FORMULATION OF DELIVERY SYSTEMS FOR CAROTENOID-RICH EXTRACTS FROM MICROALGAE

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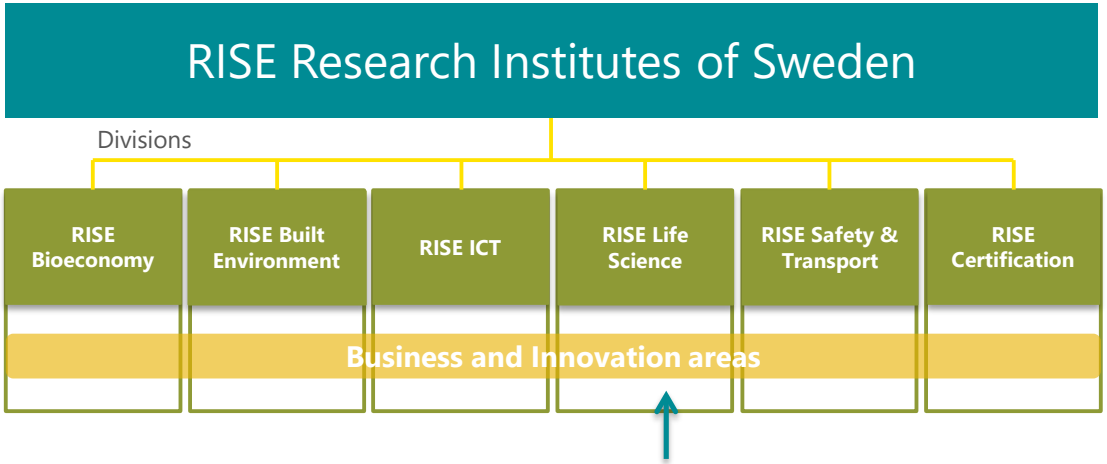
WHO WE ARE AND WHAT WE DO



- **Contract research and innovation**
  - Confidential
  - Large or small projects
  - The customer owns IP
- **Consortia projects**
  - Academia & Industry
  - Open research
  - Funding agencies
  - Non-competing members of the value chain
- **Analytical Services and Testing**
- **Courses**
  - Open training courses
  - Tailor-made, in-house

EXTRACTS FROM MICROALGAE

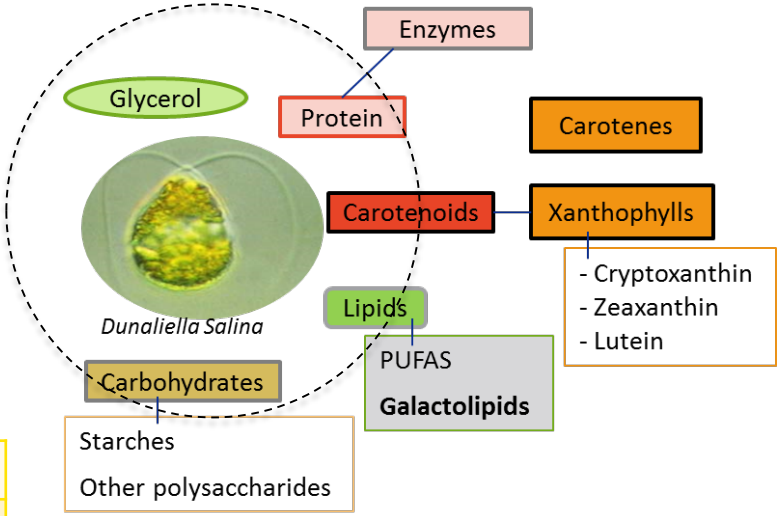
D-Factory

The Micro Algae Biorefinary

Goal Set a world benchmark for a sustainable algal biorefinery.

Start	Dec 2013
End	Nov 2017
Participants	13 partners from 8 different countries
Total budget	ca. 8 MEuro

