Nanoliposomes and Tocosomes as Nanocarriers in Food Industry

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Contributor: M. R. Mozafari

Nanoscale lipid bilayers, or nanoliposomes, are generally spherical vesicles formed by the dispersion of phospholipid molecules in a water-based medium by energy input. Nanoliposomes and tocosomes are able to provide protection and release of sensitive food-grade bioactive materials in a sustained manner. They are being utilized for the encapsulation of different types of bioactive materials (such as drugs, vaccines, antimicrobials, antioxidants, minerals and preservatives), for the enrichment and fortification of different food and nutraceutical formulations and manufacturing of functional products.

Keywords: encapsulation ; food technology ; Mozafari method ; nutraceuticals ; nanoliposome ; supplements ; tocosome

1. Introduction

Encapsulation and/or entrapment of bioactive compounds is a process in which small solid particles, or droplets of liquids or gases, are separated from other particles and from the external medium using a thin film or a vesicular system [1][2]. This process can mainly be physical (e.g., encapsulation or entrapment by colloidal carriers), chemical (e.g., by chemical conjugation of active material to the carrier molecules) or electrostatic adsorption to the surface of metal particles [1][2]. A wide range of coating materials can be employed in the field of food and nutraceuticals. These include celluloses, gums, lipids, phospholipids and proteins [3][4]. Depending on the carrier material and the process method used, encapsulation may provide several advantages to the encapsulated compounds and the finished product as well. Some of these advantages and benefits are listed in **Figure 1**.

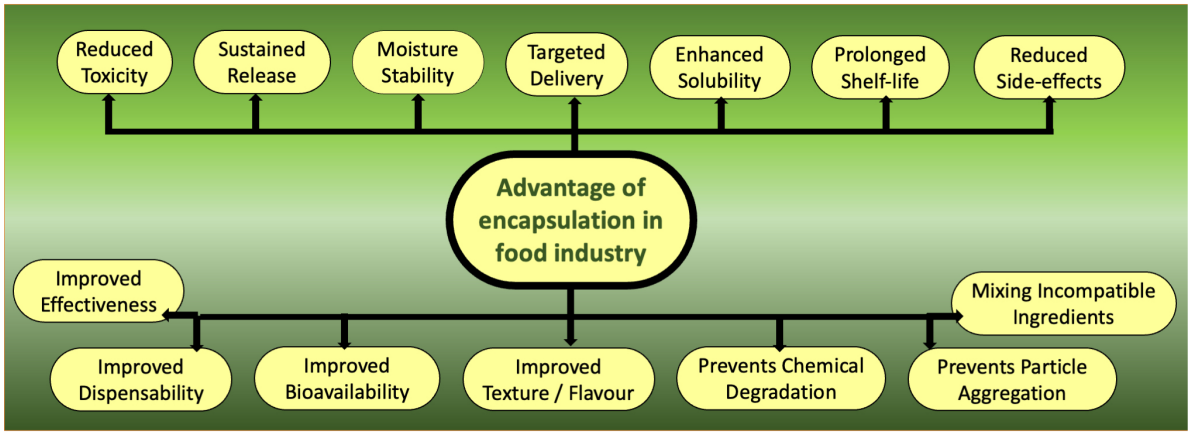

Figure 1. Advantages and benefits of using encapsulation in the food and nutraceutical industries.

In the nutraceutical, dairy and food industries, encapsulation technology is employed to stabilize sensitive components, control the release of core material and to physically separate reactive or incompatible ingredients and thereby increasing product shelf life [5][6]. Encapsulation techniques can be applied to protect the structure and function of food compounds and nutraceutical material and to improve their bioavailability. Nanovesicles and nanocarriers offer the food processor several advantages by ensuring against nutritional loss, incorporating time-release or pulsative mechanisms into the

formulation, masking or preserving aromas and flavors, and transforming liquids into the more easily handled solid products [5][6].

2. Nanoliposome Technology

Nanoliposome is a spherical or oval vesicle consisting of a phospholipid bilayer (or two or more bilayers separated by aqueous interphases) entrapping a central liquid core [7][8]. During the vesicle formation process, the hydrophilic material becomes entrapped or encapsulated within the aqueous regions (including the central core), while the hydrophobic molecules are incorporated in the bilayer membrane(s) or the lipidic domains of the vesicles. Release of the entrapped compounds can be either a gradual process resulting from diffusion through the bilayers, or almost instantaneous as a result of vesicle disruption caused by changes in pH, osmotic pressure, ionic strength or temperature. The phospholipid vesicles are ideal systems for the entrapment of different types of therapeutic and diagnostic agents (theranostics), vaccines, nucleic acid drugs and minerals (e.g., Ca^{2+}, Mg^{2+}) separately or in combination [9][10][11]. Nanoliposomes are also being considered for gene therapy applications as well as treatment of metabolic disorders [12][13]. In addition, there are a number of potential applications for nanoliposomes in the food, feed and nutraceutical sectors as well. They are able to protect sensitive material from degradation, allow incompatible ingredients to be encapsulated together, confine unpleasant odors and prevent unpleasant flavors from interacting with taste buds. Increased efficacy of the encapsulated food additives may allow significant reductions in the required amounts of these additives. The controlled release property of nanoliposomes may allow compounds such as enzymes or antimicrobial agents to be added much earlier than their action is required, without adverse effects. Applications of lipid vesicles, including nanoliposomes, in the pharmaceutical and cosmetics industries are widespread. However, issues of food safety and cost-effectiveness have limited their applications in the food systems. Nevertheless, the application of specialized techniques for large-scale manufacture of the lipid-based vesicles, and commercially available lecithin ingredients of relatively modest cost, may solve these problems [14][15].

