Nanoemulsions Prepared by Emulsification and Solvent Evaporation

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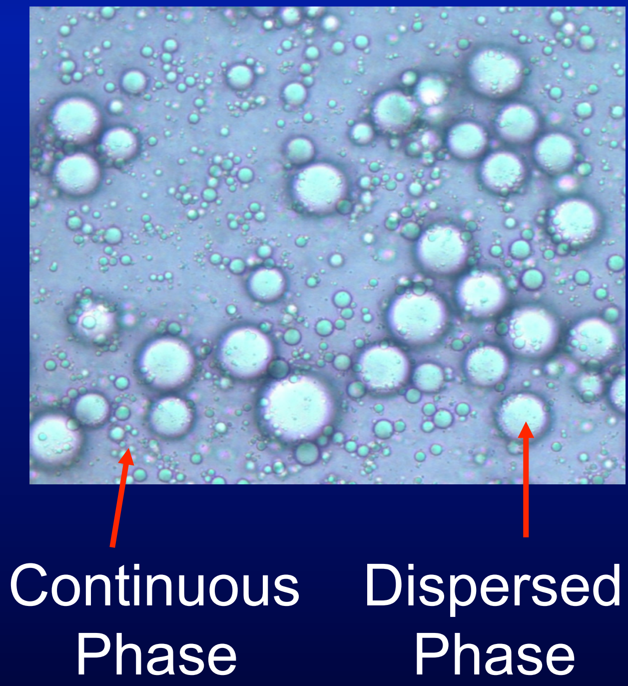
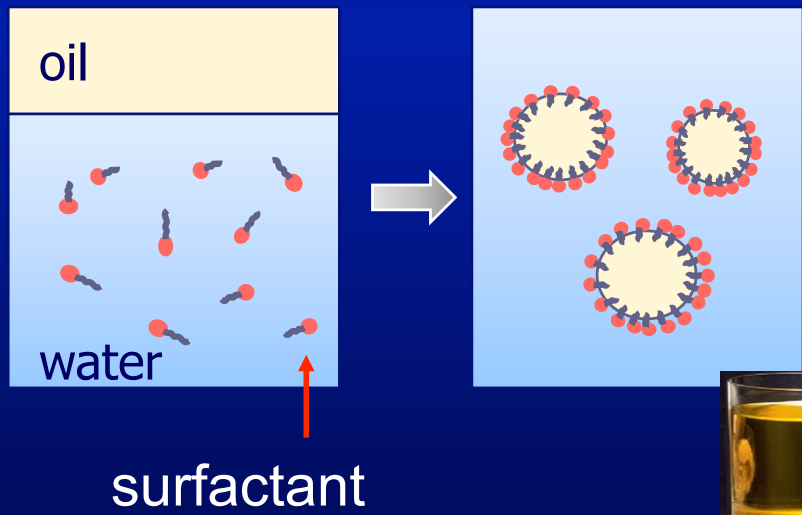
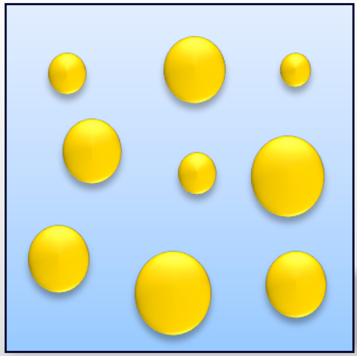
Definition of Emulsions

- An emulsion is a colloidal dispersion of two immiscible liquids (usually oil and water) with one liquid being dispersed as small droplets in the other liquid.
- The surface of droplets is covered by an interfacial layer of surface active agents (e.g. emulsifiers, proteins, polysaccharides).

