Review

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Rita Favas , Hugo Almeida , Andreia F. Peixoto , Domingos Carvalho Ferreira , Ana Catarina Silva *

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Advances in Encapsulating Marine Bioactive Compounds Using Nanostructured Lipid Carriers (NLC) and Solid Lipid Nanoparticles (SLN) for Health Applications

R. Favas[1,2], H. Almeida[1,2,3], A.F. Peixoto [4], D. Ferreira[1,2], A.C. Silva[1,2,5,*]

[1] UCIBIO, Laboratory of Pharmaceutical Technology, Faculty of Pharmacy, University of Porto, Porto, Portugal
[2] Associate Laboratory i4HB Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Porto, Portugal
[3] Mesosystem Investigação & Investimentos by Spinpark, Guimarães, Portugal.
[3] LAQV-REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Porto, Portugal
[5] FP-I3ID (Instituto de Investigação, Inovação e Desenvolvimento), FP-BHS (Biomedical and Health Sciences Research Unit), Faculty of Health Sciences, University Fernando Pessoa, Porto, Portugal
* Correspondence: author: anacatsil@gmail.com

Abstract: With increased life expectancy and improved modern lifestyles, there has been a growing concern for health, including the prevention of disease and the improvement of physical appearance. Consumers are increasingly aware of the benefits of using natural ingredients in healthcare products and at the same time are concerned about the challenges of sustainability. In this sense, the use of marine bioactive compounds as cosmetic ingredients and in food supplements has become increasingly popular due to the benefits derived from their various properties. However, some of these compounds have limited use, mainly due to their low stability and poor aqueous solubility, and solutions need to be developed to overcome these limitations. In this sense, the use of lipid nanoparticles, such as solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC), has been explored as these carriers show ability to protect and enhance the absorption of molecules. Several studies have demonstrated the efficacy of encapsulating these compounds and incorporating them into cosmetics and food supplements. However, there is still no global legislation and safety concerns have been raised. Further clinical studies in animals and humans are needed to clarify safety issues.

Keywords: healthcare; cosmetics; food supplements; marine bioactive compounds; lipid nanoparticles

1. Introduction

With increasing life expectancy and modern lifestyles, there is a growing concern for health, including the prevention of diseases and the improvement of physical appearance. Consumers are realizing the benefits of using natural compounds in health products. [1]

The marine ecosystem provides a rich source of active ingredients that can be incorporated into cosmetics and food supplements. Among marine organisms, algae, crustaceans, bacteria and fungi stand out as promising sources of bioactive compounds with different properties, such as antimicrobial and antioxidant properties, which are promising at different stages of product development, from processing to storage and formulation.[2] Within this vast source of bioactive compounds are fatty acids, carotenoids and vitamins, each with a wide range of benefits.

Fatty acids are essential components of fats and oils, playing a crucial role in regulating various physiological processes.[3] They are categorized as monounsaturated fatty acids (MUFAs) or

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polyunsaturated fatty acids (PUFAs) based on their length, degree of unsaturation, and number of bonds. PUFAs, particularly omega-3, are the predominant type of fatty acids found in the marine environment and can be sourced from algae (especially red macroalgae), marine by-products, yeasts, bacteria, and fungi, serving as alternative options to traditional seafood sources for human consumption. [3,4]

Carotenoids are isoprenoid pigments with colors within the yellow-to-red spectrum and play a crucial role in various physiological activities.[5] They are produced by all photosynthetic organisms and obtained through diet by other organisms who cannot synthesize them.[6] Carotenoids are categorized into two main groups based on their structure and chemical composition. The first group includes the carotenes, hydrocarbons without oxygen, such as β-carotene and lycopene. The second group includes the xanthophylls, which consist of oxygenated derivatives of carotenes, such as astaxanthin, fucoxanthin, and zeaxanthin.[7]

Vitamins are crucial for proper metabolism and overall health. Because the body cannot produce enough vitamins, humans must get them from food or supplements. Vitamins are classified based on their properties, whether they are water-soluble or fat-soluble. While fat-soluble (i.e. lipophilic) vitamins, such as A, D, E, and K, are easily stored upon absorption, water-soluble (i.e. hydrophilic) vitamins, such as C and B, are washed out and not stored in the body.[8,9] Common sources of these vitamins include fish, algae, bacteria, fungi, corals, sea cucumbers, and sponges.[10–13]

Various methods and carriers have been used in the cosmetic and food sector, such as microparticles (i.e. microcapsules or microspheres) and colloidal carriers (e.g. liposomes, nanoemulsions and lipid nanoparticles).[14] Due to the lipophilic nature of some of these bioactive compounds, they cannot be incorporated into aqueous formulations. Encapsulation techniques have therefore been explored to increase the solubility, stability, and activity of these compounds.[15] They are used in the food industry to preserve components from environmental conditions, to mask unpleasant tastes, or to extend shelf life.[16] In cosmetics they can improve skin hydration, epidermal delivery and increase local compound concentration with less systemic absorption.[17] Although microencapsulation has been extensively studied, nanoencapsulation techniques have been found to resolve some limitations, leading to the production of more stable carriers that improve the absorption and activity of bioactive molecules.[16] Among these, lipid nanoparticles are gaining attention for their effectiveness in encapsulating, protecting and promoting the absorption of lipophilic molecules.[15,18,19] Notwithstanding, other bioactive marine compounds, such as polysaccharides and proteins, are being used to modify the surface of lipid nanoparticles. Polysaccharides and proteins are important macromolecules in various living organisms and are critical in promoting overall body function and balance. They serve as energy sources and are essential for molecular recognition, defense, tissue formation, energy storage, and cellular transport.[20–22] Polysaccharides are derived from different marine sources and can be categorized into marine animal polysaccharides (i.e. chitin and chitosan), marine plant polysaccharides (i.e. alginate), and marine microbial polysaccharides (i.e. glucan).[20,23,24] Fish and algae are the major source of marine proteins, with the most interesting and useful being collagen and gelatin. These polymers have been applied to increase the stability and compatibility of lipid nanoparticles in different products and processes.[25]

This review aims to provide an overview of the state of the art in the use of marine bioactive compounds encapsulated in lipid nanoparticles, namely nanostructured lipid carriers (NLC) and solid lipid nanoparticles (SLN), in cosmetics and food supplements. Regulatory and safety aspects of the use of this type of nanoparticles are also addressed.

