Food Research 7 (3) : 233 - 246 (June 2023)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

REVIEW

Liposome optimisation for oral delivery of nutraceuticals in food: a review

Latrobdiba, Z.M., Fulyani, F. and *Anjani, G.

Department of Nutrition Science, Faculty of Medicine, Universitas Diponegoro, Jl. Prof Soedarto, SH, Tembalang, Semarang, Indonesia 50275

Article history:
Received: 21 January 2022
Received in revised form: 1 March 2022
Accepted: 4 March 2022
Available Online: 30 June 2023

Keywords:
Liposome,
Oral delivery,
Nutraceuticals,
Stability,
Absorption

DOI:
https://doi.org/10.26656/fr.2017.7(3).022

Abstract

A liposome is one of the most commonly used encapsulation technology with proven benefits, including high biocompatibility and improved solubility, but efficacy may be compromised when used for oral delivery, including application in food. The latter is an unfortunate but manageable setback compared to its promising compatibility with various nutraceuticals regardless of their solubility, which would be greatly useful in functional food production. Oral delivery presents several challenges, specifically the harsh environment in the gastrointestinal tract with enzymatic activities and pH changes and limited permeability of compounds across intestinal epithelia. To overcome these challenges, various efforts have been made to modify and strengthen liposomes as oral carriers. In this review, a brief introduction to liposomes and the challenges faced in the gastrointestinal tract is provided. A review of the existing modification efforts done to liposomes was to increase their stability and permeability for oral delivery.

1. Introduction

Liposome, one of the most used encapsulation technologies, is a spherical vesicle composed of a phospholipid bilayer with a hydrophilic core, making it suitable for the encapsulation of hydrophobic and hydrophilic substances (McClements, 2018). It is an encapsulation vehicle known for its functional properties, including optimum kinetic stability, efficient encapsulation capacity, and improved solubilities of encapsulated targets (Taylor *et al*., 2005). Its membrane-like structure is beneficial to target membranes with a specific composition (Taylor *et al*., 2005). Liposomes have been applied in various fields, including medical, cosmetic, pharmaceutical, agricultural, and most recently, food industries. Currently, the application of liposomes in food is mostly intended to increase shelf-life or maintain organoleptic properties (Pinilla *et al.*, 2019; Esmaeili *et al.*, 2020). Chen *et al*. (2012) reported high encapsulation efficiency of curcumin encapsulated in liposomes, ranging from 70.4% to 92.6%. The encapsulation of various vitamins in liposomes showed excellent efficiency of 48.2% to 99% (Liu and Park, 2009; Zhou *et al*., 2014; Gopi and Balakrishnan, 2020). These study results affirmed the potential of liposomes as a promising encapsulation system, especially for compounds that are sensitive and largely affected by their environment, such as nutraceuticals. Liposomes are expected to protect nutraceuticals' physical and chemical stability, minimise loss of biological functionalities, and improve bioavailability and shelf life (Mozafari *et al*., 2008; Khorasani *et al*., 2018).

For substance delivery in biological systems, liposomes are usually administered intravenously. Intravenous injections have several drawbacks, such as pain and stress for patients with repeated injection schedules, leading to overall low patient compliance. Moreover, this delivery method could lead to substance accumulation in the reticuloendothelial system, i.e., lung, liver, spleen. On the other hand, oral delivery becomes a preferable alternative method as it has several advantages, it minimises risks of infection from repeated injections, promotes mucosal and systemic immunity, is patient-friendly and easy for self-application, and is more cost-effective as it does not require professional handling (Bernkop-Schnürch, 2013; Hirlekar, 2017; Merlin *et al*., 2017). A liposome is particularly favoured for oral delivery as it is made of entirely natural ingredients, making it highly biocompatible and less likely to trigger adverse effects in the body (Patil and Jadhav, 2014; Shukla *et al*., 2017). These properties also make it more feasible as an encapsulating agent to incorporate nutrient or non-nutrient compounds with health-promoting properties in food. This could become a potential answer to the increasingly growing demand for functional food

*Corresponding author.
Email: *gemaanjani@gmail.com*

eISSN: 2550-2166

that provides health benefits without changing current dietary habits.

Nevertheless, oral delivery face problems related to the harsh gastrointestinal environment and poor permeability, limiting bioavailability and eventually reducing bioactivity (Bernkop-Schnürch, 2013; Fonte *et al.*, 2013; Hirlekar, 2017). This proves to be a concern since liposome, particularly conventional or unmodified liposome, is sensitive to endogenous chemicals in the gastrointestinal environment and has a low penetration rate across intestinal epithelial cells (Liu *et al.*, 2019; Manconi *et al.*, 2020). Conventional liposomes were reported to rapidly degrade in the simulated gastric fluid within the first two hrs (Zhang *et al.*, 2014). It is also sensitive to changes in pH and temperature, making it disadvantageous for long-term storage, as shown by eventual aggregation, fusion, and degradation over time (Shukla *et al.*, 2017). The previous researcher observed increased fluidity of liposomes which led to changes of liposome structure arrangement within less than ten days of storage, further confirming the unstable nature of conventional liposomes (Briuglia *et al.*, 2015). These drawbacks pose various challenges in using liposomes for oral delivery thus, many liposome modifications have been proposed over the years.

By modifying liposome composition and adding polymer coatings, the stability of liposomes against environmental stresses and the permeability of liposomes for intestinal epithelial cells have been tremendously improved. These improvements also include adding components that increase the rigidity of the lipid bilayer or serve as ligands for absorption. Another expert reported significantly improved payload retention in biotin-modified liposomes than conventional liposomes ($p<0.05$, 78% vs 45%) (Zhang *et al.*, 2014). This review discussed the challenges of oral delivery and modifications that can be done to optimise liposomes as an oral delivery system.

2. Methods

ScienceDirect and Scopus were used as the source for the searches using several combinations of the following keywords to refine the search: "liposome", “oral delivery”, “digestion”, “absorption”, “nutraceutical”, and “food”. Journal articles used were limited to the past ten years, with articles published in the last five years as a priority and some exclusions were made for primary source journals. Afterwards, different words were independently added to further refine the search according to the information needed.

3. Liposome

3.1 Definition and classification

A liposome is a closed lipid bilayer vesicle with an aqueous core, closely resembling a cell membrane. It is considered the best organic nanoparticle and is often used as a vehicle for orally delivered substances. Liposomes can carry hydrophilic substances in their core, while hydrophobic substances bind to the lipid bilayer (Figure 1) (McClements, 2018). Generally, the liposome can be classified according to the number of bilayers and size (Figure 2): multilamellar vesicles (MLV), large unilamellar vesicles (LUV), giant unilamellar vesicles (GUV), and small unilamellar vesicles (SUV) (Akbarzadeh *et al.*, 2013). Multilamellar liposomes have an onion-like structure where the aqueous core is enclosed by multiple concentric phospholipid bilayers, usually over 500 nm. As the name suggests, Unilamellar liposomes only have a single phospholipid bilayer surrounding the core (Akbarzadeh *et al.*, 2013). The size of GUV is about 1000 nm or more, while LUV may be slightly over 100 nm. SUV typically ranges from 20 – 100 nm (Laouini *et al.*, 2012).

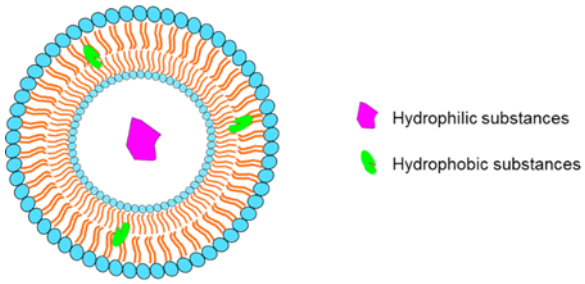

Figure 1. Illustration of liposome structure and substance loading patterns.

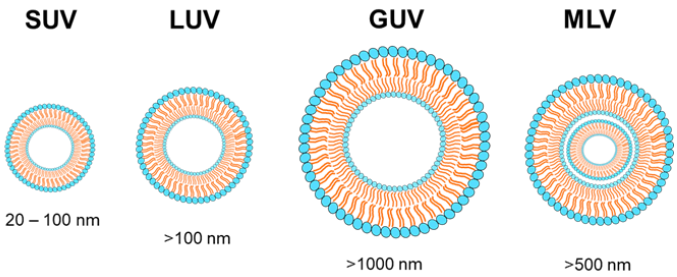

Figure 2. Liposome classification based on the size.