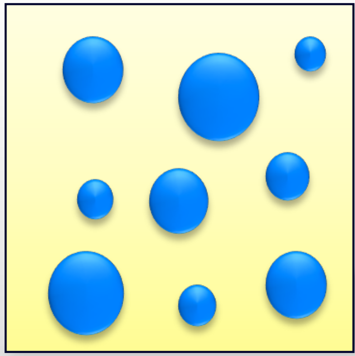




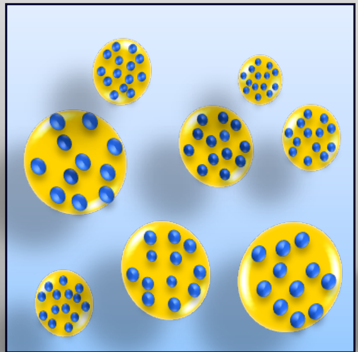
Types of Emulsions



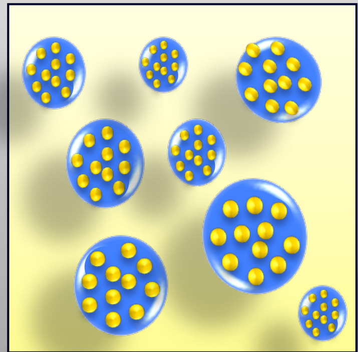
Oil-in-water (O/W)
- Milk
- Mayonnaise
- Cream
- Dressings



Water-in-oil (W/O)
- Butter
- Margarine
- Spread



W/O/W



O/W/O

Possible applications of multiple emulsions
- Encapsulation of hydrophilic component (e.g. vitamins, bioactive peptides) within the inner water phase

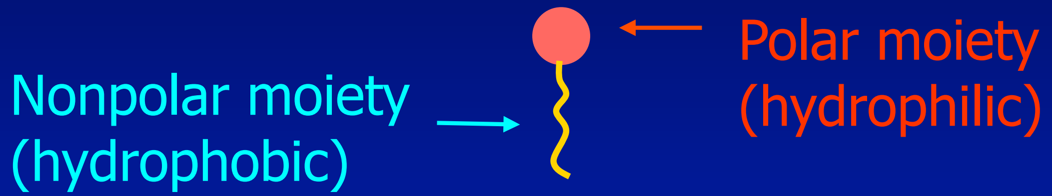
Classification of Emulsions Based on Particle Size

Emulsion type	Diameter range	Thermodynamic stability	Surface-to-mass ratio (m^2/g)	Appearance
Macroemulsion	0.1-100 μm	Unstable	0.07 – 70	Turbid/ opaque
Nanoemulsion	20-100 nm	Unstable	70 – 330	Transparent
Microemulsion	5-50 nm	Stable	130 -1300	Transparent

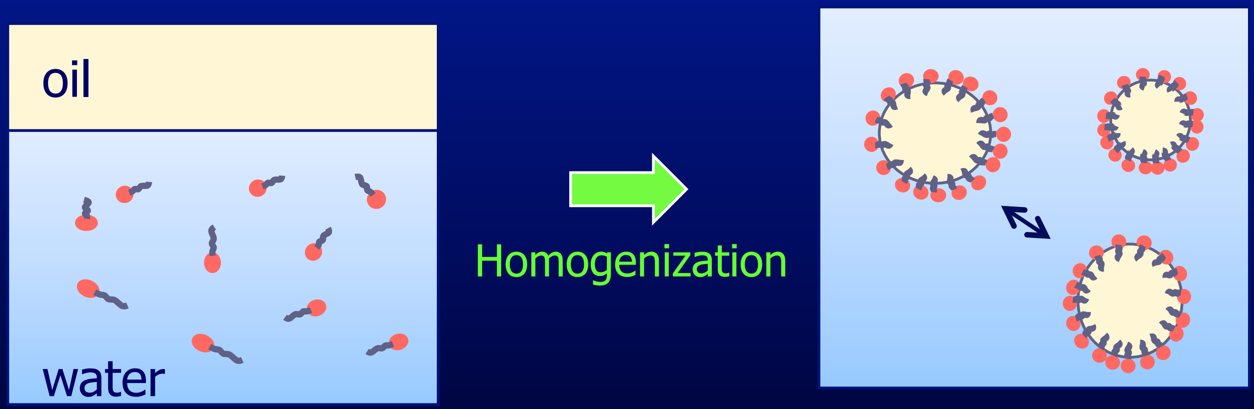
McClements (2010). Emulsion design to improve the delivery of functional lipophilic components. Annu. Rev. Food Sci. Technol. 1:241-269.

