



Pharma Group

# Open Journal of Pharmacology and Pharmacotherapeutics

DOI: <http://dx.doi.org/10.17352/ojpp>

CC BY

## Ateeq Rahman\*, Veikko Uahengo and Daniel Likius

Faculty of Science, Department of Chemistry and Biochemistry, University of Namibia, Pioneerspark, Private Bag-13301. Windhoek, Namibia

**Received:** 28 February 2018

**Accepted:** 25 October 2018

**Published:** 26 October 2018

**\*Corresponding author:** Ateeq Rahman, Faculty of Science, Department of Chemistry and Biochemistry, University of Namibia, Pioneerspark, Private Bag-13301. Windhoek, Namibia, Email: [ateeqr786@yahoo.com](mailto:ateeqr786@yahoo.com)

**Keywords:** Liposome; Drug delivery; Methods; Cell; Carriers

<https://www.peertechz.com>



## Review Article

# Mini review on emerging methods of preparation of liposome and its application as Liposome drug delivery systems

## Abstract

Liposomes, sphere-shaped vesicles consisting of one or more phospholipid bilayers, were first described in the mid-60s. Nowadays, they are a very useful reproduction, reagent, and device in various scientific disciplines, including medicine, chemistry, biochemistry, colloid science, biology, physics, biophysics, mathematics and theoretical. After the initial discoveries liposomes have made their way to the market. Among numerous brilliant new drug delivery systems developed, liposomes characterize an advanced technology to deliver active molecules to the site of action, and at present, several formulations are in clinical use. Research on liposome technology has progressed from conventional vesicles to 'second-generation liposomes', in which long-circulating liposomes are obtained by modulating the lipid composition, size, and charge of the vesicle. Liposomes with modified surfaces have also been developed using several molecules, such as glycolipids or sialic acid. This paper mini review summarizes exclusively Nano-lipids, its applications in medicine scalable techniques in treating dreadful diseases cancer, AIDS, paralysis etc and focuses on strengths, respectively, limitations in respect to industrial applicability and regulatory requirements concerning liposomal drug formulations based on FDA and EMEA documents.

## Introduction

Drug delivery systems have been envisaged and developed to deliver the drug to the site of action (targeted delivery) to avoid fluctuations in plasma drug levels (controlled release), slow release in recent years to overcome cellular barriers and enzymatic degradation, which impede absorption [1]. Development of new drug molecules is expensive and time-consuming. Improvement of the safety efficacy ratio of "old" drugs is achieved by individualizing drug therapy, dose titration and therapeutic drug monitoring. Developing drug delivery systems is another attractive option and is being pursued very briskly to improve the safety, reliability [2]. Liposome, microscopic, fluid-filled pouch whose walls are made of layers of phospholipids identical to the phospholipids that make up cell membranes. Liposomes are used to deliver certain vaccines, enzymes, or drugs (e.g., insulin and some cancer 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., the kidneys, and liver), lessening or eliminating the common side effects of nausea, fatigue, and hair loss [3]. Liposomes are especially effective in treating diseases that

affect the phagocytes of the immune system because they tend to accumulate in the phagocytes, which recognize them as foreign invaders. They have also been used experimentally to carry normal genes into a cell in order to replace defective, disease-causing genes [3-5]. Liposomes are occasionally used in cosmetics because of their moisturizing qualities. In some cases liposomes attach to cellular membranes and appear to fuse with them, releasing their contents into the cell

The artificial lipid vesicles (also called liposomes) have been acknowledged and comprehensively used as delivery vehicles for pharmaceuticals [6], as chemical microreactors [7-10], and as model biomembrane systems [10]. The phospholipid bilayer envelope is a cell-like boundary applicable for cellular investigations and affords liposomes a functional scaffold suitable for fundamental cellular functions such as motility and shape change [11], not to mention the aptitude to mimic the biophysical properties of living cells [12]. These "dynamic" behaviors refer to functions such as membrane deformation and actin polymerization which impart cell-like kinetic behavior to liposomes [8]. Innovative methodologies to construct improved liposomes for therapeutic delivery have addressed, on one end, biophysical parameters (one common

example is charge [9]. Which can be manipulated by altering the constituent bilayer phospholipids to better tailor the liposome to the required application. Additional parameters that can and have been manipulated include lamellarity [7], bilayer curvature bilayer fluidity [10–11], as well as surface modification for active or passive targeting approaches [12]. Assembly methods play a key role in defining final liposome characteristics, including encapsulation efficiency and drug release profiles. Hence, recently, the use of synthetic inorganic or polymer nanoparticles to stabilize or support liposomes has emerged as a promising strategy to create hybrid liposomal carriers [13–16]. To date, hundreds of imaging agents and drugs, including fluorophores, nanoparticles, chelating agents, peptides, and proteins, as well as anticancer and antimicrobial agents, vaccines and oligonucleotides, have been integrated into liposomes for a broad range of theranostic applications [17].

From the first liposomal pharmaceutical product–Doxil permitted in 1995 to the latest Marqibo in 2012, there are a few successful liposomal formulations (Table 1). Most of them have to be administered intravenously due to the degradation

of lipids in the gastrointestinal tract. However, some recent formulations such as Arikace (Table 2), can be intravenously injected or inhaled as aerosols. Apart from a broadened range of drugs being investigated for liposomal formulations, new strategies such as environmental sensitivity and combination therapy have been applied to the development process to achieve better efficacy. Furthermore, liposomes could be fruitfully applied to areas other than cancer therapy, such as vaccines [18–20]. Development of liposomal drugs: a typical example of doxorubicin. Doxorubicin, a kind of anthracyclines, is a potent and comprehensive spectrum anti-cancer drug and has been applied as a “first line” medicine in cancer therapy [21]. Two main mechanisms of action are involved for the drug: (1) it inhibits DNA and RNA synthesis by implanting in base pairs of DNA strands, thus preventing the replication and transcription in rapidly growing cancer cells; (2) it inhibits the enzyme topoisomerase II, which is an supplementary way for blocking DNA transcription and replication. Nevertheless, the positively charged doxorubicin is also of high affinity to negatively charged cardiolipin, which is abundant in heart tissue [22]. This damage results in the dangerous cumulative dose-dependent cardiotoxicity (i.e. irreversible congestive

**Table 1:** Approved Liposomal Formulations.

Drug	Product name	Type	Lipid Composition	Route of Administration	Approved treatment
Amphotericin B	Ambisome	Liposome	HSPC, DSPG and cholesterol	Intravenous	Sever fungal infections
Doxorubicin	Myocet	liposome	EPC and cholesterol	Intravenous	metastatic breast cancer
	Doxil	pegylated liposome	HSPC, cholesterol and DSPE-PEG2000	Intravenous	kaposi's sarcoma, ovarian and breast cancer
	Lipo-dox	pegylated liposome	DSPC, cholesterol and DSPE-PEG2000	Intravenous	kaposi's sarcoma, ovarian and breast cancer
Daunorubicin	Daunoxome	Liposome	DSPC and cholesterol	Intravenous	Blood cancer
Verteporfin	Visudyne	Liposome	EPG and DMPC	Intravenous	Age-related molecular degeneration
Cytarabine	Depocyt	liposome	DPOC, DPPG, cholesterol and triolein	Spinal	Neoplastic meningitis and lymphomatous meningitis
Morphine sulfate	Depodur	liposome	DPOC, DPPG, cholesterol and triolein	Epidural	Pain
Vincristine sulfate	Marqibo	Liposome	Egg sphingomyelin and cholesterol	Intravenous	Acute lymphoblastic leukemia

**Table 2:** Liposomal formulations in clinical trials.

Drug	Product name	Lipid Composition	Route of administration	Treatment under investigation	Trial phase
Paclitaxel	LEP-ETU	DOPC, cholesterol and cardiolipin	Intravenous	Ovarian, breast and lung cancers	I
	Endo TAG-1	DOTAP and DOPC	Intravenous	Anti-angiogenesis, breast and pancreatic cancers	II
Doxorubicin	ThermoDox	DPPC, MSPC and DSPE-PEG2000	Intravenous	Non-resectable hepatocellular carcinoma	III
Cisplatin and its analog	SPI-077	HSPC, cholesterol and DSPE-mPEG	Intravenous	Lung, head and neck cancers	I/II
	Lipoplatin	SPC, DPPG, cholesterol and DSPE-mPEG	Intravenous	Pancreatic cancer, head and neck cancer, mesothelioma, breast cancer, gastric cancer and non-small-cell lung cancer.	III
	Aroplatin	DMPC and DMPG	Intrapleural/ intravenous	Malignant pleural mesothelioma and advanced colorectal carcinoma	II
Mitoxantrone	LEM-ETU	DPOC, cholesterol and cardiolipin	Intravenous	Leukemia, breast, stomach, liver and ovarian cancers	I
Topotecan	INX-0076	Egg sphingomyelin and cholesterol	Intravenous	Advanced solid tumors	I
Vinorelbine	INX-0125	Egg sphingomyelin and cholesterol	Intravenous	Brest, colon and lung cancers	I
Lurtotecan	OSI-211	HSPC and cholesterol	Intravenous	Ovarian, head and neck cancers	II
Amikacin	Arikace	DPPC and cholesterol	Inhaled as aerosol	Lung infection	III
BLP25 lipopeptide	Stimuvax	Monophosphoryl lipid A, cholesterol, DMPG and DPPC	Subcutaneous	Non-small-cell lung carcinoma	III

failure), which significantly limits the tolerable dose range of doxorubicin. Further side effects of doxorubicin include severe myelosuppression, nausea and vomiting and mucocutaneous toxicities [23]. Consequently, liposomal formulation is proposed to overcome these toxicities. Initially, liposomal doxorubicin was prepared to be negatively charged, medium-size oligolamellar liposomes, in which the drug was passively entrapped by the lipid hydration method [24]. However, this formulation failed in following clinical trials mainly due to the rapid drug release and clearance by reticuloendothelial system *in vivo*. “Remote loading” was then used to improve the drug loading proficiency and formulation stability, bringing about Myocet and Doxil in which doxorubicin was loaded by a pH or ammonium gradient, respectively. The morphology and structure of Doxil is shown in figure 1. A major advancement of Doxil over Myocet is the coating with PEG, which significantly improves its pharmacokinetic profile. So in a pharmacokinetic study of doxorubicin loaded liposomes, free doxorubicin had an elimination half-life of 0.2 h and an area under the plasma concentration time curve (AUC) of 3.81 mg h/ml, compared with 2e3 and 46 mg h/ml for Myocet and with a further increase to 41e70 h and 902 mg h/ml for Doxil [25]. Both Myocet and Doxil considerably reduce the toxic effects of doxorubicin. In a Phase III comparison of free doxorubicin with Myocet, patients. Table 1 represents approved liposomal formulations [26] (Table 2). Liposomal formulations in clinical trials [27].

**Advance of liposomal drugs:** A characteristic example of doxorubicin Doxorubicin, a kind of anthracyclines, is a potent and wide-spectrum anti-cancer drug and has been used as a “firstline” medicine in cancer therapy [21]. Two main mechanisms of action are involved for the drug: (1) it inhibits DNA and RNA synthesis by inserting in base pairs of DNA strands, thus inhibiting the replication and transcription in swiftly growing cancer cells; (2) it inhibits the enzyme topoisomerase II, which is a surplus way for blocking DNA transcription and replication. Conversely, the positively charged doxorubicin is also of high affinity to negatively charged cardiolipin, which is plentiful in heart tissue [22]. This damage results in the dangerous cumulative dose-dependent cardiotoxicity (i.e. irreversible congestive failure), which substantially limits the tolerable dose range of doxorubicin. Other side effects of doxorubicin include severe myelosuppression, nausea and vomiting and mucocutaneous toxicities [23]. Therefore, liposomal formulation is proposed to overcome these toxicities. Originally, liposomal doxorubicin was prepared to be

negatively charged, medium-size oligolamellar liposomes, in which the drug was passively entrapped by the lipid hydration method [24]. Hence, this formulation failed in following clinical trials mainly due to the rapid drug release and clearance by reticuloendothelial system *in vivo*. “Remote loading” was then used to improve the drug loading efficiency and formulation stability, bringing about Myocet and Doxil in which doxorubicin was loaded by a pH or ammonium gradient, respectively. The morphology and structure of Doxil is shown in figure 1. A major advancement of Doxil over Myocet is the coating with PEG, which considerably improves its pharmacokinetic profile. So in a pharmacokinetic study of doxorubicin loaded liposomes, free doxorubicin had an elimination half-life of 0.2 h and an area under the plasma concentration time curve (AUC) of 3.81 mg h/ml, compared with 2e3 and 46 mg h/ml for Myocet and with a further increase to 41e70 h and 902 mg h/ml for Doxil [25]. Both Myocet and Doxil considerably reduce the toxic effects of doxorubicin. In a Phase III comparison of free doxorubicin with Myocet, patients treated with Myocet had low incidence of cardiac events (13% vs. 29%), mucositis/stomatitis (8.6% vs. 11.9%), and nausea/vomiting (12.3% vs. 20.3%) [26]. Analogous results were found in another Phase III trial of Doxil, in which the reduction of cardiotoxicity (3.9% vs. 18.8%), neutropenia (4% vs. 10%), vomiting (19% vs. 31%), and alopecia (20% vs. 66%) were found [27]. Nevertheless, equivalent survival rates between liposomes and free drugs were found in these studies, signifying the advantage of Myocet and Doxil lay only in the reduction of toxicities.

