Original Article

A Complete Review On Liposome Based Drug Delivery Sysyem

Ganesh kumar[1]*, Adarsh singh Bhadoria[2], Pragnesh patani[3]

[1*,2,3] Khyati college of pharmacy, Ahmedabad, Gujarat.
[2]Mail:- adarshadarsh586@gmail.com

***Corresponding author: -** Ganesh kumar

[*]Khyati college of pharmacy, Ahmedabad, Gujarat.
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Abstract

In this review article, we learned more about liposomes, a sort of drug delivery system utilised to target a specific tissue for the drug's effects. Liposomes can deliver medications where free medicines cannot because of the structural similarities between the lipid bilayer and the cell membrane. Because of its biocompatibility and capacity to encase hydrophobic molecules in the lipid domain, the liposomal delivery system has attracted attention as one of the noteworthy methods to improve dissolution and consequently absorption in the gastrointestinal (GI) tract. Due to its comparatively vast size, the GI tract's structural instability and low permeability across intestinal epithelia have been some of the disadvantages. In addition, despite the success of parenteral liposomes, there are yet to be any liposomal formulations authorised for oral use. However, the recent decade has seen a sharp rise in the number of published studies, which has led to a resurgence in liposomal oral administration. The majority of this research, however, has primarily involved in vitro tests, and there are not many water-insoluble medications that have in vivo evidence. People who are fairly new to this field of research should find the information in this article particularly useful.

Keywords: model lipid bilayers; bioactive compounds; membrane fluidity; membrane kinetics; essential methods for liposome/vesicle preparation oral; absorption; water-insoluble drugs; bioavailability.

INTRODUCTION

Paul Ehrlich imagined a means of delivering drugs directly to harmed cells, which he called "magic bullets." In 1906, tailored delivery was introduced as a result of this idea. [1-4].

According to the definition, "Liposomes are colloidal, vesicular structures made up of one or more lipid bilayers enclosing an equal number of aqueous compartments." Peptides, proteins, hormones, enzymes, antibiotic, antifungal, and anticancer compounds were all present in the liquid that made up the sphere-shaped shell. A free medicine injected into the blood stream frequently only maintains therapeutic levels for a brief period of time due to metabolism and excretion. Because several factors must be tuned at once to obtain clinically desirable efficacy and safety, drug research and development is extraordinarily complicated and difficult. Particularly, there has been a rising focus on the importance of taking drug-like characteristics like solubility and permeability into account during the early stages of drug discovery and development.[1]. The Biopharmaceutical Classification System (BCS) states that medications in BCS classes II and IV have low oral BA. Low BA for BCS class II medicines may be primarily the result of ineffective disintegration. In contrast, both poor solubility and limited permeability contribute to the low BA of BCS class IV medicines [5]. Water-insoluble pharmaceuticals have been delivered through a variety of methods, including salt creation, co-solvency and surfactant solubilization, solid dispersion, co-crystals, polymeric micelles, inclusion complex, size reduction, solid lipid nanoparticles, polymeric nanoparticles, and liposomes. [6–8]. Due to their ability to solubilize medications that are water-insoluble into nano-sized structures and to alter the behaviour of the drug in vivo to lessen toxicity, particle delivery systems, such as liposomes, have attracted attention. [9]. Due to its biocompatibility, liposomes have undergone the most testing of any nanoparticles because they can be administered by parenteral infusion. [10]

SIZE RANGE OF LIPOSOME

Types of liposomes	Size range (nm)
Small unilamellar vesicles	20-40
Medium unilamellar vesicles	40-80
Large unilamellar vesicles	100-1000

Multilamellar vesicles (MLV)

MLV are composed of two or more bilayers and have a size greater than 0.1 μm. The hydration of lipids in excess of an organic solvent or the thin-film hydration method are two of their straightforward and portable formulation techniques. Long-term storage doesn't affect their mechanical stability. Because of their size, they are eliminated quickly or early by the reticuloendothelial system (RES) cells, which makes them useful for various treatments that target the RES organs.

