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DRUG TARGETING: BASIC CONCEPTS AND NANOSYSTEMS IN DRUG TARGETING

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ABSTRACT

At present about 95% of all new potential therapeutic have poor pharmacokinetic and pharmacodynamics property. Either they get destroyed before going to systemic circulation or they get poorly absorbed. Hence, there is need to develop the new potential drug system which has significant good pharmacokinetic and pharmacodynamics property. For this purpose the concept of Targeted drug delivery system has been originated by "Paul Ehrlich". Among the drug carrier it includes polymer, micro particles made of synthetic polymers, microcapsules, quantum dots, magnetic microspheres, liposomes. Liposomes which are micro or nanometric vesicles composed of amphiphillic species have particle size ranging from 20nm to several micrometers. Indeed, liposomes formulations of some drugs have shown a significant increase in therapeutic efficacy and therapeutic indices compared to other non liposomal formulation. In site specific targeted drug delivery, active drug is delivered to very specific preselected compartments with maximum activity while reducing the concentration of drug to normal cells. The drug can be targeted to intracellular sites, virus cells, bacteria cell and parasites using different scientific strategies have proven highly effective. The minimum distribution of the parent drug to the non- target cells with higher and effective concentration at the targeted site certainly maximize the benefits of targeted drug delivery.

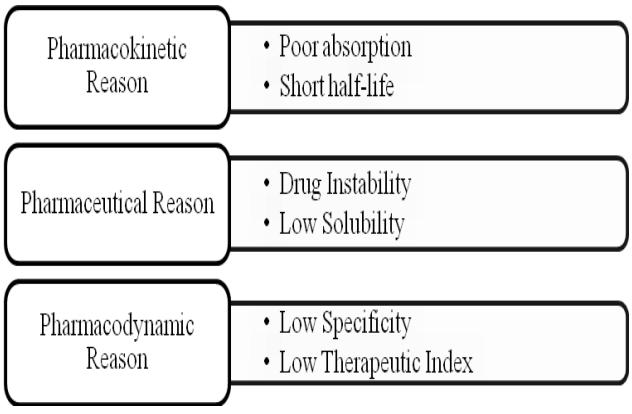
Key words: Targeted drug delivery system, Properties, Barriers, types, Liposomes.

INTRODUCTION

In the year of 1981, "Gregoriadis" described drug targeting using novel drug delivery system as ´old drug in new clothes**'.** The concept of designing targeted delivery system has been originated from the "Paul Ehrlich", who was a microbiologist, proposed the idea of drug delivery in the form of magic bullet. Selective drug targeting yet remains unachieved. Targeted drug delivery means accumulation of pharmacologically active moiety at desired target in therapeutic concentration at the same restricting its access to normal cellular lining, thus minimizing therapeutic index. In site specific targeted drug delivery, active drug is delivered to very specific pre selected compartments with maximum activity while reducing the concentration of drug to normal cells.

The drug can be targeted to intracellular sites, virus cells, bacteria cell and parasites using different scientific strategies have proven highly effective. The minimum distribution of the parent drug to the non- target cells with higher and effective concentration at the targeted site certainly maximize the benefits of targeted drug delivery [1].

Need of targeted Drug Delivery



PROPERTIES OF IDEAL TARGETED DRUG DELIVERY

- It should be nontoxic, biocompatible, biodegradable, and physically and chemically stable in vivo and in vitro.

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- Restrict drug distribution to target cells or tissue or organ or should have uniform capillary distribution.
- Controllable and predictable rate of drug release.
- The drug distribution should not be affected by drug release.
- Therapeutic amount of drug release.
- During transit there should be minimal drug leakage.
- Carrier used must be biodegradable or readily eliminated from the body without any problem and no carrier should induce modulation of diseased state.
- The preparation of drug delivery system should be easy or reasonably simple, reproductive and cost effective [2,3]

BARRIERS TO DRUG TARGETING

Targeting of drugs offers enormous advantages but isequally challenging. A better understanding of the physiological barriers which a drug needs to overcomeshould enable the pharmaceutical scientists to develop successful design of targeted drug delivery systems. Main hurdles to drug targeting include physiological barriers, biochemical challenges to identify and validate the molecular targets and the pharmaceutical challenges to devise appropriate techniques of conjugating targeting ligands to the Nano-systems. The challenge in drug targeting is not only the targeting of drug to a specific site but also retaining it for the desired duration to elicit pharmacological action.

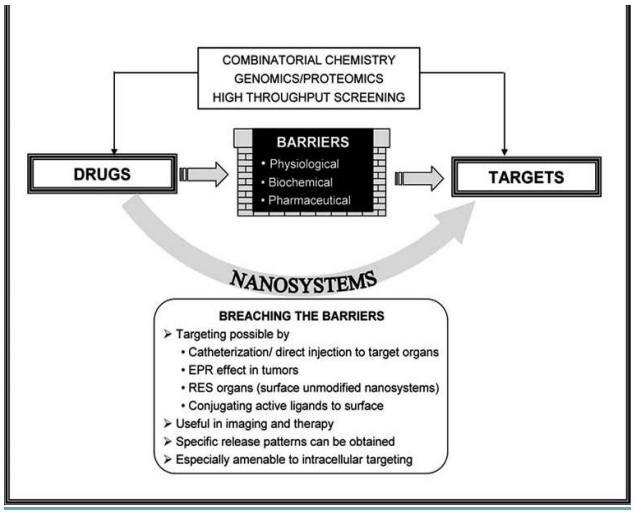
For a Nano-system administered intravenously, the first and fore most barrier is that of the vascular endothelium and the base mentmembrane. Also, plasma proteins have the ability to affect the bio-distribution of drug carrier systems introduced in the blood stream. The in vivo bio-distribution and opsonization of Nano-systems in blood circulation isgoverned by their size and surface characteristics. For the Nano-system to remain in blood circulation for a long time, the major problem is to avoid its opsonization and subsequent uptake by thephagocytic cells. The passage ofdrug molecules and drug delivery systems across the endothelium is sensitive to the molecular weight and size ofthe system, respectively.

The tight endothelial cells in brain constitute the blood brain barrier (BBB), which restricts the entry of most drugs and delivery systems. However vascular endothelium is not uniform throughout. The altered endothelia in tumors allow an enhanced permeability to macro molecules and the particulate drug delivery systems. Another barrier is that of the extracellular matrix, which should be crossed to access the target cells in a tissue. If the whole tissue constitutes a target then the uniform distribution of drug throughout the tissue is another problem.

Targeting Nano-systems to specific receptors or antigens on the cell surface provides the driving force for diffusion of the system to the specific cells. For drugs whose targets are located in the cytoplasm/nucleus of a cell, further barrier needs to be crossed to allow internalization of Nano-systems into specific cells. The barriers not end here, a number of endocytic pathways have been described for the cellular entry of Nano-systems. Drugs/Nano-systems need to diffuse through the viscous cytosol to access the particular cytoplasmic targets where site of action is located. Nuclear membrane poses another formidable barrier for drugs such as oligonucleotides, plasmid DNA and other low molecular weight drugs whose site of action is located in the nucleus of a cell.

