PKP
PUBLIC KNOWLEDGE PROJECT

Journal of Biological Studies
ISSN 2209-2560

JBS
An Open Access Peer Reviewed Journal

Research Full-Text Paper

Evaluation of *in vitro* release of zeaxanthin-containing nanocarriers

Roya Osanlou[1], Mozhgan Emtyazjoo[2]*, Aghdas Banaei[3], Mohammad Ali Hesarinejad[4], Fatemeh Ashrafi[5]

[1] *Department of Biology, Faculty of Biosciences, Islamic Azad University, North Branch, Tehran, Iran*
[2] *Department of Biology, Faculty of Marine Sciences and Technology, Islamic Azad University, North Tehran Branch, Tehran, Iran*
[3] *Department of Biology, Research Institute of Applied Science, Academic Center of Education, Culture and Research (ACECR), Tehran, Iran*
[4] *Department of Food Processing, Research Institute of Food Science and Technology (RIFST), Mashhad, Iran*
[5] *Department of Biology, Faculty of Biosciences Islamic Azad University, North Tehran Branch, Tehran, Iran*

Received: April 3, 2022; **Accepted:** April 25, 2022; **Published online:** August 30, 2022

Abstract: Many studies have shown that lipid nanocarriers can cause slow release *in vitro*. Today, zeaxanthin has become very important as a promising bioactive substance in the human diet. In addition to its vital importance as a natural dye, in particular, it is considered as a health-promoting component that has many beneficial properties. At the same time, poor water solubility, as well as poor bioavailability, are bottlenecks that limit food/drug use. Accordingly, the present study investigated the release of nanocarriers containing zeaxanthin. During this experimental-laboratory research, lipid nanocarriers were produced by combining two methods of homogenization with high shear force and ultrasound. Release of zeaxanthin was performed at gastric pH equivalent (1.2) and intestinal pH equivalent (8.6) using dialysis bag method at 37 °C. In order to evaluate the release, the data were analyzed using statistical tests. Zeaxanthin was extracted using HPLC and the purity was reported to be 91.4%. Production of zeaxanthin lipid nanocarriers was performed by combining two methods of high shear homogenization and ultrasound. Release of zeaxanthin lipid nanocarriers was performed in quasi-gastric and intestinal conditions and the release rates during the first two hours and eight hours were 40% and 70%, respectively. The results of this study showed that the lipid nanocarriers NLC had

*e-mail: M_emtyazjoo@iau-tnb.ac.ir / Moz_emtyazjoo@yahoo.com

prolonged desirable release. The chemical and physical structure of SLN and NLC nanocarriers provide a proper release making them suitable target for drug delivery system.

Keywords: Zeaxanthin, Lipid nanocarriers, Release

1 Introduction

The release of lipid nanocarriers is the process of releasing encapsulated bioactive materials targeted to particular site. Targeted delivery protects the drug against premature elimination to have an effective function in the body (Malekjani and Jafari, 2021). Lipid nanocarriers are an emerging and rapidly evolving tool for the targeted delivery of various drugs lacking the solubility, bioavailability and stability (Shukla et al., 2018). Newer lipid nanocarriers, such as solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs), are lipid pharmaceutical compounds having significant role in drug delivery system due to their low toxicity, improved bioavailability, high biocompatibility, high drug loading efficiency and slower release. Lipid nanocarriers can load both lipophilic and hydrophilic drugs (Shukla et al., 2018). Carotenoids are natural lipid-friendly pigments and have become very important as functional components in the human diet. In photosynthesizers, they are called the natural pigments of health-promoting ingredients (Rostamabadi et al., 2019). These compounds are important in increasing health, but have shown less resistance to stress. They are easily oxidized and chemically unstable, and their use in food and medicine has been limited due to some limitations such as poor bioavailability, lower solubility and rapid release. Therefore, nanocapsulation techniques can be used as a way to protect carotenoids and maintain their main properties during processing, storage and digestion, and also to improve their physicochemical properties and increase health-promoting effects (Rehman et al., 2020). Zeaxanthin is a natural carotenoid antioxidant and has been shown to contribute to eye health. Zeaxanthin and nanoparticles containing zeaxanthin have been reported to prevent the development of eye diseases such as cataracts and age-related macular degeneration (Tudor and Pintea, 2020).

