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Liposomes for oral delivery of protein and peptide-based therapeutics: Challenges, formulation strategies, and advances

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1 **Liposomes for oral delivery of protein and peptide-based therapeutics: Challenges,**
2 **formulation strategies, and advances**

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24 **Abstract**

25 Throughout the past decade, there has been a rapid growth in the development of protein/peptide-
26 based therapeutics. These therapeutics have found widespread applications in the treatment of cancer,
27 infectious diseases, and other metabolic disorders owing to their several desirable attributes, such as
28 reduced toxicity, diverse biological activities, high specificity, and potency. Most protein/peptide-
29 based drugs are still administered parenterally, and there is an unprecedented demand in the
30 pharmaceutical industry to develop oral delivery routes to increase patient acceptability and
31 convenience. Recent advancements in nanomedicine discoveries have led to the development of
32 several nano and micro-particle-based oral delivery platforms for protein/peptide-based therapeutics
33 and among these, liposomes have emerged as a prominent candidate. Liposomes are spherical vesicles
34 composed of one or more phospholipid bilayers enclosing a core aqueous phase. Their unique
35 amphiphilic nature enables encapsulation of a diverse range of bioactives/drugs including both
36 hydrophobic and hydrophilic compounds for delivery. Against this backdrop, this review provides an
37 overview of the current approaches and challenges associated with the routes and methods of oral
38 administration of protein/peptide-based therapeutics by using liposomes as a potential vehicle. First,
39 the conventional and innovative liposome formation approaches have been discussed along with their
40 applications. Next, the challenges associated with current approaches for oral delivery of protein and
41 peptide-derived therapeutics have been thoroughly addressed. Lastly, we have critically reviewed the
42 potential of liposomes utilization as vehicles for oral delivery of proteins emphasizing the current
43 status and future directions in this area.

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1. Introduction

Liposomes are spherical vesicles composed of phospholipid bilayers that enclose an aqueous phase in their core.[1] These self-assembled structures with amphiphilic nature resemble the lipid membranes found within the cellular morphology of many living organisms and thus have been used as a model of living cell membranes.[2] Based on their size and lamellarity, liposomes can be classified into three groups: (i) unilamellar vesicles (ULVs), (ii) multilamellar vesicles (MLVs), and multivesicular vesicles (MVVs) (Fig. 1). ULVs are further subdivided into three categories depending on their size: small unilamellar vesicles (SUVs), large unilamellar vesicles (LUVs), and giant unilamellar vesicles (GUVs) with sizes in the range of <100 nm, 100-1000 nm, and >1 μm, respectively.[3] The thickness of the phospholipid bilayer has been reported to be around 4 nm, which is dependent on phospholipid type, temperature, and cholesterol concentration.[4,5]

Since Bangham et al. first reported the formation of liposomes, there have been significant research efforts on their fabrication, development and applications.[6] The Bangham method, also known as the thin-film hydration (TFH) method, has been considered as the pioneer way of liposome preparation along with other traditional techniques such as ethanol injection,[7] and reverse-phase evaporation.[8] However, the use of organic solvents in these methods has raised questions about their high separation cost, residues left in the system, and safety. Consequently, in recent years, there have been many attempts to decrease or eliminate the use of organic solvents during the production of liposomes and enhance their utility. These approaches include microfluidics,[3,9] and supercritical fluid-based systems.[10,11] Carbon dioxide (CO_2) is the most common fluid used in the supercritical fluid-based liposome formation systems since it is non-toxic, abundant, and inexpensive, in addition to having a mild critical temperature (31 °C) and pressure (7.4 MPa). During liposome formation, supercritical carbon dioxide (SC-CO_2) has been used for versatile purposes, including as a solvent, cosolvent, antisolvent, or dispersing agent.[11–14]

71 In recent years, liposomes have received further attention due to their size and ability to
72 simultaneously encapsulate hydrophilic and hydrophobic bioactive compounds.[10,15,16] Liposomes have
73 been utilized to increase stability, solubility, bioaccessibility, and/or bioavailability, of bioactive
74 compounds, and to provide targeted delivery and controlled release in food, pharmaceutical, and
75 cosmetic industries. Several studies have proposed liposomal formulations for the oral delivery of
76 bioactive compounds such as curcumin,[17] icariin,[18] antidiabetic peptides,[19] exemestane (an anticancer
77 hormone therapy),[20] therapeutic peptides,[21] and asenapine maleate (an antipsychotic drug).[22] Among
78 these therapeutics, oral delivery of proteins and peptides is of great interest since (i) drugs approved
79 or in advanced-stage clinical trials are predominantly protein-based biopharmaceuticals,[23] and (ii) over
80 50% of the drugs approved are designed for oral administration.[24] However, oral delivery of proteins
81 and peptides faces many obstacles due to their susceptibility to degradation, hydrolysis, and being
82 blocked by mucus or cellular barriers in the gastrointestinal (GI) tract.

83 This review focuses on the oral liposomal delivery of protein/peptide-based therapeutics with
84 a particular emphasis on the challenges, opportunities, and recent advances. The conventional and
85 innovative liposome formation approaches, and their applications are assessed as a background for
86 further discussions. Challenges associated with the oral delivery of proteins and peptides have been
87 evaluated and critically discussed with a strong emphasis on their current status and strategies for
88 future directions.

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95 **2. Liposome synthesis methods and their applications**

96 **2.1. Conventional methods**

97 In the TFH-based Bangham method, phospholipids, cholesterol and lipophilic bioactives, are
98 first dissolved in organic solvents like chloroform, methanol, or hexane in a round-bottom flask, and
99 the organic solvents are subsequently evaporated using a rotary evaporator. The dry lipid film is
100 subsequently hydrated with an aqueous solution that may contain hydrophilic bioactives and vortexed
101 or sonicated to form liposomes. Liposomes synthesized using the TFH method are MLVs of several
102 microns in size and have relatively low bioactive (especially the hydrophilic ones) loading efficiencies.
103 [25,26]

104 The ethanol injection method is another liposome preparation technique that involves the
105 injection of ethanolic phospholipid solution into an aqueous solution.[7] This method has been reported
106 to produce SUV liposomes with sizes in the range of 73-129 nm but low solubility of hydrophobic
107 bioactives in ethanol may limit their loading capacities.[27,28] The ethanol injection process has been
108 successfully scaled up by Charcosset et al. from 60 mL to 3 L with good reproducibility and liposome
109 stability.[29]

110 Another liposome formation method, the reverse phase evaporation process, was reported by
111 Szoka and Papahadjopoulos.[8] In this method, phospholipids and hydrophobic bioactives are dissolved
112 in organic solvents such as ether, chloroform, or methanol, and an emulsion is formed by adding an
113 aqueous solution containing hydrophilic bioactives into the organic phase. The organic solvent is
114 removed from the system in a rotary evaporator, resulting in the formation of LUV liposomes in the
115 aqueous phase.[30] Even though this technique generates higher loading efficiencies compared to the
116 TFH method, the hydrophilic bioactives to be encapsulated are in contact with the organic phase,
117 which may cause denaturation of fragile molecules such as proteins and peptides.[31]

118 Furthermore, there have been several attempts to modify liposomes after they are produced
119 using the above-mentioned conventional methods. These efforts include membrane extrusion,[32]
120 sonication,[33] and freeze-thawing processes.[34] These post-formation processes can further decrease the
121 size (i.e., from LUVs to SUVs), lamellarity (i.e., from MLVs to ULVs), and heterogeneity (i.e.,
122 reduction in polydispersity index) of liposomes.[35–37] However, the conventional liposome production
123 methods continue to pose problems with regard to the removal of organic solvents, scalability, and
124 nonuniformity in the structure.

125 **2.2. Innovative methods**

126 In recent years, novel liposome production methods have been proposed to (i) minimize or
127 eliminate the use of organic solvents, (ii) increase encapsulation efficiency, (iii) overcome scalability
128 problems, (iv) increase reproducibility and homogeneity, and (v) reduce processing time. These
129 methods are discussed below in brief, along with a brief summary in Table 1.

130 Microfluidics, the science and technology of fluid systems in micron scale channels,[38] has been
131 lately adapted to produce liposomes and reviewed in detail by van Swaay and deMello.[3]
132 Electroformation, extrusion, flow focusing, pulsed jetting, and double emulsion templates are a few
133 microfluidic approaches to name. For example, Jahn et al. produced liposomes by hydrodynamically
134 focusing a stream of ethanolic phospholipid solution between two sheathed streams of aqueous
135 solutions in a microfluidic channel.[39] The resulting liposomes were ULVs with sizes in the range of 50
136 to 100 nm, which eliminated the need for post-formation processing of liposomes.[39] In another
137 microfluidic method, water-in-oil-in-water (W/O/W) double emulsions are utilized to synthesize
138 liposomes. Briefly, the oil phase is prepared by dissolving phospholipids and hydrophobic bioactives
139 in organic solvent mixtures such as chloroform and hexane. As the solvents are removed from the oil
140 phase, the phospholipid layers at the water-oil and oil-water interfaces come together and form
141 bilayers.[3,40] However, the complete removal of the organic solvents from this system is challenging.

142 This technique is reported to form monodisperse β-carotene loaded GUV liposomes with sizes around
143 100-180 μm.[41]
144 On the other hand, supercritical carbon dioxide (SC-CO_2)-based systems have been adapted
145 to generate liposomes without the use of toxic organic solvents (Table 1). Since SC-CO_2 has tunable
146 properties such as solubility, density, and viscosity, depending on pressure and temperature, it has
147 been employed in several liposome formation techniques for different purposes.[42] Recently, Tsai and
148 Rizvi and William et al. have extensively reviewed the SC-CO_2-based liposome synthesis methods.[10,11]
149 These methods include rapid expansion of supercritical solutions (RESS),[43] supercritical reverse phase
150 evaporation (SCRPE),[44] supercritical assisted liposome formation (SuperLip),[45] depressurization of an
151 expanded solution into aqueous media (DESAM),[46] depressurization of an expanded liquid organic
152 solution-suspension (DELOS-SUSP),[47] gas antisolvent (GAS),[48] supercritical antisolvent (SAS),[49] and
153 particles from gas saturated solutions (PGSS).[50] Most of these methods can address at least one of the
154 problems associated with the conventional liposome production techniques. For instance, Sharifi et
155 al. fabricated liposomes using a venturi-based RESS (Vent-RESS) process without using any organic
156 solvent.[51] In this approach, phospholipids and hydrophobic bioactives were dissolved in SC-CO_2, and
157 that stream was mixed with an aqueous cargo solution in an eductor nozzle system utilizing Bernoulli's
158 principle, where vacuum-driven cargo suction eliminated the need for an external pump to form
159 liposomes. The resulting liposomes showed a unimodal size distribution with an average particle size
160 ranging between 580 and 700 nm, and they were ULVs, MLVs, or MVVs depending upon the
161 phospholipid composition.[51] Also, using the SCRPE method, Zhao and Temelli produced liposomes
162 with superior properties compared to the ones formed by the TFH method.[52] In this SC-CO_2-based
163 method, liposomes were produced by depressurization of CO_2-expanded phospholipid suspension in
164 water, which resulted in ULV liposomes with particle sizes in the range of 214 to 265 nm, and
165 enhanced storage stability for over eight weeks. On the other hand, the TFH method generated MLV

166 liposomes with larger particle sizes (ca. 420 nm).[52] Therefore, the SC-CO_2-based liposome production
167 methods offer great potential to overcome the issues associated with the conventional methods while
168 meeting large-scale production requirements.[10,11,53]

169 **2.3. Applications of liposomes**

170 Applications of liposomes can be categorized into three main areas, namely food,
171 pharmaceutical, and cosmetics (Table 2). However, functions of liposomes across these industries can
172 be very similar, for example, as carriers for delivering bioactives with improved bioavailability and
173 storage stability, and controlled release. For food applications, liposomes have been loaded with
174 several bioactive compounds such as vitamins, minerals, antioxidants, and proteins (Table 2), where
175 the goal is to fortify foods with health-improving bioactives, protect them during food preparations,
176 and deliver them in their most bioavailable form.[16,54] For instance, Marsanasco et al. encapsulated
177 vitamins E and C in liposomes to fortify orange juice and protect these vitamins during
178 pasteurization.[55] In that study, liposomes provided a protective effect on the antioxidant activity of
179 vitamins even after pasteurization, where the heat stability of liposomes was attributed to the lipid
180 bilayer stabilizers: stearic acid and calcium stearate. Likewise, betanin, a color pigment with several
181 health benefits, was loaded into liposomes, and the liposomal formulation was incorporated into
182 gummy candy.[56] As a result, liposomes increased oxidative stability of betanin over 60 days of storage.
183 Lastly, Hong et al. developed liposomes loaded with catechin and curcumin that enhanced their
184 bioavailability and cytotoxic effects against cancer cells.[57]

185 Additionally, motivations similar to those for foods are also found in pharmaceutical
186 applications of liposomes, but the focus is more on the stability of drugs during production and
187 digestion, and their release and bioavailability in the body.[58] Therefore, in most cases, liposomes
188 developed can be used in both food and pharmaceutical products when the encapsulated bioactive
189 has similar utility. Compared to utilization in foods, applications of liposomes in pharmaceutical

products are more prevalent. Liposomes have been effectively used in delivering drugs such as exemestane,[20] asenapine maleate,[22] temozolomide,[59] irinotecan,[60] and apigenin and 5-fluorouracil.[61] Currently, more than a dozen clinically approved liposomal drugs are in the market.[62]

The cosmetic industry has adapted liposomal formulations to mainly overcome the stability and solubility problems of cosmeceuticals.[63] For instance, transdermal folic acid delivery,[64] intelligent release of L-ascorbic acid in sunscreens,[65] controlled release of proanthocyanidin,[66] increased stability and skin permeability of anthocyanin,[67] and skin delivery of vitamin K_1 oxide[68] have been achieved using various liposome-based formulations (Table 2).

3. Challenges in the oral delivery of proteins and peptides

Proteins and peptides are getting increasing attention for their use in the treatment, management, or prevention of several diseases due to their low number of side effects, diverse biological activities, and high specificity and potency.[69,70] In recent years, many protein- or peptide-based drugs have been designed to treat various diseases, including cancer, genetic disorder, diabetes, and inflammation.[71,72] The global market of protein/peptide-based therapeutics was valued at around $93.14 billion in 2018 and is expected to soon expand at a compound annual growth rate of 16.7 % and reach a market valuation of $172.87 billion (Therapeutic Proteins Global Market Report 2020).[73] Historically, parenteral administration is the predominant route for the delivery of protein/peptide-based therapeutics. Although, parenteral administration can provide higher bioavailability compared to the oral route, it has considerable disadvantages such as pain, reactions (e.g., swelling, rash, bleeding, burning, and redness) at the injection site, scarring, and cutaneous infections,[24,74] and in case of intravenous injection, a health care professional is required for administration. For those reasons, there has been a growing demand for therapeutics, especially protein- and peptide-based ones, that can be administered orally since it improves patients' compliance along with making it more cost effective,

213 and flexible in terms of design and dosage forms. Especially, oral delivery can substantially improve
214 patient compliance about treatments of chronic diseases that require long term and repeated dosing.
215 However, there are several challenges in delivering proteins and peptides orally. In addition to
216 their susceptibility to chemical degradation during production, proteins and peptides faces numerous
217 hurdles throughout the oral route including (i) biochemical, (ii) mucosal barriers in the GI tract, and
218 (iii) challenges in paracellular and transcellular transportation (Fig. 2).