Although a number of cellular and molecular targets are emerging, the real problem lies with the poor accessibility of drugs/ Nano-systems to the target tissue. The presence of such barriers leads to a poor in vitro/in vivo correlation when the targeted delivery systems are tested in receptor bearing cells in vitro and fail in vivo. Thus, to harness the potential of new targets in imaging and therapy, one would need to develop targeted systems which can successfully overcome the physiological barriers, and for drug therapy, deliver the pharmacological agent to its site of action at therapeutically relevant drug levels for a time sufficient to allow therapeutic action. Conjugation of targeting ligands to drugs or drug carrier Nano-systems is the most popular way of directing them to their target sites. To this end, various techniques have been devised, including covalent and non-covalent conjugation.

The emphasis is that the ligand must be attached stably and accessibly to the drug carrier, so that the ligand is presented in its right orientation for binding to thetarget receptors. For example, the monoclonal antibodies must bind to the drug/its carrier with their Fc part, so that their antigen binding site (Fab) is free to interact with the antigenic targets on cells. The coupling reactions must not affect the biological activity of ligand and should not adversely affect the structure of drug delivery Nano-systems. Further, such coupling reactions must be optimized so thatbinding of ligands takes place in a homogeneous manner on the surface of the drug carrier Nano-systems [4].



Types of drug targeting

An ideal targeted drug delivery approach would not only increase therapeutic efficacy of drugs but also decrease the toxicity associated with drug to allow lower doses of the drug to be used in therapy. Two approaches are used passive targeting and active targeting.

Passive targeting

Passive targeting refers to the accumulation of drug or drug-carrier system at a particular site due to physicochemical or pharmacological factors. Drug or drug carrier Nano-systems can be passively targeted making use of the patho-physiological and anatomicalopportunities.eg include targeting of anti-malarial drugs for treatment of leishmiansis, brucellosis, candiadsis [5]. This approach for liposome drug delivery exploits the natural tendency of certain cells such as Kupffer cells in the liver, and circulating macrophages of RES to phagocytosis foreign micro particulates such as liposomes. Conventional liposome (CL) formulations of drugs and immunostimulators have been successfully used for targeting the cells of RES, and exhibit significant improvement in the TI of the drugs (reviewed by Alving, 1991). In clinical trials, systemic administration of CL containing muramyl peptide derivatives caused enhancement in the tumoricidal properties of monocytes in patients with recurrent osteosarcoma (reviewed by Killion and Fidler, 1994). Liposomes have also been used to enhance the antigenicity of certain molecules for new vaccine formulations. Furthermore, CLs have also been employed for targeting of immunosuppressive drugs to lymphatic tissues such as the spleen. In a preclinical model, an increase in immunosuppressive activity, i.e. a delay in heart transplant rejection was observed with CL-encapsulated methylprednisolone [6].

Active targeting

Active targeting employs specific modification of a drug or drug-carrier Nano-systems with active. Agents having selective affinity for recognizing and interacting with a specific cell, tissue or organ in the body. Direct coupling of drugs to targeting ligand, restricts the coupling capacity to a few drug molecules. In contrast, coupling of drug carrier Nano-systems to ligands allows import of thousands of drug molecules by means of one receptor targeted ligand. Example of active targeting is use of monoclonal antibody the treatment of cancer. Active targeting of liposome encapsulated drugs may be accomplished by coupling specific antibodies to vesicles (immunoliposomes). Immunoliposomes containing diphtherin toxin (DT) have been shown to provide protection against the non-specific toxicity of DT during cancer chemotherapy. Long circulating immunoliposomes (hydrophilic polymer- coated vesicles bound to antibodies and <0.15 /~m in size) can now be designed which may be able to recognize and bind with greater specificity to target cells following systemic administration. It has been shown that long circulating immunoliposomes (LCI) enhanced therapeutic efficacy of encapsulated doxorubicin in a murine lung tumor model. The effect of size on bio distribution of LCI has been studied in a rabbit model of myocardial infarction. Small sized (0.12-0.15 /Lm) LCI containing infarct-specific antimyosin antibodies (AM) exhibited significantly lower accumulation in RES compared to CL with or without AM. However, the accumulation of LCI-AM was higher in kidneys and lungs compared to CL-AM. The accumulation of large sized (0.35 0.4 /lm) LCI in spleen was 2-fold higher than small sized LCI. Active targeting using immunoliposomes has several advantages over that of antibody-drug conjugates:
(i) immunoliposomes can carry a significantly larger number of drug molecules compared to simple conjugates;
(ii)Immunoliposomes can encapsulate drugs with widely varying physicochemical properties; and
(iii)Drugs can also reach their intracellular target by diffusion after release from immunoliposomes associated with target tissue. Therefore, unlike antibodies-drug conjugates, in some cases immunoliposomes may not have to undergo receptor mediated-endocytosis to deliver their contents intracellular [6].

This active targeting approach can be further classified into three different levels of targeting.

First order targeting

It refers to restricted distribution of the drug carrier systems to the capillary bed of a predetermined target site, organ or tissue. Example includes compartmental targeting in lymphatic's, peritoneal cavity, plural cavity, cerebral ventricles, eyes, joints, etc.

Second order targeting

Selective delivery of drugs to specific cell types such as tumorcells and not to the normal cells is referred as second order targeting E.g.includes selective drug delivery to kupffer cells in the liver.

Third order targeting

Defined as drug delivery specifically to the intracellular site of targeted cells.eg includes receptor based ligand mediated entry of a drug complex into a cell by endocytosis [5].

Recent approaches

Quantumdots

A quantum dot is a semi conductor nano structure that confines the motion of conduction and electrons, valence band holes, or excitons (bound pairs of conduction band electrons and valence band holes) in all three spatial directions. The confinement can be due to electrostatic potentials (generated by external electrodes, doping, strain, impurities), the presence of an interface between different semiconductor materials (e.g. incore-shell Nano-crystal systems), the presence of the semi conductor surface (e.g. semiconductor Nano-crystal), or a combination of these. Quantumdots are particularly significant for optical applications due to their theoretically high quantum yield. The ability to tune the size of quantum dots is advantageous for many applications and it is one of the most promising candidates for use in solid-state quantum computation and diagnosis drug delivery, tissue engineering, catalysis, filtration and also textiles technologies [7].

Quantum dots (QDs) are semiconducting materials consisting of a semiconductor core (CdSe), coated by a shell (e.g., ZnS) to improve optical properties, and a cap enabling improved solubility in aqueous buffers. They are neither atomic nor bulk semiconductors. Their

properties originate from their physical size, which ranges from 10–100 Å in radius. Due to their bright fluorescence, narrow emission, broad UV excitation and high photo stability QDs have been adopted for in vitro bio imaging for real time monitoring or tracking of intracellular process for longer time. Quantum-dots have a large impact on some important development in different medical areas like diagnostic tools (magnetic resonance imaging, MRI), in vitro and in vivo detection and analysis of biomolecules, immunoassays, DNA hybridization, development of non-viral vectors for gene therapy, transport vehicles for DNA, protein, drugs or cells, time graded fluorescence imaging of tissue, labeling of cells and as therapeutic tools for cancer treatment [8].

