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Exploring lipids: A comprehensive review on their utilization in the formulation of liposomes, phytosomes, and ethosomes for advanced drug delivery systems"

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Abstract

This comprehensive review explores the diverse applications of lipids in the formulation of advanced drug delivery systems, specifically focusing on liposomes, phytosomes, and ethosomes. Lipids, crucial components in these formulations, play a pivotal role in enhancing drug solubility, stability, and bioavailability. The review systematically examines the latest advancements in lipid-based delivery systems, shedding light on their unique characteristics and applications. In the realm of liposomes, the study delves into various lipid compositions, highlighting their influence on liposomal structure and function. Additionally, the review explores the integration of phytosomes, which involve the complexation of drugs with plant-derived phospholipids, showcasing their potential to improve therapeutic efficacy. Ethosomes, lipid vesicles containing high concentrations of ethanol, are also extensively discussed, emphasizing their ability to enhance transdermal drug delivery. Critical analyses of recent research findings, including the impact of lipid selection on vesicle stability, drug release kinetics, and pharmacokinetics, are presented. The review further examines the challenges associated with lipid-based formulations, providing insights into potential avenues for future research and development. By synthesizing current knowledge, this review serves as a valuable resource for researchers, clinicians, and pharmaceutical scientists seeking a comprehensive understanding of the role of lipids in optimizing liposomal, phytosomal, and ethosomal drug delivery systems.

Keywords: Lipid-based drug delivery; Liposomes; Phytosomes; Ethosomes; Advanced drug delivery systems.

1. Introduction

1.1. Lipids

The term 'lipid' refers to a heterogeneous set of biomolecules that are soluble in non-polar organic solvents that occur naturally. They can be classified as hydrophobic or amphiphilic small molecules in broad terms. Fats, oils, waxes, fatty acids, monoglycerides, diglycerides, triglycerides, fat-soluble vitamins, sterols, steroids, and phospholipids are all included in this category. Lipids are an important component of the cell membrane, which acts as a mechanical barrier to the cell's external environment and performs a variety of biological tasks in the body. They play a vital function in signaling as well. Subcutaneous fat slows the loss of body heat in mammals living in cold areas. Lipids and proteins combine to form lipoproteins, which are an important component of the cell membrane and aid in lipid transport.

1.2. Lipids used in the preparation of liposomes, phytosomes, and ethosomes

SPC (Soy Phosphatidylcholine), Cholesterol, Lipoid S100, Egg L-α-phosphatidylcholine (EPC), 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-polyethyleneglycol 750 (DOPE-PEG 750), Cholesteryl hemisuccinate (CHEMS), Lipoid SPC-3, Phosphotidylcholine, Phosphotidylethanolamine,

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Sphingomyelin, Lysophosphotidylcholine, Lysophosphotidylethanolamine, rhodamine-phosphatidylethanolamine (Rho-PE), NH2-PEG2000-Mal and mPEG2000-NH2, L-α-phosphotidylcholine, Deuterated 1-palmitoyl (D31)-2-oleoyl-sn-glycero-3-phosphocholine (d-POPC).

1.3. Liposomes

Liposomes were spherical-shaped concentric vesicles, derived from two Greek words lipos means fat, and soma means body. Liposomes are colloidal carriers, having a size range of 0.01–5.0μm in diameter. Liposomes are small artificial vesicles of spherical shape that can be created from cholesterol and natural non-toxic phospholipids. Due to their size and hydrophobic and hydrophilic character, liposomes are promising systems for drug delivery. Liposomes entrap drugs of both aqueous and the lipid phase and it makes them attractive drug delivery systems for hydrophilic and hydrophobic drugs. Liposomes are the novel drug delivery system that aims to deliver the drug directly to the place of action. They have the potential to accommodate both hydrophilic and lipophilic compounds to protect the drug from degradation and release the active ingredients in a controlled manner.

1.4. Phytosomes

Phytosomes are lipid-compatible molecular complexes and are little cell-like structures that are composed of “Phyto” which means plant and “some” meaning cell-like. Phytosomes are complex between natural phytoconstituents and natural phospholipids, like soy phospholipids mostly phosphatidylcholine. These complex results from the reaction of stoichiometric amounts of phospholipids with the phytoconstituents in an aprotic solvent. Phytosomes are an advanced form of herbal drug that is better absorbed and utilized and which finally leads to better results than the conventional dosage form.

1.5. Ethosomes

Ethosomes are vesicle lipid carriers containing phospholipids, having high concentrations of ethanol and water. Ethosomes have the ability to increase skin permeability. The size of ethosomes ranges from tens of nanometers to microns which have a small size related to liposome. Those have the ability to efficiently entrap various molecules, like hydrophilic, lipophilic, and amphiphilic molecules. It can be explained by the high degree of lamellarity and the presence of ethanol in the vesicles, which allows for better solubility of many drugs. Ethosomal formulations possess greater entrapment capability than liposomes. Due to their soft and flexible nature, they can penetrate the skin and allow enhanced delivery of various active agents to deeper strata of the skin or enhanced systemic circulation compared to conventional liposome or hydroethanolic solutions. The better permeability of ethosome carriers is due to the synergistic mechanism between a high concentration of ethanol, phospholipids vesicles, and skin lipids.

2. Properties of lipids

- At room temperature, lipids can be either liquids or non-crystalline solids.
- Colorless, odorless, and tasteless are the characteristics of pure fats and oils.
- They're organic compounds with a lot of energy.
- It is water-insoluble.
- Alcohol, chloroform, acetone, benzene, and other organic solvents are soluble.
- There are no ionic charges.
- Saturated fatty acids are abundant in solid triglycerols (fats).
- Unsaturated fatty acids are abundant in liquid triglycerols (oils).

2.1. Hydrolysis of tri glycerols

Triglycerols like any other esters react with water to form their carboxylic acid and alcohol– a process known as hydrolysis.

2.2. Saponification:

Triacylglycerols may be hydrolyzed by several procedures, the most common of which utilizes alkali or enzymes called lipases. Alkaline hydrolysis is termed saponification because one of the products of the hydrolysis is a soap, generally sodium or potassium salts of fatty acids.

2.3. Hydrogenation

The carbon-carbon double bonds in unsaturated fatty acids can be hydrogenated by reacting with hydrogen to produce saturated fatty acids.

