Review Paper:

Preparation methods and applications of Lipid-based nanosomes for advancement in the field of Pharmaceutical Biotechnology: A review and outlook on future

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Abstract

Nano-sized vesicles composed of a phospholipid bilayer encapsulating an aqueous core called Liposomes, have garnered substantial attention in pharmaceutical biotechnology for their capacity to safeguard and enhance drug and bioactive molecule delivery. This review presents an overview of liposome preparation methods and their applications in disease and gene therapy. Liposomes serve as drug delivery vehicles in cancer, cardiovascular and infectious diseases. In cancer therapy, they precisely transport chemotherapeutic agents to tumors, minimizing systemic toxicity. For cardiovascular diseases, liposomes ferry nitric oxide and antioxidants, ameliorating vascular function and reducing oxidative stress. In gene therapy, liposomes ferry nucleic acids (DNA, RNA) to modulate gene expression. Targeting ligands like antibodies or peptides further tailored liposomes for specific cell or tissue delivery.

With implications in diverse medical fields, particularly disease and gene therapy, liposomes are versatile drug delivery systems. Their pharmacokinetic and pharmacodynamic properties are shaped by preparation methods and composition. Advances have yielded more stable and targeted liposomes.However, further exploration is required to fully harness their potential, to ensure safer therapies and to tackle varied diseases. In conclusion, liposomes hold promise in pharmaceutical applications, efficiently delivering drugs and addressing therapeutic challenges. Continued research will unlock their potential, revolutionizing patient care and medical practices.

Keywords: Liposomes, targeted drug delivery, diseases.

Introduction

In recent decades, conventional methods of drug delivery have shown limitations in terms of effectiveness due to characteristics such as large size, restricted surface area and shape of the drugs. These methods face challenges in achieving *in vivo* stability at the target site, poor availability, solubility, absorption by cells, immune response, therapeutic effectiveness and potential adverse effects. To overcome these challenges, novel drug delivery systems, notably those using nanotechnology, have emerged as promising solutions. Among these systems, nanosomes have gained significance in the area of therapeutic and diagnostic agents.

Vesicular drug delivery mechanisms are made up of self-assembling amphiphilic building blocks comprising of one or more concentric bilayers. They perform a key role in targeting drugs to specific organs or sites while reducing undesired side effects. Liposomes, for instance, are simple spherical vesicles made up of a phospholipid layer surrounding an aqueous volume. Their size ranges from 20nm to several nanometers, making them ideal as drug delivery vehicles for pharmaceutical drugs including anti-viral vaccines, mRNA and phytoconstituents. Liposomes come in different categories such as small unilamellar vesicles, large unilamellar vesicles, multilamellar vesicles and gigantic unilamellar vesicles.

Ethosomes, on the other hand, are hydroalcoholic nonvesicular phospholipid systems containing phospholipids, ethanol and isopropanol. They act as vehicles for dermal and transdermal delivery of bioactive molecules, providing enhanced skin penetration. Phytosomes, closely related to liposomes, are phospholipid complexes of drug delivery systems. They consist of a complex mixture of active ingredients derived from herbal sources, surrounded by phospholipids.

Various novel methods, deviating slightly from conventional techniques, have been employed for making nanosomes. Examples include lipid film hydration, microfluidics, bubble method, freeze drying, detergent removal method (to remove non-encapsulated material) and mechanical dispersion method. Nanosomes can be carried via various routes such as intravenous, oral, intramuscular, nasal and transdermal, leading to differences in pharmacodynamics, pharmacokinetics and bioavailability. Liposomes, with their complex yet simple structure, can be negatively or positively charged, protecting DNA from degradative processes. Novel liposomal delivery methods have been introduced to carry large amounts of DNA, potentially as big as a chromosome. These genes loaded liposomes can be directed to specified cells or tissues.

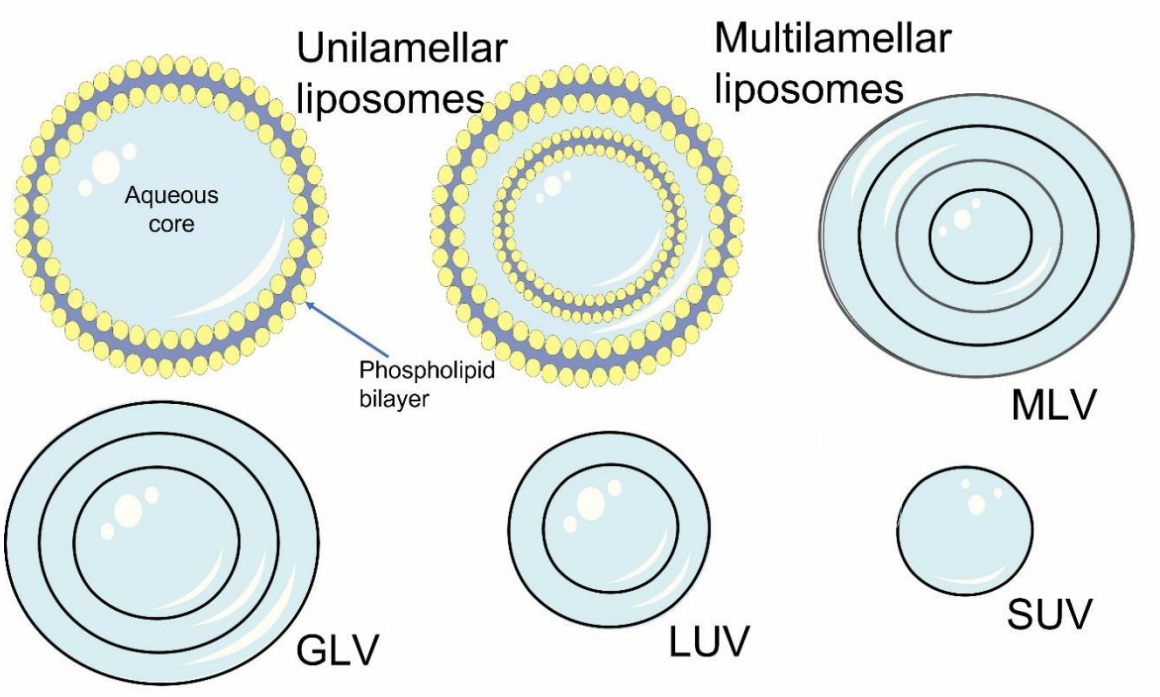

Figure 1: Types of liposomes

Liposomes, as drug delivery systems, provide advantages including improved pharmacodynamics, pharmacokinetics, reduced toxicity, enhanced therapeutic efficacy against bacteria and advanced drug-target selectivity. Current liposomal formulations span a range of applications, from clinical use to the development of multifunctional liposomal systems for treatment and diagnostics. Recent research has made significant progress in liposomal systems with enhanced drug delivery potential for cancer treatment. This includes different types of liposomal preparations such as liposomes, niosomes, ethosomes, aquasomes and phytosomes. Additionally, the various preparation methods, biological properties and recent trends in liposomes are discussed.

Nanosomes preparation methods: Nanosomes, which are nanosized particles with small and uniform dimensions, offer advanced properties compared to conventional drugs. Their large surface-to-area ratio makes them highly desirable in pharmaceutical industries, as they can enhance drug efficacy and availability. Nanosomes find applications across a wide range of diseases, where drugs are administered in nanosome form. To achieve optimal results, the preparation methods of nanosomes vary depending on factors like route of administration, desired size and encapsulation efficiency. Industries employ diverse techniques to improve production speed, to increase quantity and to reduce production costs. This review discusses on several types of nanosomes including liposomes, aquasomes, niosomes and ethosomes and provides an in-depth discussion of their respective preparation methods.

Liposomes preparation method: The bupivacaine multivesicular liposomes were prepared using a series of steps including 1st emulsification, 2nd emulsification, solvent removal and centrifugation[65]. A methanal solvent phase was prepared for the High Gravity Rotating Packed Bed (RPB) technique by mixing EPC and cholesterol at the mass proportion of 3:1 with phosphate-buffer saline at acid-base balance of 7.4. Simultaneously, an aqueous phase was produced. Both phases were injected separately into the RPB through separate inlets while the temperature was maintained by circulating water in the tank's jackets. The aqueous phase exhibited flow rates from 20 to 300 ml/min whereas the solvent part exhibited a steady flow rate of 20 ml/min. The outcome liposome suspension with a concentration of 1+0.5 mg/ml was sourced from the RPB outflow and subsequently clarified against pH 7.4 to remove residues[53].

The Super Lip method utilizes supercritical technology for lab-scale production of liposomes. The process involves the creation of a water phase containing tween 80 dissolved in distilled water and an oil phase containing a lipid mass base of 15% in isopropyl myristate. These phases are combined with carbon dioxide and an ethanolic solution in a saturator, with a 2:4 gas-to-liquid ratio. The mixture is then delivered to a third feeding line and sprayed at high pressure through an 80 µm diameter nozzle, resulting in the rapid precipitation and encapsulation of droplets by phospholipids[131].

Various hydration methods have been used for liposome preparation. In the conventional film hydration method, cholesterol and soya lecithin dissolved in ethanol are used to produce a smooth film, which is subsequently hydrated with PBS and sonicated[92]. The heating method involves the hydration of lipids with an aqueous phase containing 3% glycerol which enhances liposome stability and is

biocompatible and non-toxic[158]. The curvature-tuned preparation of nanoliposomes employs a speedy pH modification preceded by an equilibrium interval to induce spontaneous vesicle formation. This solvent-free approach involves hydrating a lipid combination with preheated PBS buffer, vertexing and adding to a pH 7.4 buffer. The resulting liposomes are isolated through centrifugation[28].

In the packed bed preparation of liposomes, non-spherical monodisperse alumina particles are used to pack capillaries. A micropipette is used to disperse the lipid mixture into the packing bed and the capillary is dried and purged with nitrogen gas. The hydration of the dried lipid bilayers is performed with a phosphate buffer[97]. The localized infrared heating method allows the formation of unilamellar vesicles from spin-coated lipid films. The process involves the use of a polydimethylsiloxane (PDMS) frame placed on a spin-coated surface, which creates a chamber. The chamber is filled with a 60 mM ionic strength PBS solution to moisturize the film and localized heating is achieved using an IR-B laser directed through an optical fiber[103].

A dual asymmetric centrifugation method shears a rich blend of cholesterol and lecithin [55:45 mol%] in a 0.90 percentage NaCl solution using centrifugal and rotational forces, resulting in the formation of liposomes[83]. Osmotic shock hydration involves the repeated rehydration of a small liposome solution with deionized water, leading to generation of enlarged and more homogenous giant unilamellar vesicles[144]. This spray drying method involves dissolving lipids in chloroform which is then sprayed into a drying chamber and subsequently moisturized and homogenized to prepare spray-dried liposomes[151]. The lyophilization method has 3 phases: frosting, initial drying and subsequent drying to obtain lyophilized liposomes[133].

The gel-assisted hydration method involves the use of a polyvinyl alcohol (PVA) solution which is spread on a coverslip and dried. Lipids dissolved in chloroform are then spread on the dried PVA and the solubilizer is vaporized under vacuum. The resulting lipid layer is rehydrated with PBS and subjected to electroformation to obtain liposomes[134]. The glass bead hydration method utilizes lipid-coated beads that are mixed with a sucrose buffer and heated at 65°C to induce liposome formation[98]. Finally, the electro formation method involves the hydration of lipid films deposited on electrodes in an electric field[161].

In a recent modified electro formation method, a 10 µL dribble of charged lipid mixture was distributed over a tin oxide-covered slide and dried for 30 minutes under vacuum. A secondary glass plate was employed to shield the vault, which was then brimmed with an accurate 0.095 molarity sucrose mixture. An alternating current voltage of 1.50V and 10.00 Hertz was supplied over a 1 mm gap within the chamber for 3 hours at 22.0 ± 1.0°C[161]. Electro formation method is also used in microfluidics where the electro formation technique is applied to micro-sized fluidic channels with a square cross-section. The channels are placed between glass slides coated with ITO electrodes and liposomes are formed within ten minutes by utilizing an alternating current voltage[3].

Traditional bulk techniques are also used to produce liposomes. In the RPEM, liposomes are formed in an inverted stage and then the inverse-phase vesicles are sonicated and prepared in a buffer solution (wet phase) containing hydrophilic molecules for encapsulation within the vesicles. In the ethanol injection method, EtOH is spontaneously delivered into an excess buffer to make liposomes. In the detergent depletion method, surfactants at the CMC's are used to solubilize fats and when the surfactant is detached, the microemulsions become increasingly enriched in phospholipids. Then, a dialysis system is used to remove detergents. Recent advancements in bulk methods include the membrane contractor method and microfluidics[73].