There is a range of synthetic and natural phospholipid ingredients available for the formulation of lipid vesicles. The most common phospholipid molecule, which is employed in nanoliposome preparation, is phosphatidylcholine (PC). The cylindrical-shaped PC molecule aggregates into bilayer planar sheets, reducing the thermodynamically unfavorable interactions between the bulk aqueous phase and the hydrocarbon chains (hence reducing the level of energy) [14]. This energy-minimization results in any hydrophobic material in the aqueous media being internalized by the bilayer membrane. Nevertheless, the bilayer arrangement still has exposed hydrophobic fatty acids at the edges of the planar sheet. To completely eliminate the unfavorable contact between these fatty acids and the water molecules, the sheet folds to bring the edges together to form a closed vesicle, hence enclosing a portion of the aqueous phase, which becomes the central core of the vesicle. Molecules dissolved in the aqueous phases may become trapped within the carrier system during this process. Consequently, nanoliposomes and tocosomes have the capacity to encapsulate both hydrophilic and hydrophobic materials within a single structure. Although the vesicular arrangement is at the minimum thermodynamic energy level [16], for vesicle formation to occur the system has first to be supplied with a minimum quantity of energy called “the activation energy”. This required energy input could be either physical, mechanical, thermal, acoustic (e.g., ultrasonication), or a combination of these [17].

3. Tocosomes

Tocosome is a colloidal and vesicular bioactive carrier system, the main constituents of which are phosphate-group-bearing alpha tocopherols [18]. However, like nanoliposomes, they can also accommodate sterols, proteins and polymers in their structure. The phosphorylated form of alpha-tocopherol, known as alpha-tocopherol phosphate (TP), is present naturally in human and some animal tissues as well as in certain food compounds [19][20]. It has recently been reported that the TP molecule is naturally present in certain fruits, green vegetables, cereals, dairy products, as well as in different nuts and seeds [21]. TP is composed of a phosphate group attached to one hydrophobic chain (phytyl tail) made of three isoprene units. Di-alpha-tocopherol phosphate (T_2P), a closely related molecule to TP, is composed of two phytyl chains. However, unlike phosphatidylcholine and some other phospholipids, the hydrophobic phytyl chains of T_2P cannot align in parallel position due to the presence of bulky isoprene side-chains (**Figure 2**). Consequently, the geometric shape of T_2P molecule is conical, while the TP molecule is cylindrical-shaped (similar to PC molecule).