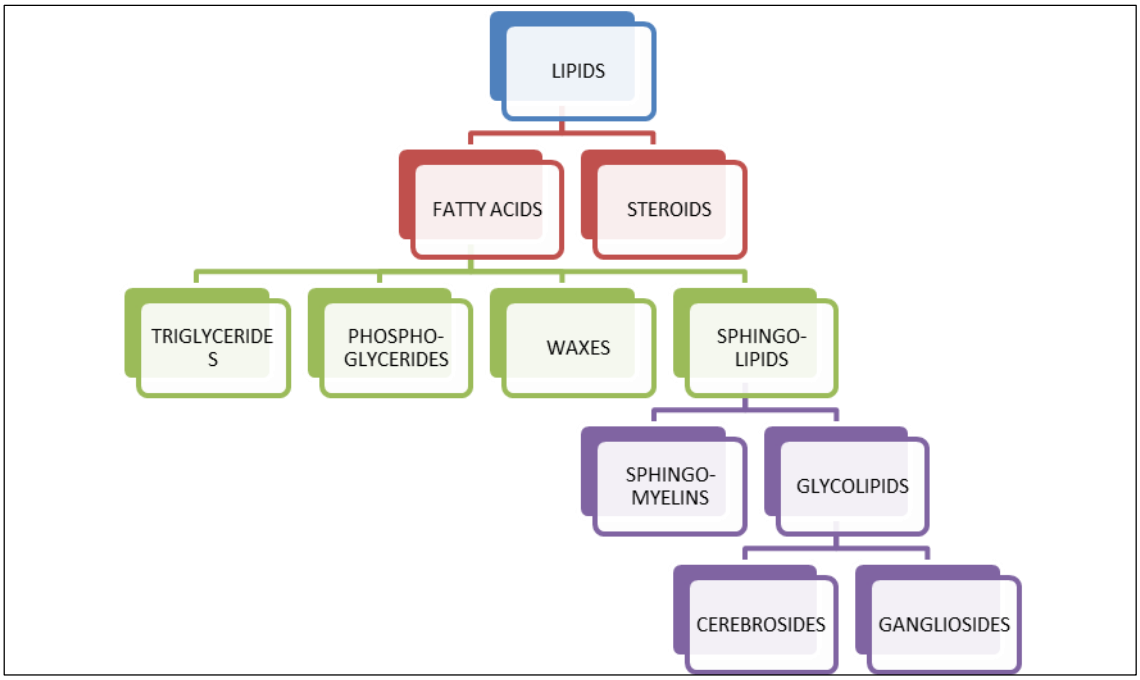
2.4. Halogenation

Unsaturated fatty acids, whether they are free or combined as esters in fats and oils, react with halogens by addition at the double bond(s). The reaction results in the decolorization of the halogen solution.

2.5. Rancidity:

The term rancid is applied to any fat or oil that develops a disagreeable odor. Hydrolysis and oxidation reactions are responsible for causing rancidity. Oxidative rancidity occurs in triacylglycerols containing unsaturated fatty acids.

3. Classification of lipids



Lipids can be classified according to their hydrolysis products and according to similarities in their molecular structures. Three major subclasses are recognized:

3.1. Simple lipids

(a) Fats and oils that yield fatty acids and glycerol upon hydrolysis.

(b) Waxes, which yield fatty acids and long-chain alcohols upon hydrolysis.

3.1.1. Fats and Oils

Both types of compounds are called triacylglycerols because they are esters composed of three fatty acids joined to glycerol, and trihydroxy alcohol.

The difference is on the basis of their physical states at room temperature. It is customary to call a lipid a fat if it is solid at 25°C, and oil if it is a liquid at the same temperature.

These differences in melting points reflect differences in the degree of unsaturation of the constituent fatty acids.

3.1.2. Waxes

Wax is an ester of long-chain alcohol (usually mono-hydroxy) and a fatty acid.

The acids and alcohols normally found in waxes have chains of the order of 12-34 carbon atoms in length.

3.2. Compound lipids

(a) Phospholipids, which yield fatty acids, glycerol, amino alcohol sphingosine, phosphoric acid, and nitrogen-containing alcohol upon hydrolysis.

They may be glycerophospholipids or sphingophospholipid depending upon the alcohol group present (glycerol or sphingosine).

(b) Glycolipids, which yield fatty acids, sphingosine or glycerol, and a carbohydrate upon hydrolysis.

They may also be glyceroglycolipids or sphingoglycolipids depending upon the alcohol group present (glycerol or sphingosine).

3.3. Derived lipids

Hydrolysis product of simple and compound lipids is called derived lipids. They include fatty acid, glycerol, sphingosine, and steroid derivatives.

Steroid derivatives are phenanthrene structures that are quite different from lipids made up of fatty acids.

4. Functions of lipids

It is established that lipids play extremely important roles in the normal functions of a cell. Not only do lipids serve as highly reduced storage forms of energy, but they also play an intimate role in the structure of cell membranes and organellar membranes. Lipids perform many functions, such as:

- Energy Storage
- Making Biological Membranes
- Insulation
- Protection – e.g. protecting plant leaves from drying up
- Buoyancy
- Acting as hormones

Act as the structural component of the body and provide the hydrophobic barrier that permits partitioning of the aqueous contents of the cell and subcellular structures.

Lipids are major sources of energy in animals and high lipid-containing seeds.

Activators of enzymes e.g., glucose-6-phosphatase, stearyl CoA desaturase ω-monooxygenase, and β-hydroxybutyric dehydrogenase (a mitochondrial enzyme) require phosphatidylcholine micelles for activation.

4.1. SPC (soy phosphatidylcholine)

- A concentrate of soybean lecithin consisting of more than 94% phosphatidylcholine and less than 2% triglycerides.
- Chemical name: Soybean lecithin
- Physical description: Dry Powder; Liquid
- Color/form: Color is nearly white when freshly made, but rapidly becomes yellow to brown in the air
- Odor: Odorless or slight nutlike odor; faint fatty odor
- Taste: Bland
- Melting point: 236-237 °C
- Solubility: Insoluble in water
- Density: 1.0305 at 24 °C/4 °C
- pH: pH 6.6

- Iodine value: 95
- Saponification value: 196

4.2. Cholesterol

Cholesterol is a sterol (or modified steroid), a type of lipid. Cholesterol also serves as a precursor for the biosynthesis of steroid hormones, bile acid, and vitamin D.

- Appearance: White crystalline powder.
- Density: 1.052 g/cm^3
- Melting point: 148 to 150 °C (298 to 302 °F; 421 to 423 K)
- Boiling point: 360 °C (680 °F; 633 K) (decomposes)
- Solubility in water: 0.095 mg/L (30 °C)
- Solubility: Soluble in acetone, benzene, chloroform, ethanol, ether, hexane, isopropyl myristate, methanol.

4.3. Phosphatidylcholine

A class of phospholipids incorporates choline. Component of biological membranes, obtained from egg yolk or soybeans, member of the lecithin group of yellow-brownish fatty substances.

Melting point: 2-oleoyl-3-stearoyl- L -1-phosphatidylcholine and 2,3-distearoyl- L -1-phosphatidylcholine both have 230°–231° C

4.4. Phosphatidylethanolamine

PE is a class of phospholipids found in biological membranes. Synthesized by the addition of cytidine diphosphate ethanolamine to diglycerides.

Melting point: di-oleoyl-phosphatidylethanolamine is -16 °C, di-oleoyl-phosphatidylcholine is -20 °C

4.5. Sphingomyelin

SPH is a type of sphingolipid found in the membranous myelin sheath that surrounds nerve cell axons. Consists of phosphocholine and ceramide or a phosphoethanolamine.

Melting point: 37 °C.

DOPE

1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) is a neutral helper lipid for cationic liposomes and combines with cationic phospholipids.