219 **3.1. Biochemical barriers**

220 Digestive enzymes and pH change are the main biochemical barriers for delivering proteins
221 and peptides throughout the GI tract (Fig. 2 (A)). Although digestion starts in the mouth with salivary
222 amylases acting on carbohydrates at pH ~6.8 for a very short time, there is almost no protein digestion
223 in this part of digestion process.[75] However, as the therapeutic proteins/peptides move to stomach,
224 they encounter low pH (1-3), and protein digesting enzyme (pepsin).[76] The time drugs exposed to
225 these conditions depends on stomach fullness, drug type, viscosity of foods ingested with, and protein
226 content.[77,78] While gastric emptying of 300 mL of water is about 1 h,[75] it is over 2 h for post meal
227 administration of paracetamol co-ingested with a glass of water.[77] Therefore, gastric emptying dictates
228 the exposure time to those harsh conditions. Furthermore, in the intestine lumen, protein/peptide-
229 based drugs may lose their activity due to several proteases such as trypsin, chymotrypsin, elastase,
230 carboxypeptidase A and B, and intestinal brush border peptidases, where each protease has a specific
231 amino acid preference.[76]

232 **3.2. Mucosal barrier**

233 The GI tract is covered with a highly complex viscous mucus layer that has multiple functions:
234 (i) lubricating ingested foods for passage, (ii) maintaining a hydrated layer on the epithelium, and (iii)
235 preventing pathogens and foreign substances reaching the epithelium.[79,80] Mucus, secreted by goblet

236 cells, is a physical hydrogel that is primarily composed of mucin (i.e., high molecular weight
237 glycoproteins). In addition to mucin, mucus contains water, lipids, surfactants, proteins, enzymes,
238 polysaccharides, and nucleic acids.[81] Also, the pH can differ significantly across the mucus layer. For
239 example, the pH changes from highly acidic (~2) at the surface of the stomach lumen to neutral at the
240 surface of the epithelium within the gastric mucus layer.[82] This poses a serious impediment to
241 designing a drug delivery vehicle.[82]

242 The permeability through mucus layer, a critical factor for the oral delivery of
243 proteins/peptides, is determined by porosity of the barrier and charge of the particles (Fig. 2 (B)),[79]
244 where the pore size varies between 25 and 200 nm.[83] Thus, to overcome these barriers, particle size
245 and charge need to be taken into account when designing a drug delivery system. For example, as
246 mucin contains negatively charged glycoproteins, particles with positive charge demonstrates better
247 penetration through mucus layer due to electrostatic interaction.[84]

248 **3.3. Challenges in paracellular and transcellular transportation**

249 After passing the mucus layer, therapeutic peptides travel either through the spaces between
250 the epithelial cells (i.e., the paracellular route), or through the epithelial cells (i.e., the transcellular
251 route) to reach the bloodstream (Fig. 2 (C)).[85] Absorption through the first route is especially
252 challenging due to tight junctions, adherens junctions, and desmosomes, with estimated pore
253 diameters ranging between 1.32 and 2.02 nm.[86] Therefore, the paracellular route is limited to small
254 molecules ($\leq$ 20 KDa).[87] Similar to the mucus layer, paracellular transport is also affected by the charge
255 of the materials, where molecules with negative charges are preferred over those that are positively
256 charged ones.[88] On the other hand, in the transcellular route, therapeutics are absorbed by the epithelial
257 cells, composed of enterocytes, goblet cells, Paneth cells, microfold cells (M-cells), etc.[89,90] Among
258 those, enterocytes are the most prominent cell types in the small intestine, and they are responsible
259 for micronutrient absorption. These cells have microvilli on their apical surface, increasing the surface

260 area for efficient absorption and diffusion.[90] Nevertheless, there are different hurdles related to the
261 transcellular transport of protein/peptide-based therapeutics. After passing through cell membrane,
262 those therapeutics may be seen as foreign molecules resulting in their degradation by intracellular
263 peptidases,[88] or expulsion back to the intestinal lumen,[91] which reduce their overall bioavailability.

264 4. Adapting liposomes for oral delivery of protein/peptide-based therapeutics

265 As discussed in the previous section, oral delivery of protein/peptide-based therapeutics is
266 largely hindered by the harsh and proteolytic environment in the GI tract. Limited absorption and
267 poor permeation of the therapeutics in the intestinal tract also pose as major obstacles. Thus, there
268 exists a clear demand for the development of new techniques to facilitate oral delivery of
269 protein/peptide-based therapeutics. These delivery media need to be made of non-toxic and
270 immunologically inert materials that would enable non-intrusive site-specific intestinal release of the
271 payload. Along with other particle-based oral delivery systems (e.g. polymeric particles, micelles,
272 inorganic nano and micro-particles, drug crystal) liposomes have emerged as a predominant delivery
273 vehicle for oral administration.[24] Fig. 3 schematically represents the fate of liposomes from oral
274 administration to passing through deleterious gastric environment and finally successful site-specific
275 delivery in the intestine. Liposomal delivery of protein/peptide-based therapeutics is preferred owing
276 to their advantageous attributes of biocompatibility, biodegradability, minimal-toxicity, and non-
277 immunogenicity. Liposomes have been effectively used to encapsulate nucleic acids, enzymes,
278 peptides, genes, and antibiotics with a narrow therapeutic index. However, conventional liposomes
279 constituting of phospholipids and cholesterol demonstrate limited efficacy in oral delivery applications
280 because of poor stability, low permeation, poor absorption, and rapid clearance by the
281 reticuloendothelial system. Some of the mechanisms proposed to enhance the stability and
282 bioavailability of liposomes under the GI tract conditions include but are not limited to enhanced
283 bilayer stability, shielding payload from enzymatic degradation, enhanced retention and better mucus

284 penetrating abilities of bioactive loaded liposomes in the intestinal tract, and improved receptor-
285 mediated uptake (Fig. 4).[92,93]

286 **4.1. Enhancing liposomal structural stability in the GI tract**

287 Since their discovery, liposomes have been extensively used in parenteral delivery of drugs and
288 bioactive compounds.[94–99] The susceptibility of liposomes' phospholipid bilayer membrane towards
289 the combined adverse effects of gastric acid, digestive enzymes (i.e. phospholipases, pancreatic lipase,
290 and cholesterol esterase) and bile salts makes it a less suitable carrier for the delivery of labile
291 bioactives.[100–104] In the GI tract, a family of enzymes named phospholipase hydrolyzes the liposomal
292 phospholipids into choline, phosphatidic acid, and lysolipids. Under the superfamily of the
293 abovementioned enzyme group, phospholipase A (A_1 and A_2) and B catalyze the hydrolysis of ester
294 linkages present in the acyl chains of phospholipids. Phospholipase C converts phospholipids into
295 diglycerols; whereas, phospholipase D cleaves the terminal phosphate ester bond which results in the
296 formation of phosphatidic acid.[105–109] Pancreatic lipase, another fat hydrolyzing enzyme present in the
297 GI tract, is also detrimental towards the liposomal structural integrity. Cholesterol esterase, an enzyme
298 secreted by the pancreas, hydrolyzes phospholipids and cholesterol esters, two major constituents of
299 the liposomal bilayer membrane.[110,111] Cholesterol esterase is a non-specific lipase and anionic
300 phospholipids (i.e., phosphatidylserine and phosphatidylinositol) are more susceptible towards its
301 enzymatic action compared to their less negatively charged counterparts (i.e.
302 phosphatidylethanolamine, phosphatidylcholine).[112–114] Furthermore, the presence of bile salts in the
303 GI tract also endangers the liposomal structural integrity. Bile salts are integral components of bile,
304 and their presence in the GI tract is crucial towards digestion, intestinal homeostasis, and
305 hepatobiliary. Due to their strong affinity towards phospholipids' hydrophobic end, bile salts adhere
306 to liposomal surface and convert them into micelles through self-assembly. Bile salts are also
307 responsible for an increased fluidity of the phospholipid bilayer by thorough permeation which

308 consequentially leads to breaking down of the liposomal structure.[115–118] Various approaches have been
309 explored to increase the stability of liposomes in GI tract during their oral administration as further
310 discussed later.

311 **4.1.1. Modification of constituent phospholipids**

312 Modification of the liposomal wall material by modulating their main constitutional
313 components, i.e. phospholipids and cholesterol, has been conducted in order to make them retain
314 their structural integrity in the GI tract.[119–125] Uhl et al. synthesized liposomes by adding
315 glycerylcaldityltetraether lipid (GCTE) (Fig. 5 (A)) to lecithin (Fig. 5 (B)) and cholesterol (Fig. 5 (C)).[126]
316 A model glycopeptide antibiotic drug vancomycin was encapsulated in synthesized liposomes.
317 Modification of liposomes with GCTE resulted in a 3-fold increase in uptake of vancomycin when
318 administered orally in Wistar rats. Menina et al. carried out liposomal encapsulation of colistin (a
319 potent antibiotic effective against multidrug-resistant infections caused by gram-negative bacteria), to
320 enhance its oral bioavailability (Fig. 6).[127] TFH was used to synthesize the liposomes and different
321 combinations of three phospholipids with saturated long acyl chains (i.e., 1,2-dipalmitoyl
322 phosphatidylcholine (DPPC) (Fig. 5 (D)), 1,2-distearoyl-sn-glycero-3-phospho-choline (DSPC) (Fig 5
323 (E)) , and 1,2-dipalmitoyl phosphatidylcholine/1,2-dipalmitoyl-sn-glycero-3-phospho-ethanolamine-
324 N-Glutaryl (DPPE-GA) (Fig 5 (F)) and cholesterols were explored to optimize liposomal drug
325 encapsulation and stability. Liposomes prepared from DSPC:DPPE-GA:cholesterol (1:0.2:1 molar
326 ratio) (L1) and DPPC:DSPC:DPPE-GA:choleterol (1:1:0.2:1 molar ratio) (L2) resulted in an
327 optimized encapsulation and loading efficiency over 55 and 50 %, respectively. The stability of colistin
328 encapsulated liposomes was evaluated in simulated biorelevant media. In fasted state-SGF and
329 simulate intestinal fluid (SIF), L1 released less than 10% of encapsulated drug. Whereas in fed state-
330 SIF along with digestive enzymes, 32% of encapsulated drug was released. For L2 less than 5 % of
331 the encapsulated antibiotic was released in fasted state-SGF and SIF conditions and maximum 20 %

332 of the drug was released in fed state-SIF along with digestive enzymes (Fig. 6 (C) and (D)). After
333 optimizing the stability of liposomes in GI tract, they functionalized the liposomes with extracellular
334 adherence protein. Functionalized liposomes were able to substantially reduce the number of
335 intracellular bacteria when treated on Human epithelial type 2 (HEp-2) and Caco-2 cells infected with
336 *Salmonella enterica* (Fig. 6 (E) and (F)).[127] Vergara et al. used rapeseed phospholipid (RP) (constituted
337 by: 1-oleoyl-2-linoleoyl-sn-glycero-3-phosphocholine (Fig. 5 (G)), 1-oleoyl-2-linoleoyl-sn-glycero-3-
338 phosphoethanolamine (Fig. 5 (H)), phosphatidic acid (Fig. 5 (I)), lysophosphatidylcholine, (Fig. 5 (J)),
339 stigmasterol (ST) (5 (K)) and/or hydrogenated phosphatidylcholine (HPC) to synthesize liposomes by
340 using TFH. They encapsulated lactoferrin (LF) as a model iron–binding glycoprotein to study the
341 potential of using these liposomes as useful oral delivery system.[128] In SGF after 2 h of incubation,
342 around 80 % of the LF remained intact when encapsulated in liposomes prepared from RP and HPC.
343 They inferred that the higher phase transition temperature of HPC (Tm ~ 55 °C) made synthesized
344 liposomes less permeable and thus more stable in SGF. In SIF condition, liposomes released around
345 80% of the encapsulated LF when incubated for 2 h in the presence of SIF. Modification of
346 constituent phospholipids to increase liposomal stability is the most direct method available because
347 of the limited number of variables to be manipulated during synthesis. It provides membrane stability
348 while maintaining the cell like bilayer structure. However, it comes with the limitations of less flexibility
349 in terms of controlling surface geometry and site-specific delivery characteristics.

350 **4.1.2. Incorporation of bile salts in bilayer**

351 Several studies have been carried out to mitigate the detrimental effects of bile salts towards
352 liposomal stability by anchoring bile salts into the bilayer membrane of liposomes.[101,129,130] Conacher
353 et al. first synthesized bile salt containing liposomes and the resulting vesicles were named
354 bilosomes.[131] Although bile salts initially disrupted liposomal structural integration, it was hypothesized
355 that prior incorporation of bile salts in bilayer would help them retain their original structure when

356 exposed to further bile salts present in the intestine. When exposed to an external bile salt
357 concentration of 5 mM, both liposomes and bilosomes were able to retain 90% of the entrapped
358 protein (bovine serum albumin, BSA). However, at a higher external concentration of bile salts (20
359 mM), bilosomes retained almost twice the amount of encapsulated BSA compared to traditional
360 liposomes.[131] The potential of using bile salts to enhance the liposomal stability has been further
361 studied by several research groups. Hu et al. compared the performance and stability of conventional
362 liposomes (Ch-L) prepared by using cholesterol and phospholipids with liposomes containing a bile
363 salt, sodium glycocholate (SGC).[101] In SGF the stability of SGC containing liposomes (SGC-L) was
364 determined by quantifying the release of fluorescent dye calcein. Inclusion of SGC resulted in
365 substantial increase in calcein release compared to Ch-L. The integrity of SGC-L encapsulating a
366 model protein insulin was investigated for both in vitro and ex vivo gastrointestinal fluids; and in both
367 these conditions SGC-L retained higher amounts of initially encapsulated insulin. In ex vivo condition,
368 after 4 h of incubation in SGF, Ch-L and SGC-L retained respectively 9 and 17 % of initial
369 encapsulated insulin. For SIF, on the other hand, those values were 9 and 20 % of the initial loading.
370 Thus, the modulation of liposomal bilayer structure provided extra protection towards encapsulated
371 insulin during its oral delivery.[101] Elnaggar et al. encapsulated Risedronate (RS), a drug that hinders the
372 onset of Osteoporosis, in bilosomes to increase its oral bioavailability.[132] They synthesized bilosomes
373 of anionic and catanionic attributes to illustrate their respective ability to enhance liposomal structural
374 integrity. Bilosomes synthesis was conducted by reversed phase evaporation technique. Phospholipid,
375 cholesterol, and bile salt molar ratio was optimized at 4:1:1 to encapsulate 10 mg/mL solution of RS.
376 In cationic bilosomes, positive charge was induced by the addition of 1, 2-Dioleoyloxy-3-
377 trimethylammonium propane chloride (DOTAP) or Stearylamine. In terms of stability in digestive
378 media, cationic bilosomes demonstrated superior results compared to their anionic counterparts.
379 However, they induced higher oral toxicity. In contrast, anionic bilosomes substantially reduced RS's