In the membrane contractor method, the system used contains a nitrogen bottle, peristaltic pump and pressurized recipient. A metal instrument with a tubular SPG membrane within 2 pressure gauges is employed. Phospholipids at a concentration of 20 mg/ml and 20% lipid (w/w) are mixed in 250.00 ml of ethanol in a pressure container. When the aqueous phase is let into it, spontaneous formation of liposomes is observed and the extracted pellets are disseminated in saline (PBS) and preserved at +4°C. Microfluidics is another bulk method that encompasses fluid movement through conduits with cross-segmental proportions typically ranging between 5–500 µm[29].

Many innovative microfluidics-focused liposome production methods have been created in recent years. The flow of a hydrophilic buffer beside two opposed walls is aided by current in a microchannel in microfluidic hydrodynamic focusing. By mixing phospholipid in EtOH and a hydrophilic buffer, a diffusive mixture is formed. The breakdown of water and alcohol reduces the concentration of alcohol, causing phospholipids to self-assemble into bilayers[(69)]. Vertical flow focusing, like other microfluidics techniques, delivers an exceptional sample homogeneity. Liposomes that are generated in microfluidic VFF devices are equipped with an elevated aspect ratio of the channel[135].

Microfluidic systems incorporating micromixer structures fall under the mixer-assisted microfluidics method of producing liposomes. The droplet method of microfluidics targets on producing specified volumes in the form of drops from immiscible fluids[130]. Pulsed microfluidic jetting involves placing a pulsating jet stream of a lyophobic solution against a flat bilayer, inducing an extension through locally distorting the bilayer. This helps to culminate in liposome formation and the formation of intact bilayers[167].

In the transient membrane ejection method, two phases are infused into a microfluidic device and squeezed through

crossflow in a T-junction, resulting in bilayers. Fluid displacement is achieved by infrared rays which produce liposomes[79]. Supercritical fluid technology involves dissolving phospholipids and other fats in hypercritical CO_2 at 60°C and 250.00 bar, with 5% ethanol as a co-solvent, to form liposomes[23]. The cDICE approach incorporates zwitterionic lipids into homogeneous giant unilamellar vesicles (GUVs) for specified size and high encapsulation efficiency[63]. The stationary phase interdiffusion approach is primarily based on the diffusible fusion of 2 intermixable phases, resulting in the self-arrangement of lipid particles to produce liposomes[64].

In conclusion, among various liposome preparation methods discussed, three standout approaches for liposome preparation are the Super Lip method using supercritical technology, the Packed Bed Preparation of liposomes and the Conventional Film Hydration. The Super Lip method demonstrates rapid and solvent-free production of liposomes, although its reliance on specialized equipment for handling supercritical fluids may limit its scalability. The Conventional Film Hydration Method offers simplicity but poses safety concerns due to the use of hydrophobic diluents.

The Packed Bed method of preparation of nanosomes allows precise size control and high uniformity, making it suited for large-scale production, albeit with equipment and temperature control requirements. While other methods discussed in the text have their advantages and limitations, factors such as complexity, specialized equipment needs, or scalability issues may hinder their suitability for massive production. Researchers should carefully consider these aspects when selecting a liposome preparation method for their specific study objectives.

Niosomes preparation method: There are several methods available to produce niosomes: The thin film hydration, ether injection, micro fluidization, bubble, transmembrane pH gradient drug uptake process and reverse phase evaporation methods[54,126]. Surface-active agents and cholesterol are dispersed in a hydrophobic solubilizer and vaporized in a thin film hydration process using a rotary evaporating unit. Surface-active agents and cholesterol are dispersed in a hydrophobic solubilizer and vaporized in a thin film hydration process using a rotary evaporating unit.

The resulting thin film is treated and rehydrated by adding a hydrophilic phase comprising the drug and the blend is sonic-wave-treated to form micelles[136]. The surfactant-cholesterol mixture is solubilized in diethyl ether and injected into the pre-warmed aqueous solution containing the drug. The temperature is kept above the boiling point of the solvent and vesicles form as the ether evaporates[17]. In the reverse-phase evaporation method, surfactant and cholesterol are solubilized in a hydrophobic solvent such as $CHCl_3$ or ether. A water-based form of the drug is then added to this mixture and the 2 immiscible phases are homogenized and sonic-wave-treated to form a dispersion. The chloroform is then removed by applying a vacuum to produce liposomes[60]. The transmembrane pH gradient method is based on niosomes using a pH gradient.

In the thin lipid method, films are hydrated with acidic compounds at pH 4. An aqueous suspension of the drug is then appended and the pH is raised to 7 using PBS to allow formation of the niosome bilayer[110]. In the bubble method, inert gas bubbles are injected into a crude dispersion of a non-hydrated mixture of phosphatidylcholine and nonionic surfactant to generate stable niosomes[61].

Table 1
Types of liposomes

Liposome Type	Size (nm)	Surface Charge	Stability	EE (%)	DLC (%)	Surface Modification	Application
Conventional	50-100	Neutral	Moderate	80%	10%	None	Drug delivery[58]
Stealth	80-200	Neutral	High	90%	20%	PEGylation	Enhanced drug delivery[12]
Cationic	100-200	Positive	Moderate	70%	15%	Positively charged	Gene therapy[107]
pH-Sensitive	80-150	Neutral	Moderate	85%	12%	pH-sensitive polymers	Controlled drug release[88]
Temperature-Sensitive	80-120	Neutral	High	95%	18%	Thermosensitive lipids	Hyperthermia-based therapy[85]
Ligand-Targeted	80-200	Neutral	High	90%	20%	Ligand conjugation	Targeted drug delivery[122]
Stimuli-Responsive	80-150	Neutral	High	95%	25%	Stimuli-responsive polymers	On-demand drug release[139]
Long-Circulating	100-200	Neutral	High	90%	22%	Stealth coating	Enhanced drug delivery[93]
Multifunctional	100-200	Variable	High	95%	30%	Multifunctional coatings	Theranostics[84]
pH-Responsive	80-150	Neutral	Moderate	88%	20%	pH-sensitive lipids	pH-triggered drug release[33]

EE-Encapsulation Efficiency, DLC-Drug Loading Capacity, PEG-Polyethylene Glycol, pH-Potential of Hydrogen

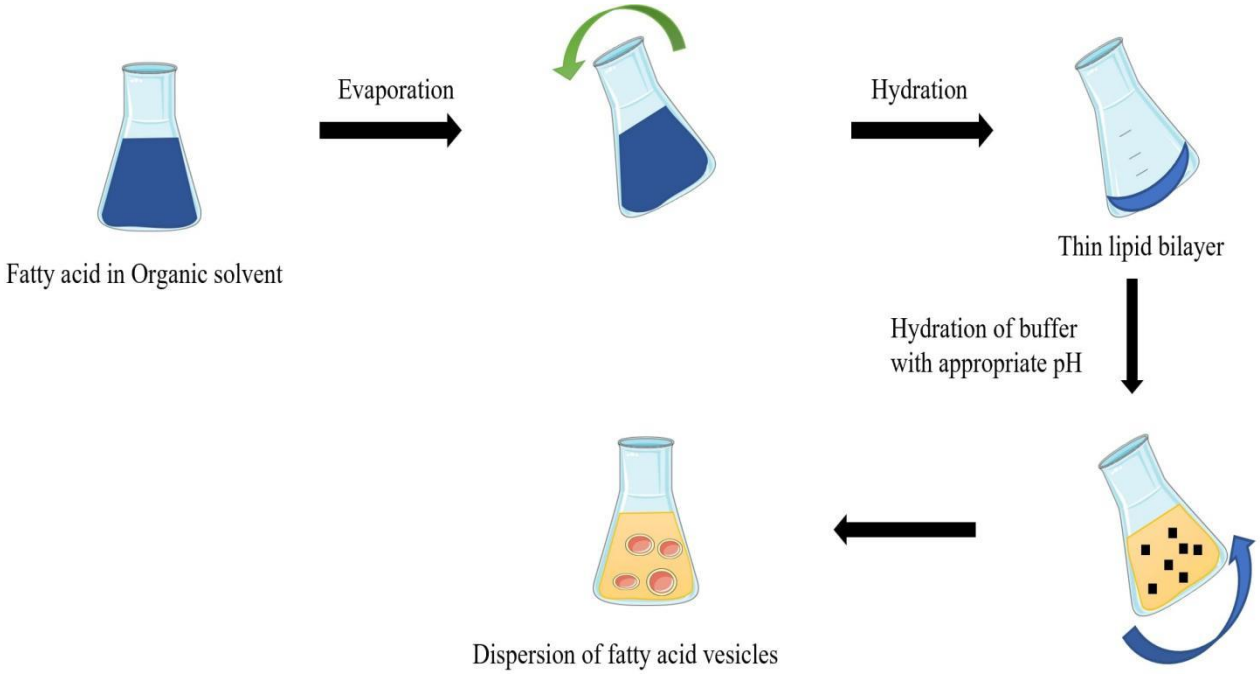

Figure 2: Thin film hydration method

The microfluidic method of niosome preparation utilizes the nonensemble system for controlled formation of vesicular structures through nanoprecipitation. A solvent mixture is used along with an acidic aqueous solution which is heated and then dialyzed against water to obtain drug-loaded niosomes[123]. Among these methods, thin film hydration, micro fluidization and reverse phase evaporation methods are considered as effective approaches. The hydration method is simple and versatile, although the use of organic solvents raises safety concerns.

The reverse phase evaporation method allows for efficient entrapment of both lipophobic and lipophilic drugs but is limited using chloroform. The micro fluidization method offers precise control over niosome formation, enhanced stability and high drug-loading capacity, but it requires specialized equipment. On the other hand, the remaining methods have certain limitations that make them less suitable for publication in scientific papers. The ether injection method poses challenges due to high temperatures and the use of volatile and hazardous solvents. The transmembrane pH gradient method may have limited applicability depending on the properties of the drugs being entrapped. The bubble method, while straightforward, may result in less stable niosomes compared to other methods. Researchers should carefully consider the advantages and limitations of each method when selecting an appropriate approach for their niosome preparation, taking into account factors such as safety, drug characteristics, scalability and equipment availability.

Aquasomes preparation method: Aquasomes are prepared through a three-step process which involves the preparation of the core material, coating of the core material and immobilization of the drug candidate. The core material can be composed of polymers or ceramics, depending on the specific requirements. Commonly used polymers include albumin, gelatin and acrylates while ceramics such as diamond particles, tin oxide and brushite are chosen for their high surface energy and absorbing properties. To enhance binding, a polyhydroxy oligomer surface film is favored. In the second step, the core material is coated with materials such as cellobiose, sucrose, or trehalose. These coating materials offer versatility in drug loading and stability. In the final step, the drug or bio actives are absorbed onto the aquasome surface and interact with the coating through noncovalent and ionic interactions[22].

The preparation method of aquasomes offers various advantages in drug delivery. The use of polymers or ceramics as core materials and coatings allows for flexibility in drug loading and enhances stability. Aquasomes exhibit improved drug solubility, bioavailability and controlled release properties. The non-covalent and ionic interactions between the drug and aquasome surface facilitate efficient drug loading and release. However, researchers should consider limitations such as potential drug leakage and the careful selection of appropriate core materials and coatings. Overall, the aquasome preparation method presents a viable and effective approach for researchers to explore controlled drug delivery systems and targeted therapies. It is a valuable topic in the field of pharmaceutical research.

Ethosomes preparation methods: Lipid-based nanovesicles, ethosomes, are used in dermal and transdermal delivery of molecules through the skin into the body. They were originally formed as an inventive lipid carrier model consisting of ethanol, water and phospholipids. Their primary application is to improve the delivery of API through the dermal medium. There are three common

methods employed for the manufacturing of ethosomes: thin-layer hydration method, cold and hot method.

In thin-layer hydration method, 500mg of PC 90G is mixed in dichloromethane and the dissolver is then vaporized using a rotary evaporating device under the lipid phase transition temperature within a nitrogen gas atmosphere. The resultant film layer is hydrated with azelaic acid, ethanol, PEG and PBS at an acid-base balance of 7.4. The composite is then sonic-treated to yield ethosomes[19]. In the cold method, PC 90G is solubilized in 3.0 mL of alcohol and azelaic acid is solubilized in 5.750 mL of alcohol. These two solutions are mixed together using a magnetic stirrer and the hydrophilic phase at pH 7.4 is added drop by drop to the hydrophobic phase while stirring at 700 rotation per minute for 30 minutes. The resulting mixture is sonicated[24].

The hot method of ethosome preparation involves maintaining a water bath at 40°C, with a beaker containing water inside it. Phospholipid is dispersed in the beaker until a colloidal solution is formed. Ethanol, PEG and drugs are mixed separately. Subsequently, the organic and aqueous phases are combined and stirred together, followed by sonication to form ethosomes[32].

