
Article:
Can, A, Tyler, AII orcid.org/0000-0003-2116-1084 and Mackie, AR orcid.org/0000-0002-5681-0593 (2021) Potential use of bile salts in lipid self-assembled systems for the delivery of phytochemicals. Current Opinion in Colloid & Interface Science, 56. 101502. ISSN 1359-0294

https://doi.org/10.1016/j.cocis.2021.101502

Potential use of bile salts in lipid self-assembled systems for the delivery of phytochemicals

Aygul Can[1], Arwen I. I. Tyler[1*], Alan R. Mackie [1*]

[1]Food Colloids and Bioprocessing Group, School of Food Science and Nutrition, University of Leeds, Leeds, United Kingdom.

*Corresponding Authors: A.I.I.Tyler@leeds.ac.uk (Dr. A.I.I. Tyler), A.R.Mackie@leeds.ac.uk (Prof. A. R. Mackie);

ABSTRACT

Phytochemicals show many desirable functions such as anti-oxidant, anti-microbial, anti-inflammatory, anti-carcinogenic, anti-aging, etc. activities. These biological functions can only be seen when the compounds are delivered at sufficient concentration. However, the bioavailability of phytonutrients suffers from their limited absorption, transformation, and rapid clearance from the circulation. In this review, we survey recent studies on the use of bile salts in lipid self-assembled systems to enhance the bioavailability of phytochemicals and consequently, their biological activity. The effect of bile salts on lipid self-assembled systems in terms of morphology, encapsulation efficiency, stability, bioavailability, and biological activities are discussed.

Keywords: Bile Salts, Lipid self-assembled system, Phytochemicals, Bioavailability, Biological activity

1. INTRODUCTION

In recent years, consumers have shown increased interest in including functional foods in their diet. Of particular interest are functional products containing health-promoting phytochemicals such as phenolic compounds, terpenes, phytosterols, etc [1, 2]. There is a growing body of literature that shows the potential of phytochemicals in the food, beverage, and pharmaceutical industries. They show many desirable biological functions including antioxidant, anti-microbial, anti-inflammatory, and anti-carcinogenic activity but these biological functions depend on their bioavailability. Phytochemicals can be included in the diet however when the amount of food and beverages that need to be consumed to reach 1 g/person/day of resveratrol intake is calculated, a problem arises. It would require consuming 795 kg of red Merlot grapes, 2584 kg of white Riesling grapes, and 505-2762 L of red wine (Figure 1). Thus, the recommended intake of these compounds may be unachievable by drinking beverages or consuming foods [3] and research is already being undertaken to improve the bioavailability of phytochemicals that are already reported as quite low after oral administration [4].

The bioavailability of these phytochemicals can be low because of their limited bioaccessibility or absorption, or because of fast metabolism in the body. The bioaccessibility of phytochemicals can be low because of limited liberation, poor solubility in the gastrointestinal tract (GIT), or through interaction with components in the GIT. Absorption can be significantly limited by the intestinal mucus layer, and also affected by innate properties of the compound including charge (ζ potential), polarity, size, and hydrophobicity. Absorbed phytochemicals pass into systemic circulation via the liver or the lymphatic system [5]. Phytochemicals and their metabolites that reach systemic circulation are absorbed by specific tissues and are finally excreted into the urine. Hence, their bioavailability may be limited by bioaccessibility, absorption, transformation, and rapid clearance from the circulation which causes low plasma concentration that is not enough to show significant pharmacological effects at target sites [6]. In order to overcome these limitations of phytochemical bioavailability , a promising solution is encapsulation technology and delivery systems [4].

Phytochemicals can be loaded into carriers and then released under controlled conditions at the target site. Encapsulation techniques are used to enhance the stability of active compounds during processing and storage, mask their undesirable taste, reduce their toxicity, preserve them from the harsh environment of the GIT, control their release and enhance their

cellular uptake and biological activities [2, 7, 8]. Different encapsulation technologies and materials can be used depending on the desired properties and function of active compounds such as increased surface area, solubility, homogeneity, stability, intracellular uptake, and bioavailability [2].

Among encapsulation techniques, there has been an expanding interest in encapsulation of bioactive compounds into the novel and promising lipid self-assembled systems (LSAS) such as phospholipid-based carriers (liposomes, bilosomes, transfersomes, cubosomes, hexosomes), surfactant-based carriers (niosomes), emulsions (single, double, microemulsions) and solid lipid nanocarriers (solid lipid nanoparticles, nanostructured lipid carriers (NLC), nano-oleogels) [9]. The properties of these LSAS can be enhanced with the use of different lipids, surfactants, or their combinations or by incorporation of several compounds such as cholesterol or bile salts (BSs) in the bilayer structure, and by modifying their surface with coatings [10]. The effect of phospholipid composition on liposome membrane properties has been investigated, showing that lipid type significantly affects the transition temperature (T_m), elasticity, and fluidity of the membrane and consequently membrane stability and rigidity [11]. The effect of sodium cholate (NaC) and sodium taurocholate (NaTC) on the membrane integrity of liposomes has been studied with a low T_m lipid (PC and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC)) and high T_m (1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), and sphingomyelin). The bile salts were shown to be less destructive to liposomes consisting of lipids with a high T_m [12]. Coating with chitosan also shows promising results during digestion. The effect of low molecular weight chitosan coating on the bioavailability of curcumin-loaded liposomes [13] during *in vitro* digestion showed that after the oral and gastric phases of digestion, slightly higher curcumin concentration was detected compared to uncoated liposomes. Curcumin concentration was higher for chitosan-coated liposomes after the intestinal phase.

Bile salts are formed from conjugated bile acids that form complexes with sodium. Primary bile acids cholic acid (CA) and chenodeoxycholic acid are synthesized from cholesterol in the liver. These primary bile acids are conjugated to either taurine or glycine at the C-24 carboxyl group to increase their hydrophilicity. Intestinal bacteria convert primary bile acids to a secondary form (deoxycholic acid and lithocholic acid) [14]. BSs such as NaC, NaTC, and sodium deoxycholate (NaDC) are characteristic amphipathic molecules that contain a steroid nucleus. The presence of hydroxyl groups provides hydrophilic properties to the

concave side of the molecule, whereas the convex side is hydrophobic due to methyl groups. Because of their steroid skeleton, bile salts have a rigid structure that is very different compared to head-tail surfactants [15]. Due to their amphiphilic nature, bile salts form primary (aggregation number 2-10) or secondary (aggregation number 10-100) micelles in aqueous solutions above the critical micelle concentration (CMC) [16, 17]. Their CMC value is also not well defined so it is given as a range. They have low aggregation numbers of 4-6 molecules for NaC and 7-12 molecules for NaDC [18].

BSs show powerful solubilisation potential against phospholipid bilayers. When bile salt concentration reaches its CMC, bile salts solubilize the vesicles to mixed micelles [19]. BSs are incorporated into the outer layer of the phospholipid bilayer below their CMC and upon further increase of BS concentration, BSs transport to the inner leaflet of the bilayer. When the total BS concentration reaches the saturation phase boundary, the system transforms to coexisting micelles and vesicles. Upon further increase of BS concentration the solubilisation phase boundary is reached and BS-rich mixed micelles are formed. During solubilisation, all steps have aggregates with BS monomers [18]. Incorporating bile salts in the vesicle bilayer results in a negatively charged surface. Through electrostatic repulsion, BS/lipid systems are thought to stabilize the bilayer against intestinal BS adsorption [20, 21] ultimately leading to a slower vesicles-to-mixed micelles transition (Figure 2) and enhanced delivery of active compounds. BSs are also used as a permeability enhancer, which changes the membrane permeability and fluidity [22].

A considerable amount of literature has been published on BS-LSAS but this review primarily focuses on BS-LSAS for the delivery of phytochemicals. Therefore, the effect of the presence of BSs in LSAS in terms of the morphology, encapsulation efficiency, release profile and stability of the LSAS, and bioavailability and biological activities of loaded phytochemicals are discussed.

2. Effect of bile salts on the morphology of lipid self-assembled systems

Liposomes are the most widely used carriers among LSAS (Figure 2). The properties of liposomes can be enhanced with the use of different compounds such as BS in the formulation. The terms used to describe BS-liposomes vary among researchers. For example, bilosomes [23, 24], transfersomes [25, 26], ultradeformables or elastic liposomes [27, 28] are all used. BS-liposomes are designed based on the structure of liposomes, which also can encapsulate both

hydrophilic and lipophilic bioactive compounds [29]. BS interactions with phospholipid membranes can have a range of effects on the morphology of LSAS.

NaDC-nanotransfersomes were designed and optimized using response surface methodology to encapsulate sodium pravastatin and naringenin [25]. The amount of omega-3 phospholipid, NaDC, and naringenin were selected as independent variables. D_h, encapsulation efficiency (EE(%)), cumulative % permeated, alanine amino transaminase (ALT) level (IU/L), and malondialdehyde (MDA) levels (mmol/mg protein) were determined as dependent variables. The impact of independent variables was reported as omega-3-phospholipid>NaDC>naringenin. Optimization results showed that increasing NaDC concentration significantly reduced the D_h of nanotransfersomes (D_h: 86-249 nm) ($p<0.0001$). For optimum composition (Table 1), 76% of sodium pravastatin and naringenin were loaded in NaDC-nanotransfersomes (D_h: 191 nm). In the same way, the increased concentration of BSs resulted in the decreased Dh of resveratrol-loaded NaC-transfersomes [26] and notoginsenoside R1-loaded NaGC-liposomes [30]. Resveratrol loaded-NaC-transfersomes were optimized using face-centred central composite design (CCD). The amount of SPC and NaC were chosen as independent variables and their effect on D_h and EE(%) were chosen to optimise NaC-transfersomes. The D_h and polydispersity index (PDI) of the optimized formulation was 178.9±12.87 nm and 0.132 respectively (Table 1). Their shape was found to be nearly spherical and uniform by transmission electron microscopy (TEM). According to the results, once the NaC concentration was increased, the D_h of transfersomes decreased [26]. D_h of notoginsenoside R1-loaded NaGC-liposomes (200 nm) was smaller than conventional liposomes and when the concentration of NaGC increased, D_h and EE(%) decreased [30]. These findings indicate that the concentration of BSs in the formulations plays an important role in the D_h of vesicles. A decrease of the D_h can result from increased BSs concentration that can decrease the surface tension of the vesicles and stabilize the bilayer and enhance the elasticity of vesicles [20, 26].