380 toxicity and increased its permeation.[132] Rizwanullah et al. synthesized bilosomes by TFH to enhance
381 the oral bioavailability of an antiviral drug acyclovir.[133] They hypothesized that use of bilosomes would
382 be effective to increase acyclovir's absorption in the intestine by providing resistance towards
383 disruption by digestive media and promoting better permeation. In SIF acyclovir containing bilosomes
384 demonstrated 95 % release, whereas for free acyclovir suspension and another commercially available
385 formulation those values were 40 and 53 %, respectively. In Wistar rat, the relative bioavailability of
386 acyclovir was 4.4 and 2.5 times higher for bilosomes when compared to free acyclovir suspension and
387 another commercially available formulation, respectively.[133] In their recent work, Deng et al. reviewed
388 how bile salts could be used for transporter mediated delivery of drugs in various forms of oral
389 administration.[134] Even though embedding bile salt in the bilayer of liposomes provides extra stability
390 in the GI tract and increases oral bioavailability of encapsulated therapeutics, it can also negatively
391 impact the intestinal internalization of liposomes and therapeutic performance of encapsulated
392 peptides or proteins.[101]

393 **4.1.3. Surface coating to enhance stability**

394 Several enteric polymers, such as natural and modified carbohydrates have been used
395 extensively to coat liposomal surface.[135,136] These materials prevent disintegration of liposomes in the
396 GI tract, and consequentially a higher proportion of liposomes are carried forward to the small
397 intestine which results in enhanced absorption of encapsulated bioactives.[92,93,137–140] In addition to
398 being resistant to low pH, most of these materials provide excellent mucoadhesive properties.[141] Costa
399 et al. used a continuous two-step microfluidic procedure to produce insulin loaded liposomes for oral
400 delivery applications.[142] Recombinant human insulin was incorporated in liposomes prepared from
401 distearoylphosphatidylethanolamine poly (ethyleneglycol)$_{2000}$ (DSPE-PEG$_{2000}$), egg-
402 phosphatidylcholine (E-PC), and cholesterol. Addition of PEG promoted an elevated blood
403 circulation of insulin loaded liposomes by the reduction of macrophagic recognition. Synthesized

404 liposomes demonstrated an average diameter of around 144 nm along with insulin encapsulation
405 efficiency of 91%. Insulin loaded liposomes were furthermore coated with another layer of chitosan
406 to enhance their stability in gastric stomach environment. Chitosan's efficacy to protect liposomes
407 from gastric degradation was validated through in vitro studies which showed that insulin release was
408 only initiated at a pH at 6.8 which is above chitosan's pKa value. Furthermore, owing to chitosan's
409 mucoadhesive properties, chitosan coated liposomes showed an enhanced uptake and permeation of
410 insulin across the intestinal epithelium.[142] Methyl methacrylate- methacrylic acid block co-polymers;
411 i.e. Eudragit L 100 and S 100 dissolves over pH 6 and 7 respectively and thus coating liposomes with
412 these polymers protect them from the acid reflux in GI tract.[143–148] Sharma et al. encapsulated
413 recombinant human insulin in liposomes prepared from soy lecithin and cholesterol. Synthesized
414 liposomes were further coated with protamine sulfate, which was used as a permeation enhancer.[149]
415 Protamine sulfate coated liposomes were encased in an Eudragit S 100 coated gelatin capsule. After 2
416 h incubation in SGF, negligible release of insulin was observed for Eudragit coated liposomes, whereas
417 in SIF, 82 % of encapsulated insulin was released. Presence of protamine sulfate coating resulted in
418 enhanced uptake of insulin in Caco-2 cells. Eudragit S 100 protected liposomes and encapsulated
419 insulin from proteolytic degradation in stomach and enabled stable release in intestinal epithelium.[149]
420 Zhao et al. in their study, aimed to increase oral absorption of sorafenib (S), a drug often used for the
421 radio therapy of colorectal cancer.[150] TFH method was used to encapsulate sorafenib in liposomes
422 (SL). The synthesized liposomes were coated with glycol chitosan (G-SL), followed by another layer
423 of coating with Eudragit S 100 (E-G-SL). Encapsulation efficiency of 97 and 90 % was observed for
424 E-G-SL and G-SL, respectively. Coated liposomes were stable in SGF and were able to retain more
425 than 80 % of the encapsulated drug compared to their uncoated counterparts, which showed only 40
426 % retention (Fig. 7 (A)). At pH 7.4, G-SL and E-G-SL both showed comparable cellular uptake(Fig.
427 7 (B)); however, when orally administered in rats, E-G-SL significantly improved systemic exposure

428 of sorafenib compared to other formulation (Fig. 7 (C)).[150] Gomaa et al. encapsulated bacteriocin
429 MccJ25 (produced by E. coli pTUC202 strain) in both cationic and anionic liposomes and coated
430 them with a dual layer comprising pectin and whey protein isolate (WPI) to develop an oral
431 administration.[151] Anionic liposomes coated with a dual layer of pectin and WPI showed substantially
432 better protection of MccJ25 under simulated gastric conditions. Mohanraj et al. coated conventional
433 liposomes containing insulin with silica nanoparticles and observed the stability and release properties
434 of synthesized hybrid silica–liposome nanocapsules.[152] Synthesized silica containing liposomes
435 demonstrated an insulin encapsulation efficiency of 70 % and negligible release of insulin was
436 observed in the SGF when incubated for 2 h. However, in SIF a two-step insulin release pattern was
437 observed; which comprised a rapid release for initial 2 h, followed by a delayed release for 8 h.[152] It is
438 evident that surface coating provides functionalization to liposomal surface and enables manipulation
439 of the surface geometry and other properties to facilitate customized and sustained release of the
440 payload. However, coated liposomes often show inconsistency in terms of shape and size, and thus it
441 is difficult to maintain monodispersity of the particles. Furthermore, the processing involves additional
442 parameters and variables that need to be optimized during coating stages.

443 **4.2. Enhancing liposomal absorption in the GI tract**

444 Another major challenge in the liposomal oral delivery of protein/peptide-based therapeutics
445 is their low bioavailability due to poor penetration through the mucus layer and low absorption in the
446 intestinal epithelial cell line. Liposomes that reach small intestine in intact condition face a second
447 challenge of poor permeation through the epithelial cells which act as the main absorption barrier.
448 Currently there is a scarcity of published work exploring the exact mechanisms of liposomal
449 absorption in the GI tract. In the first mode of absorption after their transit from the stomach to small
450 intestine, the liposomal structure is disrupted with a gradual release of the encapsulated bioactive in
451 the intestinal lumen followed by transfer into intestinal epithelia by means of micelles or other

452 secondary carriers. However, for protein/peptide-based therapeutics this is less effective because the
453 released therapeutics have to penetrate through the mucus cell lining prior to reaching the epithelia.[153]
454 Mucus is ubiquitously preset in the GI tract and acts as a biochemical barrier in between epithelial
455 lining and lumen. It is rich with several proteolytic enzymes which result in quick degradation of
456 released protein/peptide-based therapeutics before reaching the epithelia.[154] A different pathway to
457 enhance bioavailability of encapsulated therapeutics would be absorption of the liposomes along with
458 the encapsulated payload. Even though liposomes containing protein/peptide-based therapeutics pass
459 through the mucosal layer, intestinal epithelia often obstruct its entry into the circulating blood stream
460 owing to intact liposomes' relatively large particle size.[155,156] Uptake of intact liposomes by M cells,
461 situated at the surface of the follicle-associated epithelium, has been proposed as an alternative
462 pathway to enhance absorption. M cells are least protected by mucus, and possess reduced level of
463 enzymatic activities, and therefore are capable of transferring several macromolecules (i.e., antigen,
464 virus, bacteria) from lumen to lymphoid tissue. However, its limited availability and variation in
465 distribution substantially restricts its efficacy as a pathway for absorption.[157–159] One promising
466 approach of increasing absorption of liposome encapsulated protein/ peptide-based therapeutics is to
467 increase their residence time in the intestine. Several natural and synthetic polymers have been used
468 to coat liposomal surface to enhance their mucoadhesive properties. These polymers adhere to a
469 specific site of intestinal lining and develop a patch on its surface, which facilitates enhanced
470 penetration of encapsulated therapeutics into the epithelium cells along with reducing dilution effects
471 by preventing premature release.[135] Enhancement of mucoadhesion is also attainable by modulating
472 the liposomal surface charge. Liposomes with positive surface charges demonstrates better
473 mucoadhesive properties by adhering to the negatively charged moieties in mucin glycoproteins, the
474 positive charge also adds resistance towards enzymatic degradation.[19,160] In their work, Shao et al.
475 increased oral bioavailability of CoQ10, a lipophilic benzoquinone, used in the treatment of

476 cardiovascular disease.[161] Liposomes were synthesized by solvent injection method followed by
477 coating with d-alpha-tocopheryl polyethylene glycol 1000 succinate (TPGS) and chitosan (Fig. 8 (A),
478 (B), and (C)). Chitosan was used for its mucoadhesive and cationic polyelectrolytic properties. TPGS
479 was used as a permeation enhancer. It also increases cellular uptake by scavenging free radicals,
480 inhibiting P-glycoprotein -mediated drug resistance, and by prolonging circulation time. It was
481 hypothesized that liposomes coated with both chitosan and TPGS will have better GI stability and
482 intestinal absorption. TPGS and chitosan coated liposomes demonstrated CoQ10 encapsulation
483 efficiency over 75 % and good stability in acidic pH, and excellent storage stability. No significant
484 change was observed in the diameter, zeta-potential, and EE % when stored at 4 and 25 °C for 3
485 months. Coated liposome demonstrated significantly higher mucin penetration ability compared to its
486 uncoated counterpart (Fig. 8 (D)). TPGS and chitosan coated liposomes increased cellular uptake of
487 CoQ10 in Caco-2 cells by around 30-fold when compared to untreated drug. When orally administered
488 in rats, coated liposomes demonstrated an extended and sustained CoQ10 release profile for up to 24
489 h and also caused a 3.4-fold increase in systemic exposure of CoQ10 when compared to untreated
490 drug (Fig. 8 (E)).[161] In addition to enhancing stability and mucoadhesion, polymers have been also
491 used to coat liposomes to enhance its intestinal permeability. Liposomes coated with mucus
492 penetrating polymers demonstrated better uptake by epithelial cell owing to increased residence time
493 in the mucus layer.[162,163] Yamazoe et al. surface-modified liposomes with PEG and glycol chitosan
494 (GS) to increase oral bioavailability of fluorescein isothiocyanate dextran (FD), a model peptide-based
495 drug.[164] In an in vitro artificial mucus model, the PEGylated liposomes demonstrated better mucus
496 permeability compared to unmodified and liposomes modified with GS. Substantially higher cellular
497 uptake of FD was observed for PEGylated liposomes when tested in Caco-2 and mucus-secreting
498 Caco-2/HT29 co-culture. When PEGylated liposomes were combined with absorption enhancer
499 spermine maximum absorption of FD was observed during in vivo rat model.[164] To increase oral

500 bioavailability of liposomes, several absorption enhancers along with polymer coating have been
501 exploited to increase liposomal absorption through epithelial cell line by means of trans and para-
502 cellular transportation.[165,166] Parmentier et al. synthesized liposomes containing permeation-enhancers
503 to increase oral-bioavailability of human growth hormone (hGH). Liposomes were synthesized by
504 using tetraether lipid, egg-phosphatidylcholine, and cholesterol through dual asymmetric
505 centrifugation.[167] They compared the performance of several permeation-enhancers (e.g.,
506 cetylpyridinium chloride (CpCl), phenylpiperazine, sodium caprate, D-α-tocopheryl polyethylene
507 glycol 400 succinate, or octadecanethiol) individually and in combination. Liposomes containing
508 CpCL were able to achieve a 3.4 % relative bioavailability of hGH compared to subcutaneous control;
509 whereas, for oral- administration of free hGH the relative bioavailability was only 0.01 %.[167] Ligand
510 mediated endocytosis is another prospective mechanism to enhance liposomal absorption through
511 transcellular transport. Liposomal surface modification with specific nutritional ligands, allow site-
512 specific cellular uptake through ligand-receptor interaction in intestinal epithelia.[168,169] He et al.
513 investigated the feasibility of increasing insulin absorption in intestine through vitamin ligand–receptor
514 interactions by decorating liposomal surface with two different ligands, thiamine and niacin.[170] Insulin
515 loaded liposomes were prepared through reverse phase evaporation method. Thiamin and niacin were
516 first conjugated with stearamine, which facilitated the attachment of the ligands on liposomal surface.
517 Decorated liposomes demonstrated an average insulin encapsulation efficiency around 30 %. In SGF
518 and SIF after 4 h of incubation, decorated liposomes were able to protect 60 and 80 % of the
519 encapsulated insulin. In diabetic rat model induced by streptozotocin, ligand decorated liposomes
520 demonstrated a mild and sustained hypoglycemic effect. Niacin and thiamin decorated liposomes were
521 able to reduce blood glucose levels as low as 72 and 81 % of the original value, respectively.[170] Several
522 other strategies that have been explored to increase absorption of liposomes encapsulating
523 protein/peptide-based therapeutics in the GI tract have been summarized in Table 3.

524

525 **5. Conclusion and prospects**

526 In the past decade, there has been a rapid growth in the development of protein/peptide-
527 based therapeutics which has greatly reshaped the traditional pharmaceutical industry. The foundation
528 of protein/peptide-based therapeutics was laid with the discovery of insulin in 1922.[171] The huge
529 success of recombinant DNA technology to facilitate insulin's commercialization has initiated a
530 worldwide shift in the research and development of protein/peptide-based therapeutics. Currently,
531 there are more than 100 peptide-based drugs that are approved by US Food and Drug Administration
532 and a substantial new research is ongoing to evaluate their potency for the treatment of cancer,
533 infectious diseases, inflammation, and other metabolic disorders.[172–176] Though protein/peptide-based
534 drugs offer numerous advantages, parenteral administration is still the predominant route for the
535 delivery of these therapeutics. Historically oral administration of therapeutics has been considered as
536 the most suitable owing to an increased patient compliance. Key obstacles in the implementation of
537 oral of delivery of protein/peptide-based therapeutics include their low bioavailability due to the
538 hinderance caused by the harsh and proteolytic environment in the GI tract. Additionally, limited
539 absorption and poor permeation of the therapeutics in the intestinal tract also possess major challenges
540 to their adoption. Thus, development of novel oral delivery routes for protein/peptide-based drugs
541 while utilizing their full therapeutic efficacy is a topic of major interest in today's pharmaceutical
542 industry. Considerable amount of research resulted in an influx of approaches for oral delivery of
543 protein/peptide- based therapeutics, which include but not limited to smart hydrogels, tablets, ionic
544 liquids, and liposome-based systems. Among those approaches, tablet formulations of some
545 proteins/peptides such as insulin, semaglutide, and salmon calcitonin have progressed to clinical
546 trials.[177–179] The oral formulation of semaglutide (Rybelsus®) was the first oral treatment to be
547 approved by the FDA in 2019 for the control of blood sugar in adults with type 2 diabetes.[180] The
548 details of those clinical trials have been reviewed elsewhere.[24,181] However, there is not any available

549 clinical trial data for the oral delivery of protein/peptide-based therapeutics using liposomes. This
550 review illustrated some contemporary advanced techniques used to facilitate oral delivery of
551 protein/peptide-based therapeutics by using liposomes as a vehicle. As conventional liposomes are
552 susceptible to adverse effects of the pH, bile salts and enzymes of the gastric environment, several
553 attempts have been made by different researchers for the development of novel liposomal
554 formulations for protecting these therapeutics, as explained in this review. Liposomal formulations
555 have a promising potential for their clinical translation due to their low toxicity, biocompatibility, and
556 biodegradability. However, liposome-based delivery of protein/peptide therapeutics still face critical
557 challenges. Holistic research approaches are required to better elucidate the absorption mechanism of
558 liposomal formulations in the GI tract with an emphasis on understanding how different dietary
559 practices impact the inner patient variance in GI tract absorption of protein-based therapeutics. In
560 addition to this, there still exists an unmet need for optimization of formulation design to enable mass
561 production of liposome-based formulations on an industrial scale. The bottleneck of liposomal mass
562 production lies in the inconsistency in their batch-to-batch production and the absence of inexpensive
563 and efficient methodologies to develop solid dosage form.[182,183] Nonetheless, the future prospective
564 of developing liposome-based protein/peptide based therapeutic has bright potential. New sustainable
565 and scalable formulation methodologies in conjugation with a better perception of the absorption
566 mechanism will lead to the development of next generation of liposomes-based delivery platforms
567 which will enable the development and delivery of new therapies for a wide range of diseases.
568

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572

573 **Conflicts of interest**

574 There are no conflicts to declare.

575

576 **References**

577 1 V. P. Torchilin, *Nat. Rev. Drug Discov.*, 2005, **4**, 145–160.