Studies have shown that utilizing nanocarriers in drug delivery is to treat a disease effectively with minimum side effects (Chamundeeswari et al., 2019). The most common methods of drug release are tablets, injections, lotions, and suppositories. The preferred method is the oral form of the drug because it is simple, painless and self-administered. However, drugs are usually broken down in the gastrointestinal tract or are not of sufficient quality for effective absorption (Ding and Li, 2017). The use of nanocarriers containing the drug reduces the rate of metabolism and excretion of the drug from the body and increases the half-life of the drug (Perni and Prokopovich, 2014). In other words, lipid-based nanocarriers (solid lipid nanoparticles and nanostructured lipid carriers) are used to trap carotenoids and are a promising new tool in controlled release that is effective in protecting carotenoids (Rehman et al., 2020). Research suggests that nanostructured lipid carriers (NLCs) and solid lipid nanoparticles (SLNs) are safe nanocarriers and can be effective in controlled release and

provide a robust platform to protect the bioactive material against challenging conditions (Rostamabadi et al., 2019). Studies have shown that the formulation of lipid nanocarriers has been able to lead to the development of unique precursors for health products, especially in the food industry. The advantages of this system are cost reduction, improving the quality and increasing the shelf life of food products, reducing toxicity, good storage stability, synergistic effects, antioxidants and sustainable release and can easily increase production (Tan et al., 2016). Research has shown that the release of zeaxanthin-containing nanocarriers in the laboratory can create controlled, slow conditions, allowing a hydrophobic compound to disperse into a hydrophilic matrix and provide stability. It has been also suggested that nanocarriers containing zeaxanthin can be incorporated into yogurt or milk, allowing a hydrophobic compound to disperse into a hydrophilic matrix and provide stability (de Campo et al., 2019).

Considering the importance of the growing human need to consume beneficial foods and natural additives for use by all members of society, especially the elderly (Rehman et al., 2020; Tudor and Pintea., 2020), and the significant role played by zeaxanthin with coating of lipid nanocarriers in food industry (Tudor and Pintea., 2020; Ding and Li., 2017), and few past studies focusing on the hydrophilic and lipophilic nanocarriers release process, the present study was carried out to evaluate the release of zeaxanthin lipid nanocarriers *in vitro*.

2 Materials and Methods

Zeaxanthin was extracted and purified according to the method described by Chen et al., 2005. Briefly, 25 ml of 10 M potassium hydroxide solution containing 2.5% ascorbic acid was added to 10 g of dry microalgae. The resulting mixture was incubated at 60 °C for 10 minutes before cooling to ambient temperature. 10 ml of hexane-ethanol mixture was added to the mixture in a ratio of 1:1 (v:v). The mixture was then separated by centrifugation at 10,000 g for 15 minutes and the supernatant was collected. The extraction process was almost colorless until the mixture was formed, repeated in all extracts, after which all extracts were mixed together. The extract was then diluted with distilled water to twice its volume and then the two phases were separated with a separating funnel. The organic phase (n-hexane phase) was washed with 30% aqueous ethanol to keep the aqueous phase almost colorless at neutral pH. After separation, the organic phase was evaporated by rotary evaporator at 40 °C until complete drying and kept at -22 °C until use.