Quantum dots (QD) are small (2–10 nm) colloidal fluorescent semiconductor Nano crystals composed from 10–50 atoms of groups II–IV or III–V of the periodic table. Their structure consists of a metalloid crystalline core &a shell that protects the core and renders the QD available for in vivo applications. The size and shape of quantum dots can be controlled precisely, properties that determine their absorption and light emission. One of the most valuable properties of QD is their fluorescence spectrum, which make them optimal fluorophores for biomedical imaging. Fluorescent QD can be conjugated with bioactive moieties or specific ligands (e.g., receptor ligands and antibodies). QD are stable for months without degradation or alteration. QD are mostly used as long-term, high-sensitivity and multicontrast imaging agents for detection and diagnosis of cancer in vivo. Other examples of QD applications include transistors, solar cells, and quantum computing. Nevertheless, because they are composed of hazardous heavy metals, it is important to be cautious about their toxicity [9].

Preparation of Quantum Dots

Phosphor nanoparticles of CdSe/ZnS (core/shell) were synthesized using a continuous hot-soap reactor as well as a batch process. The emission luminescence wavelength of the nanoparticles prepared by the continuous route has a somewhat wider spectrum than those prepared by the batch route. However, the continuous process showed the possibility of fabricating the luminescent semiconductor nanoparticles, in which the luminescent spectra can be controlled from the range of blue to red depending on the particle size [10].

Transdermal Approach

Transdermal drug delivery system is topically administered medicaments in the form of patches that deliver drugs for systemic effects at a predetermined and controlled rate. A transdermal drug delivery device, which may be of an active or a passive design, is a device which provides an alternative route for administering medication. These devices allow for pharmaceuticals to be delivered across the skin barrier. In theory, transdermal patches work very simply. A drug is applied in a relatively high dosage to the inside of a patch, which is worn on the skin for an extended period of time. Through a diffusion process, the drug enters the bloodstream directly through the skin. Since there is high concentration on the patch and low concentration in the blood, the drug will keep diffusing into the blood for a long period of time, maintaining the constant concentration of drug in the blood flow [7].

Folate Targeting

Folate targeting is a method utilized in biotechnology for drug delivery purposes. It involves the attachment of the vitamin, folate (folic acid), to a molecule/drug to form a "folate-conjugate". Based on the natural high affinity of folate for the folate receptor protein (FR), which is commonly expressed on the surface of many human cancers, folate-drug conjugates also bind tightly to the FR and trigger cellular uptake via endocytosis. Molecules as diverse as small radio-diagnostic imaging agents to large DNA plasmid formulations have successfully been delivered inside FR positive cells and tissues. FA also displays high affinity for the folate receptor (FR), a Glycol-sylphosphatidyinositol-linked protein that captures its ligands from the extracellular milieu and transports them inside the cell via a non-destructive, recycling endosomal pathway. The FR is also a recognized tumor antigen/biomarker. Because of this, diagnostic and therapeutic methods which exploit the FR's function are being developed for cancer [7].

Brain targeted drug delivery system

The brain is a delicate organ, and evolution built very efficient ways to protect it. The delivery of drugs to central nervous system (CNS) is a challenge in the treatment of neurological disorders. Drugs may be administered directly into the CNS or administered systematically (e.g., by intravenous injection) for targeted action in the CNS. The major challenge to CNS drug delivery is the blood-brain barrier (BBB), which limits the access of drugs to the brain substance. Advances in understanding of the cell biology of the BBB have opened new avenues and possibilities for improved drug delivery to the CNS. Various strategies that have been used for manipulating the blood-brain barrier for drug delivery to the brain include osmotic and chemical opening of the blood-brain barrier as well as the use of transport/carrier systems. Other strategies for drug delivery to the brain involve bypassing the BBB. Various pharmacological agents have been used to open the BBB and direct invasive methods can introduce therapeutic agents into the brain substance. It is important to consider not only the net delivery of the agent to the CNS, but also the ability of the agent to access the relevant target site within the CNS. Various routes of administration as well as conjugations of drugs, e.g., with liposomes and Nano-particles, are considered [7].

Magnetic microspheres

There are a number carriers Microspheres, nano particles, liposome and others for which optimize technologies are under development to

a) Enhance the performance of products that have already been delivered with some success via that route and

b) Modulates the release and absorption characteristics of the drugs particularly those drugs which have shorter biological half -life.

Dosage forms that can precisely control the release rates and target drugs to a specific body site have created enormous impact on the formulation and development of novel drug delivery systems. The objective of controlled release drug delivery includes two important aspects namely spatial placement and temporal delivery of drug. Spatial placement relates to targeting a drug to a specific organ or tissue, while temporal delivery refers to controlling the rate of drug delivery to the target tissue. While a variety of devices have been used for controlled release drug delivery, biodegradable polymer microspheres are one of the most common types and hold several advantages. Microspheres can encapsulate many types of drugs including small molecules, proteins, and nucleic acids and are easily administered through a syringe needle. They are generally biocompatible, can provide high bioavailability, and are capable of sustained release for long periods of time. Magnetite offers great potential for advancements in electronics, optoelectronics, magnetic storage, biomedical, ferrofluid, separation and magnetically guided drug carriers for targeting the therapy. Small amounts of drug targeted magnetically to localized sites can replace large doses of drug that, using traditional administration methods, freely circulate in the blood and hit the target site in a generalized way only. Also, drugs within the sphere are protected from breaking down during transport and, because they are targeted instead of distributed in blood, don't harm some sensitive organs such as bone marrow. Magnetic microspheres as an alternative to traditional radiation methods which use highly penetrating radiation that is absorbed throughout the body. Its use is limited by toxicity and side effects. Magnetic radioactive microspheres are applied in methods similar to non-radioactive spheres. A magnet, placed outside the body, is directed to the target site. The magnet can be a rod-shaped permanent magnet of any size or can be contained in equipment that looks like an open magnetic resonance imaging scanner. The loaded microspheres are introduced into a blood vessel, and in as little as half an hour, they gather at the target site to emit radiation that kills surrounding cancer cells. The therapeutic action usually a couple of days or weeks, depending on the material used. If necessary, the treatment can be repeated. Spheres need to be peppered with microscopic magnetic particles, such as iron, so they will be attracted to the magnet. For applications requiring in vivo magnetic targeting, for example, magnetic drug delivery, the magnetic carriers must have a proper size range (i.e. between 200 nm and 3 mm) and high magnetizations to enable technically feasible external magnetic guidance within the vasculature. In these applications the microspheres (i.e. 1–2 mm) would be more advantageous than Nano spheres in terms of better targeting and easier capture [11].

Liposomes

These are vesicular concentric structures, range in size from a nanometer to several micrometers, containing a phospholipids bilayer and are biocompatible, biodegradable and non-immunogenic. Liposomes have generated a great interest because of their versatility and have played a significant role in formulation of potent drugs to improve therapeutics. Enhanced safety and efficacy have been achieved for a wide range of drug classes, including antitumor agents, antiviral, antimicrobials, vaccines, gene therapeutics etc. . Recently pharmaceutical science is using liposomes to reduce toxicity and side effect of drugs. The various problems like poor solubility, short half- life and poor bioavailability & strong side effect of various drugs can be overcome by employing the concept of liposomes especially in various diseases like cancer etc. Liposomes offer ample opportunities for the investigators to explore the unidentified breakthrough in the field of pharmaceutical technology [7].