578 2 T. Hamada and K. Yoshikawa, *Materials*, 2012, **5**, 2292-2305.

579 3 D. van Swaay and A. deMello, *Lab Chip*, 2013, **13**, 752–767.

580 4 S. Paula, A. G. Volkov, A. N. Van Hoek, T. H. Haines and D. W. Deamer, *Biophys. J.*, 1996, **70**,
581 339–348.

582 5 F. de Meyer and B. Smit, *Proc. Natl. Acad. Sci.*, 2009, **106**, 3654.

583 6 A. D. Bangham, M. M. Standish and J. C. Watkins, *J. Mol. Biol.*, 1965, **13**, 238-252.

584 7 S. Batzri and E. D. Korn, *Biochim. Biophys. Acta - Biomembr.*, 1973, **298**, 1015–1019.

585 8 F. Szoka and D. Papahadjopoulos, *Proc. Natl. Acad. Sci.*, 1978, **75**, 4194-4198.

586 9 S. Deshpande and C. Dekker, *Nat. Protoc.*, 2018, **13**, 856–874.

587 10 W.-C. Tsai and S. S. H. Rizvi, *Trends Food Sci. Technol.*, 2016, **55**, 61–71.

588 11 B. William, P. Noémie, E. Brigitte and P. Géraldine, *Chem. Eng. J.*, 2020, **383**, 123106.

589 12 P. Nikolai, B. Rabiyat, A. Aslan and A. Ilmutdin, *J. Therm. Sci.*, 2019, **28**, 394–430.

590 13 R. Kapoor, A. Jash and S. S. H. Rizvi, *LWT*, 2021, **135**, 110060.

591 14 J. Liu, W. R. Rodgers and M. R. Thompson, *Polym. Compos.*, 2017, **38**, 987–995.

592 15 M. R. Islam Shishir, N. Karim, V. Gowd, X. Zheng and W. Chen, *Trends Food Sci. Technol.*, 2019,
593 **85**, 177–200.

594 16 T. Subramani and H. Ganapathyswamy, *J. Food Sci. Technol.*, 2020, **57**, 3545-3555.

595 17 M.-P. Tian, R.-X. Song, T. Wang, M.-J. Sun, Y. Liu and X.-G. Chen, *Int. J. Biol. Macromol.*, 2018,
596 **120**, 702–710.

597 18 X. Sun, J. Wei, J. Lyu, T. Bian, Z. Liu, J. Huang, F. Pi, C. Li and Z. Zhong, *J. Nanobiotechnology*,
598 2019, **17**, 10.

599 19 Z. Li, A. T. Paulson and T. A. Gill, *J. Funct. Foods*, 2015, **19**, 733–743.

600 20 A. Singh, Y. R. Neupane, S. Shafi, B. Mangla and K. Kohli, *J. Mol. Liq.*, 2020, **303**, 112649.

601 21 Z. Hu, S. Nizzero, S. Goel, L. E. Hinkle, X. Wu, C. Li, M. Ferrari and H. Shen, *Sci. Adv.*, 2020,
602 **6**, eaba0145.

603 22 R. S. Managuli, J. T.-W. Wang, F. M. Faruqu, A. Pandey, S. Jain, K. T. Al-Jamal and S. Mutalik,
604 *Mater. Sci. Eng. C*, 2020, **109**, 110620.

605 23 G. Walsh, *Nat. Biotechnol.*, 2018, **36**, 1136–1145.

606 24 T. D. Brown, K. A. Whitehead and S. Mitragotri, *Nat. Rev. Mater.*, 2020, **5**, 127–148.

607 25 A. Wagner and K. Vorauer-Uhl, *J. Drug Deliv.*, 2011, **2011**, 591325.

608 26 L. Zhao and F. Temelli, *J. Food Eng.*, 2015, **158**, 104–112.

609 27 C. Jaafar-Maalej, R. Diab, V. Andrieu, A. Elaissari and H. Fessi, *J. Liposome Res.*, 2010, **20**, 228–
610 243.

611 28 P. Gentine, L. Bourel-Bonnet and B. Frisch, *J. Liposome Res.*, 2013, **23**, 11–19.

612 29 C. Charcosset, A. Juban, J.-P. Valour, S. Urbaniak and H. Fessi, *Chem. Eng. Res. Des.*, 2015, **94**,
613 508–515.

614 30 O. Mertins, M. Sebben, A. R. Pohlmann and N. P. da Silveira, *Chem. Phys. Lipids*, 2005, **138**, 29–
615 37.

616 31 A. Akbarzadeh, R. Rezaei-Sadabady, S. Davaran, S. W. Joo, N. Zarghami, Y. Hanifehpour, M.
617 Samiei, M. Kouhi and K. Nejati-Koshki, *Nanoscale Res. Lett.*, 2013, **8**, 102.

618 32 F. Olson, C. A. Hunt, F. C. Szoka, W. J. Vail and D. Papahadjopoulos, *Biochim. Biophys. Acta -*
619 *Biomembr.*, 1979, **557**, 9–23.

620 33 G. Maulucci, M. De Spirito, G. Arcovito, F. Boffi, A. C. Castellano and G. Briganti, *Biophys. J.*,
621 2005, **88**, 3545–3550.

622 34 S. Sriwongsitanont and M. Ueno, *Open Colloid Sci. J.*, 2011, **4**, 1–8.

623 35 A. Hinna, F. Steiniger, S. Hupfeld, P. Stein, J. Kuntsche and M. Brandl, *J. Liposome Res.*, 2016,
624 **26**, 11–20.

625 36 G. S. Ong, M. Chitneni, S. K. Lee, C. L. Ming and H. K. Yuen, *Pharmaceutics*, 2016, **8**, 36.

626 37 H. Zhang, in *Liposomes: Methods and Protocols*, ed. G. G. M. D'Souza, Springer New York, New
627 York, NY, 2017, pp. 17–22.

628 38 G. M. Whitesides, *Nature*, 2006, **442**, 368–373.

629 39 A. Jahn, W. N. Vreeland, D. L. DeVoe, L. E. Locascio and M. Gaitan, *Langmuir*, 2007, **23**,
630 6289–6293.

631 40 A. S. Utada, E. Lorenceau, D. R. Link, P. D. Kaplan, H. A. Stone and D. A. Weitz, *Science*, 2005,
632 **308**, 537.

633 41 M. Michelon, Y. Huang, L. G. de la Torre, D. A. Weitz and R. L. Cunha, *Chem. Eng. J.*, 2019,
634 **366**, 27–32.

635 42 A. Jash, A. Ubeyitogullari and S. S. H. Rizvi, *Green Chem.*, 2020, **22**, 5345–5356.

636 43 F. Sharifi, R. Zhou, C. Lim, A. Jash, A. Abbaspourrad and S. S. H. Rizvi, *J. CO2 Util.*, 2019, **29**,
637 163–171.

638 44 S. Yamaguchi, Z. Kimura, T. Misono, K. Tsuchiya, K. Sakai, M. Abe and H. Sakai, *J. Oleo Sci.*,
639 2016, **65**, 201–206.

640 45 R. Campardelli, P. Trucillo and E. Reverchon, *J. CO2 Util.*, 2018, **25**, 235–241.

641 46 L. A. Meure, R. Knott, N. R. Foster and F. Dehghani, *Langmuir*, 2009, **25**, 326–337.

642 47 M. Cano-Sarabia, N. Ventosa, S. Sala, C. Patiño, R. Arranz and J. Veciana, *Langmuir*, 2008, **24**,
643 2433–2437.

644 48 U. Sankar Kadimi, D. R. Balasubramanian, U. R. Ganni, M. Balaraman and V. Govindarajulu,
645 *Nanomedicine Nanotechnology, Biol. Med.*, 2007, **3**, 273–280.

646 49 I. Ahmad, S. Akhter, M. Anwar, S. Zafar, R. K. Sharma, A. Ali and F. J. Ahmad, *Int. J. Pharm.*,
647 2017, **523**, 398–409.

648 50 E. de Paz, Á. Martín and M. J. Cocero, *J. Supercrit. Fluids*, 2012, **72**, 125–133.

649 51 F. Sharifi, A. Jash, A. Abbaspourrad and S. S. H. Rizvi, *Green Chem.*, 2020, **22**, 1618–1629.

650 52 L. Zhao and F. Temelli, *J. Supercrit. Fluids*, 2015, **100**, 110–120.

651 53 L. Lesoin, C. Crampon, O. Boutin and E. Badens, *J. Supercrit. Fluids*, 2011, **60**, 51–62.

652 54 W. Liu, A. Ye and H. Singh, in *Microencapsulation and Microspheres for Food Applications*, ed. L. M.
653 C. Sagis, Academic Press, San Diego, 2015, pp. 151–170.

654 55 M. Marsanasco, A. L. Márquez, J. R. Wagner, S. del V. Alonso and N. S. Chiaramoni, *Food Res.*
655 *Int.*, 2011, **44**, 3039–3046.

656 56 S. Amjadi, M. Ghorbani, H. Hamishehkar and L. Roufegarinejad, *Food Chem.*, 2018, **256**, 156–
657 162.

658 57 S.-C. Hong, K.-M. Park, C. R. Hong, J.-C. Kim, S.-H. Yang, H.-S. Yu, H.-D. Paik, C.-H. Pan
659 and P.-S. Chang, *Colloids Surfaces A Physicochem. Eng. Asp.*, 2020, **594**, 124670.

660 58 L. Sercombe, T. Veerati, F. Moheimani, S. Y. Wu, A. K. Sood and S. Hua, *Front. Pharmacol.*,
661 2015, **6**, 286.

662 59 M. M. Nordling-David, R. Yaffe, D. Guez, H. Meirow, D. Last, E. Grad, S. Salomon, S. Sharabi,
663 Y. Levi-Kalisman, G. Golomb and Y. Mardor, *J. Control. Release*, 2017, **261**, 138–146.

664 60 M. Papi, D. Caputo, V. Palmieri, R. Coppola, S. Palchetti, F. Bugli, C. Martini, L. Digiacomo,
665 D. Pozzi and G. Caracciolo, *Nanoscale*, 2017, **9**, 10327–10334.

666 61 K. Sen, S. Banerjee and M. Mandal, *Colloids Surfaces B Biointerfaces*, 2019, **180**, 9–22.

667 62 D. J. A. Crommelin, P. van Hoogevest and G. Storm, *J. Control. Release*, 2020, **318**, 256–263.

668 63 V. Van Tran, J.-Y. Moon and Y.-C. Lee, *J. Control. Release*, 2019, **300**, 114–140.

669 64 M. S. Kapoor, A. D'Souza, N. Aibani, S. S. Nair, P. Sandbhor, D. kumari and R. Banerjee, *Sci.*
670 *Rep.*, 2018, **8**, 16122.

671 65 Y.-Q. Dai, G. Qin, S.-Y. Geng, B. Yang, Q. Xu and J.-Y. Wang, *RSC Adv.*, 2012, **2**, 3340–3346.

672 66 F. Guo, M. Lin, Y. Gu, X. Zhao and G. Hu, *Eur. Food Res. Technol.*, 2015, **240**, 1013–1021.

673 67 C. Lee and K. Na, *Macromol. Res.*, 2020, **28**, 289–297.

674 68 N. Samadi, P. Aberoomand Azar, S. Waqif Husain, H. I. Maibach and S. Nafisi, *Int. J. Pharm.*,
675 2020, **579**, 119136.

676 69 K. Fosgerau and T. Hoffmann, *Drug Discov. Today*, 2015, **20**, 122–128.

677 70 L. S. Perry and J. D. McClements, *Molecules*, 2020, **25**, 1161.

678 71 S. Marqus, E. Pirogova and T. J. Piva, *J. Biomed. Sci.*, 2017, **24**, 21.

679 72 F. Moncalvo, M. I. Martinez Espinoza and F. Cellesi, *Front. Bioeng. Biotechnol.*, 2020, **8**, 89.

680 73 Global Therapeutic Proteins Market Report 2020: Market was Valued at $93.14 Billion in 2018
681 and is Expected to Grow to $172.87 Billion through 2022 - ResearchAndMarkets.com |
682 Business Wire, https://www.businesswire.com/news/home/20191223005228/en/Global-
683 Therapeutic-Proteins-Market-Report-2020-Market-was-Valued-at-93.14-Billion-in-2018-and-
684 is-Expected-to-Grow-to-172.87-Billion-through-2022---ResearchAndMarkets.com, (accessed
685 3 November 2020).

686 74 E. Thomaidou and Y. Ramot, *Dermatol. Ther.*, 2019, **32**, e12817.

687 75 M. Minekus, M. Alminger, P. Alvito, S. Ballance, T. Bohn, C. Bourlieu, F. Carriere, R. Boutrou,
688 M. Corredig, D. Dupont, C. Dufour, L. Egger, M. Golding, S. Karakaya, B. Kirkhus, S. Le
689 Feunteun, U. Lesmes, A. Macierzanka, A. Mackie, S. Marze, D. J. McClements, O. Menard, I.

690 Recio, C. N. Santos, R. P. Singh, G. E. Vegarud, M. S. J. Wickham, W. Weitschies and A.
691 Brodkorb, *Food Funct.*, 2014, **5**, 1113–1124.

692 76 C. Philip Carsten, F. Mathias, S. Lasse, Y. Mingshi, N. Hanne Mørck and M. Huiling, *Curr.*
693 *Pharm. Des.*, 2015, **21**, 2611–2628.

694 77 C. Pentafragka, M. Vertzoni, M. Symillides, K. Goumas and C. Reppas, *Eur. J. Pharm. Sci.*, 2020,
695 **149**, 105351.

696 78 H. Vrbanac, J. Trontelj, S. Berglez, B. Petek, J. Opara, R. Jereb, D. Krajcar and I. Legen, *Eur.*
697 *J. Pharm. Biopharm.*, 2020, **149**, 113–120.

698 79 A. R. Mackie, A. N. Round, N. M. Rigby and A. Macierzanka, *Food Dig.*, 2012, **3**, 8–15.

699 80 S. Cornick, A. Tawiah and K. Chadee, *Tissue Barriers*, 2015, **3**, e982426.

700 81 C. T. Nordgård and K. I. Draget, *Adv. Drug Deliv. Rev.*, 2018, **124**, 175–183.