Surface Active Agents: Emulsifiers

Amphiphilic molecules: polar and nonpolar groups



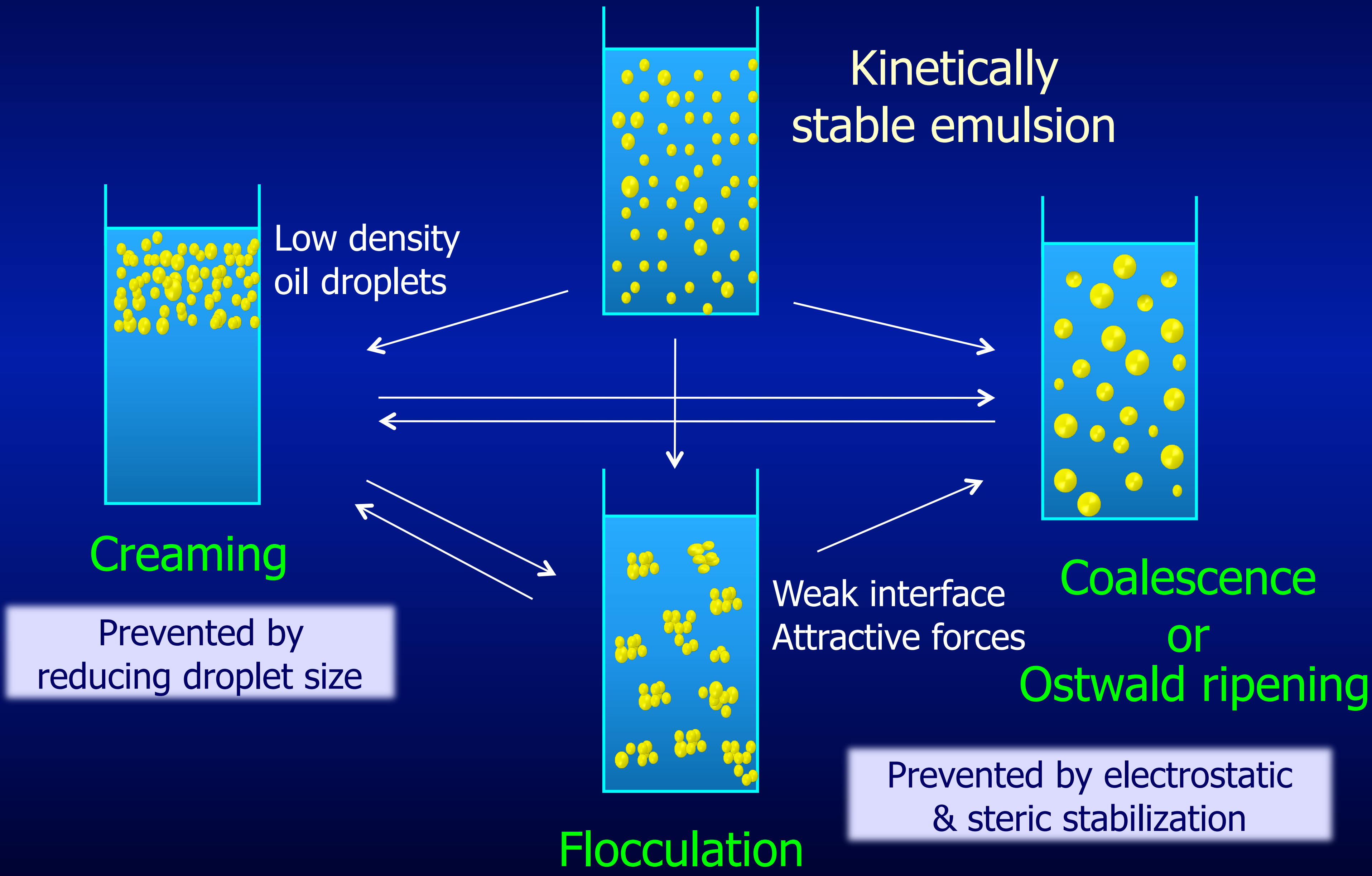
- Ability to adsorb at the oil/water interface
- Ability to reduce the interfacial tension between oil and water
- Ability to confer steric stabilization and/or electrostatic repulsion