3.2 Liposome preparation

There are several methods to generate liposomes. The first method is dissolving lipids in an organic solvent followed by adding bioactive substances and finally eliminating the solvent by evaporation. A different approach uses micelle made from a mixture of lipid and detergent. The detergent will be eventually removed using dialysis below its critical micellar concentration. The most well-known method is lipid film hydration, where phospholipid solution in chloroform is vacuum-dried to generate a lipid film. The lipid film is then hydrated using a suitable solution depending on its

https://doi.org/10.26656/fr.2017.7(3).022

target encapsulated substance (Colletier *et al*., 2002). Additional steps can also be done to increase efficiencies, such as freeze-thaw cycles or extrusion. Reversed-phase evaporation is another method of liposome formation, specifically producing smaller-sized liposomes of 20-100 nm (Patil and Jadhav, 2014). In this method, the water-in-oil emulsion is formed through vigorous water mixing added to a phospholipid solution dissolved in an organic solvent. The solvent will be removed to obtain an aqueous liposome suspension.

3.3 Liposome for nutraceutical delivery

Liposome has shown many favourable features, including simple preparation, flexible composition, low toxicity, and good biocompatibility (Ismail and Csoka, 2017). As mentioned, liposome has a unique structure that allows them to carry hydrophilic and hydrophobic substances. This advantage also makes it possible for liposomes to encapsulate more than one bioactive substance, thus generating a synergistic effect (Khorasani *et al*., 2018; Huang *et al*., 2019). A previous study about liposome co-encapsulation of nutraceuticals with contrasting polarities, specifically curcumin and resveratrol, reported that curcumin was predominantly located in the hydrophobic acyl-chain regions while resveratrol bonded with the polar head groups (Huang *et al*., 2019). Several flavonoids were found to localise and form strong bonds with a lipid membrane, resulting in more rigid and stable liposomes (Huang *et al.*, 2017). In addition, co-encapsulation produced liposome systems with higher stability during storage, heating, simulated digestion, and stronger biological activities than individually encapsulated substances (Huang *et al*., 2019; Liu *et al*., 2020).

Liposome has been used to encapsulate various substances with relatively good efficiency. Liposomes loaded with griseofulvin, a hydrophobic drug, achieved the highest encapsulation efficiency of 97.9% (Ong *et al*., 2016). Similarly, the efficiency of encapsulated vitamin D3 in liposomes was 93% (Mohammadi *et al*., 2014). Another study reported using liposomes to encapsulate bioactive salmon protein hydrolysates with an encapsulation efficiency of 71.3% (Li, Paulson and Gill, 2015). Other studies that used liposomes for oral delivery mentioned efficiency in the range of 45.0% to 94.7% (Park *et al*., 2011; Ohnishi *et al*., 2015).

Furthermore, liposome has shown favourable results in terms of cell uptake compared to free substances for oral delivery. The liposome bilayer structure gradually diffuses with the intestinal cell membrane, thus improving substance absorption and delivery to target cells (Wang *et al*., 2021). Wang et al. described increased oral bioavailability of liquiritin by 8.8 times in liposome-encapsulated liquiritin compared with free liquiritin suspension (Wang *et al*., 2021). Similarly, Hanato *et al*. found that blood serum incretin levels were 3.6 times higher in mice given liposome formulations than free incretin (Hanato *et al*., 2009).

4. Challenges in oral delivery

Oral delivery is generally preferred because of its ease, low cost, and flexible intake schedule. However, liposomes consumed orally will be exposed to the complex and harsh environment of the gastrointestinal tract before meeting their target cell or membrane. The challenges include the digestion process to absorb bioactive substances with potential effects on stability, bioavailability, solubility, and, consequently, the efficacy of encapsulated nutraceuticals.

4.1 Digestion

Two main processes occur during digestion: physical degeneration, where large molecules become small through mechanical means, and enzymatic degradation, where enzymes break down large molecules into small molecules that intestinal walls can absorb (Gropper *et al*., 2009; Lucas-González *et al*., 2018). These processes directly determine the bio-accessibility and bioavailability of orally delivered substances and put them at risk of damage and leakage (Gropper *et al*., 2009; Lucas-González *et al*., 2018). Digestion could be divided according to where it occurred, namely oral, gastric, and intestinal digestion (Guo *et al*., 2017; Liu *et al*., 2019). Oral digestion includes chewing, mastication, and enzymatic digestion by amylase and lingual lipase. Chewing and mastication break down food into small pieces and mix them with saliva to form a bolus. The strength and duration of chewing, pH of saliva, and the enzyme content of saliva affect bolus formation, which will later be sent to the stomach (Liu *et al*., 2019). In the case of liposomes, they can be consumed as supplements or part of food and swallowed with or without chewing. Considering food may only spend approximately 20 seconds in the mouth, the integrity of liposomes may not be affected much by oral digestion.

Gastric digestion can cause more damage to liposomes as it involves mechanical mixing and chemical hydrolysis in an acidic environment. Gastric juice contains hydrochloric acid, mucous, and digestive enzymes, namely pepsin and lipase. Gastric pH is around pH 5 - 6 when bolus enters the stomach before gradually declining to pH 1 - 2 over the next hour. The low pH is necessary to activate enzymes and is essential against ingested microorganisms. The conventional liposomes with specific phospholipid compositions may become unstable and eventually dissolve under low pH (Zhang *et*

https://doi.org/10.26656/fr.2017.7(3).022

al., 2014; Wu *et al*., 2015). In addition, despite that only about 30% of lipolysis occurs in the stomach, it is still likely for liposomes to be compromised by gastric lipase activities (Li *et al*., 2011).

In the intestines, digestion occurs with the help of bile salt and various enzymes released by the pancreas. Bile salt adsorbs to the oil droplet surface to promote lipid emulsification and hydrolysis. It can destroy the liposome structure by penetrating the phospholipid bilayer, thus increasing membrane fluidity and susceptibility to lipase activity (Liu *et al*., 2019). Pancreatic juice contains lipase, phospholipase, and cholesterol esterase. These enzymes may hydrolyse phospholipids and thus disrupt liposome integrity and dismantle their structure (Liu *et al*., 2012). Studies in simulated intestinal digestion revealed that liposomes showed damaged membranes and irregular forms after two hrs (Liu *et al*., 2015). Co-encapsulation of vitamin C and b-carotene showed expanded and fragmented liposomes during simulated intestinal digestion, leading to a gradual release of active substances until over 70% (Liu *et al*, 2020).

4.2 Absorption

The next challenge in the oral delivery of liposomes is ensuring that the intestinal cell walls can absorb them. The compact epithelial cell layer of the gastrointestinal tract is bound by tight junctions that allow only small molecules to pass and exclude exogenous particles, making it less likely for giant liposomes to be absorbed (Nguyen *et al*., 2016). Moreover, the epithelia secrete mucus that forms a protective coating over the intestinal walls. This mucous layer is constantly shed and rejuvenated, entrapping liposomes in the membrane layer if it cannot penetrate the mucous layer quickly enough (Ensign *et al*., 2012).

There are several proposed mechanisms for the absorption of liposomes through the intestinal cells. Liposomes may release the active substance in the gastrointestinal tract or transform it into mixed micelles that transfer ingredients across intestinal epithelia (Wu *et al*., 2015). A possible alternative route is the absorption of liposomes as a whole vesicle through the M cells on the epithelial surface of the intestines (Niu *et al*., 2012). M cells are specialised epithelial cells in the FAE of Peyer's patches and can transport particles from the intestinal lumen to lymphoid tissues (Kou *et al*., 2013). Phospholipids of liposome stimulate chylomicron production by α-glycerol-3 phosphate pathway on the rough endoplasmic reticulum or 2-monoglyceride pathway in the smooth endoplasmic reticulum (Kalepu *et al*., 2013; Ahn and Park, 2016). Liposomes are transported in the form of chylomicron and are released from the enterocyte to enter the lymphatic vessels (Ahn and Park, 2016). By entering the lymphatic vessel, encapsulated substances bypass the first-pass metabolism of the liver, thus maintaining bioavailability (Kim *et al*., 2013). This mechanism was also found in a previous study of vitamin C in liposomes where its bioavailability increased by 1.77 times compared to free vitamin C (Gopi and Balakrishnan, 2020).

Studies have reported that the uptake of liposomes by enterocytes was size-dependent. The bioavailability of small liposomes was 2.6 to 3.33 times higher than larger liposomes, attributed to the smaller size being more readily taken up by Peyer's Patches (Ong *et al*., 2016). Likewise, Andar *et al*. reported that small liposomes (40.6 nm) had significantly higher cellar uptake than larger liposomes (97.8 – 162.1 nm), nearly 12 times higher (Andar *et al*., 2014). Their study further revealed that liposomes were absorbed through different mechanisms according to their size. The smaller liposomes mainly were taken up through the dynamin-dependent pathway, a receptor-mediated pathway where endocytosis occurs with enzyme dynamin pinching vesicles from the plasma membrane (Hillaireau and Couvreur, 2009; Kou *et al*., 2013). Liposomes of about 72.3 nm or smaller were suggested to be the ideal size for cell uptake. Its absorption was shown to be affected by endocytosis inhibitors in all pathways, implying that it could be absorbed through various routes (Andar *et al*., 2014).