701 82 O. L. Lewis, J. P. Keener and A. L. Fogelson, *Am. J. Physiol. Liver Physiol.*, 2017, **313**, G599–
702 G612.

703 83 A. R. Mackie, A. Macierzanka, K. Aarak, N. M. Rigby, R. Parker, G. A. Channell, S. E. Harding
704 and B. H. Bajka, *Food Hydrocoll.*, 2016, **52**, 749–755.

705 84 S. Zhou, H. Deng, Y. Zhang, P. Wu, B. He, W. Dai, H. Zhang, Q. Zhang, R. Zhao and X.
706 Wang, *Mol. Pharm.*, 2020, **17**, 239–250.

707 85 D. J. Drucker, *Nat. Rev. Drug Discov.*, 2020, **19**, 277–289.

708 86 J. Linnankoski, J. Mäkelä, J. Palmgren, T. Mauriala, C. Vedin, A. Ungell, L. Lazorova, P.
709 Artursson, A. Urtti and M. Yliperttula, *J. Pharm. Sci.*, 2010, **99**, 2166–2175.

710 87 R. Farré, M. Fiorani, S. Abdu Rahiman and G. Matteoli, *Nutrients*, 2020, 12, 1185.

711 88 Q. Xu, H. Hong, J. Wu and X. Yan, *Trends Food Sci. Technol.*, 2019, **86**, 399–411.

712 89 L. W. Peterson and D. Artis, *Nat. Rev. Immunol.*, 2014, **14**, 141–153.

713 90 J. M. Allaire, S. M. Crowley, H. T. Law, S.-Y. Chang, H.-J. Ko and B. A. Vallance, *Trends*
714 *Immunol.*, 2018, **39**, 677–696.

715 91 T. Murakami and M. Takano, *Expert Opin. Drug Metab. Toxicol.*, 2008, **4**, 923–939.

716 92 H. He, Y. Lu, J. Qi, Q. Zhu, Z. Chen and W. Wu, *Acta Pharm. Sin. B*, 2019, **9**, 36–48.

717 93 C. Y. Wong, H. Al-Salami and C. R. Dass, *Int. J. Pharm.*, 2018, **549**, 201–217.

718 94 E. Ajorlou and A. Y. Khosroushahi, *Cancer Chemother. Pharmacol.*, 2017, **79**, 251–265.

719 95 U. Bulbake, S. Doppalapudi, N. Kommineni and W. Khan, *Pharmaceutics*, 2017, **9**, 12.

720 96 T. Nakamura and H. Harashima, *Adv. Drug Deliv. Rev.*, 2020, **167**, 78-88.

721 97 S. Shah, V. Dhawan, R. Holm, M. S. Nagarsenker and Y. Perrie, *Adv. Drug Deliv. Rev.*, 2020,
722 **154-155**, 102-122.

723 98 I. Vhora, D. Bardoliwala, S. R. Ranamalla and A. Javia, in *Novel Drug Delivery Technologies:*
724 *Innovative Strategies for Drug Re-positioning*, Springer Singapore, 2020, pp. 183–260.

725 99 Y. Wang and D. W. Grainger, *Adv. Drug Deliv. Rev.*, 2019, **151–152**, 56–71.

726 100 J. Chen, *Food Hydrocoll.*, 2009, **23**, 1–25.

727 101 S. Hu, M. Niu, F. Hu, Y. Lu, J. Qi, Z. Yin and W. Wu, *Int. J. Pharm.*, 2013, **441**, 693–700.

728 102 W. Liu, A. Ye, F. Han and J. Han, *Adv. Colloid Interface Sci.*, 2019, **263**, 52–67.

729 103 D. J. McClements and Y. Li, *Food Funct.*, 2010, **1**, 32–59.

730 104 J. N. Tian, B. Q. Ge, Y. F. Shen, Y. X. He and Z. X. Chen, *J. Agric. Food Chem.*, 2016, **64**, 1977–
731 1988.

732 105 O. Chavoshian, M. Arabsalmani, M. R. Jaafari, A. Khamesipour, A. Abbasi, Z. Saberi and A.
733 Badiee, *Curr. Drug Deliv.*, 2020, **17**, 806-814.

734 106 W. Y. Chen, L. Y. Chen, C. M. Ou, C. C. Huang, S. C. Wei and H. T. Chang, *Anal. Chem.*, 2013,
735 **85**, 8834–8840.

736 107 J. Davidsen, C. Vermehren, S. Frokjaer, O. G. Mouritsen and K. Jørgensen, in *International*
737 *Journal of Pharmaceutics*, Elsevier, 2001, vol. 214, pp. 67–69.

738 108 M. N. Holme, M. H. Rashid, M. R. Thomas, H. M. G. Barriga, K. L. Herpoldt, R. K. Heenan,
739 C. A. Dreiss, J. L. Bañuelos, H. N. Xie, I. Yarovsky and M. M. Stevens, *ACS Cent. Sci.*, 2018, **4**,
740 1023–1030.

741 109 J. L. Nieva, F. M. Goñi and A. Alonso, *Biochemistry*, 1989, **28**, 7364–7367.

742 110 W. Liu, J. Lu, A. Ye, Q. Xu, M. Tian, Y. Kong, F. Wei and J. Han, *Food Chem.*, 2018, **258**, 366–
743 373.

744 111 E. Mateos-Diaz, J. C. Bakala N'Goma, D. Byrne, S. Robert, F. Carrière and H. Gaussier, *Chem.*
745 *Phys. Lipids*, 2018, **211**, 52–65.

746 112 A. A. Jovanović, B. D. Balanč, V. B. Djordjević, A. Ota, M. Skrt, K. P. Šavikin, B. M. Bugarski,
747 V. A. Nedović and N. P. Ulrih, *Colloids Surfaces B Biointerfaces*, 2019, **183**, 110422.

748 113 E. Khanniri, N. Bagheripoor-Fallah, S. Sohrabvandi, A. M. Mortazavian, K. Khosravi-Darani
749 and R. Mohammad, *Crit. Rev. Food Sci. Nutr.*, 2016, **56**, 484–493.

750 114 W. Liu, A. Ye, W. Liu, C. Liu, J. Han and H. Singh, *Food Chem.*, 2015, **175**, 16–24.

751 115 R. N. Rowland and J. F. Woodley, *Biochim. Biophys. Acta (BBA)/Lipids Lipid Metab.*, 1980, **620**,
752 400–409.

753 116 R. Schubert, H. Jaroni, J. Schoelmerich and K. H. Schmidt, *Digestion*, 1983, **28**, 181–190.

754 117 B. Zhang, A. Xue, C. Zhang, J. Yu, W. Chen and D. Sun, *Pharmazie*, 2016, **71**, 320–326.

755 118 P. A. Dawson and S. J. Karpen, *J. Lipid Res.*, 2015, **56**, 1085–1099.

756 119 L. Luo, Q. Chen, N. Wei, Y. Liu, H. He, Y. Zhang, T. Yin, J. Gou and X. Tang, *Int. J. Pharm.*,
757 2019, **566**, 371–382.

758 120 H. Abumanhal-Masarweh, D. da Silva, M. Poley, A. Zinger, E. Goldman, N. Krinsky, R.
759 Kleiner, G. Shenbach, J. E. Schroeder, J. Shklover, J. Shainsky-Roitman and A. Schroeder, *J.*
760 *Control. Release*, 2019, **307**, 331–341.

761 121 S. Kaddah, N. Khreich, F. Kaddah, C. Charcosset and H. Greige-Gerges, *Food Chem. Toxicol.*,
762 2018, **113**, 40–48.

763 122 A. Hussain, S. Singh, D. Sharma, T. Webster, K. Shafaat and A. Faruk, *Int. J. Nanomedicine*, 2017,
764 **Volume 12**, 5087–5108.

765 123 A. K. Verma, S. Sharma, P. Gupta, D. Singodia, S. Kansal, V. Sharma and P. R. Mishra, *Mol.*
766 *Pharm.*, 2016, **13**, 2531–2542.

767 124 W. Chen, F. Cheng, C. J. Swing, S. Xia and X. Zhang, *Chem. Phys. Lipids*, 2019, **223**, 104790.

768 125 S. Müller, K. Gruhle, A. Meister, G. Hause and S. Drescher, *Pharmaceutics*, 2019, **11**, 646.

769 126 P. Uhl, S. Pantze, P. Storck, J. Parmentier, D. Witzigmann, G. Hofhaus, J. Huwyler, W. Mier

770 and G. Fricker, *Eur. J. Pharm. Sci.*, 2017, **108**, 111–118.

771 127 S. Menina, J. Eisenbeis, M. A. M. Kamal, M. Koch, M. Bischoff, S. Gordon, B. Loretz and C.
772 Lehr, *Adv. Healthc. Mater.*, 2019, **8**, 1900564.

773 128 D. Vergara, O. López, M. Bustamante and C. Shene, *Food Chem.*, 2020, **321**, 126717.

774 129 M. Niu, Y. Tan, P. Guan, L. Hovgaard, Y. Lu, J. Qi, R. Lian, X. Li and W. Wu, *Int. J. Pharm.*,
775 2014, **460**, 119–130.

776 130 G. Yang, F. Wu, M. Chen, J. Jin, R. Wang and Y. Yuan, *Int. J. Nanomedicine*, 2019, **14**, 2267–
777 2280.

778 131 M. Conacher, J. Alexander and J. M. Brewer, *Vaccine*, 2001, **19**, 2965–2974.

779 132 Y. S. R. Elnaggar, S. Omran, H. A. Hazzah and O. Y. Abdallah, *Int. J. Pharm.*, 2019, **564**, 410–
780 425.

781 133 Z. S. Rizwanullah, M. Rizwanullah, S. R. Mir and S. Amin, *J. Drug Deliv. Sci. Technol.*, 2020, **57**,
782 101634.

783 134 F. Deng and Y. H. Bae, *J. Control. Release*, 2020, **327**, 100–116.

784 135 T. X. Nguyen, L. Huang, M. Gauthier, G. Yang and Q. Wang, *Nanomedicine*, 2016, **11**, 1169–
785 1185.

786 136 M. Manconi, C. Caddeo, M. L. Manca and A. M. Fadda, *Nanomedicine*, 2020, **15**, 1795–1803.

787 137 H. Ahn and J. H. Park, *Biomater. Res.*, 2016, 20, 1–6.

788 138 S. Chen, F. Guo, T. Deng, S. Zhu, W. Liu, H. Zhong, H. Yu, R. Luo and Z. Deng, *AAPS*
789 *PharmSciTech*, 2017, **18**, 1277–1287.

790 139 W. F. Lai, W. T. Wong and A. L. Rogach, *ACS Appl. Mater. Interfaces*, 2020, **12**, 43341–43351.

791 140 A. Sarkar and A. R. Mackie, *Curr. Opin. Colloid Interface Sci.*, 2020, **48**, 40–52.

792 141 P. R. Karn, Z. Vanić, I. Pepić and N. Škalko-Basnet, *Drug Dev. Ind. Pharm.*, 2011, **37**, 482–488.

793 142 C. Costa, Z. Liu, J. P. Martins, A. Correia, P. Figueiredo, A. Rahikkala, W. Li, J. Seitsonen, J.
794 Ruokolainen, S. P. Hirvonen, A. Aguiar-Ricardo, M. L. Corvo and H. A. Santos, *Biomater. Sci.*,
795 2020, **8**, 3270–3277.

796 143 A. Catalan-Latorre, M. Ravaghi, M. L. Manca, C. Caddeo, F. Marongiu, G. Ennas, E.
797 Escribano-Ferrer, J. E. Peris, O. Diez-Sales, A. M. Fadda and M. Manconi, *Eur. J. Pharm.*
798 *Biopharm.*, 2016, **107**, 49–55.

799 144 M. Daeihamed, S. Dadashzadeh, A. Haeri and M. Faghih Akhlaghi, *Curr. Drug. Deliv.*, 2017, **14**,
800 289-303.

801 145 J. H. Kim, D. H. Shin and J. S. Kim, *Arch. Pharm. Res.*, 2018, **41**, 765–775.

802 146 E. Martí Coma-Cros, A. Biosca, E. Lantero, M. Manca, C. Caddeo, L. Gutiérrez, M. Ramírez,
803 L. Borgheti-Cardoso, M. Manconi and X. Fernàndez-Busquets, *Int. J. Mol. Sci.*, 2018, **19**, 1361.

804 147 V. De Leo, S. Di Gioia, F. Milano, P. Fini, R. Comparelli, E. Mancini, A. Agostiano, M. Conese
805 and L. Catucci, *Coatings*, 2020, **10**, 114.

806 148 C. Caddeo, M. Gabriele, X. Fernàndez-Busquets, D. Valenti, A. M. Fadda, L. Pucci and M.
807 Manconi, *Int. J. Pharm.*, 2019, **565**, 64–69.

808 149 S. Sharma, K. Jyoti, R. Sinha, A. Katyal, U. K. Jain and J. Madan, *Mater. Sci. Eng. C*, 2016, **67**,
809 378–385.

810 150 M. Zhao, S. H. Lee, J. G. Song, H. Y. Kim and H. K. Han, *Int. J. Pharm.*, 2018, **544**, 14–20.

811 151 A. I. Gomaa, C. Martinent, R. Hammami, I. Fliss and M. Subirade, *Front. Chem.*, 2017, **5**, 103.

812 152 V. J. Mohanraj, T. J. Barnes and C. A. Prestidge, *Int. J. Pharm.*, 2010, **392**, 285–293.

813 153 M. Gaber, W. Medhat, M. Hany, N. Saher, J. Y. Fang and A. Elzoghby, *J. Control. Release*, 2017,
814 254, 75–91.

815 154 L. M. Ensign, R. Cone and J. Hanes, *Adv. Drug Deliv. Rev.*, 2012, **64**, 557–570.

816 155 W. Liu, Y. Hou, Y. Jin, Y. Wang, X. Xu and J. Han, *Trends Food Sci. Technol.*, 2020, **104**, 177–
817 189.

818 156 J. E. Vela Ramirez, L. A. Sharpe and N. A. Peppas, *Adv. Drug Deliv. Rev.*, 2017, **114**, 116–131.

819 157 J.-P. Kraehenbuhl and M. R. Neutra, *Annu. Rev. Cell Dev. Biol.*, 2000, **16**, 301–332.

820 158 W. Wu, Y. Lu and J. Qi, *Ther. Deliv.*, 2015, **6**, 1239–1241.

821 159 M. A. Lopes, B. A. Abrahim, L. M. Cabral, C. R. Rodrigues, R. M. F. Seiça, F. J. de Baptista
822 Veiga and A. J. Ribeiro, *Nanomedicine Nanotechnology, Biol. Med.*, 2014, **10**, 1139–1151.

823 160 H. Takeuchi, Y. Matsui, H. Yamamoto and Y. Kawashima, *J. Control. Release*, 2003, **86**, 235–
824 242.

825 161 Y. Shao, L. Yang and H. K. Han, *Eur. J. Pharm. Biopharm.*, 2015, **89**, 339–346.

826 162 A. Huang, Z. Su, S. Li, M. Sun, Y. Xiao, Q. Ping and Y. Deng, *Drug Deliv.*, 2014, **21**, 388–396.

827 163 Q. Zhu, T. Guo, D. Xia, X. Li, C. Zhu, H. Li, D. Ouyang, J. Zhang and Y. Gan, *J. Pharm.*
828 *Pharmacol.*, 2013, **65**, 1107–1117.

