chains, with a hydrophilic polar head group. Phosphatidyl choline, also known as "lecithin", can be derived from natural and synthetic sources [3-7]. In aqueous media they align themselves closely in planner bilayers sheets in order to minimize the unfavourable action between the bulk aqueous phase and the long hydrocarbon fatty chain [i.e. they orient themselves so that the fatty acid chains face each other and the polar heads face the aqueous phase –this reduces the instability which exists when the molecule exist alone such unfavourable interactions are completely eliminated when the sheets fold on themselves to form closed sealed vesicle. Incorporation of sterols in liposome bilayer brings about major changes in preparation of these membrane. Cholesterol by itself does not forms a bilayer structure. However cholesterol acts fluidity buffer that is below the phase transition temperature it make the membrane less ordered and slightly more permeable while above the phase transition temperature it makes the membrane more ordered and stable. It can be incorporated into phospholipid membrane in very high concentration upto 1:1 or even 2:1 molar ratio of cholesterol. Cholesterol insert into the membrane with its hydroxyl groups oriented towards the aqueous surface and aliphatic chain aligned parallel to the acyl chain in the middle of the bilayer.

Mechanism of the action of cholesterol acting as fluidity buffer

Cholesterol incorporation increases the separation between the choline head groups and

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eliminates the normal electrostatic and hydrogen binding interaction thus pushing the phospholipids apart making the layer less ordered at lower temperature. However, in the higher concentration that cholesterol is used ,the membrane area occupied by the combination of acyl chains and cholesterol is greater than that taken by phosphor-cholin head group .this differences in area retards chain tilt [the phenomena responsible for phase transition –i.e trans to gauche conformation changes].above the transition temperature ,the reduction in the freedom of the acyl chains causes the membrane to remain condensed and rigid with a reduction in area through closer packing and resultant decrease in fluidity [8-12].

REVIEW OF LITERATURE

- Sarvanan M *et al*., 2021, the main perturb efflorescing and re-efflorescing of the disease which is caused by the viruses has become more frequently foremost place of health area for the public, and it is foremost of prophesy future pandemic research was.
- Yechezkel Barenholz., 2009, research on the ultra sound triggered produce of the cisplatin in murine tumours by the liposome.
- Eichhorn ME, Becker *et al*., 2006, MBT-0206 that is cationic complexes of the paclitaxel encapsulated which is the non-pairing programming of the resources of the vascular research by the increasing magnetic resonance imaging.
- Dae-Duk Kim *et al*., 2006, researched on the development of the liposomes arbutin for the topical administration.
- Gupta B, Levchenko TS, Torchilin *et al*., 2005, the bigger molecules and the smaller particles of the intracellular delivery from the penetration of the proteins as well as the peptides.
- Mahmood I, Green MD *et al.*, 2005, the main important research on the preparation of the therapeutic proteins in field of the pharmacokinetic and pharmacodynamics.
- Dark GC, Calvert AH, Grimshwar R *et al*., 2005, in this research for the trail of the ovarian cancer of the women dual intravenous schedule of the topoisomerase I inhibitor lurtotecan under national cancer institute of the Canada clinical trials.
- Adam GP, Weiner LM *et al*., 2005, research on the cancer therapy antibody of the monoclonal.
- Arfin DR, Palmer AF *et al*., 2005, physical and stability studies of the dispersion ofpoly glycol liposomal conjugation hemoglobin encapsulation.
- Asai T, Oku N *et al*., 2005, type of the oligopeptides liposome enzymol study in the cancer therapy
- Chen WC, Huang L *et al*., 2005, vector which is not viral type used as vaccine.
- Boulikas T, Stathopolous GP, Volakakis N *et al*., 2005, in human study of the tumor treatment by the lipoplatin infusion systemic.
- Bramwell VW, Eyles JE, Oya AH *et al*., 2005, vaccination of the subunit biological defense in particular drug delivery system
- Ishida T, Harada M, Wang XY *et al*., 2005, in this research when liposomal drug that is PEGylated administred by parentral which is give increasement in blood clearance.
- Zhang JA, Anyarambhatla G, Ma L *et al*., 2005, formulation of the newer cremophor EL free liposomal based paclitaxel development.
- Zamboni WC, Ramalingam S, Friedland DM, *et al*., 2005, in this research, trial of the first phase pharmacokinetic study of the liposome stealth in human with in last stage of the tumor.
- Ugwu S, Zhang A, Parmar M *et al*., 2005, development of the liposome based on the preparation of the mitoxantrone.
- Awada A, Gil T, Sales F *et al*., 2004, temodal which is temozolomide doxorubicin that is caelyx researched on the high type of solid cancers.
- Chen T, Palmer LR, Fenske DB *et al*., 2004, conjugation of the lipid larger unilameller vesicle prepared by liposome cells uptake enhancement extrusion.
- Bomgaar L, Geyer JR, Franklin J *et al*., 2004, intrathecal liposome cytarabine with neoplastic meningitis in pediatric.
- Banerjee R, Tyagi P, Li S *et al*., 2004,researched on the human prostate cancerious cells, anisamide target stealth liposomal.
- Hau P, Fabel K, Bsugmart U *et al*., 2004, in this study the more dangerous glioma pegylated liposome doxorubicin give high efficacy in patients.
- Veerareddy PR, Vobalaboina V *et al*., 2004, in this report preparation of the amphotericin B drug which is apply for the fungal infection via i.v which is formulate by the lipid based.
- Pastorino F, Bringnole C, Marimpietri D *et al*., 2003, in this research or lab work the drug which is anti-disialgangliosideimmune-liposome selective i.e. Doxorubicin-loaded fragment which is prevent the growth as well as dissemination in humans neurobastoma the experiment success on the nude mice.
- Palmer LR, Chen T, Lam AM *et al*., 2003, in this report study of the transfection properties of plasmid lipid stabilized particle having PEG Lipid form of cationic.
- Asahi S, Ram Mohan R, Sumii T *et al*., 2003, in this research the Ameliorate antiactin-targeted immune-liposome that is the activator plasminogen which is induced after focal embolic stroke, type of the haemorrhage.
- Dagar S, Krishnadas A, Rubinstein I *et al*., 2003, here stabilizing liposome which is the VIP grafted sterically applied on breast cancer.
- Mamot C, Drummond DC, Greiser U *et al*., 2003, in this research report drug i.e epidermal factor

development receptor targeted particular immune-liposome mediate to drug delivery to EGFR and EGFRv3 tumor cells which are overexpressing.