Vincristine, like the other vinca alkaloids, is very active against many of the lymphoid malignancies, including forceful non-Hodgkin’s lymphoma and all [28]. In adults with ALL, vincristine remains an integral constituent of induction chemotherapy regimens [29–32]. Vincristine acts by binding to tubulin during active mitosis, resultant in microtubule depolymerization and metaphase arrest, important to apoptosis [33–34]. Nevertheless, vincristine also binds to neuronal tubulin, disrupts axonal microtubules, and thus causes severe neurotoxicity, which has led to the clinical practice of capping the total dose of vincristine to 2.0 mg regardless of body surface area, potentially reducing clinical effectiveness. The high affinity of vincristine to both mitotic and neuronal microtubules suggests that it may be difficult to prevent neurotoxicity without reducing clinical efficacy [35].

Vincristine is an imperative and active drug in first-line therapy of all as well as in the treatment of relapsed disease [36]. Its administration is generally limited to weekly injections, usually for 2 months or less in most regimens because of severe peripheral neuropathy, including autonomic neuropathy that often leads to intestinal complications. Since of its narrow therapeutic index, the maximal antileukemia activity of free vincristine is not realized [33]. Thus, an approach to growing the dose intensity of vincristine while decreasing its toxicity through the use of liposome encapsulation technology has been considered.

**Liposome-encapsulated vincristine:** Vincristine is a vinca alkaloid with activity against a broad range of cancers, including Hodgkin’s and non-Hodgkin’s lymphoma, chronic

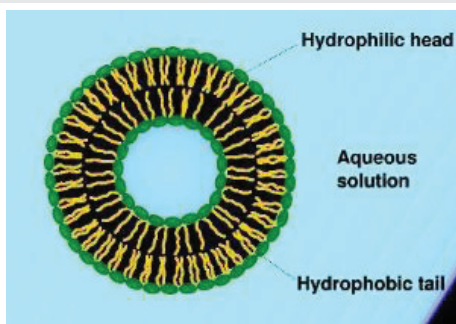


Figure 1: Liposome image [1].

lymphocytic leukemia, all, and a variety of solid tumors. Vincristine exerts its cytotoxic activity by attributing to the increasing end of microtubules and stopping their assembly, arresting cell growth in metaphase. Therefore, as a cell-cycle specific cytotoxic drug, prolonging exposure time to the drug is anticipated to be therapeutically beneficial; perhaps even more so than prolonged exposure to anthracyclines which do not act in a cell cycle-specific manner. In leukemia cell lines, exposure to vincristine from one to 72 hours resulted in a 105-fold reduction in the drug concentration wanted to cause 50% cytotoxicity (IC<sub>50</sub>), associated with only a 40-fold reduction in the IC<sub>50</sub> for doxorubicin following elongated exposure to the same extent [37–38]. Pharmacokinetic data with free vincristine comprehensive rapid tissue binding, low serum concentrations after intravenous administration, and extensive tissue spreading *in vivo* [39–40]. Limiting its potential therapeutic efficacy. Although, continuous intravenous infusion of vincristine has been inspected as a way to increase drug exposure, noteworthy neurotoxicity still occurred [41]. Furthermore, conventional vincristine is limited by significant peripheral and central nervous system neurotoxicity, which occurs at doses higher than 1.4 mg/m<sup>2</sup>. Therefore, it was hypothesized that if liposomal encapsulation and delivery results in higher levels of drug at tumor sites for longer periods of time, greatly developed efficacy may be expected with a cell cycle-specific drug like vincristine [31–38], and retention of drug in the liposome would result in lower drug concentrations in tissues where toxicity occurs, containing the peripheral and central nervous systems [33]. Preclinical development of liposome-encapsulated formulations of vincristine: The initial investigations of liposomal encapsulation of vincristine failed to demonstrate a therapeutic improvement over free vincristine sulfate in murine leukemia models [42]. Indeed, the low solubility of vincristine in aqueous solution at physiologic pH and its relatively high permeability to membranes, resulting in poor retention of drug in the liposome, initially presented significant limitations to improvement of a stable liposomal vincristine formulation. Subsequent work identified that transmembrane pH gradients, with the inside of the vesicle being acidic, can result in considerably improved trapping of drug in the liposome [43–45]. Additionally, maintenance of the pH gradient is affected, at least in part, by the lipid composition of the liposome [46]. Developing such transmembrane pH gradient drug uptake processes, almost 100% trapping of vincristine was achieved inside egg phosphatidylcholine/cholesterol and distearoylphosphatidylcholine (DSPC)/cholesterol liposomes, when the internal pH of the liposome was lowered to 4.0 [47]. However, while both DSPC/cholesterol and egg phosphatidylcholine/cholesterol preparations had similar vincristine trapping efficiency, the pH gradient across the membrane was considerably more sustained in DSPC/cholesterol vesicles with better maintenance of vincristine in the liposome [47], resulting in a longer drug circulation time and enhanced tumor delivery and antitumor activity compared with free vincristine. [47–48]. In murine models of lymphocytic leukemia, the DSPC/cholesterol liposome formulation showed greater antitumor efficacy compared with either free vincristine or the egg phosphatidylcholine/cholesterol formulation [48–49] and the LD<sub>50</sub> (median lethal dose) was significantly higher

with DSPC/cholesterol liposome-encapsulated vincristine formulation (LD<sub>50</sub> 4.8 mg/kg) compared with free vincristine (LD<sub>50</sub> 1.9 mg/kg), indicating that liposomal encapsulation increased drug delivery with less toxicity [46,47].

Preclinical models also established that vincristine encapsulated in sphingomyelin/cholesterol liposomes accumulated preferentially at tumor sites, which associated with antitumor activity [50–52]. Moreover, this was related to liposomal extravasation into the tumor rather than uptake by tumor cells of free vincristine that had been free from liposomes in the circulation [51]. In a murine model with human breast cancer xenografts, sphingomyelin/cholesterol liposome-encapsulated vincristine resulted in targeted delivery of the drug, with a four-fold intensification in concentration of drug in tumor tissue and a three-fold increase in bone marrow, with maintenance of substantial tissue drug concentrations for several days compared with free vincristine, and without increased toxicity [52]. The antitumor efficacy of sphingomyelin/cholesterol liposome-encapsulated vincristine has also been confirmed in several preclinical murine and human tumor xenograft models, signifying several cancer types [50–52,54], including human [33]. The aggregate of the above studies supports the utility of encapsulating vincristine in sphingomyelin/cholesterol liposomes to increase drug delivery while limiting release in the central blood compartment to decrease drug toxicity. In 2012, Owellan et al [34], reported an improved method for active loading of doxorubicin into the HaT formulation based on a copper (II) gradient (HaT-II) [53]. HaT-II showed improved *in vitro* stability at 37°C, together with a faster drug release rate at 41°C in the presence of serum when associated with LTSL [53]. In comparison with LTSL, HaT-II showed a 2.5-fold longer blood circulation time in mice and a 2.0-fold increase in drug delivery to the heated tumor [76]. This resulted in improved antitumor efficacy [54–55]. In 2013, Park et al reported another alleviated formulation composed of DPPC, DSPE-PEG2000, cholesterol, and fatty acid-conjugated elastin-like polypeptide 55:2:15:0.4125 (mol/mol) (STL) with encapsulated doxorubicin (Table 1) [55]. Pharmacokinetic studies in mice showed plasma half-lives of 2.03 hours and 0.92 hours for doxorubicin encapsulated in STL and LTSL [55], respectively. In combination with high intensity focused ultrasound, STL achieved significantly better tumor growth delay 7 days after injection when compared with LTSL [55]. Doxorubicin and manganese (II) form a stable complex [56], with the paramagnetic manganese (II) serving as an MRI contrast agent. Thus the release kinetics are the same for the contrast agent and the drug, agreeing for correlation between change in T<sub>1</sub> relaxation time (determined by MRI) and amount of doxorubicin (determined by high-performance liquid chromatography) [57]. Using this strategy, it was possible to show that release of doxorubicin was heterogeneously scattered in the tumor model, and that LTSL administered during hyperthermia had the greatest antitumor effect when compared with other administration strategies. Docetaxel is a semi-synthetic microtubule disrupting anti-cancer drug indicated for the treatment of breast, prostate, non-small-cell lung, head and neck, and gastric cancers [58–64]. Standard every 3-week dosages range from 60 to 100 mg/m<sup>2</sup>, with 75 mg/m<sup>2</sup> being a frequently used dose in these

diseases. Dose-limiting toxicities (DLTs) are myelosuppression and neuropathy, among others. For example, at a dose of 100 mg/m<sup>2</sup>, docetaxel causes grade 4 neutropenia in 75–86 % of patients [65]. Liposomal doxorubicin has been explored, both as monotherapy and in combination with other agents, for the treatment of several malignancies [66], including breast cancer [67–68], and platinum-refractory ovarian cancer [69]. Liposomal doxorubicin (Doxil R) is currently used in the treatment for refractory ovarian cancer and AIDS-related Kaposi's sarcoma [70–71]. Decreased incidence or severity of toxicities compared with free doxorubicin, including relative cardiotoxicity, has been reported in some studies [67–68], supporting the hypothesis of improved safety provided by liposome encapsulation of the active agent. Another approved liposomal anticancer agent, DaunoXomeR (liposomal daunorubicin), is used in the treatment for AIDS-related Kaposi's sarcoma [69].

### Methods for preparation of liposomes

An important parameter to consider when addressing the formation process of liposomes is the rigidity of the bilayer. Hydrated-single component phospholipid bilayers can be in a liquid-crystalline ('fluid') state or in a gel state. By increasing the temperature, the gel state bilayer melts and is converted into the liquid state [70]. This occurs at a temperature known as the transition temperature (T<sub>c</sub>). The T<sub>c</sub> of a bilayer depends on:

1. Acyl chain length.
2. Degree of saturation.
3. Polar head group.

The T<sub>c</sub> can vary between 15°C for egg yolk phosphatidylcholine (high degree of unsaturation) to over 50°C for fully saturated distearoyl phosphatidylcholine (DSPC) [71–72]. The raw material for liposome formation depends on the intended use of the liposome. Numerous companies supply reasonable grade and priced lipids which usually contain at least 98% phospholipid and less than 1% lysophospholipid, low endotoxin and microbial load and trace metals. It is up to the individual investigator to purify the lipid to acceptable standards [73].

There are five main groups of phospholipids that are available which is used for liposome preparation [73].

1. Phospholipid from natural sources.
2. Phospholipid modified from natural sources.
3. Semi-synthetic phospholipid.
4. Fully-synthetic phospholipid,
5. Phospholipid with non-natural head groups.

Phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylserine (PS) are commonly used phospholipids for liposome preparation. Cholesterol can be added to the bilayer

mixture to reduce the permeability of fluid crystalline state bilayers.

There are many different strategies for the preparation of liposomes, which can be classified into 3 main groups.

### Mechanical methods

**A. Film method:** The original method of Bangham et al [74], is still the simplest procedure for the liposome formation but is limited because of its low encapsulation efficiency. This technique produces liposomes by hydrating thin lipid films deposited from an organic solution on a glass wall by shaking at temperatures above the T<sub>c</sub>. The solvent is removed at reduced pressure in a rotary evaporator. The dry film of lipids which has been deposited onto the wall of a round-bottom flask is hydrated by adding a buffer with a water soluble marker. As the lipid becomes hydrated and starts to form into closed vesicles only a small amount of the solute becomes entrapped. This method yields a heterogeneous sized population of MLVs over 1µm in diameter. Further procedures must be employed to achieve a homogeneous population, which will be discussed later [75].

**B. Ultrasonication method:** Ultrasonication of an aqueous dispersion of phospholipids with a strong bath sonicator or a probe sonicator will usually yield SUVs with diameters down to 15–25nm.

### Methods based on replacement of organic solvent

**A. Reverse-phase evaporation:** In this method, several phospholipids (pure/mixed with cholesterol) can be used. The lipid mixture is added to a round bottom flask and the solvent is removed under reduced pressure by a rotary evaporator [75]. The system is purged with nitrogen and the lipids are re-dissolved in the organic phase. This is the phase that the reverse phase vesicles will form. Diethyl ether and isopropyl ether are the usual solvents of choice [71].

After the lipids are re-dissolved in this phase the aqueous phase (contains compound to be encapsulated) is added. The system is kept under continuous nitrogen and the two-phase system is sonicated until the mixture becomes a clear one-phase dispersion. The mixture is then placed on the rotary evaporator and the organic solvent removed until a gel is formed. Non-encapsulated material is removed. The resulting liposomes are called Reverse-phase evaporation vesicles (REV). The large unilamellar and oligolamellar vesicles formed have the ability to encapsulate large macromolecular vesicles with high proficiency [71].

**B. Ether vaporisation method:** In ether vaporisation method a mixture of lipids in an organic solvent (diethyl ether, ethanol, etc). Which is slowly injected into a warm aqueous solution. This results in osmotically active, unilamellar vesicles with a well-defined size distribution and high volume trapping efficiency (about ten times that of sonicated and hand shaken preparations [72].