829 164 E. Yamazoe, J. Y. Fang and K. Tahara, *Int. J. Pharm.*, 2021, **593**, 120148.1

830 165 J. Parmentier, F. J. Hartmann and G. Fricker, *Eur. J. Pharm. Biopharm.*, 2010, **76**, 394–403.

831 166 Y.-B. Huang, M.-J. Tsai, P.-C. Wu, Y.-H. Tsai, Y.-H. Wu and J.-Y. Fang, *J. Drug Target.*, 2011,
832 **19**, 709–718.

833 167 J. Parmentier, G. Hofhaus, S. Thomas, L. C. Cuesta, F. Gropp, R. Schröder, K. Hartmann and
834 G. Fricker, *J. Pharm. Sci.*, 2014, **103**, 3985–3993.

835

836 168 Y. Li, B. Yang and X. Zhang, *Int. J. Pharm.*, 2019, **568**, 118508.

837 169 A. B. Shreya, S. Y. Raut, R. S. Managuli, N. Udupa and S. Mutalik, *AAPS PharmSciTech*, 2019,
838 **20**, 1–12.

839 170 H. He, Y. Lu, J. Qi, W. Zhao, X. Dong and W. Wu, *Acta Pharm. Sin. B*, 2018, **8**, 97–105.

840 171 C. C. Quianzon and I. Cheikh, *J. Community Hosp. Intern. Med. Perspect.*, 2012, **2**, 18701.

841 172 J. Habault and J.-L. Poyet, *Molecules*, 2019, **24**, 927.

842 173 S. M. Parizadeh, R. Jafarzadeh-Esfehani, M. Ghandehari, A. Rezaei-Kalat, S. M. R. Parizadeh,
843 A. Javanbakht, S. M. Hassanian, G. A. Ferns, M. Khazaei and A. Avan, *Curr. Drug Targets*, 2019,
844 **20**, 1486–1495.

845 174 D. Sabatino, *J. Med. Chem.*, 2020, **63**, 14184-14196.

846 175 L. Bezu, O. Kepp, G. Cerrato, J. Pol, J. Fucikova, R. Spisek, L. Zitvogel, G. Kroemer and L.
847 Galluzzi, *Oncoimmunology*, 2018, **7**. e1511506.

848 176 K. Herline, E. Drummond and T. Wisniewski, *Expert Rev. Vaccines*, 2018, **17**, 707–721.

849 177 FDA approves first oral GLP-1 treatment for type 2 diabetes | FDA,
850 https://www.fda.gov/news-events/press-announcements/fda-approves-first-oral-glp-1-
851 treatment-type-2-diabetes, (accessed 26 April 2021).

852 178 Study to Evaluate the Efficacy and Safety of ORMD-0801 in Subjects With Type 2 Diabetes
853 Mellitus, https://clinicaltrials.gov/ct2/show/NCT04606576, (accessed 26 April 2021).

854 179 Long Term Comparative Effectiveness of Once Weekly Semaglutide Versus Standard of Care
855 in a Real World Adult US Population With Type 2 Diabetes - a Randomized Pragmatic Trial,
856 https://clinicaltrials.gov/ct2/show/NCT03596450, (accessed 26 April 2021).

857 180 A Study to Evaluate Oral Salmon Calcitonin in the Treatment of Osteoporosis in
858 Postmenopausal Women Taking Calcium and Vitamin D,
859 https://clinicaltrials.gov/ct2/show/NCT00525798, (accessed 26 April 2021).

860 181 D. J. Brayden, T. A. Hill, D. P. Fairlie, S. Maher and R. J. Mrsny, *Adv. Drug Deliv. Rev.*, 2020,
861 157, 2–36.

862 182 S. Franzé, F. Selmin, E. Samaritani, P. Minghetti and F. Cilurzo, *Pharmaceutics*, 2018, **10**, 139.

863 183 G. M. Jensen and D. F. Hodgson, *Adv. Drug Deliv. Rev.*, 2020, 154-155, 2-12.

864 184 S. Feng, Y. Sun, P. Wang, P. Sun, C. Ritzoulis and P. Shao, *Int. J. Food Sci. Technol.*, 2020, **55**,

865 1872–1880.

866 185 K. Tai, M. Rappolt, L. Mao, Y. Gao, X. Li and F. Yuan, *Food Hydrocoll.*, 2020, **99**, 105355.

867 186 P. Trucillo, R. Campardelli, M. Scognamiglio and E. Reverchon, *J. CO2 Util.*, 2019, **32**, 119–

868 127.

869 187 Z. Jiao, X. Wang, S. Han, X. Zha and J. Xia, *J. Drug Deliv. Sci. Technol.*, 2019, **51**, 1–6.

870 188 Y. Liu and X. An, *Colloids Surfaces B Biointerfaces*, 2019, **178**, 238–244.

871 189 P. Grad, L. Gedda and K. Edwards, *J. Colloid Interface Sci.*, 2020, **578**, 281–289.

872 190 G. Wang, B. Wu, Q. Li, S. Chen, X. Jin, Y. Liu, Z. Zhou, Y. Shen and P. Huang, *Small*, 2020,

873 **16**, 2004172.

874 191 Q. Shen, Y. Shen, F. Jin, Y. Du and X. Ying, *J. Liposome Res.*, 2020, **30**, 12–20.

875 192 A. Jash, T. Hatami and S. S. H. Rizvi, *J. Supercrit. Fluids*, 2020, **158**, 104720.

876 193 T. Ghorbanzade, S. M. Jafari, S. Akhavan and R. Hadavi, *Food Chem.*, 2017, **216**, 146–152.

877 194 T. I. Shalaby and W. M. El-Refaie, *J. Pharm. Sci.*, 2018, **107**, 2136–2143.

878 195 M. Gottesmann, F. M. Goycoolea, T. Steinbacher, T. Menogni and A. Hensel, *Appl. Microbiol.*
879 *Biotechnol.*, 2020, **104**, 5943–5957.

880 196 D. Chen, D. Xia, X. Li, Q. Zhu, H. Yu, C. Zhu and Y. Gan, *Int. J. Pharm.*, 2013, **449**, 1–9.

881 197 J. R. Yazdi, M. Tafaghodi, K. Sadri, M. Mashreghi, A. R. Nikpoor, S. Nikoofal-Sahlabadi, J.
882 Chamani, R. Vakili, S. A. Moosavian and M. R. Jaafari, *Colloids Surfaces B Biointerfaces*, 2020, **194**,
883 111203.

884 198 A. Wang, T. Yang, W. Fan, Y. Yang, Q. Zhu, S. Guo, C. Zhu, Y. Yuan, T. Zhang and Y. Gan,
885 *Adv. Healthc. Mater.*, 2019, **8**, 1801123.

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890 **Table 1.** Properties of liposomes produced using various approaches

Synthesis method	Organic solvent	Liposomal wall material	Size	Reference
Vent-RESS	No	MFGM PL	533 nm	42
Modified TFH	Yes	PC and mPEG	121-148 nm	20
Micro-fluidics	Yes	DPPC and HDA	200 nm	57
Ether injection	Yes	PC and LMP	110-160 nm	184
TFH combined with HPH	Yes	PC, PE, PS, and chitosan	190-1729 nm	185
SuperLip	Yes	PC	139 nm	186
RESS	Yes	PC	270 nm	187
TE-SC-CO_2	Yes	PC	140 nm	188
Modified TFH	Yes	HEPC and PEG	51 nm	189
Transmembrane ammonium sulfate gradient	Yes	HSPC and DOPE-GSH	65 nm	190

Modified TFH	Yes	PC and HP-β-CD	80-90 nm	191

891 Abbreviations: TFH, thin film hydration; Vent-RESS, venturi-based rapid expansion of supercritical
892 solutions; MFGM PL, milk fat globule membrane phospholipids; mPEG, methoxy polyethylene
893 glycol distearoyl ethanol-amine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS,
894 phosphatidylserine; DPPC, dipalmitoylphosphatidylcholine; HDA, hexadecylamine; EL, egg yolk
895 lecithin; AMS, 3-(4-Butyl-1H-1,2,3-triazolyl)-5β-cholan-24-oic acid, ampholytic switch; LMP, low
896 methoxyl pectin; HPH, high-pressure homogenization, SuperLip, supercritical assisted liposomes
897 formation; RESS, rapid expansion of supercritical solution process; TE-SC-CO_2, combined method
898 of thin film hydration and supercritical carbon dioxide technique; HEPC, hydrogenated egg
899 phosphatidylcholine, HSPC, hydrogenated soy phosphatidylcholine; DOPE-GSH, glutathione
900 modified 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine phospholipid; HP-β-CD, hydroxypropyl-β-
901 cyclodextrin.

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906 **Table 2.** Recent applications of liposomes in food, pharmaceutical, and cosmetic industries

Liposome synthesis method	Bioactive compounds		Liposome properties	Outcomes	Reference
	Hydrophobic	Hydrophilic			
TFH	Vitamin E	Vitamin C	- MLV - 0.5-100 μm	- Improved antioxidant activity and stability in orange juice	55
Vent-RESS	Vitamin E	Vitamin C, iron	- ULV and MLV - 580-700 nm	- Multivitamin delivery	51,192
Modified TFH	—	Betanin	- Mono-dispersed - 36 nm	- Increased oxidative stability of betanin over 60 days of storage	56
Micro-fluidics	Curcumin	Catechin	- Mono-dispersed - 200 nm	- Enhanced bioavailability	57
Heating, homogenization, and sonication	Fish oil	—	- ULV or MLV - 300-500 nm	- Increased stability of fish oil in yogurt	193
Modified TFH	Exemestane	—	- SUV - 121-148 nm	- Increased stability in gastric conditions	20
Modified TFH	Asenapine maleate	—	- SUV - 98-110 nm	- Enhanced bioavailability	22

Modified TFH	—	Temozolo-mide	- ULV - 118-145 nm	- Increased therapeutic efficacy	59
TFH + extrusion	—	Irinotecan	- Mono-dispersed - 86-168 nm	- Boost cellular uptake	60
Modified TFH	Apigenin	5-Fluorouracil	- LUV - 93-105 nm	- Increased bioavailability and therapeutic efficacy	61
Modified TFH	Folic acid	—	- ULV - 120-280 nm	- Enhanced transdermal delivery	64
Modified TFH	CDBA	L-ascorbic acid	- SUV - 143 nm	- Controlled release in sunscreens	65
REV	—	Proantho-cyanidin	- Mono-dispersed - 145 nm	- Controlled release on skin	66
Modified TFH	—	Anthocyanin	- Mono-dispersed - 123 nm	- Enhanced skin permeability	67
Modified TFH	Vitamin K_1 oxide	—	- Mono-dispersed - 127 nm	- Enhanced release and skin permeability	68

907 Abbreviations: TFH, thin film hydration; Vent-RESS, venturi-based rapid expansion of supercritical
908 solutions; MLV, multilamellar vesicle; ULV, unilamellar vesicle; SUV, small unilamellar vesicle;
909 LUV, large unilamellar vesicle; CDBA, 4-cholester-ocarbonyl-49 -(N, N'-diethylaminobutyloxy)
910 azobenzene; REV, reverse phase evaporation.

911 **Table 3.** Recent advances in increasing liposomal absorption in gastrointestinal (GI) tract

Synthesis method; encapsulated bioactive	Strategy to adapt liposomes for oral delivery	Outcomes	Properties (vesicle type, diameter, zeta potential, and micrograph)	Reference
TFH; recombinant human insulin, Humilin-N®	- DOTAP was used in bilayer for positive surface charge to facilitate higher insulin entrapment and increased residence time in the endothelial tract. - liposomes were further coated with mucoadhesive polymer chitosan.	- Insulin EE was 86 %. - In SGF and SIF, after 48 h of incubation, 19 and 73 % of loaded insulin was respectively released. - In ex vivo intestinal mucoadhesion test, chitosan coated cationic liposomes' tissue residence time was substantially higher. - A significant reduction in blood glucose level was observed within 1 h of oral administration in streptozotocin-induced diabetic mice; and the effect sustained for 8 h after administration.	ULV 439.0 nm 60.5 mV	194
TFH; amoxicillin, a penicillin antibiotic for local antibiotic therapy against Helicobacter pylori infection	- Liposomes were coated with pectin. - Pectin is mucoadhesive and also inhibits H. pylori recolonization and further infection by binding itself with H. pylori's outer membrane protein analogues (e.g. BabA, LPS). - Amoxicillin-loaded pectin-coated liposomes will be able to interact with the	- Amoxicillin EE of 83 % was observed In in vitro study 85 % of the total drug was released within 1 h. - CLSMs demonstrated site-specific binding between pectin coated liposomes and outer layer of H. pylori. Furthermore, mucoadhesive properties of pectin due to electrostatic interaction with the negatively charged mucins facilitated anchoring of liposomes with the stomach mucin and effective penetration into the	NA 517 nm -26.9 mV	195

	mucin and bind itself with the H. pylori followed by release of the antibiotic drug and subsequent inhibition of the bacterial adhesion to the host cells.	mucus layer followed by release of encapsulated amoxicillin.		
TFH followed by membrane extrusion; cyclosporine A (CyA), a polypeptide based immune suppressive agent	- Liposomes were coated with Pluronic® F127 (PF127), a hydrophilic nonionic long chain polymer. - PF127 possess mucus penetrating properties. Coated liposomes would have better ability to reach intestinal epithelial cell. Thus, encapsulation of CyA in PF127 coated liposome would increase its oral bioavailability.	- CyA EE was 90 %. - For PF127 coated liposomes an enhanced stability in SGF was observed. - PF127 coated liposomes demonstrated a ubiquitous presence throughout the mucus layer side of the GI tract and no site-specific aggregation was observed. The coated liposomes also exhibited a higher turnover at the intestinal epithelial by penetration through the mucus layer as observed under CLSM and fluorophotometry.	NA 172.8 nm - 4.3 mV NA	196

TFH followed by extrusion; insulin regular U-100, Humulin R	- The liposomes were coated with PEG to protect them from acidic stomach. - PEGylate liposomes were decorated with a folic acid (FA) ligand, FA is a form of vitamin B9 and its receptors are present in intestinal epithelial. It was hypothesized that addition of FA ligands would increase liposomal absorption through receptor-mediated endocytosis	- Insulin EE was 60 %. - PEGylated liposomes showed enhanced stability in SGF and a significantly higher amount of insulin was released in SIF when compared to uncoated liposomes. Coated liposomes demonstrated 25 and 48 % release of loaded insulin within.1 of study in SGF and SIF, respectively. - Significantly higher uptake in caco-2 cell was observed for liposomes decorated with FA ligands. - FA decorated PEGylated liposomes demonstrated higher residence time in the stomach. - When orally administered in diabetic rats, folate decorated PEGylated liposomes resulted in an increased intestinal uptake of insulin as supported by higher serum insulin content. A relative insulin bioavailability of 19.1 % was observed.	NA 167 - 208 nm -6.8 - -4.9 mV	197
TFH; insulin	- DOTAP was incorporated in liposomal bilayer to increase cellular uptake. - Synthesized liposomes were coated with BSA and were named as protein corona liposomes (PcCLs) - It was hypothesized that neutrally charged and hydrophilic BSA layer would facilitate	- The PcCLs demonstrated an insulin EE and LE of 28.7 and 1.5 %, respectively. From PcCLs, 40 % of encapsulated insulin was released in 6 h while tested using an in vitro assay, mimicking intestinal environment (pH=6.8). The PcCLs demonstrated around 21-fold higher mucus penetrating ability compared to uncoated liposomes. The BSA layer was degraded during transit through the mucus layer and only exposed	NA 194.9 nm -10.9 mV	8

mucus penetration. During transit through the mucus, BSA layer on liposomes would be enzymatically degraded while protecting the encapsulated insulin. Thus, these PcCLs would be effective to increase insulin bioavailability by overcoming the mucus and intestinal epithelium barriers.	cationic liposomes interacted with the epithelial cell line. For PcCLs, a 3.2 times higher insulin uptake was observed compared to free insulin. When administered in type 1 diabetic rats, insulin incorporating PcCLs caused around 40 % reduction in glucose level and hypoglycemic effect sustained for 12 h. For PcCLs, an oral insulin bioavailability of 11.9 % was observed.