- Moghimi SM, Szebeni J *et al*., 2003, here the report says that, nanoparticles which are circulate longer in pharmacokinetic, opsonisation and protein binding properties study generate critical issue.
- Metselaar JM, Bruin P, de Boer LW et al., 2003, in this report L amino based on acidic which are biodegradable polymer that's conjugation for the longer circulate liposome development which is capable for giving effectiveness drug-targeting.
- Stephenson SM, Low PS, Lee RJ *et al*., 2003, liposome drug which is folic receptor with apply to suitable vehicle delivery for neutron boron capturing treatment.
- Verchragen CF, Kumangai S, Davidson R *et al*., 2003, here the report says that is in first clinical trial phase as well as pharmacological studies intraperitoneal cis-bis-neodecanoato which is trans R, R-1,2 di-amino-cyclohexane 2 type of platinum entrapment in multi-lamellar vesicular liposome.
- Allen C, Dos SN, Gallagher R *et al*., 2002, the physical behaviour and biological studies in formulation of liposome apply by surface grafted poly example ethylene glycol.
- Song LY, Ahkong QF, Rong Q *et al*., 2002, the cationic lipid liposomes which is mediate by the plasmid and antisense DNA, inhibition of the effect of the PEG-lipid conjugation.
- Takeuchi H, Kojima H, Yamamoto H *et al*., 2001, the hydrophilic polymer liposome which is coated hydrophilic polymer which have in different form in albino.
- Sugano M, Egilmez NK, Yokota SJ *et al*., 2000, in this research Doxorubicin which is antibody liposome who use for the suppression of the growth as well as spread of the establishment of the human lung tumor xenografts in most chronic combine immune-deficient in mice.
- Tardi P, Choice E, Masin D *et al.*, 2000, the encapsulation of toptecan which is increase the activity of anti-cancer effects in humans.
- Drummond DC, Meyer O, Hong K *et al*., 1999, the solid tumor which is treated by the chemotherapeutic agent liposome which is optimized.
- Corvo ML, Boerman OC, Oyen WJ *et al*., in this report the superoxide dismutase which is administered by intravenous in longer way of entrapment circulating liposome 2 in rat in-vitro model of treatment of arthritis.
- Adlakha-Hutcheon G, Bally MB, Shew CR *et al.*, 1999, the liposome drug which is controlling destabilization which increase the properties of antitumor activity mitoxantrone.
- Gabizon AA, Muggia FM *et al.*, 1998, the old drugs with new therapeutics which is longer circulation liposome.
- Chonn A, Semple SC, Culis PR *et al*., 1995, the major protein beta 2-glycoprotein attached with fastly clear liposome suggest in-vivo is important action in immune clearance of non-self particles.
- Devine DV, Wong K, Serrano K *et al*., 1994, in this report implication liposomes which is interact in rat serum for survival research.
- Marjan J, Xie Z, Devine D *et al*., 1994, in this research the induction of the liposome is active for classical complement pathway which is not important for the immunoglobulin.
- Yamauchi H, Yano T, Kato T *et al*., 1994, the derivation of the sialic acid the duration of circulation is longer liposomal concentration.
- Liu D, Mori A, Huang L *et al*., 1992, the size of the liposome as well as RES blocking have role in control the biological distribution in tumor GM1-containing liposome uptake.
- Park YS, Maruyama K, Huang L *et al*., 1992, the report says that derivation of the phospholipids having some negative charge which is increase the circulation of the liposomes
- Allen TM, Hansen C, Rutledge J *et al*., 1991, the derivation of the liposome having synthetic lipid ethylene glycol which give circulation of half-life in vivo prolonged.
- Nishikawa KAHIK *et al*., 1990 *et al*., 1990, in this report acidic phospholipids of the mice which is peritoneal macrophages which is contain in scavenger receptor mediated uptake and vesicle lipids metabolism.
- Allen TM, Hansen C, Rutledge J *et al.*, 1989, in the research reticuloendothelial and other tissues which is affect the factors of the prolonger circulation duration with liposomes.
- Scherof *et al*., 1985, in this research the liposomal preparation having many types of the molecules which is in active form, Recently in clinical as well as preclinical trials in different area having most promising conclusion obtain, the main root of the difficulties in treatment by the drug in bio-distribution as well as the targeting which is overcome by the help of liposomal preparation which give protection to molecule degradation of encapsulation which is target bin passive form to tissues, organs. By administration (i.v) liposome which are fastly determin.
- Alec D Bangham *et al.*, 1961, firstly observed that smears of the egg lecithin reacted with water from quite intricate structures analysed by electron microscopy showing multitude vesicles were homogenous firstly called smecticmesophases bangham termed liposome.

History of the Liposome

The story of success of liposomes was initiated by Bangham and his colleagues in the early 1960s who observed that smears of egg lecithin reacted with water to form quite intricate structures. They were analyzed by electron microscopy showing that a multitude of vesicles were formed spontaneously. These more or less homogenous lipid vesicles were first called smecticmesophases. Later on, a colleague of Bangham termed them—more euphoniously liposomes. The physiochemical characterization of liposomes had been carried out in 1968-75. Moreover, thin lipid film hydration method had been developed to prepare multilamellar vesicles [MLVs] [13, 14]. Liposomes were widely used to study the nature of biological membrane because of close resemblance of bi-layered membrane with the biological membrane. During the late 1970s and early '80s, liposomes were re-engineered to maintain their stability so they could circulate in the blood for longer periods of time. While this was accomplished and stealth™ liposomes – ideal for delivering pharmaceutical drugs directly to cells - were developed, theyremained very difficult to produce on a large scale. In 1975 – 85Liposome's utility was improved following basic research that increased the understanding of their stability and interaction characteristic within the system [15]. This period also dealt with the discovery of various alternative methods for the preparation of liposomes. Also, due to the availability of vast knowledge about physio-chemical properties of liposomes, their behaviour within the body, their interaction with the cells, attempts had been made to improve their performance as drug carrier systems [16-18]. The development of liposomal drugs with clinical utilityrelied on the development of techniques, which allowed the rapid generation of homogeneous small liposomes and efficient accumulation of drugs into liposomes. This was made possible by the extrusion technique and the pH gradient loading techniques, which were developed in the late 1980s and early 1990s. The first liposomal drug formulation on the US market was the anticancer drug doxorubicin encapsulated in sterically stabilised liposomes [Doxil®]. Doxil® was approved by the FDA in 1995. It should be noted that it can take between 5 - 10 years and 50 - 100 million US dollars to bring a liposomal drug from the research and development stage to the market. Today, liposomes are used successfully in various scientific disciplines, including mathematics and theoretical physics [topology of two-dimensional surfaces floating in a three dimensional continuum], biophysics (properties of cell membranes and channels), chemistry [catalysis, energy conversion, photosynthesis], colloid science [stability, thermodynamic of finite systems], biochemistry [function of membrane proteins] and biology [excretion, cell function, trafficking and signal, gene delivery and function]. Ambisome TM, a parenteral amphotericin-B based liposomal product was first in the race, followed by number of other products which are either at the stage of clinical trials or are already in the market. Moreover, renaissance in the liposome research is promising many more products to come in the near future [18].

Archeosomes:- Archeosomesare vesicles consisting of anti-bacterial lipids which are chemically distinct from eukariotic and prokariotic species. They are less sensitive to oxidative stress, high temperature, and alkaline pH [19, 20].

Dendrosomes:- Dendrosomes represent a family of novel, nontoxic, neutral, biodegradable, covalent or self-assembled, hyperbranched, dendritic, spheroidal nanoparticles which are easy to prepare, inexpensive, highly stable as well as easy to handle and apply, compared with other existing synthetic vehicles for gene delivery [22].

Dried reconstituted vesicles [DRV]:- By this preparation technique, small, "empty" unilamellar vesicles, containing different lipids or mixtures of them, are prepared. After mixing those SUVs with the solubilised drug, dehydration is performed. By addition of water, rehydration leads to the formation of large quantities of rather inhomogeneous, multilamellar vesicles which need further processing [23].

Ethosomes:- Ethosomal systems are much more efficient at delivering to the skin, in terms of quantity and depth, than either conventional liposomes or hydroalcoholic solutions. Ethosomal drug permeation through the skin was demonstrated in diffusion cell experiments. Ethosomal systems composed of soy phosphatidylcholine and about 30% of ethanol were shown to contain multilamellar vesicles by electron microscopy [24].

Immunoliposomes:- Liposomes modified with antibodies, Fab's, or peptide structures on the bilayer surface were established for in vitro and in vivo application [25, 26].

Immunosomes:- Immunosomes are prepared by the anchorage of glycoprotein molecules to preformed liposomes. Under the electron microscope, immunosomes look like homogenous spherical vesicles [50–60 nm] evenly covered with spikes. Immunosomes have structural and immunogen characteristics closer to those of purified and inactivated viruses than any other form of glycoprotein lipids association [27].

Immune stimulating complex [ISCOM]:- ISCOMs are spherical, micellar assemblies of about 40 nm. They are made of the saponin mixture Quil A, cholesterol, and phospholipids. They contain amphiphilic antigens like membrane proteins. ISCOMs already have a built-in adjuvant, Quillaja saponin, which is a structural part of the vehicle [28].

Lipoplexes:- Cationic lipid-DNA complexes, named lipoplexes, are efficient carriers for cell transfection but have certain drawbacks due to their toxicity. These toxic effects may result from either cationic lipids or nucleic acids [29, 30].

LUVETs:- LUVETs are large unilamellar vesicles prepared by extrusion technique, mainly performed with high-pressure systems [31].

Niosomes:- Niosomes are small unilamellar vesicles made from nonionic surfactants also called Novasomes. Their chemical stability is comparable to that of archeosomes [32].

pH-sensitive liposomes:- Four basic classes of pH-sensitive liposomes have been described previously. The first class combines polymorphic lipids, such as unsaturated phosphatidylethanolamines, with mild acidic amphiphiles that act as stabilizers at neutral pH. This class of pH-sensitive liposomes has been the most intensively studied. The second class includes liposomes composed of lipid derivatives resulting in increased permeability to encapsulated solutes. A third class of pH-sensitive liposomes utilizes pH-sensitive peptides or reconstituted fusion proteins to destabilize membranes at low pH. The final and most current class of pH-sensitive liposomes uses pH-titratable polymers to destabilize membranes following change of the polymer conformation at low pH [33].

Polymerised liposomes:- Polymerized phosphatidyl choline vesicles (35–140 nm) have been synthesized from lipids bearing one or two methacrylate groups per monomer. Compared to nonpolymeric analogues, these vesicles exhibited improved stability and controllable time-release properties [34].

Proliposomes:- Proliposomes are defined as dry, free-flowing particles that immediately form a liposomal dispersion on contact with water [35, 36].

Proteosomes:- Vesicles of bacterial origin were solubilised, followed by ammonium sulphate precipitation and dialysis against detergent buffer. Proteins and peptides are noncovalently complexed to the membrane, making them highly immunogenic [37].