## Methods based on size transformation or fusion of preformed vesicles

**A. Freeze-thaw extrusion method:** Liposomes formed by the film method are vortexed with the solute to be entrapped until the entire film is suspended and the resulting MLVs are frozen in a dry ice/acetone bath, thawed in lukewarm water and vortexed again [73]. After two additional cycles of freeze-thaw and vortexing the sample is extruded three times. This is followed by six freeze-thaw cycles and an additional eight extrusions. The resulting liposomes are called large unilamellar vesicles by extrusion technique (LUVET) and they typically contain internal solute concentrations which are much higher than the external solute concentration's which have entrapment ratios greater than one [6]. Proteins can be effectively encapsulated using this technique [74].

**B. The dehydration-rehydration method:** This method begins with empty buffer containing SUVs (handshaken MLVs can be also be used but are usually not preferred). These are mixed with the component to be entrapped, after which they are dried. Freeze-drying is often the method of choice but other methods such as by vacuum or under a stream of nitrogen can be used. The vesicles are then rehydrated [7-74]. A mechanism has been proposed whereby as the vesicles become more concentrated during dehydration, they flatten and fuse forming multilamellar planes where the solute is sandwiched. Therefore on hydration, larger vesicles are formed. This technique is mild and simple, the main limitation being the heterogeneity of the size of the size of the liposomes [75].

**Sizing of the liposomes:** Size characteristics of liposomes have a major effect on their fate. Therefore, liposome production procedures must generate predictable and reproducible particle size distributions within a certain size range. Sizing of liposomes (if the population is heterogeneous) is usually performed by sequential extrusion at relatively low pressures through polycarbonate membranes [75]. It's easily, reproducible, no detectable degradation of the phospholipids takes place and it can double the encapsulation efficiency of the liposome preparation. Membranes of pore size  $\sim 0.2\mu\text{m}$  will yield liposomes of  $\sim 0.27\mu\text{m}$  [76]. Gel chromatography can also be used to size liposomes but more typically used to remove un-encapsulated components by separation [2]. Sonication is another process that is widely applied when sizing liposomes. Probe sonication is used rather than bath sonication and it produces small unilamellar vesicles of 20nm.

**There are many disadvantages associated with this technique:**

1. Exclusion of oxygen is difficult which results in peroxidation reactions.
2. Titanium probes shed metal particles resulting in contamination.
3. They can generate aerosol's which excludes them with from use with certain agents.

These problems can be avoided with the use of bath

sonicators but reproducible results are difficult because of the number of varying parameters associated with such baths (level and temperature of water, position of liposome in the bath, etc.) [75-76].

**Liposomes in immune assays:** The liposome immune assay system is usually based on membrane immune chemistry and the release of a detectable marker. In an enzyme linked immune sorbent assay (ELISA) the enzyme label generates a measurable amount of product which is proportional to the unknown concentration of an antigen. Liposomes are artificially prepared vesicles made of lipid bilayer. Liposomes can be filled with drugs, composite materials and used to deliver drugs for cancer and other diseases [77]. Liposomes can be composed of naturally-derived phospholipids with mixed lipid chains (like egg phosphatidylethanolamine) or other surfactants [78].

Subsequently the research conducted by new et. al. [79], much interest have been centered on the use of liposomes as a drug carrier for Amp B in the treatment of several systemic fungal and parasitic infections. It was shown that L-Amp B was as effective as free Amp B in experimental histoplasmosis [80], and cryptococcosis [81], but much less toxic [82]. Lopez-Beresteinet al. [82], carried out extensive studies on the use of L-Amp B in systemic candidiasis and paved the way for its clinical use. They used multilamellar liposomes prepared from dimyristoyl phosphatidylcholine (DMPC) and dimyristoyl phosphatidylglycerol in a 7:3 molar ratio. Hopferet.al. [83], observed that the lipid composition of the liposomes played a major role in L-Amp B activity. The presence of a sterol component (like ergosterol and cholesterol) in liposomes decreased the antifungal activity by almost 50-fold. However, Tremblay Gomdal et al and Szokaet. Al. [84-87], have found that the incorporation of cholesterol in liposomes did not result in any loss of activity. It was postulated that in multilamellar vesicles (MLV), only about 10% of the lipid is on the external monolayer and the transfer of Amp B from the internal lamellae to the fungal cell cannot take place readily. While small unilamellar vesicles (SUV) containing cholesterol have about 50-60% of the lipids on the monolayer accounting for better transfer of Amp B. With this concept, two SUV formulations were developed. Negatively charged small unilamellar vesicles made from hydrogenated soya phosphatidylcholine (SPC), cholesterol and distearoyl phosphatidyl glycerol (DSPG) in 2:1:0.8 molar ratios were tested in murine candidiasis and cryptococcosis [85-87]. The efficacy was found to be comparable with conventional Amp B on an equal dose basis. The other formulation is positively charged, prepared from SPC, cholesterol and stearyl amine in 4:3:1 molar ratio [88]. Liposomes can be prepared by disrupting biological membranes, for example by sonication. Liposomes can be composed of naturally-derived phospholipids with mixed lipid chains (like egg phosphatidylethanolamine) or other surfactants. Liposomes should not be confused with micelles and reverse micelles composed of monolayer [89].

## Liposomes act as carrier radioactive compounds as radio tracers

In addition, liposomes can be used to carry radioactive compounds as radiotracers can be linked to multiple locations

in liposomes [90]. One option is the hydrated compartment inside the liposome, another the lipid core into which especially hydrophobic conjugates can be attached, and the third option is the outer lipid leaflet where molecules can be bound by covalent linkage. Delivery of agents to the reticuloendothelial system (RES) is easily achieved, since most conventional liposomes are trapped by the RES. For the purpose of delivery of agents to target organs other than RES, long-circulating liposomes have been developed by modifying the liposomal surface [91]. Understanding of the *in vivo* dynamics of liposome-carried agents is required for the evaluation of the bio-availability of drugs encapsulated in liposomes. Amphotericin B (Amp B) remains the drug of choice in most systemic mycoses and also as a second line treatment for Kalaazar [91]. However, its toxic effects often limit its use. Although the liposome delivery system has been tried for several drugs, only a few have been used in patients due to the slow development of necessary large-scale pharmaceutical procedures. As compared to conventional Amp B, Fungisome are infused over a much shorter period requiring a smaller volume and no pre-medication. It was found to be safe in patients who had developed serious unacceptable toxicity with conventional Amp B. In renal transplant patients, fungisome did not produce any nephrotoxicity. Fungi some are effective in fungal infections resistant to fluconazole, conventional Amp B and in virgin and resistant cases of visceral leishmaniasis. Amphotericin B (Amp B) is a polyene macrolide antibiotic that is widely used for the treatment of systemic fungal infections. Disseminated fungal infections are a major cause of morbidity and mortality in patients with leukaemia receiving chemotherapy and in a variety of immuno-deficiency diseases [89]. The majority of these infections are caused by the species of *Candida* and *Aspergillus*. Despite the development of new classes of antifungal agents, Amp B remains the drug of choice. Its antimicrobial activity results from its ability to bind to the sterol component of the cell membrane, leading to the formation of transmembrane pores that allow the leakage of vital cellular constituents. Amp B binds preferentially to ergosterol, a major component of the fungal cell wall. Unfortunately, the drug also interacts with cholesterol in mammalian membrane, which probably is the basis for its profound acute and chronic toxicity. Approximately 20–50% patients treated with Amp B develop acute infusion-related reactions such as fever, chills, nausea and vomiting [90]. This is in spite of the liberal use of premedication's intended to prevent such side effects. Clements and Peaco [91], observed that nephrotoxicity is one of the most central chronic toxicities associated with Amp B usage because of its potential limiting effect on the total course of therapy. Nephrotoxicity is present in about 60–83% of patients. Additional significant encountered chronic toxicity is electrolyte disturbance secondary to renal wasting of potassium and magnesium. Ninety percent of patients on Amp B treatment require potassium supplementation [92].

**Preparation of Multi-lamellar Liposomes:** The methodology for preparation of MLVs is to use well characterized lipids in order to produce well defined liposomes [93–96]. Equally significant is the selection of bilayer components for toxicity and for shelf life optimization. The lipids normally used are the unsaturated egg phosphatidylcholine (PC),

phosphatidic acid (PA), phosphatidylglycerol (PG), and the saturated lipids DMPC, dipalmitoylphosphatidylcholine (DPPC), dipalmitoylphosphatidic acid (DPPA), and dipalmitoylphosphatidylglycerol (DMPG). Stearylamine is used when cationic liposomes are favored; and natural acidic lipids, such as phosphatidylserine (PS), PG, phosphatidylinositol (PI), PA, and cardiolipin (CL) are added when anionic liposomes are desired, while cholesterol is often involved to stabilize the bilayer. Small amounts of antioxidants such as  $\alpha$ -tocopherol or  $\beta$ -hydroxytoluidine (BHT) are included when polyunsaturated neutral lipids are used [96].

**Preparation of Lipid for hydration:** Lasic et al [97] while preparing liposomes with mixed lipid composition, the lipids must first get dissolved and mixed in an organic solvent to confirm a homogeneous mixture of lipids. In general this process is carried out using chloroform or chloroform:methanol mixtures. The intent is to obtain a clear lipid solution for complete mixing of lipids. Normally lipid solutions are prepared at 10–20mg lipid/ml organic solvent, although greater concentrations may be used if the lipid solubility and mixing are standard. When the lipids are thoroughly mixed in the organic solvent, the solvent is removed to yield a lipid film. For small volumes of organic solvent (<1mL), the solvent may be evaporated using a dry nitrogen or argon stream in a fume hood. For larger volumes, the organic solvent should be removed by rotary evaporation yielding a thin lipid film on the sides of a round bottom flask [97]. The lipid film is comprehensively dried to remove residual organic solvent by placing the vial or flask on a vacuum pump overnight. If the use of chloroform is objectionable, an alternative is to dissolve the lipids in t-butanol or cyclohexane. The lipid solution is transferred to containers and frozen by placing the containers on a block of dry ice or swirling the container in a dry ice-acetone or alcohol (ethanol or methanol) bath. Care must be taken when using the bath procedure that the container can withstand sudden temperature changes without cracking. After freezing completely, the frozen lipid cake is placed on a vacuum pump and lyophilized until dry (1–3 days depending on volume). The thickness of the lipid cake should be no more than the diameter of the container being used for lyophilization. Dry lipid films or cakes can be removed from the vacuum pump, the container close tightly and taped, and stored frozen until ready to hydrate. Basu.S et.,al., [98] proposed general methods to prepare MLV (Figure 2).

#### A general protocol to prepare MLV is as follows

1. Laughrey et al [99]. Discussed the preparation of lipid component. A suitable solution of the lipid component in a pear-shaped flask (lipid concentrations between 5 and 50 mMin either chloroform or in chloroform–methanol (3\_1, v/v), and filter the mixture to remove minor insoluble components or ultra-filter to reduce or eliminate pyrogens.

2. Employing a rotary evaporator, remove the solvent, while maintaining a temperature of  $\sim 40^{\circ}\text{C}$  in a water bath under negative pressure. Other methods of drying include spray drying and lyophilization [99]. Traces of organic solvents are removed employing a vacuum pump, normally overnight at

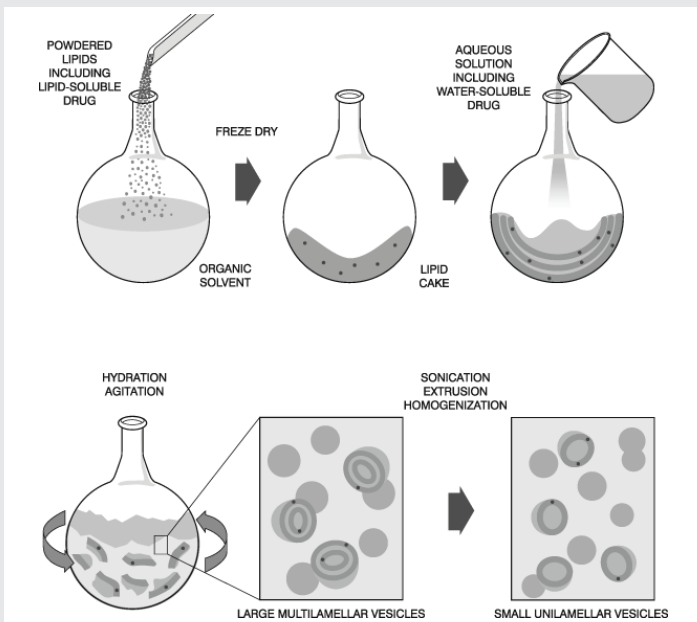


Figure 2: Preparation of lipid [118].

pressures below milliTorr ( $\sim 0.1$  Pa). Alternatively, the sample may be dried under a very low vacuum ( $< 50$   $\mu\text{mol/mg}$ ) for 1–2 h in a desiccator with drierite™ (Fisher Scientific, Malvern, PA) [98].

3. Subsequent to drying, 100  $\mu\text{L}$  of 0.5 mm glass beads are added to the 10-mL flask containing the dried lipid mixture, and hydration fluid (0.308 M glucose), which is equal to the final volume of the liposome suspension, is added. Typically, the volume of hydration fluid used is determined by the amount of liposomal phospholipid and is usually in millimolars with respect to the hydration fluid [98].