Liposomes in targeted drug delivery system

Recent advances in biomedical science and combinatorial chemistry have resulted in the design and synthesis of hundreds of new agents with potential activity against a wide range of therapeutic targets in vitro. However, most of these new drugs fail to live up to their potential in the clinic. For instance, although there are numerous anticancer agents that are highly cytotoxic to tumor cells in vitro, the lack of selective antitumor effect in vivo precludes their use in clinic. One of the major limitations of antineoplastic drugs is their low therapeutic index (TI), i.e. the dose required to produce anti-tumor effect is toxic to normal tissues. The low TI of such drugs may be due to:

(i) Their inability to achieve therapeutic concentrations at the target site (solid tumors)

(ii) Nonspecific cytotoxicity to critical normal tissues such as bone marrow, renal, GI tract and cardiac tissue

(iii) Problems associated with formulation of the drug, for example, low solubility in pharmaceutically suitable vehicles, leading to the use of surfactants or organic co-solvents which have their own undesirable side effects.

Thus, there is a need for effective delivery systems that not only act as a formulation aid butalter the bio-distribution of drugs in such a way that a greater fraction of the dose reaches the target site. Liposomes are micro-particulate or colloidal carriers, usually 0.05-5.0 /~m in diameter which form spontaneously when certain lipids are hydrated in aqueous media. Liposomes are composed of relatively biocompatible and biodegradable material, and they consist of an aqueous volume entrapped by one or more bilayers of natural and/or synthetic lipids. Drugs with widely varying lipophilicities can be encapsulated in liposomes, either in the phospholipid bilayer, in the entrapped aqueous volume or at the bilayer interface. Liposomes have been investigated as carriers of various pharmacologically active agents such as antineoplastic and antimicrobial drugs, chelating agents, steroids, vaccines and genetic material. Due to recent developments in liposome technology, more effective strategies are now availablefor controlling the stability and reactivity of liposomes after systemic administration. On

the basis of the ability of liposomes to interact with cells and/or blood components, at least two types of liposomes currently can be designed including:

(i) non-interactive sterically stabilized (long-circulating)liposomes (LCL) and; (ii) highly interactivecationic liposomes. Sterically stabilized liposomes can be formulated by incorporating hydrophilic long-chain polymers in the bilayer which can form a coat on the liposome surface and repel opsonin penetration and adsorption. Reduction in 'marking' by opsonins leads to slower uptake of these liposomes (LCL) by the cells of reticulo endothelial system (RES). Thus, LCL exhibit extended circulation half-life compared to the so-called 'conventional liposomes' (CL) because of their reduced recognition and uptake by the RES. Furthermore, LCL can be designed to exhibit specific interaction with target cells by attaching target specific ligands. In contrast to LCL, cationic liposomes exhibit high affinity to cell membranes and can be used to deliver exogenous genetic material intracellularly via fusion with the cell membranes. Cationic liposome formulations provide a promising non-viral delivery system for transfection of cells by exogenous plasmids, RNA and oligonucleotides [12].

Liposomes are micro or nanometric vesicles composed of amphiphilic species, such as lipids or phospholipids, that spontaneously form one (unilamellar) or more (multilamellar) concentric bilayers separated by water compartments. Lipids expose their hydrophilic head outwards, while the hydrophobic tail is directed inwards in the bilayer. Depending on the number of bilayers, the particle size can range from about 20 nm to several micrometers.

Liposomes show very versatile properties in terms of size, surface charge and lipid composition, and their ability to incorporate almost any drug independent of its solubility in water makes these micro reservoir systems useful for delivery purposes. There is a large variety of lipids employable for the preparation of liposomes, comprising for instance mixtures of stearic acid and Tween 80, mixtures of distearoyl phosphatidylcholine, distearoyl phosphatidylglycerol, and cholesterol, diplasmenylcholine, mixtures of distearoyl phosphatidylcholine, dimyristoyl phosphatidylglycerol, and cholesterol, mixtures of castor oil, phosphatidylcholine and polyethylene glycol coupled to distearoylphosphatidylethanolamine. The stability of protein-based drugs can be maintained using adequate incorporation processes such as reverse-phase evaporation, injection and freeze-thaw. Liposomes also proved to be safe in vivo and were even tested as possible candidates to improve the safety in prescribing drugs during pregnancy. Liposomes can be exploited for drug delivery via pulmonary route. This method exhibits numerous benefits as an alternative for repeated injection of drugs like insulin. Encapsulation of insulin into liposomal carriers for pulmonary delivery (dry powder inhalation) showed good hypoglycemic effect with low blood glucose level and long-lasting period and a relatively high pharmacological bioavailability. Liposomes are suitable carriers for the delivery of pro-apoptotic membrane proteins into cancer cells to induce cell death. Voltage-dependent anionic channel (VDAC) and Bak are two mitochondrial outer membrane proteins involved in the activation of the intrinsic apoptotic pathway that have been successfully conjugated to liposomes and delivered into cells. It is well known that the use of liposomes as suitable carriers for drug delivery is prone to issues connected with the phagocytic activity of macrophage. The clearance of liposomes from the blood stream depends not only on mechanical filtration and membrane fusion events, but also on interactions with serum proteinsm and cellular receptors. Cellular receptors do not directly recognize the liposome as a foreign body, but they recognize specific serum proteins that bind the liposome surface (opsonins). Once the liposome has been internalized into a cell by endocytosis, several strategies can be used to achieve endosomal escape of liposome-encapsulated drugs. A recent technique consists of providing the cell with photosensitizer molecules, which primarily accumulate in endosomal membranes, and then exposing the system to light. Upon illumination, reactive singlet oxygen species form and damage the endosomal membrane, which becomes permeable to endocyted molecules (photochemical internalization). This method was successfully used to induce cytotoxicity in EGFreceptor positive human ovarian cancer cells, thanks to the plant toxin saporin encapsulated into targeted liposomes. Liposomes can also undergo lipid exchange with high-density lipoproteins in blood, leading to liposome disintegration. The intrinsic instability of liposomes in the body environment causes fast release of the loaded drug. This means a peak in drug concentration a short time after administration (burst effect). Liposomes can be protected against the burst effect, e.g., by encapsulation in alginate shells cross-linked with Ba^{2+} ions. They can also be properly designed in order to minimize the macrophage uptake and prolong their circulation time after intravenous injection. The most exploited modification used to reach this goal is PEGylation. When the linear, non-toxic, flexible and hydrophilic PEG polymer that shows anti-opsonizing properties is grafted on the liposome surface, it forms a hydrophilic layer that shields the liposome surface charge. Its steric hindrance and hydrophilicity prevent opsonins from settling on the liposome surface, making it difficult for RES cells to recognize and interact with liposomes. For this reason, such liposomes are commonly named stealth liposomes, long-circulating liposomes or sterically stabilized liposomes. It has been reported that free PEG and bound PEG produce opposite effects: free PEG causes fusion of the particles, while bound PEG protects them. PEG-functionalized lipids exhibit dose-independent pharmacokinetics in animals and humans, and the ability to cross biological barriers in vivo. These features allow PEGylated liposomes improved delivery and therapeutic efficacy of anti-cancer drugs. An important aspect to be considered in liposome PEGylation is the choice of the PEG-lipid conjugate alkyl chain, which can in some cases produce immunogenicity after repeated administration. Woodle *et al* investigated the value of novel systemically long-circulating liposomes to prolong