High added-value products



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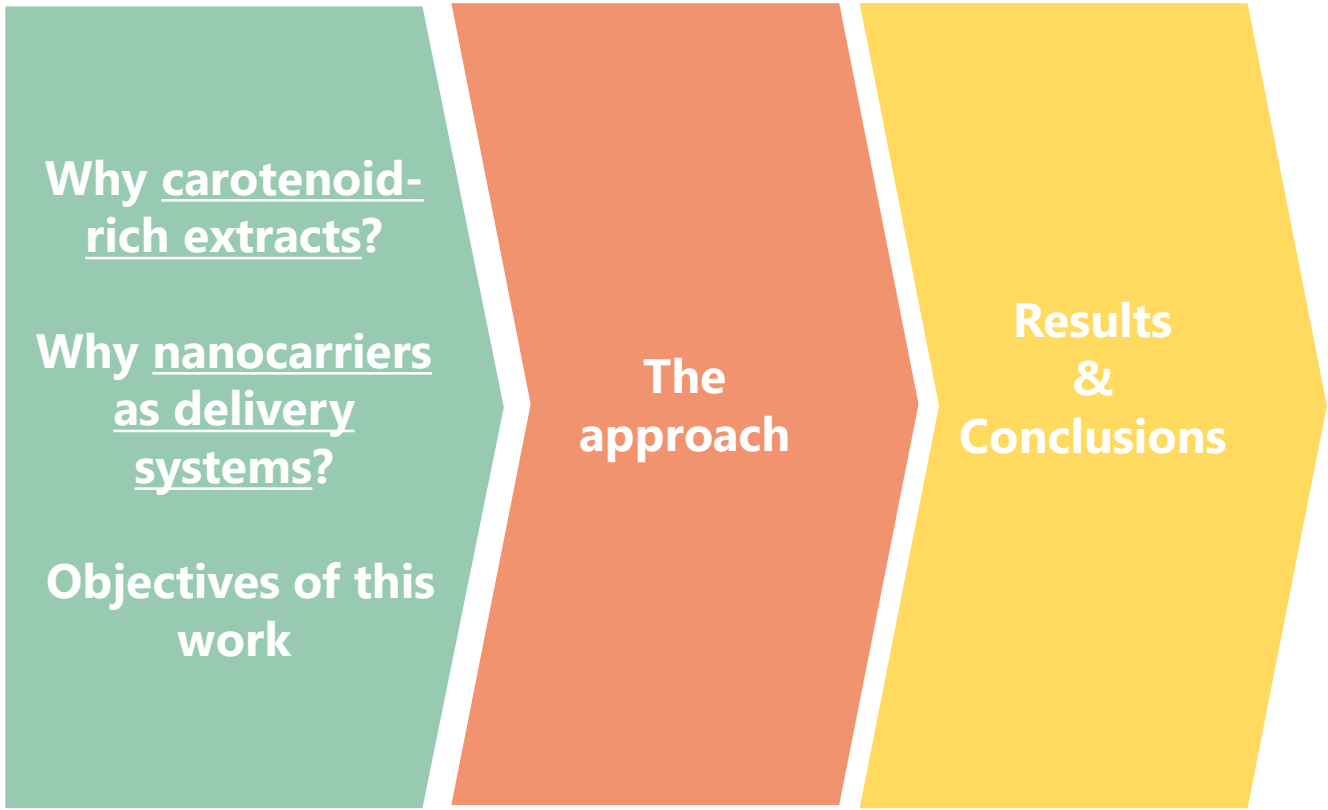
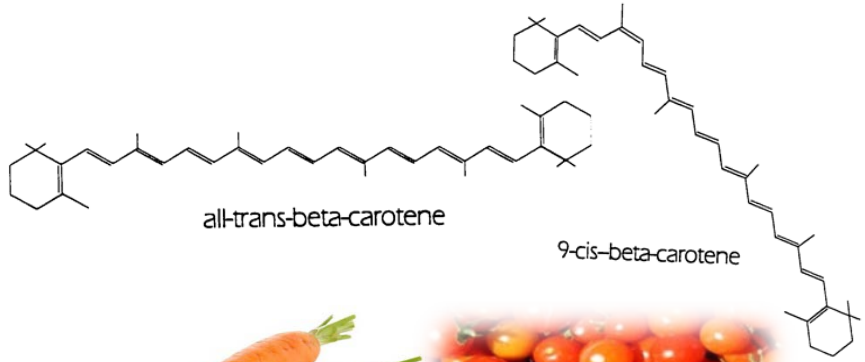
OUTLINE


Why carotenoid-rich extracts?
Why nanocarriers as delivery systems?
Objectives of this work
The approach
Results & Conclusions

WHY CAROTENOID-RICH EXTRACTS?