4. Vortex mixing the flask for 1–2 min causes all of the dried lipid from the flask to be dispensed into the hydration fluid. Alternative hydration mediums are distilled water, buffer solution, saline, or non-electrolytes such as a sugar solution. For an *in vivo* preparation, physiological osmolality (290 mosmol/kg) is recommended and can be achieved using 0.6% saline, 5% dextrose, or 10% sucrose solution. MLVs of tens of micrometers to several tenths of a micrometer are spontaneously formed when an excess volume of aqueous buffer is added to the dry lipid and the flask is agitated.

5. The “dry” lipid mixture is then hydrated in an aqueous medium containing buffers, salts, chelating agents, and the drug to be entrapped [98].

**Preparation of small unilamellar liposomes:** High-energy sonic fragmentation processes were introduced in the early 1960s [100]. Modifications of these procedures using a high-pressure homogenization device followed [101]. SUVs are prepared by the following methodology to dissolve phospholipids in water to form optically clear suspensions.

### Sonication

Methods for the preparation of sonicated SUVs have been reviewed in detail by Bangham et al [74]. Classically the MLV

dispersion is placed in test tubes and sonicated either in a bath sonicator or by tip sonication. Normally a 5–10-min sonication procedure (above  $T_c$ ) is sufficient to prepare SUVs with radii  $< 50$  nm. With some lipids, radii  $< 20$  nm are also possible while some diacyl cationic lipids (including 1-[2-(oleoyloxy)-ethyl]-2-oleoyl-3-(2-hydroxyethyl) imidazolium chloride (DOIC) and dioctadecylamidoglycylspermine (DOGS) can even form micelles. Dioctadecyldiammonium bromide (DOBAD) neutral lipid liposomes cannot be sized  $< 130$  nm [101–102].

### Extrusion

Pre-filtering the LMV solution through a filter with pores  $\sim 1$   $\mu\text{m}$  is followed by pre-filtering the solution five times through 0.4- and 0.2- $\mu\text{m}$  pores. This is followed by 5–10 extrusions through a filter with a pore size of 100 nm. Allowing the formation of LUVs with diameters slightly above pre-sizes ( $\sim 110$ – $120$  nm) [98,102]. If smaller vesicles are desired, continued filtering through 80- and 50-nm pores is needed. Extrusion through smaller pores (30nm) or in the case of some more rigid bilayers, 50 nm, does not reduce the size further but rather increases it owing to the imposition of too high a curvature to vesicles. The extrusion method yields the best vesicles with respect to the homogeneity of size distribution and to control the size distribution of vesicles, especially for larger (100–500 nm) diameters [102].

**Preparation of Large Uni-lamellar Liposomes:** Large unilamellar liposomes denote to vesicles  $> 100$  nm in diameter restricted to a single bilayer membrane. LUVs provide a number of benefits compared to MLVs, including high encapsulation of water-soluble drugs, economy of lipid, and reproducible drug release rates [101]. These liposomes are the most difficult type of liposomes to produce; however, a number of techniques for producing LUVs such as freeze-thaw cycling, slow swelling in non-electrolytes, dehydration followed by rehydration, and the dilution or dialysis of lipids have been reported. The two primary methods used are one involving detergent dialysis, while the other uses the formation of a water-in-oil emulsion [101]. Detergents commonly used for this purpose exhibit a relatively high critical micelle concentration (CMC) such as bile salts and octylglucoside. During dialysis, when the detergent is removed, the micelles become progressively richer in phospholipid levels and finally coalesce to form closed, single-bilayer vesicles [101–102]. Liposomes (100 nm in diameter) are formed within a few hours. Uniform single-layered phospholipid vesicles of 100 nm are formed when sonicated, small phospholipid vesicles or dry phospholipid films are mixed with deoxycholate at a molar ratio of 1:2. Subsequently, the detergent is removed by passing over a Sephadex G-25 column [103]. This procedure separates 100-nm vesicles from small sonicated vesicles [103]. The phospholipid solution is layered onto a sucrose gradient and subjected to high-speed centrifugation. The SUVs form as a sediment, leaving behind detergent in the supernatant layer. This procedure involves the removal of a nonionic detergent, Triton X-100, from detergent/phospholipid micellar suspensions. Bio-Beads SM-2 have the ability to absorb Triton X-100 rapidly and selectively. Following absorption of the detergent, the beads are removed by filtration. The final liposome size depends



on the conditions used including lipid composition, buffer composition, temperature, and, most importantly, the amount and the efficacy of the detergent-binding capacity of the beads. Additional procedure to prepare LUVs employs water-in-oil emulsions of phospholipids and buffer in excess. This method is principally useful to encapsulate a large amount of a water-soluble drug [103]. Two phases are usually emulsified by sonication. Removal of the organic solvent under the vacuum causes the phospholipid-coated droplets to coalesce and ultimately form a viscous gel. The removal of the final traces of solvent under a high vacuum or mechanical disruption results in the collapse of the gel into a smooth suspension of LUVs. To prepare reverse phase evaporation vesicle (REV)-type liposomes, the phospholipids are first dissolved in either diethyl ether isopropyl ether or mixtures of two solvents such as isopropyl ether and chloroform. Emulsification is most easily accomplished if the density of the organic phase is  $\sim 1$ . The aqueous phase containing the material to be entrapped is added directly to the phospholipid-solvent mixture, forming a two-phase system. The ratio of aqueous phase to organic phase is maintained as 1:3 for ether and 1:6 for isopropyl ether-chloroform mixtures. The two phases are sonicated for a few minutes, forming a water-in-oil emulsion, and the organic phase is carefully removed on a rotary evaporator at 20–30°C. The removal of the last traces of solvent transforms the gel into large unilamellar liposomes [103–104] (Table 3).

### Preparation of ferrous sulfate liposomes

**Preparation of empty liposomes:** Preparation of empty PC-cholesterol liposomes: The empty PC-cholesterol liposomes were prepared by reverse-phase evaporation method (REV) [104]. The lipid mixture, containing egg PC (20mmol L<sub>-1</sub>) and various quantities of cholesterol (from 0 to 50 mol%), was dissolved in 30 mL diethyl ether. One-third volume (10 mL) of buffer solution composed of 10 mmol citric acid and 10 mmol Na<sub>2</sub>HPO<sub>4</sub> (pH being adjusted to 6.8) was added to the organic phase [104]. Ultra-sonication with a probe sonicator (VCX400, Sonics & Material Vibra Cell, 400 W, 20 kHz) in an ice bath for 5 min resulted in a homogeneous w/o emulsion. After the removal of the organic solvent at controlled reduced pressure with a rotary evaporator, a gel was formed. Upon continued rotary evaporation the gel was broken, and then the remaining aqueous phase (20 mL) was added with gentle vortexing. The remaining ether was evacuated at 0.01 MPa. The liposomes were stored at 4°C in a refrigerator [104].

**Preparation of empty Tween-grafted liposomes:** The empty Tween-grafted liposomes were also prepared by REV method. The lipid mixture contained egg PC (20mmol L<sub>-1</sub>) and cholesterol (2 mmol L<sub>-1</sub>). When the gel was broken, the remaining aqueous phase containing various quantities of Tween 80 surfactants (molar ratio from 0 to 1.0) was added. The other procedures were the same as the above [104–105].

**Preparation of ferrous sulfate liposomes:** Based on the study of empty liposomes, the basic composition of the membrane fraction of the liposomes was fixed at 80% (mol/mol) egg lecithin, 10% (mol/mol) cholesterol and 10% (mol/mol) Tween 80. Ascorbic acid was used as an anti-oxidant to protect the ferrous ion against oxidation. Four kinds of methods were used to prepare ferrous sulfate liposomes in order to compare their encapsulation efficiency (EE) [104–105]. The most suitable method was selected according to the EE. Then parameters such as weight ratio of iron to lipids, hydrating media and sonication strength were optimized. The effect of weight ratio of iron to lipids (0.04, 0.06 and 0.1) on the EE was investigated. The effect of hydrating media on the EE was studied by encapsulating the ferrous sulfate solution (weight ratio of iron to lipids was 0.04) with different hydrating media (deionized water, 0.01 mol L<sub>-1</sub> citric acid–Na<sub>2</sub>HPO<sub>4</sub> buffer solution and 0.01 mol L<sub>-1</sub> citric acid–sodium citrate buffer solution). In addition, the effect of sonication strength (60%, 70%, 80% and 90%) on the EE was studied [105].

**Reverse-phase evaporation (REV):** Ferrous sulfate liposome's were prepared by Fukuhi H et al [104] slightly differently from empty liposomes. In this system, one-third volume of aqueous solution containing ferrous sulfate and ascorbic acid (15:1 weight ratio of ferrous sulfate to ascorbic acid) was added to the organic phase. Final lipid concentration was about 30 mg mL<sub>-1</sub>. Thin-film hydration (TF) [104]: Lipids, at the above-indicated ratios, were dissolved in diethyl ether, in the adequate proportions, to obtain a lipid solution. Soon, the solvent was evaporated from the lipid solution in a rotary evaporator at controlled reduced pressure until a fine film was formed on the walls of the receptacle. It was compulsory to establish that no residue of the organic solvent remained, for that reason the lipid samples were placed under a high vacuum for at least 4 h to remove any residual solvent. Ferrous sulfate solution, containing Tween 80, was introduced into the aforementioned receptacle and stirred up vigorously at ambient temperature. The agitation was continuous until there was no lecithin deposited on the wall, to give a final lipid concentration of approximately 30 mg mL<sub>-1</sub>. Thin-film and sonication (TFS) [106]. After the thin-film hydration, the liposomes suspension was submitted to a probing sonication (Sonics & Material Vibra Cell, 400 W, 20 kHz) process at 40% (sonication strength) in an ice bath for 5 min with a sequence of 1s of sonication and 1s rest. The liposomes were stored at 4°C. Freeze-thawing (FT) [105]. When the thin-film sonication liposome suspension had been obtained, it was rapidly frozen in a freezer at 180 oC and left overnight. The next day, the suspension was spontaneously thawed at ambient temperature. The liposome suspension was subjected to three freeze-and-thaw cycles. The final liposomes were stored at 4°C.

**Table 3:** The main types are listed and their characteristics are outlined [1-2].

Vesicle Types	Abbrev	Diameter Size	Number of lipid bilayers
Small unilamellar vesicles	SUV	Diameter of 20-100nm.	One lipid bilayer
Large unilamellar vesicles	LUV	Diameter of >100nm.	One lipid bilayer
Multilamellar vesicles	MLV	Diameter of >0.5µm.	Five to twenty lipid bilayers
Oligolamellar vesicles	OLV	Diameter of 0.1-1µm.	Approximately five lipid bilayers
Multivesicular vesicles	MMV	Diameter of >1µm.	Multicompartmental structure [2]

**Preparation of biotin-peliposomes:** Large unilamellar liposomes (LUVs; egg phosphatidylcholine [EPC]; Avanti Polar Lipids, Alabama) containing biotin-PE (0.1 mol%; Molecular Probes, Oregon, or Avanti Polar Lipids, Alabama), are prepared by extrusion techniques as described by Hope et al [106]. Concisely, appropriate amounts of lipid mixtures dissolved in chloroform are deposited in a tube and dried to a lipid film under a stream of nitrogen followed by high vacuum for 2 h. Lipid samples are routinely hydrated in 150 mM NaCl, 25 mM N-(2-hydroxyethyl) piperazine-N-3-propanesulfonic acid (EPPS), pH 8. Fortargeting experiments, a fluorescent marker such as carboxyfluorescein is included in the hydration buffer (15 mM). The resulting multi-lamellar vesicles are frozen and thawed 5 times and extruded 10 times through 2 stacked 100-nm filters [107].

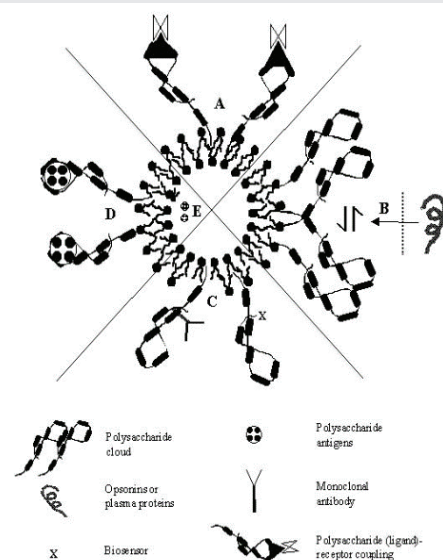
**Binding of streptavidin to biotin-Pe-Liposomes:** Liposomes (99.9 mol% EPC, 0.1 mol% biotin-PE; 1 pmol total lipid) are normally incubated with streptavidin (1.1 KiWmg, 4 mg/ml in 20 mM EPPS, 150 mM NaCl pH 8.0; Sigma Chemical Co.) at room temperature for 30 min. Samples are then chromatographed on Sepharose CL-4B (Pharmacia, Uppsala, Sweden) which is equilibrated with EPPS, pH 8.0 to separate liposomal bound streptavidin from free. The ratio of streptavidin bound, lipid is determined by counting  $^{125}I$  for streptavidin and a standard phosphate assay for phospholipid. Under the above conditions, a maximum of 5.8 kg of streptavidin binds per pmole of lipid [108]. The amount of streptavidin associated with vesicles is increased further by increasing the mol% of biotin PE in the vesicles (up to 0.3 mol% of the total lipid) or by the inclusion of cholesterol (Chol) in the vesicle preparation (50 mol%). To maximize the coupling efficiency, a constant ratio of streptavidin to total lipid is maintained. Streptavidin-liposomes with up to 30  $\mu$ g of protein bound per mol of lipid can readily be achieved in this manner. Further increases in the levels of biotin-PE in liposomes lead to a significant loss of lipid due to aggregation and precipitation of vesicles. Under the optimal incubation conditions outlined above. Protein to lipid coupling is rather inefficient. Only 3% of the initial protein becomes lipid associated. For this reason the rapid interaction of streptavidin with biotinylated components may be more efficiently exploited by indirect targeting procedures. Whereby cells are sequentially labeled with a biotinylated ligand, streptavidin and finally, biotin-PE liposomes [106-108].