the duration of an antidiuretic hormone, arg8-vasopressin (VP), as a representative of low molecular weight peptides with rapid clearance. The cholesterol content was found to have a controlling effect on VP release in serum. Three types of liposomes were tested in VP-deficient Brattleboro rats. One contained phosphatidylserine (PS), which was rapidly cleared from the circulation. In the other two liposomes, PS was replaced by either phosphatidylglycerol or a novel phospholipid derivatized with polyethylene glycol (PE-PEG); both showing prolonged circulation. The duration of the prolonged bioactivity was not dose dependent, but the amplitude was. This is attributed to VP release from liposomes, which were distributed intact to another compartment without being taken up by the RES. The authors concluded that liposomes could be applied to prolong the biological activity of a therapeutic peptide by balancing liposome circulation time, release rate, and dose. Kedar et al demonstrated that recombinant human interleukin-2 (IL-2) can be successfully encapsulated in unilamellar, long circulating, sterically stabilized liposomes. They also compared the immunomodulatory and anti-tumor effects of IL-2, pegylated IL-2 (PEG-IL-2) and liposome encapsulated IL-2 (SSL-IL-2) in mice. They found that SSL-IL-2 was significantly more effective than IL-2 in increasing leukocyte number in the blood and spleen and triggering spleen lymphokine activated killer cell activity. The survival of mice with advanced metastatic carcinoma (previously treated with cyclophosphamide chemotherapy) was two to six times greater following administration of SSL-IL-2 than IL-2. Moreover, successful treatment with SSL IL-2 required lower cumulative doses and fewer administrations. PEG-IL-2 was a more potent immune stimulator than SSL-IL-2 in normal mice, and as effective as SSL-IL-2 in tumor-bearing mice. PEG-IL-2, however, caused marked toxicity, including severe thrombocytopenia. PEGylation is also frequently used to prepare radiolabeled liposomes for imaging techniques. PEGylated liposomes labeled with 111In administered intravenously to rats affected by Staphylococcus aureus showed that their clearance from the blood stream is similar to that of control 111In-IgG. On the contrary, the uptake by the inflammatory site was twice that of the control, making the inflammation visible by scintigraphy one hour after injection. However, PEGylation is not the only modification reaction that can be carried out on liposomes. To improve the efficacy of ligand binding to a liposome membrane, Yagi et al. developed a novel lipid analog based on amino acids for liposome modification. This lipid consists of three peptide derivatives and two fatty acids, and it was used to prepare liposomes incorporating the HIV-TAT peptide (domain of human immunodeficiency virus TAT protein). This is a protein transduction domain commonly employed to investigate the delivery of macromolecules, nucleic acids and liposomes into cells. Liposomes containing the lipid analog bearing HIV-TAT peptide exhibited efficient cellular uptake. Other peptides can be used to modify liposomes surface. Octaarginineoligopeptide (R8) bound on the surface of liposomes can enhance cell internalization by macropinocytosis. R8-modified liposomes can escape from macropinosomesinto the cytosol, preserving the encapsulated drug from degradation. Green fluorescence protein was chosen as a model protein and efficiently delivered into mitochondria thanks to highly mitochondrion-fusogenic lipid formulation of the liposomes. Lipid modification can also alter the drug-loading efficacy by inducing changes in the membrane properties, such as micropolarity, microviscosity and free volume. Incorporation of cholesterol proved to reduce the partitioning of porphyrins, while methyl oleate and PEGylated lipids noticeably increased the value of the relevant binding constants. Another important feature deriving from lipid modification is the insertion of functional groups able to bind ligands to the surface of liposomes. Ligands can react with the functional groups either before or after the liposome formation [13].

Applications of liposomes in drug delivery

New drug delivery systems such as liposomes are developed when an existing formulation is not satisfactory and reformulation offers superior therapeutic efficacy and safety over the existing formulation. Indeed, liposome formulations of some drugs have shown a significant increase in therapeutic efficacy and/or therapeutic indices in preclinical models and in humans, compared to their non-liposomal formulations. The therapeutic applications of liposomes generally fall into several categories briefly described below.

Formulation aid

Hydrophobic drugs such as cyclosporin and paclitaxel are usually formulated in surfactants and organic co-solvents for systemic administration in humans. These solubilizers may cause toxicity at the doses needed to deliver the drug. Incontrast, liposomes are made up of lipids which are relatively non-toxic, non-immunogenic, biocompatible and biodegradable molecules, and canen capsulate a broad range of water-insoluble (lipophilic) drugs. Currently, liposomes or phospholipid mixtures are being used as excipients for preparing better-tolerated preclinical and clinical formulations of several lipophilic, poorly water soluble drugs such as amphotericin B. In preclinicalstudies, liposomes have been evaluated as avehicle for the delivery of paclitaxel and its analogs as an alternative to the cremophor/ethanol vehicle. Paclitaxel liposomes were able to deliver the drug systemically and increase the therapeutic index of paclitaxel in human ovarian tumor models.

Intracellular drug delivery

Drugs with intracellular targets/receptors are required to cross the plasma membrane for pharmacological activity. Liposomes can be used to increase cytosolic delivery of certain drugs such asN-(phosphonacetyl)-L-aspartate (PALA) which are normally poorly taken up into cells. PALA is taken up into the tumor cells through fluid-phase endocytosis (pinocytosis) and it diffuses out into the cytoplasmas the endosome pH drops. However, pinocytosis is very limited in its

efficiency. Liposomal delivery of drugs which normally enter the cells by pinocytosis can be very effective because liposomes can contain greater concentrations of drug compared to the extracellular fluid and the endocytosis process by which negatively charged liposomes are predominantly taken up by the cells, is more efficient than pinocytosis. For example, the potency of PALA encapsulated liposomes was up to 500-fold greater against human ovarian tumor cell lines than that of free PALA.

Sustained release drug delivery

Sustained release systems are required for drugs such as cytosine arabinoside (Ara-C) that are rapidly cleared in vivo and require plasma concentrations at therapeutic levels for a prolonged period for optimum pharmacological effects. It is now possible to design sustained release liposome formulations with an extended circulation half-life and an optimized drug release rate in vivo. For example, Ara-C encapsulated in LCL is effective as a prolonged release system in the treatment of murine L1210/C2 leukemia. Conventional liposomes which localize by phagocytosis in the cells of RES may also act as a sustained release depot by slowly leaking drugs from RES into the general circulation.

Gene therapy

A number of systemic diseases are caused by lack of enzymes/factors which are due to missingor defective genes. In recent years, several attempts have been made to restore gene expression by delivery of the relevant exogenous DNA orgenes to cells (reviewed by Crystal. Cationic liposomes (Table 1) have been considered as potential non-viral human gene delivery system. They are usually composed of a cationic lipid derivative and a neutral phospholipid (DOPE).The latter is required by certain cationic lipids to form stable liposomes. Some of the widely usedcationic liposome formulations are: lipofectin(DOTMA:DOPE, 1:1); lipofectamine (DOSPA:DOPE, 3:1); transfectace (DDAB:DOPE, 1:3); cytofectin(DMRIE:DOPE); transfectam (DOGS)and DC-cholesterol. The negatively charged genetic material (e.g., plasmid) is not encapsulated in liposomes but complexed with cationic lipids by electrostatic interactions. Plasmid-liposome complexes are thought to enter the cell by fusion with the plasma or endosome membrane. Allovectin-7, a gene transfer product is currently in clinical trials (phase I/II) as an immune therapeutic agent for the treatment of metastatic melanoma, renal cell and colorectal carcinoma (Table 3). Allovectin-7 is composed of a plasmid containing the gene for the major histo compatibility complex antigen HLA-B7 with fl-2microglobulin formulated with the cytofect in (DMRIE:DOPE). The ongoing clinical trials have indicated that intralesional injection of Allovectin- 7 can be performed safely and have demonstrated antitumor activity in some patients. Plasmid-liposome complexes have many advantages as gene transfer vehicles over viral-based vectors.