2. Lipid Nanoparticles

Lipid nanoparticles are spherical particles composed of one or more lipids, with molecules solubilized or dispersed in the matrix and are stabilized by emulsifying agents. Depending on their internal structure and composition, these nanoparticles can be classified as solid lipid nanoparticles (SLN) or nanostructured lipid carriers (NLC).[17,26,27]

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SLN were first described and consist of one solid lipid (5-40%), one or two emulsifiers (0.5-5%), the encapsulated molecules, and water (q.s. 100%). The use of solid lipids creates a matrix that enhances physical-chemical stability and delays the release of the encapsulated molecules. The lipid matrix remains solid at both body and room temperatures and acts as a protective barrier, protecting the molecules from degradation caused by environmental factors such as light, heat, pH, and enzymes. Being composed of physiological lipids (know as Generally Recognized as Safe - GRAS), this type of lipid nanoparticles are biocompatible, exhibit good permeation through various body tissues, and can be easily incorporated into a different pharmaceutical dosage forms for topical, oral and parenteral administration.[19,26,27] However, SLN have limitations, mainly related to a low capacity to encapsulate molecules in the lipid matrix and excessive crystallization of the lipids during storage, leading to leakage of the encapsulated molecules. Thereby, more advanced nanocarriers derived from SLN have been developed, the nanostructured lipid carriers (NLC), which consist of a solid matrix resulting from the combination of a solid lipid and a liquid lipid, the former in a higher percentage. Compared to SLN, NLC have been shown to have a higher loading capacity and a greater ability to prevent the release of molecules during storage by inhibiting or reducing the crystallization of lipids. These advantages have been attributed to the presence of the liquid lipid, which results in a more disorganized lipid matrix with more space to accommodate molecules. [17,26–30]

Interested readers can consult the references provided for very comprehensive reviews of the characteristics of SLN and NLC.

2.1. Relevant Studies with Bioactive Marine Compounds in SLN and NLC

Given the various applications of marine bioactive compounds in health care and the possibility of encapsulating them in SLN and NLC to overcome their main limitations, the analysis of published studies will provide good prospects for their potential use. Although most of the research focuses on the encapsulation of these compounds, some studies also present information on their potential health applications.

Additionally, marine bioactive compounds such as chitosan, alginate, and gelatin have been tested as coating polymers to modify the surface of SLN and NLC. They can increase their stability, performance, delivery efficiency, absorption rate and biocompatibility.[25] Table 1 provides a summary of the key findings from the most relevant studies in this area.

Table 1. Relevant studies and applications of the main bioactive marine compounds encapsulated in SLN and NLC.

Marine bioactive compound	Type of lipid nanoparticle	Type of study	Relevant results	Healthcare application	References
Docosahexaenoic and α-linolenic acid	SLN	*In vitro*	Docosahexaenoic acid **Particle size:** 100 ± 1.8 nm **PDI:** 0.220 ± 0.020 **EE:** 100% α-linolenic acid **Particle size:** 842.2 ± 1.3 nm **PDI:** 0.126 ± 0.017	Food supplement	[31]

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			EE: 77%		
Docosahexaenoic acid	NLC	*In vitro* and *in vivo*	**Particle size:** 163.7 ± 2.0 nm **ZP:** 40.1 ± 1.3 mV **PDI:** 0.118 ± 0.01 **EE:** 78.13 ± 1.85% **DL:** 28.09 ± 0.48% **DPPH:** 0.57 ± 0.03	Food supplement	[32]
β–carotene	NLC	*In vitro* and *in vivo*	**Particle size:** 222.8 ± 87.3 nm **ZP:** -43.46 ± 1.74 mV **PDI:** 0.666 **EE:** 23.96 ± 3.13% 34% *in vivo* penetration of β–carotene in deeper skin layers in humans	Cosmetic	[33]
		In vitro	**Particle size:** 166.3 ± 0.19 nm **ZP:** -26.9 ± 0.17 mV **PDI:** 0.35 ± 0.1 **EE:** 91.2 ± 0.15% **ABTS:** 91.47 ± 1.9% **DPPH:** 24.72 ± 0.38% **IC_{50}:** 7.0 µg/mL	Food supplement	[34]
Astaxanthin	NLC	*In vitro*	**Particle size:** 67.4 ± 2.1nm **PDI:** 0.26 **EE:** 94.3 ± 0.5% **CR:** 83.0 ± 3.4% at 48h **CP:**174.10 ± 4.38 µg/cm[2] **Retention:** 18.60 ± 1.62 µg/cm[2]	Cosmetic	[35]

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		In vitro **Particle size:** 114.4 nm **ZP:** -34.1 mV **EE:** 85.67% **ROS reduction:** 81.6% **DNA damage reduction:** 41.6% *In vivo* Protection of 6/6 mice from skin damage	*In vitro* and *in vivo*	Cosmetic	[36]
		Particle size: 145.3 nm **ZP:** -30.8 ± 0.3 mV **PDI:** 0.468 ± 0.036 **EE:** 94.8 ± 1.0% **Stability:** 28 days at 25ºC	*In vitro*		[37]
		Particle size: 142.8 ± 5.02 nm **ZP:** -32.2 ± 7.88 mV **PDI:** 0.247 ± 0.016 **EE:** 94.1 ± 2.26% **DL:** 23.5 ± 1.48% **Stability:** 6 months at 4-8 ± 2ºC	*In vitro* and *in vivo*	Food supplement	[38]
	SLN and NLC	SLN **Particle size:** 106.967 ± 2.515 nm **ZP:** −24.133 ± 0.379 mV **PDI:** 0.220 ± 0.017 **EE:** 99.99 ± 0.00% NLC **Particle size:** 117.300 ± 2.163 nm **ZP:** 23.267 ± 0.451 mV **PDI:** 0.222 ± 0.016 **EE:** 99.61 ± 0.04%	*In vitro*	Food supplement	[39]
Lycopene	NLC	**Particle size:** 166 ± 4 nm **ZP:** -74.6 ± 2.0 mV **PDI:** 0.15 ± 0.05 **EE:** 100 ± 0%	*In vitro*	Cosmetic	[40]

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			FRS: 36.6 ± 0.4mM/mg NLC **AA:** 14.1 ± 0.6mg/mL		
Fucoxanthin	NLC	*In vitro*	**Particle size:** 427.3 ± 5.7 nm **ZP:** 21.21 ± 1.23 mV **PDI:** 0.309 ± 0.11 **EE:** 90.12 ± 2.51% **DL:** 1.62 ± 0.12%	Cosmetic	[41]
	SLN		**Particle size:** 168 nm **PDI:** 0.162		[42]
Vitamin A	SLN	*In vitro* and *ex vivo*	**Particle size:** 224 nm **PDI:** 0.205 **Vitamin A concentration in the in the stratum corneum:** 3400 ng	Cosmetic	[43]
		In vitro and *in vivo*	**Particle size:** 350 nm **DR:** 54.38% up to 24h **Unabsorbed vitamin A:** 67% **PII:** 0.00		[44]
	SLN and NLC	*In vitro*	SLN **Particle size:** 223 ± 10 nm **ZP:** -25 ± 1 mV **PDI:** 0.171 ± 0.008 **EE:** 97 ± 3% **DL:** 7% NLC **Particle size:** 228 ± 7 nm **ZP:** -22 ± 1 mV **PDI:** 0.146 ± 0.007 **EE:** 90 ± 1% **DL:** 7%	Food supplement	[45]
Vitamin D	NLC	*In vitro*	**Particle size:** 87 ± 5 nm **ZP:** -12.2 ± 4.86 mV **PDI:** 0.24	Food supplement	[46]
		In vitro	**Particle size:** 110 ± 4 nm **ZP:** -17.10 ± 0.30 mV **PDI:** 0.23 ± 0.01		[47]