Boiling point: 759.2±70.0 °C at 760 mmHg

Table 1 Problems and solutions

	Problems	**Solutions**
T. Limsuwan et al. 2012	Low permeability of MPA.	Development of ethosome containing MPA for the enhancement of skin permeation after topical administration by film hydration method using L-α-phosphatidylcholine (SPC), CHOL, and PEG.
Selenia Ternullo et al. 2019	Curcumin's low solubility and poor bioavailability limit its oral administration.	Development of an efficient liposomal formulation for dermal delivery of curcumin focusing on its potential for the treatment of inflamed and infected wounds. The neutral, cationic, and anionic deformable liposomes (NDLs, CDLs, and ADLs, respectively) containing curcumin were prepared by conventional film method using SPC.
Yang Liu et al. 2021	Transdermal delivery takes a long onset time which prolongs	Improvement of the transdermal delivery of lidocaine hydrochloride (LidH) using elastic nano-liposomes (ENLs) and

Noemia Penoy et al. 2020	the preparation time for surgery. Homogenization of liposomes.	microneedle (MN) array pretreatment. LidH-containing ENLs were prepared by a reverse-phase evaporation method using soybean phosphatidylcholine and cholesterol. Conventional methods need an additional step such as extrusion to homogenize the size of liposomes, the supercritical process does not require additional extrusion or ultrasonication. By phospholipid dispersion using SPC, Egg L-α-phosphatidylcholine (EPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 3β – [N-(N′, N′ - dimethylaminoethane)-carbamoyl] cholesterol hydrochloride (DC cholesterol), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N- [methoxy (polyethylene glycol)-2000] (ammonium salt) (DSPE PEG 2000) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N- [methoxy (polyethylene glycol)-750] (ammonium salt) (DSPE PEG 750), and cholesteryl hemisuccinate (CHEMS) and cholesterol (CHOL), the supercritical fluid technology developed.
Thanaporn Amnuaikit et al. 2018	Phenylethyl resorcinol (PR) has low solubility and skin irritation.	Elastic vesicle carriers such as transferosomes and invasomes, useful for the PR delivery system, which is highly effective for skin-lightening products than conventional liposomes, using l-α-phosphatidylcholine from soybean (SPC) and cholesterol (CHOL).
Darshan Telange et al. 2020	Low solubility and permeability of ranolazine.	Enhancement of the aqueous solubility and permeability of ranolazine, by solvent method using LIPOID SPC-3.
Pajaree Sakdiset et al. 2019	Low permeability of indomethacin.	Ethosomes containing indomethacin were prepared by thin-film hydration technique using soybean phosphatidylcholine (SPC).
Chander Prakash Dora et al. 2017	Low solubility of erlotinib (ERL).	Complex of erlotinib (ERL) with phospholipid (PC) for enhancement of solubility and thus bioavailability, therapeutic efficacy, and reducing the toxicity of erlotinib. The phospholipid complex of the drug was prepared by a solvent evaporation method using Lipoid SPC3.
Yang Li et al. 2014	Encapsulation efficiency, regulation of drug release, and a systematic evaluation of the MTX prodrug acting as a targeting ligand have not been previously reported.	MTX prodrug-targeted and MMC-loaded PEG–PE–PLA NP improve therapeutic efficacy and reduce the side effects of MMC. MTX prodrug-targeted and MMC-loaded PLA–lipid–PEG hybrid NPs double emulsion solvent evaporation method for MMC–soybean phosphatidylcholine complex or DSPE–PEG–MTX, in which the MTX prodrug can be exploited as a targeting ligand.
Fei Yu et al. 2016	Poor gastrointestinal absorption and low oral bioavailability of berberine.	Phytosomes loaded with berberine-phospholipid complex (P-BER) were prepared by a rapid solvent evaporation method followed by a self-assembly technique for BER drug delivery system using Soybean phosphatidylcholine (SPC) LIPOID S-100.
Seong-Min Kim et al. 2019	Low bioavailability, low efficacy of chrysin.	Chrysin-loaded phytosomes (CP) were prepared by the solvent evaporation method using either soya phosphatidylcholine (SPC) or egg phospholipid (EPL).
Jinyuan Ma et al. 2018	The single therapeutic strategy may not always cope with cancer efficiently and thoroughly.	Zinc phthalocyanine-soybean phospholipid (ZnPc-SPC) complex-based drug delivery system with doxorubicin (Dox), additional chemotherapy while the carrier itself could serve as multifunctional and switchable theranostic agent. By solvent evaporation method followed by a self-assembly technique, ZS and DZSM were constructed using Soybean phosphatidylcholine (SPC), 1, 2-diacyl-SN-glycero-3-phosphoethanolamine-N-[methoxy (poly (ethylene glycol))-2000] (DSPE-PEG-OCH3), 1,2-

		distearoyl-snglycero-3-phosphoethanolamine-N- [amino (polyethylene glycol)- 2000] (DSPE-PEG-NH2).
Dao Shi et al. 2021	Lack of self-targeting capacity prevents nano therapy from efficiently accumulating in tumor tissue and internalizing into tumor cells, resulting in a suboptimal therapeutic effect.	Methotrexate (MTX)-soybean phospholipid (SPC) inclusion complex (MTX-SPC)-modified graphene oxide (CGO) nano therapy (CGO-MTX-SPC) is constructed by CGO nanosheets as a supporter for MTX-SPC
Xiangru Yang et al. 2019	OEA is a water-insoluble molecule.	Neuroprotective nanoformulation (OEA–SPC NPs) via the combination of the nanoparticle drug delivery system with the endogenous N-oleoylethanolamine (OEA). By forming a hydrogen bond between OEA and the carrier—soybean phosphatidylcholine (SPC), the form of OEA was turned into an amorphous state when loading to the nanoparticles, improving its bioavailability.
Yao Quin et al. 2011	Due to the presence of the blood-brain barrier (BBB) delivery of drugs to the brain is a major challenge	TAT-modified liposome (TAT-LIP) was developed to overcome the ineffective delivery of normal drug formulation to the brain. By film hydration method using Soybean phospholipids (SPC) and cholesterol (CHO). Dioleoyl phosphatidylethanolamine (DOPE) and rhodamine-phosphatidylethanolamine (Rho-PE)
Narumon Changsan et al. 2008	Stability problem of RF.	Liposome suspensions were prepared using cholesterol (CH) and soybean L-1-p hosphatidylcholine (SPC) by the chloroform film method.
Xiaojuan Zhao et al. 2013	Low activity of DCs and low immune response.	Salidroside liposome formulation promotes the maturation of DCs, the stimulation of DCs on MLR proliferation, and the antigen-presenting ability, inducing the sustained cellular immune and humoral immune response. By ammonium sulfate gradient method using soybean phospholipid, cholesterol.
Elena Valeria Fuior et al. 2020	Naringenin, reduced water solubility and bioavailability.	Naringenin was loaded into lipid nanoemulsions by using soybean oil, sodium 4-[2-[(1E,3E,5E,7Z)-7-[1,1-dimethyl-3-(4-sulfonatobutyl) benzo[e]indol-2-ylidene]hepta-1,3,5-trienyl]-1,1-dimethyl benzo[e]indol-3-ium-3-yl]butane-1-sulfonate (Indocyanine Green), lipopolysaccharide (LPS, serotype E coli O111:B4), SYBR-Green; soy phosphatidylcholine (SPC), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[Maleimide(PolyethyleneGlycol) 2000](Ammonium salt) (Mal-PEG-DSPE) and 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt) (Rhodamine-PE).
Shichao Wa et al. 2021	Low solubility, low bioavailability.	OEASPC & DSPE-PEG lipid nanoparticles (OSDP LNPs) developed. The hydrogen bond within OEA-SPC totally changed the form of OEA and enhanced its solubility. The crystallization of OEA inside the OSDP LNPs further enhanced the drug loading; hence, the bioavailability of OEA would highly increase.

5. Development of Ethosomes Containing Mycophenolic Acid T. Limsuwan et al. 2012

5.1. Materials

Mycophenolic acid (MPA). L-α-phosphatidylcholine from soybean (t30%, SPC) and stearylamine (SA), Cholesterol, deoxycholic acid (DA), and triethylamine. Triton X-100. Polyoxyethylene sorbitan monooleate (Tween80).