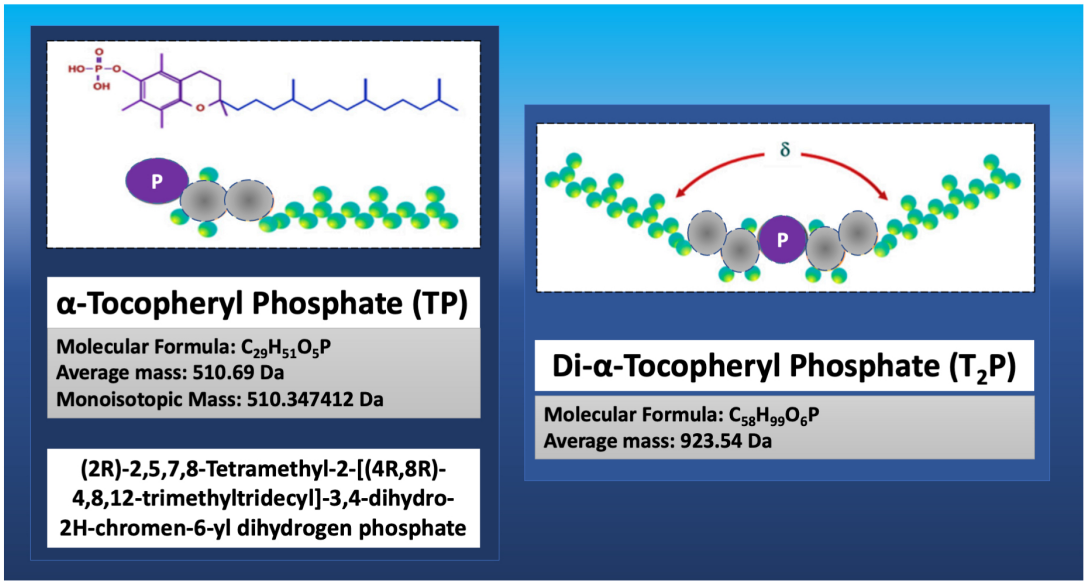

Figure 2. Chemical structure of alpha tocopheryl phosphate (TP) and di-alpha tocopheryl phosphate (T_2P). The angle of alignment of hydrocarbon chains (phytyl tails) of T_2P molecule (δ) results in an "inverted truncated cone" molecule with a critical packing parameter (cpp) of greater than 1.

Clinical investigations have shown that TP and T_2P molecules possess many health benefits such as anti-inflammatory, atherosclerotic-preventing and cardioprotective properties [22][23]. In addition, the inhibitory effect of TP molecule towards tumor invasion has also been reported [24]. Studies also showed that α-TP protected primary cortical neuronal cells from glutamate-induced cytotoxicity in vitro and reduced the levels of lipid peroxidation products in the plasma and liver of mice in vivo [25]. Tocosomal formulations containing different phospholipid molecules (in addition to TP and T_2P components), and varying combinations of cholesterol, have been employed recently for the entrapment and controlled-release of the anticancer drug 5-fluorouracil [18]. Being composed of molecules with exquisite health benefits and strong antioxidant activities, tocosomes have great potential for applications in the formulation of food and nutraceutical products, as explained in the following sections.

4. Differences between Tocosomes and Nanoliposomes

The main underlying difference between a tocosome and a nanoliposome is the distinction between their main ingredients TP/T_2P and phospholipids. Alpha-tocopherol phosphate (also called α-tocopheryl phosphate or tocopherol phosphate ester) is in fact a phosphoric acid ester of α-tocopherol (vitamin E), possessing the hydroxyl group of tocopherol [26]. The phosphate ester of alpha-tocopherol is present in certain animal and plant tissues [21]. The TP molecule can exist in either the natural form (i.e., RRR, d) or the synthetic form (i.e., all-racemic, dl), as is the case with the vitamin E molecule. One of the stereoisomers in all-racemic alpha-tocopherol molecule is 2R, 4′R, 8′R (designated as RRR) that is the only stereoisomer found in nature. Another tocopherol derivative has also been identified as the bis-tocopherol phosphate ester, or di-alpha-tocopherol phosphate (i.e., T_2P, **Figure 2**). The T_2P molecule can be synthesized through esterification of two tocopherol molecules and one phosphate molecule. A number of other synthetic derivatives of vitamin E have also been synthesized which have novel or tocopherol-related biological activities. These synthetic molecules can be converted by the esterase enzymes to the natural form of vitamin E [18][22].

Over recent years, explicit effects for each individual vitamin E-analogue have been defined at cellular level. These effects are resulted from either modulating signal transduction and/or gene expression. They possibly reflect particular interactions of each of the vitamin E-analogues with lipids, structural proteins, enzymes and/or transcription factors. There are ambiguities with respect of classification of tocopheryl-phosphate molecules and in some cases, they are erroneously referred to as phospholipids. This is while phospholipids are similar to triglycerides except that the first hydroxyl moiety of the glycerol molecule has a polar phosphate group instead of the fatty acid chain. Phospholipids are amphipathic (amphiphilic), being both hydrophilic and hydrophobic. The head group of a phospholipid molecule is hydrophilic and its fatty acids (acyl chains) are hydrophobic. The phosphate moiety of the phospholipid head group is anionic (possessing negative zeta potential). Phospholipids are a class of lipids, which are a major ingredient of all bio-membranes. Each phospholipid molecule contains a diglyceride, a phosphate group, and in addition a simple organic molecule such as a

choline moiety. A phospholipid molecule can also be defined as a lipid, which in its simplest form is composed of a glycerol bonded to two fatty acid chains and a phosphate-bearing group [22][23][24].

It is noticeable that while definition of a phospholipid molecule necessitates presence of a phosphate group, two fatty acid tails and a glycerol linker, the tocopherol phosphates are composed of a chroman head (with two rings: one phenolic and the other heterocyclic) and a phytyl tail with 3 isoprene side-chains. Moreover, tocopherols and their derivatives (i.e., TP and T_2P molecules) do not contain glycerol group, that is an indispensible chemical part of phospholipids [27]. Chemical structure of a tocopheryl phosphate molecule, in comparison with a phospholipid molecule, is depicted in **Figure 3**. Although differences in the chemical constituents of nanoliposomes and tocosomes are undeniable, these carrier systems behave pretty much the same with respect of their drug delivery mechanisms and release behavior. They are both bilayer colloidal systems composed of amphiphilic molecules and as such can be utilized for the encapsulation, entrapment and controlled release of different types of bioactive compounds. Consequently, both of these carrier systems can be used successfully in the food, feed and nutraceutical industries for the same applications keeping in mind that the ingredients of tocosomes possess more health benefits compared to phospholipids [18][8].