**Liposomal delivery of other drugs:** The most active drugs against breast cancer are currently the anthracyclines and taxanes (paclitaxel and docetaxel). Schemes for the delivery of taxanes are under active research to increase tumor exposure and/or to reduce adverse effects such as neurotoxicity, edema, asthenia, and alopecia. In addition, special issues with the taxanes provide further rationale for the application of delivery systems [109]. Both paclitaxel and docetaxel are poorly soluble in aqueous solutions, and have consequently been formulated with vehicles Cremaphor EL and polysorbate 80 (TWEEN), respectively. These formulations are highly allergenic, require extensive premedication, and are responsible for most of the acute toxicities observed with taxane therapy, rather than the taxanes themselves. Delivery strategies in clinical trials include

liposome-encapsulated paclitaxel] and poly(L-glutamic acid)-paclitaxel, a polymer conjugate [109-110] (Figure 3).

**Liposome-based drug delivery in breast cancer treatment:** Liposomal anthracyclines have achieved highly effective drug encapsulation, resulting in substantial anticancer activity with reduced cardio toxicity, and include versions with greatly prolonged circulation such as liposomal daunorubicin and pegylated liposomal doxorubicin [109]. Pegylated liposomal doxorubicin has shown substantial efficacy in breast cancer treatment both as monotherapy and in combination with other chemotherapeutics. Additional liposome constructs are being developed for the delivery of other drugs. The next generation of delivery systems which include true molecular targeting; immune liposomes and other ligand-directed constructs represent an integration of biological components capable of tumor recognition with delivery technologies [109-110].

**Delivery of anthracyclines:** Anthracyclines demonstrate the case of potent anticancer activity that is guarded by highly problematic systemic toxicities. The most studied drug delivery applications in oncology have involved anthracyclines to reserve or to enhance efficacy against tumor cells while limiting exposure to critical target sites such as myocardium and bone marrow. Anthracyclines have been encapsulated in a number of liposomal constructs [109]. Current varieties exploit ion trapping methods to achieve extremely efficient loading of doxorubicin or daunorubicin within the aqueous interior of unilamellar (single bilayer) liposomes, reaching 10<sup>4</sup> drug molecules per liposome particle. Liposome-encapsulated doxorubicin (TLC D-99, Myocet™; Elan Pharmaceuticals, Inc., Cedar Knolls, NJ, USA) have developed a multiple vial kit, with discrete vials containing moderately sized (~190 nm)



**Figure 3:** Various drug delivery, targeting and biotechnological areas where polysaccharide-bearing liposomes could be exploited as tools for future therapy. (A) Macrophage targeting based upon the ligand-receptor interaction. (B) Stealth behavior of the polysaccharide cloud, which further provides a hydrophilic environment to deter them from the opsonins and plasma proteins. (C) Polysaccharides as bio-sensors (targeting ligands) and offering bio-protection. (D) Vaccination potential of anchored bacterial polysaccharides or lipopolysaccharides. (E) Encapsulating polysaccharide antigens for vaccination [109].

liposomes, lyophilized doxorubicin, and citric acid buffer. The components are mixed at a point of care, resulting in highly efficient (>99%) loading of doxorubicin into the liposomes. Myocet™ provides a limited degree of prolonged circulation as likened with free drug. However, the liposome encapsulation significantly alters the bio-distribution of doxorubicin, resulting in some reduction in toxicities [110].

**Application's for delivery systems in breast cancer:** Additional application's for delivery systems in breast cancer include approved chemotherapy drug's such as vinca alkaloid's, platinum's and camptothecin's. In each case, it's possible that delivery systems such as liposomes or polymers could improve pharmacokinetics, could increase tumor accumulation, and/or could reduce limiting toxicities. Polymers capable of recognizing certain compounds by using the technique of molecular imprinting, which is more often associated with chromatography. The molecule that the polymer will sense is used as a template around which the monomers are allowed to polymerize. The template molecule is then extracted from the polymer [110-111].

**Potential of polysaccharide anchored liposomes in drug delivery, targeting and immunization:** Recently the prominence has been laid upon the carbohydrate mediated liposomal interactions with the target cells. Among the various carbohydrate ligands, such as glycoproteins, glycolipids, viral proteins, polysaccharides, lipo-polysaccharides and other oligosaccharides, this section deals with the polysaccharide anchored liposomal system for their potential in drug delivery, targeting and immunization [112-113]. Over the years, various schemes have been developed which include coating of the liposomal surface with natural or hydrophobized polysaccharides, namely manna, pullulan, amylopectin, dextran etc., or their palmitoyl or cholesteroyl derivatives. The polysaccharide(s) coat tends vesicular constructs physicochemical stable in bio-environments and site-specific. The aim of improving the physical and biochemical stability of liposomes and the ability to target liposomes to specific organs and cells, were the major attributes of the polysaccharide anchored liposomes [111-113] (Figure 4).

**Polysaccharide Anchored Liposomes:** In the development of polysaccharide anchored liposomes for therapeutic purposes, it is important to consider the mechanisms and methodologies of the polysaccharide link with the bilayer membrane and resultant effect on the bilayer permeability, fluidity, and integrity [114]. The affinity and the selectivity of the anchored polysaccharide towards it's paired ligand's is a desirable pre-requisite that makes the system site specific and target oriented. The impact of carbohydrate and polysaccharide specific recognition domains on the cell surface has stimulated the research quantitatively towards exploitation of technology to develop systems for drug's and/or antigen's [111-113]. Since the potential of natural or hydrophobized polysaccharides, methods have been developed to link polysaccharides to the surface of liposomes [106]. Earlier methods were attempted to anchor polysaccharides on the surface of the liposomes through adsorption, however recently spacer activated covalent coupling or hydrophobic anchoring have been valued as

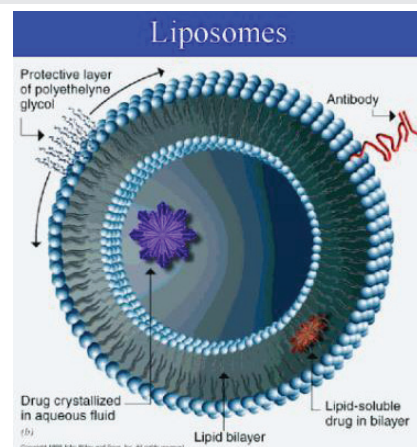


Figure 4: Liposomes used in cosmetic industry [112].

methods of anchoring. Earlier methods of anchoring exploited possible interaction of liposomes and polysaccharides. Sunamoto at. al., [106-107] investigated communications of simple polysaccharides and liposomal membranes and revealed that simple and naturally occurring polysaccharides, such as dextran, chitosan, pullulan, mannan, or amylopectin, powerfully adhere on to the liposomal surface mostly via hydrophobic interactions inducing subsequent aggregation and fusion of liposomes. Under specific conditions however, which do not allow for aggregation or fusion, the adsorption of polysaccharides over liposomal membranes may be due to diffusion controlled mechanism of constitutive components and coat, followed by lateral diffusion and subsequent inter-digitization of adsorbed polysaccharide molecules into bilayers. This theory was later confirmed and substantiated by fluorescence depolarization technique using FITC-dextran as marker probe [106].

Polysaccharide anchoring by adsorption was found to be thermodynamically unstable and pharmaceutically unacceptable due to the following reasons:

1. The polysaccharides adsorbed on the liposomal surfaces easily desorb/delodge on dilution or on mechanical agitation.
2. Peptization or coagulation of the polysaccharides could lead to successive destabilization of the liposomal bilayer
3. Stoichiometric ligand density is often non-reproducible.

In order to prevent adsorptive coating related limitations, Sunamoto and Iwamoto [107] employed chemically modified polysaccharides, i.e., palmitoylated polysaccharides, to coat the liposomes. These partially hydrophobized polysaccharides were allowed to react covalently and subsequently integrate with the lipid constituents of liposomal membranes.

The surface modification of liposomes is mediated through the hydrophobic legs. Coating of liposomes with these hydrophobized polysaccharides can be achieved by incubation of aqueous solutions of polysaccharide derivative's with pre-formed liposomal dispersion. In some cases, Chol substituted

polysaccharide was used to conjugate sensory devices like salicylic acid derivative or an IgM fragment [108]. The salicylic acid conjugated cholesterol substituted polysaccharide or immuno-polysaccharide derivatives were subsequently anchored over liposomes by dispersion-incubation technique under optimized standard conditions. The method have been used in the preparation of newly designed immune liposomes where PC based large oligolamellar vesicles were anchored to the polysaccharide pullulan. The system has been modified to carry both, i.e., cholesterol as a hydrophobic anchor, and monoclonal antibody fragment (anti-sialosyl Lewis x IgMs) as a sensory device. Various studies moreover reflect that polysaccharides anchored on liposomes using above-mentioned methods retained their ligand affinity and specificity [108].

### Therapeutic and clinical applications

Polysaccharides have been developed in recent years for various delivery and targeting strategies, either they provided stabilization and formed a skeleton on which suitable sensory molecules were detached or otherwise they themselves behave as sensory devices to bring out the resultant targeted therapeutic effects. Some of the therapeutic benefits that polysaccharide anchored vesicles offer is well discussed [108].

**Lung Therapeutics:** Increased lung accumulation of polysaccharides anchored liposome promises for their selectivity and potential as drug delivery system for the therapy of lung diseases [109]. The alveolar macrophages selectively sequester the O-palmitoyl amylopectin anchored liposomes. The macrophage uptake have been confirmed with the help of fluorescent probe marker, as marker was traced mainly in macrophages after IV injection [110]. Moreover, OPA anchored liposomes are reported to be sequestered and retained selectively in lungs by anionic-scavenging receptors [110-111]. Liposomes appended with O-palmitoylpullulan (OPP) and O-palmitoyl amylopectin (OPA) are swiftly cleared from the blood as compared to 'naked' liposomes. However, they have a relatively wide tissue circulation including liver and spleen, it's found that OPA anchored liposomes are selectively intercepted sequestered and internalized by the lung macrophages and monocytes. Subsequent to this observation, investigations were made on OPA anchored liposomes to explore their potential as a delivery system for sisomycin treatment of lung diseases in guinea pigs infected with *Legionella pneumophila* [110]. The therapeutically beneficial results of these studies subsequently promoted further investigations, where OPA anchored liposomes were tested for targeted delivery of antimicrobial agents against intracytoplasmic pathogens and fungus. Specifically, amylopectin anchored liposomes were found to be effective for the delivery of Ceftazidime to *L. Pneumophila* infected guinea pigs where relative to treatment with free drug the survival rate achieved following the liposome treatment was 30%. The liposomal encapsulated drug accumulation in the lung was two-fold higher compared to lung drug concentration following free drug administration. Miyazaki and coworkers [111] revealed that coating liposomes with amylopectin negotiates targeting of the incorporated amphotericin B to the lungs. The LD50 of amylopectin-anchored liposomal amphotericin B in normal mice was more than 10.0 mg/kg,

whilst for conventional amphotericin B, LD50 recorded to be 1.2 mg/kg. Amylopectin-anchored liposomes showed two-fold higher accumulation in the lungs as compared to conventional liposomes. These workers further studied *in vivo* efficacy of the system using murine model of pulmonary candidiasis. *Candida albicans* was inoculated into BALB/C mice and the number of *Candida* in the lungs of mice treated with amylopectin-anchored liposomes and conventional liposomes were compared. The amylopectin-anchored liposomes improved the survival rate of inoculated mice [111].

**Nano pores:** Nanopores or micropores that optimistically remember only the specific template. When a solution of glucose, sucrose, and galactose, this particular compound would recognize only the glucose. In the example of glucose-sensing molecularly imprinted nanoparticles, the detection of glucose would trigger the release of insulin from within the particle. As drugs become larger and less water soluble, the importance of new delivery systems will only increase [112].

**Nano-liposomes and Their Applications in Food Nanotechnology:** Food nanotechnology involves the exploitation of nano-carrier system's to stabilize the bio-active materials against a wide range of environmental and chemical changes as well as to improve their bio-availability [112]. Nano-liposome technology presents exciting opportunities for food technologists in areas such as encapsulation and controlled release of food materials, as well as the enhanced bio-availability, stability, and shelf-life of sensitive ingredients. Liposomes and nano-liposomes have been used in the food industry to deliver flavor's and nutrients and more recently, have been investigated for their ability to integrate antimicrobial's that could aid in the protection of food product's against microbial contamination [112]. The main physic-chemical properties of liposomes and nano-liposomes are industrially applicable methods for their manufacture are reviewed. There are quite huge applications of nano-liposomes as carrier vehicles of nutrients, enzymes, food additives, and food antimicrobials.