Biflavonoid extract from *Selaginella doederleinii* was encapsulated using liposomes [31]. The SPC/biflavonoid extract ratios (15:1, 10:1 and 5:1 w/w) and SPC/NaDC ratios (15:1, 10:1 and 5:1 w/w) were chosen for optimization to see the effect on EE(%). The SPC/biflavonoid extract ratios showed the lowest effect among the four variables and 10:1 w/w ratio was chosen for optimum formulation (Table 1). The SPC/NaDC ratio had the second-highest effect among the four variables. Optimized liposomes (SPC/NaDC, 10:1 w/w) had a

D_h of 249.77±15.68 nm with PDI of 0.184±0.002. 91.39±0.88% of the extract was encapsulated in the optimized liposomes. These results are similar to those reported by Yang *et al.* who studied tripterine-loaded hyaluronic acid (HA)-coated NaDC-bilosomes. Effects of concentration of NaDC (1, 2, and 3 mg/mL), on the D_h and EE(%) of bilosomes were evaluated [23]. With increased NaDC concentration from 1 mg/mL to 2 mg/mL, D_h decreased from ~140 nm to ~90 nm [25, 26, 30]. However, when NaDC concentration was increased to 3 mg/mL, the D_h of bilosomes increased to ~110 nm. A possible explanation for these results, up to a specific concentration of BSs reduces the surface tension that induces membrane curvature and stabilizes the system and leads to decreased D_h. Further increase in BS concentration and an increase in the D_h may be related to aggregation in the system and increased medium viscosity [23, 24, 32]. The 2 mg/mL NaDC in the bilosome formulation had the lowest D_h (~90 nm) and the highest EE(%) (~100%.). For the HA-coated particles, 2 mg/mL of NaDC was chosen and the D_h was 95.3 nm and 118.4 nm for the bilosomes and HA-functionalized bilosomes, respectively.

Barone *et al.* studied ammonium glycyrrhizate-loaded liposomes composed of SPC and NaC. Loading of ammonium glycyrrhizate did not affect the D_h, PDI, ζ potential, and the deformability index (DI) of vesicles significantly. D_h increased from 109±3.8 to 128±4.6 nm and the ζ potential decreased from -17±0.5 to −22±0.6 mV [27]. A decrease in the ζ potential can result from the negative charge on the liposome surface arising from the interaction between hydrophilic groups of ammonium glycyrrhizate and water molecules and an increased number of hydrogen bonds. Moreover, the solvent layer can be increased with the interaction of hydrophilic groups and water that may result in an increased D_h [27, 33]. The lowering of the ζ potential mainly results from the presence of NaC in the formulation. The LSAS with ζ potential values more than ±30 mV shows moderate stability. As the ζ potential comes close to zero, the system lost its stability, and agglomeration and precipitation were seen [34].

Surface-modified bilosomes were prepared by Waglewska *et al.* and triblock copolymer Pluronic P123 was used to stabilise the surface. Three different concentrations of NaC were added into formulations (0.25, 0.50, and 1.00 wt %) [24]. The effect of the presence of NaC in the bilosome formulation was similar to those shown by others [27]. The negative ζ potential of the formulation results from the negative charge of NaC [35]. Sulphated polysaccharide-protein complexes of *Enteromorpha intestinalis*-loaded bilosomes were prepared using three different BSs (NaC, NaDC, NaTDC) and two different surfactants including Span 40

(hydrophilic-lipophilic balance (HLB): 6.7 [36] and Span 65 (HLB: 2.1) [37]. Span 40-bilosomes had more than double the D_h compared to Span 65-bilosomes for all BSs formulations. The lower D_h of Span 60-bilosomes resulted from the lower HLB value of Span 65. Low HLB results in a decrease in the surface free energy and shows increased lipophilic affinity that leads to smaller vesicles [38]. Higher HLB values lead to water uptake into the vesicles and cause increased vesicle size due to hydrophilicity [21]. BSs had a significant effect on the D_h of bilosomes. The D_h of bilosomes were reported as NaTDC-bilosomes >NaDC-bilosomes>NaC-bilosomes. The EE(%) were ranged from 69.66% to 71.60% ($p>0.05$). Span 65/NaC-bilosomes had the highest EE(%) (71.60±0.25%) with Dh: 181±16.80 nm [39]. In phenylethyl resorcinol-loaded transfersomes, NaDC (HLB: 16), Tween 80 (HLB: 15), Tween 20 (HLB: 16.7), Span 80 (HLB: 4.3), Span 20 (HLB: 8.6) were used as skin enhancers in the formulation [40]. In contrast to previous work [39], the D_h of NaDC-transfersomes was 353.70±10.90 nm, and the PDI, ζ potential, and EE(%) of NaDC-transfersomes were 0.111±0.038, −41.86±0.73, and 92.49±0.01 respectively. TEM and SEM analysis also revealed that NaDC-transfersomes had a unilamellar to multilamellar structure with a smooth surface [40].

Catechin-loaded hexosomes for topical application have been prepared using NaC, NaDC, and NaTC in the formulations and the D_h of hexosomes was 148 nm without BSs but the D_h decreased to 107–110 nm after 0.6 wt% BS addition [41]. Moreover, small-angle X-ray scattering (SAXS) measurements, showed that after the addition of 0.02 wt% of BSs in the formulation of an inverse bicontinuous cubic phase dispersion (cubosomes) completely transitioned to unilamellar vesicles. The phase transition could have been caused by the amphiphilic structure of BSs that is in contact with the bulk water so decreases the negative interfacial curvature of the system [41].

Surfactant concentration (NaGC and Polysorbate 80) was chosen as one of the independent variables for the optimization of gypenosides loaded NLCs [42]. While the surfactant concentration did not show a significant effect on D_h it had a significant effect on EE(%). The EE(%) increased with increased surfactant concentration and the D_h, PDI, and ζ potential of the optimized NLCs formulation (Table 1) were reported as 146.7±6.8 nm, 0.137±0.011, and −56.0±3.4 mV respectively with 74.22% in EE(%).

D_h of LSAS is a considerable parameter in the delivery of active compounds and, consequently, their passage through a biological membrane. According to the results, a

decrease of D_h is correlated to an increase in BSs up to a specific concentration (Figure 3). BSs stabilize the vesicles by reducing the interfacial tension of the vesicles. Further increased BSs concentration, BSs begin to show disruption effect on the LSAS.

3. Effect of bile salts on encapsulation efficiency, release, and stability of lipid self-assembled systems

The encapsulation efficiency of LSAS varies depending on the hydrophobicity or hydrophilicity of encapsulated phytochemicals, stability of the lipid membrane, the surface area of LSAS and the methods and parameters chosen for vesicle loading. The general stability of LSAS comprises the stability of active material and the retention capacity of the membranes. Membrane stability that provides integrity of the system has a direct effect on the encapsulation efficiency [43].

NaDC-liposomes and HA-coated NaDC-bilosomes were prepared to encapsulate tripterine [23]. Both formulations showed a narrow PDI and the ζ potential decreased from 4.8 mV to -34.2 mV due to the use of the negatively charged polysaccharide coating. EE(%) and DL(%) were 99.56% and 8.15% respectively. Doxorubicin-silybin-loaded liposomes were prepared using cholic acid (CA) to improve the oral treatment of actives [44]. The D_h of CA-liposomes was 97.03±2.17 nm with a narrow PDI and both active compounds had an EE(%) of approximately 95%. *In vitro* release was investigated in PBS buffers (pH: 2 and 7.4) to mimic the simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) conditions. While ~25% of silybin was released from liposomes at pH 2, ~50% of silybin was released in pH 7.4 in 12 h. In addition, the stability was determined in rat gastric fluid, rat intestinal fluid, and serum. After a 12 h incubation, the EE(%) of all liposomes in different steps was still more than 80% which shows that CA-liposomes protect their integrity during different steps of digestion. However, some active material leakage problems were also reported because of the presence of a high concentration of BSs. When the effect of NaC concentration on EE(%) of resveratrol-loaded transfersomes was studied, high EE(%) was seen at intermediate NaC levels and a decrease in the EE(%) was seen as NaC was increased. Higher NaC amounts also can cause leakage of active material because of the formation of lipid pores in the membrane. The ζ potential of the optimized formulation (Table 1) was reported as -23.2±1.4 mV [26].

The EE(%) of the system is affected by hydrophobicity or hydrophilicity of encapsulated phytochemicals. In vesicle preparations the EE(%) of hydrophilic phytochemicals is related to

236 the aqueous phase trapping ability of LSAS. When the desired rigidity of the lipid membrane
237 could not be achived, encapsulated hydrophilic phytochemicals may leak through the lipid
238 membrane by diffusion [45]. The EE(%) of hydrophilic phytochemicals are generally reported
239 as less than 30%. Hydrophobic phytochemicals can be incorporated into the lipid phase of
240 LSAS during vesicle formation. Thus, the EE(%) of hydrophobic phytochemicals in LSAS
241 mainly depends on the interaction between phytochemicals and lipids. High EE(%) can be
242 reached with optimised lipid type and concentration in the formulation [46]. The EE(%)
243 increases with increased lipid concentration and increases the Dh of particles so a higher
244 amount of phytochemicals can be loaded [47].