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		and in vivo			
Vitamin E	NLC	*In vitro*	**Particle size:** 105 to 328 nm	Cosmetic	[48]
		In vitro and in vivo	**Particle size:** 386 ± 0.00 nm **OF:** 80%		[49]
			Particle size: 82 nm **ZP:** -28.6 mV **PDI:** 0.261 **EE:** 95.83 ± 0.02%		[50]
	SLN	*In vitro*	**Particle size:** 228.2 ± 3.5 nm **ZP:** -8.92 ± 2.2 mV **PDI:** 0.34 ± 0.02 **EE:** 99.9 ± 0.1%	Food supplement	[51]
Vitamin K	SLN	*In vitro*	**Particle size:** 132 nm **ZP:** -26.83 ± 2.83 mV **PDI:** 0.17 ± 0.02 **EE:** 98%	Food supplement	[52]
Chitosan coating	NLC	*In vitro* and *ex vivo*	**Particle size:** 231.5 ± 5.8 nm **PDI:** 0.18 ± 0.01 **ZP:** 19.9 ± 0.3 mV **EE:** 96%	Cosmetic	[53]
Alginate coating	NLC	*In vitro*	**Particle size:** 321.2 ± 18.3 nm **PDI:** 0.23 ± 0.02 **ZP: -**16.0 ± 3.0 mV **EE for quercetin:** 85% **EE for alpha-tocopherol:** 92%	Cosmetic	[54]
Gelatin coating	NLC	*In vitro*	**Particle size:** 100.4 nm **PDI:** 0.36 **ZP:** -18.4 mV **EE:** 80%	Food supplement	[55]

Abbreviations: AA: antioxidant activity; ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay; CP: cumulative permeability; CR: cumulative release; DL: drug loading; DPPH: 2,2-diphenyl-1-pyridylohydrazinyl assay; DR: drug release; EE: encapsulation efficiency; FRS: free radical scavenging; NLC: nanostructured lipid carriers; OF: occlusion factor; PDI: polydispersity index; PII: primary irritation index; SLN: solid lipid nanoparticles; ZP: zeta potential.

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2.2 Omega 3

Research on SLN and NLC loaded with omega-3 mostly focuses on their use as a component of the oil phase rather than as a bioactive compound. However, a few studies have explored the latest application. For instance, Serini *et al.* encapsulated α-linolenic and docosahexaenoic acid in SLN. In the experiments, the researchers evaluated the physiochemical properties of SLN and their effect on human colorectal cancer cells (HT-29 and HCT116 cell lines). Regarding the docosahexaenoic acid and α-linolenic acid encapsulation, the particle size was 100 ± 1.8 nm and 842.2 ± 1.3 nm, the polydispersity index (PDI) was 0.220 ± 0.020 and 0.126 ± 0.017, and the encapsulation efficiency (EE) was 100% and 77%, respectively. The results showed that after encapsulation, there was a significant increase in the uptake of α-linolenic acid in cancer cells after 24 hours of incubation. The incorporation of docosahexaenoic acid was also notably higher at 24 hours when encapsulated in the SLN. At all tested concentrations, free docosahexaenoic acid and docosahexaenoic acid-loaded SLN demonstrated a time-dependent inhibition of colorectal cancer cell growth. After 48 and 72 hours, the 50 μM docosahexaenoic acid-loaded SLN demonstrated a significantly greater inhibition of cell growth ($p < 0.01$ and $p < 0.001$ in HT29 and HCT116 cells, respectively) compared to the free docosahexaenoic acid at the same concentration. In addition, α-linolenic acid exhibited a significantly inhibitory effect on the growth of HT-29 and HCT116 cells after 48 hours, with a more pronounced effect observed after 72 hours. Notably, in HT-29 cells, α-linolenic acid-loaded SLN was significantly more effective ($p < 0.02$) in inhibiting tumor cell growth than free α-linolenic acid at all the concentrations tested. In HCT116 cells, α-linolenic acid-loaded SLN demonstrated significantly enhanced efficacy than free α-linolenic acid at concentrations of 10 and 50 μM ($p < 0.05$ and $p < 0.001$, respectively).[31]

The potential of using docosahexaenoic acid encapsulated in NLC for peri-implantitis treatment was studied. The docosahexaenoic acid-loaded NLC had particle size of 163.7 ± 2.0 nm, PDI of 0.118 ± 0.01, zeta potential (ZP) of 40.1 ± 1.3 mV, and EE of 78.13% ± 1.85%. The release of docosahexaenoic acid from the NLC showed a gradual and steady pattern over 144 hours. According to the 2,2-diphenyl-1-pyridylohydrazinyl (DPPH) assay *in vitro* studies, the free-radical-scavenging rate of the docosahexaenoic acid-loaded NLC was significantly higher (0.57 ± 0.03) than that of pure docosahexaenoic acid (0.17 ± 0.003, $p < 0.001$). Moreover, the docosahexaenoic acid-loaded NLC exhibited a superior inhibitory effect on the expression of cellular inflammatory factors compared to the pure form. *In vivo* studies on rats also showed that the docosahexaenoic acid-loaded NLC group displayed the most effective suppression of gingival inflammation after a 2-week reagent treatment.[32]

2.3. β–Carotene

In the study conducted by Maretti *et al.* on encapsulated β-carotene, two different NLC formulations were prepared, with particle sizes ranging between 200 and 800 nm. The researchers evaluated the skin penetration of these carriers in humans (n = 4) by measuring the concentration of β-carotene in the stratum corneum. The results showed that pure β-carotene was mainly retained in the outer layers of the stratum corneum (45%). In contrast, one of the β-carotene-loaded NLC formulations demonstrated improved penetration into deeper skin layers, with 34% of β-carotene detected. The characterization studies revealed that the β-carotene loaded NLC were spherical and showed a particle size of 222.8 ± 87.3 nm, PDI of 0.666, ZP of -43.46 ± 1.74 mV, and EE of 23.96 ± 3.13%.[33]