**The Preparation and Testing of Liposomes and its Applications in Cosmetics:** Liposome is one of the emerging in pharmaceutical and cosmetics in recent years. Existence of biological carrier, liposome loaded with active ingredients is very effective in treating disease and skin care. The result of Freeze fracture electromicrography showed the presence of liposomes. In the forgone conclusion that liposomes had good stability by measuring their particle size and microscopic structure and M. R. Muzafarriet. al., [111] also tested the encapsulation rate to the water soluble substance and discussed the application of liposomes in cosmetics as well. Liposomal formulations are the first Nano-Pharmaceuticals introduced to market, Doxil® PEGylated liposomal formulation for doxorubicin is the first product based on liposomes. These liposomes are called as "Stealth" liposomes with size <200nm which are long circulation with hydrophilic (PEG) surface. These long circulating liposomes found to target to tumour tissue by a mechanism known as enhanced permeation and retention (EPR). Hence liposomal formulation of doxorubicin considerably reduced the cardio-toxicity of drug [112-113].

**Novel Nano-liposomal CPT-11 Infused by Convection-Enhanced Delivery in Intracranial Tumors: Pharmacology and Efficacy:** J. W. Park et. al., [112] postulated that combining convection-enhanced delivery (CED) with a novel, highly stable nanoparticle/liposome containing CPT-11 (nano-liposomal CPT-11) would provide a dual drug delivery strategy for brain tumor treatment. Following CED in rat brains, tissue retention of nano-liposomal CPT-11 was greatly prolonged, with >20% injected dose remaining at 12 days for all doses. Tissue residence was dose dependent, with doses of 60 µg (3 mg/mL), 0.8 mg (40 mg/mL), and 1.6 mg (80 mg/mL) resulting in tissue half-life ( $t_{1/2}$ ) of 6.7, 10.7, and 19.7 days, respectively [113]. In contrast, CED of free CPT-11 resulted in rapid drug clearance (tissue  $t_{1/2}$  = 0.3 day). At equivalent CED doses, nano-liposomal CPT-11 increased area under the time-concentration curve by 25-fold and tissue  $t_{1/2}$  by 22-fold over free CPT-11; CED in intracranial U87 gliomaxenografts showed even longer tumor retention (tissue  $t_{1/2}$  = 43 days). Plasma levels were undetectable following CED of nano-liposomal CPT-11. Importantly, prolonged exposure to nano-liposomal CPT-11 resulted in no measurable central nervous system (CNS) toxicity at any dose tested (0.06–1.6 mg/rat), whereas CED of free CPT-11 induced severe CNS toxicity at 0.4 mg/rat. In the intracranial U87 gliomaxenograft model, a single CED infusion of nano-liposomal CPT-11 at 1.6 mg resulted in significantly improved median survival (>100 days) compared with CED of control liposomes (19.5 days;  $P = 4.9 \times 10^{-5}$ ) or free drug (28.5 days;  $P = 0.011$ ). The CED of nano-liposomal CPT-11 greatly prolonged tissue residence while also substantially reducing toxicity, resulting in a highly effective treatment strategy in preclinical brain tumor models [113].

**Nano-liposome Delivers Anticancer Drug to Brain Tumors, Avoids Healthy Tissue:** Uniqueness is why patients with brain cancer face such a poor diagnosis is that there are so few anticancer drugs that can actually cross the so-called blood-brain barrier and reach tumors growing in the brain [114]. But using a nano-scale, drug-loaded liposome and a pressure-driven drug administration technique known as convection-enhanced delivery, investigators at the University of California at San Francisco (UCSF) have developed an efficient method of getting anticancer drugs into the brain and keeping them there. This new approach can also reduce the therapy-limiting toxicities that are often associated with the most potent anticancer agents [114] (Figure 5).

**Loading nanoliposome therapeutics in red blood cells using electroporation:** Amrita Mehta et.al., [114] reported prepared a novel drug delivery system by loading nanoliposome therapeutics in human red blood cells (RBCs) by electroporation, and to enhance drug circulation profiles *in vivo*. During the studies conducted by A. Mehta et. Al., [114] rigorous evaluation of delivery approach, A. Mehta et al [114], have determined that these specific type and concentration of liposomes, as well as the number of electroporation pulses used are important considerations for efficient loading of nano-systems in RBCs. They [114] also investigated the role of a cell preservative solution on the viability of electroporated red blood cells, in an effort to increase the life span of the cells following reinjection.

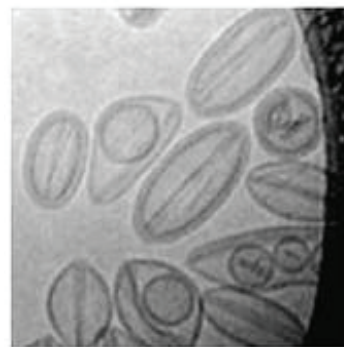


Figure 5: Nanoliposome Delivery May Improve Cancer Drug Activity [114].

**Nano-liposome Delivery May Improve Cancer Drug Activity:** The use of nano-carriers will allow the creation of formulations that are highly active against solid tumors and can be targeted to cell-surface receptors, the researchers said. “Our technology forms a remarkably stable complex between the drug and a high-charge-density polyanion in the liposomal lumen [115–116]. The stability of this complex helps govern the rate of release of the drug from the carrier, thus ensuring that it can localize to the tumor to a significant extent before it is released,” said Daryl C. Drummond, PhD, senior director for liposomal discovery at Merrimack Pharmaceuticals in Cambridge [115–118].

## Conclusion

Mini review articulates the significance of liposome drug delivery systems are designed to be stable and efficiently carry anticancer agents to tumor sites. This has been accomplished with some success by liposomal versions of anthracyclines. Current liposomal agents, nanolipids, anthracyclines provide improved pharmacokinetics, provide reduced toxicities to a number of organ sites, and provide potentially increased tumor uptake. The next generation of delivery systems in development combine these features with tumor cell recognition, and include antibody-targeted and cell-internalizing systems. Such systems will enable drug delivery to move beyond pharmacokinetic-driven and bio-distribution-driven mechanisms to true molecular targeting. This trend can also be viewed as an integration of biological therapeutics and drug delivery technologies. Various methods of preparation of liposomes have been depicted which could give good activity for drug delivery systems, ex: nano liposomes or nanophospholipids. It is probable that such integrated approaches will include biologically targeted delivery systems for small molecule drugs as well as for biological agents with anticancer activity, and will be an increasingly important theme in the development of new treatments of breast cancer, heart ailments, paralysis, AIDS and dreadful diseases which is the need of the hour.

## References

1. D. Chapman (1983) *Liposomal technology* 1: 1-18.
2. HAH Rongen, A Bult, WP van Bennekom (1997) Liposomes and immunoassays. *J Immuno Methods* 204: 105-133. [Link: https://tinyurl.com/y9kax4gx](https://tinyurl.com/y9kax4gx)
3. DJA Crommelin (1978) *Liposomes* 3: 73-190 4194-4198.

4. D Deamer, AD Bangham (1976) Large volume liposomes by an ether vaporization method *Biochem Biophys Acta* 443: 629-634. [Link: https://tinyurl.com/y8rplmz2](https://tinyurl.com/y8rplmz2)
5. Lipids For Liposome Formation. [Link: https://tinyurl.com/yb383knn](https://tinyurl.com/yb383knn)
6. Karmali PP, Chaudhuri A (2007) Cationic liposomes as non-viral carriers of gene medicines: resolved issues, open questions, and future promises. *Med Res Rev* 27: 696–722. [Link: https://tinyurl.com/yaznt8x3](https://tinyurl.com/yaznt8x3)
7. Deepthi V, Kavitha A (2014) Liposomal drug delivery system: a review. *RGUHS J Pharm Sci* 4: 47–56. [Link: https://tinyurl.com/y9cj2uor](https://tinyurl.com/y9cj2uor)
8. Lemie`re J, Carvalho K, Sykes C (2015) Cell-sized liposomes that mimic cell motility and the cell cortex. *Methods Cell Biol* 128: 271–285. [Link: https://tinyurl.com/yazo3hns](https://tinyurl.com/yazo3hns)
9. Sercombe L, Veerati T, Moheimani F, Wu SY, Sood AK, et al. (2015) Advances and challenges of liposome assisted drug delivery. *Front Pharmacol* 6: 286. [Link: https://tinyurl.com/ycqcob5](https://tinyurl.com/ycqcob5)
10. Mouritsen O (2011) Lipids, curvature, and nano-medicine. *Eur J Lipid Sci Technol* 113: 1174–1187. [Link: https://tinyurl.com/y7ddhadj](https://tinyurl.com/y7ddhadj)
11. Sharma PH, Powar PV, Sharma SS (2014) Pharmacosomes: a novel drug delivery system. *Pharma Innov J* 3: 94–100. [Link: https://tinyurl.com/y9vwtqry](https://tinyurl.com/y9vwtqry)
12. Hua S, Wu SY (2013) The use of lipid-based nanocarriers for targeted pain therapies. *Front Pharmacol* 4: 143. [Link: https://tinyurl.com/y9j8hdn2](https://tinyurl.com/y9j8hdn2)
13. Zhang LF, Granick S (2006) How to stabilize phospholipid liposomes (using nanoparticles). *Nano Lett.* 6: 694-698. [Link: https://tinyurl.com/yd2nuoq5](https://tinyurl.com/yd2nuoq5)
14. Michel R, Plostica T, Abezgauz L, Danino D , Gradzielski M (2013) Control of the stability and structure of liposomes by means of nanoparticles. *Soft Matter* 9: 4167-4177. [Link: https://tinyurl.com/yc968dqh](https://tinyurl.com/yc968dqh)
15. Liu JW, Jiang XM, Ashley C, Brinker CJ (2009) Electrostatically Mediated Liposome Fusion and Lipid Exchange with a Nanoparticle-Supported Bilayer for Control of Surface Charge, Drug Containment, and Delivery. *J Am Chem Soc.*131: 7567-7569. [Link: https://tinyurl.com/y9kouojt](https://tinyurl.com/y9kouojt)
16. Liu JW, Stace-Naughton A, Jiang XM, Brinker CJ (2009) Porous Nanoparticle Supported Lipid Bilayers (Protocells) as Delivery Vehicles. *J Am Chem Soc.* 131: 1354-1355. [Link: https://tinyurl.com/ybn25gdl](https://tinyurl.com/ybn25gdl)
17. Gao WW, Hu CMJ, Fang RH, Zhang LF (2013) Liposome-like nanostructures for drug delivery. *J Mater Chem B.* 1: 6569-6585. [Link: https://tinyurl.com/ycmqwq3ee](https://tinyurl.com/ycmqwq3ee)
18. Batist G, Sawyer M, Gabrail N, N.Christiansen , J.L.Marshall, et al. (2008) A multicenter, phase II study of CPX-1 liposome injection in patients (pts) with advanced colorectal cancer (CRC). *J Clin Oncol* 26: 4108. [Link: https://tinyurl.com/y8e7nzt0](https://tinyurl.com/y8e7nzt0)
19. Batist G, Sawyer M, Hart K , A Louie (2010) Phase 2 study of CPX-1 liposome injection: UGT1A1 and prediction of severe toxicities. *Ann Oncol* 19: 103.20. *Rodríguez-Antona C Pharmacogenomics of paclitaxel. Pharmacogenomics* 11: 621-623. [Link: https://tinyurl.com/y75fygno](https://tinyurl.com/y75fygno)
20. Minotti G, Menna P, Salvatorelli E, Cairo G , Gianni L (2004) Anthracyclines:molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. *Pharmacol Rev* 56:185-229. [Link: https://tinyurl.com/ycre5u6t](https://tinyurl.com/ycre5u6t)
21. Rahman AM, Yusuf SW, Ewer MS (2007) Anthracycline-induced cardiotoxicity and the cardiac-sparing effect of liposomal formulation. *Int J Nanomedicine* 2: 567-583. [Link: https://tinyurl.com/yc7you6b](https://tinyurl.com/yc7you6b)
22. Barenholz Y. Doxil\_ e (2012) the first FDA-approved nano-drug: lessons learned. *J Control Release* 160:117e134. [Link: https://tinyurl.com/ybaf55gt](https://tinyurl.com/ybaf55gt)
23. Gabizon A, Goren D, Fuks Z, et al (1983) Enhancement of adriamycin delivery to liver metastatic cells with increased tumoricidal effect using liposomes as drug carriers. *Cancer Res* 43:4730e4735. [Link: https://tinyurl.com/y8t3fr9x](https://tinyurl.com/y8t3fr9x)
24. Hoffhein RD, Gnad-Vogt SU, Beyer U, et al (2005) Liposomalencapsulated anti-cancer drugs. *Anticancer Drugs* 16: 691e707. [Link: https://tinyurl.com/yb8juj3y](https://tinyurl.com/yb8juj3y)
25. Harris L, Batist G, Belt R, et al (2002) Liposome-encapsulated doxorubicin compared with conventional doxorubicin in a randomized multicenter trial as first-line therapy of metastatic breast carcinoma. *Cancer* 94:25e36. [Link: https://tinyurl.com/yczofedr](https://tinyurl.com/yczofedr)
26. O'Brien ME, Wigler N, Inbar M, et al (2004) Reduced cardiotoxicity and comparable efficacy in a phase III trial of pegylated liposomal doxorubicin HCl (CAELYX/Doxil) versus conventional doxorubicin for first-line treatment of metastatic breast cancer. *Ann Oncol* 15:440e449. [Link: https://tinyurl.com/y85evq7x](https://tinyurl.com/y85evq7x)
27. Carbone PP, Bono V, Frei E 3rd, Brindley CO (1963) Clinical studies with vincristine. *Blood* 21:640–647. [Link: https://tinyurl.com/ycuclblg](https://tinyurl.com/ycuclblg)
28. Kantarjian HM, O'Brien S, Smith TL, et al (2000) Results of treatment with hyper-CVAD, a dose-intensive regimen, in adult acute lymphocytic leukemia. *J Clin Oncol* 18: 547–561. [Link: https://tinyurl.com/ybmnprb4](https://tinyurl.com/ybmnprb4)
29. Larson RA, Dodge RK, Burns CP, et al (1995) A five-drug remission induction regimen with intensive consolidation for adults with acute lymphoblastic leukemia: Cancer and Leukemia Group B study 8811. *Blood* 85: 2025–2037. [Link: https://tinyurl.com/ycl8fhoq](https://tinyurl.com/ycl8fhoq)
30. Linker CA, Levitt LJ, O'Donnell M, Forman SJ, Ries CA (1991) Treatment of adult acute lymphoblastic leukemia with intensive cyclical chemotherapy: a follow-up report. *Blood* 78: 2814–2822. [Link: https://tinyurl.com/y95aa5qq](https://tinyurl.com/y95aa5qq)
31. Lamanna N, Heffner LT, Kalaycio M, et al (2013) Treatment of adults with acute lymphoblastic leukemia: do the specifics of the regimen matter? Results from a prospective randomized trial. *Cancer* 119: 1186–1194. [Link: https://tinyurl.com/y9d5qwx6](https://tinyurl.com/y9d5qwx6)
32. Millar JL, Millar BC, Powles RL, et al (1998) Liposomal vincristine for the treatment of human acute lymphoblastic leukaemia in severe combined immuno deficient (SCID) mice. *Br J Haematol* 102: 718–721. [Link: https://tinyurl.com/yczfjvn5](https://tinyurl.com/yczfjvn5)
33. Owellen RJ, Hartke CA, Dickerson RM, Hains FO (1976) Inhibition of tubulin-microtubule polymerization by drugs of the vinca alkaloid class. *Cancer Res* 36:1499–1502. [Link: https://tinyurl.com/y8kajy2v](https://tinyurl.com/y8kajy2v)
34. Sarris AH, Hagemester F, Romaguera J, et al (2000) Liposomal vincristine in relapsed non-Hodgkin's lymphomas: early results of an ongoing phase II trial. *Ann Oncol* 11:69–72. [Link: https://tinyurl.com/yc46fb38](https://tinyurl.com/yc46fb38)
35. Hoelzer D (1994) Treatment of acute lymphoblastic leukemia. *Semin Hematol* 31:1–15.
36. Mayer LD, Nayar R, Thies RL, Boman NL, Cullis PR, Bally MB (1993) Identification of vesicle properties that enhance the antitumor activity of liposomal vincristine against murine L1210 leukemia. *Cancer Chemother Pharmacol* 33:17–24. [Link: https://tinyurl.com/yag5gz5l](https://tinyurl.com/yag5gz5l)
37. Boman NL, Bally MB, Cullis PR, Mayer LD, Webb MS (1995) Encapsulation of vincristine in liposomes reduces toxicity and improves its anti-tumor efficacy. *J Liposome Res* 5: 523–541. [Link: https://tinyurl.com/y8xyemh](https://tinyurl.com/y8xyemh)
38. Owellen RJ, Root MA, Hains FO (1977) Pharmacokinetics of vindesine and vincristine in humans. *Cancer Res* 37: 2603–2607. [Link: https://tinyurl.com/yd338dul](https://tinyurl.com/yd338dul)
39. Sethi VS, Jackson DV Jr, White DR, et al (1981) Pharmacokinetics of vincristine sulfate in adult cancer patients. *Cancer Res* 41: 3551–3555. [Link: https://tinyurl.com/yanq76wj](https://tinyurl.com/yanq76wj)