245 The addition BSs below the CMC into the formulation can also enhance the EE(%) of
246 LSAS (Figure 3). The flexibility of vesicles increases with the presence of BSs and the bilayer
247 and the aqueous core can be flexed to encapsulate the drug more [48]. BSs above the CMC
248 show solubilisation effect on vesicles so vesicles transit to micelles and increase the drug
249 solubility consequently decrease the EE(%). In addition, drug leakage can be seen because of
250 opened lipid membrane pores [49].

251 *In vitro* release studies are important to give a prediction about the *in vivo* release profile
252 [33]. *In vitro* dissolution profiles of NLCs and NaGC-NLCs were compared with gypenosides
253 powders. Cumulative dissolution of gypenosides powders (29.4%) was slightly higher than
254 both NLCs in 6 h. Although NaGC-NLCs showed slightly higher release, the release of NLCs
255 and NaGC-NLCs were not significantly different after 48 h. While ~55% of gypenosides was
256 released from both NLCs at 48 h, the cumulative dissolution of gypenosides powders did not
257 change significantly after 6 h. The most likely cause of slow-release from NLCs is due to lipid
258 layers slowing the diffusion of the active compounds [42]. Sulphated polysaccharide-protein
259 complexes-loaded bilosomes were prepared. Span 65-NaC-bilosomes and Span 40-NaC-
260 bilosomes released ~70% and ~40% of active compounds respectively, while other
261 formulations prepared with NaDC or NaTDC released less than ~20% in PBS (pH 6.8) at 37°C
262 in 24 h [39]. It was reported that while ~50% of [6]-gingerol released, using NaC-liposomes
263 significantly increase the cumulative release of [6]-gingerol (~90% in 24 h) ($p<0.01$) [50]. *In*
264 *vitro* release from bilosomes and HA-coated bilosomes were investigated [23]. Bilosomes
265 released only 40.51% of tripterine in phosphate-buffered saline (PBS) (pH 7.4) in 24 h, which
266 may be due to the hydrophobic nature of tripterin. The HA-coating also slowed the release of
267 actives from bilosomes; 11.35% and 23.24% of tripterine were released at 8 h and 24 h,

respectively. The release of active compounds can be controlled by loading the active into the system that acts as a barrier. Also, these barriers can be modified using coatings which provide additional layers to slow down the release. The presence of BSs in the self-assembled formulation enhances the physical stability of the system and thus can slow down the release [51].

The physical and chemical stability is important and to provide long-term shelf-life and to protect the structure of LSAS from the harsh conditions of the GIT and keep the structure in circulation longer[52]. Several factors such as lipid type, PDI, and ζ potential of the system, etc. are known to affect the stability of LSAS. BSs are only one of many factors that can enhance stability.

Phenylethyl resorcinol-loaded NaDC-transfersomes were stored at 4°C, 30°C and 45°C for 4 months (RH: 75%). While NaDC-transfersomes was stable for 4 months at 4±1°C and 30±1°C, they could retain their stability for 2 months at 45±1°C [40]. The accelerated stability of NLCs and NaGC-NLCs was monitored and both NLCs showed stable structures. The D_h and EE(%) of NLCs did not change significantly during storage at 40±2°C, relative humidity: 75±5% for 6 months [42]. Likewise, methylene blue and curcumin-loaded NaC-bilosomes showed minimum changes during storage at 4ºC for 28 days. The D_h and PDI of the formulations were reported as ~100 nm and <0.25. EE(%) were 85% and 70% for methylene blue and curcumin, respectively [24]. The physical stability of ammonium glycyrrhizate-loaded NaC-liposomes (EE(%): 57.3±3.7%) was determined at 25°C and 37°C for 4 weeks. While the D_h of vesicles did not change significantly at 25°C, a significant increase was observed at 37°C after 2 weeks, which could be related to the gel to fluid lamellar phase transition at 37°C [27].

PDI and ζ potential of LSAS are important parameters that reflect the homogeneity (PDI<0.3) of the particles and stability (more than ±30 mV) of particles respectively. Aggregation, flocculation, or precipitation problems can be seen during storage with the decrease of ζ potential below ±30 mV [53]. The net surface charge of particles comes from the combination of lipid, active compounds, and other compounds that are used to modify the structure. The addition of BSs in LSAS increases ζ potential negatively and physically stabilizes the system due to the increased repulsion between particles. An increase of negative values of ζ potential is correlated to an increased BS concentration due to the negative charge of BSs.

4. Effect of bile salts on the bioavailability of lipid self-assembled systems

The biological fate of LSAS that aims to enhance the bioavailability of active compounds differs depending on their administration. While digestion is one of the main steps for the oral delivery of active materials, skin penetration is taken into consideration for the topical application of active materials. Much research is already being undertaken on LSAS for further exploiting the biological activities of phytochemicals.

After oral administration, LSAS pass through the oral cavity and reach the stomach. In the gastric compartment, LSAS interact with gastric fluids (pH 1-3) and a high concentration of calcium and sodium salts. Physical properties begin to change during this interaction and some lipophilic compounds in the structure begin to get digested by gastric lipases in the stomach (10–30%). After the gastric phase, LSAS that can survive the acidic environment of the stomach enter the small intestine (pH 6-7.5). Compounds mix with bile salts from the gall bladder and pancreatic secretions. Following hydrolysis of lipophilic compounds to free fatty acids and monoglycerides by pancreatic lipases in the small intestine (70–90%), bile salts, free fatty acids, monoglycerides, and phospholipids form mixed micelles which can be absorbed by the intestinal enterocytes [54, 55]. LSAS that are composed of digestible compounds lose their integrity after the gastric and intestinal phases. Hydrophilic phytochemicals pass to the enterocytes for absorption and are transported to the portal vein [54, 56]. Highly hydrophobic phytochemicals (partition coefficient (log P)>5) are attached to chylomicrons. Chylomicrons enter the lymphatic system and carry these hydrophobic phytochemicals to the tissues. Hydrophobic phytochemicals are released from chylomicrons by hydrolysis of lipoprotein by lipases for the absorption of phytochemicals at the tissue [54, 56].

LSAS that are composed of indigestible coatings like chitosan, pectin, etc can remain intact through the small intestine and pass to colon where they may be hydrolysed by colonic microflora, encapsulated phytochemicals can then be absorbed in the colon [54].

Gypenosides are triterpenoid saponins from *Gynostemma pentaphyllum*. Gypenosides are used for medical purposes for many years however they are sensitive to the environment and suffer low solubility both in lipid and water [42]. In a study designed to assess the delivery of gypenosides for oral application, the time to peak plasma concentration (T_{max}), maximum plasma concentration (C_{max}), area under the plasma concentration ($AUC_{0-\infty}$), and mean retention time ($MRT_{0-\infty}$) were investigated in rats after oral administration of gypenosides

powders, NLCs and NaGC-NLCs. Loading of gypenosides into NLCs prolonged the absorption time 1.8-fold and retention time 1.9-fold ($p<0.05$). The C_{max} of NLCs (0.671±0.073 mg/mL.h) also increased ~1.5-fold compared to the powder so it shows that NLCs remained longer in the circulation. The addition of NaGC in the NLC formulation increases the T_{max} and $MRT_{0-\infty}$ significantly ($p<0.05$). The plasma concentration of gypenosides increased compared to gypenosides powders and NLCs ~1.9-fold and 1.3-fold, respectively. Intestinal absorption of NLCs and NaGC-NLCs was also compared with the gypenosides powder. The apparent absorption coefficient (P_{app}) of NLCs showed increased permeability and bioavailability compared to gypenosides powder ($p<0.05$) in all sections of the intestine. The presence of NaGC in NLCs increased the P_{app} 5.11-fold compared to gypenosides powder and ~2-fold compared to NLCs in the jejunum ($p<0.05$). In addition, Caco-2 cell viability was increased from ~70% to ~90% at 100 µg/mL gypenosides due to loading the gypenosides into NaGC-NLCs that reduced the cytotoxicity of gypenosides [42].

The delivery of tripterin isolated from *Tripterygium wilfordii* was investigated [23]. Tripterin, also known as celastrol, shows antioxidant, anti-angiogenic, and anti-rheumatic effects. However, it is poorly soluble in water and consequently shows low solubility in biological fluids [57]. Cellular uptake of free tripterine, bilosomes, and HA-coated bilosomes in RAW264.7 cells was compared. Loading tripterine into bilosomes doubled its cellular uptake from ~75 ng/mg of protein to ~150 ng/mg of protein ($p<0.01$). Compared to uncoated bilosomes, HA enhanced the internalization of bilosomes and provided higher cellular uptake (~275 ng/mg protein) ($p<0.01$). This enhanced internalization ability was further confirmed by confocal laser scanning microscopy (CLSM). Bioavailability and biodistribution results of free tripterine, bilosomes, and HA-coated bilosomes showed that C_{max}, $AUC_{0-\infty}$ and the elimination time of HA-coated bilosomes was significantly different from free tripterine and bilosomes. The C_{max} and $AUC_{0-\infty}$ of bilosomes (23.57±0.49 µg/mL, 76.19±1.13 µg/mL.h) and coated bilosomes (25.24±0.57 µg/mL, 112.19±0.85 µg/mL.h) were higher than the C_{max} of free tripterine (22.62±0.71 µg/mL, 35.86±0.53 µg/mL.h). In addition, the elimination time of the drug decreased 2.13-fold using bilosomes and 3.13 fold using coated bilosomes. Encapsulation improved the resistance of tripterinein in circulation. The relative bioavailability of tripterine in bilosomes and coated bilosomes was 480.3% and 799.9% respectively, compared to free tripterine [23].

Notoginsenoside R1 derived from *Panax notoginseng* poorly dissolves in water and cannot exhibit its biological functions sufficiently [58]. For the oral application of notoginsenoside R1, *in vitro* cellular uptake in Caco-2 cells and intestinal absorption of notoginsenoside R1-loaded NaGC-liposomes and liposomes were compared. NaGC-liposomes showed 1.5-fold higher cellular uptake compared to liposomes ($p<0.01$) so the presence of NaGC enhanced the cellular uptake of notoginsenoside R1.