Specifically, the ratio of aqueous volume to lipid content in MLV is moderate. By using a slower rate of hydration and gentle mixing, the drug entrapment or corporate into the vesicles can be improved. It is also simple to increase encapsulation effectiveness by hydrating thin films of dry lipids. After combining with the aqueous phase (which contains the drug), further lyophilization and rehydration can produce MLV with a good encapsulation efficiency, or 40%.

Large unilamellar vesicles (LUV)

Donaruma and coworkers, 1985 Large unilamellar vesicles, in particular, belong to this type of liposomes and have a size higher than 0.1µm. They are composed of a single bilayer. Being able to store a big volume of solution in their cavity increases their encapsulation efficiency [11-12]. They can be used to encapsulate hydrophilic medications because of their high trapped volume. The most practical benefit of LUV is that it requires less lipid to encapsulate a lot of medication. Due to their bigger size, they are quickly eliminated by RES cells, just like MLV. LUV can be created using a variety of procedures, including ether injection, detergent dialysis, and reverse phase evaporation techniques. In addition to these techniques, LUV can be created by freezing and thawing liposomes, dehydrating and rehydrating SUV, and slowly swelling lipids in non-electrolyte solutions.

Small unilamellar vesicles (SUV)

(Abra et al., 1981) Compared to MLV and LUV, SUV are smaller (less than 0.1µm) in size and feature a single bilayer. They are distinguished by having a lengthy circulation half life and having a low entrapped aqueous volume to lipid ratio[13]. SUV can be made via the solvent injection method (ethanol or ether injection methods) or, alternatively, by shrinking the size of MLV or LUV using the sonication or extrusion process in an inert atmosphere like nitrogen or Argon. Either a probe-type sonicator or a bath-type sonicator can be used to perform the sonication. SUV can also be accomplished by applying high pressure on MLV while it passes through a small aperture. These SUVs can be combined and fused at little or no cost.

Methods of liposome preparation

General methods of preparation

All the methods of preparing the liposomes involve four basic stages:
1. Drying down lipids from organic solvent.
2. Dispersing the lipid in aqueous media.
3. Purifying the resultant liposome.
4. Analyzing the final product.

Method of liposome preparation and drug loading

The following methods are used for the preparation of liposome:
1. Passive loading techniques
2. Active loading technique.

Passive loading techniques include three different methods:

1. Mechanical dispersion method.
2. Solvent dispersion method.
3. Detergent removal method (removal of nonencapsulated material) [14,15].

Mechanical dispersion method

The following are types of mechanical dispersion methods:
1.1. Sonication.
1.2. French pressure cell: extrusion.
1.3. Freeze-thawed liposomes.
1.4. Lipid film hydration by hand shaking, non-hand. shaking or freeze drying.
1.5. Micro-emulsification.
1.6. Membrane extrusion.
1.7. Dried reconstituted vesicles [14,15].

Table no 1:-Types of Liposomes.[16-17]