Among these methods, the thin-layer hydration method and cold method are considered effective approaches for ethosome preparation in the field of topical drug administration. The thin-layer hydration method offers simplicity and efficient encapsulation of drugs, resulting in the formation of ethosomes with enhanced capabilities for delivering drugs to the skin. The cold method, although requiring longer mixing times, facilitates the inclusion of both water-liking and lipophilic drugs while maintaining the integrity of the vesicles. On the other hand, the hot method is regarded as less effective due to its limitations. Although it provides convenience in terms of temperature control, the hot method may result in decreased stability and compromised integrity of the ethosomes.

Overall, the development of ethosomes and the optimization of their preparation methods present promising avenues for researchers in the field of topical drug delivery with the prospective to improve patient experiences and therapeutic outcomes.

Route of administration of nanosomes: Nanosomes find extensive applications across numerous industries, specifically in the pharmaceutical sector, where they offer potential therapeutic benefits for the treatment of diverse diseases like cancer, dystrophy, dementia and more. It is important to consider that the properties of nanosomes may vary depending on the method of their preparation. Moreover, the route of administration plays a significant role in governing the distribution, bioavailability, permeability and mode of action of drugs encapsulated within nanosomes.

The selection of the appropriate route of administration is considered by several factors inclusive of the patient's preference and the severity of the disease. Several routes of administration are available such as nasal, oral, transdermal and intravenous, among others. Each route of administration has distinct implications for drug delivery and subsequent therapeutic outcomes.

Oral administration: The oral route of drug administration is predominantly used for the treatment of various chronic diseases and nanosomes can also be administered via this route. In a therapeutic setting, it has been observed that curcumin-loaded nanosomes, coated with the natural alkaline polysaccharide chitosan, demonstrated an higher absorption rate of 7.26% and better bioavailability[166].

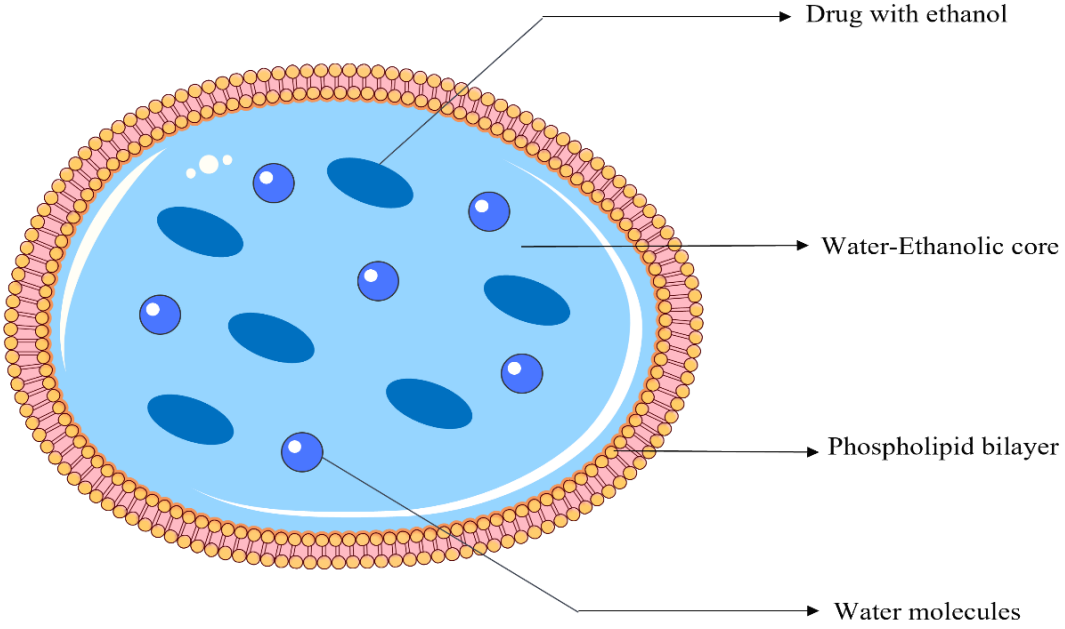

Figure 3: Structure of Ethosomes

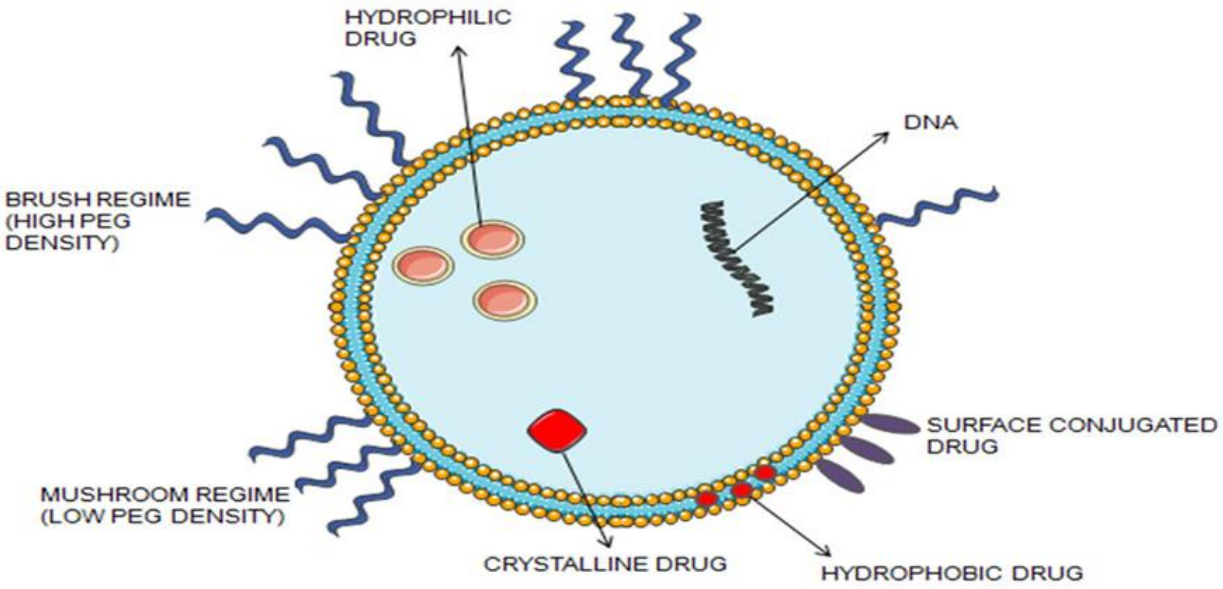

Figure 4: Drug entrapped inside a liposome

Another example involves a nanosomal formulation of resveratrol, utilizing lecithin in a lyophilized form, which exhibited antioxidant and anti-inflammatory properties with a bioavailability of 61%. Notably, it demonstrated a four-fold increase in peak plasma concentration, thereby showing promise for the treatment of diabetes types 1 and 2[108].

For the administration of docetaxel, a novel nanosomal lipid suspension was used as a one-hour intravenous infusion every three weeks, resulting in a potentially promising overall response. However, the most prevalent non-hematological toxicity was nausea with vomiting[18]. To enhance therapeutic efficiency and mitigate the toxic effects of drugs, receptor-specific ligand-crafted active targeting nanocarriers have been employed[127]. Utilizing lipidic nanocarriers cloaked with polysaccharides, botanical anticancer drugs produced from the natural polyphenol curcumin showed an 8.94-fold increase in bioavailability, while limiting the effectiveness of CD133 and ABCG2 by 38% and 32% respectively[155]. Insulin-loaded nanosomes with WGA-N-glut-PE exhibited high entrapment efficiency of 60% and a bioavailability of 7.11% when administered orally[163].

In male rats, an oral administration of vinpocetine, used to treat dementia and stroke, incorporated solid lipid nanoparticles produced by ultrasonic-solvent emulsification. This approach demonstrated a rapid diffusion rate and enhanced bioavailability in the bloodstream[94]. Lipid nano capsules delivering biocompatible ibuprofen achieved an incorporation efficiency of 95%, thereby aiding in the treatment of postoperative pain due to their antinociceptive efficacy[87]. Furthermore, simvastatin-loaded lipid nanoparticles, characterized by their spherical shape and encapsulation efficiency over 95%, exhibited enhanced intestinal absorption and oral bioavailability, making them valuable for the treatment of high cholesterol and coronary heart disease[165].

Solid lipid nanoparticles (SLNs) of buspirone HCl prepared using emulsification evaporation followed by sonication, demonstrated a loading efficiency of 32.8%. Oral administration of 15 mg/kg resulted in a relative increase in bioavailability with approximately 90% of buspirone released within 4.5 hours[147]. Praziquantel-loaded SLNs, produced through ultrasound techniques, showed improved oral bioavailability with an encapsulation efficiency of 80%. These SLNs hold promise as a drug carrier for PZQ and Schistosoma treatment[154].

Transdermal administration: Rice bran oil nanosomes produced via the reverse phase evaporation method contain various antioxidants such as gamma-oryzanol, tocopherols and tocotrienols. These nanosomes exhibit an antioxidant activity of 4.3 mg/mL. They possess excellent entrapment efficiency and permeation capacity, making them suitable for use in cosmetic creams for their anti-aging properties[95]. Soya lecithin-based emulsome nanoparticles have been primarily used for the percutaneous administration of lornoxicam in the treatment of inflammatory joint disorders. These nanoparticles have an entrapment efficiency of 67.75% and an optimized formulation with a release rate of 88%[75]. Cosmetical nanosomes made from oleic acid-conjugated peptides have shown high skin penetration capacity, with potential benefits in anti-aging properties and skin repair[13].

In clinical trials, nano retinol for the treatment of facial acne vulgaris, has been found to be more effective and better tolerated compared to other formulations. Additionally, it demonstrates a reduction in both total and inflammatory acne lesion count[44]. Furthermore, the topical delivery of

PEG-based liposomes loaded with tofacitinib citrate has shown enhanced skin permeability by 4-11 folds. These liposomes remain stable for a period of 6 months, highlighting their effectiveness for topical drug delivery[81].

Nasal administration: To treat Parkinson's disease, L-DOPA was incorporated into a polymer matrix composed of a biodegradable copolymer of glycolic acids. Pharmacokinetic studies were conducted and it was observed that nasal administration resulted in a remarkable increase in drug bioavailability by 244.4%[106]. In the context of Alzheimer's disease treatment, hydrogels loaded with donepezil HCl were prepared. The liposomes exhibited an entrapment efficiency of 62.5% and a vesicle diameter of 438 nm. Intranasal delivery of donepezil demonstrated a significant increase in drug content by 107%[4]. For the treatment of cerebral malaria, nanocomposite liposomes modified with glucose were utilized. These nanosomes had a particle size of 86.2 ± 0.9 nm and an encapsulation efficacy exceeding 90%[137].

Additionally, anionic liposomes incorporated with chitosan DNA complex were found to be capable of delivering anti-caries DNA. These developed nanoparticles provide a potential platform for packaging and delivering DNA vaccines, enabling strong elicitation of mucosal immunity[34]. In the case of valproic acid, an anticonvulsant, it was entrapped in soy-based cholesterol liposomes. This formulation is useful for delivering valproic acid to the brain through intranasal administration with an entrapment efficiency exceeding 85%. Furthermore, it exhibited 2.7 times higher bioavailability compared to the free drug[78].

Intravenous administration: Nanosomal tacrolimus, comprising of castor oil hydrogenated with polysorbate 60, was delivered and analyzed using frozen-fracture electron micrograph. The findings exposed an existence of a homogeneous group of nano-sized matter. Animal toxicity studies demonstrated no mortality, indicating that it is a safer alternative treatment for patients undergoing organ relocation[9]. In the case of Nanosomal Docetaxel Lipid Suspension, IV injection for an hour per cycle of 21 days showed improved clinical efficiency in subjects with stage 4 breast tumor. Each patient received a maximum of 6 cycles of treatment[2]. A combination therapy involving ginsenosides and paclitaxel loaded in a unique nanocarrier exhibited the ability to inhibit human gastric cancer cell proliferation when administered via the intravenous route[68].

In the case of Doxorubicin loaded vesicles, they are capable of specifically targeting Her2+ breast cancer cells, inducing a cytotoxic effect. These liposomes have a size of 101 nm and an entrapment efficacy of 88%[68]. Furthermore, Decoquinate loaded liposomes, when administered intravenously, can effectively suppress the activity of the parasite *P. berghei*, which causes severe malaria. These liposomes exhibit an entrapment efficiency of more than 95% and demonstrate excellent DQ IC50 (half maximal inhibitory concentration) of 1.33 nM[160].