Overall, it appears that composition and size greatly determine the absorption pathway of the liposome (Nguyen *et al*., 2016). Chemical composition also affects liposome stability in the gastrointestinal tract. Therefore, adjusting and modifying liposome composition and formulation may bring enhancements to the digestion behaviours of the liposome.

5. Modifications of liposome for oral delivery

Liposome has flexible physicochemical properties, which allows modifications to improve their structure and stability. Over the years, various changes have been made, including adjustments to liposome composition, surface coating, incorporation of absorption enhancers, improvement of muco-adhesiveness, and ligand-mediated targeting to intestinal epithelia (Figure 3) (Wu *et al*., 2015; He *et al*., 2019). These modifications have been shown to increase liposome stability against oxidation and deamination, provide protection from enzymatic degradation, and enhance the absorption of substrates. Many studies have been done in improving liposome stability for oral delivery with lots of promising results (Table 1).

https://doi.org/10.26656/fr.2017.7(3).022

Table 1. Various modifications of liposomes with improved properties

Modification Type	Modification Material	Encapsulated compounds	Key findings
Liposome composition	Rapeseed phospholipid combined with hydrogenated PC	Lactoferrin (LF)	Increased resistance against gastric (79.9% LF retained) and intestinal (32.19% LF retained) digestion (Vergara *et al*., 2020)
	SPC + DPPG combined with either cholesterol, B-Sitosterol, ergosterol, lanosterol, stigmasterol	Recombinant human insulin (rhINS)	Significant protection of rhINS in gastric digestion (74.8%) by ergosterol liposomes (Cui *et al*., 2015)
Surface coating	Whey protein isolate	Quercetin	Stable and unchanged particle size and seta potential during incubation in simulated gastric digestion (Frenzel *et al*., 2015)
	Chitosan	Curcumin	Enhanced protection of curcumin in the digestion phases of stomach (~75% retained) and intestines (~55% retained raw digesta); Improved bioavailability (~75% retained in micelles)(Cuomo *et al*., 2018)
	Pectin	Bufalin	Improved mucin adsorption up to 4 times higher than uncoated liposomes (Ying Li *et al*., 2014).
	Polyethylene glycol	Fluorescein isothiocyanate dextran (FD4)	Higher intracellular uptake in Caco-2 cells by PEG-liposomes than unmodified liposomes; Increased blood concentration of FD4 in rats given PEG-liposomes (Yamazoe *et al*., 2021)
Absorption enhancer	Sodium deoxycholate	Cyclosporine A (CyA)	Enhanced bioavailability of CyA (120.3%) (Guan *et al*., 2011)
Ligand mediator	Folic acid	Insulin	Improved intestinal cells uptake by 1.2 to 1.5 times compared to unmodified liposomes (Yazdi *et al*., 2020)
	Biotin	Insulin	Increased protection in gastric (78% retained) and intestinal (85% retained) digestion; Increased permeability of insulin by 1.85 times (Zhang *et al*., 2014)

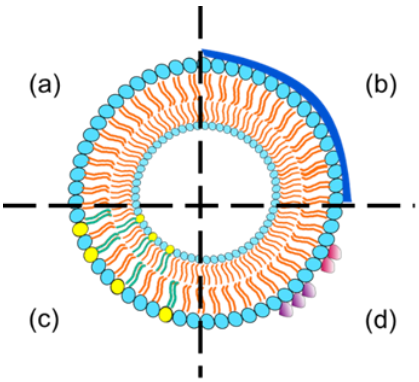

Figure 3. Types of liposome modifications, (a) conventional (unmodified) liposome; (b) surface coating; (c) modification of liposomal composition; (d) modifications with ligands

5.1 Liposomal composition

Modifications to liposomal composition mostly led to decreased membrane fluidity, resulting in liposomes with rigid membranes and better stability. Liposome composition can be optimised with the use of certain types or combinations of phospholipids or the addition of cholesterol.

5.1.1 The use of specific phospholipids

The major phospholipids found in liposomes are glycerophospholipids, such as phosphatidylcholine and phosphatidylethanolamine. An essential quality of phospholipids that influences liposome stability is phase transition temperature (T_m), i.e., the temperature where phospholipids switch from a solid gel to a liquid crystalline state. At these temperatures, liposome becomes more prone to leakage and release of encapsulated materials. The use of phospholipids with high T_m generated liposomes with rigid membranes and good resistance to harsh gastrointestinal conditions (da Silva Malheiros *et al*., 2010). Higher T_m can also be achieved using a combination of phospholipids. For example, liposomes from pure dipalmitoyl phosphatidylcholine (DPPC) have a T_m of 41°C, combining it with distearoyl phosphatidylcholine (DSPC) can increase it to 45°C (Li *et al*., 2015). Furthermore, bile salt disrupted liposomes made from phospholipids with T_m lower than 37°C, while those with higher T_m were less affected (He *et al*., 2019). The substitution of common lipids with hydrogenated phospholipids, which have high T_m, increased liposome rigidity (Abraham *et al*., 2005). In another study, liposomes made of hydrogenated phospholipids maintained nearly 80% of encapsulated lactoferrin after 120 mins of artificial gastric digestion. The exact formulation did not show a significant release of free fatty acids during simulated intestinal digestion, implying that the liposome could remain intact (Vergara

https://doi.org/10.26656/fr.2017.7(3).022

et al., 2020).

5.1.2 The use of cholesterol

Cholesterol is another element that can be added to increase liposome stability. When used as a liposome component, the hydroxyl group of cholesterol binds to the lipid-water interface with the whole molecule perpendicular to the membrane surface or aligned with the phospholipids (Liu *et al*., 2019). This interaction impacted the physical properties of liposomes, specifically: increased bilayer thickness, decreased permeability, increased resistance for aggregation, increased the packing of phospholipid molecules and reduced bilayer mobility (Liu *et al*., 2019). Briuglia *et al.* reported that the combination of lipids and cholesterol at the ratio of 2:1 (70% lipids:30% cholesterol) is an ideal formulation to obtain stable liposomes with well-organised structures (Briuglia *et al*., 2015). Liu *et al.* reported that liposome formulations that incorporated cholesterol generated less free fatty acids under simulated intestinal digestion than those that did not (42% vs. 62%) (Liu *et al.*, 2017). Cholesterol could easily integrate into lipid bilayers with its easy-to-fit structure, leading to increased cohesion of lipids, reduced membrane fluidity, and consequently, enhanced stability during digestion (Liu *et al*., 2017). Another study affirmed that the addition of cholesterol in liposomes effectively protected liposomes from pepsin activity (Hwang *et al*., 2010).

Structurally similar sterols may also be used as alternatives to cholesterol for liposome modifications. Ergosterol, a microorganism-derived sterol, served as a membrane stabiliser in liposome formation, producing an almost spherical vesicle with physical characteristics closely similar to the cholesterol-added liposome (Cui *et al*., 2015). The double bonds in ergosterol made liposomes more rigid with larger cross-sectional areas in bilayers (Tai *et al*., 2018). Liposomes made of a mix of lipids and ergosterol exhibited great protection capacity for encapsulated substances, retaining more than 74.8±9.6% of insulin after an hour of artificial stomach digestion (Cui *et al*., 2015). Interestingly, ergosterol-modified liposome managed to preserve higher insulin concentration than cholesterol-modified liposome after four hrs of simulated digestion (56.3±1.8% vs 14.8±3.6%) (Cui *et al*., 2015). Furthermore, incorporating ergosterol to liposomes was found to enhance insulin transport across Caco-2 monolayer, displaying better permeability than free insulin solution due to its high affinity with the cell membrane (Cui *et al*., 2015).

5.2 Surface coating

Liposome surfaces can be coated to protect against digestive enzymes or improve permeability for intestinal cells. There are four mechanisms of liposome surface coating: Insertion, where coating materials insert their hydrophobic chains into the hydrophobic chains of the phospholipids; Scaffold, where electrostatic or hydrogen bonds are formed between the coating material and the liposome surface; Intercalation, where small particles half-embed on the surface of liposome; and Phospholipid modification, where the head group of phospholipids is modified to form functionalised lipids (Sriraman *et al*., 2016; Wibroe *et al*., 2016). Coating materials that have been used for liposomes include polysaccharides, whey protein, alginate, chitosan, dextran sulfate, and silica nanoparticles.