The application of β-carotene in the food industry was also investigated by Rohmah *et al.*. The study aimed to evaluate the bioavailability and antioxidant activity of β-carotene-loaded NLC. For the experiments, was used an *in vitro* gastrointestinal tract model to simulate the biological course of ingested food through mouth, gastric, and intestinal phases. The particle size was 166.3 ± 0.19 nm, the PDI was 0.35 ± 0.1, the ZP was -26.9 ± 0.17 mV, and the EE was 91.2 ± 0.15%. The results showed that NLC have a superior capacity to encapsulate β-carotene compared to the other tested systems, such as a β-carotene emulsion, a β-carotene-tween 80 phosphate buffered solution, and a β-carotene

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and phosphate buffered solution ($p < 0.05$). After 4 hours of incubation, the release of β-carotene in the small intestine was significantly higher for the β-carotene-loaded NLC (233 μg/mL) compared to the other formulations, indicating that NLC effectively released the β-carotene in the small intestine, reducing degradation in the digestive tract. Furthermore, the bioavailability of β-carotene-loaded NLC (60.7%) in the intestine exceeded the ones obtained from the emulsion (34.1%), the β-carotene-tween 80 solution (23.4%), and β-carotene-phosphate buffered solution (8.7%). In terms of antioxidant activity, β-carotene-loaded NLC exhibited moderate to strong antioxidant properties, with the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and DPPH assay values of 91.47 ± 1.9 and 24.72 ± 0.38%, respectively. Additionally, the IC_{50} of the β-carotene-loaded NLC was 7.0 μg/mL, while the pure β-carotene solution (standard) was 10.0 μg/mL, indicating enhanced antioxidant activity for the encapsulated β-carotene. The study also evaluated the radical scavenging activity during the digestive process and consistently demonstrated that β-carotene-loaded NLC exhibited higher radical scavenging activity compared to the other tested systems. These results suggest that NLC may be a promising vehicle for application in functional food and beverages.[34]

2.4. Astaxanthin

Geng *et al.* optimized and assessed the stability, skin retention ability, and permeability of astaxanthin-loaded NLC. The optimization process involved adjusting different independent variables, such as the solid lipid: liquid lipid ratio, total amount of lipids, astaxanthin concentration, and type and amount of emulsifiers, while the dependent variables were particle size, PDI, and EE. For the experiments, were prepared 21 formulations, and the optimized formulation showed stable nanoparticles with a spherical surface, a particle size of 67.4 ± 2.1 nm, PDI of 0.26 and EE of 94.3 ± 0.5%. This formulation was also non-irritating, homogeneous, and exhibited excellent stability. The *in vitro* release studies indicated that the cumulative release rate of astaxanthin from the NLC was 83.0 ± 3.4% for 48 hours, while pure astaxanthin dissolved completely within 4 hours. Moreover, the antioxidant and anti-linoleic lipid peroxidation activities, were effectively preserved following encapsulation in the NLC. The results of the skin penetration studies indicated that the cumulative permeability within 24 hours was 174.10 ± 4.38 μg/cm² and the retention 18.60 ± 1.62 μg/cm² for astaxanthin-loaded NLC, while pure astaxanthin demonstrated permeability and retention of 295.20 ± 6.04 μg/cm² and 8.00 ± 1.62 μg/cm², respectively. Although the encapsulation of astaxanthin showed a disadvantageous reduction in skin permeation, the NLC formulation showed a sustained release profile compared with the free astaxanthin, reducing the amount of free astaxanthin that can permeate through the skin. However, the skin retention of astaxanthin was effectively improved by the encapsulation in the NLC, maximizing the local astaxanthin concentration in the skin while reducing its systemic delivery. These findings suggest that NLC has potential to serve as a carrier for astaxanthin, offering enhanced stability and improved skin retention capabilities.[35] Another study tested the ability of astaxanthin-loaded NLC to reduce skin damage caused by radiotherapy. In the experiments, were tested the effects of the formulation on human fibroblasts and in mice after exposure to X-irradiation. The results showed that the astaxanthin-loaded NLC had particle size of 114.4 nm, ZP of −34.1 mV, and EE of 85.67%. Furthermore, astaxanthin-loaded NLC (0.25 μg/mL) successfully reduced reactive oxygen species (ROS) production by 81.6%, decreased DNA damage by 41.6%, and lowered cell death by 62.69%, compared to the control. In the *in vivo* experiments, all six mice treated with astaxanthin-loaded NLC were protected from acute skin damage after nine days of X-irradiation. In contrast, 5 out of 6 untreated mice exhibited grade-1 skin damage. Furthermore, after 28 days of treatment, histological images indicated significant skin recovery with minimal differences in collagen fibers and sebaceous glands compared to normal skin.[36] Other researches have suggested that astaxanthin-loaded NLC could be employed in the production of food supplements.[37] For example, researchers encapsulated astaxanthin in NLC and analyzed its behavior during *in vitro* digestion. The particle size was 145.3 nm, the PDI was 0.468 ± 0.036, and the ZP was -30.8 ± 0.3 mV. The EE was 94.8 ± 1.0%, and a long-term stability study revealed that around 75% of astaxanthin remained encapsulated, after 28 days of storage at 25ºC. In addition, the antioxidant activity test demonstrated that the astaxanthin retained its biological activity after

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encapsulation in the NLC. The *in vitro* release study showed that free astaxanthin was rapidly released in the initial 6 hours, reaching approximately 90% after 12 hours, while encapsulated astaxanthin exhibited a more sustained release profile, with 88% released in 24 hours. The results of the formulation's digestion test indicated that the particle size and PDI remained stable during oral and stomach digestion. However, during intestine digestion, the particle size increased significantly ($p < 0.05$) to 227.6 ± 1.8 nm. This change was attributed to the presence of anionic components in the micelle mixture, such as bile salts, phospholipids, and free fatty acids. Additionally, the digestion process in the intestine initially occurred rapidly but then slowed down. The researchers concluded that astaxanthin was trapped in the inner oil phase and suggested that the lipid matrix might contribute to increased stability. These results suggest that NLC can be a promising delivery system for astaxanthin, offering enhanced stability and prolonged release characteristics.[37]