Table 2
Modes of administration of liposomes

Mode of Administration	Commercial Name	Targeted Disease	Liposome Composition	DEE (%)	Stability	Encapsulation Method
Intratumoral	Doxorubicin Liposomal	Various cancers	DSPC, cholesterol, PEG-lipid	95%	Stable	Thin film hydration[10]
Inhalation	Amikacin Liposome Inhalation	Pulmonary infections	DPPC, cholesterol	80%	Stable	Freeze-drying[140]
Subcutaneous	Naltrexone Liposomal	Substance abuse treatment	DSPC, cholesterol	90%	Stable	pH-gradient[38]
Intraperitoneal	Cisplatin Liposome	Peritoneal cancers	DSPC, cholesterol	85%	Stable	Reverse phase evaporation[23]
Topical	AmBisome	Fungal skin infections	Lipid-based	70%	Stable	Ethanol injection[112]
Intravitreal	Triamcinolone Acetonide	Ocular inflammation	DSPC, cholesterol, PEG-lipid	92%	Stable	Bubble method[21]
Intrathecal	DepoCyt	Central nervous system cancers	DSPC, cholesterol	88%	Stable	Microfluidic method[59]
Intramuscular	Morphine Sulfate Liposomal	Pain management	DSPC, cholesterol, PEG-lipid	95%	Stable	Ether injection method[27]
Intravesical	Bacillus Calmette-Gurin	Bladder cancer	Lipid-based	75%	Stable	Transmembrane pH gradient[132]
Intratumoral	Vincristine Sulfate Liposomal	Acute lymphoblastic leukemia	DSPC, cholesterol, PEG-lipid	90%	Stable	Ether injection method[40]

DSPC-Distearoylphosphatidylcholine, PEG-Polyethylene Glycol

Applications of liposomes in field of medical: Liposomes find extensive applications in various domains in the field of pharmacology and medicine, serving as valuable tools for therapeutic and diagnostic purposes. Liposomes can be loaded with drugs or different markers, allowing for targeted delivery and enhanced efficacy. Moreover, liposomes serve as essential models for studying cell interactions, recognition mechanisms and the mode of action of specific substances, providing valuable insights into fundamental biological processes.

One of the significant challenges in drug therapy is the narrow therapeutic window exhibited by many medications where the effective therapeutic concentration is not significantly lower than the potentially harmful concentration. However, by employing suitable drug carriers such as liposomes, it is possible to modulate the pharmacokinetics and biodistribution of drugs. This approach offers several advantages including the potential reduction of toxicity associated with the drug and the enhancement of its therapeutic efficacy[39]. Using appropriate drug carriers, liposomes can effectively optimize the delivery of drugs, striking a balance between effectiveness and safety, thus providing a promising avenue for advancing medical treatments.

Application of liposomes in treating bacterial infections: Liposomes have emerged as versatile tools in the medical field, finding applications in the treatment of various diseases caused by bacteria, viruses, fungi and protozoa. They offer unique advantages such as targeted drug delivery, reduced systemic side effects, improved solubility and prolonged drug release, thereby enhancing the efficacy of therapeutic interventions. For instance, negatively charged deformable rigid bilayer liposomes loaded with the broad-spectrum antibiotic Azithromycin (AZI) have been administered orally to effectively treat cervicovaginal bacterial infections caused by planktonic and biofilm-forming *E. coli* and intracellular chlamydia. The encapsulation of the antibiotic within liposomes enhances its solubility, antibiotic properties and biofilm inhibition capabilities[145].

In the case of *Staphylococcus aureus*, a gram-positive bacterium known for causing systemic infections, phospholipid liposomes containing vancomycin, levofloxacin and rifabutin have been utilized to penetrate and accumulate within biofilms formed by this bacterium. The liposomes demonstrate efficient release of the antibiotics in *in situ* conditions, offering potential therapeutic benefits[49].

Similarly, liposomal vaccines derived from glycosphingolipids (GSLs) have shown promise in combating *Acinetobacter baumannii*, a common pathogen responsible for a range of infections. GSLs liposomes have been found to induce antibody secretion, cytokine production and lymphocyte proliferation, leading to a reduction in biofilm production and improved survival rates[82]. Cationic liposomes, prepared with CMC-PE via amidation reactions, are loaded with a derivative of CSD to target amoxicillin-resistant *Staphylococcus aureus* and exhibit significant antibacterial effects. These liposomes demonstrate high encapsulation efficiency, favorable zeta potential and effective inhibition of biofilm formation[162].

Additionally, liposomes containing ParELC3 have been employed to inhibit the growth of *E. coli* strains responsible for infections. These liposomes exhibit high purity, solubility and the ability to inhibit key bacterial enzymes, thereby displaying potential antibacterial activity[121]. Liposomes have also been used to treat specific bacterial infections. For instance, fusogenic liposomes containing methicillin and Tat47-57 cell-penetrating peptides have shown effective antimicrobial activity against *Neisseria meningitidis*, a bacterium associated with brain inflammation. The liposomes exhibit successful penetration into bacterial cells, highlighting their potential therapeutic value[51]. Combating streptococcal toxic shock syndrome caused by *Streptococcus pneumoniae*, *Streptococcus dysgalactiae* and *Streptococcus pyogenes* subspecies has been explored using liposomes composed of high levels of cholesterol and choline-containing phospholipids. These liposomes demonstrate antagonistic properties against exotoxins produced by these pathogens[25].

Furthermore, liposomes loaded with curcumin have exhibited antagonistic properties against *Aeromonas sobria*, a Gram-negative bacterium associated with gastrointestinal and extraintestinal infections. These liposomes demonstrate potential antibacterial activity against *A.sobria*[26]. To address human salmonellosis, liposomes loaded with geraniol have shown anti-virulence properties, inhibiting the colonization of Salmonella bacteria. The liposomes exhibit high encapsulation efficiency and hold promise for combatting this infectious disease[43]. In the treatment of endophthalmitis, sustained-release liposomes containing moxifloxacin have been investigated as a therapeutic approach. These liposomes, produced by the AL method, demonstrate high encapsulation efficiency and antimicrobial properties, offering potential benefits in treating this intraocular inflammation[105]. Liposomes have also shown promise in targeting specific bacterial toxins. For example, liposomes capable of absorbing cholesterol-dependent toxins such as pneumolysin have been explored for combating *Streptococcus pneumoniae*-induced inflammation and associated responses[52].

Immunization strategies against tuberculosis have been developed using liposomes loaded with tuberculosis antigens Ag85B and ESAT-6. These liposomes, combined with poly:IC adjuvant and phosphatidylserine, effectively reduce bacterial loads in the lungs and spleen, offering a unique vaccination strategy[41]. To tackle *Cutibacterium acnes*, a Gram-negative bacterium known for biofilm production and skin infections, positively charged liposomes

encapsulating enzymes have been developed. These liposomes exhibit high encapsulation efficiency and demonstrate significant inhibition of biofilm growth including effective penetration within the biofilm structure[45].

Application of liposomes in treating fungal infections

Candidiasis, cryptococcosis and aspergillosis are among the diseases responsible for numerous annual fatalities. Amphotericin B, a potent antifungal agent, exhibits effectiveness against *Aspergillus fumigatus*. However, the limited efficacy of antifungal drugs can lead to reduced fungicidal effects. To enhance drug efficiency, dectin-2-coated amphotericin B-loaded liposomes were developed, specifically targeting fungal cells. Dectin-2, a mammalian innate immune membrane receptor, binds to mannans as a dimer to signal fungal infections, with the estimated MICs for AmB species ranging from 0.06 to 1.3 µM[14]. Amphotericin B is a lipophilic macrolide used in antifungal therapy, but its solubility in water is low and it poorly absorbs through the gastrointestinal mucosa and skin. Direct drug release in the body can cause nephrotoxicity.

However, liposome drug delivery systems mitigate amphotericin B's toxicity by controlling the drug's transfer rate from the carrier to the cell membrane[66]. A promising drug, gene and tissue engineering delivery system is the fibrin-based delivery system. Plasma beads, as efficient drug and antigen carriers, effectively load and encapsulate the antifungal compound amphotericin B, releasing the drug at a slower rate compared to liposomal drug delivery. In animal studies, AmpB impregnated plasma beads showed kidney drug levels of 2.3 µg/g and 2.7 µg/g at 24 h[48]. In combination therapies, antifungal drugs like fluconazole, voriconazole, caspofungin and micafungin are used along with amphotericin B and lipid formulations. Fluconazole, in particular, is advanced for treating invasive candidiasis and is well-tolerated, but certain Candida species like *C. krusei* and *C. glabrata* are resistant to it. Dendrimers serve as drug carriers, consisting of core, dendrons and surface-active groups, with drug encapsulation occurring in the dendrimer's inner part. However, these dendrimers have high cytotoxicity and lower stability and biocompatibility[149].

The FDA has approved three amphotericin B liposomes: Ambisome, Abelcet and Amphotec. Ambisome efficiently penetrates the fungal cell wall, binding to membrane sterol components before releasing amphotericin B. Abelcet, a preservative-free phospholipid, is sterile and used to treat invasive and difficult-to-respond-to fungal infections. Finally, amphotec is a parenteral liposome formulation of amphotericin B, characterized by a small, spherical particle size of 100nm[67].

Applications of liposomes in cancer treatment

The liposomes have been manifested as an efficient promising technique for drug delivery in cancer therapy due to their unique chemical and physical traits. These versatile structures can encapsulate both water-repellent and hydrophilous drugs, granting protection against deterioration as well as increasing the drugs bioavailability. Moreover, liposomes can either passively target cancerous growth through the intensified penetration and containment effect, or vigorously target specific cells via ligand-mediated binding mechanisms. One notable example of liposomal drug delivery in cancer treatment is Doxil® (doxorubicin hydrochloride liposome injection), a vesicular formulation of doxorubicin for chemotherapy. Doxil® is used for the management of ovary and breast cancers that have shown resistance to prior chemotherapy[57]. Another liposomal formulation is Irinotecan liposome injection (Onivyde®), which encapsulates the chemotherapy drug irinotecan. It has gained authorization for the therapy of spreading stage of pancreatic cancer that has worsened during gemcitabine-based therapy, usually administered in combination with other drugs[20].

A newer liposomal formulation of Irinotecan called nano liposomal Irinotecan (nal-IRI) has been proposed to enhance its pharmacokinetics and biodistribution. It is approved for use in the treatment of stage 4 pancreatic tumor in mix with 5-FU and leucovorin[55]. Marqibo® is a liposomal combination of the chemotherapy drug oncovin, specifically approved for the diagnosis of recurrent ALL in aged people[124]. Cytarabine liposome injection is another liposomal formulation that contains the chemotherapy drug cytarabine, primarily used to treat lymphomatous meningitis, a complication caused by certain types of lymphomas[120]. Vyxeos® is a liposomal blend of two cancer drugs, daunorubicin and cytarabine. It is employed for treatment of freshly diagnosed AML[141].

In the realm of metastatic colorectal cancer treatment, an oral liposomal formulation combining trifluridine and tipiracil hydrochloride has shown efficacy in patients who have previously received chemotherapy[86]. L-MTP-PE is a liposomal formulation that incorporates muramyl dipeptide, a molecule that stimulates the immune system. It is currently under investigation for the therapy of different cancers, comprising osteosarcoma and mesothelioma, often in combination alongside chemotherapy and radiation therapy. Furthermore, liposome-based drugs have been used in the treatment of numerous other tumor types[104].

Applications of liposomes in autoimmune disease

Inflammation plays a key role in various number of diseases ranging from infections and autoimmune disorders to cancer and organ rejection. Within the site of inflammation, increased vascular permeability is one of the notable vascular consequences[16]. This augmented permeability allows fluid, proteins and leukocytes, particularly leukocytes, to migrate from the blood vessels into the interstitial space at specific locations where the endothelial barrier is more permeable[113]. Due to this phenomenon, liposomes are unable to traverse the intact endothelium barrier in healthy tissues but can selectively extravasate into

inflamed tissues, a process known as passive targeting. Macrophages, as antigen-presenting cells, play a substantial role in inflammation throughout the stages of injury, repair and tissue regeneration[109]. *In vitro* studies have demonstrated that macrophages possess phagocytic activity and can uptake liposomes within a short span of 8 hours[101].