NLCs and SLNs were prepared according to previous studies with some modification (Zardini et al., 2018). Briefly, the aqueous phase, containing tween 80 emulsifier, and the lipid phase, containing lecithin and zeaxanthin, were prepared separately. The lipid phase was the same at 5 wt. % for all nanocarriers. To engineer nanostructured lipid carriers, 20% of the solid lipid was replaced with MCT (medium chain triglycerides). The lipid or mixture of lipids was heated at 80 °C in the water bath to prepare the lipid phase. Zeaxanthin was then added in two different loading levels: no zeaxanthin loading and 0.8 mg/g lipid zeaxanthin loading. A load of zeaxanthin with a concentration of 0.8 mg of zeaxanthin per gram of lipid was added to glycerol monostearate, glycerol diastearate (GMS, GDS). The mixture was stirred at 80 °C for 5

min to ensure complete zeaxanthin dissolution in lipid, and the dark stage started (all tubes and dishes were covered with aluminum foil to prevent the effect of light). Once the aqueous solution containing 3% tween 80 was prepared, both phases were placed at 80 °C for 15 min to be homogenized in terms of the temperature. After that, the aqueous solution was added to the oil phase and homogenized with a homogenizer (T25 digital ULTRA-TURRAX, IKA, Germany) at 20,000 rpm. Prior to the experiment, the probe was carefully washed with ethanol for few seconds to clean the potential contaminations and then dried using oven at 50 °C. The pre-emulsion solution produced by the ultrasonic probe (XL 2020, Misonix, USA) with 25% output power and 50 cycles (4 sec on and 1sec off) was treated with ultrasound. Finally, samples were homogenized again for 1 min by the homogenizer at 20,000 rpm. The produced samples were placed in an ice bath for 30 min to reduce the samples' temperature while forming lipid nanoparticles. To investigate the nanocarriers' thermal properties, some of the produced nanodispersion was dried by freeze-drying at -30 °C and 0.1 mbar for 48 hours (Christ Alpha LD, Germany) (Mehnert and Mäder., 2012). The composition of produced nanocarriers is shown in Table 1.

The release of zeaxanthin was evaluated according to previous studies (Varshosaz et al., 2010; Tamjidi et al., 2014) in gastric pH (1.2) and intestinal pH (8.6) using dialysis bag method at 37 ° C. Before use, dialysis bag was kept in water for 24 hours. 3 ml of the dispersion containing the nanocarriers was transferred into a 12 kDa dialysis bag (D0530, Sigma, Canada) with the two ends closed. It was placed in gastric buffer for 2 hours and then transferred to intestinal buffer for 6 hours. Due to the hydrophobic nature of zeaxanthin, it was not possible to investigate its release in aqueous environments of the stomach and intestine without the use of emulsifiers. In order to solve this problem and create sink conditions, twine 80 (0.5% by weight-volume) was added to the release medium. In addition, for the volume of release media at each stage was equal to 60 ml and the stirring speed of the release environment was equal to 140 rpm. At regular intervals, 5 ml of the release medium was removed and, accordingly, fresh buffer was added to the medium at 37 °C.

The amount of zeaxanthin in the release medium was measured using a spectroscopy at 425 nm. Mini-tab software was used for statistical analysis. Kolmogorov-Smirnov test was used to determine the normality of the data. *P*-value of 5% or lower was considered to be statistically significant.

3 Results and Discussions

3. 1. Extraction of zeaxanthin by spirulina platensis

Identification of zeaxanthin was performed using HPLC (High Performance Liquid Chromatography). The purity of zeaxanthin was 91.4% (Figure 1).