SR NO.	TYPES	METHODS
1	**BASED ON STRUCTURAL PARAMETERS**	**1.Unilamellar vesicles**: Small unilamellar vesicles (SUV): size ranges from 20- 40 nm Medium unilamellar vesicles (MUV}: size ranges from 40-80 nm. Large unilamellar vesicles (LUV): size ranges from 100 nm-1,000 nm **2.Oligolamellar vesicles (OLV)**: These are made up of 2-10 bilayers of lipids surrounding a large internal volume **3.Multilamellar vesicles (MLV)**: They have several bilayers. They can compartmentalize the aqueous volume in an infinite numbers of ways. They differ according to way by which they are prepared. The arrangements can be onion like arrangements of concentric spherical bilayers of LUV/MLV enclosing a large number of SUV etc.
2	**BASED ON METHOD OF LIPOSOME PREPARATION**	1. REV: Single or oligolamellar vesicles made by Reverse- Phase Evaporation Method. 2. MLV-REV: Multilamellar vesicles made by Reverse-Phase Evaporation Method. 3. SPLV: Stable Plurilamellar Vesicles 4. FATMLV: Frozen and Thawed MLV. 5. VET: Vesicles prepared by extrusion technique 6. DRV: Dehydration-rehydration method.
3	**BASED UPON COMPOSITION AND APPLICATION**	1. Conventiuonal Liposome (CL): Neutral or negatively charged phospholipids and Cholesterol. 2. Fusogenic Liposomes (RSVE): Reconstituted Sendai virus envelopes 3. pH sensitive Liposomes: Phospholipids such as PE or DOPE with either CHEMS or OA 4. Cationic Liposomes: Cationic lipids with DOPE 5. Long Circulatory (Stealth) Liposomes (LCL): They have polyethylene glycol (PEG) derivatives attached to their surface to decrease their detection by phagocyte system (reticuloendothelial system; RES). The attachment of PEG to liposomes decreases the clearance from blood stream and extends circulation time of liposomes in the body. The attachment of PEG is also known as pegylation. 6. Immuno-Liposomes: CL or LCL with attached monoclonal antibody or recognition sequence.

EVALUATION OF LIPOSOMES [18-12]

Liposomal formulation and processing are described for a specific purpose to ensure their consistent in vitro and in vivo performance. Three major categories, including physical, chemical, and biological factors, can be used to classify the characterization parameters for evaluation purposes.

- Physical characterisation assesses a number of factors, such as size, shape, surface characteristics, lamellaty, phase behaviour, and drug release profile.
- Chemical characterisation entails investigations that determine the potency and purity of specific lipophillic components.
- Biological characterisation parameters can be used to determine if a formulation is safe and appropriate for use in therapeutic applications.

Some of parameters are:

1. Vesicle shape and lamellarity: Electron microscopic techniques can be used to analyse vesicle shape. Using Freeze-Fracture Electron Microscopy and P-31 Nuclear Magnetic Resosance Analysis, the lamellarity of vesicles, or the number of bilayers present in liposomes, is ascertained.

2. Vesicle size and size distribution: For the purpose of determining size and size distribution, various methodologies are discussed in the literature. Light microscopy, fluorescent microscopy, electron microscopy (especially transmission electron microscopy), laser light scattering photon correlation spectroscopy, field flow fractionation, gel permeation, and gel exclusion are some of these. Since it allows for close inspection of each liposome and the precise gathering of data regarding the population profile of liposomes over the entire spectrum of sizes, electron microscopy is the most accurate means of determining liposome size. Unfortunately, it takes a long time and requires equipment that may not always be available. In contrast, the disadvantage of the laser light scattering method is that it cannot measure the average property of the bulk of the liposomes. All of these techniques call for pricey equipment. Gel exclusion chromatography procedures are advised if only a rough concept of the size range is needed, as the only costs involved are those of the buffers and gel material. Atomic force microscopy, a more recent development in microscopic methods, has been used to examine the morphology, size, and stability of liposomes. Numerous categories, including microscopic, diffraction, scattering, and hydrodynamic approaches, can be used to classify the majority of size, shape, and distribution study techniques.

(a)Microscopic Techniques

1) **Optical Microscopy:** The microscopic method includes use of Bright-Field, Phase Contrast Microscope and Fluorescent Microscope and is useful in evaluating vesicle size of large vesiscle.
2) **Negative Stain TEM:** Electron Microscopic Techniques used to assess liposome shape and size are mainly negative-stain TEM and Scanning Electron Microscopy. The latter technique is less preferred. Negative Stain Electron Microscopy visualizes bright areas against dark background (hence termed as negative stain)

3) The negative stains used in TEM analysis are ammonium molybdate or Phosphotungstic acid (PTA) or uranyl acetate. Both PTA and ammonium molybdate are anionic in nature while uranyl acetate are cationic in nature.

4) **Cryo-Transmission Electron Microscopy Techniques (cryo-TEM):** This technique has been used to elucidate the surface morphology and size of vesicles.