5.2. Formulation and preparation of ethosome containing MPA

According to the thin film hydration method, the formulation and preparation of ethosome containing MPA were prepared, using the concentration of MPA 10mg/ml. The main composition of ethosome includes 2-6% w/v SPC and hydroethanolic solution (mixture of phosphate buffer pH 7.4 with 10-50% v/v ethanol). The properties of ethosome are improved by additives such as CHOL, TWEEN 80, PEG, and surface charge agents (SA and DA). In terms of type, concentration, and ratio, all the components of ethosomes were varied.

5.3. Liposomes augment biological benefits of curcumin for multitargeted skin therapy Selenia Ternullo et al. 2019

Materials

Curcumin (≥94% curcuminoid content; ≥80% curcumin). Lipoid S 100 (> 94% soybean phosphatidylcholine, SPC). Polysorbate 20 (P20), stearylamine (SA), sodium deoxycholate (SDC), propylene glycol (PG), methanol, disodium hydrogen phosphate dihydrate, monobasic potassium phosphate, sodium chloride, ammonium molybdate, Fiske-Subbarow reducer agent, phosphorus standard solution, RPMI 1640 medium, lipopolysaccharide (LPS) and Iscove's modified Dulbecco's medium (IMDM). Hydrogen peroxide 30%, sulphuric acid, sulfanilamide, naphthyl ethylenediamine dihydrochloride phosphoric acid, and Albunorm® (human serum albumin, 200 mg/mL). HFF (CCD-1112Sk, ATCC® CRL-2429™) and murine macrophage RAW 264.7 cell lines.

5.3.1. Preparation of deformable liposomes

Using the conventional film method, the DLs were prepared. NDLs with SPC and P20 (total 200 mg) in a weight ratio of (85:15, respectively). By addition of SA in the same lipid mixture as used for NDLs, CDLs were prepared. The ratio of SA to SPC was 1:9 (w/w). ADLs were made of SPC and SDC, with the same weight ratio between lipid and surfactant (85:15, respectively) as for NDLs and CDLs. Curcumin (20 mg) and lipids (200 mg: SPC and, when applicable, P20, SA, and SDC), were dissolved in methanol. After evaporation of the solvent for 1 h in a rotary vacuum evaporator (Büchi Rotavapor R-124 with Büchi Vacuum Pump V-700) a thin lipid film was obtained. For an additional 1h, the lipid film was kept under vacuum (55 mbar) at 55 °C and subsequently resuspended in 10 mL of phosphate buffer saline (PBS) (pH 7.4; 2.98 g/L Na2HPO4 2H2O, 0.19 g/L KH2PO4, 8 g/L NaCl). The DLs were stored at 4 °C overnight. The empty (curcumin-free) NDLs, CDLs, and ADLs were prepared using the same liposomal composition as for curcumin DLs for assessment of liposomes' elasticity, antibacterial and anti-inflammatory activities of curcumin-DLs, and in vitro cell viability study. By hand extrusion through the polycarbonate membrane (Nuclepore® Track-Etched Membranes, Whatman House) the size of all DLs was reduced. To obtain liposomes between 200 and 300 nm in size, the pore size of the membranes and the number of extrusion cycles were optimized for each formulation. For all DLs, five cycles of extrusion through an 800 nm pore-size membrane were performed. Additionally, NDLs were extruded four times through a 400 nm pore-size membrane, while CDLs and ADLs were extruded through the same pore-size membrane two and seven times, respectively. CDLs (both empty and curcumin-containing) were maintained at 55 °C for 10 min in a thermostat.

5.4. Transdermal Delivery of Lidocaine-Loaded Elastic Nano-Liposomes with Microneedle Array Pretreatment Yang Liu et al. 2021

Materials

Dulbecco's modified Eagle's medium (DMEM). Polyoxyethylene sorbitan monooleate (Tween 80) and sorbitan monooleate (Span 80). Soybean phosphatidylcholine (SPC) and cholesterol and the Cell Counting Kit-8 (CCK-8). Red blood cell lysis buffer. LidH. Protamine sulfate and rhodamine B. Pentobarbital sodium.

5.5. LidH SLNs and ENLs Preparation

Using the reverse-phase evaporation method, the LidH SLNs and ENLs were prepared. In the mass ratio 5:1 SPC and cholesterol were dissolved in trichloromethane and mixed evenly. According to the SPC: surfactant mass ratio of 10:1, two surfactants, Span 80 or Tween 80, were chosen and weighed in the ENL formulations, while no surfactant was added in the SLN formulation. The solution of LidH (2%, w/w) was added to the mixture (1:3, v/v). Until the dispersion became milky and monophasic, the mixture was sonicated in an ice bath for 3 min. The solvent was removed from the reverse micelle dispersion by rotary evaporation under reduced pressure (200 rpm, 45 ◦ C, 355 mmHg) if the dispersion was not layered after at least 30 min. 1× phosphate-buffered saline (PBS; 10 mM phosphate buffer, 137 mM NaCl, pH 7.4) was added to hydrate the suspension under reduced pressure after a thin and viscous film formed on the inner wall of

the round-bottom flask and collapsed into a suspension. Under 3 s sonication with 5 s intervals, the emulsion was sonicated for 3 min in an ice bath.

5.6. A supercritical fluid technology for liposome production and comparison with the film hydration method No´emie Penoy et al. 2020

Materials

Soy phosphatidylcholine (SPC), Egg L-α-phosphatidylcholine (EPC), 1,2-dioleoyl-3-trimethylammonium-propane (chloride salt) (DOTAP), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 3β – [N-(N′, N′ - dimethylaminoethane)-carbamoyl] cholesterol hydrochloride (DC cholesterol), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N- [methoxy (polyethylene glycol)-2000] (ammonium salt) (DSPE PEG 2000) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N- [methoxy (polyethylene glycol)-750] (ammonium salt) (DSPE PEG 750), and cholesteryl hemisuccinate (CHEMS) and cholesterol (CHOL). 4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) ≥ 99.5%.

5.7. Preparation of the phospholipid dispersion

By dispersing lipids in HEPES buffer (10 mM, pH value 7.4) at 65 ◦ C a phospholipid dispersion was prepared and stirred at 1200 rpm on a hot plate stirrer for 15 min. According to the experimental strategy, the total lipid concentration of the dispersion was fixed.

Vesicular carriers containing phenyl ethyl resorcinol for topical delivery system; liposomes, transfersomes, and invasomes Thanaporn Amnuaikit et al. 2018

Materials

l-α-phosphatidylcholine from soybean (SPC) and cholesterol (CHOL). Absolute ethanol. Tween 20 and Tween 80. Span 20 and Span 80. Kojic acid, tyrosinase enzyme, and dimethyl sulfoxide (DMSO). Citral, (R)-(+)-Limonene and (1R)- (−)-Fenchone, Sodium deoxycholate.

5.8. Methods

5.8.1. Preparation of PR-loaded vesicle carrier

The main composition of liposomes is 0.5% (w/v). The 0.5% (w/v) CHOL, 3% (w/v) SPC, and water up to 100% (v/v). In terms of concentrations and ratios, the skin enhancers were varied. Fenchone, citral, and d-limonene mixed with 10% (v/v) ethanol as skin enhancers, are used in the invasome formulations. Tween 80, Tween 20, Span 80, Span 20, and SDC as skin enhancers, are used in transfersome formulations. By thin-film hydration method, all compositions were prepared as follows: First, the oil phase which included SPC, CHOL, PR, and skin enhancer was dissolved in absolute ethanol. The aqueous phase used was water for liposomes and transfersomes; a hydro-ethanolic solution consisting of water and 10% (v/v) absolute ethanol for invasomes. Second, until homogeneity, the oil phase and aqueous phase were separately sonicated at 60 °C for 30 min. Via evaporation by a rotary evaporator (Model Eyela N-1000 series), the absolute ethanol was removed from the oil phase. while the other components formed a thin lipid film. The lipid film was hydrated with 10 ml of aqueous phase, followed by shaking for 5 min. To obtain the complete formulations, the mixtures were sonicated at 60 °C for 30 min.