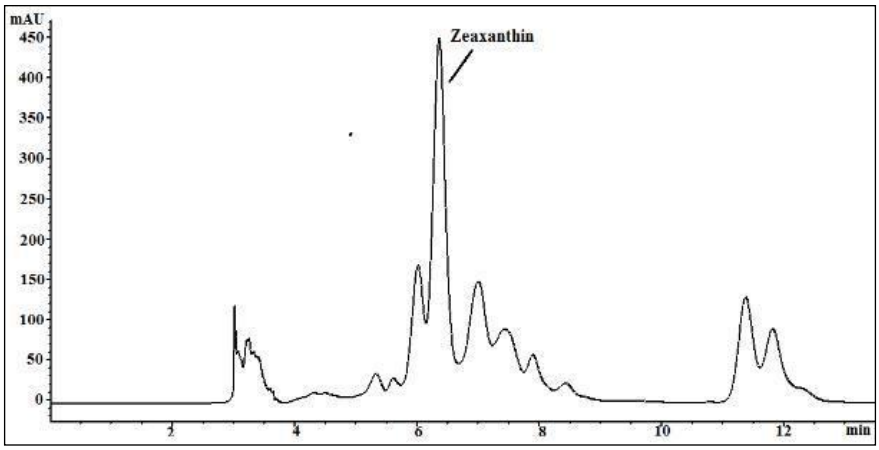

Figure 1: Chromatogram of the extracted sample of zeaxanthin at 435 nm (mAU: milli-Absorbance Units).

3. 2. Production of zeaxanthin lipid nanocarriers

The results of the production of zeaxanthin lipid nanocarriers by combining two methods of high shear homogenization and ultrasound showed that nanocarriers containing glycerol di stearate had better performance and loading than glycerol mono stearate (Tables 1 and 2).

Table 1. Compounds (w/w) used to produce nanocarriers (3% tween 80 and 6% lecithin were used in all samples).

Sample code	Zeaxanthin%	Glycerol mono-stearate	Glycerol de-stearate	MCT
SLN1	0	5	0	0
SLN2	0.8	5	0	0
SLN3	0	0	5	0
SLN4	08	0	5	0
NLC1	0	4	0	1
NLC2	0.8	4	0	1
NLC3	0	0	4	1
NLC4	0.8	0	4	1

In all samples, 3% tween 80 and 6% lecithin were used.

Table 2. PS, PDI, zeta potential, EE, and DL of nanocarriers immediately after production.

Sample Code	PS (nm)	PDI	Zeta Potential (mV)	EE	DL
SLN1	91.78 ± 3.18	0.23 ± 0.01	-13.02 ± 0.57	-	-
SLN2	179.16 ± 0.94	0.34 ± 0.01	-19.44 ± 1.19	81.14 ± 3.06	7.07 ± 0.28
SLN3	77.49 ± 1.70	0.24 ± 0.01	-12.11 ± 0.80	-	-
SLN4	119.03 ± 1.85	0.30 ± 0.01	-20.03 ± 0.48	85.38 ± 3.47	7.21 ± 0.23
NLC5	79.92 ± 1.14	0.23 ± 0.01	-16.88 ± 0.61	-	-
NLC6	129.94 ± 3.07	0.29 ± 0.01	-22.23 ± 0.41	88.10 ± 2.19	7.26 ± 0.18
NLC7	71.13 ± 0.88	0.24 ± 0.01	-17.01 ± 0.30	-	-
NLC8	130.16 ± 1.58	0.30 ± 0.01	-21.49 ± 1.13	90.43 ± 2.85	7.61 ± 0.19

PS (particle size), PDI (Particle dispersion index), EE (Encapsulation efficiency), DL (drug loading).

3. 3. Release of nanocarriers containing zeaxanthin

The releasing rate of zeaxanthin lipid nanocarriers in gastrointestinal and gastrointestinal

conditions showed that during the first two hours, the release rate was 40% during the first two hours, and was 70% during eight hours (Figure 2).