Intestine absorption of notoginsenoside R1 was reported as duodenum>jejunum>ileum>colon. The P_{app} of notoginsenoside R1 was NaGC-liposomes> liposomes> notoginsenoside R1 solution for the same drug concentration (20 μg/mL and 100 μg/mL) and in the same section of the intestine ($p < 0.05$). Addition of NaGC increased P_{app} 1.85-fold (20 μg/mL drug) and 3.19 fold (100 μg/mL drug) compared to free drug. NaGC-liposomes enhanced the C_{max} 1.85-fold and $T_{1/2}$ 2.52-fold compared to liposomes. The presence of NaGC also improved AUC_{0-t} 1.32-fold and 2.68-fold compared to liposomes and notoginsenoside R1 solution [30]. Loading into NaC-liposomes increased the relative bioavailability (16.4-fold) and elimination half-time ($T_{1/2}$) of [6]-gingerol (~2.5-fold) which shows that [6]-gingerol can stay longer in the circulation [50]. Similarly, an *in vivo* pharmacokinetic study showed that while C_{max}, $AUC_{0-\infty}$ and $MRT_{0-\infty}$ of all biflavonoids were increased, C_L decreased when loaded into NaDC-liposomes. Encapsulation did not show a significant difference in the $T_{1/2}$ of biflavonoids. A pharmacokinetic study showed that the relative bioavailability of biflavonoids in the extract was enhanced between 191%-995% due to loading into NaDC-liposomes. Moreover, the cytotoxicity of the biflavonoids extract-loaded liposomes was evaluated on HT-29 cells. Encapsulation into NaDC-liposomes reduced the half-maximal inhibitory concentration (IC_{50}) of the extract by 37% [31].

Ammonium glycyrrhizinate is isolated from *Glycirrhiza glabra* and is used for supplements and medical purposes. Ammonium glycyrrhizinate shows an anti-inflammatory effect against inflammation on the skin [59]. The applicability of the ammonium glycyrrhizate-loaded NaC-liposomes for topical application was investigated and at 24 h, only 40% of ammonium glycyrrhizate was released from vesicles and showed pseudo-first-order kinetics [27]. Permeation and skin tolerability studies were done with the topical application of vesicles on humans. 134.9 μg/cm^2 ammonium glycyrrhizate penetrated through the *stratum corneum* and epidermis and permeation through the skin layers showed zero-order kinetics. For topical application, drugs need to pass through the *stratum corneum* and epidermis as well as protect

their structure [27]. Erythema index (ΔEI) values measured were below 4.5 and show that ammonium glycyrrhizate-loaded NaC-liposomes can be tolerated by human skin without any toxicity. In order to determine the effect of NaC on the deformability of dipalmitoylphosphatidylcholine (DPPC) liposomes, the deformability of liposomes and NaC-liposomes (DPPC/NaC 4:1) were compared using the vesicle-pore model where both liposomes are pushed to pass through narrow pores and deformability parameters are measured [28]. According to the results, NaC improves the flexibility of liposomes and decreases the deformability through pores.

Resveratrol (3,5,4′-trihydroxytrans-stilbene) is a well-known natural antioxidant [60]. Spreadability, skin permeation, and deposition of a resveratrol-loaded NaC-transfersome cream was compared with a conventional resveratrol cream [26]. The spreadability of the vesicular cream was 47.90±3.65% spread by weight (conventional resveratrol cream: 39.14±1.59%). The permeation flux of the resveratrol-loaded NaC-transfersomes cream and conventional cream was determined as 4.95±0.69 and 2.70±0.73 μg/cm^2 respectively (at 24 h). When deposition in the skin layer was evaluated, drug deposition of the NaC-transfersome cream (335.2±4.12 μg/cm^2 with skin retention: 70.16±0.87%) was reported to be significantly higher than conventional cream (67.12±19.63 μg/cm^2 with skin retention: 14.05±4.11%). *In vitro* skin permeation and deposition of phenylethyl resorcinol-loaded NaDC-transfersomes were investigated. Compared to liposomes (skin permeation: 20.65 μg/cm^2, accumulation: 28.18 μg/cm^2), transfersomes (skin permeation: 72.66 μg/cm^2, accumulation: 71.21 μg/cm^2) provided higher permeation and accumulation of phenylethyl resorcinol in newborn pig skin ($p<0.05$) [40]. According to the results, the enhanced permeation ability of the resveratrol-loaded NaC-transfersome cream [26] and phenylethyl resorcinol-loaded NaDC-transfersomes [40] is possibly due to the presence of BSs that can enhance the penetration [16]. Sodium pravastatin and naringenin-loaded NaDC-transfersomes were optimized and NaDC showed a significant impact on the cumulative % permeated (<0.0001). When NaDC concentration (20-60 mg) increased, cumulative (%) permeated increased from 37% to 59%. Results also showed that NaDC did not show a considerable effect on ALT (p: 0.8767) and MDA (p: 0.4490) levels [25]. The deformability of phenylethyl resorcinol-loaded NaDC-transfersomes and liposomes was compared before and after filtering through a 200 nm polycarbonate filter [40]. While transfersomes (D_h: 398.37±9.82 nm, PDI: 0.06±0.08) passed through the filter, liposomes (D_h: 600.23±11.92 nm, PDI: 0.21±0.07) couldn't pass because of their rigid structure. After filtration, the D_h, PDI, and DI of NaDC-transfersomes were 371.97±8.72 nm, 0.13±0.09, and

6.63% respectively. These results confirm that incorporating NaDC into the lipid bilayers enhances the flexibility of vesicles.

Catechin is a widely studied phenolic compound that shows mainly antioxidant activity. *In vitro* skin permeation performance of catechin-loaded NaTC-hexosomes was tested on the skin of newborn pigs and compared with catechin-loaded hexosomes and vesicles. Due to the presence of NaTC, NaTC-hexosomes showed better permeation through skin layers and higher drug accumulation in the skin layers including *stratum corneum*:~14%, epidermis:~7%, dermis:~8%, and receptor compartment:~9%. While liposomes showed max accumulation (~9%) in the dermis, no drug accumulation was detected in the receptor compartment. Hexosomes showed maximum of ~5% drug accumulation both in the *stratum corneum* and the receptor compartment [41].

Loading of active compounds into LSAS prolonged the absorption time and retention time compared to free active compounds. The addition of BSs to the LSAS further increases the absorption time and retention time of the active compounds compared to BS-free LSAS. Due to the presence of BSs, the C_{max} and $AUC_{0-\infty}$ of circulating active compounds are increased, therefore improved relative bioavailability has been reported (Figure 3). In addition, BSs increase the elasticity of particles and enhance the penetration and accumulation of active compounds [48].

5. Effect of bile salts on the biological activity of phytochemicals loaded into lipid self-assembled systems

Phytochemicals show numerous biological activities such as antioxidant, anti-inflammatory, anti-tumour, anti-arthritic, anti-tyrosinase activity, etc (Figure 4). LSAS can provide increased solubility, enhanced diffusion properties, and stability to phytochemicals and promote these biological functions [61].

In vivo anti-tumour activity of biflavonoids extract-loaded liposomes was determined on HT-29 colon cancer cells. NaDC-liposomes almost doubled the antitumor activity of biflavonoid extracts ($p<0.001$) without systemic toxicity [31]. In the same way, the anti-tumour activity of [6]-gingerol (100 µg/mL, 15% inhibition) on HepG2 cells was improved significantly due to loading into NaC-liposomes (inhibition rate:~100%) ($p<0.01$) [50]. In addition, the *in vitro* anti-tumour effect of curcumin-loaded polyethylenimine-ethosomes (PEI) and doxorubicin-loaded NaC-ethosomes (7:3 v/v) on B16 cells was determined. PEI-loaded

NaC-ethosomes and doxorubicin-loaded NaC-ethosomes showed similar inhibition (~55%) against B16 cells. PEI-ethosomes and doxorubicin-loaded NaC-ethosomes (7:3 v/v) exerted stronger inhibition against B16 cells and cell survival was decreased to ~30% ($p<0.01$). Similar results were also seen for *in vivo* anti-tumor effects. Melanoma tumor inhibition rates were 21.9% and35.5% for curcumin-loaded PEI and doxorubicin-loaded NaC-ethosomes, respectively. PEI-ethosomes and doxorubicin-loaded NaC-ethosomes (7:3 v/v) inhibited 46.38% of Melanoma tumor ($p<0.01$) [62]. Loading the phytochemicals in the BS-contining system enhanced their anti-tumour activity significantly.

The anti-inflammatory activity of ammonium glycyrrhizate-loaded NaC-liposomes was investigated in human volunteers [27]. Free compound and liposomes were applied topically on chemically (0.2% w/v methyl nicotinate) stimulate erythema and NaC-liposomes showed ~10-30-fold increase in anti-inflammatory activity compared to an equivalent of ammonium glycyrrhizate solution.

Anti-arthritic activity of tripterine-loaded HA-coated NaDC-bilosomes on arthritic mice was studied [23]. Mediators that show the inflammation level was measured and coated and uncoated bilosomes were compared with the model control. While bilosomes reduced the concentration of mediators in serum approximately double times compared to model control, HA-coated bilosomes reduced mediators approximately fourfold compared to model control ($p<0.01$).

Anti-tyrosinase activity of phenylethyl resorcinol-loaded NaDC-transfersomes and liposomes were evaluated after topical application on pig skin. Kojic acid was chosen as a positive control. Liposomes (95.54 ±0.13%) and NaDC-transfersomes (91.09 ±1.23%) showed better anti-tyrosinase activity than kojic acid (87.35±0.76%). Accumulation of phenylethyl resorcinol from NaDC-transfersomes (80.47±0.22%) was nearly the same as liposomes (79.53±0.45%). Tyrosinase activity and melanin content in B16 melanoma cells was also measured after NaDC-tranfersome and liposome application. Compared to liposomes (trosinase activity: 71.27% and melanin content: 82.11%), NaDC-transfersomes decreased trosinase activity to 64.36% and melanin content to 64.85%. NaDC-transfersomes showed effective skin lightening properties [40].