Reverse-phase evaporation vesicles:- [REV] Vesicles are formed by evaporation of oil in water emulsions resulting in large unilamellar liposomes [38].

Stealth liposomes:- In the early 1990s, this liposome engineering process culminated with the observation that coating of liposomes with polyethylene glycol [PEG], a synthetic hydrophilic polymer, would improve their stability and lengthens their half-lives in circulation, rendering the use of glycolipids obsolete. PEG coating inhibits protein adsorption and opsonization of liposomes, thereby avoiding or retarding liposome recognition by the reticuloendothelial system [RES]. These PEG-coated liposomes are also referred to as sterically stabilized or stealth liposomes. The PEG stabilizing effect results from local surface concentration of highly hydrated groups that sterically inhibit both hydrophobic and electrostatic interactions of a variety of blood components at the liposome surface [39, 40].

Temperature-sensitive liposomes: Temperature-sensitive liposomes are considered to be a promising tool to achieve site-specific delivery of drugs. Such liposomes have been prepared using lipids which undergo a gel-to-liquid crystalline phase transition a few degrees above physiological temperature. However, temperature sensitization of liposomes has been attempted using thermosensitive polymers. So far, functional liposomes have been developed according to this strategy whose content release behavior, surface properties, and affinity to cell surface can be controlled in a temperature-dependent manner [41, 42].

Transfersomes:- Transfersomes consist of phosphatidylcholine and cholate and are ultra-deformable vesicles with enhanced skin-penetrating properties [43].

Virosomes:- Virosomes are small unilamellar vesicles containing influenza hemagglutinin, by which they became forsenic with endocytic membranes. Co-incorporation of other membrane antigens induces enhanced immune responses [44].

Classification of liposome:

Liposomes are spherical, self-closed structures formed by one or several concentric lipid bilayers with an aqueous phase inside and between the lipid bilayers. There are a number of different types of liposomal vesicle:

Based on structural parameter:

1. Multilamellar vesicles: these range in size from 500 to 5,000 nm and consist of several concentric bilayers.
2. Small unilamellar vesicles: around 100 nm in size and formed by a single bilayer.
3. Large unilamellar vesicles: range in size from 200 to 800 nm.

Classification of commonly known lipid vesicles according to their structures and/or preparation:

Mechanism of action of liposome:

Liposomes as drug delivery systems can offer several advantages over conventional dosage forms especially for parenteral [i.e. local or systemic injection or infusion], topical, and pulmonary route of administration. The preceding discussion shows that liposomes exhibit different biodistribution and pharmacokinetics than free drug molecules. In several cases this can be used to improve the therapeutic

efficacy of the encapsulated drug molecules. The limitations can be reduced bioavailability of the drug, saturation of the cells of the immune system with lipids and potentially increased toxicity of some drugs due to their increased interactions with particular cells. The benefits of drug loaden liposomes, which can be applied as [colloidal] solution, aerosol, or in [semi] solid forms, such as creams and gels, can be summarized into seven categories-

- Improved solubility of lipophilic and amphiphilic drugs. Examples include Porphyrins, Amphotericin B, Minoxidil, some peptides, and anthracyclines, respectively; furthermore, in some cases hydrophilic drugs, such as anticancer agent Doxorubicin or Acyclovir can be encapsulateded in the liposome interior at concentrations several fold above their aqueous solubility. This is possible due to precipitation of the drug or gel formation inside the liposome with appropriate substances encapsulated [45].
- Passive targeting to the cells of the immune system, especially cells of the mononuclear phagocytic system [in older literature reticuloendothelial system]. Examples are antimonials, Amphotericin B, porphyrins and also vaccines, immunomodulators or [immune-supress] [45].
- Sustained release system of systemically or locally administered liposomes. Examples are doxorubicin, cytosine arabinose, cortisones, biological proteins or peptides such as vasopressin [46].
- Site-avoidance mechanism: liposomes do not dispose in certain organs, such as heart, kidneys, brain, and nervous system and this reduces cardio-, nephro-, and neuro-toxicity. Typical examples are reduced nephrotoxicity of Amphotericin B, and reduced cardiotoxicity of Doxorubicin liposomes [47].
- Site specific targeting: in certain cases liposomes with surface attached ligands can bind to target cells [‘key and lock’ mechanism], or can be delivered into the target tissue by local anatomical conditions such as leaky and badly formed blood vessels, their basal lamina, and capillaries. Examples include anticancer, antiinfectionand antiinflammatory drugs [48].
- Improved transfer of hydrophilic, charged molecules such as chelators, antibiotics [48]
- Improved penetration into tissues, especially in the case of dermally [48].

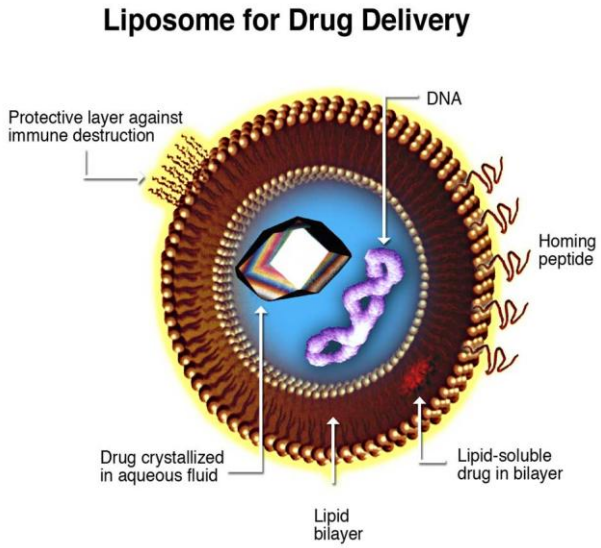

Figure-1

Liposome in vivo: -Over the last 30 years much information has been gained concerning the behaviour of liposomes *in vivo:*

- It was found that clearance of liposomes from the circulation and their biodistribution depend on the physicochemical properties of the liposomes such as liposome size, surface charge and bilayer packing, as wellas on other factors such as dose and route of administration [49-51].
- In order to transport drugs to or into tumour cells liposomes must avoid interactions with circulating cells and proteins in the blood, and uptake by phagocytic cells, which are responsible for their removal from the circulation.
- Then they must leave the vasculature [extravasate] at the site of tumour growth.
- Liposomes have then to cross the space between the vasculature and the tumour [interstitial space] and enter the tumour mass. There, dependent on the drug being delivered,the liposomes have to be taken up into the tumour cells and facilitate the delivery of the drug to its intracellular site of action [52, 53]
- For conventional drugs there is no absolute need for the liposomes to associate with the tumour cells and to be taken up into the cells.

Drug-Encapsulation

Spherical phospolipid-based liposomes can be used to carry large amount of small water-soluble molecules [therapeutic drugs] in their aqueous core or lipophilic ones in their lipid bilayer membrane through co-valent, non-covalent or avidin-biotin binding [54].

The active delivery of therapeutics to targeted sites [disease sites] of the body is largely dependent on sufficient local concentration of a therapeutic agent.

Resistance to the rapid clearance by the reticuloendothelial system9, amount of polyethylene glycol[PEG] moiety10 incorporated into the liposome bilayer and adequate amount of transport agents when crossing the endothelial barriers through various mechanisms [e.g. the transport via receptor-mediaton] [55, 56]. PEG molecules, which are used as a spacer [gure 3], results in a better exibility of the targeting vector bound.

That particular binding increases the efficiency of targeting to specefic receptors or in general transport of drugs into targeting tissues [10]. The size of liposomes has also been shown to be important factor in the efficient delivery of therapeutic drugs to the disease sites [e.g. antitumor drugs to a tumor site] [57].

Shematic representation of drug encapsulation in liposomes. Hydrophilic drugs [yellow] are encapsulated in the interrior of the liposome, while hydrophobic ones [violet] are bound in the interior of phospholipid membrane. Vesicles [red], bound on PEG molecules [blue] represent targeting vectors which bind to a special_ cell receptors [57].

Fig-Liposome-cell interaction. The _first process is liposomal adsorbtion to the cell membrane [lower left]. Some of the contents may be released into the extracellular uid and some fraction may pass through the membrane. The second possible process is the uptake of liposomes by endocytosis [upper left], which then degrade in organelles called lysosomes and release the content into the cytoplasm. The third possible process is fusion, where the liposome's content enters the cytoplasm directly [upper right]. The last mechanism is the exchange of lipids [lower right].