(b)Diffraction and Scaterring Techniques

1) **Laser Light Scattering:** Photon correlation spectroscopy (PCS) is analysis of time dependence of intensity fluctuation in scattered laser light due to Brownian motion of particles in solution/suspension. Since small particles diffuse more rapidly than large particles, the rate of fluctuation of scattered light intensity varies accordingly. Thus, the translational diffusion coefficient (D) can be measured, which in turn can be used to determine the mean hydrodynamic radius (Rh) of particles using the Stoke-Einstein equation. Using this technique one can measure particles in range of about 3nm.

2) **Hydrodynamic Techniques:** This technique includes Gel Permeation and Ultracentrifuge. Exclusion chromatography on large pure gels was introduced to separate SUVs from radial MLVs. However, large vesicles of 1-3μm diameter usually fail to enter the gel and are retained on top of column. A thin layer chromatography system using agarose beads has been introduced as a convient, fast technique for obtaining a rough estimation of size distribution of liposome preparation. However, it was not reported if this procedure was sensitive to a physical blockage of pores of the agarose gel as is the more conventional column chromatography

(3)Encapsulation Efficiency and Trapped Volume: These determine amount and rate of entrapment of water soluble agents in aqueous compartment of liposomes.

a) **Encapsulation Efficiency:** It describes the percentage of the aqueous phase, and thus, the percentage of water-soluble drugs, that are finally captured during the creation of liposomes, and is often given as % entrapment/mg lipid. Minicolumn centrifugation and the Protamine Aggregation Method are two procedures used to evaluate encapsulation efficiency. Liposomes are typically purified and separated using minicolumn centrifugation on a small scale. In the tiny column centrifugation method, the hydrated gel is placed in a barrel of a 1 ml syringe without a plunger that is plugged with a Whatman GF/B filter pad. This barrel is resting inside of a centrifuge tube. To clear the gel of extra saline solution, this tube is spun for three minutes at 2000 rpm. The gel column should have dried after centrifugation and should have separated from the barrel side. After then, the collection tube is empty of the eluted salt. In order to eject the liposome-containing void volume into the centrifugation tube, 0.2 ml of liposome suspension is dropped dropwise onto the top of the gel bed. The column is then spun at 2000 rpm for three minutes. The elute is then taken out and placed away for analysis. Liposomes that are neutral or negatively charged can be aggregated using protamine.

b) **Trapped volume:** It is a crucial factor that controls the shape of vesicles. Aqueous entrapped volume divided by the amount of lipids constitutes the trapped or internal volume. Between 0.5 and 30 microliters/micromol may be used. To calculate trapped/internal volume, a variety of materials are utilised, such as spectroscopically inert fluid, radioactive markers, and fluorescent markers.

The best technique to measure internal volume is to measure the amount of water directly by substituting a spectroscopically inert fluid (deuterium oxide) for the external medium (water), and then measuring the water signal using NMR.
By distributing lipid in an aqueous solution containing a non-permeable radioactive solute, one can also experimentally measure the trapped volume. Centrifugation is used to remove external radioactivity, and the residual activity per lipid is then calculated to indicate the percentage of solute trapped.

4)Phase Response and Transitional Behaviour: Liposomes and lipid bilayers show a variety of phase transitions that are investigated for their function in triggered drug release or stimulus-mediated fusion of liposomal components with target cells. Understanding phase transitions, the fluidity of phase transitions, and the fluidity of phospholipid membranes is crucial for producing and using liposomes because the properties of the liposomal membrane depend on their phase behaviour, including permeability, fusion, aggregation, and protein binding.
Utilizing freeze fracture electron microscopy, the phase transition has been assessed. By using differential scanning colorimeter (DSC) analysis, they are more thoroughly confirmed.