The *in vitro* antioxidant activity of catechin-loaded NaTC-hexosomes was measured. Using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, NaTC-hexosomes, hexosomes,

vesicles, and catechin in methanol solution could all inhibit 88% of DPPH radicals [41]. Similarly, the antioxidant activity of a resveratrol solution (95%) and resveratrol-loaded NaC-transfersomes (~92%), were not significantly different ($p>0.05$). In addition, empty tranfersomes also showed the ability to inhibit ~21% of 25 µM DPPH radicals [26]. The presence of BSs in formulations and loading into BSs-systems did not show a significant effect on the antioxidant activity of catechin and resveratrol.

In general, the presence of BSs in LSAS increased the biological activity of the phytochemicals. Although increased permeation and accumulation of catechin and increased skin retention of resveratrol were reported due to loading into BS-LSAS, no significant changes were observed in the biological activities of resveratrol and catechin.

6. CONCLUSION

This review focused on the effect of BSs on phytochemicals-loaded-LSAS. The presence of BSs in LSAS showed effects on the morphology, encapsulation efficiency, stability, bioavailability, and biological activities of phytochemicals. Up to a specific concentration that differs depending on the BSs, the D_h of LSAS decreased as the concentration of BSs increased. This was due to BSs reducing the surface tension of the vesicles and stabilize the bilayers. While the EE(%) of hydrophilic phytochemicals is related to the trapping ability of the lipid membrane to hydrophilic phytochemicals dissolved in aqueous phases, the EE(%) of hydrophobic phytochemicals is mainly related to the interaction between the lipid membrane and phytochemicals. The addition of BSs to the formulations affected the EE(%) significantly. Increased BS concentration up to a specific concentration, resulted in increased EE(%). Upon further increase in BS concentration, BSs showed a solubilisation effect resulting in the transition from vesicles to micelles and disruption of the vesicles. It has been reported that EE(%) may decrease due to membrane pore formation caused by BSs which may cause leakage of phytochemicals. *In vitro* release studies showed that encapsulation of phytochemicals into BS-LSAS slows their release. The addition of negative charged BSs improved the stability of LSAS due to an increase negative ζ potential that increased repulsion between particles. Pharmacokinetics studies reported that the addition of BSs increased the absorption time and plasma concentration and decreased the elimination time of phytochemicals. BSs also enhanced the flexibility of particles and favored the penetration and accumulation of phytochemicals. Increased relative bioavailability of phytochemicals was reported due to the loading of the compounds into BS-LSAS. With enhanced bioavailability, in general,

phytochemicals showed higher biological activity. The application of LSAS has potential in the food, beverage, and pharmaceutical industries. Hence, exploring the possible use of BSs in LSAS for the delivery of phytochemicals was outlined.

Author Contributions

AC: Conceptualization, Investigation, Identification, Methodology, Screening, Writing-Original draft preparation; AIIT: Project Administration, Supervision, Validation, Writing-Review and editing; ARM: Project Administration, Supervision, Validation, Writing-Review and editing.

Conflicts of interests

The authors declare no competing financial interest.

Acknowledgments

Funding: This study was supported by the Republic of Turkey-The Ministry of National Education.

REFERENCES

Papers of particular interest, published within the period of review, have been highlighted as: * of special interest, ** of outstanding interest

[1] Mouhid L, Corzo-Martinez M, Torres C, Vazquez L, Reglero G, Fornari T, et al. Improving In Vivo Efficacy of Bioactive Molecules: An Overview of Potentially Antitumor Phytochemicals and Currently Available Lipid-Based Delivery Systems. J Oncol. 2017;2017:7351976.

[2] Shishir MRI, Xie L, Sun C, Zheng X, Chen W. Advances in micro and nano-encapsulation of bioactive compounds using biopolymer and lipid-based transporters. Trends in Food Science & Technology. 2018;78:34-60.

[3] Weiskirchen S, Weiskirchen R. Resveratrol: how much wine do you have to drink to stay healthy? Advances in Nutrition. 2016;7:706-18.

[4] Amri A, Chaumeil JC, Sfar S, Charrueau C. Administration of resveratrol: What formulation solutions to bioavailability limitations? J Control Release. 2012;158:182-93.

[5] McClements DJ, Li F, Xiao H. The Nutraceutical Bioavailability Classification Scheme: Classifying Nutraceuticals According to Factors Limiting their Oral Bioavailability. Annu Rev Food Sci Technol. 2015;6:299-327.

[6] Walle, Hsieh F, DeLegge MH, Oatis JE, Walle UKJDm, disposition. High absorption but very low bioavailability of oral resveratrol in humans. 2004;32:1377-82.

[7] Esfanjani AF, Assadpour E, Jafari SM. Improving the bioavailability of phenolic compounds by loading them within lipid-based nanocarriers. Trends in Food Science & Technology. 2018;76:56-66.

[8] Jafari SM, McClements DJ. Nanotechnology Approaches for Increasing Nutrient Bioavailability. Adv Food Nutr Res. 2017;81:1-30.

[9] Assadpour E, Jafari SM. An overview of lipid-based nanostructures for encapsulation of food ingredients. In: Jafari SM, editor. Lipid-Based Nanostructures for Food Encapsulation Purposes: Academic Press.; 2019. p. 1-34.

[10] Kotla NG, Chandrasekar B, Rooney P, Sivaraman G, Larrañaga A, Krishna KV, et al. Biomimetic Lipid-Based Nanosystems for Enhanced Dermal Delivery of Drugs and Bioactive Agents. ACS Biomaterials Science & Engineering. 2017;3:1262-72.

[11] Bouarab L, Maherani B, Kheirolomoom A, Hasan M, Aliakbarian B, Linder M, et al. Influence of lecithin–lipid composition on physico-chemical properties of nanoliposomes loaded with a hydrophobic molecule. 2014;115:197-204.

[12] Kokkona M, Kallinteri P, Fatouros D, Antimisiaris SG. Stability of SUV liposomes in the presence of cholate salts and pancreatic lipases: effect of lipid composition. European Journal of Pharmaceutical Sciences. 2000;9:245-52.

[13] Cuomo F, Cofelice M, Venditti F, Ceglie A, Miguel M, Lindman B, et al. In-vitro digestion of curcumin loaded chitosan-coated liposomes. Colloids Surf B Biointerfaces. 2018;168:29-34.

[14] Ridlon JM, Harris SC, Bhowmik S, Kang DJ, Hylemon PB. Consequences of bile salt biotransformations by intestinal bacteria. Gut Microbes. 2016;7:22-39.

[15] Pabois O, Lorenz CD, Harvey RD, Grillo I, Grundy MM-L, Wilde PJ, et al. Molecular insights into the behaviour of bile salts at interfaces: a key to their role in lipid digestion. Journal of colloid and interface science. 2019;556:266-77.

[16] Moghimipour E, Ameri A, Handali S. Absorption-Enhancing Effects of Bile Salts. Molecules. 2015;20:14451-73.

[17] Pabois O, Ziolek RM, Lorenz CD, Prévost S, Mahmoudi N, Skoda MW, et al. Morphology of bile salts micelles and mixed micelles with lipolysis products, from scattering techniques and atomistic simulations. Journal of Colloid and Interface Science. 2021;587:522-37.

[18] Garidel P, Hildebrand A, Knauf K, Blume A. Membranolytic activity of bile salts: Influence of biological membrane properties and composition. Molecules. 2007;12:2292-326.

[19] Wüstner D, Herrmann, A., Müller, P. Head group-independent interaction of phospholipids with bile salts: a fluorescence and EPR study. Journal of lipid research. 2000;41 (3).

[20] Aburahma MH. Bile salts-containing vesicles: promising pharmaceutical carriers for oral delivery of poorly water-soluble drugs and peptide/protein-based therapeutics or vaccines. Drug Deliv. 2016;23:1847-67.

[21] Al-Mahallawi AM, Abdelbary AA, Aburahma MH. Investigating the potential of employing bilosomes as a novel vesicular carrier for transdermal delivery of tenoxicam. Int J Pharm. 2015;485:329-40.

[22] Hu S, Niu M, Hu F, Lu Y, Qi J, Yin Z, et al. Integrity and stability of oral liposomes containing bile salts studied in simulated and ex vivo gastrointestinal media. Int J Pharm. 2013;441:693-700.

**[23] Yang H, Liu Z, Song Y, Hu C. Hyaluronic acid-functionalized bilosomes for targeted delivery of tripterine to inflamed area with enhancive therapy on arthritis. Drug delivery. 2019;26:820-30.
This study reports successfully the enhanced stability and bioavailability of tripterine due to the loading NaDC-bilosomes and further improved relative bioavailability with loading hyaluronic acid-functionalized with surface modification. (relative bioavailability of NaDC-bilosomes: 480.3% and hyaluronic acid-functionalized NaDC-bilosomes: 799.9% compared to free tripterine).

[24] Waglewska E, Pucek-Kaczmarek A, Bazylińska U. Novel Surface-Modified Bilosomes as Functional and Biocompatible Nanocarriers of Hybrid Compounds. Nanomaterials. 2020;10:2472.

*[25] Hosny KM, Alharbi WS, Almehmady AM, Bakhaidar RB, Alkhalidi HM, Sindi AM, et al. Preparation and optimization of pravastatin-naringenin nanotransfersomes to enhance bioavailability and reduce hepatic side effects. Journal of Drug Delivery Science and Technology. 2020;57:101746.
This study shows the effect and importance of NaDC concentration on the morphology of sodium pravastatin and naringenin-loaded transfersomes (concentration of NaDC increased, D_h of transfersomes decreased).