- Carotenoid - organic pigments
- Flowers, plants, vegetables and some (micro)algae
- Two main classes: Carotenes & Xanthophylls
- **Many associated health benefits**
- **Food supplements**, **food colourants**
- Stable in their natural form
  Extracted carotenoids need protection



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- Poorly soluble subtance
- Prone to chemical degradation /oxidation

- Autoxidation
- Thermal Degradation
- Photodegradation
- Singlet Oxygen
- Acid
- Iron and Iodine
- Free Radicals

CAROTENOID-RICH EXTRACTS FROM MICROALGAE (*Dunaliella Salina*)

- 25-30% carotenoids (mostly β- and α-carotene)
- 2-5% chlorophylls
- 5-10% triglycerides
- Traces of mono and diglycerides
- 50-60% likely lipid-bound carotenoids

Fatty acid profile	
C16:0	24.2 %
C18:3	27.3 %
C18:1	12.6 %
C18:2	7.3 %

DELIVERY SYSTEMS: WHY NANOCARRIERS?

- Common way of incorporating lipophilic actives in foods/nutraceuticals:
  - oil-in-water (O/W) emulsions
  - Solid lipid carriers (SLNC, NLC)
- Beverage formulations
- Drops/particles < 0.5 um (500 nm)
- Improved physical stability, sensory properties, release profile
- Recommended daily intake of β-carotene: 11mg

DELIVERY SYSTEMS: WHY NANOCARRIERS?

Solid Lipid Nanoparticles as Delivery Systems for Bioactive Food Components

June 200

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Article

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Transparent Dispersions of Milk-Fat-Based Nanostructured Lipid Carriers for Delivery of β-Carotene

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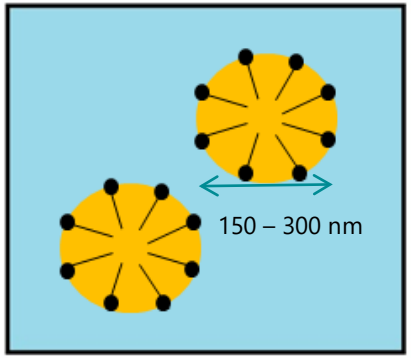


Solid lipid carriers (SLNC, NLC)

- Active dispersed in **solid particles** of solid lipid/oil mixture.
- The addition of **oil** leads to a **less crystalline** matrix
- Degree of **crystallinity** → **loading capacity** and **physical stability**
  - Degradation can be much reduced
  - Reduced mobility for diffusing to the interface

OBJECTIVES

- Develop o/w nanoemulsions and nanostructured lipid carriers, NLC, for encapsulation of the carotenoid-rich algal extract.
- Evaluate and compare the two delivery systems in terms of their ability to protect the carotenoids from degradation.

EXPERIMENTAL APROACH

Lipid phase: solution of extract in oil or melted lipid

↓

Emulsification via microfluidisation

↓

Storage conditions at different conditions

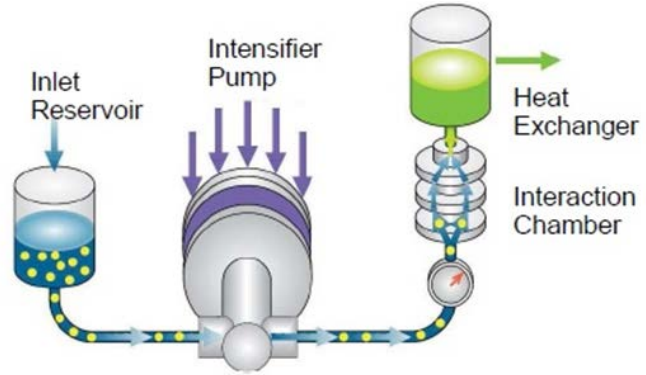
↓

Assessment of carotenoid concentration
UV absorbance
UV-vis spectrophotometry, 454nm