(i) These complexes are relatively non immunogenic because they lackproteins; (ii) liposomes or lipid complexes can be used for transfection of large - sized genetic material; and

(iii) Viruses, unlike plasmid-liposome complexes, may replicate and cause infections.

However, there are several problems limiting the application of liposomes as a gene delivery system.

Site-avoidance delivery

Drugs used in the treatment of diseases like cancer usually have a narrow therapeutic index (TI) and can be highly toxic to normal tissues. The toxicity of these drugs may be minimized by decreasing delivery to critical normal organs. It has been shown that even a small reduction in distribution of the drug to critical organs by encapsulation in liposomes can significantly reduce the drug toxicity. Liposomes are taken up poorly by tissues such as heart, kidney, and GI tract, which are major sites for toxic side-effects of a variety of antineoplastic drugs. Thus, liposome formulation may improve the TI by altering the bio-distribution of drug away from drug sensitive normal tissues. For instance, free amphotericin B and doxorubicin produce severe dose-limiting nephrotoxicity and cardiac toxicity, respectively. Reformulation of these drugs in liposomes results in reduced toxicity with no change in therapeutic efficacy. Liposome formulations of amphotericin B and doxorubicin have now been approved for clinical use.

Site-specific targeting

Site-specific delivery, the concept first proposed by Paul Ehrlich involves the delivery of a larger fraction of drug to the target site and therefore, reducing exposure to normal tissues. Liposomes have been employed for accomplishing both passive and active targeting of drugs.

Intraperitoneal administration

Direct administration of antineoplastic agents into the intraperitoneal (i.p.) cavity has been proposed to be therapeutically advantageous for cancers that develop in or metastasize to the peritoneal cavity. Intraperitoneal chemotherapy has been somewhat unsuccessful for free drugs because of relatively fast clearance of the drugs from the i.p. cavity resulting in lowered concentrations at the site of action

However, the clearance of liposomes from the peritoneal cavityis significantly slower than that of free drug and therefore, higher drug concentrations can be achieved in the proximity of the target site for extended periods of time with the use of liposome formulations. Furthermore, reformulation of erosive drugs in liposomes has been shown to reduce local drug toxicity such as dermal toxicity of doxorubicin. An increase in TI of paclitaxel in liposomes after administration may also be due to a reduction in local (abdominal) toxicity of the drug. The tendency of liposomes to interact with macrophages in RES is exploited in this approach (passive targeting).The mechanism by which liposomes cause increasesin antigens' immune response is not fully understood. However, augmentation of liposomal adjuvanticity can be achieved by co-administration of liposome encapsulated antigen with other adjuvants such

as lipid A, lipopolysaccharides, muramyldipeptide and interleukin (IL-2). Furthermore, antibody-mediated targeting of liposomal to antigen-presenting cells may also improve immune stimulatory effects. The influence of physicochemical properties of the liposomes such as charge density, membrane fluidity and epitope density, on the immune response of the antigen has been extensively studied. For instance, liposome formulations of inactivated encephalo myocarditis and Semliki Forest viruses were significantly more immunogenic when charged phospholipids were used compared to neutral lipids. The phase transition temperature (To) of the lipids also appears to influence immunogenicity. For example, immunogenicity of haptens was higher in liposomes composed of lipids with a high Tc than in those with a low T. Recently, the first liposome-based vaccine (liposomes containing inactivated hepatitis Avirions) was approved for human use in Switzerland and currently, several other liposome-based vaccines are in clinical trials.

Immunological adjuvants in vaccines

Liposomes can encapsulate antigens in theiraqueous space or incorporate in the bilayer depending on the lipophilicity of the antigen. Liposomes were first used as immunological adjuvants in order to enhance the immune response to encapsulated diphtheria toxoid. Since then, liposomeshave been used as nontoxic adjuvants with bacterial, viral, protozoan, tumor and other antigens. The tendency of liposomes to interact with macrophages in RES is exploited in this approach (passive targeting). The mechanism by which liposomes cause increases in antigens' immune response is not fully understood. However, augmentation of liposomal adjuvanticity can be achieved by co-administration of liposome encapsulated antigen with other adjuvants such as lipid A, lipopolysaccharides, muramyldipeptide and interleukin (IL-2). Furthermore, antibody-mediated targeting of liposomal to antigen-presenting cells may also improve immune stimulatory effects. The influence of physicochemical properties of the liposomes such as charge density, membrane fluidity and epitope density, on the immune response of the antigen has been extensively studied (reviewed by Kersten and Crommelin, 1995). For instance, liposome formulations of inactivated encephalomyocarditis and Semliki Forest viruses were significantly more immunogenic when charged phospholipids were used compared to neutral lipids. The phase transition temperature (To) of the lipids also appears to influence immunogenicity. For example, immunogenicity of haptens was higher in liposomes composed of lipids with a high Tc than in those with a low T. Recently, the first liposome-based vaccine (liposomes containing inactivated hepatitis A virions) was approved for human use in Switzerland and currently, several other liposome-based vaccines are in clinical trials [14].

Method of preparation

Classical Technique

There are four classical methods of liposome manufacture. The difference between the various methods is the way in which lipids are drying down from organic solvents and then redispersed in aqueous media. These steps are performed individually or are mostly combined.

Hydration of a Thin Lipid Film

Bangham Method. This is the original method which was initially used for liposomes production. A mixture of phospholipid and cholesterol were dispersed in organic solvent. Then, the organic solvent was removed by means of evaporation (using a Rotary Evaporator at reduced pressure). Finally, the dry lipidic film deposited on the flask wall was hydrated by adding an aqueous buffer solution under agitation at temperature above the lipid transition temperature. This method is widespread and easy to handle, however, dispersed-phospholipids in aqueous buffer yields a population of multilamellar liposomes (MLVs) heterogeneous both in size and shape (1–5 _m diameter). Thus, liposome size reduction techniques, such as sonication for SUVs formation or extrusion through polycarbonate filters forming LUVs25_ 26 were useful to produce smaller and more uniformly sized population of vesicles.

Reverse-Phase Evaporation (REV) Technique

A lipidic film is prepared by evaporating organic solvent under reduced pressure. The system is purged with nitrogen and the lipids are re-dissolved in a second organic phase which is usually constituted by diethyl ether and/or isopropyl ether. Large unilamellar and oligolamellar vesicles are formed when an aqueous buffer is introduced into this mixture. The organic solvent is subsequently removed and the system is maintained under continuous nitrogen. These vesicles have aqueous volume to lipid ratios that are 30 times higher than sonicated preparations and 4 times higher than multilamellar vesicles. Most importantly, a substantial fraction of the aqueous phase (up to 62% at low salt concentrations) is entrapped within the vesicles, encapsulating even large macromolecular assemblies with high efficiency.