[26] Arora D, Khurana B, Nanda S. DoE directed optimization, development and evaluation of resveratrol loaded ultradeformable vesicular cream for topical antioxidant benefits. Drug development and industrial pharmacy. 2020;46:227-35.

*[27] Barone A, Cristiano MC, Cilurzo F, Locatelli M, Iannotta D, Di Marzio L, et al. Ammonium glycyrrhizate skin delivery from ultradeformable liposomes: a novel use as an anti-inflammatory agent in topical drug delivery. Colloids and Surfaces B: Biointerfaces. 2020;193:111152.
This study reports the anti-inflammatory activity of ammonium glycyrrhizate-loaded NaC-liposomes on human volunteers and ~10-30-fold increased anti-inflammatory activity.

*[28] Wu Z, Yang C, Chen L, Ma L, Wu X, Dai X, et al. A Multiscale Study on the Effect of Sodium Cholate on the Deformation Ability of Elastic Liposomes. AAPS PharmSciTech. 2019;20:1-10.
This study reports clearly the effect of the presence of NaC on the flexibility of DPPC liposomes by the vesicle-pore model.

[29] Van Tran V, Moon JY, Lee YC. Liposomes for delivery of antioxidants in cosmeceuticals: Challenges and development strategies. J Control Release. 2019;300:114-40.

[30] Fan Q, Zhang Y, Hou X, Li Z, Zhang K, Shao Q, et al. Improved oral bioavailability of notoginsenoside R1 with sodium glycocholate-mediated liposomes: preparation by supercritical fluid technology and evaluation in vitro and in vivo. International journal of pharmaceutics. 2018;552:360-70.

*[31] Chen B, Wang X, Lin D, Xu D, Li S, Huang J, et al. Proliposomes for oral delivery of total biflavonoids extract from Selaginella doederleinii: formulation development, optimization, and in vitro–in vivo characterization. International journal of nanomedicine. 2019;14:6691.
This study highlights the importance of NaDC concentration in the liposome formulations in terms of morphology, cytotoxicity of biflavonoids (Encapsulation into NaDC-liposomes reduced the IC_{50} of the biflavonoids extract by 37%).

[32] Madsen KL, Herlo R. Recursive alterations of the relationship between simple membrane geometry and insertion of amphiphilic motifs. Membranes. 2017;7:6.

[33] Nkanga CI, Bapolisi AM, Okafor NI, Krause RWM. General perception of liposomes: formation, manufacturing and applications. Liposomes-advances and perspectives. 2019.

[34] Riddick TM. Control of colloid stability through zeta potential: with a closing chapter on its relationship to cardiovascular disease. Published for Zeta-Meter, inc., by Livingston Pub. Co.; 1968.

[35] Holm R, Østergaard J, Schönbeck C, Jensen H, Shi W, Peters GH, et al. Determination of stability constants of tauro-and glyco-conjugated bile salts with the negatively charged sulfobutylether-β-cyclodextrin: comparison of affinity capillary electrophoresis and isothermal titration calorimetry and thermodynamic analysis of the interaction. Journal of inclusion phenomena and macrocyclic chemistry. 2014;78:185-94.

[36] Foo KS, Bavoh CB, Lal B, Mohd Shariff A. Rheology Impact of Various Hydrophilic-Hydrophobic Balance (HLB) Index Non-Ionic Surfactants on Cyclopentane Hydrates. Molecules. 2020;25:3725.

[37] Thakare M, Israel Be, Garner S, Ahmed H, Elder D, Capomacchia A. Nonionic surfactant structure on the drug release, formulation and physical properties of ethylcellulose microspheres. Pharmaceutical development and technology. 2017;22:418-25.

[38] Yoshioka T, Sternberg B, Florence AT. Preparation and properties of vesicles (niosomes) of sorbitan monoesters (Span 20, 40, 60 and 80) and a sorbitan triester (Span 85). International journal of pharmaceutics. 1994;105:1-6.

[39] Matloub AA, Salama AH, Aglan HA, AbouSamra MM, ElSouda SSM, Ahmed HH. Exploiting bilosomes for delivering bioactive polysaccharide isolated from Enteromorpha intestinalis for hacking hepatocellular carcinoma. Drug development and industrial pharmacy. 2018;44:523-34.

[40] Amnuaikit T, Limsuwan T, Khongkow P, Boonme P. Vesicular carriers containing phenylethyl resorcinol for topical delivery system; liposomes, transfersomes and invasomes. Asian journal of pharmaceutical sciences. 2018;13:472-84.

*[41] Fornasier M, Pireddu R, Del Giudice A, Sinico C, Nylander T, Schillén K, et al. Tuning lipid structure by bile salts: Hexosomes for topical administration of catechin. Colloids and Surfaces B: Biointerfaces. 2021;199:111564.
This study highlights that BSs (NaC, NaGC and NaTC) interact with catechin in the formulations differently consequently their effects on the stability of lipid self-assembled systems differ. (While NaTC-hexosomes that contain 0.2-0.5 wt% NaTC were stable for 2 months, NaC and NaDC-hexosomes only continued their stability for ~2 weeks. (NaTC: 0.2-0.5 wt%)).

*[42] Yang G, Wu F, Chen M, Jin J, Wang R, Yuan Y. Formulation design, characterization, and in vitro and in vivo evaluation of nanostructured lipid carriers containing a bile salt for oral delivery of gypenosides. International journal of nanomedicine. 2019;14:2267.
This study emphasizes of enhanced release and bioavailability of compounds due to encapsulation into NLCs and the addition of NaGC in the NLCs formulations (Plasma concentration of gypenosides increased compared to gypenosides powder and NLCs as ~1.9-fold and 1.3-fold, respectively).

[43] Uchegbu IF, Florence AT. Non-ionic surfactant vesicles (niosomes): physical and pharmaceutical chemistry. Adv Colloid Interface Sci. 1995;58:1-55.

*[44] Li Y, Yang D, Wang Y, Li Z, Zhu C. Co-delivery doxorubicin and silybin for anti-hepatoma via enhanced oral hepatic-targeted efficiency. International journal of nanomedicine. 2019;14:301.
This study reports the release profile of cholic acid-liposomes in PBS buffers (pH: 2 and 7.4) to mimic the SGF and SIF conditions and stability of liposomes in rats gastric fluid, rat intestinal fluid, and serum.

[45] Nii T, Ishii F. Encapsulation efficiency of water-soluble and insoluble drugs in liposomes prepared by the microencapsulation vesicle method. International journal of pharmaceutics. 2005;298:198-205.

[46] Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo S, Zarghami N, Hanifehpour Y, et al. Liposomes: Classification, preparation and applications. Nanoscale Res Lett 8 (1): 102. Springer; 2013.

[47] Boseila AA, Abdel-Reheem AY, Basalious EB. Design of bile-based vesicles (BBVs) for hepatocytes specific delivery of Daclatasvir: Comparison of ex-vivo transenterocytic transport, in-vitro protein adsorption resistance and HepG2 cellular uptake of charged and β-sitosterol decorated vesicles. PLoS ONE. 2019;14.

[48] Ahad A, Raish M, Ahmad A, Al-Jenoobi FI, Al-Mohizea AM. Development and biological evaluation of vesicles containing bile salt of telmisartan for the treatment of diabetic nephropathy. Artificial Cells Nanomedicine and Biotechnology. 2018;46:S532-S9.

[49] Mahmood S, Taher M, Mandal UK. Experimental design and optimization of raloxifene hydrochloride loaded nanotransfersomes for transdermal application. Int J Nanomedicine. 2014;9:4331-46.

**[50] Wang Q, Wei Q, Yang Q, Cao X, Li Q, Shi F, et al. A novel formulation of [6]-gingerol: Proliposomes with enhanced oral bioavailability and antitumor effect. International journal of pharmaceutics. 2018;535:308-15.
This study reports enhanced relative bioavailability (16.4-fold), reduced elimination time (~2.5-fold), and improved antitumor activity (~2-fold) of [6]-gingerol due to loading into NaC-liposomes.

[51] Zhang B, Xue A, Zhang C, Yu J, Chen W, Sun D. Bile salt liposomes for enhanced lymphatic transport and oral bioavailability of paclitaxel. Pharmazie. 2016;71:320-6.

[52] Hashemzadeh H, Javadi H, Darvishi M. Study of Structural stability and formation mechanisms in DSPC and DPSM liposomes: A coarse-grained molecular dynamics simulation. Scientific reports. 2020;10:1-10.

[53] Danaei M, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A, et al. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics. 2018;10:57.

[54] Wang T, Luo Y. Biological fate of ingested lipid-based nanoparticles: current understanding and future directions. Nanoscale. 2019;11:11048-63.

[55] Elnaggar YS. Multifaceted applications of bile salts in pharmacy: an emphasis on nanomedicine. International journal of nanomedicine. 2015;10:3955.

[56] Roger E, Lagarce F, Garcion E, Benoit J-P. Biopharmaceutical parameters to consider in order to alter the fate of nanocarriers after oral delivery. Nanomedicine. 2010;5:287-306.

[57] Ng SW, Chan Y, Chellappan DK, Madheswaran T, Zeeshan F, Chan YL, et al. Molecular modulators of celastrol as the keystones for its diverse pharmacological activities. Biomedicine & Pharmacotherapy. 2019;109:1785-92.

[58] Liang F, Hua JX. Absorption profiles of sanchinoside R1 and ginsenoside Rg1 in the rat intestine. European journal of drug metabolism and pharmacokinetics. 2005;30:261-8.