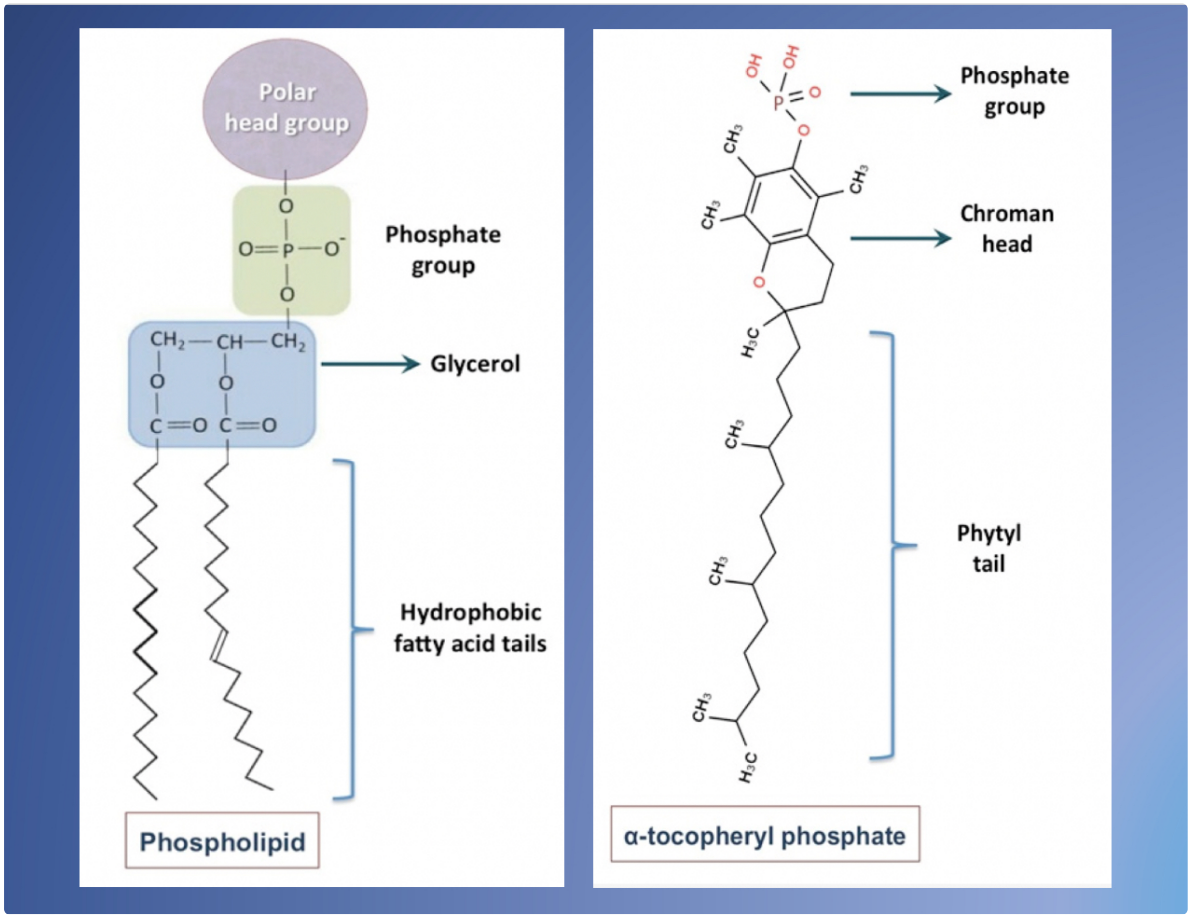

Figure 3. Comparison of the chemical structure of a tocopheryl phosphate molecule with a phospholipid molecule. A phospholipid molecule is composed a phosphate group, 2 fatty acid tails and a glycerol linker. However, alpha-tocopheryl phosphate consists of a chroman head (with two rings: one phenolic and one heterocyclic) and a phytyl tail with 3 isoprene side-chains.

5. Mechanism of Formation of Nanoliposomes and Tocosomes

Nanoliposomes and tocosomes are formed through the assembly of amphipathic molecules, mainly but not exclusively phospholipids (in the case of nanoliposome) and tocopheryl phosphates (in the case of tocosome), in an aqueous environment, as a result of hydrophilic/hydrophobic interactions and van der Waals forces [28][29]. In addition to lipids, phospholipids, TP and T_2P molecules, nanoliposomes and tocosomes may contain other molecules including proteins and carbohydrates in their structure to increase their stability or as a targeting strategy [30][31]. When the amphipathic ingredients are churned in an aqueous medium, the shape of the molecules is a major factor in determining which of a variety of different structures is most likely to be produced. The most abundant phases found in the amphiphilic systems of interest are the micellar solutions with regular (L_1) or reversed (L_2) aggregate structures, lamellar (Lα) liquid-crystalline phases, normal (H_1) or reversed (H_2) hexagonal liquid-crystalline phases and a number of different cubic liquid-crystalline phases [32], some of which are depicted in **Figure 4**.