Recent researches have suggested that encapsulating astaxanthin in NLC or SLN can enhance the treatment of neurodegenerative diseases through the nose-to-brain route. For instance, researchers conducted comparative *in vitro* experiments to evaluate the compatibility of astaxanthin-loaded NLC and astaxanthin-loaded SLN with nasal and neuronal cells. They also assessed their antioxidant activity (neuroprotective effect) and the cellular uptake of astaxanthin. The results revealed that astaxanthin-loaded SLN particle size of 106.967 ± 2.515 nm, PDI of 0.220 ± 0.017, ZP of −24.133 ± 0.379 mV, and EE of 99.99 ± 0.00%; while the astaxanthin-loaded NLC exhibited particle size of 117.300 ± 2.163 nm, PDI of 0.222 ± 0.016, ZP of 23.267 ± 0.451 mV, and EE of 99.61 ± 0.04%. Additionally, both astaxanthin-loaded NLC and astaxanthin-loaded SLN were found to be safe for nasal and neuronal cells at concentrations up to 100 µg/mL. Regarding the neuroprotective effects, both formulations exhibited ability to inhibit neurodegenerative pathways, such as oxidative stress. Notably, the astaxanthin-loaded NLC demonstrated superior neuroprotective effects against cytotoxicity induced by aggressors in comparison to the astaxanthin-loaded SLN.[39] In addition, in the nose-to-brain route, a different study involving astaxanthin-loaded NLC was carried out to test the *in vivo* effectiveness of this approach in slowing the progression of Alzheimer's disease. The optimized astaxanthin-loaded NLC had particle size of 142.8 ± 5.02 nm, PDI of 0.247 ± 0.016, ZP of -32.2 ± 7.88 mV, and EE of 94.1 ± 2.46%, and remained stable at 4-8 ± 2°C for six months. When administered to rats with Alzheimer's disease-like symptoms, the astaxanthin-loaded NLC led to a significant decrease in oxidative stress, amyloidogenic pathway, neuroinflammation, and apoptosis, while also showing improvement in cholinergic neurotransmission compared to a free astaxanthin solution.[38]

2.5. Lycopene, Fucoxanthin and Zeaxanthin

Okonogi *et al*. developed lycopene-loaded NLC for skin use, which had particle size of 166 ± 4 nm, PDI of 0.15 ± 0.05, ZP of -74.6 ± 2.0 mV and EE of 100 ± 0%. The *in vitro* release studies indicated a biphasic profile with faster release in the first 6 hours followed by sustained release over the following 18 hours. The *in vitro* occlusion test revealed increased occlusive properties with higher lycopene loading in the NLC. Additionally, lycopene-loaded NLC exhibited higher antioxidant capacity (36.6 ± 0.4 mM/mg) compared to the placebo NLC (26.6 ± 0.1 mM/mg). The IC_{50} obtained by the DPPH assay was 14.1 ± 0.6 mg/mL for the lycopene-loaded NLC, and was 17.7 ± 0.4 mg/mL for the placebo NLC. Physical stability studies showed that the particle size, PDI, and ZP of lycopene-loaded NLC remained stable over 120 days, when stored at different temperatures (4, 30, and 40ºC). Chemical stability data suggested that encapsulation in NLC improved the stability of lycopene, particularly at lower temperatures (4ºC), which was demonstrated by the delay in lycopene degradation (0.4 µg/mL/day).[40]

Two separate studies investigated the potential use of fucoxanthin encapsulated in SLN and NLC for cosmetic applications. Cordenonsi *et al.* investigated the potential of fucoxanthin-loaded NLC to prevent skin hyperproliferative diseases, particularly psoriasis. The developed fucoxanthin-loaded NLC had a particle size of 427.3 ± 5.7 nm, PDI of 0.309 ± 0.11, ZP of 21.21 ± 1.23 mV and EE of 90.12 ± 2.51%. *In vitro* experiments on fibroblast cultures showed that up to a concentration of 5 mM fucoxanthin there was no reduction in cell viability. Additionally, fucoxanthin-loaded NLC

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decreased the expression of psoriatic markers by approximately 40%, suggesting its potential in managing skin hyperproliferation and inflammation.[41] In the other study, Lee *et al.* assessed the efficacy of fucoxanthin-loaded SLN to increase the effectiveness of sun protection. The results showed that fucoxanthin-loaded SLN had particle size of 168 nm and PDI of 0.162, and could effectively transport this active ingredient. In addition, the sun protection factor (SPF) of the fucoxanthin-loaded SLN was significantly higher (1.85 times) than that of the other formulations tested (3 different chemical sunscreens), demonstrating a remarkable sun protection enhancing effect.[42]

The only study found on zeaxanthin involved optimizing zeaxanthin-loaded SLN and zeaxanthin-loaded NLC, were the respective particle size were 179.16 ± 0.94 nm and 130.16 ± 1.58 nm; the PDI were 0.34 ± 0.01 and 0.30 ± 0.01; the ZP were -19.44 ± 1.19 mV and -21.49 ± 1.13 mV; and the EE were 81.14 ± 3.06% and 90.43 ± 2.85%.The researchers suggested that both SLN and NLC could have a wide range of applications, particularly as carriers for bioactive compounds in nutraceutical beverages.[56]

2.6. Vitamin A

The first documented studies of vitamin A-loaded SLN date back to the early 2000s. Jenning *et al.* developed vitamin A-loaded SLN and tested its efficacy in permeating porcine skin, comparing it to a vitamin A nanoemulsion. The results revealed that SLN significantly enhanced the stability of vitamin A compared to the nanoemulsion. The vitamin A-loaded SLN had a particle size of 224 nm and a PDI of 0.205. Six hours after application, a substantial concentration of vitamin A (approximately 3400 ng) was detected in the stratum corneum and the upper epidermis. In contrast, the vitamin A nanoemulsion only transported 2500 ng to the upper skin layers ($p < 0.05$). Surprisingly, after 24 hours, a change in the distribution pattern was observed. The concentration of vitamin A in the upper epidermis decreased significantly to 900 ng ($p < 0.05$), while the concentration in the deeper skin layers increased from 0 to 250 ng ($p < 0.05$). In contrast, the distribution pattern of the vitamin A nanoemulsion did not change.[43] In another study, Pople *et al.* developed vitamin A-loaded SLN with a particle size of 350 nm. The *in vitro* release studies showed that the vitamin A-loaded SLN had a prolonged release of vitamin A for up to 24 hours, with 54.38% of the compound released, compared to a 70% released observed with a vitamin A hydrogel. This extended release was attributed to the compound being embedded in the solid lipid core. In *in vitro* penetration studies, after 24 hours, the vitamin A concentration in the skin was almost 2 times higher with the vitamin A-loaded SLN formulation compared to the one obtained with the vitamin A hydrogel. Only 1.2 % of the compound was detected in the receptor compartment of the vitamin A-loaded SLN, while almost 10 times more was detected in the vitamin A hydrogel, resulting in a significantly higher amount of unabsorbed compound (around 72 %) compared to the vitamin A-loaded SLN (around 67 %). The skin hydration studies in albino rats showed that the application of the conventional vitamin A hydrogel resulted in a slight change in the thickness of the stratum corneum, while the application of the vitamin A-loaded SLN led to a substantial increase in the thickness of the stratum corneum: 3-fold compared to the hydrogel and 3.5-fold compared to the untreated skin. Additionally, the developed vitamin A-loaded SLN showed a primary irritation index of 0.00, with no observed erythema or edema.[44]