Encapsulation of the drug in liposome has several advantages-

- Stable encapsulation of drugs in liposomes changes the drugelimination characteristics [pharmacokinetics] and bio-distribution.For example, free drugs injected into the blood stream usually have a large volume of distribution and as a consequence exhibit significant toxicity for healthy tissues.
- Encapsulation of drugs in liposomes can reduce the volume of distribution and decrease toxic side effects in healthy tissues. Furthermore, increased circulation life times result in higher levels of accumulation at disease sites as compared to free drug.
- This can result in increased efficacy if the drug is bioavailable [released from the liposomes] [57, 58].

Two problems become immediately obvious when trying to encapsulate drugs into liposomes.

* **First**, encapsulation becomes more difficult and inefficient as the size of the drug increases. For example, the longest dimension of a 4490 bp plasmid is between 300 - 500 nm and exceeds the diameter of a 100 nm liposome

* **Second**, the encapsulation efficiencies and drug-to-lipid ratios achieved by 'passive' encapsulation techniques such as lipid film hydration are low [57, 58]

Simplest and most widely used method of Physical disperse

Hand-shaking-method-

- Dissolution of the lipid mixture and charge components in chloroform methanol solvent
- Evaporation of the solvent in rotate evaporator or by hand shaking to form a film
- Further drying of the film by attaching the flask to the manifold of the lypo-pholizer
- Casted film is then dispersed in an aqueous medium.
- Upon hydration-lipid swell and peel off the wall of the flask and vesiculate forming multilamellervesic

Non-Shaking-method-

- solution of lipid in chloroform :methenol mixture is spread over the flat bottom conical flask
- The solution is evaporated at room temprature by flow of nitrogen through the flask without disturbing the solution.
- After drying water saturated nitrogen is passed through the flask untill the opacity of the dried lipid film disappears[15-20min]
- After hydration ,lipid is swelled by addition of bulk fluid .the flask is inclined to one side ,10-20 ml of 0.2m sucrose in distilled water [degassed]is introduced down the side of the flask and the flask is slowly returned to upright orientation
- The fluid is allowed to run gently over the lipid layer on the bottom of the flask.
- The flask is flushed with nitrogen sealed and allowed to stand for 2 hrs at 37 degree celceius .take care not distribution the flask in any way.
- After swelling the vesicle are harvested by swirling the contents of the flask gently to yield a milky suspension

Proliposome:

- Method devised to increase the surface of dry lipid while keeping the low aqueous volume.
- In this method ,the lipid are dried down to afinely divided particulate support such as powdered sodium chloride or sorbital or other polysaccharide to give pro liposome
- The lipid are swelled upon adding water to dried lipid coated powder [pro liposome] where the support rapidly dissolves to give a suspension of in aqueous solution.
- The size of the carrier influences the size and heterogeneity of the liposome
- The method overcomes the problems encountered when storing liposomes themselves in either liquid ,dry or frozen from and is ideally suited for

preparation where the material to be entrapped incorporates into lipid membrane.

- In case where 100% entrapement of aqueous component is not essential ,this method is also of value
- For preparing proliposome a special equipment i.e. buchirotatry evaporator' R' with water cooled condenser coil and a stainless steel covered thermocouple connected to a digital thermocouple is required.
- The end of the glass solvent inlet tube is modified to a fine point so that the solvent is introduced into the flask as a fine.

Method of the preparation of proliposome-

- The lipid solution in chloroform [60 mg per ml] is prepared and sorbital powder is introduced into 100 ml flask.
- The flask is then fitted into the evaporate and rotated slowly so that the powder tumbles gently off the walls to ensure good mixing andthe solvent is evaporated.
- The flask is lowered into a water bath at 50-55 degree celcius when a good vaccum is developed
- An aliquot of 5 ml of lipid solution is introduced into the flask via the solvent inlet tube.
- The solvent is absorbed completely by the powder and the temperature of the bed is monitored.
- An evaporation process ,the temperature will decrease
- A second aliquot is introduced slowly when the temperature begins to rise again
- the temperature is allowed to rise to 30 degree celcius ,the vacuum is released and the dryning process is completed by connecting the flask containing the powder to lyophilizeer and leaving it evacuated overnight at room temperature
- The powder is transfered into a 10 ml glass vial containing 600mg solid each [100 mg lipid and 500mg sorbital support] flused with nitrogen and sealed well and stored.

Sonication-

- At high energy level ,the average size of the vesicles is further reduced
- This was first achieved onexposer of MLV_S fultra-sonic irradiation and still remains the method most widely used for producing small vesicles.
- There are two methods of sonication based on the use of either probe or bath ultrasonic disintegrators.
- The probe is employed for dispersions. Which require higher energy in a small volume while the bath is more suitable for large volume of diluted lipids.

French Pressure cell liposome-

- The French press [42] originally was established for breaking up cells under milder and more appropriate conditions compared to the ultrasound techniques, because lipids as well as proteins or other sensitive compounds might be degraded during the sonication procedure.
- This system is normally used in the volume of 1 to 40 mL and therefore is not suitable for large-scale production.
- However, a scale-up-based strategy on this technique was established as the micro-fluidization. This continuous and scalable variation of the French press technique enforcesdownsizing of Liposomes by collision of larger vesicles at high pressure in the interaction chamber of the micro-fluidizer.

Membrane-extrution-

- The most prominent scalable downsizing method is the extrusion. Size reduction is managed under mild and more reproducible conditions compared to those discussed above.
- In this method, preformed vesicles are forced through defined membranes by a much lower pressure as described in the French press method. Extrusion through polycarbonate filters was first published by Olson et al. in 1979 [45]. Mayer et al. [19]
- Performed extensive studies on varying lipid compositions and the influence on extrusion behaviour and membrane properties.
- Depending on the apparatus and scale, the diameters of these membranes range from 25 to 142 mm. LipexBio-membranes Inc., now Northern Lipids Inc., invented a vessel system for extrusion which is marketed from the mL scale to several liter. As suggested for all downsizing methods, liposomes should be extruded above the Tc of the lipid composition; this system can be tempered. The Lipex extruder system is available in a jacketed mode to allow extrusion at higher temperatures.

Freeze thaw method-

- The method is based upon freezing of a unilamellar dispersion and then thawing by standing at room temperature for 15 min and finally subjecting to brief sonication cycle.
- Thus the process rupture and fuses suv_s during which the solute equilibrates between inside and outside and the liposome themselves fuse and increase markedly in size.

Solvent Dispersion Method-
Ethanol-Injection-

This method has been reported as one of the alternatives used for the preparation of suv_s without sonication.

- An ethenol solution of lipids is injected rapidly through a fine needle into an excess of saline or other aqueous medium.
- The rate of the injection is generally sufficient to achieve complete mixing so that the ethanol is

diluted almost stantaneously in water and phospholipid molecule are dispersed evenly throughout the medium.
- This procedure yields a high proportion of suv_s
- This method is extremely simple and has low risk of degradation of sensitive lipids.

Ether-Injection-
- Ether injection method is similar to the ethanol injection in many respects.
- It involve injecting immiscible organic solution very slowly into an aqueous phase through a narrow needle at the temperature of vapourizing the organic solvent
- This method may also treat sensitive lipids very gently
- It has little risk of causing oxidative degradation provided ether is free from peroxides.

Reverse phase evaporation method-
- Similarly to the above presented injection methods, lipid is hydrated via solubilization in an organic phase followed by introduction into an aqueous phase.
- The organic phase should be immiscible with the aqueous phase, thus an oil/water emulsion is created, which is diluted with further aqueous phase for liposome formation
- The advantage of this very popular preparation technique is a very high encapsulation rate up to 50%. One variation of the microemulsion technique, the double emulsion technique, further improves the encapsulation rates and results in unilamellar-liposome.
- A possible drawback of this efficient method is the remaining solvent or the proof of their absence especially for using them for pharmaceutical purposes.
- The other important issue is large-scale production which might be feasible if appropriate shear mixing devices for the creation of the microemulsion and pumps for the dilution step are available.

Double emulsion method-
- This method requires two steps for the preparation of liposomes,
- First the inner leaflet of the bilayer then the outer half.
- The common features of this method is the formation of" water In oil" emulsion by introduction of a small quantity of aqueous medium containing material to be entrapped into large volume of immiscible organic solution of lipid .this was followed by mechanical agitation to break up the aqueous phase into microscopic water droplets
- These droplets are stabilized by the presence of phospholipid monolayer at the interface.
- The size of droplet is determined by the intensity of mechanical energy used to form the emulsion and amount of lipid relative to the volume of aqueous phase since each droplet requires a complete monolayer of phospholipid covering its surface in order to prevent the possible coalescence with other droplets[59,60]

APPLICATIONS OF LIPOSOMES IN THE SCIENCES
- Mathematics: Topology of two-dimensional surfaces in three-dimensional space governed only by bilayer elasticity
- Physics: Aggregation behaviour, fractals, soft and high-strength materials
- Biophysics: Permeability, phase transitions in two-dimensions, photo-physics
- Physical Chemistry: Colloid behaviour in a system of well-defined physical characteristics, inter and intra- aggregate forces, DLVO
- Chemistry: Photochemistry, artificial photosynthesis, catalysis,
- Biochemistry: Reconstitution of membrane proteins into artificial membranes
- Biology: Model biological membranes, cell function, fusion, recognition
- Pharmaceutics: Studies of drug action
- Medicine: Drug-delivery and medical diagnostics, gene therapy [61].