5) Drug Release: A properly calibrated in vitro diffusion cell can be used to evaluate the process of drug release from liposomes. Before conducting expensive and time-consuming in vivo experiments, the liposome-based formulation can benefit from using in vitro assays to estimate drug pharmacokinetics and bioavailability. The dilution-induced drug release in buffer and plasma was used as a predictor for the pharmacokinetic performance of liposomal formulations, and a different assay that determined intracellular drug release caused by liposome degradation in the presence of mouse-liver lysosome lysate was used to determine the drug's bioavailability.

STABILITY OF LIPOSOME

The stability of the produced formulation is a crucial factor for developing liposomal medicinal products. The stability of the liposomes during all stages of production, storage, and distribution controls the drug's therapeutic action. A stable dosage form is one that preserves the physical stability and chemical integrity of the active substance throughout its creation process and storage. A well-designed stability study covers the assessment of the product's physical, chemical, and microbiological properties as well as the assurance of the product's integrity during the storage duration. Therefore, a stability protocol is necessary to examine the physical and chemical integrity of the drug product while it is being stored. (Johnston et al., 2007)

Physical stability

When phospholipids are hydrated in water, bilayered vesicles called liposomes are created. Different sizes of vesicles are produced during this process. In order to reach a thermodynamically advantageous condition during storage, the vesicles tend to congregate and grow in size. In 2011 (Andreas et al.) The physical stability of the liposomal medicinal product can be compromised during storage by drug leakage from the vesicles as a result of fusion and breaking of the vesicles. In order to evaluate the physical stability, it is crucial to consider the shape, size, and size distribution of the vesicles. A number of methods, including light scattering and electron microscopy, can be used to gauge this, including estimating the morphology (visual appearance) and size of the vesicles.

Chemical stability

Phospholipids are chemically unsaturated fatty acids that are vulnerable to oxidation and hydrolysis, which could change how stable a medicinal product is. A liposomal formulation is also greatly influenced by factors such as pH, ionic strength, solvent system, and buffered species. In fact, substances like light, oxygen, heat, and heavy metal ions can all cause chemical reactions. Since free radicals are produced throughout the oxidation process, degradation from oxidation includes the production of cyclic peroxides and hydroxyperoxidases. By shielding them from light, adding antioxidants like butylated hydroxyl toluene (BHT) or alpha-tocopherol, manufacturing the product in an inert environment (the presence of nitrogen or argon), or adding EDTA to remove trace amounts of heavy metals, liposomes can be protected from oxidative degradation. Lyso-phosphatidylcholine (lysoPC), which increases the permeability of the liposomal contents, is produced as a result of hydrolysis of the ester bond at the carbon position of the glycerol moiety of phospholipids. As a result, it becomes vital to regulate the maximum amount of lysoPC present in the liposomal medicinal product. This can be done by creating liposomes with phosphatidylcholine that is lysoPC-free. (Johnston et al., 2007)

In vivo behavior of liposomes:

During the optimization of liposomal formulation, a number of physico-chemical parameters are changed to obtain the desired bio-distribution and cellular uptake of pharmaceuticals. The following list of factors describes how they impact liposomes' in vivo (biological) performance. the following are factor’s that liposomes' in vivo (biological) performance.

Liposome size (Allen.1997)

Since it controls the portion cleared by RES, the size of the vesicle controls the fate of liposomes in vivo. With increasing vesicle size, RES can absorb liposomes at a faster rate. In comparison to liposomes smaller than 0.1μm, those larger than 0.1μm are opsonized (taken up) by RES more quickly. Liposome extravasations are also influenced by the vesicle's size. Capillaries within a tumour are more permeable than those within healthy tissue. Liposomal medication accumulation in tumour tissue is increased as a result of the leaky vasculature, which allows fluids and small liposomes to pass through the gaps and accumulate. A driving force for the extravasations of small sized liposomes is the differential between intravascular hydrostatic and interstitial pressure.