40. Haim N, Epelbaum R, Ben-Shahar M, Yarnitsky D, Simri W, Robinson E (1994) Full dose vincristine (without 2-mg dose limit) in the treatment of lymphomas. *Cancer* 73: 2515–2519. [Link: https://tinyurl.com/ybcrtwrh](https://tinyurl.com/ybcrtwrh)
41. Layton D, Trouet A (1980) A comparison of the therapeutic effects of free and liposomally encapsulated vincristine in leukemic mice. *Eur J Cancer* 16: 945–950. [Link: https://tinyurl.com/y9v5nj8s](https://tinyurl.com/y9v5nj8s)
42. Mayer LD, Tai LC, Ko DS (1989) Influence of vesicle size, lipid composition, and drug-to-lipid ratio on the biological activity of liposomal doxorubicin in mice. *Cancer Res* 49: 5922–5930. [Link: https://tinyurl.com/ybbd9zdq](https://tinyurl.com/ybbd9zdq)
43. Bally MB, Mayer LD, Loughrey H (1988) Dopamine accumulation in large unilamellar vesicle systems induced by transmembrane ion gradients. *Chem Phys Lipids* 47: 97–107. [Link: https://tinyurl.com/yb8dugyc](https://tinyurl.com/yb8dugyc)
44. Mayer LD, Wong KF, Menon K, Chong C, Harrigan PR, et al. (1988) Influence of ion gradients on the transbilayer distribution of dibucaine in large unilamellar vesicles. *Biochemistry* 27: 2053–2060. [Link: https://tinyurl.com/yblsbb72](https://tinyurl.com/yblsbb72)
45. Mayer LD, Bally MB, Loughrey H, Masin D, Cullis PR (1990) Liposomal vincristine preparations which exhibit decreased drug toxicity and increased activity against murine L1210 and P388 tumors. *Cancer Res* 50: 575–579. [Link: https://tinyurl.com/y9c932ny](https://tinyurl.com/y9c932ny)
46. Mayer LD, Nayar R, Thies RL, Boman NL, Cullis PR, et al. (1993) Identification of vesicle properties that enhance the antitumor activity of liposomal vincristine against murine L1210 leukemia. *Cancer Chemother Pharmacol* 33: 17–24. [Link: https://tinyurl.com/y9pxh9wv](https://tinyurl.com/y9pxh9wv)
47. Krishna R, Webb MS, St Onge G, Mayer LD (2001) Liposomal and nonliposomal drug pharmacokinetics after administration of liposome-encapsulated vincristine and their contribution to drug tissue distribution properties. *J Pharmacol Exp Ther* 298: 1206–1212. [Link: https://tinyurl.com/y9gywgf2](https://tinyurl.com/y9gywgf2)
48. Webb MS, Harasym TO, Masin D, Bally MB, Mayer LD (1995) Sphingomyelin-cholesterol liposomes significantly enhance the pharmacokinetic and therapeutic properties of vincristine in murine and human tumour models. *Br J Cancer* 72: 896–904. [Link: https://tinyurl.com/ycmeg8b8](https://tinyurl.com/ycmeg8b8)
49. Wong M, Kantarjian H, Appelbaum F (2007) Vincristine sulfate liposomes injection concentrates vincristine in tumor tissue and bone marrow of tumor-bearing mice. American Association for Cancer Research - National Cancer Institute - European Organisation for Research and Treatment of Cancer: International Conference on Molecular Targets and Cancer Therapeutics 22-26. [Link: https://tinyurl.com/y9dsufp3](https://tinyurl.com/y9dsufp3)
50. Kanter PM, Klaich GM, Bullard GA, King JM, Bally MB, et al. (1994) Liposome encapsulated vincristine: preclinical toxicologic and pharmacologic comparison with free vincristine and empty liposomes in mice, rats and dogs. *Anticancer Drugs* 5: 579–590. [Link: https://tinyurl.com/yad97rdc](https://tinyurl.com/yad97rdc)
51. Leonetti C, Scarsella M, Semple SC (2004) *In vivo* administration of liposomal vincristine sensitizes drug-resistant human solid tumors. *Int J Cancer* 110: 767–774. [Link: https://tinyurl.com/y8ky9tcc](https://tinyurl.com/y8ky9tcc)
52. Silverman JA, Deitcher SR (2013) Marqibo® (vincristine sulfate liposome injection) improves the pharmacokinetics and pharmacodynamics of vincristine. *Cancer Chemother Pharmacol* 71: 555–564. [Link: https://tinyurl.com/y995qly](https://tinyurl.com/y995qly)
53. Tagami T, May JP, Ernsting MJ, Li SD (2012) A thermosensitive liposome prepared with a Cu<sup>2+</sup> gradient demonstrates improved pharmacokinetics, drug delivery and antitumor efficacy. *J Control Release* 161: 142–149. [Link: https://tinyurl.com/y863vrc4](https://tinyurl.com/y863vrc4)
54. Park SM, Kim MS, Park SJ (2013) Novel temperature-triggered liposome with high stability: formulation, *in vitro* evaluation, and *in vivo* study combined with high-intensity focused ultrasound (HIFU). *J Control Release* 170: 373–379. [Link: https://tinyurl.com/y89aru7x](https://tinyurl.com/y89aru7x)
55. Chiu GN, Abraham SA, Ickenstein LM (2005) Encapsulation of doxorubicin into thermosensitive liposomes via complexation with the transition metal manganese. *J Control Release* 104: 271–288. [Link: https://tinyurl.com/y7kxp2s9](https://tinyurl.com/y7kxp2s9)
56. Viglianti BL, Ponce AM, Michelich CR (2006) Chemodosimetry of *in vivo* tumor liposomal drug concentration using MRI. *Magn Reson Med* 56: 1011–1018. [Link: https://tinyurl.com/yalrvz6a](https://tinyurl.com/yalrvz6a)
57. Nabholz JM, Falkson C, Campos D, Szanto J, Martin M (2003) Docetaxel and doxorubicin compared with doxorubicin and cyclophosphamide as first-line chemotherapy for metastatic breast cancer: results of a randomized, multicenter, phase III trial. *J Clin Oncol* 21: 968–975. [Link: https://tinyurl.com/yct6bw6r](https://tinyurl.com/yct6bw6r)
58. Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A (2004) Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *N Engl J Med* 351: 1502–1512. [Link: https://tinyurl.com/y9pev8w4](https://tinyurl.com/y9pev8w4)
59. Fossella FV, DeVore R, Kerr RN, Crawford J, Natale RR (2000) Randomized phase III trial of docetaxel versus vinorelbine or ifosfamide in patients with advanced non-small cell lung cancer previously treated with platinum-containing chemotherapy regimens. The TAX 320 Non-Small Cell Lung Cancer Study Group. *J Clin Oncol* 18: 2354–2362.
60. Fossella F, Pereira JR, von Pawel J, Pluzanska A, Gorbounova V (2003) Randomized, multinational, phase III study of docetaxel plus platinum combinations versus vinorelbine plus cisplatin for advanced non-small-cell lung cancer: the TAX 326 study group. *J Clin Oncol* 21: 3016–3024. [Link: https://tinyurl.com/y76m8t7c](https://tinyurl.com/y76m8t7c)
61. Vermorken JB, Remenar E, van Herpen C, Gorlia T, Mesia R (2007) Cisplatin, fluorouracil, and docetaxel in unresectable head and neck cancer. *N Engl J Med* 357: 1695–1704. [Link: https://tinyurl.com/y97btuw5](https://tinyurl.com/y97btuw5)
62. Posner MR, Hershock DM, Blajman CR, Mickiewicz E, Winquist E, et al. (2007) Cisplatin and fluorouracil alone or with docetaxel in head and neck cancer. *N Engl J Med* 357: 1705–1715. [Link: https://tinyurl.com/y89l9qqo](https://tinyurl.com/y89l9qqo)
63. Van Cutsem E, Moiseyenko VM, Tjulandin S, Majlis A, Constenla M, et al. (2006) Phase III study of docetaxel and cisplatin plus fluorouracil compared with cisplatin and fluorouracil as first-line therapy for advanced gastric cancer: a report of the V325 Study Group. *J Clin Oncol* 24: 4991–4997. [Link: https://tinyurl.com/ybmcv4ya](https://tinyurl.com/ybmcv4ya)
64. Taxotere package insert. [Link: https://tinyurl.com/y9z2l4h](https://tinyurl.com/y9z2l4h)
65. Sharpe M, Easthope SE, Keating GM, Lamb HM (2002) Polyethylene glycol-liposomal doxorubicin: a review of its use in the management of solid and hematological malignancies and AIDS-related Kaposi's sarcoma. *Drugs* 62: 2089–2126. [Link: https://tinyurl.com/yaza2qyo](https://tinyurl.com/yaza2qyo)
66. Park J (2002) Liposome-based drug delivery in breast cancer treatment. *Breast Cancer Res* 4: 95–99. [Link: https://tinyurl.com/y7n3v75f](https://tinyurl.com/y7n3v75f)
67. Schwonzen M, Kerbacher C, Mallmann P (2000) Liposomal doxorubicin and weekly paclitaxel in the treatment of metastatic breast cancer. *Anticancer Drugs* 11: 681–685. [Link: https://tinyurl.com/y9brfyhb](https://tinyurl.com/y9brfyhb)
68. Johnston S, Gore M (2001) Caelyx: phase II studies in ovarian cancer. *Eur J Cancer* 37(Suppl 9): S8–S14. [Link: https://tinyurl.com/y83lf6dp](https://tinyurl.com/y83lf6dp)
69. Gordon AN, Fleagle JT, Guthrie D, Parkin DE, Gore ME, et al. (2001) Recurrent epithelial ovarian carcinoma: a randomized phase III study of pegylated liposomal doxorubicin versus topotecan. *J Clin Oncol* 9: 3312–3322. [Link: https://tinyurl.com/yar9vbu5](https://tinyurl.com/yar9vbu5)
70. Stewart S, Jablonowski H, Goebel FD, Arasteh K, Spittle M, et al. (1998) Randomized comparative trial of pegylated liposomal doxorubicin versus bleomycin and vincristine in the treatment of AIDS-related Kaposi's sarcoma. International Pegylated Liposomal Doxorubicin Study Group. *J Clin Oncol* 16: 683–691. [Link: https://tinyurl.com/yct7qkdg](https://tinyurl.com/yct7qkdg)