912

913 Abbreviations: TFH, thin film hydration; ULV, unilamellar vesicle; DOTAP , N-[1-(2, 3-dioleoyloxy)
914 propyl]-N,N,N-trimethylammonium methyl-sulfate; CLSM, confocal laser scanning microcopy; GI,
915 gastrointestinal; PEG, polyethylene glycol; EE, encapsulation efficiency; LE, loading efficiency; SGF,
916 simulated gastric fluid; SIF, simulated intestinal fluid; BSA, bovine serum albumin.

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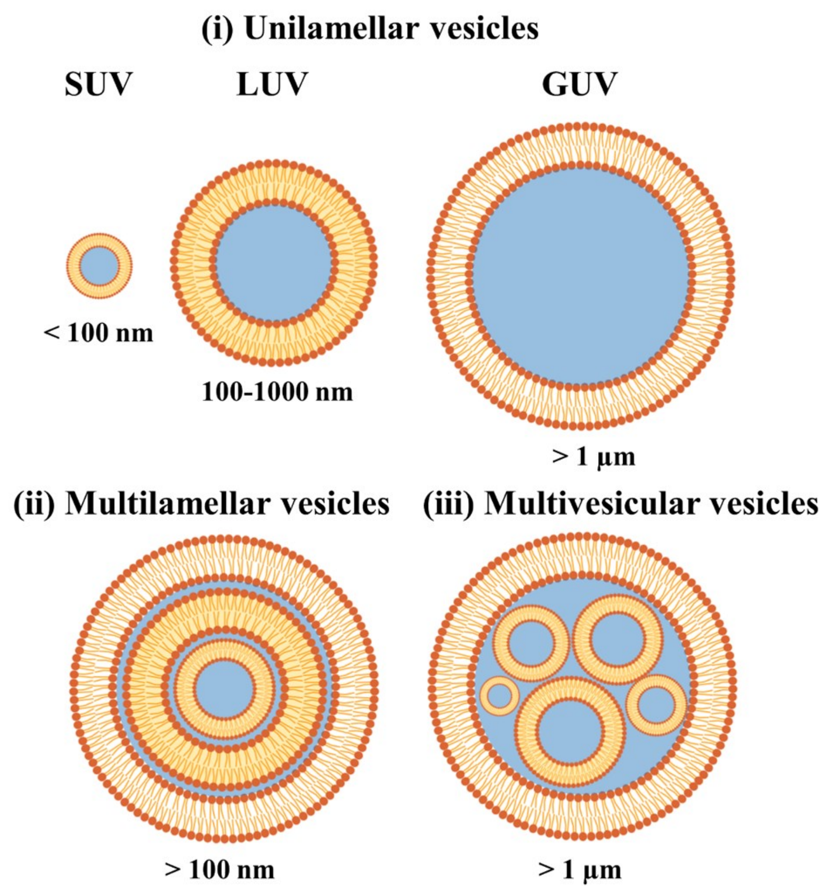

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920 **Fig. 1.** Classification of liposomes based on their lamellarity and size.

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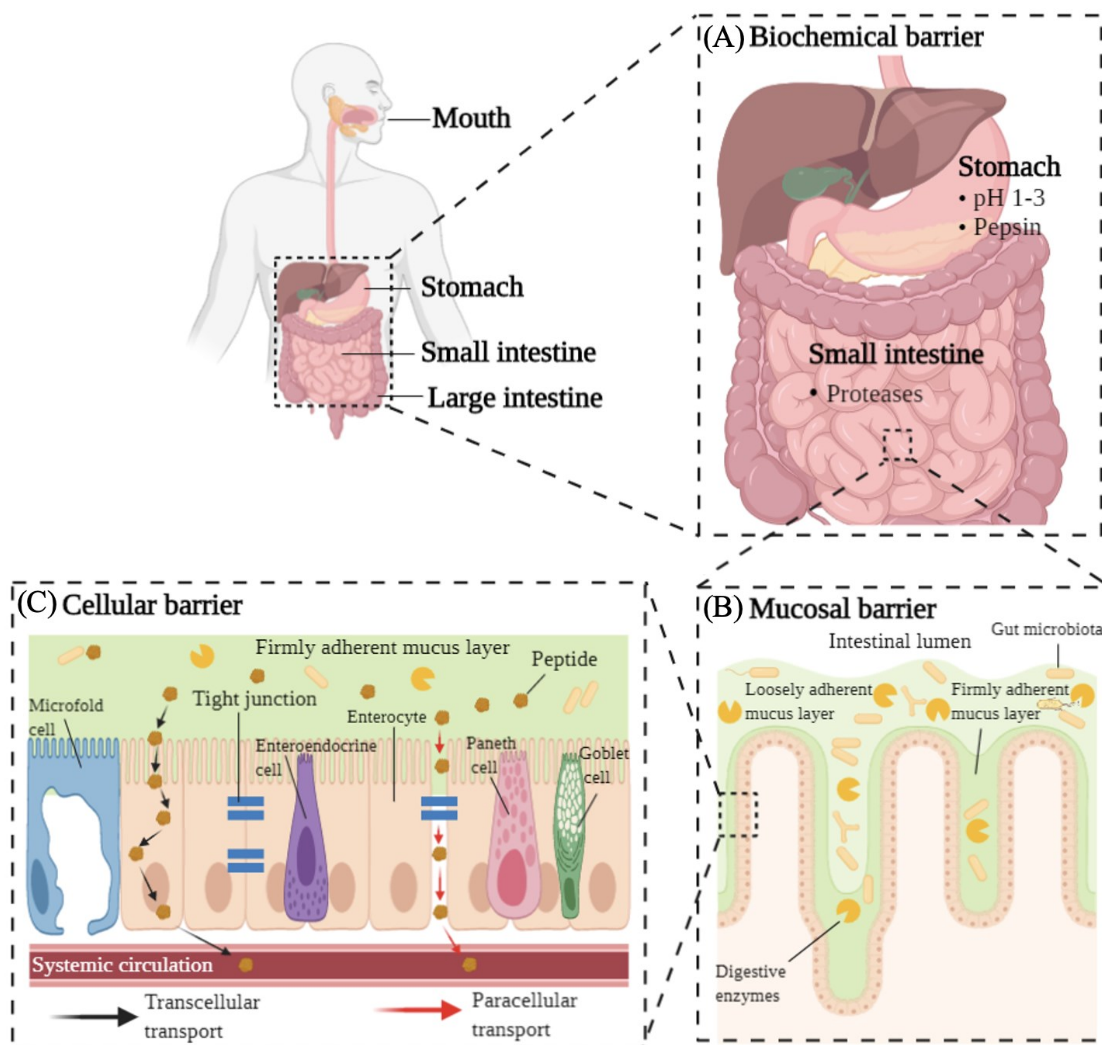

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924 **Fig. 2**. Physiological barriers during oral delivery of proteins and peptides. (A) biochemical barrier,
925 (B) mucus barrier, and (C) cellular barrier. Adapted from Brown et al. [24]

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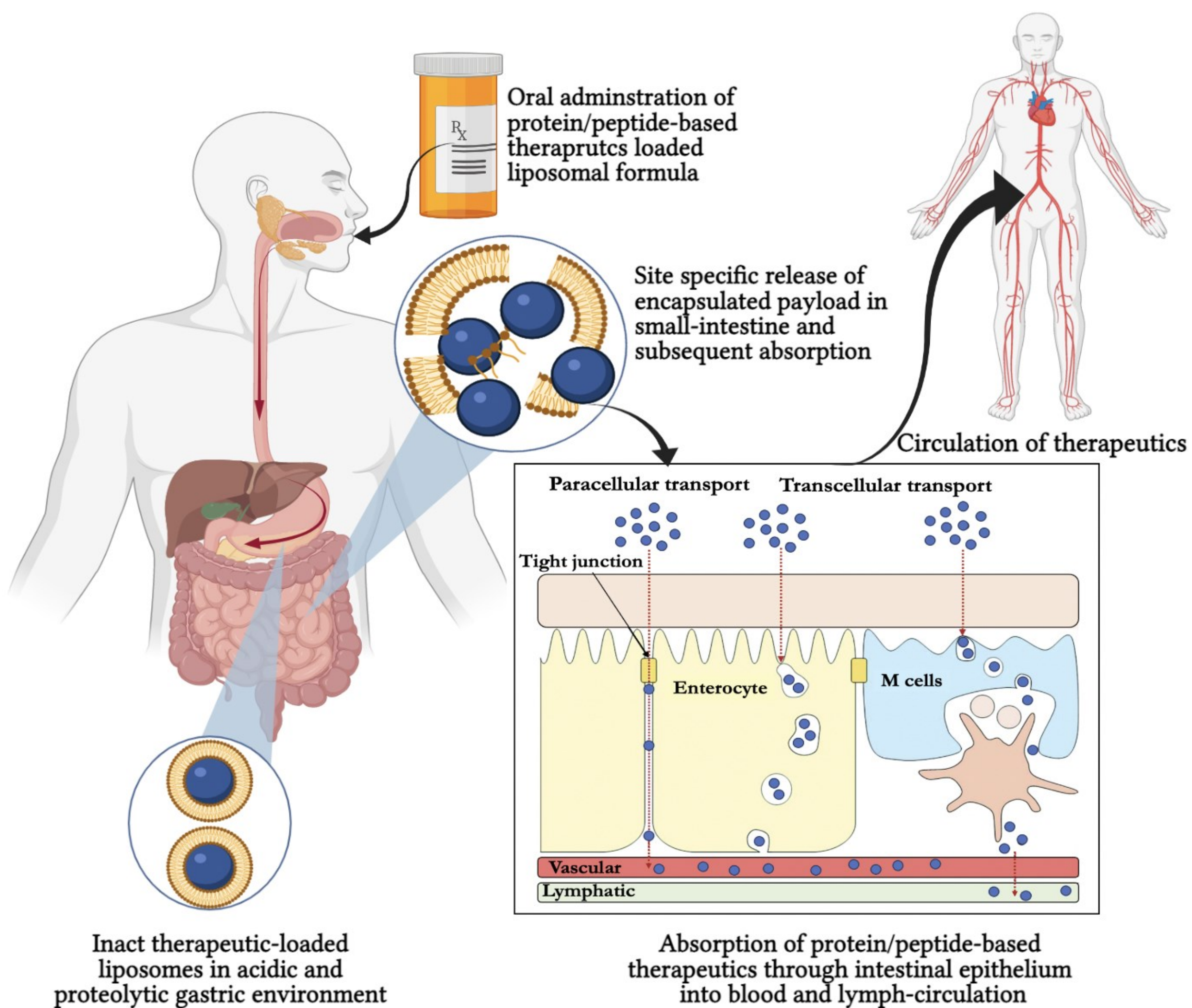

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929 **Fig. 3.** Schematic representation describing the fate of protein/peptide-based therapeutics loaded liposomes
930 from oral administration to site-specific intestinal delivery to circulation.

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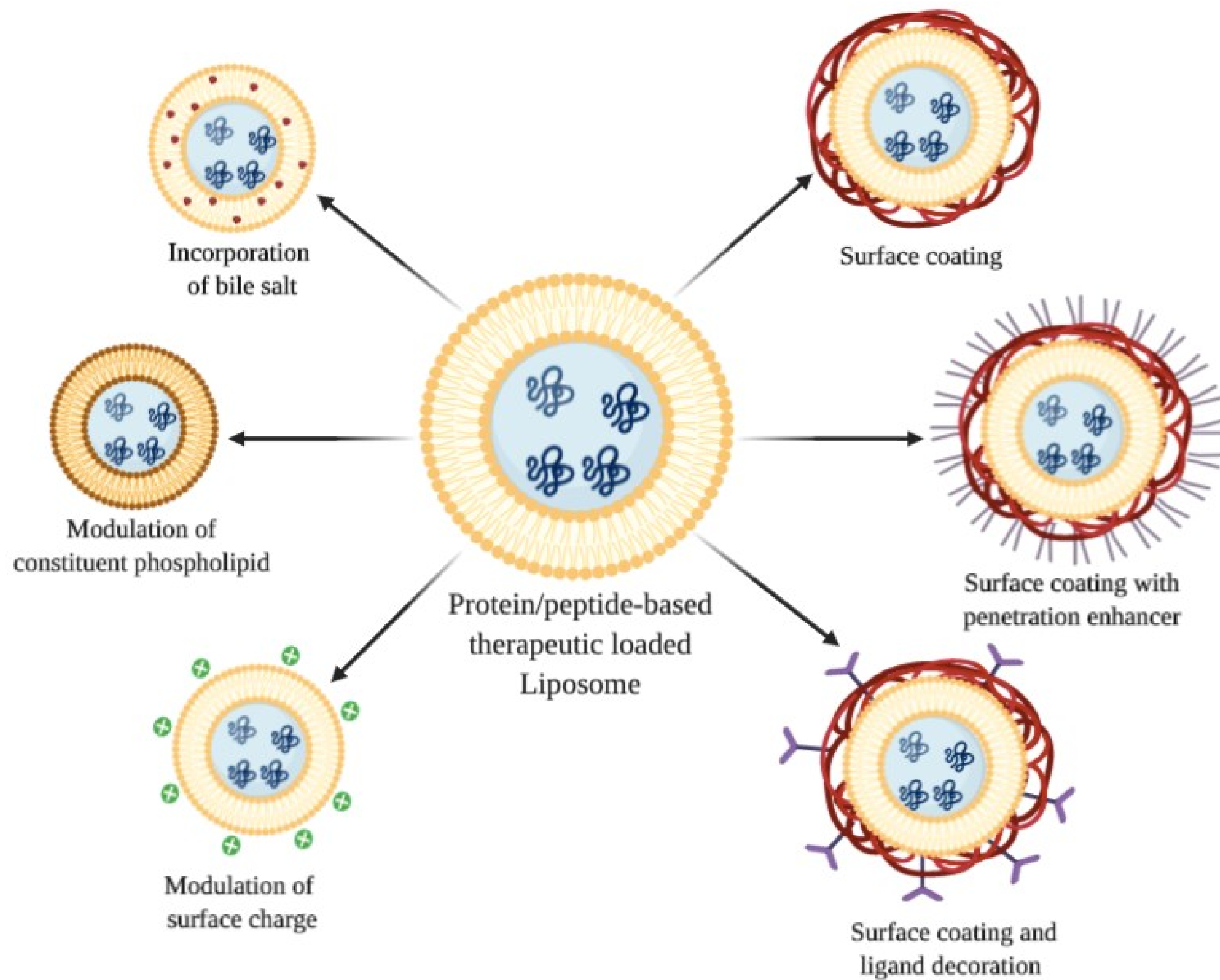

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935 **Fig. 4.** Strategies for adapting liposomes for oral protein/peptide-based therapeutics delivery.