Conventional Liposome-

For historical reasons we shallconventional liposomes distinguish between conventional liposomes and liposomes with altered surface properties.

First generation of liposomes includes various lipid compositions which changed the physicochemical properties of liposomes in a variety of different ways, but could not significantly alter their biological properties upon intravenous administration which is the most widely used route in medical applications.

Therefore, the optimistic goals of antibody sensitised liposomes [immunoliposomes as 'guided missiles'], which gave often very encouraging results in in vitro studies – which are in general performed in the absence of immunoglobulins, complement components, and macrophages – failed in in vivo applications.

The first condition for the immunoliposome concept to work is therefore that the escape the clearance by the mononuclear phagocytic system.

This was made possible by the introduction of sterically stabilized liposomes in which the presence of surface grafted hydrophilic polymers substantially prolongs the liposome blood circulation times, probably due to reduced interactions with the components of the immune system.

This reduction arises from the presence of a steric barrier which prevents adsorption or hydrophobic

binding of immune system components onto the foreign body. The liposomes with altered surfaces therefore include sterically stabilized liposomes and immune-liposomes.

With respect to sterically stabilized immunoliposomes one should add a note of caution. Even liposomes with prolonged circulation in blood are not likely to be as widely applicable as many researchers envision [62].

Liposome in parasitic disease and injection-

Since conventional liposomes are digested by phagocytic cells in the body after intravenous administration, they are ideal vehicles for the targeting of drug molecules into these macrophages.

The best known examples of this 'Trojan horse-like' mechanism are several parasitic diseases which normally reside in the cell of mononuclear phagocytic system. They include leishmaniasis and several fungal infections. Leishmaniasis is a parasitic infection of macrophages which affects over 100 million people in tropical regions and is often fatal.

The efficacious dose of drugs, mostly different antimonials, is not much lower than the toxic one. Liposomes accumulate in the very same cell population which is infected and therefore offer an ideal drug delivery vehicle [63]. Indeed, the therapeutic index was increased in rodents as much as several hundred times upon administration of the drug in various liposomes.

The best results reported so far in human therapy are probably liposomes as carriers for Amphotericin B in antifungal therapies.

This drug is the drug of choice in disseminated fungal infections which often parallel compromised immune system, chemotherapy, or AIDS and are frequently fatal. Unfortunately, the drug itself is very toxic and its dosage is limited due to its nephro- and neuro-toxicity.

These toxicities are normally correlated with the size of the drug molecule or its complex and obviously liposome encapsulation prevents accumulation of drug in these organs and drastically reduces toxicity [63].

Similar approaches can be implemented in antibacterial, and antiviral therapy [64]. Most of the antibiotics, however, are orally available and liposome encapsulation can be considered only in the case of very potent and toxic ones which are administered parenterally.

The preparation of antibiotics loaded liposomes at reasonably high drug to lipid ratios may not be easy because of the interactions of these molecules with bilayers and high densities of their aqueous solutions which often force liposomes to float as a creamy layer on the top of the tube. Several other routes, such as topical or pulmonary [by inhalation] are being considered also.

Liposome encapsulated antivirals such as acyclovir, ribavarin, or azide thymidine [AZT] have also shown reduced toxicity and currently more detailed experiments are being performed with respect to their efficacy [65].

Macrophage activation and vaccination-

Some natural toxins induce strong macrophage response which results in macrophage activation. This can be duplicated and improved by the use of liposomes because small molecules with immunogenic properties [haptens] cannot induce immune response without being attached to a larger particle.

For instance, liposomes containing muramyl tripeptide, the smallest bacterial cell wall subunit with immunogenic properties cause macrophage activation. Activated macrophages are larger and contain more granulomae and lysosome material. Their state lasts for a few days during which they show enhanced tumouricidal, virocidal, and microactarial activity.

Early expectations in anti-tumour activity turned out to be too optimistic due to the simple fact that the number of free circulating macrophages is too small for an effective therapy. In cancer therapy, however, surgery or radiotherapy often does not remove all the tumour cells and in these cases, when tumour burden is low, this therapy is very promising for complete eradication of malignant cells. Activation of macrophages was proven useful in the treatment of viral, bacterial, and fungal infections as well. Synergy between encapsulated immunomodulators and other activating factors such as cytokines and lymphokines, including interferon [66]. Macrophages are involved also in the process of immunisation. Many molecules, however, do not induce an immune response because they are too small. In order to do so, they must be attached to larger particles. Normally this is done by administration of alum or killed bacteria and obviously liposomes offer an elegant alternative [67]. Indeed, liposomes are used in animal vaccination already since 1988, while human vaccinations against malaria are now in clinical trials [68].

Liposome in anti-cancer therapy-

Many different liposome formulations of various anticancer agents were shown to be less toxic than the free drug [69]. Anthracyclines are drugs which stop the growth of dividing cells by intercalating into the DNA and therefore kill predominantly quickly dividing cells. These cells are in tumours, but also in gastrointestinal mucosa, hair, and blood cells and therefore this class of drugs is very toxic. The most

used and studied is Adriamycin [commercial name for Doxorubicin HCl]. In addition to the above mentioned acute toxicities its dosage is limited by its cumulative cardiotoxicity. Many different formulations were tried. In most cases the toxicity was reduced about 50%. This includes both, short term and chronic toxicities because liposome encapsulation reducesthe distribution of the drug molecules towards those tissues. For the same reason, on the other hand, the efficacy was in many cases compromised due to the reduced bioavailability of the drug, especially if the tumour was not phagocytic, or located in the organs of mononuclear phagocytic system. In some cases, such as systemic lymphoma, the effect of liposome encapsulation showed enhanced efficacy due to the sustained release effect, i.e. longer presence of therapeutic concentrations in the circulation [70] while in several other cases the sequestration of the drug into tissues of mononuclear phagocytic system actually reduced its efficacy. Applications in man showed in general reduced toxicity, better tolerability of administration with not too encouraging efficacy. Several different formulations are in different phases of clinical studies and show mixed results [71].

Liposome formulation for clinical application-

According to Crommelin and Storm212, the following quality-control assays should be applied to liposomal formulations:

Basic characterization assays: pH; molarity; trapped volume; phospholipid concentration; phospholipid composition; phospholipid acyl chain composition; cholesterol concentration; active compound concentration; residual organic solvents and heavy metals; active compound/phospholipid ratio; proton or ion gradient before and after remote loading.

Chemical stability assays: phospholipid hydrolysis; non-esterified fatty acid concentration; phospholipid acyl chain auto-oxidation; cholesterol autoxidation; active compound degradation.

Physical characterization assays: appearance; vesicle size distribution; sub-micron range;micron range; electrical surface potential and surface pH; zetapotential; thermotropicbehaviour,phase transition, and phase separation; percentage of free drug [72].

Magnetic liposome-

An interesting approach for targeted drug delivery under the action of magnetic field is the use of liposomes loaded with a drug and a ferromagnetic material. In one example, magnetic liposomes containing doxorubicin were intravenously administered to osteosarcoma-bearing hamsters. When the tumour-implanted limb was placed between two poles of a 0.4 Tesla magnet, the application of the field for 60 minutes resulted in a fourfold increase in drug concentration in the tumour [73]. In the same osteosarcoma model in which the magnet was implanted into the tumour, magnetic liposomes loaded with adriamycin demonstrated better accumulation in tumour vasculature and resulted in enhanced tumour-growth inhibition] [74]. Intravenous injection in rats of liposomes loaded with 99mTc-albumin and magnetite resulted in a 25-fold increase in accumulated radioactivity in the right kidney, near which a SmComagnet was implanted, compared with the control left kidney [75]. This might become a promising way of drug targeting by liposomes.

Liposome in diagnostic imaging

The use of liposomes for the delivery of imaging agents for all imaging modalities has a long history[76]. The relative efficacy of entrapment of contrast materials into different liposomes, as well as the advantages and disadvantages of various liposome types, have been discussed by Tilcock[77]. Liposomal contrast agents have been used for experimental diagnostic imaging of liver, spleen, brain, cardio-vascular system, tumours, inflammation and infections151,153.GAMMA-SCINTIGRAPHY and MRI both require a sufficient quantity of radionuclide or paramagnetic metal to be associated with the liposome.