Types of Emulsifiers

Natural (macromolecules)	Synthetic
Phospholipids •Lecithin from soy bean and egg yolk **Proteins** •Milk proteins (caseins, whey proteins, β -lg, lactoferrin, etc), soy proteins, egg white proteins **Hydrocolloids** • Gum Arabic •Chemically modified hydrocolloids (e.g. pectin, cellulose)	• Mono-diglycerides • Mono-diglycerides derivatives: DATEM, CITREM, LACTEM, etc • Propylene Glycol Esters (PGE) • Sorbitan esters (Spans) • Ethoxylated sorbitan esters (Tweens) • Polyglycerol esters • Sucrose esters

Emulsion Stability: Instability Mechanisms

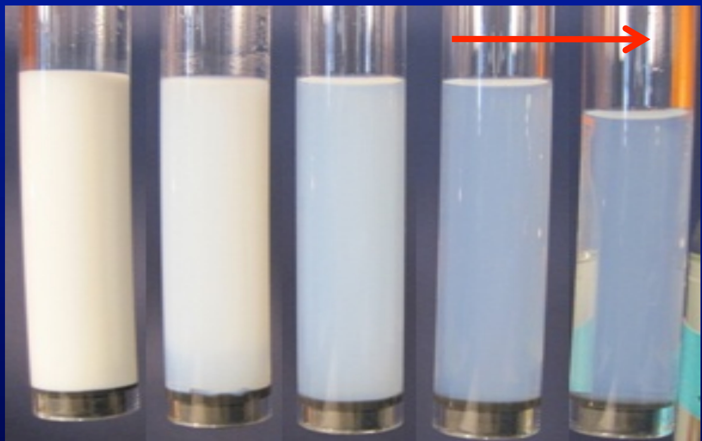


What is Nanoemulsion?

- Size range: Very small droplets (20 -100 nm)
- Stability: High kinetic stability against creaming or sedimentation
- Optical appearance: Transparent or translucent

- There is a growing interest in the use of nanoemulsions
  - e.g. pharmaceutical, cosmetics and food industry

< 100 nm



Size decreasing

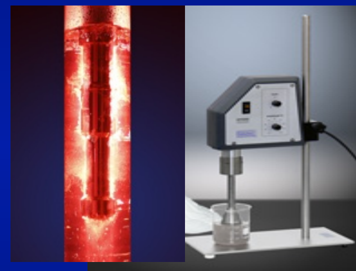

Applications of Nanoemulsions

In the food applications,

- Incorporation of lipophilic components into clear beverages.
- Improve the solubility and bioavailability of many functional components
  - e.g. carotenoids, omega-3 FAs, phytosterols, etc

- Functional properties of nanoemulsions can be tailored by structurally designing and fabricating emulsion systems (composition, structure, interfacial layer) using appropriate ingredients and processing operations.