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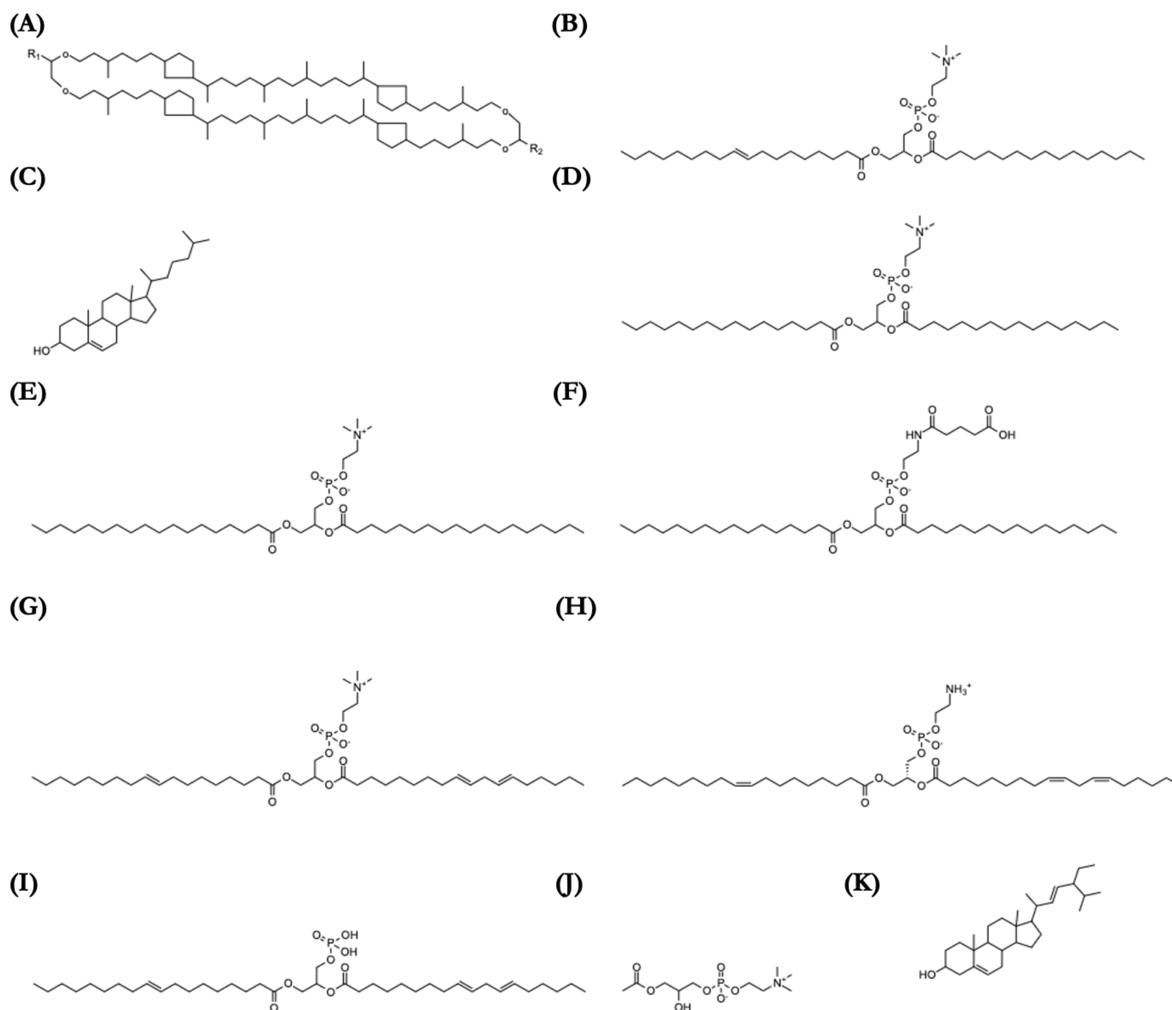
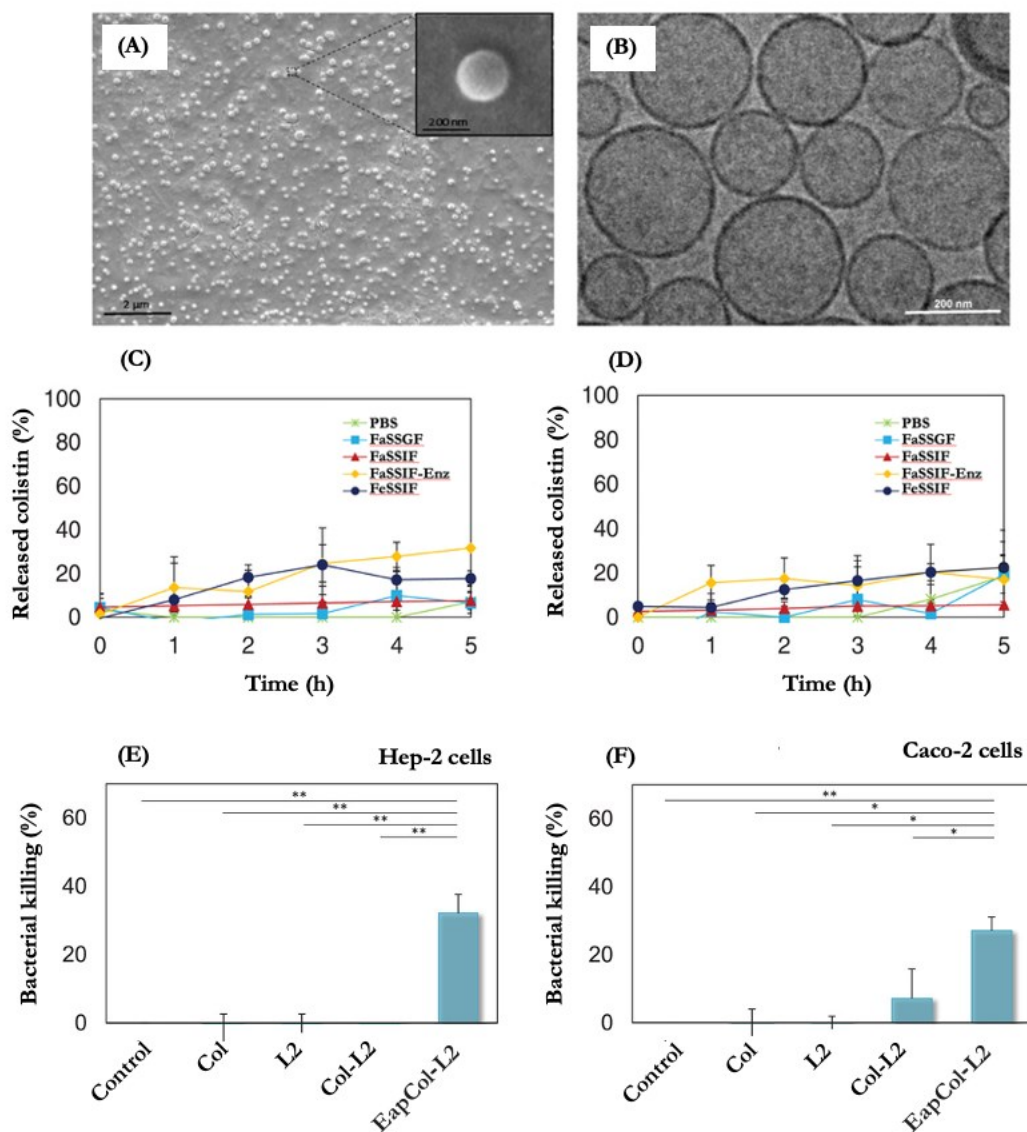


941

942 **Fig. 5.** Structure of phospholipids and modified-phospholipids that have been used to improve
943 liposomal stability in GI tract (A) glycerylcaldityltetraether lipid (GCTE), (B) lecithin, (C) cholesterol,
944 (D) 1,2-dipalmitoyl phosphatidylcholine (DPPC), (E)1,2-distearoyl-sn-glycero-3-phosphocholine
945 (DSPC), (F) 1,2-dipalmitoyl-sn-glycero-3-phospho-ethanolamine-N-Glutaryl (DPPE-GA), (G) 1-
946 oleoyl-2-linoleoyl-sn-glycero-3-phosphocholine, (H) 1-oleoyl-2-linoleoyl-sn-glycero-3-
947 phosphoethanolamine, (I) phosphatidic acid, (J) lysophosphatidylcholine, and (K) stigmasterol.



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949 **Fig. 6.** (A) Scanning electron microscopy and (B) cryo-transmission electron microscopy images of
950 L2 containing 4 mg mL-1 colistin. Cumulative release of colistin from (C) Col-L1 and (D) Col-L2 over
951 a duration of 5 h in different media (FaSSGF: fasted state simulated gastric fluid, FaSSIF: fasted state
952 simulated intestinal fluid, FaSSIF-Enz: fasted state simulated intestinal fluid containing enzymes,
953 FeSSIF: Fed state simulated intestinal fluid, PBS: phosphate buffer solution. Effect of treating
954 Salmonella enterica infected (E) HEp-2 cells and (F) Caco-2 cells with free colistin (Col), empty
955 liposomes (L2), nonfunctionalized colistin loaded liposomes (Col-L2), and extracellular adherence
956 protein-functionalized colistin loaded liposomes (EapCol-L2). A colistin concentration of 30 µg
957 mL−1 was used in all cases and extracellular adherence protein concentration of 20 µg mL−1 was
958 used for EapCol-L2. *$P < 0.05$ and **$P < 0.01$. Reproduced from Menina et al.[127]

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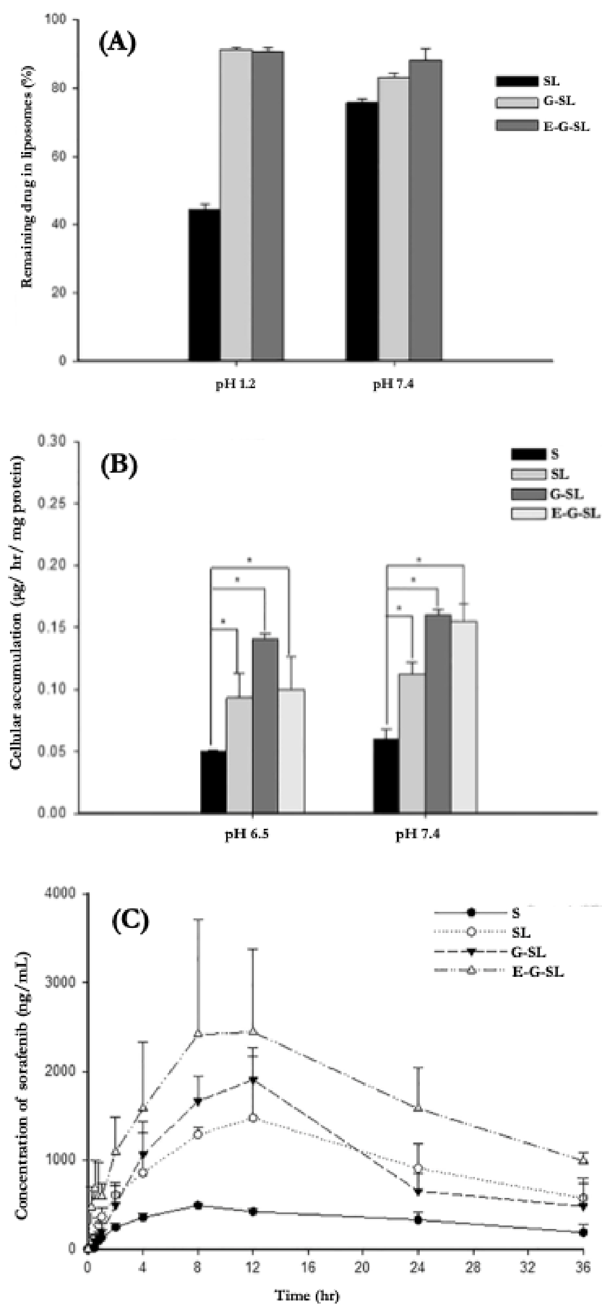

961

962 **Fig. 7**. (A) Stability of different liposomal formulations at pH mimicking gastric (pH 1.2) and intestinal
963 environment (pH 7.4) after respective incubation of 2 and 8 h. (Mean ± SD, n = 3). (B) Cellular uptake of
964 sorafenib by Caco-2 cells from different liposomal formulations at pH 6.5 and 7.4 (Mean ± SD, n = 3). *p < .05.
965 (C) Sorafenib's pharmacokinetic profile after oral administration to rats (Mean ± SD, n = 4–6) from different
966 liposomal formulations, dose was equivalent to 10 mg/kg of sorafenib. Reproduced with permission from
967 Zhao et al.[150]

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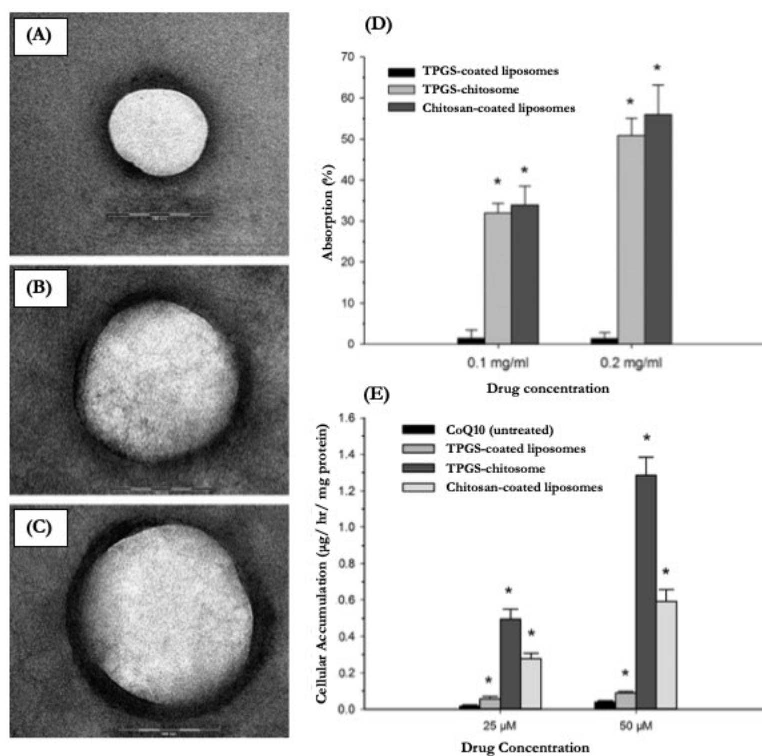

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972 **Fig. 8.** Transmission electron microscopy images of (A) TPGS-coated liposomes, (B) TPGS-
973 chitosome, (C) chitosan-coated liposomes (scale bar = 200 nm). (D) Muco-adhesiveness of TPGS-
974 coated liposomes, TPGS-chitosome, and chitosan-coated liposomes (mean ± SD, n = 3), $*p < 0.05$.
975 (E) Cellular accumulation of CoQ10 from different liposomal formulations compared to untreated
976 CoQ10 control (mean ± SD, n = 5), $*p < 0.05$. Reproduced with permission from Shao et al.[161]