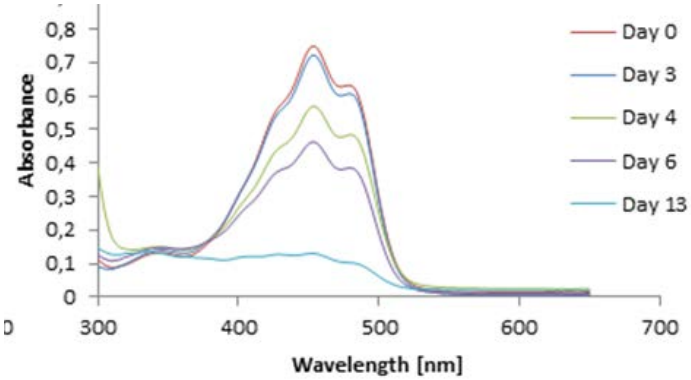
Chemical stability upon storage

↓

Drop size distribution
Physical stability



- RT and 40^{0}C (no light)
- RT + UV

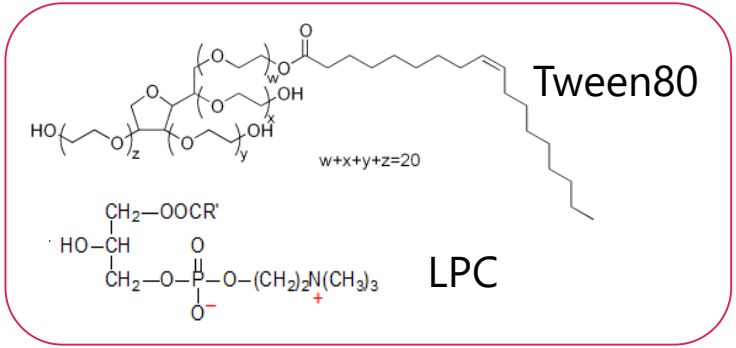


NANOEMULSION DEVELOPMENT - APPROACH

Choice of oil

- Medium chain tryglycerides (MCT)
- Sunflower oil

- Extract solubility
- Extract chemical stability -2 weeks



Choice of Emulsifier

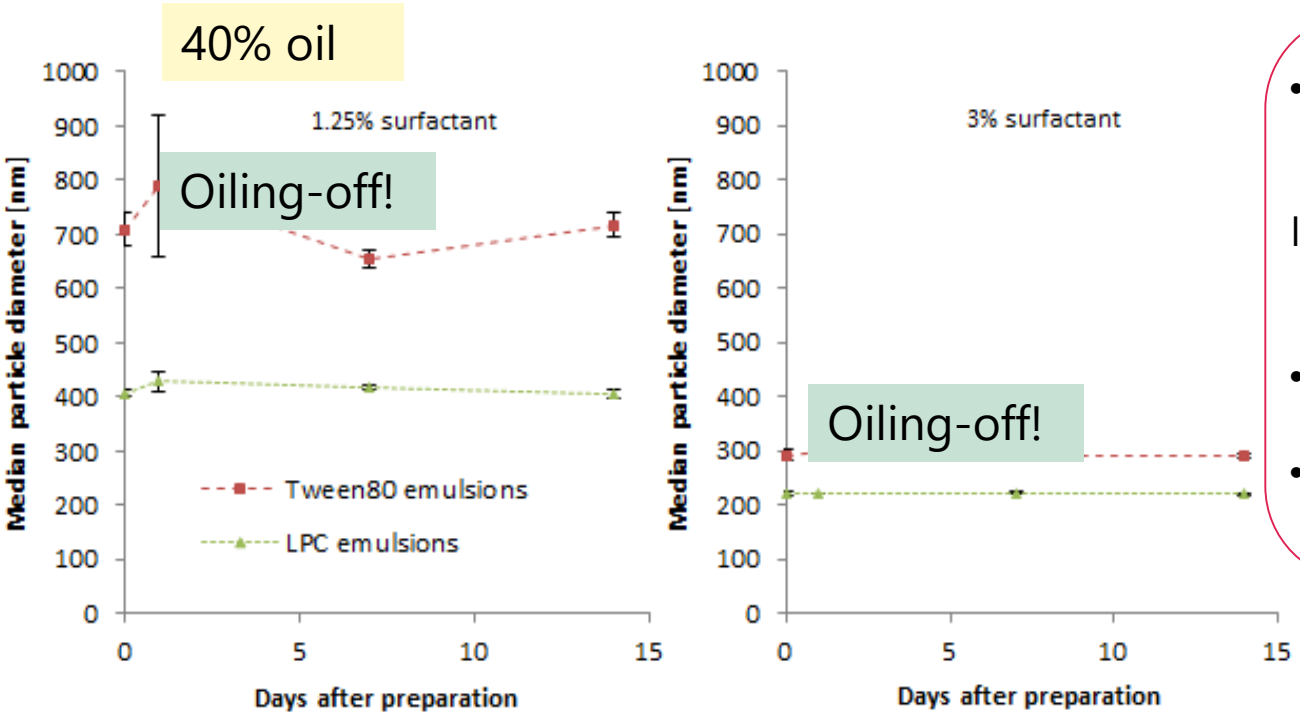
- Ethoxylated sorbitan monooleate (Tween80)
- Soybean lysolecithin (Lipoid LPC S80)

- Two different concentrations

Physical stability

- Particle size (2 weeks)
- Visual observations (4 weeks)