71. J A Treat, C Huang, N Damjanov, S Walker, P Drobins, et al. (2000) Phase I trial in advanced malignancies with liposome encapsulated paclitaxel (LEP). Clin Cancer Res 6: 4492s. [Link: https://tinyurl.com/ya57r4fn](https://tinyurl.com/ya57r4fn)
72. Chapman CJ, Erdahl WE, Taylor RW, Pfeiffer DR (1991) Effects of solute concentration on the entrapment of solutes in phospholipid vesicles prepared by freeze-thaw extrusion. Chem Physic Lipid 60: 201-208. [Link: https://tinyurl.com/y8epegkq](https://tinyurl.com/y8epegkq)
73. Bangham AD, Standish MM, Watkin JC (1965) Diffusion of univalent ions across the lamellae of swollen phospholipids. J Mol Biol 13: 238-252. [Link: https://tinyurl.com/yaqoju59](https://tinyurl.com/yaqoju59)
74. Kirby C, Gregoriadis G (1984) Dehydration-Rehydration Vesicles: A Simple Method for High Yield Drug Entrapment in Liposomes. Biotechnology 2: 979-984. [Link: https://tinyurl.com/yaxvk9j2](https://tinyurl.com/yaxvk9j2)
75. Kimball's Biology Pages, "Cell Membranes." [Link: https://tinyurl.com/yb3ta78n](https://tinyurl.com/yb3ta78n)
76. S Stryer (1981) Biochemistry. 213.
77. New RR, Chance ML, Heath S (1981) Antileishmanial activity of amphotericin and other antifungal agents entrapped in liposomes. J antimicrob Chemother 8: 371-381. [Link: https://tinyurl.com/ydf2htb4](https://tinyurl.com/ydf2htb4)
78. Taylor RL, Williams DM, Craven PC, Graybill JR, Drutz DJ, et al. (1982) Amphotericin B in liposomes: a novel therapy for histoplasmosis. Am Rev Respir Dis 125: 610-611. [Link: https://tinyurl.com/ybp7xd4v](https://tinyurl.com/ybp7xd4v)
79. Graybill JR, Craven PC, Taylor RL, Williams DM, Magee WE (1982) Treatment of murine cryptococcosis with liposome-associated amphotericin B. J Infect Dis 145: 748-752. [Link: https://tinyurl.com/yaw54kkw](https://tinyurl.com/yaw54kkw)
80. Lopez-Berestein G, Mehta R, Hopfer RL, Mills K, Kasi L, et al. (1983) Treatment and prophylaxis of disseminated infection due to *Candida albicans* in mice with liposome-encapsulated amphotericin B. J Infect Dis 147: 939-945. [Link: https://tinyurl.com/y8zml5e3](https://tinyurl.com/y8zml5e3)
81. Lopez-Berestein G, Mehta R, Hopfer RL, Mills K, Kasi L, et al. (1983) Treatment and prophylaxis of disseminated infection due to *Candida albicans* in mice with liposome-encapsulated amphotericin B. J Infect Dis 147: 939-945. [Link: https://tinyurl.com/y8zml5e3](https://tinyurl.com/y8zml5e3)
82. Hopfer RL, Mills K, Mehta R, Lopez-Berestein G, Fainstein V, et al. (1984) In vitro antifungal activities of amphotericin B and liposome-encapsulated amphotericin B. Antimicrobial Agents Chemother 25: 387-389. [Link: https://tinyurl.com/ydg7udby](https://tinyurl.com/ydg7udby)
83. Adler-Moore JP, Chiang SM, Satorius A, Guerra D, Andrews B, et al. (1984) J Antimicrob Chemother; 28: 63-71.
84. Abra RM, Hunt CA, Lau DT (1984) Liposome disposition *in vivo*. VI: Delivery to the lung. J Pharm Sci 73: 203-206. [Link: https://tinyurl.com/yccak2dm](https://tinyurl.com/yccak2dm)
85. Szoka Jr FC, Milholland D, Barza M (1987) Effect of lipid composition and liposome size on toxicity and *in vitro* fungicidal activity of liposome-intercalated amphotericin B. Antimicrob Agents Chemother 31: 421-429. [Link: https://tinyurl.com/y9bd2to3](https://tinyurl.com/y9bd2to3)
86. Bangham AD, Standish MM, Watkin JC (1965) Diffusion of univalent ions across the lamellae of swollen phospholipids. J Mol Biol 13: 238-252. [Link: https://tinyurl.com/yaqoju59](https://tinyurl.com/yaqoju59)
87. Gold JW (1984) Am J Med 76: 485-63.
88. Gilman, Rall TW, Nies AS, Taylor P (1985) (New York Macmillan), Sande MA, Mandell GL In The Pharmacological Basis of Therapeutics. Editors AG. 1219-1222.
89. Clements JS, Peacock JE (1990) Amphotericin B revisited: reassessment of toxicity. J Med Am 88: 22N-7N. [Link: https://tinyurl.com/yb8clpex](https://tinyurl.com/yb8clpex)
90. Meunier F, Prentic HG, Ringden O (1991) J Antimicrob Chemother 28: 83-91.
91. Alvin CR, Sivartz GM., Jr. (1984) Liposome Technology, vol. II. CRC Press, Boca Raton, FL, 55-69.
92. Szoda FC, Papahadjopoulos D (1981) Liposomes: preparation and characterization, in Liposomes: From Physical Structure to Therapeutic Application Knight, C. G.ed. Elsevier 51-82. [Link: https://tinyurl.com/y80xe4va](https://tinyurl.com/y80xe4va)
93. Payne NL, Browning I, Haynes CA (1986) Characterization of Proliposomes. J. Pharmaceut. Sci. 75: 330-333. [Link: https://tinyurl.com/ybxwzkw](https://tinyurl.com/ybxwzkw)
94. Saunders L, Perrin J, Gammack DB (1962) Ultrasonic irradiation of some phospholipids sols. J. Pharmaceut. Pharmacol. 14: 567-572. [Link: https://tinyurl.com/y9cqyne6](https://tinyurl.com/y9cqyne6)
95. Huang CH (1969) Studies on phosphatidylcholine vesicles. Formation and physical characteristics. Biochemistry 8: 344-351. [Link: https://tinyurl.com/ycqaa33r](https://tinyurl.com/ycqaa33r)
96. Lasic DD (1993) Liposomes: from physics to application. Netherlands: Elsevier 575. [Link: https://tinyurl.com/yadposnx](https://tinyurl.com/yadposnx)
97. Paoli DT, Hager AA, Adelina V (1996) Liposomes containing bioavailable Iron (II) and process for obtaining them. US patent 5,534,268. [Link: https://tinyurl.com/ydghjaa6](https://tinyurl.com/ydghjaa6)
98. Basu S, Basu M, (2008) Liposome methods and protocols. 199.
99. Laughrey L, Baily MB, Ctdlis PR (1987) preparation of streptavidin-liposomes for use in ligand-specific targeting applications. Biop@. Acra. 901. 157. [Link: https://tinyurl.com/y8zuvh5e](https://tinyurl.com/y8zuvh5e)
100. Matsumura Y, Maeda H (1986) A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumorotropic accumulation of proteins and the antitumor agent smancs. Cancer Res 6: 193-210. [Link: https://tinyurl.com/y84a92fb](https://tinyurl.com/y84a92fb)
101. (2002) Liposome-based drug delivery in breast cancer treatment. UCSF Comprehensive Cancer Center, San Francisco, California, USA Breast Cancer Res 4: 95-99 [Link: https://tinyurl.com/y873xa7o](https://tinyurl.com/y873xa7o)
102. (2001) Pharm PharmaceutSci (www.ualberta.ca/~cps) 4: 138-158.
103. Iwamoto K, Kato T, Kawahara M, Koyama N, Watanabe S, et al. (1991) Polysaccharide-anchored oil droplets in oil-in-water emulsions as targetable carriers for lipophilic drugs. J Pharm Sci 80: 219-224. [Link: https://tinyurl.com/yc86c46b](https://tinyurl.com/yc86c46b)
104. Fukui H1, Akiyoshi K, Sunamoto J (1996) O/w-emulsion of alpha-linolenic acid stabilized with hydrophobized polysaccharide. Its effect on the growth of human colon cancer cells. J BiomaterSciPolym Ed 7: 829-838. [Link: https://tinyurl.com/yblrwgfy](https://tinyurl.com/yblrwgfy)
105. Akiyoshi K, Kobayashi S, Shichibe S, Mix D, Baudys M, et al. (1998) Self-assembled hydrogel nanoparticle of cholesterol-bearing pullulanas a carrier of protein drugs: complexation and stabilization of insulin. J Control Rel, 54: 313-320. [Link: https://tinyurl.com/y93o9tuj](https://tinyurl.com/y93o9tuj)
106. Sunamoto J, Sato T, Taguchi T, Hamazaki H (1992) Macromolecules, 25: 5665-5670. [Link: https://tinyurl.com/ycxfgh3s](https://tinyurl.com/ycxfgh3s)
107. Sunamoto J, Iwamoto K, Takada M, Yujuriha T, Katayama K (1984) Recent Advances in Drug Delivery Systems. Plenum Press, NY 153-162.
108. Takada M, Yuzuriha T, Katayama K, Iwamoto K, Sunamoto J (1984) Biochim BiophysActa, 802: 237-244. [Link: https://tinyurl.com/ybdll8zv](https://tinyurl.com/ybdll8zv)
109. Sunamoto J, Iwamoto K, Takada M, Yuzuriha T, Katayama K (1983) Polym Sci Technol 23: 157-168.
110. Miyazaki T, Kohno S, Sasayama K, Inoue Y, Hara K, et al. (1992) Tohoku J Exp Med 168: 483-490. [Link: https://tinyurl.com/y73jeczz](https://tinyurl.com/y73jeczz)
111. Mozafari MR, Johnson C, Hatziantoniou S, Demetzos C (2008)





Nanoliposomes and Their Applications in Food Nanotechnology DOI: 10.1080/08982100802465941. [Link: https://tinyurl.com/y9ejjerl](https://tinyurl.com/y9ejjerl)

112. John W Park, Kirpotin DB, Drummond DC, Yi Shao, Shalaby MR, et al. (2006) Cancer Res 66: 2801-2806. [Link: https://tinyurl.com/ycnhhno7](https://tinyurl.com/ycnhhno7)
113. www.nanotechwire.com. [Link: https://tinyurl.com/odx97yg](https://tinyurl.com/odx97yg)
114. Mehta A (2009) Pharmaceutical Science Master's Theses, Northeastern University. [Link: https://tinyurl.com/yc4urav9](https://tinyurl.com/yc4urav9)
115. Drummond DC, Noble CO, Guo Z, Connolly-Ingrama C, Gabriela SB et al.

(2010) J Control Release 141: 13-21. [Link: https://tinyurl.com/ybtncskw](https://tinyurl.com/ybtncskw)

116. Basu S, Basu M. (2008) Liposome methods and protocols 199. [Link: https://tinyurl.com/y88yeo4x](https://tinyurl.com/y88yeo4x)
117. [Link: https://tinyurl.com/y9m7gg9c](https://tinyurl.com/y9m7gg9c)
118. Hope MJ, Bally MB, Webb G, Cullis PR (1985) Production of large unilamellar vesicles by a rapid extrusion procedure: characterization of size distribution, trapped volume and ability to maintain a membrane potential. Biochim Biophys Acta 812: 55-65. [Link: https://tinyurl.com/ycc47m4u](https://tinyurl.com/ycc47m4u)

**Copyright:** © 2018 Rahman A, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Citation:** Rahman A, Uahengo V, Likius D (2018) Mini review on emerging methods of preparation of liposome and its application as Liposome drug delivery systems. Open J Pharmacol Pharmacother 3(1): 005-021. DOI: <http://dx.doi.org/10.17352/ojpp.000007>