5.9. LIPOID SPC-3-Based Coprecipitates for the Enhancement of Aqueous Solubility and Permeability of Ranolazine Darshan R. Telange et al. 2020

Materials

Ranolazine (purity more than 99.86%). LIPOID SPC-3. Absolute ethanol, acetone, chloroform, dichloromethane, diethyl ether, and 1,4-dioxane, potassium bromide, potassium chloride, potassium dihydrogen phosphate, sodium hydroxide, sodium dihydrogen phosphate, and sodium chloride.

5.10. Preparation of Ranolazine-LIPOID SPC-3 Coprecipitate

Using the solvent evaporation method, the RNZ-SPC-CP was prepared according to stoichiometric ratios (1:1, 1:2, 1:3, 1:4, and 1:5). The required quantity of ranolazine and LIPOID SPC-3 (LSPC-3) was accurately weighed and transferred into a 50 -mL beaker. The ingredients were mixed and then dissolved in 10 mL of absolute ethanol using a magnetic

stirrer until a homogenous solution was formed. The prepared solution was allowed to evaporate at room temperature, solid mass of RNZ-SPC-CP was formed. Solid mass was collected from the beaker and dried under vacuum at 40 °C for 24 h. The dried RNZ-SPC-CP formulation was sieved and placed into a light-resistant amber-colored glass bottle, flushed with N2, and kept in desiccators for further characterization.

5.11. Formulation development of ethosomes containing indomethacin for transdermal delivery Pajaree Sakdiset et al. 2019

Materials

Indomethacin and the reference standard of indomethacin. Mycophenolic acid, is the internal standard. The commercial indomethacin solution (Elmetacin® spray, Batch number 806034). For ethosomal formulations, 3-sn-phosphatidylcholine from soybean (SPC), cholesterol from lanolin (CHOL), deoxycholic acid (DA), and stearylamine (SA). Triton® X-100. Acetic acid was purchased from BHD AnalaR and sodium chloride. Sodium dihydrogen orthophosphate, disodium hydrogen orthophosphate anhydrous, and sodium acetate. Absolute ethanol, hydrochloric acid sodium chloride, and sodium hydroxide. Acetonitrile, hexane, and methanol.

5.12. Optimization and preparation of ethosomes containing indomethacin

Using indomethacin at a concentration of 8 mg/mL, the ethosomes containing indomethacin were prepared. In terms of the type, concentration, and ratio the compositions of the ethosomal formulations, including SPC, dispersion medium, and additives (e.g., CHOL and surface charge modifying agents) were varied. By using the thin-film hydration technique, all ethosomes were prepared. First, indomethacin, SPC, and the other additives were dissolved in a mixture of chloroform and methanol (2:1 v/v) in a round-bottomed flask and then evaporated at 60 °C for 1 h using a rotary evaporator (Eyela N-1000 series) to obtain the thin lipid film and ensure complete evaporation of the organic solvents. The hydroethanolic mixture (5 mL) was added to hydrate the lipid film, followed by sonication in an ultrasonic bath (HT Crest) at 60 °C for 30 min in a well-sealed flask. In triplicates, all formulations were prepared. Liposomes were prepared using 2%-6% SPC and phosphate buffer pH 7.4 without other additives.

5.13. Improved oral bioavailability and therapeutic efficacy of erlotinib through molecular complexation with phospholipid Chander Prakash Dora et al. 2017

Materials

Erlotinib hydrochloride (ERL). Lipoid® SPC3. Dialysis membrane (14 kDa MWCO), Dulbecco's modified Eagle's medium (DMEM), antibiotic antimycotic solution, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), ethylenediaminetetraacetic acid (EDTA), and trypsin. Fetal bovine serum (FBS). 7,12-Dimethylbenz[a]anthracene (DMBA). Commercial kits for estimation of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Blood Urea Nitrogen (BUN), and Creatinine level. High-performance Liquid Chromatography (HPLC) grade reagents such as acetonitrile, and methanol. Purified water, degassed and filtered through 0.45 μm hydrophilic PVDF filters (Millipore MillexHV), was used in all experiments. Caco-2 (Human epithelial colorectal adenocarcinoma), MIA PaCa-2, and PANC-1 (Human Pancreas adenocarcinoma) cell lines.

5.14. Preparation of erlotinib phospholipid complex

By solvent evaporation with slight modifications, the ERL-Phospholipid (ERL-PC) complex was developed. Drug: Phospholipids with different ratios (2:1, 1:1, 1:2, and 1:4) were screened on the basis of complexation efficiency. For the preparation of phospholipid complex, 0.3 mM ERL was dissolved in 20 ml of methanol and then 0.6 mM of Lipoid® SPC3 was added, which further was kept for reflux at a temperature not exceeding 60 ◦ C for 4 h. By using rotavapor (R-210) the resultant mixture was evaporated to generate a molecular complex of drug and phospholipid, which was further dried under vacuum (overnight) to remove traces of solvents and stored at below 20°C.

5.15. Novel methotrexate prodrug-targeted drug delivery system based on PEG–lipid–PLA hybrid nanoparticles for enhanced anticancer efficacy and reduced toxicity of mitomycin C Yang Li et al. 2014

Materials

MMC, purity 99.5%, SPC. PLA, 10 kDa. DPPE. DSPE–PEG–NH2 and DSPE–PEG–COOH. MTX and FA. DCC and NHS. DMSO, DCM, THF, and TEA. DiD, DiR, and Lysotracker green. Cell Counting Kit-8 (CCK-8). Dialysis bag (Mw = 8000 to 12000 Da). FBS. 0.25% trypsin–EDTA and penicillin–streptomycin solution. HeLa cells, A549 cells, Caco-2 cells, and MC 3T3-E1 cells.

5.16. Preparation of MTX-PEG-PE-PLA NPs/MMC-SPC

By a solvent evaporation method, the MMC–SPC complex was prepared. MTX–PEG–PE–PLA NPs/MMC–SPC were formulated by a modified double-emulsion solvent evaporation technique. The internal water phase and the oil phase were formed by weighing the quantity of MMC–SPC (mol ratio MMC–SPC ¼ 1:1) was reconstituted in DI water and PLA was dissolved in DCM respectively. For the formation of external water phase DPPE, DSPE–PEG–COOH (DSPE–PEG) and DSPE–PEG–MTX (weight ratio of DPPE–DSPE–PEG–DSPE–PEG– MTX ¼ 2:1:0.6) were dispersed in DI water by sonication. The internal water phase was then added to the oil phase and the mixture was emulsified by sonication in an ice-water bath; the first water in oil emulsion was formed. After that, the emulsified mixture was added to the external water phase, and the mixture vortexed and then sonicated, to form a double emulsion. By magnetic stirring, the organic solvent was separated from the emulsion and finally removed by evaporation under reduced pressure in a rotary evaporator. The suspension was centrifuged at 15,000 rpm for 20 min at 4 ◦ C to collect the MTX–PEG–PE–PLA NPs/MMC–SPC. After washing three times with deionized water, the NPs were resuspended in deionized water with 3% w/w sucrose as cryo-protectant and lyophilized at 80 ◦ C for 24 h. At 4 ◦ C the lyophilized products were stored. On the one hand, PEG–PE–PLA NPs/MMC–SPC was prepared using the identical procedure as for DSPE–PEG–MTX, replaced by DSPE–PEG at the equivalent DSPE molar concentration in most of the experiments for comparison. On the other hand, the MTX–PEG–PE–PLA NPs/MMC or MTX–PEG–PE–PLA NPs/MMC + SPC were respectively prepared using the same method, except that MMC–SPC was replaced by MMC, or by a physical mixture of MMC and SPC at the equivalent MMC or SPC mass, for comparison with in vitro drug release. PEG–PE–PLA NPs/SPC and MTX–PEG–PE–PLA NPs/SPC were each prepared using the same technique but with MMC–SPC replaced by SPC at the equivalent mass, for comparison of cytocompatibility and hemocompatibility.