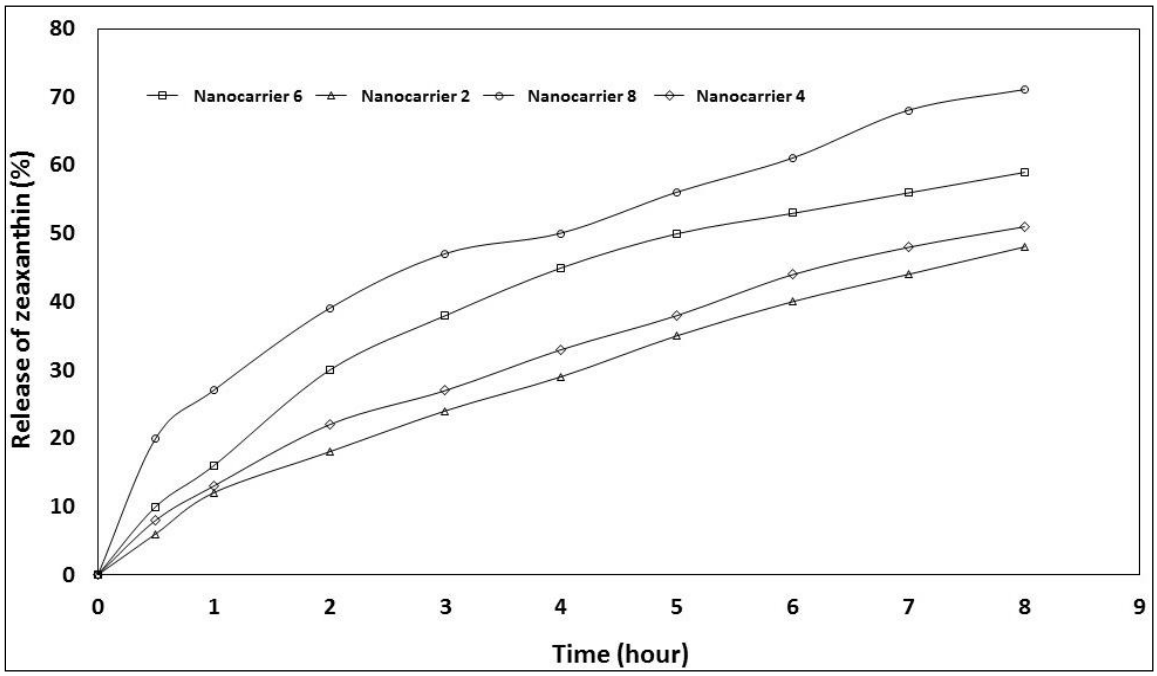

Figure 2: Release kinetics from manufactured nanocarriers.

3. 4. Production of zeaxanthin lipid nanocarriers

The results of this study showed that solid lipid nanocarriers and nanostructured nanocarriers were produced with a combination of high shear homogenization and sonographic method without any organic solvent. Production of nanoparticles with higher PDI (Particle dispersion index) is one of the disadvantages of probe ultrasound technique (Tamjidi et al., 2014). Therefore, high shear homogenization was performed in order to produce a uniform mixture of ingredients. The results of our initial experiments showed that high shear homogenization after ultrasound reduced the amount of PDI nanoparticles.

Fat and emulsifier concentrations were kept constant and the effect of applied lipids (GMSs, GDSs), type of nanocarriers (SLNs and NLCs) and zeaxanthin loading were investigated. Concomitant use of lipophilic and hydrophobic surfactants created a more stable dispersion, and therefore in this study twine 80 and lecithin were used simultaneously as a hydrophilic and lipophilic surfactant accoring to past studies (Zardini et al., 2018). To produce NLC, part of the solid lipids in the SLN were replaced by MCT as liquid lipids. The thermodynamic stability of MCT and the high solubility of bioactive were the most important reasons for choosing MCT to produce NLC.

3. 5. Release of zeaxanthin-containing nanocarriers *in vitro*

The release behavior of zeaxanthin loaded nanoparticles under simulated gastrointestinal conditions was investigated using a dialysis bag method at 37 °C and a size of 200 nm, and the SLN release system was reported differently to the NLC system at a given time. Shape, size,