The potential use of vitamin A-loaded SLN and vitamin A-loaded NLC in the food industry has recently been investigated. The study focused on ensuring the stability and oral bioavailability of these nanoparticles under food processing conditions. Six formulations were developed for the experiments, from which two were selected for further investigation. The particle size, PDI, ZP and EE for the vitamin A-loaded SLN were 223 ± 10 nm, 0.171 ± 0.008, -25 ± 1 mV and 97 ± 3%, respectively. For vitamin A-loaded NLC, the corresponding values were 228 ± 7 nm, 0.146 ± 0.007, -22 ± 1 mV and 90 ± 1%. The results of the stability tests showed that these nanoparticles remained stable for one month after storage at room temperature (25ºC). Furthermore, exposure to typical food processing temperatures (60 and 70°C) did not significantly change the nanoparticle properties, although these temperatures being above the melting point of the solid lipid used in the nanoparticles (43°C). The nanoparticles were also tested in different media, including phosphate buffer at pH 5, highly concentrated sucrose solution, and high ionic strength sodium chloride solution. Both the vitamin A-

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loaded SLN and vitamin A-loaded NLC showed stability in all the tested media, with variations of < 10% compared to fresh formulations not exposed to the various media. Afterwards, to simulate gastric conditions, the nanoparticles were subjected to a simulated gastric environment containing an acidic pH, salts, and gastric enzymes.

Despite an increase of 0.03 in the PDI after two hours, the size of NLCs remained roughly the same. A similar situation was observed for SLNs. Furthermore, after two hours in a simulated gastric environment, approximately 80% of the encapsulated vitamin A remained intact in the SLN and NLC, suggesting their potential to deliver the compound to the intestine where it can be absorbed.[45]

2.7. Vitamin D, E and K

The potential of using NLC to improve the effectiveness of vitamin D3 in fighting breast cancer cells has been investigated. The developed vitamin D3-loaded NLC showed that a particle size of 87 ± 5 nm, PDI of 0.24, and ZP of -12.2 ± 4.86 mV. The 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay showed that vitamin D3-loaded NLC was more successful in inhibiting cancer cell proliferation compared to free vitamin D3 ($p < 0.05$), reducing cell proliferation from 49 ± 7.2% to 37 ± 5.1%, respectively. Furthermore, vitamin D3-loaded NLC increased the percentage of cells in the apoptotic phase to 40 ± 3.45% ($p < 0.05$). These findings suggest that vitamin D3-loaded NLC can improve the efficacy of chemotherapeutics in breast cancer patients.[46] Another study suggested using oral vitamin D3-loaded NLC to improve the management of inflammatory bowel disease. The vitamin D3-loaded NLC had a particle size of 110 ± 4 nm, PDI of 0.23 ± 0.01 and ZP of -17.10 ± 0.30 mV. When vitamin D3-loaded NLC was orally administered to mice, the concentration of vitamin D3 in the colon was observed to be three times higher than the basal level, persisting for at least 12 hours ($p < 0.01$). Subsequently, the researchers assessed the effects of vitamin D3-loaded NLC on intestinal inflammation. The results demonstrated that although the symptoms worsened in mice treated with free vitamin D3 and vitamin D3-loaded NLC, the group tested with the NLC experienced a progressive mitigation of clinical symptoms from day 3, and suppressed the decrease in body weight from day 6. Additionally, the histological examination of colonic tissue showed significantly reduced inflammatory manifestations, immune cell infiltration, and crypt destruction in the group treated with vitamin D3-loaded NLC ($p < 0.01$).[47]

Eiras *et al.* developed a vitamin E-loaded NLC and assessed its potential for skin application. The characterization of the NLC loaded with vitamin E showed that the particles had a consistent size in the nanometer range, with 90% having a size of less than 328 nm and 50% having a size of less than 105 nm, even after 7 months of storage. The results of *in vitro* studies indicated that the vitamin E-loaded NLC is biocompatible and non-irritant (with a score of 0.00), being suitable for skin application. From these findings, the researchers proposed using vitamin E-loaded NLC in cosmetic and dermatological formulations to improve skin hydration.[48] Latter, the same researchers conducted a more comprehensive study with this formulation, where they found no significant differences in particle size after incorporating vitamin E-loaded NLC into a hydrogel (D90= 386 ± 0.00 nm *vs*. D90= 397 ± 0.021 nm). The biocompatibility studies were assessed by the MTT assay, where it was observed that the vitamin E-loaded NLC was more cytotoxic to human keratinocytes before incorporation in the hydrogel (IC_{50} = 14.38 μg/mL *vs*. IC_{50} = 28.74 μg/mL, $p < 0.001$). *In vitro* and *in vivo* tests, revealed that vitamin E-loaded NLC demonstrated superior occlusive properties (80% *vs*. 67%) and significantly reduced the skin transepidermal water loss (TEWL) compared to a vitamin E nanoemulsion ($p < 0.05$). In this study, the researchers demonstrated the safety and hydration potential of the hydrogel containing vitamin E-loaded NLC for skin application. However, it was emphasized the need for more *in vivo* experiments over extended periods and involving more volunteers to confirm these evidences.[49] In another study, the effectiveness of vitamin E-loaded NLC was examined for its moisturizing and anti-aging properties. The optimized vitamin E-loaded NLC had a particle size of 82 nm, a PDI of 0.261, a ZP of -28.6 mV, and an EE of 95.83 ± 0.02%. The stability study revelated that the nanoparticles maintained their spherical shape and physical stability for 12 weeks, after storage at room temperature. The release profile indicated that 30% of the vitamin E was released within the first 5 hours, with 70% released after 24 hours. The vitamin E-loaded NLC

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was incorporated into a hydrogel base and *in vivo* tests were performed on 13 human volunteers over 12 weeks to assess skin moisture content and mechanical properties. In the skin irritation test, all volunteers showed no signs of erythema after 48 hours of exposure to the hydrogel containing the vitamin E-loaded NLC. However, 3 out of 13 volunteers experienced mild erythema when exposed to the vitamin E hydrogel alone. This suggests that the free form of vitamin E can be irritating to the skin, but the NLC provides a protective effect against this irritation. Additionally, throughout the 12-week study period the hydrogel containing the vitamin E-loaded NLC enhanced skin elasticity capacity (p = 0.0319) and consistently increased skin moisture levels by 80%, while a vitamin E hydrogel only resulted in a 20% increase in moisture levels ($p < 0.01$).[50] In a different approach, the researchers evaluated the effectiveness of encapsulating vitamin E in SLN to reduce the negative effects of anemia treatment. The characterization of the vitamin E-loaded SLN revealed a particle size of 228.2 ± 3.5 nm, a PDI of 0.34 ± 0.02, a ZP of -8.92 ± 2.2 mV and an EE of 99.9 ± 0.1%. The *in vitro* release studies revealed that approximately 65% of the encapsulated vitamin E was released after 24 hours in simulated gastrointestinal media at 37 °C. Furthermore, the vitamin E-loaded SLN demonstrated good hemocompatibility at various concentrations. After 3 hours of incubation, there was minimal hemolysis (less than 0.3%). Even after 24 and 48 hours, the level of hemolysis remained low, with less than 3.0% at the highest SLN concentration. It's important to note that biomaterials causing hemolysis of less than 5% are generally considered safe for human use, according to ISO/TR 7406. These results indicate their potential for clinical applications. Notably, the vitamin E-loaded SLN significantly boosted lymphocyte cell proliferation by approximately 150% and decreased DNA damage caused by the iron treatment.[51]