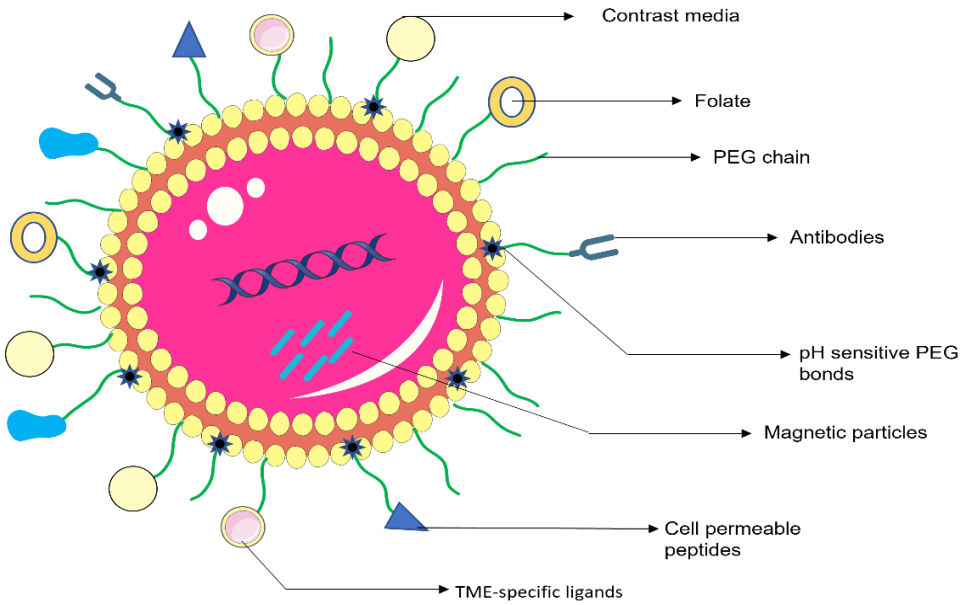

Figure 5: Multi-functional liposomes for targeting tumors

Table 3
Application of liposomes in cancer treatment

Application	Liposome Used	Imaging Modality	Targeting Ligand	Liposome Composition
Photodynamic Therapy	Folate-targeted	Fluorescence imaging, MRI	Folate receptor	Lipid bilayers + Photosensitizing agent[37]
Radiotherapy Enhancement	Radiosensitizer-loaded	Computed tomography (CT) imaging	HER2 receptor	Lipid bilayers + Radiosensitizing agent[31]
Immunomodulation	Cationic	Fluorescence imaging	Mannose receptor	Lipid bilayers + Immunostimulating agent[72]
Gene Delivery	pH-sensitive	Bioluminescence imaging	Transferrin receptor	Lipid bilayers+ Nucleic Acid + Cationic agent[100]
Targeted Drug Delivery	PEGylated	Positron emission tomography (PET)	Epidermal growth factor receptor	Lipid bilayers + Drug + Surface-modifying agent[11]
Vaccine Delivery	Cationic	Fluorescence imaging	Mannose receptor	Lipid bilayers + Antigen + Cationic agent[128]
Controlled Release	pH-sensitive	Near-infrared fluorescence imaging	Transferrin receptor	Lipid bilayers + Drug + pH-sensitive component[138]
Theranostics	Multifunctional	Magnetic resonance imaging (MRI)	HER2 receptor	Lipid bilayers + Drug + Imaging agent[150]
Nanotheranostics	PEGylated	Fluorescence imaging, MRI	Transferrin receptor	Lipid bilayers + Drug + Imaging agent[71]
Combination Therapy	PEGylated	Computed tomography (CT) imaging	Epidermal growth factor receptor, Transferrin receptor	Lipid bilayers + Drugs + Nanoparticle[111]

PEG-Polyethylene Glycol, CT-Computed Tomography, MRI-Magentic Resonance Imaging, PET-Positron emission Tomography, HER2-Human Epidermal Growth Factor Receptor

Inflammatory conditions are well-suited for passive liposomal drug delivery due to the localized extravasation of liposomes and subsequent cellular absorption. Several pre-clinical investigations have explored the potential advantages of liposomal drug delivery in the treatment of inflammatory conditions. Particularly, researchers studied liposomal formulations of conventional drug for the management of rheumatoid arthritis[50,115,142]. These studies consistently demonstrate improved efficacy or better localization of the liposomal drugs when compared to conventional therapies. Furthermore, significant progress has been made in the development of liposomal medications that are designed for local release triggered by factors such as pH, ultrasound, or thermosensitivity, or modified for enhanced accumulation through peptide binding or folate conjugation[35,56,129,153].

Additionally, liposomes have been utilized for co-delivery of conventional drugs to explore potential synergistic effects[42,102,148,164]. Liposomal formulations have also provided a new perspective on drugs that were previously discontinued due to severe adverse effects. For example, liposomal administration of a sulfapyridine prodrug resulted in improved intraarticular retention and increased therapeutic efficacy[80]. In a clinical evaluation involving psoriasis patients, an ethosomal gel demonstrated superior efficacy compared to a liposomal formulation[47]. The utilization of liposomal encapsulation for biologics, employing spherical nucleic acids targeting the IL-17 receptor, has shown promising outcomes in a mouse model with psoriatic plaque induced by imiquimod[91]. Combination therapy involving laser irradiation and controlled liposomal drug release exhibited encouraging results, with treated mice showing no recurrence of symptoms[89].

Furthermore, liposomal encapsulation of dithranol demonstrated positive outcomes, achieving complete lesion clearance in five out of nine treated patients and a 50% reduction in lesions in the remaining two[77]. More recently, a liposomal formulation of prednisolone was tested in a mouse model of arteriovenous fistulas (AVF) revealing the suppression of vascular inflammation and promotion of outward remodeling[152]. In a randomized clinical trial, the administration of liposomal prednisolone was found safe for patients after AVF surgery, although no significant improvement in effective AVF maturation was observed[143].

Applications of Phytosomes

Phytosomes have emerged as advanced delivery systems within the field of phytotherapy, attracting considerable attention due to their remarkable enhancements in bioavailability and therapeutic efficacy. These specialized complexes comprise of plant extracts and phospholipids, which synergistically create unique structures capable of enhancing the solubility and permeability of active constituents. Notably, phospholipids like phosphatidylcholine play a crucial role in creating phytosomes by encapsulating the hydrophobic elements found in plant extracts. This process results in improved stability and absorption properties. Phytosomes bring a multitude of benefits including enhanced cellular uptake, extended circulation time and the ability to target specific tissues or organs.

Researchers have conducted extensive investigations into the potential uses of phytosomes across various therapeutic fields including their effectiveness in anti-inflammatory, anticancer and antioxidant interventions. Moreover, phytosomes have shown promising results in enhancing the delivery of herbal drugs with low solubility, effectively addressing the limitations of conventional herbal formulations. This comprehensive review aims to offer a detailed overview of the diverse applications of phytosomes, highlighting their potential in optimizing phytotherapeutic approaches and opening doors for innovative drug delivery strategies.

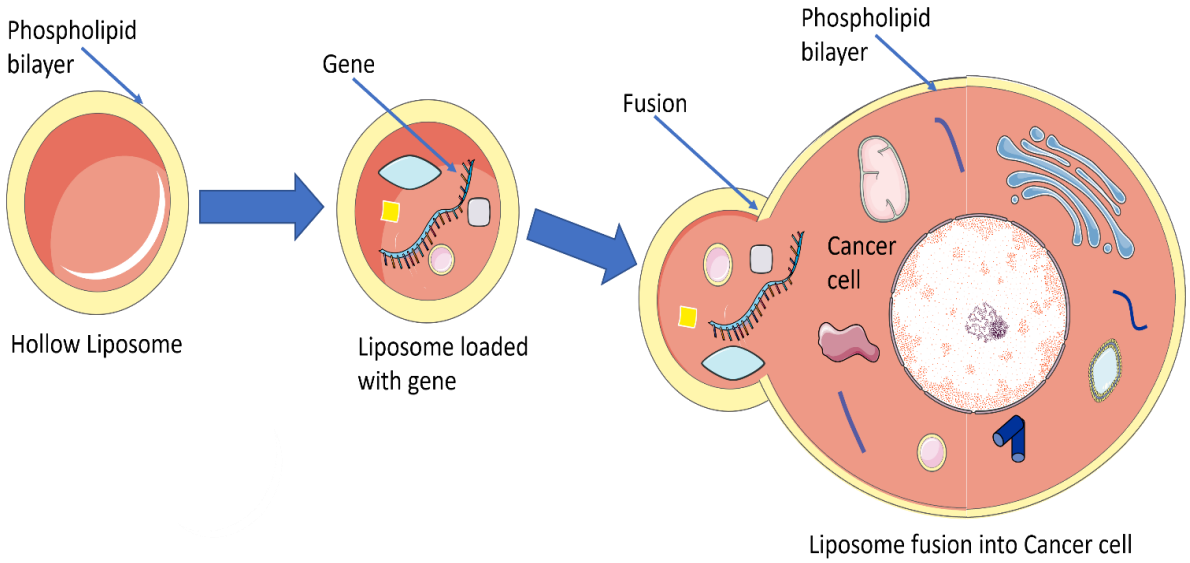

Figure 6: Surface topology of phytosomes

Table 4
Application of Phytosomes

Phytosome	Phyto-constituent	Lipid composition used	Pharmaceutical activity
Scorpion Venom-Functionalized Quercetin Phytosomes	Scorpion Venom peptide and Quercetin	Phospholipo® 90H	Anti breast cancer activity[7]
Optimized Icariin Phytosomes	Icariin	Phospholipo® 90H	Cytotoxic and apoptosis enhancing activities in ovarian cancer cells[8]
Thymoquinone-Loaded Soy-Phospholipid phytosomes	Thymoquinone	Phospholipo® 90H	Anti-cancer activity in human lung cancer cells[5]
Crocetin phytosomes from NAT	crocetin	phosphatidyl choline and cholesterol	Wound and cut healing properties in rat model[5]
Piper longum and Abutilon indicum loaded phytosomes	*Ethanolic extract of Abutilon indicum.*and *Piper longum*	phospholipid (soy PC)	Hepatoprotective effect[125]
Cisplatin and curcumin coloaded lipid nanosomes	Cisplatin and curcumin	DMPC AND DOPA	High cytotoxic effect on hepG2 cells[36]
Cholate modified polymer-lipid nanoparticles	Quercetin	PLGA and lecithin	Antileukemic effect[159]
Piceatannol loaded bilosome	piceatannol	cholesterol	Cytostatic and apoptotic activity in lung cancer cells[6]
Targeted lipid nano structured lipid crystal of folate	letrozol	compritol	Improve efficiency of LTZ on breast cancer cells[119]
2-Methoxy 3-estradiol loaded polymeric micelle	2-Methoxy 3-estradiol	EG-PLGA and ALA	Enhancing anti-cancer activity in prostate cancer[6]

DMPC-Dimyristoyl-sn-glycero-3-phosphocholine, PLGA-Poly Lactic co Glycolic Acid, ALA-Alanine

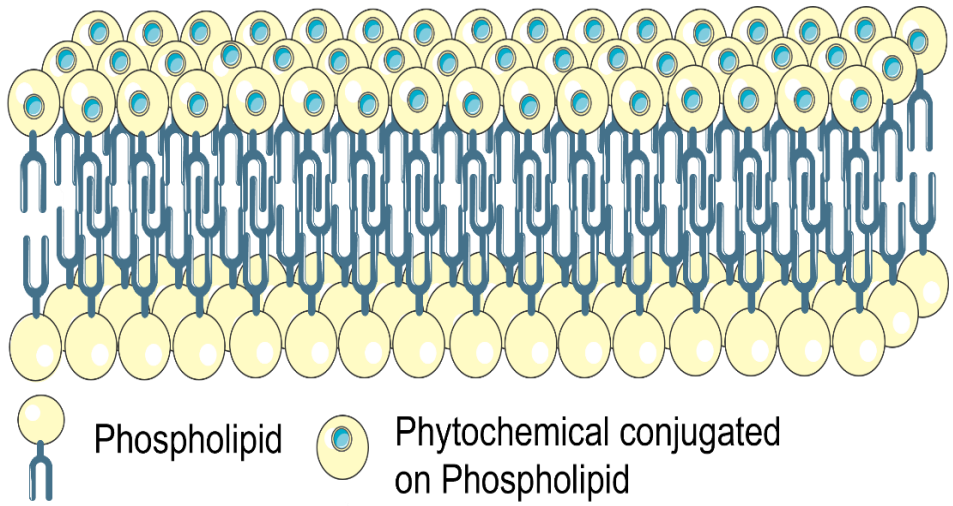

Figure 7: Gene delivery through liposomes

Gene delivery through nanosomes: Gene therapy has arisen as a promising clinical intervention for the treatment of various conditions including cancer, cardiovascular disorders, dermatological disorders, infectious diseases and neurological ailments. Its substantial development over the recent decades has propelled it into the forefront of medical research[156]. Currently, gene transfer methods can be categorized into three major groups: viral (transduction) and physical and direct microinjection[76]. Notably, liposome-based DNA delivery systems have been developed, some of which incorporate molecular components for targeted cell surface receptor recognition or evasion of the lysosomal compartment. Additionally, a recently developed technique utilizing cationic lipids has gained significant interest for its potential in gene transfer[118].