Nanoemulsion Formation



- High energy method
  - High pressure homogenizer
  - Microfluidizer
  - Ultrasonic device

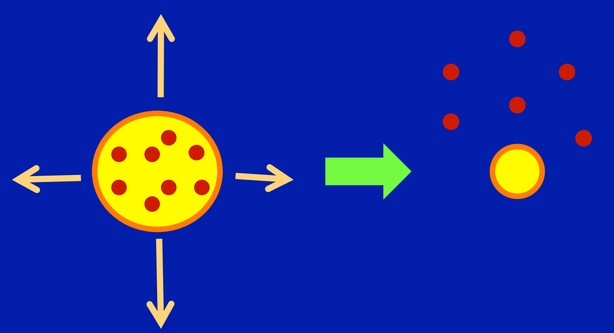
- Low energy method
  - Phase inversion temperature (PIT) method

High energy method	Low energy method
High energy methods alone normally do not yield oil droplets (<100 nm).	The limitations • Synthetic surfactants • Complex • Precise approach required

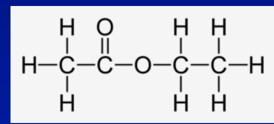
Preparation of Nanoemulsions by Emulsification and Solvent Evaporation

In recent years, a combined method of emulsification and solvent evaporation has been used for nanoparticles and nanoemulsions.

- Type of organic solvent
- Water immiscible
- Low boiling point
- (e.g. acetone, hexane, etc)



- In our study
- Ethyl acetate



  - Amphiphilic volatile
  - US FDA: GRAS for use in foods and beverages as a flavoring agent
  - Used for the production of nanoemulsions in the pharmaceutical industry
- Food-grade nanoemulsions

Materials & Methods

Homogenization & Solvent Evaporation

Materials

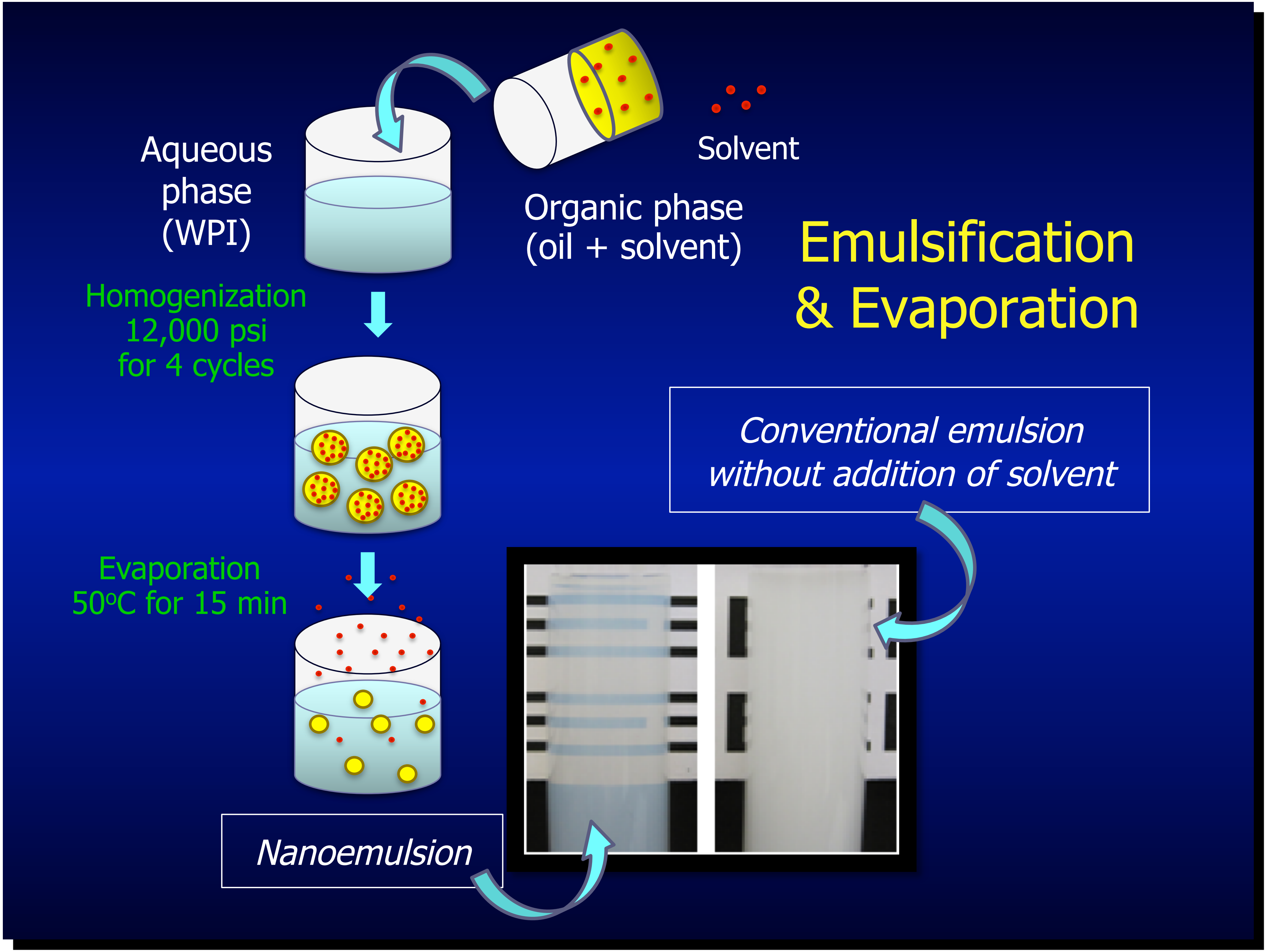
- Whey protein isolate (WPI)
- Corn oil & Ethyl acetate