bioactivity and diffusion in the environment, polymorphic state of lipid nanocarriers, ambient pH, DL, porosity and biological ratio between media receiver and carriers are variables that may affect the release of nanoparticles (Fathi et al., 2012). The results of this study showed that the maximum release rate was 40% during first two hours. The rate was higher when higher amount of zeaxanthin was present in the carrier. Consistent with these findings, other studies have shown that this formulation has led to unique precursors to health products, particularly the food industry. The advantages of this system are cost reduction, improving the quality and increasing the shelf life of food products, reducing toxicity, good stability, storage, synergistic effects, antioxidants and sustainable release and can easily increase the production rate (Behbahani et al., 2019; Tan et al., 2016). It has also been reported that the release of zeaxanthin-containing nanocarriers in the laboratory can create controlled and slow conditions, allowing a hydrophobic compound to disperse into a hydrophilic matrix and provide stability. Nnanocarriers containing zeaxanthin can be incorporated into yogurt or milk, allowing a hydrophobic compound to disperse into a hydrophilic matrix and provide stability (de campo et al., 2019). In this regard, the results of a study on zeaxanthin-containing formulations indicated that the releasing rate of zeaxanthin-containing lipid nanocarriers in the laboratory was favorably acceptable (Behbahani et al., 2019). In contrast, some research findings showed that the release of bioactive material containing lipid nanocarriers, due to the small size below 200 nm, had a weak diffusion in the first two hours of release (Behbahani et al., 2019; Gaur et al., 2014).

SLNs are made of fat or a mixture of solid fats and NLCs are made of a mixture of liquid lipid (MCT) with solid lipid. Due to the presence of solid lipid in the structure of NCLs, they provide slow release fo nanostructure materials (Das and Chaudhury, 2011). In NLCs, the space between fatty acid chains and defective crystals provides greater compatibility for drug molecules. In addition, some nanocarriers have higher solubility in MCT (Feng and Mumper, 2013; Campos et al., 2015).

4 Conclusion

Overall, the results of this study showed that the lipid nanocarriers SLN and NLC have prolonged release and this release was more desirable in NLC nanocarriers. The chemical and physical structure of SLN and NLC nanocarriers provide a proper release making them suitable target for drug delivery system.

Acknowledgments

This research was carried out with support of the Vice Chancellor for Research, Islamic Azad University, North Tehran Branch. We thank all our colleagues for their stellar efforts and valuable contributions.

Conflict of interests

The authors state that there are no conflicts of interest regarding the publication of this article.

References

Behbahani, E. S., Ghaedi, M., Abbaspour, M., Rostamizadeh, K., & Dashtian, K. (2019). Curcumin loaded nanostructured lipid carriers: In vitro digestion and release studies. *Polyhedron, 164*. 113-122. https://doi.org/10.1016/j.poly.2019.02.002

Campos, E. V. R., de Oliveira, J. L., da Silva, C. M. G., Pascoli, M., Pasquoto, T., Lima, R., & Fraceto, L. F. (2015). Polymeric and solid lipid nanoparticles for sustained release of carbendazim and tebuconazole in agricultural applications. *Scientific Reports, 5*(1), 1-14. https://doi.org/10.1038/srep13809

Chamundeeswari, M., Jeslin, J., & Verma, M. L. (2019). Nanocarriers for drug delivery applications. *Environmental Chemistry Letters, 17*(2), 849-865. https://doi.org/10.1007/s10311-018-00841-1

Chen, F., Li, H. B., Wong, R. N. S., Ji, B., & Jiang, Y. (2005). Isolation and purification of the bioactive carotenoid zeaxanthin from the microalga Microcystis aeruginosa by high-speed counter-current chromatography. *Journal of Chromatography A, 1064*(2), 183-186. https://doi.org/10.1016/j.chroma.2004.12.065

Das, S., & Chaudhury, A. (2011). Recent advances in lipid nanoparticle formulations with solid matrix for oral drug delivery. *Aaps Pharmscitech, 12*(1), 62-76. doi: 10.1208/s12249-010-9563-0

de Campo, C., Assis, R. Q., da Silva, M. M., Costa, T. M. H., Paese, K., Guterres, S. S., & Flôres, S. H. (2019). Incorporation of zeaxanthin nanoparticles in yogurt: Influence on physicochemical properties, carotenoid stability and sensory analysis. *Food Chemistry, 301*, 125230. https://doi.org/10.1016/j.foodchem

Ding, C., & Li, Z. (2017). A review of drug release mechanisms from nanocarrier systems. *Materials Science and Engineering: C, 76*, 1440-1453. https://doi.org/10.1016/j.msec.2017.03.130.