Surface charge (Andreas et al.,2011)

The type and density of the charge on the liposome surface can control how the lipid interacts with the cell. The nature and charge of the liposome can be changed by charging the lipid content. Lack of charge in SUV liposomes can cause them to aggregate, decreasing the liposome's stability; in contrast, neutrally charged liposomes interact with cells in a remarkably minimal way. A liposome with a high electrostatic surface charge may benefit the promotion of lipid-cell contact. The degree of lipid-cell interactions is influenced by density that is negatively charged, and target cells are more likely to take liposomes up inside their cells. But following systemic delivery, positively charged liposomes are eliminated more quickly. Cationic liposomes, as opposed to negatively charged liposomes, convey their contents to cells via fusing with cell membranes. (Johnston et al., 2007)

Surface hydration (Andreas et al., 2011)

The absorption of liposomes by RES cells is decreased because hydrophilic liposomes are less susceptible to opsonization. This is explained by the hydrophilic coating on the liposomes' surface, which lessens their interaction with blood and cell components. Compared to liposomes coated with hydrophobic coatings, these sterically stabilised liposomes have greater circulation half-lives and are more stable in the biological environment. The steric stability of liposomes is achieved by hydrophilic groups such as monogangliosides, hydrogenated phosphotidyl inositol, and polyethylene glycol.

Bilayer fluidity (Chrai et al., 2001)

Different physical states of lipid are present both above and below the phase transition temperature (Tc). They are solid and well-ordered below Tc but are in a fluid-like liquid-crystalline state above Tc, which represents the temperatures at which different phospholipids go through their phase transitions. When the temperature is physiological, the fluid-like nature of low Tc liposomes (less than 37°C) makes them vulnerable to drug content leakage. However, at physiological temperatures, the liposomes with high Tc (higher than 37°C) are stiff and less leaky. The contact between liposomal cells is also controlled by the phase transition temperature. When compared to liposomes with high Tc lipids, those with low Tc lipids have a higher degree of RES uptake. Liposome stability is provided by the incorporation of cholesterol into the bilayer, which can reduce membrane fluidity at temperatures above the phase transition point. New drug delivery systems are created when a conventional dose form fails to have the desired therapeutic effect. One of these systems, which offers greater therapeutic efficacy and safety in compared to current formulations, is liposomes. The following are some of the main therapeutic uses for liposomes in medication delivery: (Johnston et al., 2007)

Table 1. Characteristics and in vivo bioavailability of liposomal formulations for various water-insoluble drugs.

Drugs (Therapeutic category)	Liposome composition	Encapsulation efficiency (%)	Physical forms	Study subject	Relative BA (fold)	Comparator	Reference
BCS Class II drugs							
Apigenin (herbal supplement)	Phospholipid 90H	93.3%	Solid: proliposome (mannitol)	Rat	1.5	Free drug suspension	[23]
Carbamazepine (antiepilectic)	Drug:DMPG (1:1)	ND	Solid: co-precipitate	Rabbitt	1.2 (NS)	Tegretol suspenstion	[24]
Carvedilol (cardiovascular)	EPC:CH:Labrasol (65:15:20)	79.8%	Liquid: liposome dispersion	Rat	2.3	Free drug suspension	[25]
Docetaxel (anticancer)	EPC:SA (1:0.2) with SDC and coating with Eudragit L100/S100(4:1)	33.6%	Solid: Freeze-dried liposomes (trehalose, mannitol)	Rat	3.1	Free drug solution in polysorbate 80 /ethanol/saline (20:13:67)	[26]
Dronedarone (antiarrhythmic)	DMPG Na:CH (1:2)	84%	Solid: proliposomes (MCC)	Rat	1.5	Free drug suspension	[27]
Fenofibrate (antilipidemic)	SPC:SDC (4:1)	88.2%	Liquid: liposomal dispersion	Dog	5.1	Micronized fenofibrate in capsule	[28]
Flutamide (antiandrogen)	SPC:CH (4:1 w/w)	70.6%	Liquid: liposomal dispersion	Rat	0.9	Free drug suspension	[29]
Halofantrine (antimalarial)	DSPC:Drug (3:1) Coating with CAP	ND	Solid: proliposomes (enteric coating)	Rat	1.4	Free drug suspension	[30]
Indomethacin (NSAID)	DSPC:DCP:CH (8:2:1) coating with chitosan	ND	Liquid: liposomal dispersion	Rat	1.8	Free drug solution	[31]
Isradipine (calcium antagonist)	HSPC:CH (1:1)	96.8%	Solid: proliposomes (enteric coating)	Rat	2.0	Free drug suspension	[32]
Lovastatin (antilipidemic)	SPC:CH (9:1)	85.8%	Solid: proliposomes (silicified MCC)	Rat	1.6	Free drug suspension	[33]
Nisoldipine (calcium channel blocker)	DMPC:CH (4:1)	85.6%	Solid: proliposome (MCC)	Rat	3.0	Free drug suspension	[34]
Piroxicam (NSAID)	DMPG	ND	Solid: solid dispersion	Rat	1.3 (NS)	Free drug suspension	[35]
Raloxifen	HSPC:CH with DCP or SA	94.2% (cationic) 93.2% (anionic)	Solid: proliposomes (mannitol)	Rat	3.4 (cationic); 2.6 (anionic);	Free drug suspension	[36]
estrogen receptor modulator		93.9% (neutral)				(processed without lipids)	