Positively charged liposomes, known as cationic liposomes, have gained recognition in the field of gene therapy because of their beneficial interactions with negatively charged DNA and cell membranes[90]. The transfection process using cationic liposomes is highly efficient, as the complex readily binds to and is internalized by cells. The versatile nature of cationic liposomes makes them attractive for DNA delivery into mammalian cells[46]. Liposomes have also been applied for ultrasound technique and enhanced drug delivery, with gas and drugs being encapsulated within the liposomal structure. Echogenic liposomes, produced through various techniques like lyophilization, pressurization and biotin-avidin binding, have demonstrated notable *in vivo* uses in the treatment of cardiac disease, stroke and tumor therapy[70].

Additionally, liposomes have showcased their efficacy as delivery systems for gene therapy and drug transport to skin tissues. The application of the PINC polymer and a mixture of propylene glycol, alcohol and water have shown success in delivering beta-galactosidase or luciferase DNAs[114]. Encapsulation of DNA and conjugation with monoclonal antibodies (MAbs) has consistently shown high efficiency. Furthermore, the stability of liposomal complexes and their ability to block transfection of N2A cells have been maintained even after 48 hours in a physiological buffer[117].

Limitations of Nanosomal delivery of drugs

Nanosomal drug delivery has emerged as a promising technique to improve the therapeutic effectiveness of drugs through their encapsulation within nanoscale vesicles. These nanosomes are typically composed of lipids, polymers, or other biocompatible materials and can be engineered to accommodate various payloads including small molecule drugs, nucleic acids and proteins. However, despite their potential advantages, there are several limitations associated with the utilization of nanosomal drug delivery systems that necessitate careful consideration. This study aims to provide an encompassing overview of these limitations, encompassing biocompatibility, cost, stability and clearance concerns. Thorough comprehension of these limitations is vital for the development of safe and efficacious nanosomal drug delivery systems for clinical implementation[74].

One key limitation of nanosomal drug delivery systems is their constrained drug loading capacity due to their small size and surface area. For instance, a study demonstrated that nanosomes loaded with curcumin exhibited a loading efficiency of merely 17.9% owing to the limited solubility of the drug in the lipid bilayer[15]. Additionally, nanosomal drug delivery systems can display instability and a restricted shelf life, particularly if they are not stored under appropriate conditions. Research has reported that nanosomes containing docetaxel possessed a short shelf life due to drug degradation and aggregation[116]. Furthermore, the targeting ability of nanosomal drug delivery systems can be limited, as they may struggle to differentiate between healthy and diseased cells. An investigation revealed that nanosomes containing doxorubicin targeted both cancerous and healthy cells, leading to toxicity in normal cells[99]. The production of nanosomes can be costly, time-consuming and challenging to scale up for clinical use. For instance, the production of nanosomes containing siRNA was limited, posing challenges in translating the technology into clinical practice[62]. Additionally, nanosomes may elicit immune responses and provoke toxicity in the body due to their foreign nature and potential interactions with biological components. Studies have demonstrated that nanosomes containing siRNA induced an immune response in mice, resulting in liver toxicity[96].

Conclusion

In summary, liposomes have revolutionized the fields of disease management, pharmaceutical biotechnology and gene therapy. These lipid-based nanoparticles have showcased remarkable versatility and effectiveness as carriers for various therapeutic agents, allowing for precise drug delivery to specific locations within the body. Furthermore, liposomes have played a crucial role in advancing gene therapy by facilitating the efficient delivery of genetic materials, opening up new avenues for treating a wide range of medical conditions.

As technology continues to advance and our understanding of liposomes deepens, their potential in drug delivery and therapeutic applications remains incredibly promising. Therefore, it is crucial for researchers and scientists to continue exploring and refining the utilization of liposomes, offering innovative solutions to current and future healthcare challenges.

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References

1. Abkarian M., Loiseau E. and Massiera G., Continuous droplet interface crossing encapsulation (cDICE) for high throughput monodisperse vesicle design, *Soft Matter*, **7(10)**, 4610–4614 **(2011)**

2. Ahmad A. et al, Therapeutic efficacy of a novel Nanosomal Docetaxel lipid suspension compared with Taxotere in locally advanced or Metastatic breast cancer patients, *Clinical Breast Cancer*, **14(3)**, 177–181 **(2014)**

3. Akbarzadeh A. et al, Liposome: Classification, preparation and applications, *Nanoscale Research Letters*, **8(1)**, 1–9 **(2013)**

4. Al Harthi S., Alavi S.E., Radwan M.A., El Khatib M.M. and AlSarra I.A., Nasal delivery of donepezil HCl-loaded hydrogels for the treatment of Alzheimer's disease, *Scientific Reports*, **9(1)**, DOI:10.1038/s41598-019-46032-y **(2019)**

5. Alhakamy N.A. et al, Optimized Icariin Phytosomes: Enhanced cytotoxic and apoptosis enhancing activities in ovarian cancer cells, *Nanomaterials*, **10(9)**, 1802 **(2020)**

6. Alhakamy N.A. et al, Piceatannol loaded bilosome: formulation optimization, characterization and evaluation of its cytostatic and apoptotic activity in lung cancer cells, *Pharmaceutics*, **13(1)**, 94 **(2021)**

7. Alhakamy N.A. et al, Scorpion Venom-Functionalized Quercetin Phytosomes for Breast Cancer Management: In Vitro Response Surface Optimization and Anticancer Activity against MCF-7 Cells, *Polymer*, **14(1)**, 93 **(2022)**

8. Alhakamy N.A. et al, Boosting curcumin activity against human prostatic cancer PC3 cells by utilizing scorpion venom conjugated phytosomes as promising functionalized nanovesicles, *Drug Delivery*, **29(1)**, 807 **(2022)**

9. Ali S.M. et al, Polyoxyl 60 hydrogenated castor oil free nanosomal formulation of immunosuppressant Tacrolimus: Pharmacokinetics, safety and tolerability in rodents and humans, *International Immunopharmacology*, **10(3)**, 325–330 **(2010)**

10. Allen T.M. and Cullis P.R., Drug delivery systems: Entering the mainstream, *Science*, **303(5665)**, 1818-1822 **(2002)**

11. Alsouz Mohammed A.K. and Al-jibouri Mahmoud Najim, Synthesis and Characterization of New Azo dye and applications in colorimetric analysis of trace metal ions in Aqueous Medium, *Res. J. Chem. Environ.*, **27(5)**, 22-26 **(2023)**

12. Allen T.M., Hansen C., Martin F. and Redemann C., Attachment of antibodies to sterically stabilized liposomes: Evaluation, comparison and optimization of coupling procedures, *Biochimica et Biophysica Acta Biomembranes*, **1239(2)**, 133-144 **(1995)**

13. Alvarez-Román R., Naik A., Kalia Y.N., Fessi H. and Guy R.H., Visualization of skin penetration using confocal laser scanning microscopy, *European Journal of Pharmaceutics and Biopharmaceutics*, **58(2)**, 301-316 **(2004)**

14. Ambati S. et al, Dectin-2-Targeted Antifungal Liposomes Exhibit Enhanced Efficacy, *mSphere*, **4(5)**, e00715-19 **(2019)**

15. Anand P., Kunnumakkara A.B., Newman R.A. and Aggarwal B.B., Bioavailability of curcumin: Problems and promises, *Molecular Pharmaceutics*, **4(6)**, 807-818 **(2007)**

16. Antwi S. et al, Prophylactic or therapeutic administration of *Holarrhena floribunda* hydro ethanol extract suppresses complete Freund's adjuvant-induced arthritis in Sprague-Dawley rats, *Journal of Inflammation (London)*, **19(1)**, doi:10.1186/s12950-022-00301-2 **(2022)**

17. Aparajay P. and Dev A., Functionalized niosomes as a smart delivery device in cancer and fungal infection, *European Journal of Pharmaceutical Sciences*, **168**, 106052 **(2022)**

18. Ashraf M. et al, 156P Efficacy and safety of a novel nanosomal docetaxel lipid suspension (NDLS) as an anti-cancer agent - a retrospective study, *Annals of Oncology*, **27**, ix49 **(2016)**

19. Babaie S. et al, Nanoethosomes for dermal delivery of lidocaine, *Advanced Pharmaceutical Bulletin*, **5(4)**, 549 **(2015)**

20. Baker D.E. and Levien T.L., Irinotecan liposome injection, *Hospital Pharmacy*, **52(2)**, 144-150 **(2017)**

21. Bakri S.J. et al, Pharmacokinetics of intravitreal triamcinolone acetonide in silicone oil-filled eyes, *Retina*, **29(5)**, 692-699 **(2009)**

22. Banerjee S. and Sen K.K., Aquasomes: A novel nanoparticulate drug carrier, *Journal of Drug Delivery Science and Technology*, **43**, 446-452 **(2018)**

23. Bannon P.G. et al, Encapsulation of cisplatin in lipid micelles improves drug delivery to ovarian cancer, *Journal of Controlled Release*, **269**, 352-361 **(2018)**

24. Barupal A.K., Gupta V. and Ramteke S., Preparation and characterization of ethosomes for topical delivery of aceclofenac, *Indian Journal of Pharmaceutical Sciences*, **72(5)**, 582 **(2010)**

25. Besançon H. et al, Tailored liposomal nanotraps for the treatment of Streptococcal infections, *Journal of Nanobiotechnology*, **19(1)**, DOI:10.1186/s12951-021-00775-x **(2021)**

26. Bhaskar S. et al, Liposomes as a drug carrier for the treatment of pain, *Journal of Controlled Release*, **269**, 18-24 **(2018)**

27. Bhuvaneshwari R., Johnny Subakar Ivin J., Kanmani Bharathi J., Joshi J.L. and Anandan R., In vitro regeneration through organogenesis in Costus igneus–an important herbal insulin plant, *Res. J. Biotech.*, **18(3)**, 70-78 **(2023)**

28. Billerit C., Jeffries G.D.M., Orwar O. and Jesorka A., Formation of giant unilamellar vesicles from spin-coated lipid films by localized IR heating, *Soft Matter*, **8(42)**, 10823-10826 **(2012)**

29. Carugo D. et al, Liposome production by microfluidics: potential and limiting factors, *Scientific Reports*, **6(1)**, 25876 **(2016)**

30. Carvalho K. et al, Giant unilamellar vesicles containing phosphatidylinositol(4,5) bisphosphate: Characterization and functionality, *Biophysical Journal*, **95(9)**, 4348-4360 **(2008)**

31. Caster J.M. et al, Nanoparticle delivery of chemosensitizers improves chemotherapy efficacy without incurring additional toxicity, *ACS Nano*, **8(8)**, 9758-9766 **(2014)**

32. Chauhan N. et al, Ethosomes: A novel drug carrier, *Annals of Medicine and Surgery*, **82**, 104595 **(2022)**

33. Chen C.K. et al, pH-Responsive drug delivery systems for tumor targeting, *Theranostics*, **9(26)**, 7866-7883 **(2019)**

34. Chen L. et al, Enhanced nasal mucosal delivery and immunogenicity of anti-caries DNA vaccine through incorporation of anionic liposomes in chitosan/DNA complexes, *PLoS One*, **8(8)**, 71953 **(2013)**

35. Chen M. et al, Folate receptor-targeting and reactive oxygen species-responsive liposomal formulation of methotrexate for treatment of rheumatoid arthritis, *Pharmaceutics*, **11(11)**, 582 **(2019)**

36. Cheng Y. et al, Cisplatin and curcumin co-loaded lipid nanosomes for the treatment of HepG2 cells, *International Journal of Nanomedicine*, **13**, 2047-2063 **(2018)**

37. Cheng Z. et al, Multifunctional nanoparticles: Cost versus benefit of adding targeting and imaging capabilities, *Science*, **338(6109)**, 903-910 **(2012)**

38. Comer J., Gilmartin B. and Shen F., Naltrexone formulations for substance abuse treatment: Recent advances and future prospects, *Expert Opinion on Drug Delivery*, **3(3)**, 361-374 **(2006)**

39. Daraee H. et al, Application of liposomes in medicine and drug delivery, *Artificial Cells Nanomedicine and Biotechnology*, **44(1)**, 381-391 **(2016)**

40. DeAngelo D.J. et al, Long-term outcome of a pediatric-inspired regimen used for adults aged 18–50 years with newly diagnosed acute lymphoblastic leukemia, *Leukemia*, **24(11)**, 2062-2067 **(2010)**

41. Diogo G.R. et al, Immunization with *Mycobacterium tuberculosis* antigens encapsulated in phosphatidylserine liposomes improves protection afforded by BCG, *Frontiers in Immunology*, **10**, 1349 **(2019)**