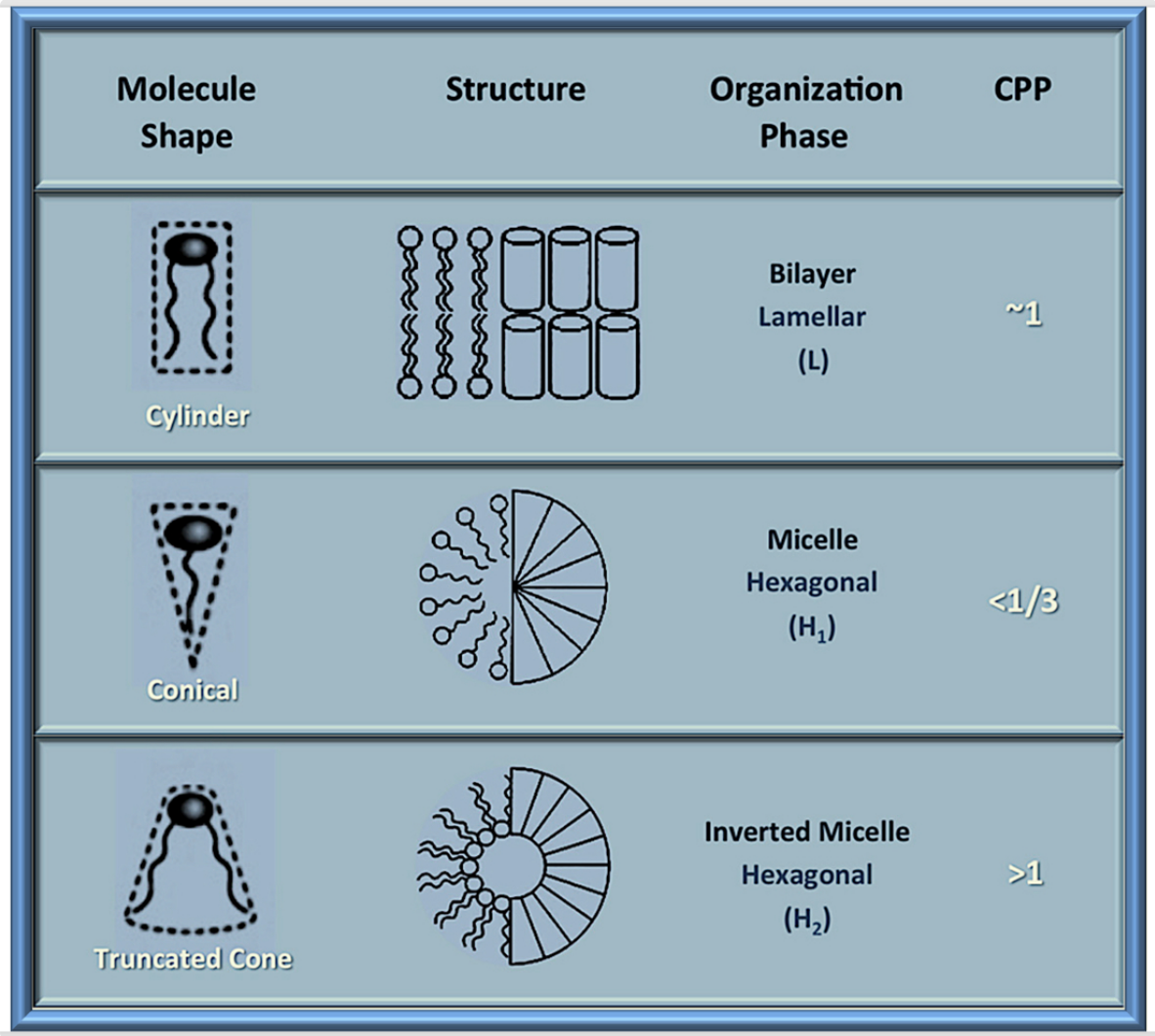

Figure 4. Schematic diagram of the effect of molecular shape on the structure of the three main types of amphiphilic aggregates. CPP: critical packing parameter (adapted with modifications from Kulkarni 2016; Lasic 1998).