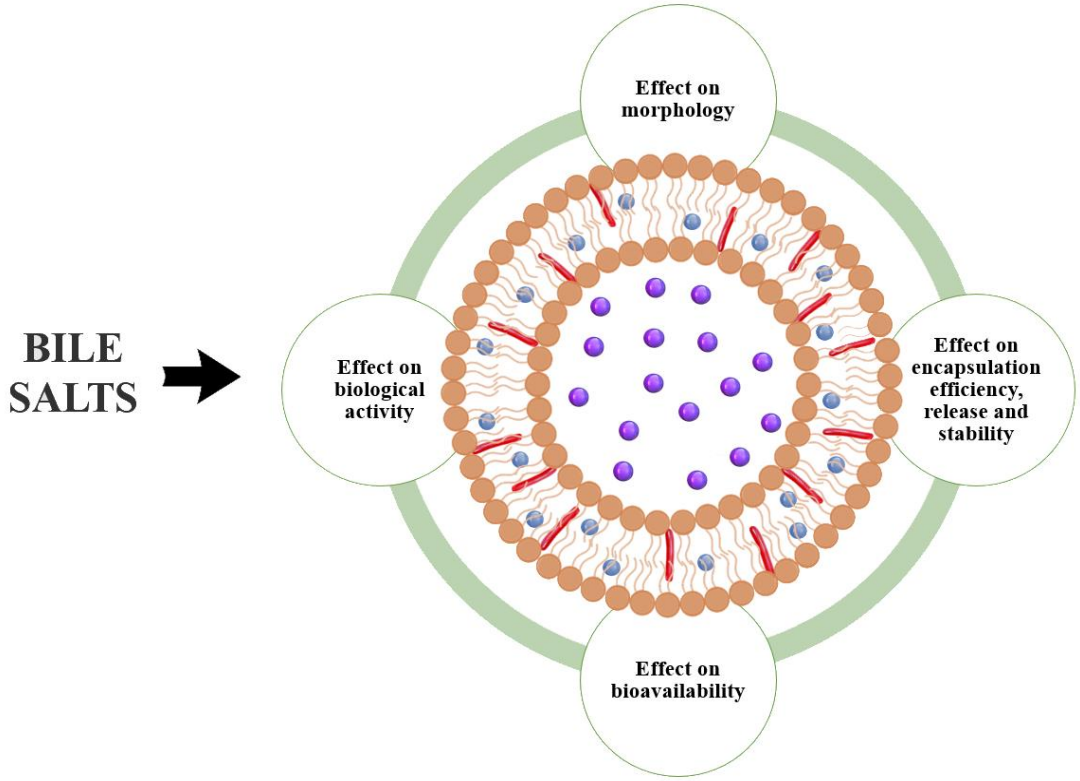
[59] Kubo Y, Nogita T, Kimura I, Chiba M, Sakakibara K. Interventional evaluation of monoammonium glycyrrhizinate-glycine/DL-methionine combination tablets in mild alopecia areata. Journal of Clinical & Experimental Dermatology Research. 2016;7:322.

[60] Huang X, Dai Y, Cai J, Zhong N, Xiao H, McClements DJ, et al. Resveratrol encapsulation in core-shell biopolymer nanoparticles: Impact on antioxidant and anticancer activities. Food Hydrocolloids. 2017;64:157-65.

[61] Can A, Kiraz A, Pala CU. In vitro Evaluation of the Antibacterial Potential of Niosome-Encapsulated Olive Leaf Extract. International Journal of Scientific and Technological Research. 2019;5:1-8.

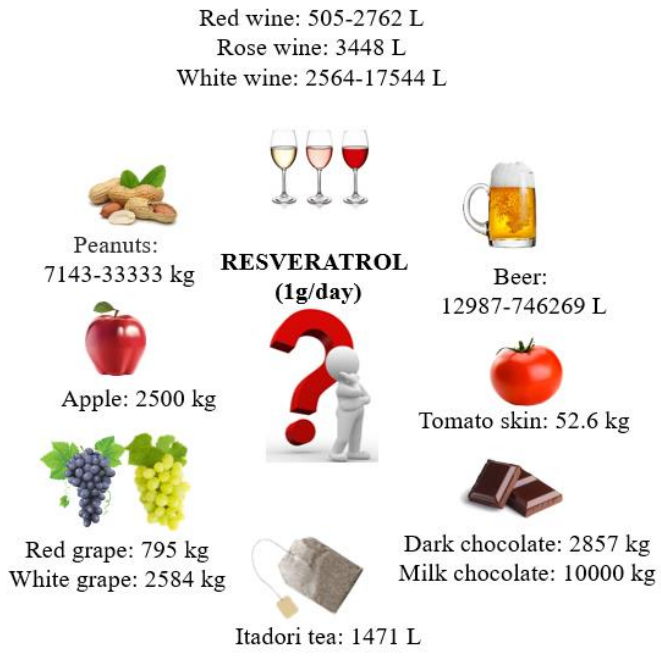
*[62] Ma L, Wang X, Wu J, Zhang D, Zhang L, Song X, et al. Polyethylenimine and sodium cholate-modified ethosomes complex as multidrug carriers for the treatment of melanoma through transdermal delivery. Nanomedicine. 2019;14:2395-408.
This study reports synergistic effects of doxorubicin-loaded ethosomes and curcumin-loaded ethosomes and both in vitro and in vivo antitumor effect. (Melanoma tumor inhibition rates of curcumin-loaded PEI-ethosomes (21.9%), doxorubicin-loaded NaC-ethosomes (35.5%) and curcumin-loaded PEI-ethosomes/ doxorubicin-loaded NaC-ethosomes (7:3 v/v) (46.38%)).

[63] Langasco R, Fancello S, Rassu G, Cossu M, Cavalli R, Galleri G, et al. Increasing protective activity of genistein by loading into transfersomes: A new potential adjuvant in the oxidative stress-related neurodegenerative diseases? Phytomedicine. 2019;52:23-31.



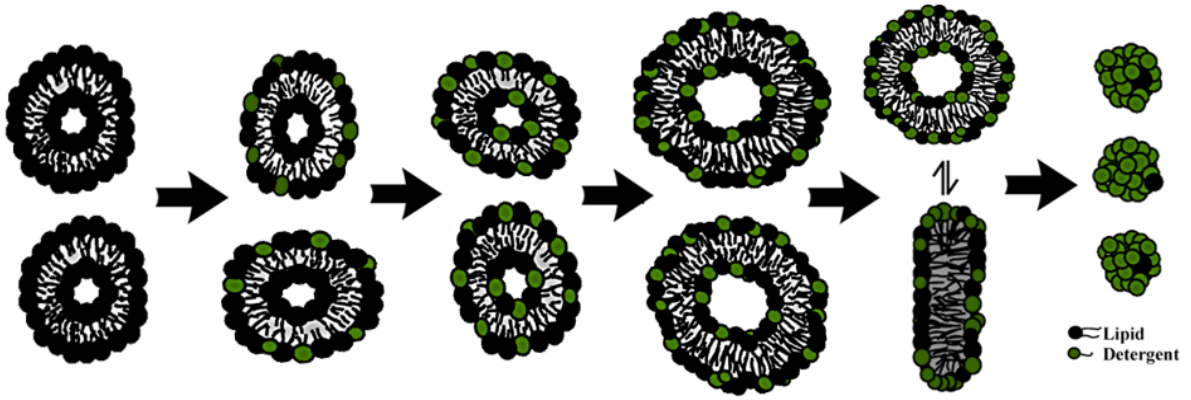
765

766 **Graphical abstract**



767

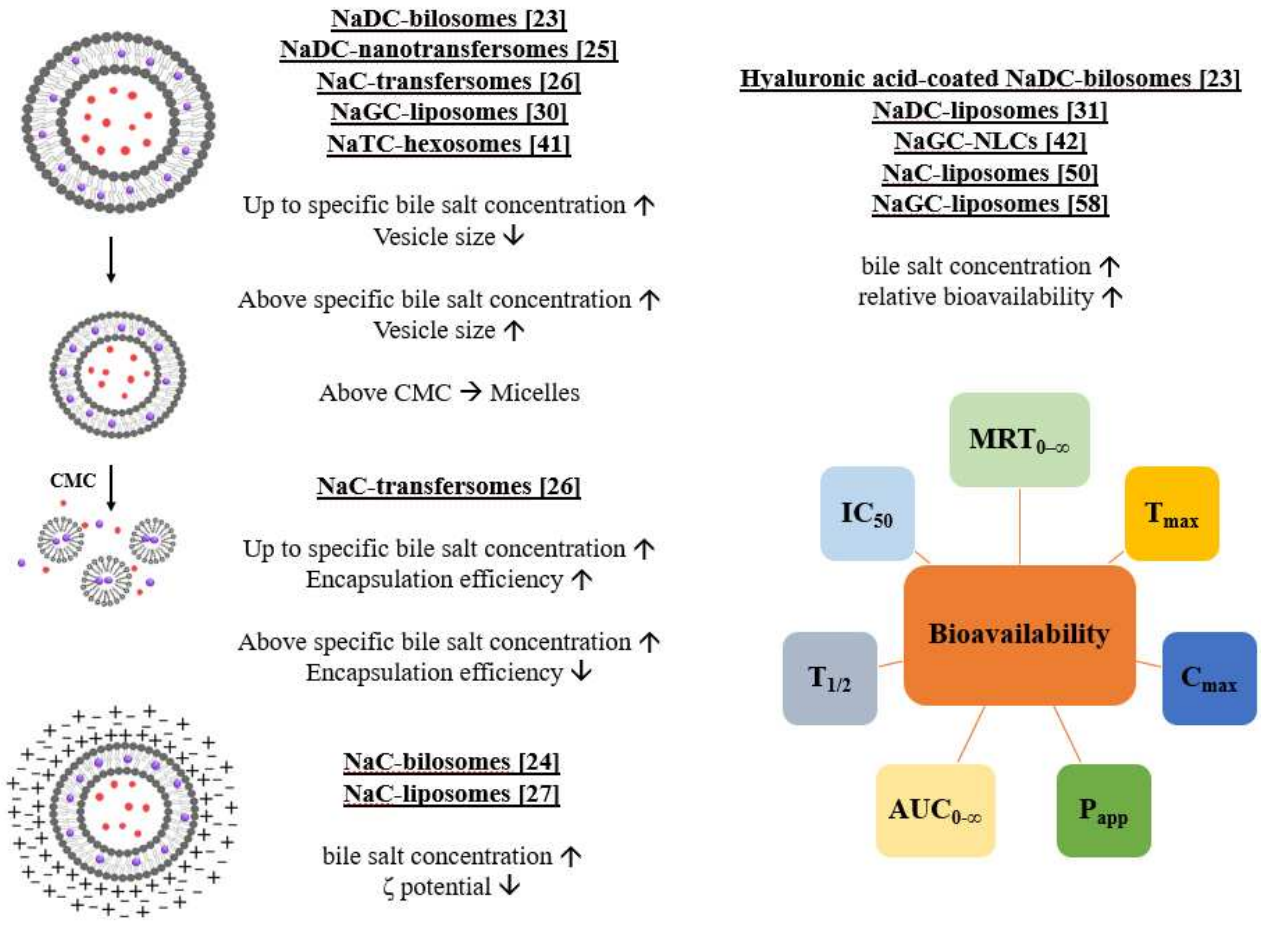
768 **Figure 1.** Quantities of food and beverages that must be consumed to reach therapeutic
769 doses of reseveratrol. If a person intends to ingest 1 g of resveratrol each day, this would require
770 consuming the depicted quantities of foods or beverages [3].