42. Duan W. and Li H., Combination of NF-kB targeted siRNA and methotrexate in a hybrid nanocarrier towards the effective treatment in rheumatoid arthritis, *Journal of Nanobiotechnology*, **16(1)**, 58 **(2018)**

43. Ekonomou S.I. et al, Development of Geraniol-Loaded Liposomal Nanoformulations against *Salmonella* colonization in the pig gut, *Journal of Agricultural and Food Chemistry*, **70(23)**, 7004 **(2022)**

44. El-Samahy M. et al, Effect of topical application of nano retinol on mild to moderate acne vulgaris, *Egyptian Journal of Hospital Medicine*, **68(1)**, 1049–1058 **(2017)**

45. Ekonomou S.I. et al, Development of Geraniol-Loaded Liposomal Nanoformulations against *Salmonella* colonization in the pig gut, *Journal of Agricultural and Food Chemistry*, **70(23)**, 7004 **(2022)**

46. Fang J.Y. et al, Facile biofilm penetration of cationic liposomes loaded with DNase I/proteinase K to eradicate *Cutibacterium acnes* for treating cutaneous and catheter infections, *International Journal of Nanomedicine*, **16**, 8121 **(2021)**

47. Farhood H. et al, Cationic liposomes for direct gene transfer in therapy of cancer and other diseases, *Annals of the New York Academy of Sciences*, **716(1)**, 23-35 **(1994)**

48. Fathalla D. et al, Liposomal and ethosomal gels for the topical delivery of anthralin: Preparation, comparative evaluation and clinical assessment in psoriatic patients, *Pharmaceutics*, **12(5)**, 446 **(2020)**

49. Fatima M.T. et al, Plasma bead entrapped liposomes as a potential drug delivery system to combat fungal infections, *Molecules*, **27(3)**, 1105 **(2022)**

50. Ferreira M. et al, Liposomes as a nanoplatform to improve the delivery of antibiotics into *Staphylococcus aureus* biofilms, *Pharmaceutics*, **13(3)**, 1–25 **(2021)**

51. Ferreira-Silva M. and Faria-Silva C., Liposomal nanosystems in rheumatoid arthritis, *Pharmaceutics*, **13(4)**, 454 **(2021)**

52. Flaifel Mustafa Khaled, Qaralleh Haitham, Dmour Saif M., Alqaraleh Moath and Al-Kabariti Aya Yassin, Azadirachta indica (Neem) Leaf: chemical composition and antibacterial activity as determined by different polarity solvents, *Res. J. Biotech.*, **18(2)**, 55-62 **(2023)**

53. Garcia C.B. and Shi D., Tat-functionalized liposomes for the treatment of meningitis: an in vitro study, *International Journal of Nanomedicine*, **12**, 3009–3021 **(2017)**

54. Genç R. and Ortiz M., Curvature-tuned preparation of nanoliposomes, *Langmuir*, **25(21)**, 12604–12613 **(2009)**

55. Gharbavi M. and Amani J., Niosome: a promising nanocarrier for natural drug delivery through blood-brain barrier, *Advances in Pharmacological Sciences*, doi: 10.1155/2018/6847971.6847971 **(2018)**

56. Glassman D.C. et al, Nanoliposomal irinotecan with fluorouracil for the treatment of advanced pancreatic cancer, a single institution experience, *BMC Cancer*, **18(1)**, 693 **(2018)**

57. Gouveia V.M. et al, Hyaluronic acid-conjugated pH-sensitive liposomes for targeted delivery of prednisolone on rheumatoid arthritis therapy, *Nanomedicine*, **13(9)**, 1037–1049 **(2018)**

58. Gregoriadis G., Liposome technology: liposome preparation and related techniques, *Journal of Drug Targeting*, **3(6)**, 479-487 **(1995)**

59. Groves M.J. and Mawas F., Microencapsulation of opioid-containing liposomes for intrathecal administration: a drug delivery system for pain management, *Journal of Controlled Release*, **107(3)**, 542-553 **(2005)**

60. Guinedi A.S. and Mortada N.D., Preparation and evaluation of reverse-phase evaporation and multilamellar niosomes as ophthalmic carriers of acetazolamide, *International Journal of Pharmaceutics*, **306(1–2)**, 71–82 **(2005)**

61. Gupta Jeetendra Kumar, Implications of Glutathione-S-transferases in Mitogen-activated protein kinase pathway: Risk associated with anticancer drug resistance, Res. J. Chem. Environ., **26(12)**, 185-190 **(2022)**

62. Hachi Mohamed, Hamidi Mohamed, Touati Mostefa, Berrabah Yasmina and Korichi Ayoub, Phytoremediation potential of spontaneous plant species in soils contaminated by hexavalent chromium in Djelfa city (Algeria), *Res. J. Chem. Environ.*, **26(1)**, 66-74 **(2022)**

63. Hardee C.L. and Arévalo-Soliz L.M., Advances in non-viral DNA vectors for gene therapy, *Genes*, **8(7)**, 65 **(2017)**

64. Has C. and Phapal S.M., Rapid single-step formation of liposomes by flow assisted stationary phase interdiffusion, *Chemistry and Physics of Lipids*, **212**, 144–151 **(2018)**

65. Has C. and Sunthar P., A comprehensive review on recent preparation techniques of liposomes, *Journal of Liposome Research*, **30(4)**, 336–365 **(2020)**

66. He Y. et al, Liposomes and liposome-like nanoparticles: from anti-fungal infection to the COVID-19 pandemic treatment, *Asian Journal of Pharmaceutical Sciences*, **17(6)**, 817 **(2022)**

67. Hoang Lam Minh, Tuyet Le Thi Vi and Ha Tran Le Bao, Platelet-rich Plasma Extract promoting Migration of Mouse Bone Marrow Cells, *Res. J. Biotech.*, **17(3)**, 42-47 **(2022)**

68. Hong C. et al, Novel ginsenoside-based multifunctional liposomal delivery system for combination therapy of gastric cancer, *Theranostics*, **9(15)**, 4437–4449 **(2019)**

69. Hood R.R. and Devoe D.L., High-throughput continuous flow production of nanoscale liposomes by microfluidic vertical flow focusing, *Small*, **11(43)**, 5790–5799 **(2015)**

70. Huang S.L., Liposomes in ultrasonic drug and gene delivery, *Advanced Drug Delivery Reviews*, **60(10)**, 1167-1176 **(2008)**

71. Huang Y. and Goel S., Vascular normalization as an emerging strategy to enhance cancer immunotherapy, *Cancer Research*, **77(3)**, 681-686 **(2017)**

72. Irvine D.J. and Hanson M.C., Synthetic nanoparticles for vaccines and immunotherapy, *Chemical Reviews*, **115(19)**, 11109-11146 **(2015)**

73. Jaafar-Maalej C. and Charcosset C., A new method for liposome preparation using a membrane contactor, *Journal of Liposome Research*, **21(3)**, 213-220 **(2011)**

74. Jain A. and Thakur K., Limitations of nanosomal delivery of drugs: a review, *Journal of Nanoscience and Nanotechnology*, **19(10)**, 6057-6074 **(2019)**

75. Jatav M.P. and Ramteke S., Formulation and evaluation of lecithin organogel for treatment of arthritis, *International Journal of Scientific World*, **3(2)**, 267 **(2015)**

76. Jin L. and Zeng X., Current progress in gene delivery technology based on chemical methods and nano-carriers, *Theranostics*, **4(3)**, 240-255 **(2014)**

77. Jovanović M. and Golušin Z., Nonsteroidal topical immunomodulators in allergology and dermatology, *Biomedical Research International*, **2016**, 2016 **(2016)**

78. Jufri M. and Yuwanda A., Study of valproic acid liposomes for delivery into the brain through an intranasal route, *Heliyon*, **8(3)**, e09030 **(2022)**

79. Kankala R.K. and Zhang Y.S., Supercritical fluid technology: an emphasis on drug delivery and related biomedical applications, *Advanced Healthcare Materials*, **6(16)**, 1700433 **(2017)**

80. Kapoor B. et al, Fail-safe nano-formulation of prodrug of sulfapyridine: preparation and evaluation for treatment of rheumatoid arthritis, *Materials Science and Engineering C: Materials for Biological Applications*, **118**, 111332 **(2021)**

81. Kathuria H. and Nguyen D.T.P., Proposome for transdermal delivery of tofacitinib, *International Journal of Pharmaceutics*, **585**, 119558 **(2020)**

82. Khan M.A. and Allemailem K.S., Glycosphingolipids (GSLs) from *Sphingomonas paucimobilis* increase the efficacy of liposome-based nanovaccine against *Acinetobacter baumannii*-associated pneumonia in immunocompetent and immunocompromised mice, *Molecules*, **27(22)**, 7790 **(2022)**

83. Kikuchi H. and Yamauchi H., A spray-drying method for mass production of liposomes, *Chemical and Pharmaceutical Bulletin (Tokyo)*, **39(6)**, 1522–1527 **(1991)**

84. Kim J. and Piao Y., Multifunctional nanostructured materials for multimodal imaging and simultaneous imaging and therapy, *Chemical Society Reviews*, **47(6)**, 1805-1819 **(2018)**

85. Kono K., Thermosensitive polymer-modified liposomes, *Advanced Drug Delivery Reviews*, **53(3)**, 307-319 **(2001)**

86. Kotani D. et al, Retrospective cohort study of trifluridine/tipiracil (TAS-102) plus bevacizumab versus trifluridine/tipiracil monotherapy for metastatic colorectal cancer, *BMC Cancer*, **19(1)**, 1253 **(2019)**

87. Krupa S. and Siddalinga Murthy K.R., Phenolics, proteins, antioxidant activity and GC – MS analysis of Artocarpus heterophyllus Lam seeds, *Res. J. Biotech.*, **17(3)**, 129-133 **(2022)**

88. Lamprecht A. and Saumet J.L., Lipid nanocarriers as drug delivery system for ibuprofen in pain treatment, *International Journal of Pharmaceutics*, **278(2)**, 407–414 **(2004)**

89. Lasic D.D., Doxorubicin in sterically stabilized liposomes, *Nature*, **380(6574)**, 561-562 **(1996)**

90. Liu C. et al, Barriers and strategies of cationic liposomes for cancer gene therapy, *Molecular Therapy Methods and Clinical Development*, **18**, 751-764 **(2020)**

91. Liu H. et al, Targeting the IL-17 receptor using liposomal spherical nucleic acids as topical therapy for psoriasis, *Journal of Investigative Dermatology*, **140(2)**, 435-444.e4 **(2020)**

92. Liu Y. and Wu K., Continuous production of antioxidant liposome for synergistic cancer treatment using high-gravity rotating packed bed, *Chemical Engineering Journal*, **334**, 1766–1774 **(2018)**

93. Lukyanov A.N. and Elbayoumi T.A., Tumor-targeted liposomes: doxorubicin-loaded long-circulating liposomes modified with anti-cancer antibody, *Journal of Controlled Release*, **100(2)**, 135-144 **(2004)**

94. Luo Y.F. and Chen D.W., Solid lipid nanoparticles for enhancing vinpocetine's oral bioavailability, *Journal of Controlled Release*, **114(1)**, 53–59 **(2006)**

95. Maharachatahtakul P., Development of Rice Bran Oil Nanosome for Using in Cosmetic, Master's thesis, Faculty of Pharmaceutical Sciences, Prince of Songkla University **(2008)**

96. Maruyama K., Intracellular targeting delivery of liposomal drugs to solid tumors based on EPR effects, *Advanced Drug Delivery Reviews*, **63(3)**, 161-169 **(2011)**

97. Massing U. and Cicko S., Dual asymmetric centrifugation (DAC) - a new technique for liposome preparation, *Journal of Controlled Release*, **125(1)**, 16–24 **(2008)**

98. Mertins O. and Da Silveira N.P., Electroformation of giant vesicles from an inverse phase precursor, *Biophysical Journal*, **96(7)**, 2719 **(2009)**

99. Minchinton A.I. and Tannock I.F., Drug penetration in solid tumours, *Nature Reviews Cancer*, **6(8)**, 583-592 **(2006)**

100. Mintzer M.A. and Simanek E.E., Nonviral vectors for gene delivery, *Chemical Reviews*, **109(7)**, 259-302 **(2009)**

101. Minutolo R. et al, Hyporesponsiveness to erythropoiesis-stimulating agents and renal survival in non-dialysis CKD patients, *Nephrology Dialysis Transplantation*, **27(7)**, 2880–2886 **(2012)**

102. Mohanty S. et al, Naringin in combination with isothiocyanates as liposomal formulations potentiates the anti-inflammatory activity in different acute and chronic animal models of rheumatoid arthritis (Erratum), *ACS Omega*, **6(4)**, 3434 **(2021)**

103. Motta I. et al, Formation of giant unilamellar proteo-liposomes by osmotic shock, *Langmuir*, **31(25)**, 7091 **(2015)**