Regarding vitamin K, only one significant study involving the encapsulation of vitamin K1 in SLN for oral delivery was found. In the experiments, seventeen different formulations were prepared and the most effective one was chosen for further tests. The particle size, PDI, ZP and EE for the vitamin K-loaded SLN were 132 nm, 0.17 ± 0.02, and -26.83 ± 2.83 mV, 98%, respectively. This formulation was shown to be stable in a 54-hour *in vitro* release study in simulated gastric and intestinal media and after 4 months storage at 25°C. [52]

2.8. Chitosan

Almeida *et al.* aimed to evaluate the efficacy of chloroaluminium phthalocyanine-loaded NLC, optimized and coated with chitosan, in photodynamic therapy of skin cancer. Chloroaluminium phthalocyanine-loaded NLC coated with chitosan showed a particle size of 231.5 ± 5.8 nm, a PDI of 0.18 ± 0.01, a ZP of +19.96 mV and an EE of 96%. It is important to highlight that the chitosan coating resulted in larger, but equally viable, nanoparticles. *Ex vivo* studies showed that the phthalocyanine-loaded NLC coated with chitosan significantly retained the compound in the skin after 2 h (5.8 ng) and 4 h (581 ng), with no detection in the bloodstream, indicating limited systemic exposure and no potential adverse events in patients. The biocompatibility test performed on L929 fibroblasts showed that the phthalocyanine-loaded NLC coated with chitosan did not induce cytotoxicity at any of the concentrations tested, indicating that the chitosan coating did not affect the biocompatibility of the NLC. It was also observed that the phthalocyanine-loaded NLC coated with chitosan were localized around the cellular nucleus. The photodynamic therapy tests performed on BF16-F10 melanoma cells showed that phthalocyanine-loaded NLC coated with chitosan did not have toxic effects when not exposed to irradiation. However, after irradiation there was a 50% reduction in cell viability ($p <$ 0.001). These results highlight that the chitosan coating improved stability, biocompatibility, and facilitated drug passage through the skin.[53]

2.9. Alginate

Costa-Fernandez *et al.* developed an NLC co-encapsulated with vitamin E and quercetin and coated with alginate to investigate its potential to promote wound healing and to evaluate the influence of the alginate coating in enhancing the penetration of the encapsulated compounds into the skin damaged by an incision. The results showed that the alginate-coated NLC co-encapsulated with vitamin E and quercetin had a particle size of 321.2 ± 18.3 nm, a PDI of 0.23 ± 0.02 a ZP of -16.0

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± 3.0 mV and an EE of 85% **for quercetin and 92% for vitamin E.** The irritation potential of the developed alginate-coated NLC was assessed by the Hen's Egg Test - chorioallantoic membrane, showing that they did not induce bleeding, lysis or coagulation. Regarding the *in vitro* release, it was observed 44.2% of quercetin released and 32.3% of **vitamin E** released after 16 h. Furthermore, the release of vitamin E from NLC increased 1.9-fold and the release of quercetin from NLC increased 2.3-fold compared to intact skin. The TEWL was also reduced when using alginate-coated NLC co-encapsulated with vitamin E and quercetin. The results highlight the advantages of using polysaccharides, such as alginate, as bioadhesive polymers to promote the penetration of compounds into the upper layers of the skin.[54]

2.10. Gelatin

Gelatine, a natural biopolymer (i.e. a protein composed of long chains of amino acids), is widely used in the food industry because of its advantages such as non-toxicity, low cost, availability, biocompatibility, and biodegradability. Researches has shown that gelatin can stabilize nanocarriers due to its amine and carboxylic groups. Malekmohammadi *et al.* conducted a study aiming to synthesize gelatin-coated NLC to encapsulate sage extract for inhibiting microbial growth and lipid oxidation in beef burgers. The optimized sage extract-loaded NLC coated with gelatin had a particle size of 100.4 nm, PDI of 0.36, ZP of −18.4 mV, and EE of 80%. The results of the DPPH assay showed that the sage extract-loaded NLC coated with gelatin exhibited significantly higher antioxidant activity than the free extract, after 30 days of storage at 25ºC ($p < 0.05$). It also observed a higher inhibitory effect against *E. coli* compared to the free extract in the minimum inhibitory concentration (0.1 ± 0.00 *vs*. 0.2 ± 0.00 mg/mL) and the minimum bactericidal concentration (0.1 ± 0.00 *vs*. 0.3 ± 0.00 mg/mL) tests. Furthermore, incorporating the formulation into beef burgers increased their oxidation stability during 90 days of storage, at 4 and −18 °C ($p < 0.05$). The sage extract-loaded NLC coated with gelatin also effectively decreased the total counts of various bacteria, yeasts, and molds in treated beef burger samples during storage in comparison to the controls (all $p < 0.05$). The sensory tests showed no significant differences in the color, odor, texture and flavor attributes of the beef burger samples immediately after preparation. However, over time, the treated samples showed greater acceptability. These improved sensory properties were attributed to reduced proteolytic and lipolytic reactions, as well as reduced microbial activities. Overall, the study suggests that sage extract-loaded NLC coated with gelatin can be an effective preservative for extending the shelf life of beef burgers.[55]

3. Regulatory and Safety Concerns of Lipid Nanoparticles for Healthcare Applications

3.1. Cosmetics

Despite the regulatory frameworks governing the global market for nanocosmetics, the recognition of nanomaterials as cosmetic ingredients remains inconsistent across jurisdictions.[57] Consequently, each country adheres to its legal system. Given that the European Union (EU) and the United States of America (USA) represent the two most significant markets for cosmetics products, their respective regulatory frameworks are of considerable importance. However, there are notable discrepancies between the two. Prior to 2023, the USA did not require cosmetic product registration, while the EU has mandated registration since 2013, especially for products containing nanoparticles.