Sorafenib tosylate (anticancer)	DPPC:DPPG: TPGS:CH (8:1:2:4) Coating with Glycol chitosan & Eudragit S100	94.6%(uncoated) 96.6% (glycol chitosan-coated) 89.7% (double layer coated	Liquid: liposome dispersion	Rat	2.9(uncoated); 3.0 (glycol chitosancoated); 5.1 (EudragitS100/ glycol chitosan coated)	Free drug	[37]
Silymarin (hepatoprotective)	Phospholipid (82% PC)	92.6%	Solid: proliposomes (mannitol)	Dog	3.4	Powder	[38]
Dehydrosilymarin (hepatoprotective)	SPC 0.3g CH 0.075g IPM 0.2 g Sodium cholate 0.2g	70–80%	Solid: proliposomes (mannitol)	Rabbit	2.2	Free drug suspension	[39]
Tacrolimus (immunosuppressant)	DSPC:CH (4:1)	approx. 70–80%	Solid: proliposomes	Rat	1.9	Free drug suspension	[40]
Vinpocetine (Cardiovascular)	SPC:CH (9:1, w/w	86.3%	Solid: proliposomes (sorbitol)	Rabbit	3.5	Free drug suspension	[41]
Zaleplon (hypnotic)	HSPC:CH (1:1) with DCP or SA	93.8% (cationic) 92.5% (anionic) 94.6% (neutral)	Solid: proliposomes (mannitol)	Rat	4.6 (cationic) 3.0 (anionic) 2.0 (neutral)	Free drug suspension (processed without lipids)	[42]
BCS class IV drugs							
Curcumin (herbal supplement)	SPC:SDC (85:15 w/w) Coating with Silica	93.3%	Liquid: liposome dispersion	Rat	2.3(uncoated);3.3(silica-coated)	Free drug suspension	[43]
	SPC:CH:TPGS:drug (20:2:12:1) Coating with TMC	86.7%	Liquid: liposome dispersion	Rat	6.7(uncoated); 10.6(TMC-coated)	Free drug suspension	[44]
	SPC:SDC (70:25 w/w) Coating with TMC and CMCS	ND	Liquid: liposome dispersion	Rat	6.3(CMCS/TMC -coated); 2 (TMC-coated)	Uncoated liposomes	[45]
Cyclosporine A (immunosuppressant)	ePC:Cremophor EL (10:0.5)	96.3%	Solid: proliposomes (lactose)	Rat	9.6	Free drug suspension	[46]
	SPC:SDC (3:1)	94.0%	Liquid: liposome dispersion	Rat	1.2 (NS)	Sandimmune Neoral®®	[47]
	SPC:CH (20:1) Coating with OACS	98.0%	Liquid: liposome dispersion	Rat	1.7 (uncoated); 3.4 (OACScoated)	Free drug suspension	[48]
	EPC:CH (28:5) with Pluronic F127	90.0%	Liquid: liposome dispersion	Rat	1.8	Unmodified liposomes	[49]
Daidzein (natural compound)	SPC:CH:DSPEPEG2000 (55:40:5)	80.2%	Solid: freeze dried liposomes with 3% sucrose	Rat	2.5	Free drug suspension	[50]
Lopinavir (antiviral)	HSPC, CH (7:3)	Approx. 89%	Solid: proliposome (mannitol)	Rat	2.2	Free drug suspension	[51]
Paclitaxel (anticancer)	SPC:CH:SA (24.5:11.5:2 w/w) Coating with PAA and then PAH	81.3%	Solid: freeze dried liposomes with mannitol	Rat	4.0 (double-layer coated)	Free drug suspension	[52]