Solvent (Ether or Ethanol) Injection Technique

The solvent injection methods involve the dissolution of the lipid into an organic phase (ethanol or ether), followed by the injection of the lipid solution into aqueous media, forming liposomes. The ethanol injection method was first described in 1973.The main relevance of the ethanol injection method resides in the observation that a narrow distribution of small liposomes (under 100 nm) can be obtained by simply injecting an ethanolic lipid solution in water, in one step, without extrusion or sonication. The ether injection method differs from the ethanol injection method since the ether is immiscible with the aqueous phase, which is also heated so that the solvent is removed from the liposomal product. The method involves injection of ether-lipid solutions into warmed aqueous phases above the boiling point of the ether. The ether vaporizes upon contacting the aqueous phase, and the dispersed lipid forms primarily unilamellar liposomes. An advantage of the ether injection method compared to the

ethanol injection method is the removal of the solvent from the product, enabling the process to be run for extended periods forming a concentrated liposomal product with high entrapment efficiencies.

Detergent Dialysis

Liposomes, in the size range of 40–180 nm, are formed when lipids are solubilized with detergent, yielding defined mixed micelles.32 As the detergent is subsequently removed by controlled dialysis, phospholipids form homogeneous unilamellar vesicles with usefully large encapsulated volume.

Other methods have been already used for liposome's preparation such as: calcium induced fusion, Nano-precipitation, and emulsion techniques. However, these classical techniques require large amounts of organic solvent, which are harmful both to the environment and to human health, requiring complete removal of residual organic solvent. Furthermore, conventional methods consist of many steps for size homogenization and consume a large amount of energy which is unsuitable for the mass production of liposomes.

New Large-Scale Liposome Technique:

Since industrial scale production of liposomes has become reality, the range of liposome preparation methods has been extended by a number of techniques such as Heating Method, Spray drying, Freeze Drying, Super Critical Reverse Phase Evaporation (SCRPE), and several modified ethanol injection techniques which are increasingly attractive.

Heating Method

A new method for fast production of liposomes without the use of any hazardous chemical or process has been described. This method involves the hydration of liposome components in an aqueous medium followed by the heating of these components, in the presence of glycerol (3% v/v), up to 120C.

Glycerol is a water-soluble and physiologically acceptable chemical with the ability to increase the stability of lipid vesicles and does not need to be removed from the final liposomal product. Temperature and mechanical stirring provide adequate energy for the formation of stable liposomes. Reza Mozafari et al. confirmed by TLC that no degradation of the used lipids occurred at the above mentioned temperatures. The particle size can be controlled by the phospholipid nature and charge, the speed of the stirring and the shape of the reaction vessel. Otherwise, employment of heat abolishes the need to carry out any further sterilisation procedure reducing the time and cost of liposome production.

Spray-Drying

Since spray-drying is a very simple and industrially applicable method, the direct spray-drying of a mixture of lipid and drug was applied in the preparation of liposomes. The spray-drying process is considered to be a fast single-step procedure applied in the nanoparticles formulation. Hence, liposomes were prepared by suspending lecithin and mannitol in chloroform. The mixture was sonicated for 8 min (bath sonicator) and subjected to spray-drying on a Buchi 190 M Mini Spray Dryer. The spray-drying conditions were as follows: inlet and outlet temperatures were 120 C and 80 C, respectively; airflow rate was 700 NI/hr; and the flow rate was 1000 ml/hr. The dried product was hydrated with different volumes of phosphate buffered saline (PBS; pH 7.4) by stirring for 45 min. The main factor influencing the liposomal size was the volume of aqueous medium used for hydration of the spray-dried product. However, mannitol plays an important role in increasing the surface area of the lipid mixture, enabling successful hydration of the spray-dried product.

Freeze Drying.

This new method was described for the preparation of sterile and pyrogen-free submicron narrow sized liposomes.39_ 40 It is based on the formation of a homogenous dispersion of lipids in water-soluble carrier materials. Liposome-forming lipids and water-soluble carrier materials such as sucrose were dissolved in tert-butyl alcohol/watercosolvent systems in appropriate ratios to form a clear isotropic monophase solution. Then the monophase solution was sterilized by filtration and filled into freeze-drying vials. In recent study, a laboratory freeze drier was used and freeze-drying process was as follows: freezing at −40 C for 8 h; primary drying at −40 C for 48 h and secondary drying at 25 C for 10 h. The chamber pressure was maintained at 20 Pascal during the drying process. On addition of water, the lyophilized product spontaneously forms homogenous liposome preparation. After investigation of the various parameters associated with this method it is found that the lipid/carrier ratio is the key factor affecting the size and the polydispersity of the liposome preparation.39 Therefore, TBA/water cosolvent system was used for economy concerns.

Super Critical Reverse Phase Evaporation (SCRPE)

The SCRPE is a one-step new method that has been developed for liposomes preparation using supercritical carbon dioxide. This method allowed aqueous dispersions of liposomes to be obtained through emulsion formation by introducing a given amount of water into a homogeneous mixture of supercritical carbon dioxide/LR-dipalmitoyl phosphatidyl choline/ ethanol under sufficient stirring and subsequent pressure reduction. Transmission electron microscopy observations revealed that vesicles are large unilamellar with diameters of 0.1–1.2 m. The trapping efficiency of these liposomes indicated more than 5 times higher values for the water-soluble solute compared to multilamellar vesicles prepared by the Bangham method. The trapping efficiency for an oil soluble substance, the cholesterol, was about 63%.Resultsshowed that the SCRPE is an excellent technique that permits one-step preparation of large unilamellar liposomes exhibiting a high trapping efficiency for both water-soluble and oil-soluble compounds.

Modified Ethanol Injection Method

Novel approaches based on the principle of the ethanol injection technique such as the micro fluidic channel method, the cross flow-injection technique, and the membrane contactor method51 were recently reported for liposome production.

The Cross flow Injection Technique

The concept of continuous cross flow injection is a promising approach as a novel scalable liposome preparation technique for pharmaceutical application. Wagner et al. used a cross flow injection module made of two tubes welded together forming a cross. At the connecting point, the modules were adapted with an injection hole. The influencing parameters such as the lipid concentration, the injection hole diameter, the injection pressure, the buffer flow rate, and system performance were investigated. A minimum of buffer flow rate is required to affect batch homogeneity and strongly influencing parameters are lipid concentration in combination with increasing injection pressures. After exceeding the upper pressure limit of the linear range, where injection velocities remain constant, the vesicle batches are narrowly distributed, also when injecting higher lipid concentrations. Reproducibility and scalability data show similar results with respect to vesicle size and size distribution and demonstrate the stability and robustness of the novel continuous liposome preparation technique.

Microfluidization

By using a microfluidic hydrodynamic focusing (MHF) platform, Jahn et al. generated liposomes by injecting the lipid phase and the water phase into a microchannel. Microfluidic flow is generally laminar due to the small channel dimensions and relatively low flow rates. Well-defined mixing is then obtained by interfacial diffusion when multiple flow streams are injected in a microchannel. The size of the liposomes was mainly controlled by changing the flow rate.