104. Nardin A. and Lefebvre M., Liposomal muramyl tripeptide phosphatidylethanolamine: targeting and activating macrophages for adjuvant treatment of osteosarcoma, *Current Cancer Drug Targets*, **6(2)**, 123–133 **(2006)**

105. Natsaridis E. et al, Moxifloxacin liposomes: effect of liposome preparation method on physicochemical properties and antimicrobial activity against *Staphylococcus epidermidis*, *Pharmaceutics*, **14(2)**, 370 **(2022)**

106. Nedorubov A.A. et al, Pharmacokinetics of nanosomal form of levodopa in intranasal administration, *Open Access Macedonian Journal of Medical Sciences*, **7(21)**, 3509 **(2019)**

107. Niidome T. and Huang L., Polyethylene glycol-conjugated liposomes for gene delivery, *Advanced Drug Delivery Reviews*, **56(11)**, 1649-1659 **(2004)**

108. Oganesyan E.A. et al, Use of nanoparticles to increase the systemic bioavailability of trans-resveratrol, *Pharmaceutical Chemistry Journal*, **44(2)**, 74-76 **(2010)**

109. Oishi Y. and Manabe I., Macrophages in inflammation, repair and regeneration, *International Immunology*, **30(11)**, 511–528 **(2018)**

110. Okore V.C. et al, Formulation and evaluation of niosomes, *Indian Journal of Pharmaceutical Sciences*, **73(3)**, 323 **(2011)**

111. Peer D. et al, Nanocarriers as an emerging platform for cancer therapy, *Nature Nanotechnology*, **2(12)**, 751-760 **(2007)**

112. Piet A. et al, Synthesis of (2'-deoxy) thymidine triphosphate (dTTP) lipid conjugates for solid-phase oligonucleotide synthesis, *Tetrahedron*, **74(9)**, 884-892 **(2018)**

113. Pober J.S. and Sessa W.C., Inflammation and the blood microvascular system, *Cold Spring Harbor Perspectives in Biology*, **7(1)**, a016345 **(2014)**

114. Raghavachari N. and Fahl W.E., Targeted gene delivery to skin cells in vivo: a comparative study of liposomes and polymers as delivery vehicles, *Journal of Pharmaceutical Sciences*, **91(3)**, 615-622 **(2002)**

115. Rahman M. et al, Emergence of liposome as targeted magic bullet for inflammatory disorders: current state of the art, *Artificial Cells Nanomedicine Biotechnology*, **44(7)**, 1597–1608 **(2016)**

116. Rapoport N., Physical stimuli-responsive liposomes for controlled drug delivery, *Biomaterials*, **31(27)**, 7124-7140 **(2010)**

117. Rivest V. et al, Novel liposomal formulation for targeted gene delivery, *Pharmaceutical Research*, **24(5)**, 981-990 **(2007)**

118. Ropert C., Liposomes as a gene delivery system, *Brazilian Journal of Medical and Biological Research*, **32(2)**, 163-169 **(1999)**

119. Sabzichi M. et al, Targeted lipid nanocarriers for delivery of letrozole to breast cancer cells, *Journal of Liposome Research*, **26(3)**, 233-243 **(2016)**

120. Salehi B. et al, Liposomal cytarabine as cancer therapy: from chemistry to medicine, *Biomolecules*, **9(12)**, 773 **(2019)**

121. Sanches B.C. et al, Rhamnolipid-based liposomes as promising nano-carriers for enhancing the antibacterial activity of peptides derived from bacterial toxin-antitoxin systems, *International Journal of Nanomedicine*, **16**, 925 **(2021)**

122. Sawant R.R. and Torchilin V.P., ROS-responsive drug delivery systems, *Drug Development Research*, **69(8)**, 441-448 **(2008)**

123. Seleci D.A. et al, Rapid microfluidic preparation of niosomes for targeted drug delivery, *International Journal of Molecular Sciences*, **20(19)**, 4696 **(2019)**

124. Shah N.N. et al, Vincristine sulfate liposomes injection (VSLI, Marqibo®): results from a phase I study in children, adolescents and young adults with refractory solid tumors or leukemias, *Pediatric Blood & Cancer*, **63(6)**, 997-1005 **(2016)**

125. Sharma S. et al, *Piper longum* and *Abutilon indicum* loaded phytosomes: hepatoprotective potential, *Scientifica (Cairo)*, **2016**, 3096953 **(2016)**

126. Sheikhi A., Emerging cellulose-based nanomaterials and nanocomposites, Nanomaterials and Polymer Nanocomposites: Raw Materials to Applications, Chapter 9, 307-351 **(2019)**

127. Shreya A.B. et al, Active targeting of drugs and bioactive molecules via oral administration by ligand-conjugated lipidic nanocarriers: recent advances, *AAPS Pharm Sci Tech*, **20(1)**, 7 **(2019)**

128. Sloat B.R. et al, Targeted gadolinium-loaded liposomes for potential application in imaging and therapy, *Magnetic Resonance in Medicine*, **64(2)**, 252-261 **(2010)**

129. Souto E.B. et al, Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery, *International Journal of Pharmaceutics*, **278(1)**, 71–77 **(2004)**

130. Stachowiak J.C. et al, Unilamellar vesicle formation and encapsulation by microfluidic jetting, Proceedings of the National Academy of Sciences of the United States of America, **105(12)**, 4697–4702 **(2008)**

131. Sundar S.K. and Tirumkudulu M.S., Synthesis of sub-100-nm liposomes via hydration in a packed bed of colloidal particles, *Industrial & Engineering Chemistry Research*, **53(1)**, 198–205 **(2013)**

132. Sylvester R.J. et al, Predicting recurrence and progression in individual patients with stage Ta T1 bladder cancer using EORTC risk tables: a combined analysis of 2596 patients from seven EORTC trials, *European Urology*, **49(3)**, 466-477 **(2005)**

133. Tanasescu R. et al, Facile and rapid formation of giant vesicles from glass beads, *Polymers (Basel)*, **10(1)**, 54 **(2018)**

134. Tanapichatsakul Chutima, Prachya Surasak and Pripdeevech Patcharee, Antibacterial and cytotoxic activities of endophytic fungus Diatrypella sp. MFLUCC 19-0492 *Res. J. Biotech.*, **16(4)**, 59-66 **(2021)**

135. Teh S.Y. et al., Droplet microfluidics, *Lab on a Chip*, **8(2)**, 198–220 **(2008)**

136. Thabet Y. et al, Methods for preparation of niosomes: a focus on thin-film hydration method, *Methods*, **199**, 9–15 **(2022)**

137. Tian Y. et al, Establishment and evaluation of glucose-modified nanocomposite liposomes for the treatment of cerebral malaria, *Journal of Nanobiotechnology*, **20(1)**, 318 **(2022)**

138. Torchilin V.P. and Weissig V., Liposomes: a practical approach, Oxford University Press **(2013)**

139. Torchilin V.P., Multifunctional nanocarriers, *Advanced Drug Delivery Reviews*, **63(9)**, 738-745 **(2011)**

140. Toumani M. et al, Development of amikacin liposome inhalation for pulmonary infections: formulation optimization using design of experiments and its characterization, *Pharmaceutics*, **11(9)**, 450 **(2019)**

141. Tzogani K. et al, EMA review of daunorubicin and cytarabine encapsulated in liposomes (Vyxeos, CPX-351) for the treatment of adults with newly diagnosed, therapy-related acute myeloid leukemia or acute myeloid leukemia with myelodysplasia-related changes, *Oncologist*, **25(9)**, e1414–e1420 **(2020)**

142. Van Alem C.M.A. et al, Local delivery of liposomal prednisolone leads to an anti-inflammatory profile in renal ischaemia-reperfusion injury in the rat, *Nephrology Dialysis Transplantation*, **33(1)**, 44–53 **(2018)**

143. Van Alem C.M.A. et al, Recent advances in liposomal-based anti-inflammatory therapy, *Pharmaceutics*, **13(7)**, 1004 **(2021)**

144. Van Winden E.C.A., Freeze-drying of liposomes: theory and practice, *Methods in Enzymology*, **367**, 99–110 **(2003)**

145. Vanić Ž. et al, Azithromycin-liposomes as a novel approach for localized therapy of cervicovaginal bacterial infections, *International Journal of Nanomedicine*, **14**, 5957–5976 **(2019)**

146. Varadkar M. et al, Crocetin phytosomes from *Nannochloropsis oceanica* microalgae, *Journal of Applied Pharmaceutical Science*, **11(3)**, 177-186 **(2021)**

147. Varshosaz J. et al, Formulation and optimization of solid lipid nanoparticles of buspirone HCl for enhancement of its oral bioavailability, *International Journal of Pharmaceutics*, **20(4)**, 286–296 **(2010)**

148. Verma A. et al, Folate-conjugated double liposomes bearing prednisolone and methotrexate for targeting rheumatoid arthritis, *Pharmaceutical Research*, **36(8)**, 123 **(2019)**

149. Voltan A.R. et al, Fungal diseases: could nanostructured drug delivery systems be a novel paradigm for therapy?, *International Journal of Nanomedicine*, **11**, 3715 **(2016)**

150. Wang Z. et al., Nanoparticle delivery of cancer drugs, *Annual Review of Medicine*, **67**, 121-137 (**2016**).

151. Weinberger A. et al, Gel-assisted formation of giant unilamellar vesicles, *Biophysical Journal*, **105(1)**, 154 **(2013)**

152. Wong C. et al, Liposomal prednisolone inhibits vascular inflammation and enhances venous outward remodelling in a murine arteriovenous fistula model, *Scientific Reports*, **6(1)**, 30439 **(2016)**

153. Wu H. et al, Near-infrared fluorescence imaging-guided focused ultrasound-mediated therapy against rheumatoid arthritis by MTX-ICG-loaded iRGD-modified echogenic liposomes, *Theranostics*, **10(22)**, 10092–10105 **(2020)**

154. Xie S. et al, Formulation, characterization and pharmacokinetics of praziquantel-loaded hydrogenated castor oil solid lipid nanoparticles, *Nanomedicine*, **5(5)**, 693–701 **(2010)**

155. Xie X. et al, Oral administration of natural polyphenol-loaded natural polysaccharide-cloaked lipidic nanocarriers to improve efficacy against small-cell lung cancer, *Nanomedicine*, **29**, 102261 **(2020)**

156. Xu H. et al, Nanocarriers in gene therapy: a review, *Journal of Biomedical Nanotechnology*, **10(12)**, 3483-3507 **(2014)**

157. Xu Y. et al, Preparation and characterization of tea tree oil solid liposomes to control brown rot and improve quality in peach fruit, *LWT*, **162**, 113442 **(2022)**

158. Yarce C.J. et al, Development of antioxidant-loaded nanoliposomes employing lecithins with different purity grades, *Molecules*, **25(22)**, 5344 **(2020)**

159. Yin J. et al, Cholate-modified polymer-lipid nanoparticles for enhanced oral absorption of quercetin: preparation and evaluation, *Pharmaceutics*, **11(11)**, 610 **(2019)**

160. Zeng S. et al, Decoquinate liposomes: highly effective clearance of *Plasmodium* parasites causing severe malaria, *Malaria Journal*, **21(1)**, 24 **(2022)**

161. Zhang G. and Sun J., Lipid in chips: a brief review of liposome formation by microfluidics, *International Journal of Nanomedicine*, **16**, 7391–7416 **(2021)**

162. Zhang J. et al, Preparation and antibacterial effects of carboxymethyl chitosan-modified photo-responsive camellia sapogenin derivative cationic liposomes, *International Journal of Nanomedicine*, **14**, 8611 **(2019)**

163. Zhang N. et al, Lectin-modified solid lipid nanoparticles as carriers for oral administration of insulin, *International Journal of Pharmaceutics*, **327(1–2)**, 153–159 **(2006)**

164. Zhang Y. et al, Dimeric artesunate phospholipid-conjugated liposomes as promising anti-inflammatory therapy for rheumatoid arthritis, *International Journal of Pharmaceutics*, **579**, 119166 **(2020)**

165. Zhang Z. et al, The characteristics and mechanism of simvastatin loaded lipid nanoparticles to increase oral bioavailability in rats, *International Journal of Pharmaceutics*, **394(1–2)**, 147–153 **(2010)**

166. Zhao J. et al, Natural oral anticancer medication in small ethanol nanosomes coated with a natural alkaline polysaccharide, *ACS Applied Materials & Interfaces*, **12(14)**, 16159–16167 **(2020)**

167. Zhou-Lian Z. et al, A new method—ejection method for nondestructive online monitoring of the pretension of building membrane structure, *Structural Control and Health Monitoring*, **20(4)**, 445–464 **(2013)**.

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