5.2.1 Whey protein

Whey protein is a particularly attractive material for liposome coating because it is resistant to gastric digestion due to its lower sensitivity to pH and resilience to pepsin (Frenzel and Steffen-Heins, 2014). It also exhibits functional activities that are beneficial for health, including antibacterial and hypoglycemic activities (Al-Baarri *et al.*, 2010; Jacubowicz and Froy, 2013). A major fraction (60%) of whey protein is ß-lactoglobulin which contains a rigid beta-sheet structure, resulting in low molecule flexibility that hinders pepsin from associating with the substance (Teng *et al*., 2015). In addition, ß-lactoglobulin mostly consists of polar and charged amino acids, whereas the site of action for pepsin is the peptide bonds at the non-polar areas (Teng *et al*., 2015). Frenzel and Steffen-Heins (2014) investigated the use of whey protein isolate for liposome coating and found favourable results. They postulated that whey protein isolates partially insert into the glycerol/phosphate residue region up to the hydrocarbon region of the liposome. As a result, the liposome membrane became more rigid and physically stable. This was affirmed in later experiments in simulated gastric digestion, where uncoated liposomes showed enlarged particle sizes within 30 mins while whey protein-coated liposomes remained unchanged for 120 mins (Frenzel and Steffen-Heins, 2014).

In another study, Gomaa *et al*. used differently charged liposomes and tried coating them with whey protein and/or pectin, discovering various mechanisms of liposome coating depending on its charge. Anionic liposomes coated with negatively charged pectin formed interactions between amide groups of pectin and liposomes, while electrostatic and ionic interactions occurred between cationic liposomes with negatively charged pectin and whey protein (Gomaa *et al*., 2017).

https://doi.org/10.26656/fr.2017.7(3).022

These firm associations resulted in a liposome system that was resistant to gastrointestinal digestion. Single coated liposomes, either by whey protein or pectin, maintained a significantly higher amount of antimicrobial peptide than uncoated liposomes during simulated digestion. The best protection effect was observed in liposomes with dual coating with whey protein as the first layer and pectin as the outer layer, preserving the most amount of encapsulated peptide after 30 mins to the end of digestion (2 hrs in gastric digestion and 4 hrs in intestinal digestion) in comparison to uncoated liposomes (Gomaa *et al*., 2017).

5.2.2 Chitosan

A commonly used material for liposome coating is chitosan, due to its biocompatibility and mucoadhesive properties. Chitosan inhibits lipid digestion by electrostatic repulsion with lipase and bile salts (Li *et al*., 2016). Moreover, chitosan can form thick protective layers with a highly strong positive charge (~20 mV) that prevents the destabilisation of the colloidal system and slow down the rate and extent of lipid digestion (Jo *et al*., 2019). Li *et al.* found that chitosan-coated liposomes were more stable in both simulated gastric and intestinal digestion than uncoated liposomes (Li *et al*., 2015). They also reported a lower release of salmon protein hydrolysates in simulated intestinal fluids than uncoated liposomes, confirming its improved stability (Li *et al.*, 2015). Chitosan improves the mucoadhesive properties of liposomes by performing chemical coupling of physical coating (Huang *et al*., 2011; Gradauer *et al*., 2013; Cuomo *et al*., 2018). Coating with chitosan was shown to promote the absorption of encapsulated substances and liposomes through interference on the tight junctions of epithelia (Huang *et al*., 2011; Chen *et al.*, 2012).

The positively charged surfaces of chitosan favour adhesion to typically negatively charged cell membranes, leading to a longer retention time in the gastrointestinal tract and promoting penetration into the mucus layer (Mady and Darwish, 2010). Huang *et al.* confirmed that liposomes modified with N-trimethyl chitosan had high affinities to mucin particles. This modification can cover their surfaces in acidic and neutral environments, with N -trimethyl chitosan-modified liposomes showing prolonged retention time and penetrative behaviours across intestinal segments that were stronger than uncoated liposomes even after 4 hrs (Huang *et al*., 2011). Oral administration of curcumin-loaded chitosan-modified liposomes to rats also showed supporting results. Higher plasma concentration of curcumin was observed for rats given chitosan-coated liposomes (46.13 μg/L) than uncoated liposomes (32.12 μg/L) (Chen *et al*., 2012).

5.2.3 Pectin

Pectin is an example of a popular choice of mucoadhesive polysaccharides for nanovesicle coating. The effect of pectin on the stability of liposomes is influenced by the type of pectin used. High methoxylated pectin for liposome coating was shown to have the best physical properties compared to other types of pectin (Klemetsrud *et al*., 2013). Pectin-coated nanoliposomes were reported to have an initial high release rate before transcending into a more steady, sustained release rate, suggesting that it would have extended time in the gastrointestinal tract, increasing its absorption (Lopes *et al*., 2017). This was confirmed by Li *et al*. that found that bufalin release in pectin-coated liposomes started at 6 hrs and reached a cumulative release of 50% in the first 12 hrs, which is more sustainable than uncoated liposomes that started drug release at 2 hrs and reached 90% in 12 hrs (Li *et al*., 2014). They also reported that the adsorption of mucin on pectin-coated liposomes was four times higher than uncoated liposomes, implying enhanced mucoadhesive properties and prolonged retention time. Pectin-coated liposomes also showed a more efficient uptake of liposomes through endocytosis, as the inhibitory effects of bufalin were enhanced with pectin-coated liposomes than free bufalin or uncoated liposomes (Li *et al*., 2014).

5.2.4 Polyethylene glycol

Liposome, coated with polyethylene glycols, was reported to increase liposome's resilience to bile salts as well as its protective capacity for encapsulated ingredients (Li *et al*., 2003). It is known that polyethylene glycol chains penetrate deeply into the intestinal mucous layer, thus prolonging the residence time of liposome in the gastrointestinal tract. As a result, liposome uptake by M cells is enhanced, and bioactive substances can be absorbed (D'souza and Shegokar, 2016). Yamazoe *et al*. (2021) have confirmed that liposomes, modified with 10% polyethylene glycol, had the highest intracellular uptake in Caco-2 cell cultures and the highest blood concentrations of encapsulated substances in rat models. Similarly, during 24 hrs of gastric and intestinal digestion simulation, Yazdi found that polyethylene glycol-modified liposome was releasing insulin differently ($p < 0.001$) (Yazdi *et al*., 2020). During the first hour, insulin release was 25% and 48% for modified liposomes in gastric and intestinal digestion. While the non-modified liposome recorded was 39% and 64%, with a drastic increase in the following hrs (Yazdi *et al*., 2020). These results affirmed that modifications are necessary to preserve encapsulated materials during digestion and to achieve high

https://doi.org/10.26656/fr.2017.7(3).022

concentration for absorption.

5.3 Other modifications

Other modifications mainly include the addition of various materials that either improve liposome properties or permeability for intestinal absorption. Intestinal absorption can be improved by increasing liposome affinity to the intestinal epithelial cells, increasing retention time, and achieving specific targeting. These can be achieved using materials such as absorption enhancers and ligand mediators.

5.3.1 Absorption enhancers

Absorption enhancers that can be added are surfactants such as Tween-80 and bile salt. Sodium cholate, sodium taurocholate, sodium deoxycholate, and sodium glycocholate are among the commonly used bile salts for liposome modifications. The addition of bile salt in the lipid bilayers was reported to stabilise liposomes. Bile salt improved membrane rigidity which made it more stable against the gastrointestinal tract (Hu *et al*., 2013). Sodium glycocholate was shown to inhibit the activity of pepsin or pancreatin (Niu *et al*., 2011). Bilosomes, or bile salt-modified liposomes, have been reported to have increased bioavailability from facilitated absorption (Guan *et al*., 2011). Liposomes modified with sodium glycocholate retained 74% of insulin after 4 hrs of simulated gastric digestion, indicating decent protective capability in the early steps of digestion (Cui *et al*., 2015). Another study used spermine as the absorption enhancer and reported the highest bioavailability of substances encapsulated in liposome (Yamazoe *et al*., 2021). Spermine functions as an activator of extracellular calcium-sensing receptors and triggers the opening of tight junctions in the epithelial cell layer, thus enhancing the absorption of substances. Spermine also showed partial protection of substances against enzymatic degradation, particularly elcatonin from trypsin degradation (Yamazoe *et al*., 2021).