Table 2. Major liposome-specific characteristics.

Characteristics	Representative techniques
Particle size and size distribution Morphology, lamellarity	Dynamic light scattering (DLS), Electron microscopy Electron microscopy
Surface charge	Zeta potential analysis
Encapsulation efficiency	Separation of free drug (dialysis, ultrafiltration, size exclusion chromatography) and drug analysis (HPLC etc.)
Release rate Physical stability	Release in physiological media or storage buffer Particle size change in physiological media or storage buffer

Table 3. Advantages and disadvantages of liposomes as an oral delivery system.

Advantages	Disadvantages
Biocompatibility	Physical instability in liquid state
Versatility for drug encapsulation	Lysolipid formation by chemical degradation
Flexibility of membrane components	Drug leakage
Capability of surface modification	Disruption in the stomach
Proposed enhanced permeation	Low permeability of intact liposome in the GI tract
Modifiable pharmacokinetic behavior	Difficulty in mass production and quality control

Researchers	Formulations	Dose	F	AUC0–∞ (ng·h/L) (* AUC0–12h)	Cmax (ng/L)
Li et al. 2012	Free drug suspension	50 mg/kg (oral)	-	86.65 *	71.35
	Liposomes (SPC:SDC)	50 mg/kg (oral)	-	203.64*	128.78
	Silica-coated liposome	50 mg/kg (oral)	-	673.79*	446.66
Chen et al. 2012	Free drug suspension	250 mg/kg (oral)	-	244,770	46,130
	Liposomes (SPC:CH:TPGS)	40 mg/kg (oral)	-	263,770	32,120
	TMC-coated liposomes	40 mg/kg (oral)	-	416,580	35,460
Tian et al., 2018	Liposomes (SPC:SDC)	10 mg/kg (oral)	6%	528,900*	48,200
	TMC-coated liposomes	10 mg/kg (oral)	12%	1,218,200*	78,300
	CMCS/TMC-coated Liposomes	10 mg/kg (oral)	38%	3,021,200*	167,800
Wang et al. 2020	Intravenous	40 mg/kg (i.v.)	-	268,900	-
	Commercial product 1 (tablet)	250 mg/kg (oral)	0.9%	20,000	12,600
	Commercial product 2 (capsule)	250 mg/kg (oral)	0.6%	10,740	9,920
		250 mg/kg (oral)	3.1%	45,600	17,800

CONCLUSIONS

There is a lot of in vivo data showing that liposomes can boost penetration and sustain release in the GI tract while also solubilizing certain water-insoluble medicines to increase their BA. Solid proliposomes and freeze-dried liposomes were developed to accelerate the rate of dissolution while maintaining medication stability during storage and in the GI tract by presenting the drug in an amorphous state rather than a crystalline one. The hostile stomach environment was avoided by covering the liposomes with enteric material. To increase the permeability of liposomal medications, mucoadhesive polymers, mucus-penetrating polymers, and bile salts were also added. To comprehend the in vivo fate of liposomal medications, however, more thorough mechanistic investigations are required. Despite initial scepticism, in vivo research has shown that liposomes can be effective oral delivery systems with easily adaptable characteristics.

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