5.17. Monodisperse microparticles loaded with self-assembled berberine-phospholipid complex-based phytosomes to improve oral bioavailability and enhance hypoglycemic efficiency Fei Yu et al. 2016

Materials

Berberine (C20H18NO4, MW 336). Soybean phosphatidylcholine (SPC) LIPOID S-100. Fetal bovine serum (FBS) and Dulbecco' modified Eagle' medium (DMEM). Human intestinal epithelial Caco-2 cells.

5.17.1. Preparation of BER-SPC

By a rapid solvent evaporation technique, the BER-SPC was prepared. At first, 5 mg of BER and 25 mg of SPC were dissolved in hot ethanol (55 °C) and dichloromethane at a volume ratio of 1: 9 independently (the weight ratio of BER to SPC and the volume ratio of ethanol to dichloromethane were optimized in the preliminary experiments). Then, they were mixed together into a round-bottom flask very quickly. At last, by rotary evaporation, the organic solvent was removed in a vacuum yielding a thin film on the wall of the flask. To remove the residual organic solvents, the film was thoroughly vacuum-dried. Before conducting further studies, the crude BER-SPC was sealed and stored at 4 °C. For the testing of the complexing effect of BER with SPC in the formation of the BER-SPC complex, the crude BER-SPC complex was dissolved in chloroform (the BER insoluble solvent), followed by the vigorous vortexing, and filtered through a 0.2 μm membrane filter (PTFE, Millipore) to remove the excess and uncomplexed BER. The filtrate was evaporated.

5.18. Characteristics and Glucose Uptake Promoting Effect of Chrysin-Loaded Phytosomes Prepared with Different Phospholipid Matrices Seong-Min Kim et al. 2019

Materials

SPC (>98% purity), EPL (phosphatidylcholine 84.5%, phosphatidyl-ethanolamine 9%, sphingomyelin 3%, lysophosphatidylcholine 3%, lysophosphatidyl-ethanolamine 0.5%. 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG). Chrysin and the solvents. Taqman®Universal master mix, Taqman® probes (50 -fluorescein-based reporter dye; 30 -TAMRA quencher) and the high-capacity RNA-to-cDNA kit.

5.18.1. Preparation of CP

Using the solvent evaporation method, the CP was prepared at a molar ratio (chrysin: phospholipid) of 1:2 and 1:3 using SPC or EPL, respectively. Chrysin (159 mg, 50 mM) and SPC or EPL (969 and 1453 mg, 100 and 150 mM) were dissolved in 12.5 mL tetrahydrofuran and stirred for 4 h at 40 ◦ C. Using a rotary evaporator at 40 ◦ C, the solvent was removed. Using 12 mL distilled water, CP in a round bottom flask was recovered. Without the addition of chrysin, the empty phytosome was prepared through the same procedure.

Zinc phthalocyanine-soybean phospholipid complex-based drug carrier for switchable photoacoustic/fluorescence image, multiphase photothermal/ photodynamic treatment and synergetic therapy Jinyuan Ma et al. 2018

Deionized (DI) water. Zinc phthalocyanine (ZnPc). Soybean phosphatidylcholine (SPC). Doxorubicin. Methotrexate (MTX). 1, 2-diacyl-SN-glycero-3-phosphoethanolamine-N- [methoxy (poly(ethyleneglycol))-2000] (DSPE-PEG-OCH3), 1,2-distearoyl-snglycero-3-phosphoethanolamine-N- [amino (polyethylene glycol)- 2000] (DSPE-PEG-NH2), and N-hydroxysuccinimide (NHS). Dicyclohexylcarbodiimide (DCC). Dimethyl formamide (DMF) and tetrahydrofuran (THF). HeLa, MCF-7, and 4T1 cells. RPMI medium modified and Dulbecco's minimum essential medium/high glucose (DMEM/high glucose). Fetal bovine serum (FBS). Trypsin and penicillin-streptomycin. Methylthiazolyldiphenyl-tetrazolium bromide (MTT). DCFH-DA. The dialysis bags (MWCO = 3500).

5.18.2. Preparation and characterization of ZnPc-SPC complex

By co-solvent method with modification, the ZnPc-SPC complex was synthesized. At first, 5 mg of ZnPc and 35 mg of SPC were dissolved in 10 mL of tetrahydrofuran (THF) and magnet stirred at 40 °C for at least 6 h to obtain the ZnPc-SPC complex. To evaluate the phase of the ZnPc-SPC complex X-ray diffractometer was performed using Phillips X' pert Pro Super, Panalytical. Via a Bruker AV400 MHz NMR spectrometer, a 1 H NMR spectrum was displayed. On a Bruker IFS-55 infrared spectrometer, FTIR analysis was performed to determine the interaction between ZnPc and SPC. Using a Perkin Elmer Lambda 750 UV–vis-near-infrared spectrophotometer, the UV–vis-NIR absorption spectrum of the ZnPc-SPC complex was scanned.

5.18.3. Preparation and characterization of ZS and DZSM

By solvent evaporation method followed by a self-assembly technique, ZS, and DZSM were constructed. A solvent containing 1 mg of ZnPc was taken and THF was removed later by reduced pressure evaporation using a rotary evaporator (N-1300). ZnPc-SPC complex thin film was formed and 3 mL of DI water was added followed by ultrasonic dispersion using an ultrasonic bath cleaner (SK7200HP). Then ZS was formed and a clear colloidal suspension was formed. The suspension was diluted to 10 mL and ZS colloid suspension containing ZnPc equivalent to 0.1 mg/ mL was obtained. Then ZnPc-SPC complex dissolved in THF containing 1 mg of ZnPc was taken and by reduced pressure evaporation, THF was removed. ZnPc-SPC complex thin film was formed and 3 mL of 40 μg/mL ammonium sulfate solutions was added followed by ultrasonic dispersion. The clear colloidal suspension was added into a dialysis bag (MW = 3000) and dialyzed for at least 1h. Through amide reaction between DSPE-PEG-NH2 and MTX, DSPE-PEGMTX was synthesized. The above dialyzed colloidal suspension, 1 mg of Dox, 0.5 mg of DSPE-PEG-MTX, and 0.5 mg of DSPE-PEG-OCH3 were mixed, diluted to 10 mL, and stirred overnight to get DZSM colloid suspension containing ZnPc equivalent to 0.1 mg/mL. DZS was synthesized with the same method, DSPE-PEG-MTX, and DSPE-PEG-OCH3 should not be added. By the replacement of 0.5 mg of DSPE-PEG-MTX with 0.5 mg of DSPE-PEG-OCH3, DZSP was synthesized with the same method. By transmission electron microscope (TEM, Tecnai G2 Spirit) the morphology of DZS, DZSP, and DZSM were observed.