When churned in aqueous media under certain conditions of temperature, pH, pressure and agitation, the amphipathic molecules arrange themselves as spherical bilayer structures via hydrophilic/hydrophobic and Vander Waals interactions and form tocosomes or nanoliposomes. As a consequence, the hydrophilic groups of the ingredient molecules face the interior and exterior aqueous phases of the vesicles while the hydrophobic fragment of each of the monolayers face each other in an attempt to avoid contact with water molecules. It should seriously be considered that, although generally suggested, formation of drug delivery vesicles, such as tocosomes and lipid bilayers, is not a spontaneous process. An adequate quantity of energy, in the right form, must be provided to the system for these carrier systems to form [17]. The underlying biophysical principles and the mechanism of formation of bioactive carrier systems, including tocosomes and nanoliposomes, are described in detail by Lasic [33][34][35] as well as Mozafari et al. [36][17]. In brief, bilayered colloidal carriers are formed when their ingredient molecules are placed in aqueous media, such as distilled water, buffer or an isotonic solution, and afterwards form bilayer vesicles once sufficient level of energy is supplied. This is a strictly required stage in order to overcome an energy barrier for curving the planar lipid/phospholipid/tocopheryl phosphate bilayers and form spherical vesicles. Input of energy (e.g., through homogenization, agitation, heating, sonication, microfluidization, etc.) results in the arrangement of the amphipathic molecules, in the form of bilayer structures, to achieve a thermodynamic equilibrium in the aqueous media [36][17][33][34][35]. Based on these principles, several methods have been invented and introduced for the manufacture of lipid vesicles and tocosomes, some of which are listed in **Table 1**. These methods are generally classified as low-energy and high-energy techniques. Low-energy consuming procedures include solvent injection, solvent diffusion and Mozafari method. High-energy consuming techniques include microfluidization, high-pressure homogenization (hot and cold), and the sonication methods [37][38].

Table 1. Some of the commonly used preparation methods of bioactive carriers (including nanoliposomes and tocosomes). From References [2][36][17][31][37][38].

Method	Advantages	Disadvantages
Thin-film hydration method	High solubility of ingredients in the initial stage of the process	Use of potentially toxic solvents, time consuming, difficult to scale-up
Ethanol/ether injection	Simple procedure	Organic solvent residue, nozzle blockage in ether system, time consuming, sterilization issue
Reverse phase evaporation	Simple design, acceptable encapsulation efficiency	Not suitable for the encapsulation of sensitive material due to large quantity of organic solvent use, time consuming, sterilization issue
Microfluidisation	Control of particle size, large volume manufacture in a continuous and reproducible manner	Employment of high pressures (up to l0,000 psi)
Supercritical Fluid Process (SFP)	Control of particle size, possibility of in situ sterilization, low organic solvent consumption	High cost, low yield, high pressure up to 350 bar used
Dual asymmetric centrifugation	Simple method, yields products with narrow size distribution, high encapsulation efficiency	Not suitable for bulk production, high pressure and high shear force
Sonication	Simple and fast technique	Overheating of the sample causing degradation, sonicator tips releases metal particles into the product
Heating Method	Organic solvent free, scalable	High temperature requirement
Mozafari Method	Simple design, safe and mild procedure, organic solvent free, easily scalable	New method, Reproducibility need to be attested under different conditions
Binary Nanodispersions	Organic solvent free, not requiring secondary emulsifier	Requires ultrasonication

6. Applications in the Food Industry

There has been less development of nanoliposome and tocosome technology in the food and diet industries compared with that seen in the pharmaceutical and cosmetics industries. Despite this, it is believed that the agrifood industry has the largest number of potential applications for nanobiotechnology [39]. The limited development to date has not been due to a lack of potential applications, but to difficulties in finding safe, low-cost ingredients and economical processing methods suitable for producing large volumes of nanoliposomes/tocosomes with batch-to-batch consistency. The recent development of Mozafari method [40][41] and pro-liposome techniques [42] offers possible solutions to many of the food processing problems. Moreover, ongoing research into the application of cost-effective commercial lecithin ingredients may lead to suitably low product costs [43].

The application of tocosomes and nanoliposomes as potential carriers to encapsulate and deliver food ingredients and nutraceutical compounds is relatively an innovative technology. Studies thus far indicate the potential of carrier systems for improving the flavor of ripened cheese using accelerated methods, the targeted delivery of functional food ingredients, the synergistic delivery of tocopherols and ascorbic acid for enhancing antioxidant activities in foods, and the stabilization

of minerals, such as calcium and iron, in milk and other drinks [44]. In the food, diet and nutraceutical industries, nanoliposomes and other lipidic carriers have been employed to encapsulate flavoring and nutritive agents. They have also been suitable candidates to entrap and deliver antimicrobial preservatives in order to improve product shelf life.