771

772 **Figure 2.** Schematic representation of the transition from vesicles to mixed micelles upon an
773 increase in bile salt concentration. Adapted from reference [18].

774



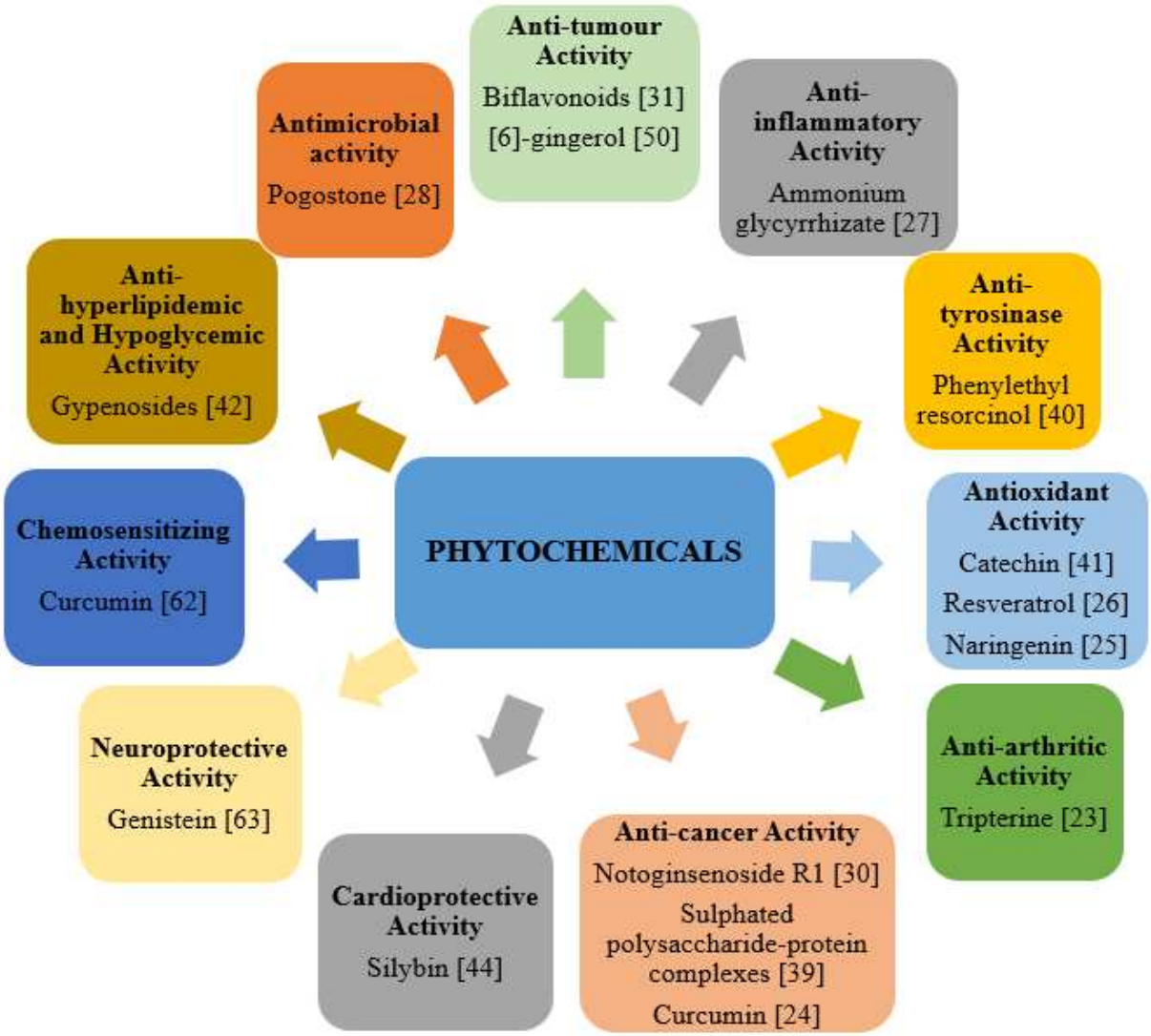
775

776 **Figure 3.** Effect of presence of bile salts on D_h, EE(%), ζ potential of lipid self-assembled
777 systems and relative bioavailability of phytochemicals.

778

779

780



781

782 **Figure 4.** Biological activities of phytochemicals.

783 **Table 1.** A summary of the self-assembled system for delivery of phytochemicals.

Self-assembled System	Application	Active Compound	Biological Activity	Bile Salts	Composition	Reference
Liposomes	Topical Application	Ammonium glycyrrhizinate	Anti-inflammatory Activity	NaC	SPC: 88mg NaC:12 mg in 6 mL water/EtOH Ammonium glycyrrhizate : 0.3% w/v of the drug in the lipid	[27]
Liposomes	Oral Application	Doxorubicin and Silybin	Cardioprotective Activity	Cholic acid	PC: 40 mg/mL DSPE-PEG- Cholic acid : 3 mg/mL Chol: 8 mg/mL Silybin:5 mg/mL Doxorubicin: 1 mg/mL	[44]
Liposomes	Oral Application	Biflavonoids extract from *Selaginella doederleinii*	Anti-tumour Activity	NaDC	Optimum: SPC: 30 mg/mL Chol: 3 mg/mL NaDC: 3 mg/mL Extract: 3 mg/mL Isomalto-oligosaccharides/lipid, 2:1, w/w)	[31]
Liposomes	Oral Application	[6]-gingerol	Anti-tumour Activity	NaC	Optimum: PC: 75 mg Isopropyl myristate: 0.08 mg NaC: 0.04 mg [6]-gingerol: 5 mg	[50]
Liposomes	Oral Application	Notoginsenoside R1	-	NaGC	Optimum: SPC: 50 mg Chol: 10 mg DSPE-PEG2000: 10 mg NaGC: 6 mg Notoginsenoside R1: 8 mg	[30]
Liposomes	Topical Application	Pogostone	-	NaC	SPC, NaC, Chol, Vitamin E, Pogostone: 0.07%	[28]
Transfersomes	Oral Application	Genistein	Antioxidant and Neuroprotective Activity	NaDC	PC: 693 mg NaDC: 297 mg Genistein: 10 mg in 50 mL water	[63]

Transfersomes	-	Resveratrol	Antioxidant Activity	NaC	Optimum: SPC: 90.7 mg NaC: 12.5 mg Resveratrol: 100 mg in 5 mL PBS buffer (pH 7.4)	[26]
Transfersomes	Topical Application	Phenylethyl resorcinol	Anti-tyrosinase activity	NaDC	SPC: 3% (w/v) CHOL: 0.5% (w/v) phenylethyl resorcinol: 0.5% (w/v) NaDC: 15% (w/w) in water: up to 100% (v/v)	[40]
Transfersomes	Oral Application	Sodium pravastatin and Naringenin	Anti-hyperlipidemic and Antioxidant Activity	NaDC	Desirability values of the numerical optimization: Omega-3 PC: 0.7908 NaDC: 1 Naringenin: 1	[25]
Bilosomes	-	Tripterine	Anti-arthritic Activity	NaDC	SPC: 80 mg DOTAP: 20 mg NaDC: 2 mg/mL Tripterine: 10 mg HA: 10 mg in water: 10 mL	[23]
Bilosomes	Oral Application	Sulphated polysaccharide-protein complexes of *Enteromorpha intestinalis*	Anti-cancer activity	NaC, NaDC and NaTDC	Chol/ Span 65: 1:5 molar ratio Active compounds: 10 mg NaC: 0.5 M in 10 mL saline solution (0.9% w/v NaCl)	[39]
Surface-modified Bilosomes	Intravenous and Topical Application	Methylene blue and Curcumin	Anticancer Activity	NaC	Optimum: L-α-PC: 1.00 wt% Chol: 0.30 wt% Pluronic P123: 0.60 wt% NaC: 0.50 wt% in water: 97.60 wt%	[24]
Hexosomes	Topical Application	Catechin	Antioxidant Activity	NaC, NaGC and NaTC	Optimum: GMO: 3.0 wt% Oleic acid: 0.5 wt% NaTC: 0.3 wt% Pluronic F108: 0.3 wt% in water: 95.9 wt%	[41]
Ethosomes	Transdermal Application	Doxorubicin and Curcumin	Cytotoxic and Chemosensitizing Activity	NaC	Lecithin: 100 mg Chol: 10 mg PEI or SC: 5 mg in 10 ml of water/ethanol	[62]
Nanostructured Lipid Carriers	Oral Application	Gypenosides	-	NaGC	Oleoyl macrogolglycerides, glyceryl monolinoleate, glycerol monostearate, SPC, Gypenosides, NaGC, Polysorbate 80	[42]

785