Furthermore, a notification to the European Commission (EC), including information on the identification, specifications, toxicological profile, and safety of the nanomaterials, is required. In cases of uncertainty regarding the safety of a nanomaterial, the EC can request an opinion from the Scientific Committee on Consumer Safety (SCCS).[58] Under the stipulations set in the EC Regulation 1223/2009, the term "nanomaterial" is defined to regulate cosmetics products as a deliberately insoluble or bio-persistent manufactured material that has external dimensions (one or more) or an internal structure within the range of 1 to 100 nanometers. On the other hand, in the USA, the Food and Drug Administration (FDA) oversees the use of nanomaterials in cosmetics through the Federal Food, Drug, and Cosmetic Act (FFDCA). However, the FDA does not have a legal definition for

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nanotechnology, does not approve the ingredients in cosmetic formulations, and does not require manufacturers to disclose the presence of nanomaterials in their products. The FDA has thus far refrained from making a definitive determination regarding the inherent safety or potential risks associated with nanotechnology.[59] In consequence, the FDA has created the National Nanotechnology Initiative (NNI) and the Nanotechnology Task Force (NTF) intending to assess the restrictions required for nanotechnology products. These entities have published two documents addressing the safety issues of nanotechnology and cosmetics, which merely make recommendations. The first document is concerned with the determination of a material's classification as a nanomaterial based on the size of the particles and their properties/phenomena. In particular, it considers whether the material or final product is: a) intentionally designed to have at least one external dimension, internal dimension, or surface structure within the nanoscale range (approximately 1 nm to 100 nm); b) meant to exhibit properties or phenomena, including physical, chemical, or biological effects, attributed to its size, even if these dimensions extend beyond the nanoscale range to one micrometer (1000 nm). It is important to highlight that the properties of nanomaterials relevant to safety, effectiveness, performance, quality evaluation, public health impact, and product regulatory status can be linked to materials with one or more dimensions larger than the 1–100 nm range. In a succeeding document, the FDA advises a thorough assessment of safety by characterizing the nanomaterial itself and examining a wide range of chemical and physical properties. This involves evaluating the toxicity, absorption, distribution, metabolism, and excretion of the particles.[57,60]

The scientific community has been debating whether the use of insoluble nanoparticles in cosmetics poses a health risk. The results so far have been inconclusive due to inconsistent findings and a lack of long-term toxicological studies. Safety evidence originates mainly from the EU, which has strict regulations.[60] When it comes to using SLN and NLC in cosmetics, these can be classified as nanomaterials according to EU standards, as they consist of water-insoluble materials, such as solid and liquid lipids. However, these lipids are similar to the body's natural lipids. They can readily adhere to and interact with the outer layer of the skin, causing lipid rearrangement and allowing the encapsulated compounds to penetrate deeper layers of the skin.

Furthermore, the nanoscale dimensions of the particles facilitate enhanced adhesion and surface contact area, which in turn improves the permeation of the compound through the skin.[17] Accordingly, they are regarded as soluble substances.[60] Moreover, the utilization of SLN and NLC in topical cosmetics has been documented to diminish the probability of these carriers causing systemic toxicity.[29] In light, SLN and NLC in cosmetic products are not subject to any objections or safety concerns, as stipulated by the pertinent legislation.

3.2. Food Supplements

The regulation No 2015/2283 of the EC defines engineered nanomaterials in food as novel foods, categorizing them as intentionally produced materials with dimensions of approximately 100 nm or less. These materials can have discrete functional parts at the surface or internally, as well as structures, aggregates, or agglomerates, which may maintain nanoscale-specific properties despite having a size above 100 nm. Such properties may be related to specific physicochemical properties that differ from those of the non-nano form of the same material and/or to the large specific surface area of the materials considered. Nevertheless, the EU legislation applicable to novel food is the same as to all food (Regulation EC No 178/2002).[61] Concerning this legislation, certain safety stipulations give rise to uncertainty, particularly the potential adverse effects of the novel food on the health of both the current and future generations.[62] Regarding long-term effects, there is a gap in data to substantiate the safety of nanoparticles. However, the European Food Safety Authority (EFSA) has issued an exceptional document that provides an overview of the risk assessment of nanomaterials in food and the requisite information.[63] The document elucidated that lipid nanoparticles can facilitate the delivery of select food components, particularly those exhibiting pronounced lipophilicity. Moreover, the components of the nanoparticle can be derived from naturally occurring body lipids or approved food additives, such as emulsifiers. The primary safety concerns pertain to

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the extent of degradation of the encapsulation materials within the gastrointestinal tract. This encompasses not only the active ingredient itself but also the encapsulating material and the entire encapsulate/nanocarrier. In this regard, it has been proposed that specific adaptations in hazard characterization are required, including the assessment of the quantity of the encapsulated compound and the amount present in its free form in the food. Moreover, it may be advisable to examine the pertinent chemical constituents of a nanocarrier system and present evidence on how intestinal cells absorb and transport them.[64]

In the USA, the FDA adheres to the same stance as the EFSA. They monitor the safety and effectiveness of nanotechnology products. As well as regulate these products within its current regulatory framework, customized to the specific standards for each product category under its authority. As a result, the industry is committed to ensuring compliance with all applicable legal requirements and is encouraged to collaborate with the FDA to address any concerns regarding the safety or regulatory status of these products.[59]

The safety of SLN and NLC in food supplements has been reported. Once ingested, these nanoparticles undergo the same physiological processes as the lipids present in food. The process includes digestion in the stomach, absorption primarily in the small intestine, and systemic blood uptake. In the small intestine, SLN and NLC can be broken down by lipase enzymes, releasing and facilitating the absorption of the compounds. Additionally, lipid nanoparticles have adhesive properties, allowing them to adhere to the gut wall, specifically the enterocyte surface, leading to prompt absorption of the compounds within the enterocytes.

Additionally, oral lipid nanoparticles are generally larger than 100 nm and biodegradable, indicating that their cellular uptake is not expected. Therefore, it is reasonable to conclude that no significant toxicological concerns are anticipated.[65,66]

4. Conclusions

In recent years, concerns about sustainability and well-being have motivated research into the discovery of bioactive compounds from natural sources that are safe for human consumption. In this context, the potential health benefits of marine bioactive compounds have been described by several researchers. However, most of these molecules have stability problems and low water solubility, so the use of lipid nanoparticles such as SLN and NLC has proved effective in overcoming these problems. Indeed, the data presented in this review work demonstrates the potential of these nanoparticles to improve the use of bioactive marine compounds in healthcare applications, particularly in cosmetics and food supplements.

Noteworthy, most of the current studies are based on *in vitro* experiments and there is a lack of information on regulatory and safety standards for human use. It is therefore important to give priority in the future to:

- Standardization of clinical trial protocols to allow comparisons between different studies.
- Carrying out *in vivo* studies to obtain more information on the absorption, distribution and excretion of bioactive compounds and their nanocarriers.
- Conducting additional tests in human volunteers to confirm that the results observed in animal studies apply to humans.
- Sharing "bad results" to avoid redundant efforts, promote transparency and accelerate collective learning.

Academia and industry must work together to overcome technological and financial constraints. This partnership is essential to drive innovation, bridge the gap between theory and practice and ensure that research addresses real-world challenges. It is also essential to ensure that regulatory standards are kept up to date with the latest scientific advances to ensure safety and progress.

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