5.3.2 Ligand mediators

Another modification that can be done is the addition of ligands with specific targets on intestinal epithelial cells. Ligand-mediated liposomes were proven to enhance the absorption and uptake of liposomes or their substrate into intestinal epithelial cells. Lectin, for example, has specific recognition and binding to glycans, which are found attached to intestinal cell membranes. Due to that, lectin has been viewed as a promising material for oral delivery systems (Zhang *et al*., 2005).

Folic acid can also be used as a target mediator as it has receptors on the intestinal tract cells, and its conjugates can be endocytosed through those receptors (Agrawal *et al*., 2014). Other vitamins like vitamin B_{12}, biotin, thiamine, and niacin may also be used as ligands for active targeting of intestinal epithelial (Zhang *et al*., 2014; He *et al*., 2018). The addition of folic acid at 1% and 2% to liposome showed significantly improved intestinal cells uptake ($p < 0.01$), multiplying by 1.2 to 1.5 times compared to unmodified liposomes (Yazdi *et al*., 2020). The increased absorption led to higher blood concentrations of substrate, as observed at 3-hour post-gavage. Furthermore, using insulin as the substrate, the study also reported that folic acid-modified liposome exhibited blood glucose-lowering effects after 4 hrs, while insulin injections began after 2 hrs (Yazdi *et al*., 2020). He *et al.* investigated the use of thiamine and niacin decorated liposomes to encapsulate insulin and found protective effects against degradation in simulated gastric and intestinal fluids (He *et al*., 2018). Approximately only 40% and 20% of insulin were degraded in artificial gastric and intestinal digestion, respectively (He *et al*., 2018). The niacin-decorated liposome had better hypoglycemic effects than the thiamine-added liposome, owing to stronger bioactivity for endocytosis of niacin receptors (GPR 109a) than thiamine receptors (Th Tr-1 and Th Tr-2) in intestinal epithelial cells (He *et al*., 2018).

Likewise, liposomes modified with biotins as the target ligand were significantly better at protecting encapsulated insulin than conventional liposomes, exhibiting a slow degradation rate during simulated digestions. In the study, free insulin was completely degraded within 5 mins of digestion, while biotinylated liposome could retain significantly ($p < 0.05$) higher concentrations of insulin in simulated digestion than conventional liposome (Zhang *et al*., 2014). About 78% and 85% of insulin could be preserved by biotinylated liposomes in simulated gastric and intestinal digestion, respectively, whereas conventional liposomes could only maintain 45% and 65%, respectively (Zhang *et al*., 2014). The permeability of insulin through the Caco-2 cell monolayer was improved by 1.85 times higher for biotinylated liposomes than conventional liposomes (Zhang *et al*., 2014). Biotin receptors are non-specific and distributed throughout the small intestine, and further examinations revealed that biotinylated liposomes were absorbed through active uptake. Biotinylated liposomes travelled from the stomach to the duodenum in the first 30 mins after oral administration, then spread through the small intestine at 1 hour before being detected weakly after 4 hrs, implying that liposomes were almost identical entirely absorbed (Zhang *et al*., 2014). This was also confirmed through fluorescent imaging of transepithelial absorption, where intense immunofluorescence could be detected in all intestinal segments examined for biotinylated liposomes.

https://doi.org/10.26656/fr.2017.7(3).022

These results show the importance of ligand-targeted mechanisms for improving liposomes' absorption rate and payload.

6. Conclusion and future prospect

The rising interest in functional food boosted the development of encapsulation technologies as carriers for nutraceuticals that are generally unstable and sensitive to environmental factors. The liposome is one of the most promising encapsulation technologies that can be applied for the protection of nutraceuticals, but challenges still exist in the implementation of liposomes for oral delivery. Liposome fragility in the gastrointestinal environment and low permeability across intestinal epithelia remain major obstacles that need to be overcome to be able to achieve decent bioavailability that can support the efficacy of ingested nutraceuticals. Various modification efforts have been made by altering the liposomal composition, adding surface coating, and incorporating other compounds with mucoadhesive properties. Despite the positive results, most of these liposome modification studies were done with *in vitro* models that may not completely represent the complexity of in vivo settings. Therefore, it is necessary to further confirm the effects of these modifications on liposome stability with various *in vivo* experiments and clinical trials.

Furthermore, studies regarding improved liposome absorption from modifications have shown that there are various options for absorption pathways, but not many have discussed the types of substances that may be supported by these pathways. Granted, no delivery system can be universally used as each bioactive substance come with unique properties, but there is a need for more works that compare the effects of each type of modification on the efficacy and permeability of orally administered nutraceuticals.

As the general interest leans towards increasing the health-promoting effects of food, there is potential for liposomes to be used as a nutraceutical carrier that is incorporated in food. Current food application of liposomes showed relatively decent functionality of bioactive substances in the food matrix to improve

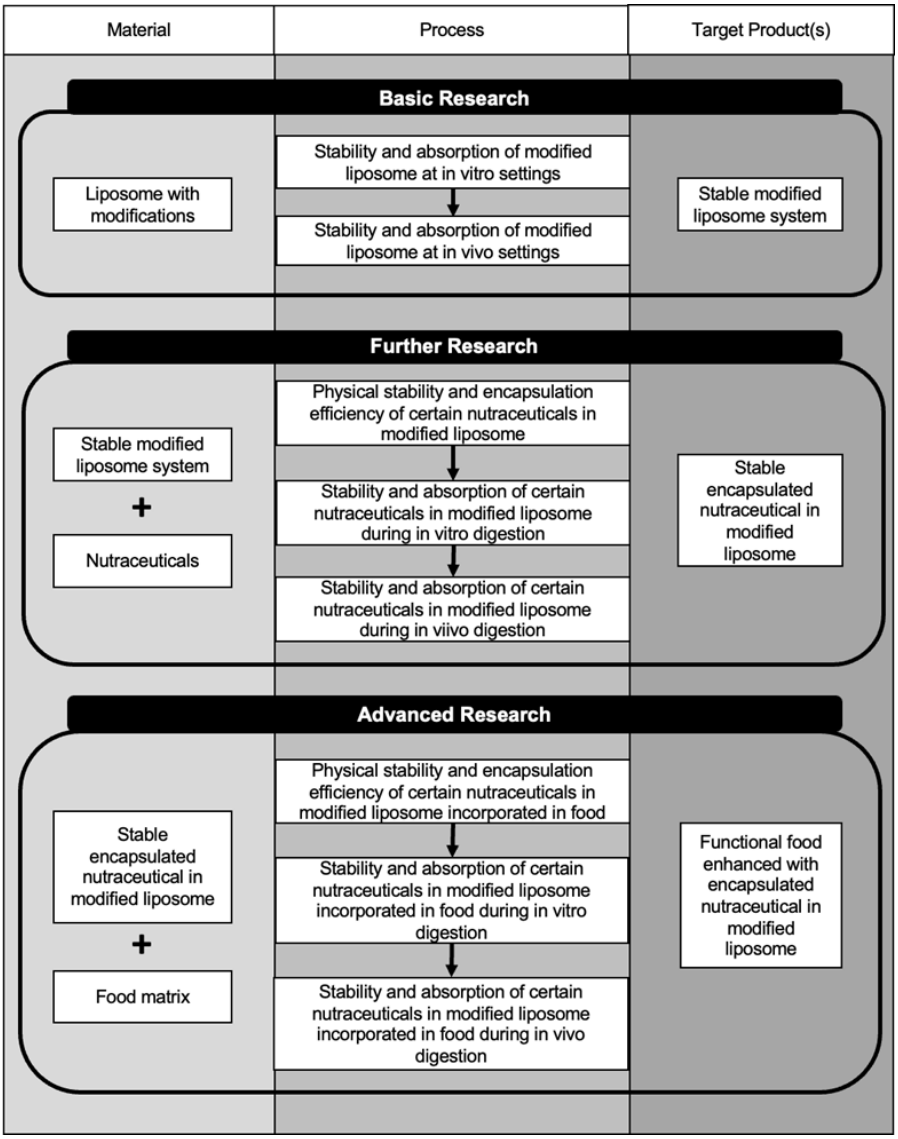

Figure 4. Future prospects of research about liposome optimisation for oral delivery of nutraceuticals in food

https://doi.org/10.26656/fr.2017.7(3).022

sensory properties, but there is no guarantee that the same properties will persist when consumed orally. This raises concerns about the stability of liposome-encapsulated nutraceuticals that are digested along with food, including potential interactions that may occur with nutrients or other food components and whether further modifications are required to minimise any adverse interactions. Accordingly, this will open more works about liposome modifications that may enhance its functionality and stability as a valid food additive. The future prospect is summarised in Figure 4.