NANOEMULSION DEVELOPMENT



- Smaller particle size with LPC

lower interfacial tension and/or more elastic interfacial film

- Better stability with LPC
- Particle size ↓ as emulsifier concentration ↑

Nanoemulsion Formulation Development

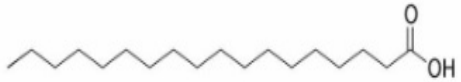
40% oil

	1	2	3	4	5
Emulsifier	Tween 80	Tween 80	LPC	Tween 80	LPC
Emulsifier concentration in water phase	1.25%	1.25%	1.25%	3%	3%
Extract concentration in oil phase	1%	2.5%	2.5%	2.5	2.5%

NANOSTRUCTURED LIPID CARRIER (NLC) DEVELOPMENT - APPROACH

Type and Concentraion Emulsifier - Fixed

3% LPC



80% C56-C60 saturated wax esters
20% fatty acids, fatty alcohols and hydrocarbons.

Choice of lipid matrix composition

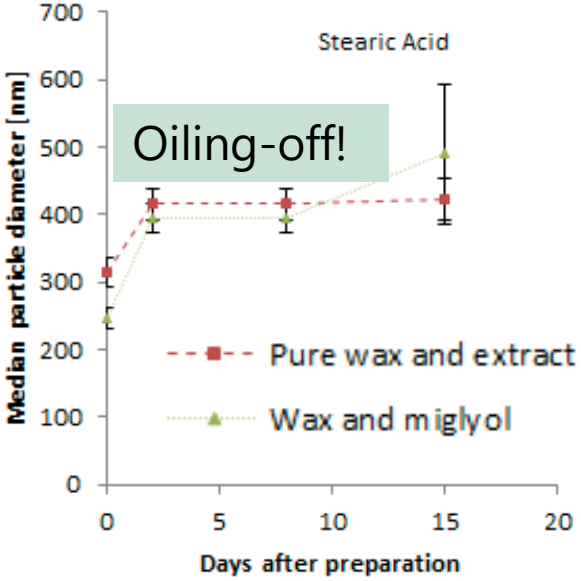
- Stearic Acid
- Carnauba Wax

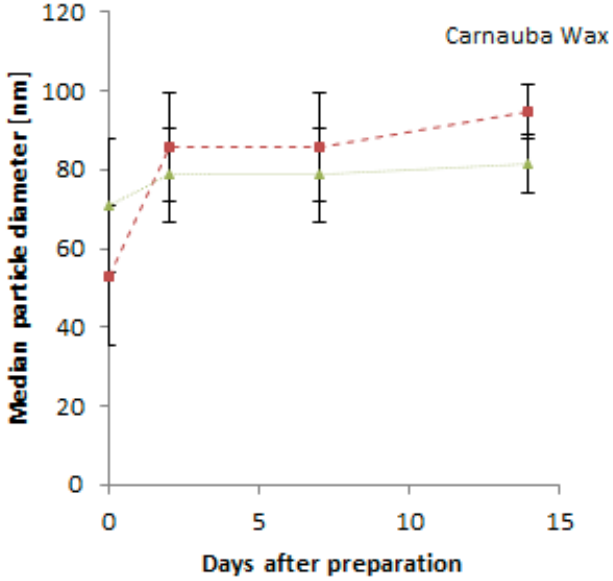
- + 15% MCT

2 week physical stability

- Particle size (2 weeks)
- Visual observation (4 weeks)

NANOSTRUCTURED LIPID CARRIER (NLC) DEVELOPMENT





- Much lower sizes with Carnuba wax
- MCT in matrix: no significant difference in size
- Stearic acid:
  oiling-off at day 0
- Carnuba wax :
  - lower crystallinity - better encapsulation efficiently

Selected Delivery Systems

40% o/w Nanoemulsion

Oil: MCT
Emulsifier: LPC
Extract in oil : 2.5%
Overall extract : 1%
D (0.5): ca. 220 nm

10% o/w Nanoemulsion

Oil: MCT
Emulsifier: LPC
Extract in oil : 2.5%
Overall extract: 0.25%
D (0.5): ca. 134 nm