Solutions

- Aqueous phase: WPI solutions (0.25 - 1 wt%)
- Organic phase: Solvent (ethyl acetate) + corn oil with different ratios (9.5:0.5, 9:1, 8.5:1.5, 8:2, 5:5, 3:7, 1:9 and 0:10)

Emulsification and evaporation

- 10 wt% organic phase: 90 wt% aqueous phase
- Emulsification: Microfluidizer (12,000 psi & 4 times)
- Evaporation: 50^{o}C for 15 min/reduced pressure

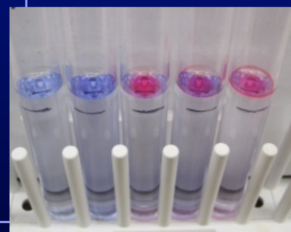


Aqueous phase (WPI)
Organic phase (oil + solvent)
Solvent
Emulsification & Evaporation
Homogenization 12,000 psi for 4 cycles
Conventional emulsion without addition of solvent
Evaporation 50ºC for 15 min
Nanoemulsion

Characterization of Emulsions

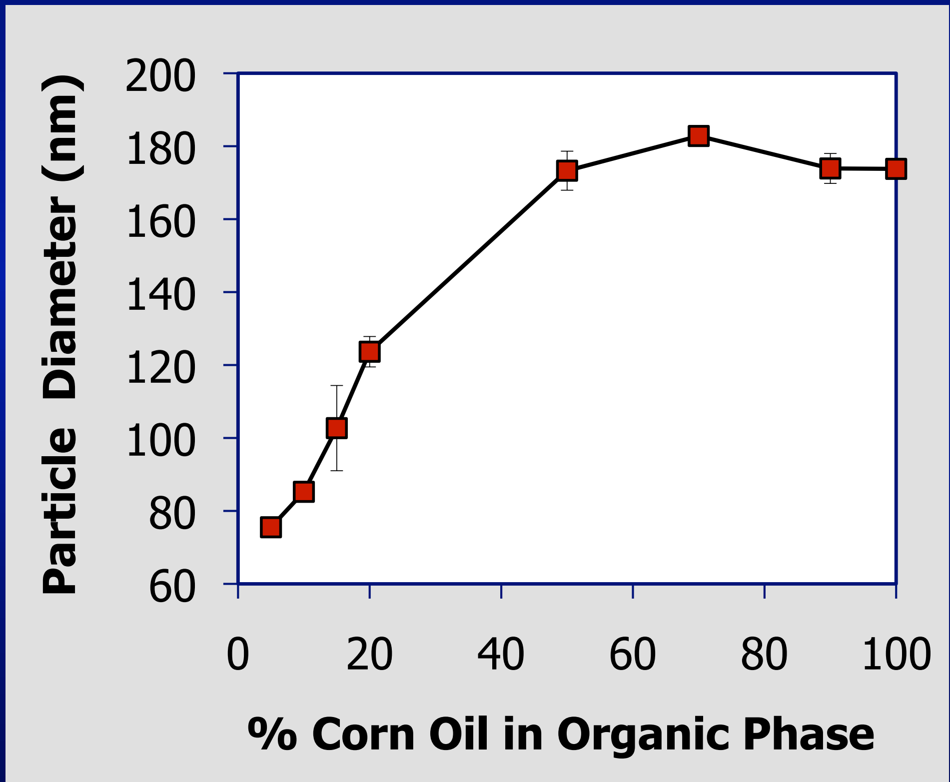
Both nanoemulsions and conventional emulsions were diluted to 0.5 wt% oil after solvent evaporation and then analyzed.

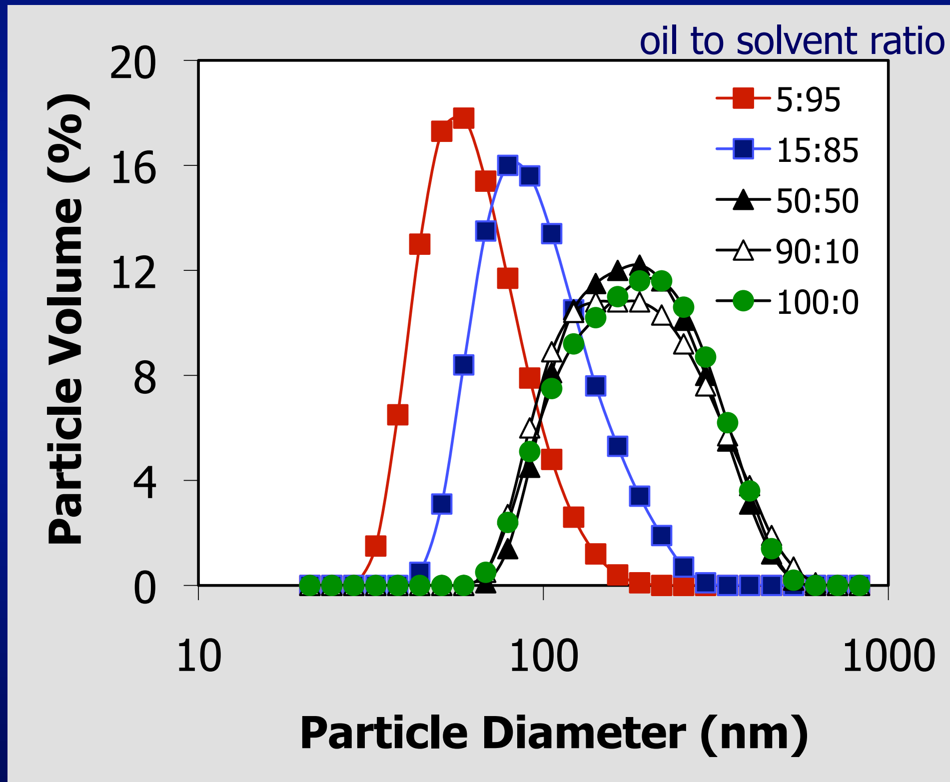
- Particle size and size distribution
- Zeta potential
- Turbidity
- Emulsion stability affected by environmental factors (pH, ionic strength (NaCl), thermal treatment)
- Emulsion digestibility in SIF
- Oxidative stability: TBARS at $38^{o}C$