In conclusion, there is still much room to explore in terms of establishing liposome formulations for the oral delivery of nutraceuticals. Moreover, as the research about functional compounds continues to grow, more studies might be needed to compose liposome formulations that can also cater to the delivery of these newly discovered substances.

Conflict of interest

The authors declare no conflict of interest.

References

Abraham, S.A., Waterhouse, D.N., Mayer, L.D., Cullis, P.R., Madden, T.D. and Bally, M.B. (2005). The Liposomal Formulation of Doxorubicin. *Methods in Enzymology*, 391, 71-97. https://doi.org/10.1016/S0076-6879(05)91004-5

Agrawal, A.K., Harde, H., Thanki, K. and Jain, S. (2014). Improved Stability and Antidiabetic Potential of Insulin Containing Folic Acid Functionalized Polymer Stabilized Multilayered Liposomes Following Oral Administration. *Biomacromolecules*, 15(1), 350–360. https://doi.org/10.1021/bm401580k

Ahn, H. and Park, J.H. (2016). Liposomal delivery systems for intestinal lymphatic drug transport. *Biomaterials Research*, 20, 36. https://doi.org/10.1186/s40824-016-0083-1

Akbarzadeh, A., Rezaei-Sadabady, R., Davaran, S., Joo, S.W., Zarghami, N., Hanifehpour, Y., Samiei, M., Kouhi, M. and Nejati-Koshki, K. (2013). Liposome: classification, preparation, and applications. *Nanoscale Research Letters*, 8(1), 102. https://doi.org/10.1186/1556-276X-8-102

Al-Baarri, A., Agawa, M. and Hayakawa, S. (2010). Scale-Up Studies on Immobilization of Lactoperoxidase Using Milk Whey for Producing Antimicrobial Agent. *Journal of the Indonesian Tropical Animal Agriculture*, 35(3), 185-191. https://doi.org/10.14710/jitaa.35.3.185-191

Andar, A.U., Hood, R.R., Vreeland, W.N., DeVoe, D.L. and Swaan, P.W. (2014). Microfluidic preparation of liposomes to determine particle size influence on cellular uptake mechanisms. *Pharmaceutical Research*, 31(2), 401–413. https://doi.org/10.1007/s11095-013-1171-8

Bernkop-Schnürch, A. (2013). Nanocarrier systems for oral drug delivery: do we really need them? *European Journal of Pharmaceutical Sciences*, 49 (2), 272–277. https://doi.org/10.1016/j.ejps.2013.03.008

Briuglia, M.L., Rotella, C., McFarlane, A. and Lamprou, D.A. (2015). Influence of cholesterol on liposome stability and on in vitro drug release. *Drug Delivery and Translational Research*, 5(3), 231–242. https://doi.org/10.1007/s13346-015-0220-8

Chen, H., Wu, J., Sun, M., Guo, C., Yu, A., Cao, F., Zhao, L., Tan, Q. and Zhai, G. (2012). N-trimethyl chitosan chloride-coated liposomes for the oral delivery of curcumin. *Journal of Liposome Research*, 22(2), 100–109. https://doi.org/10.3109/08982104.2011.621127

Colletier, J.P., Chaize, B., Winterhalter, M. and Fournier, D. (2002). Protein encapsulation in liposomes: Efficiency depends on interactions between protein and phospholipid bilayer. *BMC Biotechnology*, 2(1), 9. https://doi.org/10.1186/1472-6750-2-9

Cui, M., Wu, W., Hovgaard, L., Lu, Y., Chen, D. and Qi, J. (2015). Liposomes containing cholesterol analogues of botanical origin as drug delivery systems to enhance the oral absorption of insulin. *International Journal of Pharmaceutics*, 489(1–2), 277–284. https://doi.org/10.1016/j.ijpharm.2015.05.006

Cuomo, F. Cofelice, M., Venditti, F., Ceglie, A., Miguel, M., Lindman, B. and Lopez, F. (2018). In-vitro digestion of curcumin loaded chitosan-coated liposomes. *Colloids and Surfaces B: Biointerfaces*, 168, 29–34. https://doi.org/10.1016/j.colsurfb.2017.11.047

D'souza, A.A. and Shegokar, R. (2016). Polyethylene glycol (PEG): a versatile polymer for pharmaceutical applications. *Expert Opinion on Drug Delivery*, 13 (9), 1257–1275. https://doi.org/10.1080/17425247.2016.1182485

da Silva Malheiros, P., Daroit, D.J. and Brandelli, A. (2010). Food applications of liposome-encapsulated antimicrobial peptides. *Trends in Food Science and Technology*, 21(6), 284–292. https://doi.org/10.1016/j.tifs.2010.03.003

Ensign, L.M., Cone, R. and Hanes, J. (2012). Oral drug delivery with polymeric nanoparticles: The gastrointestinal mucus barriers. *Advanced Drug*

Delivery Reviews, 64(6), 557–570. https://doi.org/10.1016/j.addr.2011.12.009

Esmaeili, H., Cheraghi, N., Khanjari, A., Rezaeigolestani, M., Basti, A.A., Kamkar, A. and Aghaee, E.M. (2020). Incorporation of nanoencapsulated garlic essential oil into edible films: A novel approach for extending shelf life of vacuum-packed sausages. *Meat Science*, 166, 108135. https://doi.org/10.1016/j.meatsci.2020.108135

Fonte, P., Araújo, F., Reis, S. and Sarmento, B. (2013). Oral insulin delivery: how far are we? *Journal of Diabetes Science and Technology*, 7(2), 520–531. https://doi.org/10.1177/193229681300700228

Frenzel, M., Krolak, E., Wagner, A.E. and Steffen-Heins, A. (2015). Physicochemical properties of WPI coated liposomes serving as stable transporters in a real food matrix. *LWT-Food Science and Technology*, 63(1), 527–534. https://doi.org/10.1016/j.lwt.2015.03.055

Frenzel, M. and Steffen-Heins, A. (2014). Whey protein increases bilayer rigidity and stabilities of liposomes in food-like matrices. *Food Chemistry*, 173, 1090–1099. https://doi.org/10.1016/j.foodchem.2014.10.076

Gomaa, A.I., Martinent, C., Hammami, R., Fliss, I. and Subirade, M. (2017). Dual Coating of Liposomes as Encapsulating Matrix of Antimicrobial Peptides: Development and Characterization. *Frontiers in Chemistry,* 5, 103. https://doi.org/10.3389/fchem.2017.00103

Gopi, S. and Balakrishnan, P. (2020). Evaluation and clinical comparison studies on liposomal and non-liposomal ascorbic acid (vitamin C) and their enhanced bioavailability. *Journal of Liposome Research*, 31(4), 356-364. https://doi.org/10.1080/08982104.2020.1820521

Gradauer, K., Barthelmes, J., Vonach, C., Almer, G., Mangge, H., Teubl, B., Roblegg, E., Dünnhaupt, S., Fröhlich, E., Bernkop-Schnürch, A. and Prassl, R. (2013). Liposomes coated with thiolated chitosan enhance oral peptide delivery to rats. *Journal of Controlled Release*, 172(3), 872–878. https://doi.org/10.1016/j.jconrel.2013.10.011

Gropper, S.S., Smith, J.L. and Groff, J.L. (2009). Advanced Nutrition and Human Metabolism. Wadsworth, Ohio, USA: Cengage Learning.

Guan, P., Lu, Y., Qi, J., Niu, M., Lian, R., Hu, F. and Wu, W. (2011). Enhanced oral bioavailability of cyclosporine A by liposomes containing a bile salt. *International Journal of Nanomedicine*, 6, 965—974. https://doi.org/10.2147/IJN.S19259

Guo, Q., Ye, A., Bellissimo, N., Singh, H. and Rousseau, D. (2017). Modulating fat digestion through food structure design. *Progress in Lipid Research*, 68, 109–118. https://doi.org/10.1016/j.plipres.2017.10.001

Hanato, J., Kuriyama, K., Mizumoto, T., Debari, K., Hatanaka, J., Onoue, S. and Yamada, S. (2009). Liposomal formulations of glucagon-like peptide-1: improved bioavailability and anti-diabetic effect. *International Journal of Pharmaceutics*, 382(1–2), 111–116. https://doi.org/10.1016/j.ijpharm.2009.08.013

He, H., Lu, Y., Qi, J., Zhu, Q., Chen, Z. and Wu, W. (2019). Adapting liposomes for oral drug delivery. *Acta Pharmaceutica Sinica B*, 9(1), 36–48. https://doi.org/10.1016/j.apsb.2018.06.005

He, H., Lu, Y., Qi, J., Zhao, W., Dong, X. and Wu, W. (2018). Biomimetic thiamine-and niacin-decorated liposomes for enhanced oral delivery of insulin. *Acta Pharmaceutica Sinica B*, 8(1), 97–105. https://doi.org/10.1016/j.apsb.2017.11.007

Hillaireau, H. and Couvreur, P. (2009). Nanocarriers' entry into the cell: relevance to drug delivery. *Cellular and Molecular Life Sciences*, 66(17), 2873–2896. https://doi.org/10.1007/s00018-009-0053-z

Hirlekar, R.S. (2017). Oral Insulin Delivery: Novel Strategies. *Asian Journal of Pharmaceutics*, 11(3), S434.