There are two possible routes to improve the efficacy of liposomes as contrast mediums for gamma-scintigraphy and MRI:

A. Increasing the quantity of carrier-associated reporter metal [such as 111In or Gd], and/or enhancing the signal intensity.

B. To increase the load of liposomes with reporter metals, amphiphilic chelating polymers, such as *N*,α-[DTPA polylysyl] glutaryl phosphatidylethanolamine, were introduced [78].

These polymers easily incorporate into the liposomal membrane and markedly increase the number of chelated Gd or In atoms attached to a single lipid anchor. In the case of MRI, metal atoms chelated into these groups are directly exposed to the water environment, which enhances the signal intensity of the paramagnetic ions and leads to corresponding enhancement of the vesicle contrast properties. The overall performance of chelating polymer-bearing liposomes might be further improved by additional incorporation of amphiphilic PEG into the liposome membrane, because of the presence of the increased concentration of PEG-associated water protons in the close vicinity of chelated Gd ions located on the liposomal membrane. In addition to the enhanced RELAXIVITY, the coating of liposome surface with PEG polymer can help in preventing the contrast agent being taken up at the site of injection by resident phagocytic cells. This approach results in efficient liposomal contrast agents for MRI of the blood pool [79]. MRI using pH-responsive contrast liposomes allows for the visualization of pathological areas with decreased pH values [80]. Liposomes loaded with

contrast agent have also used for the *in vivo* monitoring of tissue pharmacokinetics of liposomal drugs in mice [81].

ATP Liposome-

There is interest in liposomal forms of 'bio-energic' substrates, such as ATP, and some encouraging results with ATP-loaded liposomes in various *in vitro* and *in vivo* models have been reported ATP liposomes were shown to protect human endothelial cells from energy failure in a cell culture model of sepsis191. In a brain ischaemia model, the use of the liposomal ATP increased the number of ischaemic episodes tolerated before brain electrical silence and death192. In a HYPOVOLEMIC shock-reperfusion model in rats, the administration of ATP liposomes provided effective protection to the liver [82]. ATP liposomes also improved the rat liver energy state and metabolism during the cold storage preservation [83]. Similar properties were also demonstrated for the liposomal coenzyme Q10. Interestingly, biodistribution studies with the ATP liposomes demonstrated significant accumulation in the damaged myocardium196. Recently ATP-loaded liposomes were shown to effectively preserve mechanical properties of the heart under ischaemic conditions in an isolated rat heart model [84]. ATP-loaded immunoliposomes have also been prepared that possess specific affinity towards myosin — that is, which are capable of specifically recognizing hypoxic cells [85].

9. Liposomes in photo-dynamic therapy:-

Photo-dynamic therapy [PDT] is a rapidly developing modality for the treatment of superficial tumours, in which photosensitizing agents are used for the photo-chemical eradication of malignant cells. In PDT, liposomes are used both as drug carriers and enhancers, and a review on the use of liposomes in PDT has recently been published [86]. Targeting as well as the controlled release of photosensitizing agent in tumours might still further enhance the outcome of the liposome-mediated PDT. A benzoporphyrin derivative encapsulated in polycation liposomes modified with cetyl-polyethyleneimine was used for antiangiogenic PDT. This drug, encapsulated in such liposomes, was better internalized by human umbilical vein endothelial cells and was found in the intranuclear region and associated with mitochondria [87]. The commercial liposomal preparation of the benzoporphyrin derivative monoacid ring A [Visudyne; Novartis] is active against tumours in sarcoma-bearing mice [88]. PDT with liposomal photofrin provides better results against human gastric cancer in mice than is achieved with free drug [89]. Another porphyrin derivative [SIM01] in dimyristoylphosphatidylcholineiposomes also produces better results in PDT, mainly due to better accumulation in the tumour [human adenocarcinoma in nude mice] [90]. Liposomal meso-tetrakis-phenylporphyrin is effective in PDT of human amelanotic melanoma in nude mice [91]. The interest in this area of liposome is still growing.

Liposome in photo-dynamic therapy-

A new approach to drug delivery has recently emerged, which is based on the use of certain viral proteins that have the ability to penetrate into cells [the so-called 'protein transduction'phenomenon]. The transactivating transcriptional activator [TAT] protein from HIV-1 enters various cells when added to the surrounding media [92]. Recent data indicate that there is more than one mechanism used by cell-penetrating peptides and proteins [CPP] and CPP-mediated intracellular delivery of various molecules and particles. TAT-mediated intracellular delivery of large molecules and nanoparticles occurs through energy-dependent macropinocytosis, with subsequent enhanced escape from endosome into the cell cytoplasm [93], whereas individual CPPs or CPPconjugated small molecules penetrate cells via electrostatic interactions and hydrogen bonding and and the penetration does not seem to be associated with metabolic energy [that is, it is a purely physical, not biological, process] [94]. Traversing cellular membranes represents amajor barrier for the efficient delivery of macromolecules into cells, and therefore CPPs, whatever their mechanism of action, could serve to transport various drugs and even drug-loaded pharmaceutical carriers into mammalian cells *in vitro* and *in vivo*. It has been demonstrated that relatively large particles, such as liposomes, can be delivered into various cells by several TAT-peptide or other CPP molecules attached to the liposome surface [94, 95]. Complexes of TAT-peptide liposomes with a plasmid [plasmid pEGFP-N1, which encodes the green fluorescent protein] were used for successful *in vitro* transfection of various tumour and normal cells, as well as for *in vivo* transfection of tumour cells in mice bearing Lewis lung carcinoma [96]. Whatever mechanisms underlie the TAT-mediated delivery of large cargo such as liposomes into cells, the covalent coupling of TAT-peptides to microparticulate drug carriers could provide an efficient tool for the cytosolic delivery of various drugs and DNA *in vitro* and even *in vivo* in certain protocols of local treatment.

CONCLUSION

liposome are now used to deliver certain vaccines,enzymes and drugs to the body .when used in the delivery of certain cancer drugs, liposomes help to shield healthy cells from the drugs toxicity and prevent their concentration in vulnerable tissues (e.g kidney, liver), lessening or elimination the common side effects of nausea fatigue and hair lose. Liposomes are one of the classical specific drug delivery system, which can be used for controlled and targeted action. These systems can be administered through oral, parenteral as well as topical route. This wide range of selection of route of administration makes its flexible in designing the drug delivery system. Also these systems provide as an effective carrier for cosmetic formulations also. The

major problem in the formulation of liposome is its stability problem. These problems can be overcome by employing modification in the preparation method and also by using some specialized carriers. Nowadays liposomes are used as carrier for wide variety of drugs. In spite of its few disadvantages liposomes serve as versatile carrier for wide range of drugs. Liposome are especially effective in treating diseases that effects phagocytes. Also used to carry genes into cells and can be administered by various route.