References

1. Bratovcic, A.; Suljagic, J. Micro-and nano-encapsulation in food industry. Croat. J. Food Sci. Technol. 2019, 11, 113–121.
2. Jafari, S.M. Lipid-Based Nanostructures for Food Encapsulation Purposes (Vol. 2); Academic Press: Cambridge, MA, USA, 2019.
3. Costa, S.S.; Machado, B.A.S.; Martin, A.R.; Bagnara, F.; Ragadalli, S.A.; Alves, A.R.C. Drying by spray drying in the food industry: Micro-encapsulation, process parameters and main carriers used. Afr. J. Food Sci. 2015, 9, 462–470.
4. Srivastava, Y.; Semwal, A.D.; Sharma, G.K. Application of various chemical and mechanical microencapsulation techniques in food sector-A. review. Int. J. Food Ferment. Technol. 2013, 3, 1–13.
5. Cerqueira, M.A.; Pinheiro, A.C.; Ramos, O.L.; Silva, H.; Bourbon, A.I.; Vicente, A.A. Advances in food nanotechnology. In Emerging Nanotechnologies in Food Science; Busquets, R., Ed.; Elsevier: Oxford, UK, 2017; pp. 11–38.
6. Yurdugul, S.; Mozafari, M.R. Recent advances in micro-and nanoencapsulation of food ingredients. Cell. Mol. Biol. Lett. 2004, 9, 64–65.
7. Mozafari, M.R.; Khosravi-Darani, K.; Borazan, G.G.; Cui, J.; Pardakhty, A.; Yurdugul, S. Encapsulation of food ingredients using nanoliposome technology. Int. J. Food Prop. 2008, 11, 833–844.
8. Khorasani, S.; Danaei, M.; Mozafari, M.R. Nanoliposome technology for the food and nutraceutical industries. Trends Food Sci. Technol. 2018, 79, 106–115.
9. Lokhande, S.S. Liposome drug delivery: An update review. Pharma Sci. Monit. 2018, 9, 188–202.
10. Mozafari, M.R.; Zareie, M.H.; Piskin, E.; Hasirci, V. Formation of supramolecular structures by negatively charged liposomes in the presence of nucleic acids and divalent cations. Drug Deliv. 1998, 5, 135–141.
11. Zareie, M.H.; Mozafari, M.R.; Hasirci, V.; Piskin, E. Scanning tunnelling microscopy investigation of liposome-DNA-Ca2+ complexes. J. Liposome Res. 1997, 7, 491–502.
12. Arya, M.; Yale University. Compositions and Methods for Treating Metabolic Disorders. U.S. Patent Application 15/781,606, 2017.
13. Mozafari, M.R.; Omri, A. Importance of divalent cations in nanolipoplex gene delivery. J. Pharm. Sci. 2007, 96, 1955–1966.
14. Edris, A.E. Formulation and shelf life stability of water-borne lecithin nanoparticles for potential application in dietary supplements field. J. Diet. Suppl. 2012, 9, 211–222.
15. Mozafari, M.R.; Johnson, C.; Hatziantoniou, S.; Demetzos, C. Nanoliposomes and their applications in food nanotechnology. J. Liposome Res. 2008, 18, 309–327.
16. Martin, F.J. Pharmaceutical manufacturing of liposomes. Drugs Pharm. Sci. 1990, 41, 267–316.
17. Mozafari, M.R. Nanoliposomes: Preparation and analysis. In Liposomes; Humana Press: Totowa, NJ, USA, 2010; pp. 29–50.
18. Mozafari, M.R.; Javanmard, R.; Raji, M. Tocosome: Novel drug delivery system containing phospholipids and tocopheryl phosphates. Int. J. Pharm. 2017, 528, 381–382.
19. Azzi, A. Tocopheryl phosphate, a novel natural form of vitamin E: In vitro and in vivo studies. FASEB J. 2006, 20, LB79–LB80.
20. Gianello, R.; Libinaki, R.; Azzi, A.; Gavin, P.D.; Negis, Y.; Zingg, J.M.; West, S.M. α-Tocopheryl phosphate: A novel, natural form of vitamin E. Free Radic. Biol. Med. 2005, 39, 970–976.
21. Ogru, E.; Gianello, R.; Libinaki, R.; Smallridge, A.; Bak, R.; Geytenbeek, S.; West, S. Vitamin E phosphate: An endogenous form of vitamin E. In Free Radicals and Oxidative Stress: Chemistry, Biochemistry and Pathophysiological Implications; Galaris, D., Ed.; Medimond Pub. Inc.: Bologna, Italy, 2003; pp. 127–132.
22. Munteanu, A.; Zingg, J.M.; Ogru, E.; Libinaki, R.; Gianello, R.; West, S.; Azzi, A. Modulation of cell proliferation and gene expression by α-tocopheryl phosphates: Relevance to atherosclerosis and inflammation. Biochem. Biophys. Res. Commun. 2004, 318, 311–316.