Fathi, M., Mozafari, M. R., & Mohebbi, M. (2012). Nanoencapsulation of food ingredients using lipid based delivery systems. *Trends in Food Science & Technology, 23*(1), 13-27. https://doi.org/10.1016/j.tifs.2011.08.003

Feng, L., & Mumper, R. J. (2013). A critical review of lipid-based nanoparticles for taxane delivery. *Cancer Letters, 334*(2), 157-175. https://doi.org/10.1016/j.canlet.2012.07.006

Gaur, P. K., Mishra, S., Bajpai, M., & Mishra, A. (2014). Enhanced oral bioavailability of efavirenz by solid lipid nanoparticles: in vitro drug release and pharmacokinetics studies. *BioMed Research International, 2014*. https://doi.org/10.1155/2014/363404

Malekjani, N., & Jafari, S. M. (2021). Modeling the release of food bioactive ingredients from carriers/nanocarriers by the empirical, semiempirical, and mechanistic models. *Comprehensive Reviews in Food Science and Food Safety, 20*(1), 3-47. https://doi.org/10.1111/1541-4337.12660

Mehnert, W., & Mäder, K. (2012). Solid lipid nanoparticles: production, characterization and applications. *Advanced Drug Delivery Reviews, 64*, 83-101. https://doi.org/10.1016/J.ADDR.2012.09.021

Perni, S., & Prokopovich, P. (2014). Continuous release of gentamicin from gold nanocarriers. *RSC Advances, 4*(94), 51904-51910. https://doi.org/10.1039/c4ra10023a.

Rehman, A., Tong, Q., Jafari, S. M., Assadpour, E., Shehzad, Q., Aadil, R. M., Iqbal, M. W., Rashed, M. M. A., Mushtaq, B. S., & Ashraf, W. (2020). Carotenoid-loaded nanocarriers: A comprehensive review. *Advances in Colloid and Interface Science, 275*, 102048. https://doi.org/10.1016/j.cis.2019.102048

Rostamabadi, H., Falsafi, S. R., & Jafari, S. M. (2019). Nanoencapsulation of carotenoids within lipid-based nanocarriers. *Journal of Controlled Release, 298*, 38-67. https://doi.org/10.1016/j.jconrel.2019.02.005

Shukla, T., Upmanyu, N., Pandey, S. P., & Gosh, D. (2018). Lipid nanocarriers. In *Lipid Nanocarriers for Drug Targeting* (pp. 1-47). William Andrew Publishing.

Tamjidi, F., Shahedi, M., Varshosaz, J., Nasirpour, A. (2014). Design and characterization of astaxanthin-loaded nanostructured lipid carriers. *Innov Food Sci Emerg Technol 26*, 366-374. https://doi.org/10.1016/j.ifset.2014.06.012

Tan, C., Feng, B., Zhang, X., Xia, W., & Xia, S. (2016). Biopolymer-coated liposomes by electrostatic adsorption of chitosan (chitosomes) as novel delivery systems for carotenoids. *Food Hydrocolloids, 52*, 774-784. https://doi.org/10.1016/j.foodhyd.2015.08.016

Tudor, C., & Pintea, A. (2020). A brief overview of dietary zeaxanthin occurrence and bioaccessibility. *Molecules, 25*(18), 4067. https://doi.org/10.3390/molecules25184067

Varshosaz, J., Eskandari, S., & Tabakhian, M. (2010). Production and optimization of valproic acid nanostructured lipid carriers by the Taguchi design. *Pharmaceutical Development and Technology, 15*(1), 89-96. https://doi.org/10.3109/10837450903013568

Zardini, A. A., Mohebbi, M., Farhoosh, R., & Bolurian, S. (2018). Production and characterization of nanostructured lipid carriers and solid lipid nanoparticles containing lycopene for food fortification. *Journal of Food Science and Technology, 55*(1), 287-298. https://doi.org/10. 1007/s13197-017-2937-5