REFRENCES

1. Vyas, S. P., & Khar, R. K. (2004). *Targeted & controlled drug delivery: novel carrier systems*. CBS publishers & distributors.
2. Jain, S. K., & Jain, N. K. (2005). Controlled and Novel Drug Delivery Systems, Chapter 15 Liposomes As Drug Carriers, 2.
3. Jain, S. K., & Jain, N. K. (2005). Controlled And Novel Drug Delivery Systems, Chapter 15 Liposomes As Drug Carriers, 3.
4. Jain, S. K., & Jain, N. K. (2005). Controlled And Novel Drug Delivery Systems, Chapter 15 Liposomes As Drug Carriers, 4.
5. Jain, S. K., & Jain, N. K. (2005). Controlled And Novel Drug Delivery Systems, Chapter 15 Liposomes As Drug Carriers, 5.
6. Jain, S. K., & Jain, N. K. (2005). Controlled And Novel Drug Delivery Systems, Chapter 15 Liposomes As Drug Carriers, 6.
7. Jain, S. K., & Jain, N. K. (2005). Controlled And Novel Drug Delivery Systems, Chapter 15 Liposomes As Drug Carriers, 7.
8. Jain, S. K., & Jain, N. K. (2005). Controlled And Novel Drug Delivery Systems, Chapter 15 Liposomes As Drug Carriers, 8.
9. Jain, S. K., & Jain, N. K. (2005). Controlled And Novel Drug Delivery Systems, Chapter 15 Liposomes As Drug Carriers, 9.
10. Jain, S. K., & Jain, N. K. (2005). Controlled And Novel Drug Delivery Systems, Chapter 15 Liposomes As Drug Carriers, 10.
11. Jain, S. K., & Jain, N. K. (2005). Controlled And Novel Drug Delivery Systems, Chapter 15 Liposomes As Drug Carriers, 11.
12. Jain, S. K., & Jain, N. K. (2005). Controlled And Novel Drug Delivery Systems, Chapter 15 Liposomes As Drug Carriers, 12.
13. Lichtenberg, D., & Barenholz, Y. (1988). In: Glick, D. [Ed.] Methods of biological analysis, Vol. 33, John Wiley and Sons Inc., New York, pp. 337-461.
14. Nassander, U. K., Storm, G., Peeters, P. A. M., & Crommelin, D. J. A. (1990). Liposomes. In: Chosin, M. and Langer, R., [Eds.], Biodegradeable Polymers as Drug Delivery Systems, Marcel Dekker Inc., New York, pp. 261-338.
15. Lasic, D. D. (1998). Novel application of liposomes. *Tibitech*, 16, 307-321.
16. Gregoriadis, G. (1988). In: Gregoriadis, G., [Ed.], Liposomes as drug carriers: recent trends and progress, Chicester, John Wiley, pp. 52-61.
17. Szoka, F. C. (1991), In: Wilschut, L., Hockstra, D., [Eds], Membrane Fusion, Marcel Dekker Inc., New York, pp. 845-890.
18. Chrai, S. S., Murari, R., & Ahmad, I. (2001). Liposomes [a review] part one: manufacturing issues. *Bio Pharm*, 11, 10-14.
19. Lasic, D. D. (1993). In: Liposomes: From Biophysics to applications, Amsterdam, Elsevier, NY, pp. 9-11.
20. Lipowsky, R. (1991). The confirmation of membrane. *Nature*, 349, 475-481.
21. Sharma Vijay, K., Mishra, D., Sharma, A., & Srivastava, B. (2010). Liposomes: present prospective and future challenges. *International journal of current pharmaceutical review & research*, *1*(2), 6-16.
22. Hope, M. J., & Kitson, C. N. (1993). Liposomes. A perspective for dermatologists. *Dermatol Clin*, 11, 143-154.
23. Eibl, H. (1980). Synthesis of glycerophospholipids, Chem. *Phys Lipids*, 26, 405-429.
24. Weiner, N., Martin, F., & Riaz, M. (1989). Lipoomes as drug delivery system. *Drug Develop Ind Pharm*, 15, 1523-1554.
25. Szoke, F. Jr., & Papahadjopoulos, D. (1980). Comparative properties and methods of preparation of lipid vesicles [liposomes]. *Ann Rev Biophys Bioeng*, 9, 467-508.
26. New, R. R. C. (1990). Preparation of liposomes. In: New R. R. C., [Ed.], Lipsomes: A Practical Approach, IRL Oxford University Press, New York, pp. 33-104.
27. Mezei, M. (1993). Liposomes and the skin. In: Gregoriadis, G., Florence, A. T., Patel, H. M., [Eds.], Liposomes in Drug Delivery, Harwood Academic Publishers pp. 125-135.
28. Mayer, L. D., Hope, M. J., Cullis, P. R., & Janoff, A. S. (1985). Solute distributions and trapping efficiencies observed in freeze-thawed multilmellar vesicles. *Biochim Biophys Acta*, 817, 193-196.
29. Vyas, S. P., & Khar, R. K. (2006). Targeted And Controlled Drug Delivery: Novel Carrier Systems. Edition 1, CBS Publishers & Distributor, New Delhi. pp. 421-427.
30. Bangham, A. D., & Horne, R. W. (1964). Negative staining of phospholipids and their structured modofication by surface active agents as observed in the electron microscope, *J Mol Biol*, 8, 660–668.
31. Papahadjopoulos, D. ed., (1978). Liposomes and their use in biology and medicine, Ann. NY Acad Sci. 308, 1–412.
32. Lasic, D. D. (1992). Liposomes, *Am Sci*, 80, 20–31.
33. Lipowsky, R. (1992). The conformation of membranes, *Nature*, 349, 475–481.
34. Lasic, D. D. (1993). Liposomes: From Physics to Applications [Elsevier, Amsterdam] 1993. see

articles by E. Sackmann, R. Lipowsky, V.A. Parsegian, E. Evans and Helfrich, in this volume.
35. Svetina, S., & Zeks, B. (1982). Bilayer couple as a possible mechanism of biological shape formation, *Biomed Biophys Acta*, 44, 979–986.
36. Berndt, K., Kaes, J., Lipowsky, R., Sackmann, E., & Seifert, U. (1990). Shape transformations of giant vesicles: extreme sensitivity to bilayer asymmetry, *Europhys Lett*, 13, 659–664.
37. Kaes, J., & Sackmann, E. (1991) Shape transitions and shape stability of giant phospholipid vesicles in pure water induced by area-to-volume changes, *Biophys J*, 60, 825–844.
38. Bloom, M., Mouritsen, O., & Evans, E. (1991). Physical properties of the fluid lipid bilayer component of cell membranes: A perspective, *Q Rev Biophys*, 24, 293–397.
39. Helfrich, W. (1974). The size of sonicated vesicles, *Phys Lett*, 50a, 115–116.
40. Lasic, D. D. (1991). Formation of membranes, *Nature*, 351, 163.
41. Fendler, J. H. (1987). Atomic and molecular clusters in membrane mimetic chemistry, *Chem Rev*, 87, 877–899.
42. Cornelius, F. (1991). Functional reconstitution of the sodium pump. Kinetics and exchange reactions performed by reconstituted Na/K ATPase, *Biochim Biophys Acta*, 1071, 19–66.
43. Villalobo, A. (1991). Reconstitution of ion-motive transport ATPases in artificial lipid membranes, *Biochim Biophys Acta*, 1071, 1–48.
44. Lasic, D. D., Frederik, P. M., Stuart, M. C. A., Barenholz, Y., & McIntosh, T. J. (1992). Gelation of liposome interior. A novel method for drug encapsulation, *FEBS Lett*, 312, 255–258.
45. Nässander, U. K., Steerenberg, P. A., Poppe, H., Storm, G., Poels, L. G., De Jong, W. H., & Crommelin, D. J. (1992). In vivo targeting of OV-TL 3 immunoliposomes to ascitic ovarian carcinoma cells (OVCAR-3) in athymic nude mice. *Cancer research*, *52*(3), 646-653.
46. New, R. R. C., Chance, S. M., Thomas, S. C., & Peters, W. (1978). Nature antileshmanial activity of antimonials entrapped in liposomes, 272, 55–58.
47. Lopez-Berestein, G., Fainstein, V., Hopfer, R., Mehta, K., Sullivan, M. P., Keating, M., ... & Bodey, G. P. (1985). Liposomal amphotericin B for the treatment of systemic fungal infections in patients with cancer: a preliminary study. *Journal of Infectious Diseases*, *151*(4), 704-710.
48. Lasic, D. D. (1992). Mixed micelles in drug delivery. *Nature*, *355*(6357), 279-280.
49. Fidler, I. J., Fan, D., & Ichinose, Y. (1989). Potent in situ activation of murine lung macrophages and therapy of melanoma metastases by systemic administration of liposomes containing muramyltripeptide phosphatidylethanolamine and interferon gamma. *Invasion & metastasis*, *9*(2), 75-88.
50. Gregoriadis, G. (1990). Immunological adjuvants: a role for liposomes. *Immunology today*, *11*, 89-97.
51. Alving, C. R. (1991). Liposomes as carriers of antigens and adjuvants. *Journal of immunological methods*, *140*(1), 1-13.
52. Poste, G., Kirsh, R., & Bugelski, P. (1984). Liposomes as a drug delivery system in cancer therapy. In *Novel approaches to cancer chemotherapy* (pp. 165-230). Academic Press.
53. Storm, G., Roerdink, F. H., Steerenberg, P. A., De Jong, W. H., & Crommelin, D. J. A. (1987). Influence of lipid composition on the antitumor activity exerted by doxorubicin-containing liposomes in a rat solid tumor model. *Cancer research*, *47*(13), 3366-3372.
54. Lopez-Berestein, G., & Fidler, I. J. (1989). Liposomes in the Treatment of Infectious Diseases and Cancer (A. Liss, New York).
55. Williams, B. D., O'sullivan, M. M., Saggu, G. S., Williams, K. E., Williams, L. A., & Morgan, J. R. (1986). Imaging in rheumatoid arthritis using liposomes labelled with technetium. *British medical journal (Clinical research ed.)*, *293*(6555), 1143-1145.
56. McCalden, T. A. (1990). Particulate systems for drug delivery to the lung. *Advanced drug delivery reviews*, *5*(3), 253-263.
57. Fielding, R. M., & Abra, R. M. (1992). Factors affecting the release rate of terbutaline from liposome formulations after intratracheal instillation in the guinea pig. *Pharmaceutical research*, *9*(2), 220-223.
58. Hamori, C. J., Lasic, D. D., Vreman, H. J., & Stevenson, D. K. (1993). Targeting zinc protoporphyrin liposomes to the spleen using reticuloendothelial blockade with blank liposomes. *Pediatric research*, *34*(1), 1-5.
59. Allen, T. M., & Chonn, A. (1987). Large unilamellar liposomes with low uptake into the reticuloendothelial system. *FEBS letters*, *223*(1), 42-46.
60. Gabizon, A., & Papahadjopoulos, D. (1988). Liposome formulations with prolonged circulation time in blood and enhanced uptake by tumors. *Proceedings of the National Academy of Sciences*, *85*(18), 6949-6953.
61. Woodle, M. C., & Lasic, D. D. (1992). Sterically stabilized vesicles, *Biochim Biophys Acta*, 1113, 171–199.
62. Lasic, D. D., Martin, F. J., Gabizon, A., Huang, S. K., & Papahadjopoulos, D. (1991). Sterically stabilized liposomes: a hypothesis on the molecular origin of the extended circulation times. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *1070*(1), 187-192.
63. De Gennes, P. G. (1987). Polymers at an interface; a simplified view. *Advances in colloid and interface science*, *27*(3-4), 189-209.
64. Needham, D. T. J. D. D., McIntosh, T. J., & Lasic, D. D. (1992). Repulsive interactions and