23. Libinaki, R.; Tesanovic, S.; Heal, A.; Nikolovski, B.; Vinh, A.; Widdop, R.E.; Ogru, E. Effect of tocopheryl phosphate on key biomarkers of inflammation: Implication in the reduction of atherosclerosis progression in a hypercholesterolaemic rabbit model. Clin. Exp. Pharmacol. Physiol. 2010, 37, 587–592.
24. Saitoh, Y.; Yumoto, A.; Miwa, N. α-tocopheryl phosphate suppresses tumor invasion concurrently with dynamic morphological changes and delocalization of cortactin from invadopodia. Int. J. Oncol. 2009, 35, 1277–1288.
25. Nishio, K.; Ishida, N.; Saito, Y.; Ogawa-Akazawa, Y.; Shichiri, M.; Yoshida, Y.; Niki, E. α-Tocopheryl phosphate: Uptake, hydrolysis, and antioxidant action in cultured cells and mouse. Free Radic. Biol. Med. 2011, 50, 1794–1800.
26. Mukherjee, S.; Lekli, I.; Das, M.; Azzi, A.; Das, D.K. Cardioprotection with α-tocopheryl phosphate: Amelioration of myocardial ischemia reperfusion injury is linked with its ability to generate a survival signal through Akt activation. Biochim. Biophys. Acta (BBA)–Mol. Basis Dis. 2008, 1782, 498–503.
27. Kamal-Eldin, A.; Appelqvist, L.A. The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids 1996, 31, 671–701.
28. Mohammadabadi, M.R.; Mozafari, M.R. Enhanced efficacy and bioavailability of thymoquinone using nanoliposomal dosage form. J. Drug Deliv. Sci. Technol. 2018, 47, 445–453.
29. Mohammadabadi, M.R.; Mozafari, M.R. Development of nanoliposome-encapsulated thymoquinone: Evaluation of loading efficiency and particle characterization. J. Biopharm. 2019, 11, 39–46.
30. Aveling, E.; Zhou, J.; Lim, Y.F.; Mozafari, M.R. Targeting lipidic nanocarriers: Current strategies and problems. Pharmakeftiki 2006, 19, 101–109.
31. Maherani, B.; Arab-Tehrany, E.; Mozafari, M.R.; Gaiani, C.; Linder, M. Liposomes: A review of manufacturing techniques and targeting strategies. Curr. Nanosci. 2011, 7, 436–452.
32. Kulkarni, C. Lipid self-assemblies and nanostructured emulsions for cosmetic formulations. Cosmetics 2016, 3, 37.
33. Lasic, D.D. Mechanisms of liposome formation. J. Liposome Res. 1995, 5, 431–441.
34. Lasic, D.D. Novel applications of liposomes. Trends Biotechnol. 1998, 16, 307–321.
35. Lasic, D.D.; Walker, S.; Bode, C.J.; Paquet, K.J. Kinetic and thermodynamic effects on the structure and formation of phosphatidylcholine vesicles. Hepatology 1991, 13, 1010–1013.
36. Mozafari, M.R.; Mortazavi, S.M. Nanoliposomes: From Fundamentals to Recent Developments; Trafford Pub. Ltd.: Oxford, UK, 2005.
37. Katouzian, I.; Esfanjani, A.F.; Jafari, S.M.; Akhavan, S. Formulation and application of a new generation of lipid nano-carriers for the food bioactive ingredients. Trends Food Sci. Technol. 2017, 68, 14–25.
38. Wang, T.; Xue, J.; Hu, Q.; Zhou, M.; Luo, Y. Preparation of lipid nanoparticles with high loading capacity and exceptional gastrointestinal stability for potential oral delivery applications. J. Colloid Interface Sci. 2017, 507, 119–130.
39. Verma, M.L. Nanobiotechnology advances in enzymatic biosensors for the agri-food industry. Environ. Chem. Lett. 2017, 15, 555–560.
40. Colas, J.C.; Shi, W.; Rao, V.M.; Omri, A.; Mozafari, M.R.; Singh, H. Microscopical investigations of nisin-loaded nanoliposomes prepared by Mozafari method and their bacterial targeting. Micron 2007, 38, 841–847.
41. Poudel, A.; Gachumi, G.; Wasan, K.M.; Dallal Bashi, Z.; El-Aneed, A.; Badea, I. Development and Characterization of Liposomal Formulations Containing Phytosterols Extracted from Canola Oil Deodorizer Distillate along with Tocopherols as Food Additives. Pharmaceutics 2019, 11, 185.
42. Patel, S.; Patel, R.; Dharamsi, A. A review: Proliposomes as a stable novel drug delivery. World J. Pharm. Res. 2019, 8, 638–651.
43. Sanguansri, L.; Augustin, M.A. Microencapsulation in functional food product development. Funct. Food Prod. Dev. 2010, 1, 1–23.
44. Khanniri, E.; Bagheripoor-Fallah, N.; Sohrabvandi, S.; Mortazavian, A.M.; Khosravi-Darani, K.; Mohammad, R. Application of liposomes in some dairy products. Crit. Rev. Food Sci. Nutr. 2016, 56, 484–493.