5.19. Self-targeting nano therapy based on functionalized graphene oxide for synergistic thermochemotherapy Dao Shi et al. 2021

Materials

MTX (98%), SPC (98%). Graphite powder (300 mesh). In addition, both 4-chl oro-7-nitro-1,2,3-benzoxadiazole (NBD-Cl, 98%) and 1,1-dioctade cyl-3,3,3,3-tetramethylindotricarbocyanine iodide (DiR, 98%). Sulfuric acid (H2SO4, 98%), potassium permanganate (KMnO4, 99%), sodium nitrate (NaNO3, 98%), hydrogen peroxide (H2O2, 30%), and hydrochloric acid (HCl, 36.5%). Cell culture medium. Dimethyl sulfoxide (DMSO, 99.9%) and tetrahydrofuran (THF, 99.8%). The dialysis membrane (molecular weight cutoff of 3500).

5.19.1. Synthesis and characterization of MTX-SPC

10 mg of MTX and 40 mg of SPC were added to 5 mL of THF and stirred at 50 °C for 5 h. Purification of MTX-SPC was done by filtration with a 0.22 μm organic membrane. When the solution became transparent without precipitation in 24 h, implying that the MTX-SPC dispersion was successfully synthesized.

5.20. Integration of phospholipid-complex nanocarrier assembly with endogenous N-oleoyl ethanolamine for efficient stroke therapy Xiangrui Yang et al. 2019

Materials

OEA (purity grade>95.0%) and Cy 5.5-NHS. SPC (soybean phosphatidylcholine, purity grade>90%). Dichloromethane (DCM), dimethyl sulfoxide (DMSO), and tetrahydrofuran (THF).

5.20.1. Preparation and characterization of OEA–SPC complex (OEA–SPC)

By a solvent evaporation method, the OEA–SPC was prepared. 6 mg of OEA powder and 30 mg of SPC were dissolved in a glass pressure vessel with 15 mL of THF, accompanied by vigorous agitation at 40 °C for 10 h. Via vacuum rotary evaporation with a rotary evaporator (N-1001S-W), THF was removed. By grinding with an agate mortar, the physical mixture of OEA and SPC (OEA & SPC) was prepared at the same weight ratio.

5.20.2. Preparation of OEA–SPC nanoparticles (OEA–SPC NPs)

By a nanoprecipitation technique, the OEA–SPC NPs were synthesized. 10 mL of DCM was added to the OEA–SPC (6 mg of OEA), and then the clear homogeneous solution was dropwise (0.2 mL/min) introduced into 40 mL of distilled water under magnetic stirring (200 rpm/min). The dispersed phase gradually evaporated with stirring overnight to remove the DCM, clear suspension was produced and resulting in the formation of the OEA–SPC NPs.

5.20.3. Liposome formulated with TAT-modified cholesterol for enhancing brain delivery Yao Qin et al. 2011

Materials

Soybean phospholipids (SPC) and cholesterol (CHO). Dioleoyl phosphatidylethanolamine (DOPE) and rhodamine-phosphatidylethanolamine (Rho-PE). NH2-PEG2000-Mal and mPEG2000-NH2. TAT peptide with terminal cysteine (Cys-AYGRKKRRQRRR). Coumarin-6 and didecyldimethylammonium bromide (DDAB). DIR. The BCA protein assay kit.

5.20.4. Preparation of the liposomes

By the thin film hydration method, Rho-labeled liposomes were prepared. Lipid materials were dissolved in chloroform in various amounts. By rotary evaporation, chloroform was then removed. For the complete removal of organic solvent, the lipidic thin film was kept in a vacuum for over 6 h. In 5% glucose solution (pH 7.2) the thin film was hydrated for 1 h at 37 ◦ C. And further sonicated by a probe sonicator at 100W for 50 seconds. To select the optimal prescription of TAT-LIP, Rho-labeled liposomes with different amounts of CHO–PEG2000–TAT and different amounts of CHO–mPEG2000 were prepared. As the method described above, Coumarin-6-loaded liposomes and DIR-labeled liposomes were prepared. Lipid materials with various amounts and coumarin-6/DIR were dissolved in chloroform. Operations were the same as the Rho-labeled liposomes. 10 g/ml and 20 g/ml respectively were the final concentration of coumarin-6 and DIR of the liposome.

5.21. Physicochemical Characterization and Stability of Rifampicin Liposome Dry Powder Formulations for Inhalation NARUMON CHANGSAN et al. 2008

Materials

Rifampicin (RIF). Rifampicin standard, cholesterol (CH) from lanolin, D-(þ)-lactose monohydrate, L-a-phosphatidylcholine from soybean (SPC), and D-mannitol. D-(þ)-Trehalose dehydrate and Triton-X 100. Deuterated 1-palmitoyl (D31)-2-oleoyl-sn-glycero-3-phosphocholine (d-POPC). Sodium dihydrogen phosphate. Chloroform, Acetonitrile, and methanol. All the solvents were high-performance liquid chromatography (HPLC) grade. Milli-Q-water (Millipore, Bedford, MA).

5.21.1. Preparation of RIF Encapsulated Liposome Suspensions

By the chloroform-film method, liposome suspensions were prepared. All ingredients including RIF (2.5 mg) were dissolved in a solvent mixture (10 mL) of chloroform and methanol (2:1, v/v). With different millimole ratios of SPC and CH, four suspension formulations (numbered 1–4) were prepared. To remove the solvent in the mixture a rotary evaporator was used until a dry film was formed. To produce liposome suspensions containing 0.05 mM of RIF, the hydrating solution consisted of 0.2 M phosphate buffer pH 7.4:1 mM EDTA (1:1, v/v) and 60 mL was added

Salidroside liposome formulation enhances the activity of dendritic cells and immune responses Xiaojuan Zhao et al. 2013

6. Materials and methods.

6.1. Preparation of salidroside liposome

By ammonium sulfate gradient method, the salidroside (with purity of 98% HPLC, Mol. wt: 300.01, liposomes were prepared. Soybean phospholipid, cholesterol, and tween-80 (6: 1: 1, w: w:w) were dissolved into ethanol. The solution was injected into 0.26 mol/L (NH4)2SO4 solution in a 43 °C water bath with the speed of 3 mL/min. Under lipid transition temperature, ethanol was removed by rotary vacuum evaporation. With the bag filter in PBS for 24 h the blank liposomes were dialyzed to remove (NH4)2SO4 in the external environment of liposomes, which could form the pH gradient between the inside and outside of the blank liposomes. By the pH gradient generated from the ammonium sulfate within the liposomes, the loading of the salidroside was driven. To monitor the uptake of the drug, Salidroside solution was added into the blank liposome suspension at a drug: lipid = 1:20 (w/w), followed by incubation at 50 °C. With ultrasonic cleaner, the mixture was homogenized for 30 min to form the small single-chamber liposomes. The solution was filtered with 0.45 μm and 0.22 μm millipore membrane successively.

Novel theranostic zinc phthalocyanine–phospholipid complex self-assembled nanoparticles for imaging-guided targeted photodynamic treatment with controllable ROS production and shape-assisted enhanced cellular uptake Jinyuan Ma et al. 2017

6.2. Materials

Zinc phthalocyanine (ZnPc). Soybean phosphatidylcholine (SPC). 6 Methotrexate (MTX). 1, 2-diacyl-SN-glycero-3-phosphoethanolamine-N-[methoxy(poly(ethyleneglycol))-200 0] (DSPE-PEG2000-OCH3, DSPE-mPEG), 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-N- [amino (polyethylene glycol)-2000] (DSPE-PEG2000-NH2), and N-hydroxysuccinimide (NHS). Dicyclohexylcarbodiimide (DCC). Dimethyl formamide (DMF) and tetrahydrofuran (THF). HeLa, MCF-7, and 4T1 cells. RPMI medium modified and Dulbecco's minimum essential medium/high glucose (DMEM/high glucose). Fetal bovine serum (FBS). Trypsin and penicillin-streptomycin. The dialysis bags (MWCO = 3,500).