mechanical stability of polymer-grafted lipid membranes. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *1108*(1), 40-48.
65. Needham, D., Hristova, K., McIntosh, T. J., Dewhirst, M., Wu, N., & Lasic, D. D. (1992). Polymer-grafted liposomes: physical basis for the "stealth" property. *Journal of Liposome Research*, *2*(3), 411-430.
66. de Gennes, P. G. (1991). A second type of phase separation in polymer solutions, C.R. *Acad Sci*, 313-II, 1117–1122.
67. De Gennes, P. G. (1992). Private communication.
68. Lasic, D. D., & Needham, D. Stealth liposomes: A prototypic biomaterial, Chem. Rev., in press.
69. Gabizon, A. A. (1992). Selective tumor localization and improved therapeutic index of anthracyclines encapsulated in long-circulating liposomes. *Cancer research*, *52*(4), 891-896.
70. Papahadjopoulos, D., Allen, T. M., Gabizon, A., Mayhew, E., Matthay, K., Huang, S. K., ... & Redemann, C. (1991). Sterically stabilized liposomes: improvements in pharmacokinetics and antitumor therapeutic efficacy. *Proceedings of the National Academy of Sciences*, *88*(24), 11460-11464.
71. Mayhew, E. G., Lasic, D., Babbar, S., & Martin, F. J. (1992). Pharmacokinetics and antitumor activity of epirubicin encapsulated in long- circulating liposomes incorporating a polyethylene glycol-derivatized phospholipid. *International journal of cancer*, *51*(2), 302-309.
72. Vaage, J., Mayhew, E., Lasic, D., & Martin, F. (1992). Therapy of primary and metastatic mouse mammary carcinomas with doxorubicin encapsulated in long circulating liposomes. *International journal of cancer*, *51*(6), 942-948.
73. Huang, S. K., Mayhew, E., Gilani, S., Lasic, D. D., Martin, F. J., & Papahadjopoulos, D. (1992). Pharmacokinetics and therapeutics of sterically stabilized liposomes in mice bearing C-26 colon carcinoma. *Cancer research*, *52*(24), 6774-6781.
74. Allen, T. M., Mehra, T., Hansen, C., & Chin, Y. C. (1992). Stealth liposomes: an improved sustained release system for 1-β-D-arabinofuranosylcytosine. *Cancer Research*, *52*(9), 2431-2439.
75. Bakker-Woudenberg, I. A., Lokerse, A. F., Marian, T., & Storm, G. (1992). Enhanced localization of liposomes with prolonged blood circulation time in infected lung tissue. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, *1138*(4), 318-326.
76. Woodle, M. C., Storm, G., Newman, M. S., Jekot, J. J., Collins, L. R., Martin, F. J., & Szoka Jr, F. C. (1992). Prolonged systemic delivery of peptide drugs by long-circulating liposomes: illustration with vasopressin in the Brattleboro rat. *Pharmaceutical research*, *9*(2), 260-265.
77. Martin, F., Gabizon, A., & Martin, F. J. (1992). Human pharmacokinetics of doxil (stealth liposome encapsulated doxorubicin). In *UCLA Symposium: Prevention and Treatment of AIDS, Book of Abstracts, Keystone, CO*.
78. Bogner, J. R., & Goebel, F. D. (1995). Phase II open-label trial of doxil (stealth liposomal doxorubicin) in advanced AIDS-KS. In *VIII International Conference on AIDS, in ref* (Vol. 51).
79. Northfelt, D. W., Kaplan, L., Russell, J., Volberding, P. A., & Martin, F. J. Single dose pharmacokinetics, safety and tumor localization study of doxil in AIDS patients with Kaposi's sarcoma. In *VIII International Conference on AIDS, in ref* (Vol. 51).
80. Nicolau, C., & Cudd, A. (1989). Liposomes as carriers of DNA. *Critical reviews in therapeutic drug carrier systems*, *6*(3), 239-271.
81. Fraley, R., Subramani, S., Berg, P., & Papahadjopoulos, D. (1980). Introduction of liposome-encapsulated SV40 DNA into cells. *Journal of Biological Chemistry*, *255*(21), 10431-10435.
82. Lurquin, P. F. (1979). Entrapment of plasmid DNA by liposomes and their interactions with plant protoplasts. *Nucleic acids research*, *6*(12), 3773-3784.
83. Felgner, P. L., Gadek, T. R., Holm, M., Roman, R., Chan, H. W., Wenz, M., ... & Danielsen, M. (1987). Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. *Proceedings of the National Academy of Sciences*, *84*(21), 7413-7417.
84. Rose, J. K., Buonocore, L., & Whitt, M. A. (1991). A new cationic liposome reagent mediating nearly quantitative transfection of animal cells. *BioTechniques*, *10*(4), 520-525.
85. Gao, X., & Huang, L. (1991). A novel cationic liposome reagent for efficient transfection of mammalian cells. *Biochemical and biophysical research communications*, *179*(1), 280-285.
86. Stribling, R., Brunette, E., Liggitt, D., Gaensler, K., & Debs, R. (1992). Aerosol gene delivery in vivo. *Proceedings of the National Academy of Sciences*, *89*(23), 11277-11281.
87. Law, B. A., & King, J. S. (1985). Use of liposomes for proteinase addition to Cheddar cheese. *Journal of Dairy Research*, *52*(1), 183-188.
88. Alkhalaf, W., PIARD, J. C., El Soda, M., GRIPON, J. C., Desmazeaud, M., & Vassal, L. (1988). Liposomes as proteinase carriers for the accelerated ripening of Saint- Paulin type cheese. *Journal of Food Science*, *53*(6), 1674-1679.
89. Kirby, C. (1990). Delivery systems for enzymes. *Chemistry in Britain*, *26*, 847-850.
90. Tahibi, A., Sakurai, J. D., Mathur, R., & Wallach, D. F. H. (1991). Novasome vesicles in extended pesticide formulation. In *Proc. Symp. Contr. Rel. Bioact. Mat* (Vol. 18, pp. 231-232).

91. Gatt, S., Bercovier, J. H., & Barenholz, Y. (1991). Use of liposomes to combat oil spills and their potential application to bioreclamation, in: On Site Bioreclamation, eds Hinchee, R. E., & Olfenbuttel, R. F., [Butterworth, Stoneham] pp. 293–312.
92. Dutton, G. (1993). The promise of liposomes, Gen. Engin News, 13, 6–9.
93. Boroske, E., Elwenspoek, M., Helfrich, W. (1981). Osmotic shrinkage of giant egg-lecithin vesicles, *Biophys J*, 34, 95–109.
94. Kuhl, T. L., Leckband, D. E., Lasic, D. D., & Israelachvili, J. N. (1994). Modulation of interaction forces between bilayers exposing short-chained ethylene oxide headgroups. *Biophysical Journal*, *66*(5), 1479-1488.
95. Tsuchida, E. (1994). Stabilized hemoglobin vesicles. *Artificial Cells, Blood Substitutes, and Biotechnology*, *22*(3), 467-477.
96. Zheng, S., Zheng, Y., Beissinger, R., & Martin, F. J. (1994). Efficacy, Physical Properties and Pharmaco-Kinetics of Sterically-Stabilized Liposome-Encapsulated Hemoglobin. *Artificial Cells, Blood Substitutes, and Biotechnology*, *22*(3), 487-501.

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