Hu, S., Niu, M., Hu, F., Lu, Y., Qi, J., Yin, Z. and Wu, W. (2013). Integrity and stability of oral liposomes containing bile salts studied in simulated and ex vivo gastrointestinal media. *International Journal of Pharmaceutics*, 441(1–2), 693–700. https://doi.org/10.1016/j.ijpharm.2012.10.025

Huang, A., Makhlof, A., Ping, Q., Tozuka, Y. and Takeuchi, H. (2011). N-trimethyl chitosan-modified liposomes as carriers for oral delivery of salmon calcitonin. *Drug Delivery*, 18(8), 562–569. https://doi.org/10.3109/10717544.2011.596585

Huang, M., Su, E., Zheng, F. and Tan, C. (2017). Encapsulation of flavonoids in liposomal delivery systems: the case of quercetin, kaempferol and luteolin. *Food and Function*, 8(9), 3198-3208. https://doi.org/10.1039/C7FO00508C

Huang, M., Liang, C., Tan, C., Huang, S., Ying, R., Wang, Y., Wang, Z. and Zhang, Y. (2019). Liposome co-encapsulation as a strategy for the delivery of curcumin and resveratrol. *Food and Function*, 10(10), 6447–6458. https://doi.org/10.1039/C9FO01338E

Hwang, J.S., Tsai, Y.L. and Hsu, K.C. (2010). The feasibility of antihypertensive oligopeptides encapsulated in liposomes prepared with

REVIEW

phytosterols-β-sitosterol or stigmasterol. *Food Research International*, 43(1), 133–139. https://doi.org/10.1016/j.foodres.2009.09.007

Ismail, R. and Csoka, I. (2017). Novel strategies in the oral delivery of antidiabetic peptide drugs–Insulin, GLP 1 and its analogs. *European Journal of Pharmaceutics and Biopharmaceutics*, 115, 257–267. https://doi.org/10.1016/j.ejpb.2017.03.015

Jo, M., Ban, C., Goh, K.K.T. and Choi, Y.J. (2019). Influence of chitosan-coating on the stability and digestion of emulsions stabilised by waxy maise starch crystals. *Food Hydrocolloids*, 94, 603–612. https://doi.org/10.1016/j.foodhyd.2019.04.010

Kalepu, S., Manthina, M. and Padavala, V. (2013). Oral lipid-based drug delivery systems – an overview. *Acta Pharmaceutica Sinica B*, 3(6), 361–372. https://doi.org/10.1016/j.apsb.2013.10.001

Khorasani, S., Danaei, M. and Mozafari, M.R. (2018). Nanoliposome technology for the food and nutraceutical industries. *Trends in Food Science and Technology*, 79, 106–115. https://doi.org/10.1016/j.tifs.2018.07.009

Kim, H., Kim, Y. and Lee, J. (2013). Liposomal formulations for enhanced lymphatic drug delivery. *Asian Journal of Pharmaceutical Sciences*, 8(2), 96–103. https://doi.org/10.1016/j.ajps.2013.07.012

Klemetsrud, T., Jonassen, H., Hiorth, M., Kjøniksen, A.L. and Smistad, G. (2013). Studies on pectin-coated liposomes and their interaction with mucin. *Colloids and Surfaces B: Biointerfaces*, 103, 158–165. https://doi.org/10.1016/j.colsurfb.2012.10.012

Kou, L., Sun, J., Zhai, Y. and He, Z. (2013). The endocytosis and intracellular fate of nanomedicines: Implication for rational design. *Asian Journal of Pharmaceutical Sciences*, 8(1), 1–10. https://doi.org/10.1016/j.ajps.2013.07.001

Laouini, A., Jaafar-Maalej, C., Limayem-Blouza, I., Sfar, S., Charcosset, C. and Fessi, H. (2012). Preparation, Characterisation and Applications of Liposomes: State of the Art. *Journal of Colloid Science and Biotechnology*, 1(2), 147–168. https://doi.org/10.1166/jcsb.2012.1020

Li, H., Song, J.H., Park, J.S. and Han, K. (2003). Polyethylene glycol-coated liposomes for oral delivery of recombinant human epidermal growth factor. *International Journal of Pharmaceutics*, 258 (1–2), 11–19. https://doi.org/10.1016/S0378-5173(03)00158-3

Li, J., Wang, X., Zhang, T., Wang, C., Huang, Z., Luo, X. and Deng, Y. (2015). A review on phospholipids and their main applications in drug delivery systems. *Asian Journal of Pharmaceutical Sciences*, 10(2), 81–98. https://doi.org/10.1016/j.ajps.2014.09.004

Li, J., Hwang, I.C., Chen, X. and Park, H.J. (2016). Effects of chitosan coating on curcumin loaded nano-emulsion: Study on stability and in vitro digestibility. *Food Hydrocolloids*, 60, 138–147. https://doi.org/10.1016/j.foodhyd.2016.03.016

Li, Y., Hu, M. and McClements, D.J. (2011). Factors affecting lipase digestibility of emulsified lipids using an in vitro digestion model: Proposal for a standardised pH-stat method. *Food Chemistry*, 126 (2), 498–505. https://doi.org/10.1016/j.foodchem.2010.11.027

Li, Y., Zhao, H., Duan, L.R., Li, H., Yang, Q., Tu, H.H., Cao, W. and Wang, S.-W. (2014). Preparation, characterisation and evaluation of bufalin liposomes coated with citrus pectin. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 444, 54–62. https://doi.org/10.1016/j.colsurfa.2013.12.006

Li, Z., Paulson, A.T. and Gill, T.A. (2015). Encapsulation of bioactive salmon protein hydrolysates with chitosan-coated liposomes. *Journal of Functional Foods*, 19(Part A), 733–743. https://doi.org/10.1016/j.jff.2015.09.058

Liu, N. and Park, H.J. (2009). Chitosan-coated nanoliposome as vitamin E carrier. *Journal of Microencapsulation*, 26(3), 235–242. https://doi.org/10.1080/02652040802273469

Liu, W., Wei, F., Ye, A., Tian, M. and Han, J. (2017). Kinetic stability and membrane structure of liposomes during in vitro infant intestinal digestion: Effect of cholesterol and lactoferrin. *Food Chemistry*, 230, 6–13. https://doi.org/10.1016/j.foodchem.2017.03.021

Liu, W., Ye, A., Han, F. and Han, J. (2019). Advances and challenges in liposome digestion: Surface interaction, biological fate, and GIT modeling. *Advances in Colloid and Interface Science*, 263, 52–67. https://doi.org/10.1016/j.cis.2018.11.007

Liu, W., Ye, A., Liu, C., Liu, W. and Singh, H. (2012). Structure and integrity of liposomes prepared from milk-or soybean-derived phospholipids during in vitro digestion. *Food Research International*, 48(2), 499–506. https://doi.org/10.1016/j.foodres.2012.04.017

Liu, W., Ye, A., Liu, W., Liu, C., Han, J. and Singh, H. (2015). Behaviour of liposomes loaded with bovine serum albumin during in vitro digestion. *Food Chemistry*, 175, 16–24. https://doi.org/10.1016/j.foodchem.2014.11.108

Liu, X., Wang, P., Zou, Y.X., Luo, Z.G. and Tamer, T.M. (2020). Co-encapsulation of Vitamin C and β-Carotene in liposomes: Storage stability, antioxidant

activity, and in vitro gastrointestinal digestion. *Food Research International*, 136, 109587. https://doi.org/10.1016/j.foodres.2020.109587

Lopes, N.A., Pinilla, C.M.B. and Brandelli, A. (2017). Pectin and polygalacturonic acid-coated liposomes as novel delivery system for nisin: Preparation, characterisation and release behavior. *Food Hydrocolloids*, 70, 1–7. https://doi.org/10.1016/j.foodhyd.2017.03.016

Lucas-González, R., Viuda-Martos, M., Pérez-Alvarez, J.A. and Fernández-López, J. (2018). In vitro digestion models suitable for foods: Opportunities for new fields of application and challenges. *Food Research International*, 107, 423–469. https://doi.org/10.1016/j.foodres.2018.02.055

Mady, M.M. and Darwish, M.M. (2010). Effect of chitosan coating on the characteristics of DPPC liposomes. *Journal of Advanced Research*, 1(3), 187–191. https://doi.org/10.1016/j.jare.2010.05.008

Manconi, M., Caddeo, C., Manca, M.L. and Fadda, A.M. (2020). Oral delivery of natural compounds by phospholipid vesicles. *Nanomedicine*, 15(18), 1795–1803. https://doi.org/10.2217/nnm-2020-0085

McClements, D.J. (2018). Encapsulation, protection, and delivery of bioactive proteins and peptides using nanoparticle and microparticle systems: a review. *Advances in Colloid and Interface Science*, 253, 1–22. https://doi.org/10.1016/j.cis.2018.02.002

Merlin, M., Pezzotti, M. and Avesani, L. (2017). Edible plants for oral delivery of biopharmaceuticals. *British Journal of Clinical Pharmacology*, 83(1), 71–81.