10% Carnauba Wax NLC

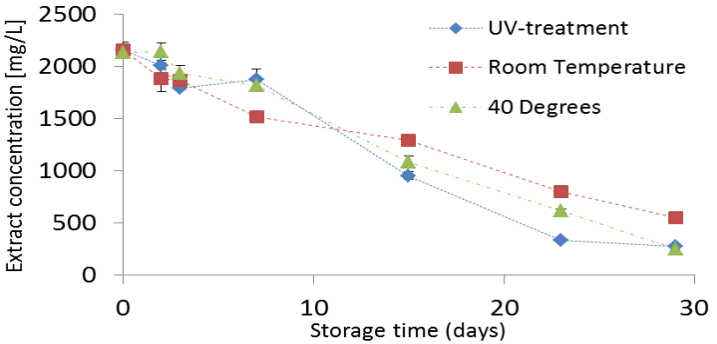
Lipid : C.W/MCT (15%)
Emulsifier: LPC
Extract in lipid: 2.5%
Overall extract : 0.25%
D (0.5): ca 80 nm

10% Stearic Acid NLC

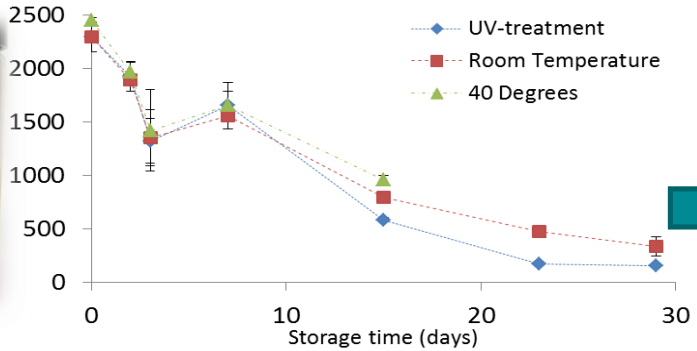
Lipid: S.A./MCT (15%
Emulsifier: LPC
Extract in lipid: 2.5%
Overall extract: 0.25%
D (0.5): ca 193 nm

Stability of extract in nano-carriers

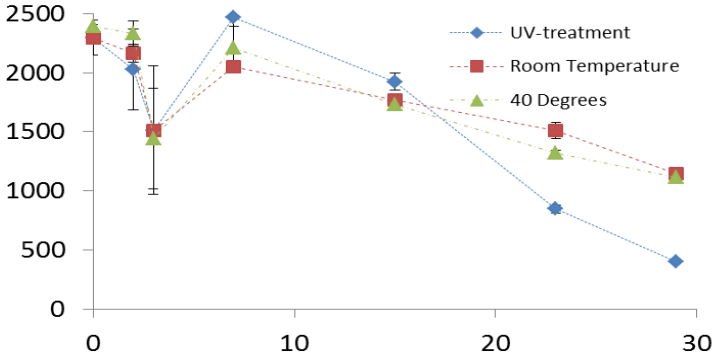
Stearic acid nano dispersion



Carnuba wax nano dispersion



MCT nano emulsion

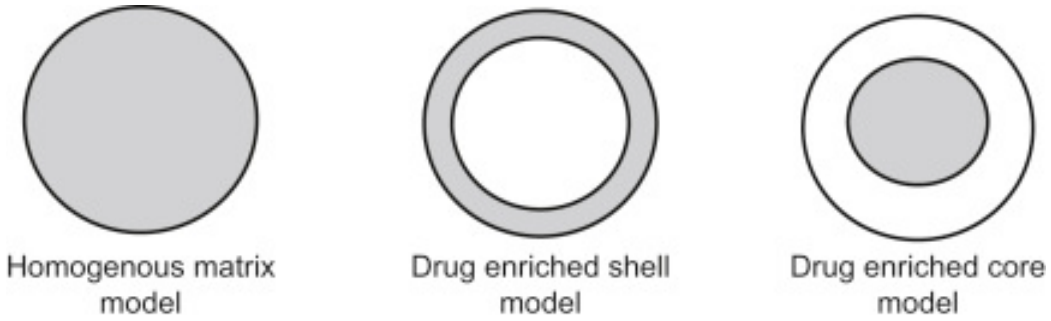


10% lipid/oil

2500 mg/L extract (0.025%)

Emulsifier: Soy Lyso Lecithin

NANOSTRUCTURE IN NLC



CONCLUSIONS

- Not possible to directly extrapolate behaviour of single carotenoids to that of complex carotenoid mixtures.
- Limited mobility of actives in NLC´s not enough to prevent degradation.
- Better understanding oh how to control internal structure and crystallisation process in NLC´s is essential.

Thanks to:

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Karin Persson
Anna Fureby

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www.d-factoryalgae.eu

and to you for your attention!