Membrane Contactor

Recently applied the ethanol injection technique while using a membrane contactor for large scale liposomes production. In this method, a lipid phase (ethanol, phospholipid and cholesterol) was pressed through the membrane with a specified pore size. Nitrogen gas at pressure below 5 bar was sufficient for passing the organic phase through the membrane. At the same time, the aqueous phase flew tangentially to the membrane surface and swept away the formed liposomes within the membrane device. The new process advantages are the design simplicity, the control of the liposome size by tuning the process parameters and the scaling-up abilities. As a result, these techniques lead from the conventional batch process to potential large scale continuous procedures [15].

Table 1. Advantages and disadvantages of liposomes

Advantages of liposome	Disadvantages of liposome
Liposomes increased efficacy and therapeutic index of drug (actinomycin-D)	Low solubility
Liposome increased stability via encapsulation	Short half-life
Liposomes are non-toxic, flexible, biocompatible, completely biodegradable, and nonimmunogenic for systemic and non-systemic administrations	Sometimes phospholipid undergoes oxidation and hydrolysis-like reaction
Liposomes reduce the toxicity of the encapsulated agent (amphotericin B, Taxol)	Leakage and fusion of encapsulated drug/ Molecules
Liposomes help reduce the exposure of sensitive tissues to toxic drugs	Production cost is high
Site avoidance effect	Fewer stables
Flexibility to couple with site-specific ligands to achieve active targeting	

Fig 1. A LIGHT IN DARK PLACES: Spectral imaging of quantum dots. Orange-red fluorescence signals indicate a prostate tumor growing in a live mouse

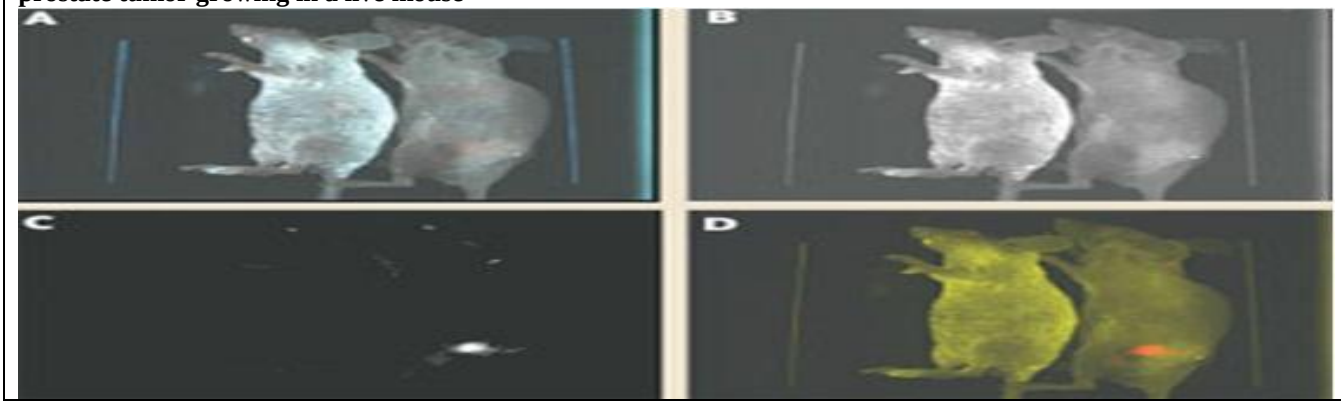


Fig 2. Structure of a unilamellar liposome for drug delivery

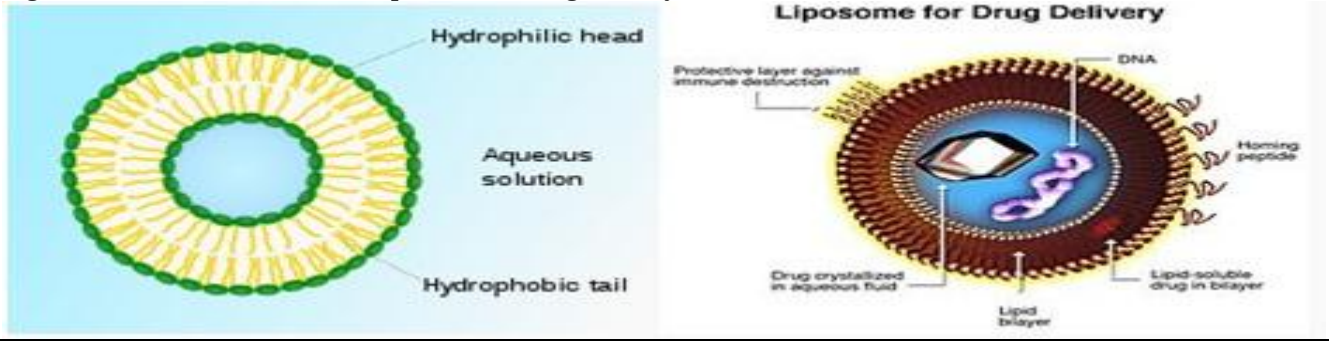
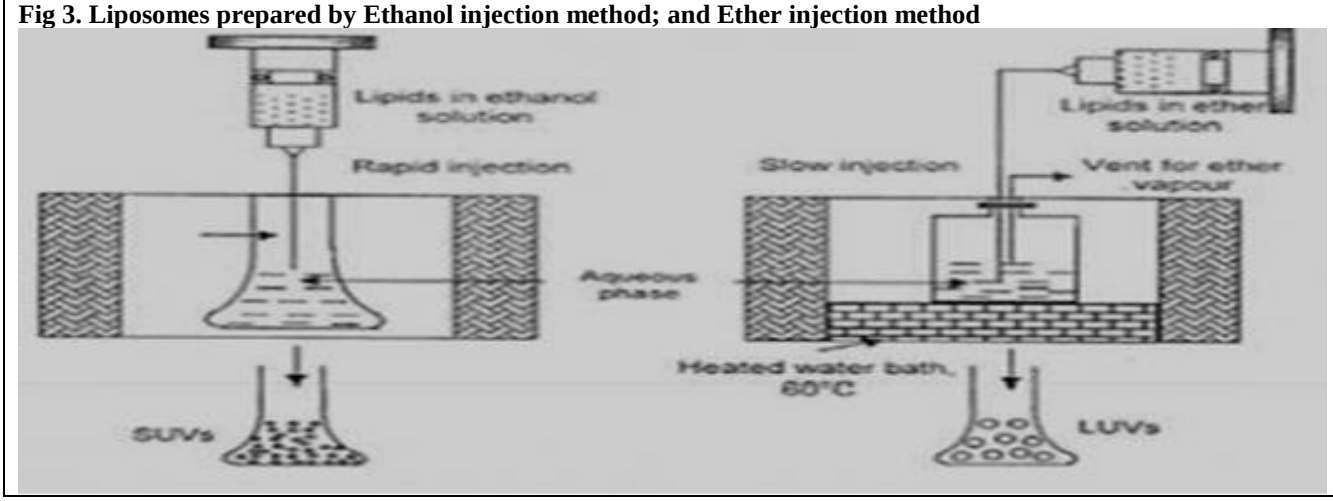


Fig 3. Liposomes prepared by Ethanol injection method; and Ether injection method



CONCLUSION

Targeted drug delivery system offer opportunity to achieve drug targeting with newly discovered disease, specific targets. Targeted delivery of drugs, as the name indicates is to assist the drug molecule to reach preferably to the desired sites. It is a challenging area for future research in the drug targeting so; more research in the drug targeting so more researches, long term toxicity study and characterization will insure the improvement.

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