6.2.1. ZnPc-SPC complex synthesis

Using the co-solvent method, the ZnPc-SPC complex was synthesized with magnetic stirring at 40 ◦C. 5 mg of ZnPc and 35 mg of SPC were added to 10 mL of tetrahydrofuran (THF). By reduced pressure evaporation using a rotary evaporator, the solvent was removed. To disperse the product N-hexane, a poor solvent for ZnPc was added. In n-hexane, ZnPc, SPC, and a physical mixture of both were added respectively, with the equivalent amount of ZnPc and stirred vigorously.

6.2.2. The preparation and characterization of ZnPc-SPC self-assembled nanoparticles

By solvent evaporation method followed by a self-assembly technique, the ZnPc-SPC complex self-assembled nanoparticles were constructed. THF containing 1 mg of ZnPc was taken and ZnPc-SPC complex dispersed in it. A uniform and thin film was formed by removing the solvent by reduced pressure evaporation. A small quantity of deionized water was added by ultrasonic dispersion using an ultrasonic bath cleanser until the clear colloidal solution was formed. ZS solution containing ZnPc equivalent to 0.1 mg/mL was obtained by diluting the suspension to 10 mL. By amide reaction between DSPE-PEG $_{2000}$ - NH2 and MTX, DSPE-PEG$_{2000}$-MTX was synthesized. Complex dispersed in THF containing equivalent to 1 mg of ZnPc was taken and then solvent was evaporated. To the dispersion, 0.5 mg of DSPE-PEG-MTX and 0.5 mg of DSPE-mPEG were added and diluted to 10 mL and stirred overnight.

6.3. Evaluation of VCAM-1 Targeted Naringenin/Indocyanine Green-Loaded Lipid Nanoemulsions as Theranostic Nanoplatforms in Inflammation Elena Valeria Fuior et al. 2020

6.3.1. Reagents and Consumables

The main reagents and consumables used in this study were provided by commercial suppliers, as specified: naringenin (catalog no. N5893, purity > 95%), hesperetin (catalog no. W431300, purity > 95%), soybean oil, sodium 4-[2-[(1E,3E,5E,7Z)-7-[1,1-dimethyl-3-(4-sulfonatobutyl) benzo[e]indol-2-ylidene]hepta-1,3,5-trienyl]-1,1-dimethyl benzo[e]indol-3-ium-3-yl]butane-1-sulfonate (Indocyanine Green), lipopolysaccharide (LPS, serotype E coli O111:B4), SYBR-Green; soy phosphatidyl choline (SPC), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-

[Maleimide(PolyethyleneGlycol) 2000](Ammonium salt) (Mal-PEG-DSPE) and 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt) (Rhodamine-PE) from Avanti Polar Lipids (Alabaster, AL, USA); VCAM-1 recognition peptide with sequence VHPKQHRGGSKGCC from GeneCust (Dudelange); fetal bovine serum (FBS); glycerin; Tris (2-carboxyethyl) phosphine (TCEP); SpectraPor dialysis membrane (cut-off 500-1000 Da); 100 kDa cutoff Amicon centrifugal filter columns. Trizol; primers; M-MLV Reverse Transcriptase and Taq DNA Polymerase. Deionized water (18.2 MΩ/cm) was prepared in-house using the Milli-Q system.

6.4. Preparation of Lipid Nanoemulsions

6.4.1. Preparation of Naringenin-Loaded Lipid Nanoemulsions

The organic phase, containing lipids dissolved in chloroform and a fixed volume of ethanol containing naringenin, was evaporated in a vacuum on a rotary evaporator (Laborota 4000) at 40 ◦C. Using a UPH200H probe-type sonicator (Hielscher), the residual was reconstituted in the aqueous phase, containing water and glycerin, followed by sonication for 10 min. 9.8 mM SPC, 0.2 mM Mal-PEG-DSPE and 0.5% v/v soybean oil, and 10% glycerin, the final formula of nanoemulsions. For the fluorescent labeling of the lipid nanoemulsions, 1.5 mol % Rhodamine-PE was added after LN preparation from an ethanol stock solution and incubated for 30 min at room temperature in the dark.

Endogenous Oleoylethanolamide Crystals Loaded Lipid Nanoparticles with Enhanced Hydrophobic Drug Loading Capacity for Efficient Stroke Therapy Shichao Wu et al. 2021

Materials and Methods

OEA (purity grade >90.0%) and SPC (soybean phosphatidylcholine, purity grade >90.0%). DSPE-PEG (MWCO 2000). Dichloromethane (DCM) and tetrahydrofuran (THF). 2,3,5-Triphenyltetrazolium chloride (TTC).

6.4.2. Preparation of OEA-SPC Complex (OEASPC)

Three milligrams of OEA powder and 15 mg of SPC were dissolved in a glass pressure vessel with 8 mL of THF, accompanied by vigorous agitation at 40°C for 8 h. Then, via vacuum rotary evaporation with a rotary evaporator (RE-5299), THF was removed.

6.4.3. Preparation of OSDP LNPs

By a nanoprecipitation technique, the OSDP LNPs were synthesized. OEA (5 mg) and OEA-SPC (3 mg of OEA) were dissolved in 10 mL of DCM, and then the clear homogeneous solution was dropwise (0.2 mL/min) introduced into distilled water (40 mL) containing DSPEPEG (20 mg) under magnetic stirring (200 rpm/min). After stirring for 1 hour, the system turned into a stable, white O/W suspension. By rotary evaporation, the dichloromethane was rapidly removed, producing a clear suspension. Then, through polycarbonate membranes (0.22 μm pore diameter) the suspension was extruded, and the OSDP LNPs were completely prepared. The OEA-SPC LNPs were prepared in the same way except no extra OEA was added in this procedure and DSPE-PEG was replaced by SPC.

7. Conclusion

In conclusion, this comprehensive review has illuminated the multifaceted applications of lipids in the development of advanced drug delivery systems, with a specific focus on liposomes, phytosomes, and ethosomes. The pivotal role played by lipids in enhancing drug solubility, stability, and bioavailability has been systematically explored, providing a nuanced understanding of their unique characteristics and applications. The investigation into various lipid compositions within liposomes has underscored their profound influence on structure and function. Additionally, the integration of phytosomes, utilizing plant-derived phospholipids, has been highlighted for its potential to enhance therapeutic efficacy. The extensive discussion on ethosomes, lipid vesicles enriched with ethanol, has emphasized their notable contribution to transdermal drug delivery. Critical analyses of recent research findings, including the impact of lipid selection on vesicle stability, drug release kinetics, and pharmacokinetics, have been presented, offering valuable insights for researchers, clinicians, and pharmaceutical scientists.

Furthermore, this review has addressed the challenges associated with lipid-based formulations, paving the way for potential avenues of future research and development. By synthesizing current knowledge, the review serves as a comprehensive resource for those seeking a deeper understanding of the intricate role of lipids in optimizing liposomal, phytosomal, and ethosomal drug delivery systems. Overall, the findings presented herein contribute to advancing the

field and inspire further exploration into the intricacies of lipid-based drug delivery systems for improved therapeutic outcomes.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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