Mohammadi, M., Ghanbarzadeh, B. and Hamishehkar, H. (2014). Formulation of nanoliposomal vitamin D3 for potential application in beverage fortification. *Advanced Pharmaceutical Bulletin*, 4(Suppl. 2), 569.

Mozafari, M.R., Khosravi-Darani, K., Borazan, G.G., Cui, J., Pardakhty, A. and Yurdugul, S. (2008). Encapsulation of Food Ingredients Using Nanoliposome Technology. *International Journal of Food Properties*, 11(4), 833–844. https://doi.org/10.1080/10942910701648115

Nguyen, T. X., Huang, L., Gauthier, M., Yang, G. and Wang, Q. (2016). Recent advances in liposome surface modification for oral drug delivery. *Nanomedicine*, 11(9), 1169–1185. https://doi.org/10.2217/nnm.16.9

Niu, M., Lu, Y., Hovgaard, L., Guan, P., Tan, Y., Lian, R., Qi, J. and Wu, W. (2012). Hypoglycemic activity and oral bioavailability of insulin-loaded liposomes containing bile salts in rats: the effect of cholate type, particle size and administered dose. *European Journal of Pharmaceutics and Biopharmaceutics*, 81(2), 265–272. https://doi.org/10.1016/j.ejpb.2012.02.009

Niu, M., Lu, Y., Hovgaard, L. and Wu, W. (2011). Liposomes containing glycocholate as potential oral insulin delivery systems: preparation, in vitro characterisation, and improved protection against enzymatic degradation. *International Journal of Nanomedicine*, 6, 1155-1166. https://doi.org/10.2147/IJN.S19917

Ohnishi, N., Tanaka, S., Tahara, K. and Takeuchi, H. (2015). Characterisation of insulin-loaded liposome using column-switching HPLC. *International Journal of Pharmaceutics*, 479(2), 302–305. https://doi.org/10.1016/j.ijpharm.2014.12.056

Ong, S.G.M., Ming, L.C., Lee, K.S. and Yuen, K.H. (2016). Influence of the encapsulation efficiency and size of liposome on the oral bioavailability of griseofulvin-loaded liposomes. *Pharmaceutics*, 8(3), 25. https://doi.org/10.3390/pharmaceutics8030025

Park, S.J., Choi, S.G., Davaa, E. and Park, J.S. (2011). Encapsulation enhancement and stabilisation of insulin in cationic liposomes. *International Journal of Pharmaceutics*, 415(1–2), 267–272. https://doi.org/10.1016/j.ijpharm.2011.05.061

Patil, Y.P. and Jadhav, S. (2014). Novel methods for liposome preparation. *Chemistry and Physics of Lipids*, 177, 8–18. https://doi.org/10.1016/j.chemphyslip.2013.10.011

Pinilla, C.M.B., Thys, R.C.S. and Brandelli, A. (2019). Antifungal properties of phosphatidylcholine-oleic acid liposomes encapsulating garlic against environmental fungal in wheat bread. *International Journal of Food Microbiology*, 293, 72-78. https://doi.org/10.1016/j.ijfoodmicro.2019.01.006

Shukla, S., Haldorai, Y., Hwang, S.K., Bajpai, V.K., Huh, Y.S. and Han, Y.K. (2017). Current Demands for Food-Approved Liposome Nanoparticles in Food and Safety Sector. *Frontiers in Microbiology*, 8, 2398. https://doi.org/10.3389/fmicb.2017.02398

Sriraman, S.K., Salzano, G., Sarisozen, C. and Torchilin, V. (2016). Anti-cancer activity of doxorubicin-loaded liposomes co-modified with transferrin and folic acid. *European Journal of Pharmaceutics and Biopharmaceutics*, 105, 40–49. https://doi.org/10.1016/j.ejpb.2016.05.023

Tai, K., Liu, F., He, X., Ma, P., Mao, L., Gao, Y. and Yuan, F. (2018). The effect of sterol derivatives on properties of soybean and egg yolk lecithin liposomes: Stability, structure and membrane characteristics. *Food Research International*, 109, 24–34. https://doi.org/10.1016/j.foodres.2018.04.014

REVIEW

Taylor, T.M., Weiss, J., Davidson, P.M. and Bruce, B.D. (2005). Liposomal nanocapsules in food science and agriculture. *Critical Reviews in Food Science and Nutrition*, 45(7–8), 587–605. https://doi.org/10.1080/10408390591001135

Teng, Z., Xu, R. and Wang, Q. (2015). Beta-lactoglobulin-based encapsulating systems as emerging bioavailability enhancers for nutraceuticals: a review. *Rsc Advances*, 5(44), 35138–35154. https://doi.org/10.1039/C5RA01814E

Vergara, D., López, O., Bustamante, M. and Shene, C. (2020). An in vitro digestion study of encapsulated lactoferrin in rapeseed phospholipid–based liposomes. *Food Chemistry*, 321, 126717. https://doi.org/10.1016/j.foodchem.2020.126717

Wang, Q., Wei, C., Weng, W., Bao, R., Adu-Frimpong, M., Toreniyazov, E., Ji, H., Xu, X.M. and Yu, J. (2021). Enhancement of oral bioavailability and hypoglycemic activity of liquiritin-loaded precursor liposome. *International Journal of Pharmaceutics*, 592, 120036. https://doi.org/10.1016/j.ijpharm.2020.120036

Wibroe, P.P., Ahmadvand, D., Oghabian, M.A., Yaghmur, A. and Moghimi, S.M. (2016). An integrated assessment of morphology, size, and complement activation of the PEGylated liposomal doxorubicin products Doxil®, Caelyx®, DOXOrubicin, and SinaDoxosome. *Journal of Controlled Release*, 221, 1–8. https://doi.org/10.1016/j.jconrel.2015.11.021

Wu, W., Lu, Y. and Qi, J. (2015). Oral delivery of liposomes. *Therapeutic delivery*, 6(11), 1239–4. https://doi.org/10.4155/tde.15.69

Yamazoe, E., Fang, J.Y. and Tahara, K. (2021). Oral mucus-penetrating PEGylated liposomes to improve drug absorption: Differences in the interaction mechanisms of a mucoadhesive liposome. *International Journal of Pharmaceutics*, 593, 120148. https://doi.org/10.1016/j.ijpharm.2020.120148

Yazdi, J.R., Tafaghodi, M., Sadri, K., Mashreghi, M., Nikpoor, A.R., Nikoofal-Sahlabadi, S., Chamani, J., Vakili, R., Moosavian, S.A. and Jaafari, M.R. (2020). Folate targeted PEGylated liposomes for the oral delivery of insulin: In vitro and in vivo studies. *Colloids and Surfaces B: Biointerfaces*, 194, 111203. https://doi.org/10.1016/j.colsurfb.2020.111203

Zhang, N., Ping, Q.N., Huang, G.H. and Xu, W.F. (2005). Investigation of lectin-modified insulin liposomes as carriers for oral administration. *International Journal of Pharmaceutics*, 294(1–2), 247–259. https://doi.org/10.1016/j.ijpharm.2005.01.018

Zhang, X., Qi, J., Lu, Y., He, W., Li, X. and Wu, W. (2014). Biotinylated liposomes as potential carriers for the oral delivery of insulin. *Nanomedicine*, 10(1), 167–176. https://doi.org/10.1016/j.nano.2013.07.011

Zhou, W., Liu, W., Zou, L., Liu, W., Liu, C., Liang, R. and Chen, J. (2014). Storage stability and skin permeation of vitamin C liposomes improved by pectin coating. *Colloids and Surfaces B: Biointerfaces*, 117, 330–337. https://doi.org/10.1016/j.colsurfb.2014.02.036