SiF: Simulated intestinal fluid
TBARS: Thiobarbituric acid reactive substances



Effect of oil to solvent ratios in the organic phase on the particle size and size distribution of emulsions

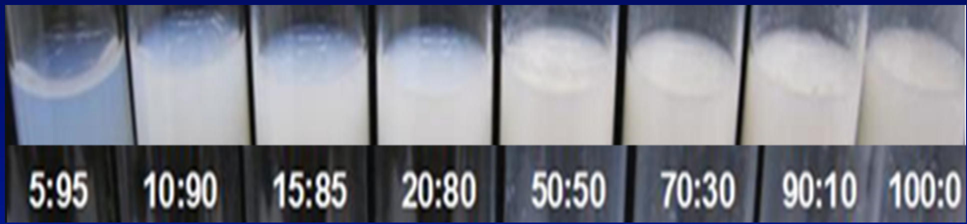


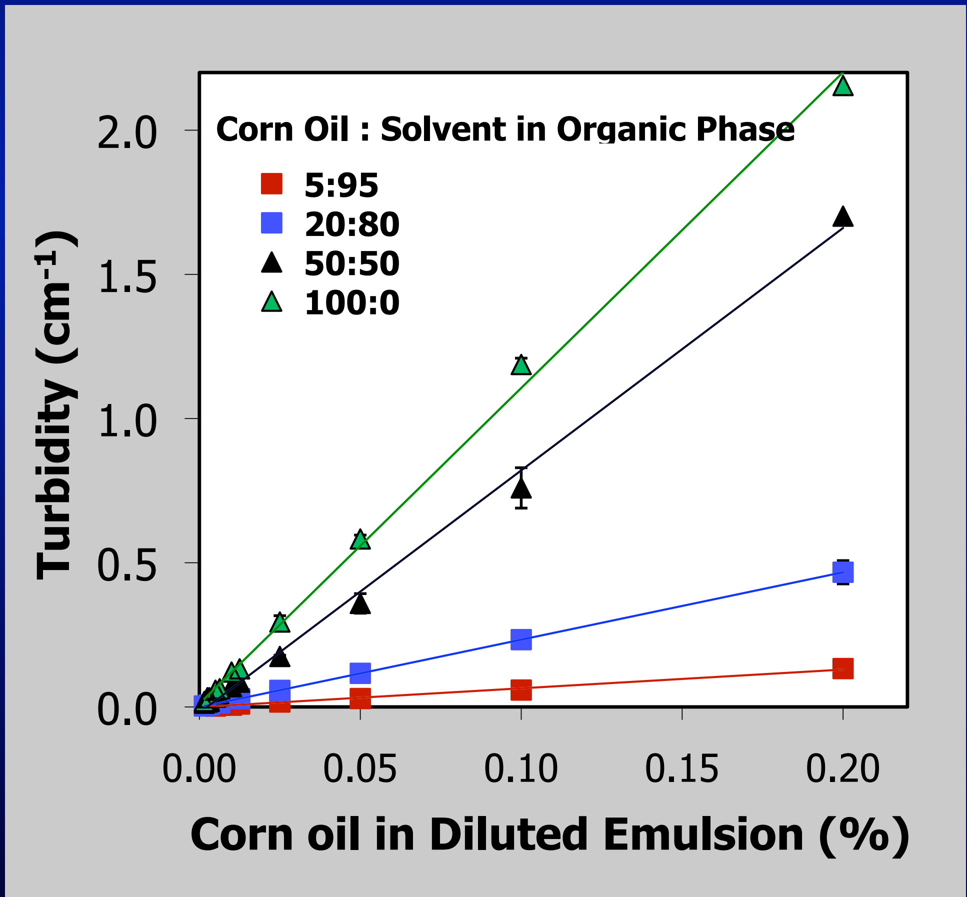


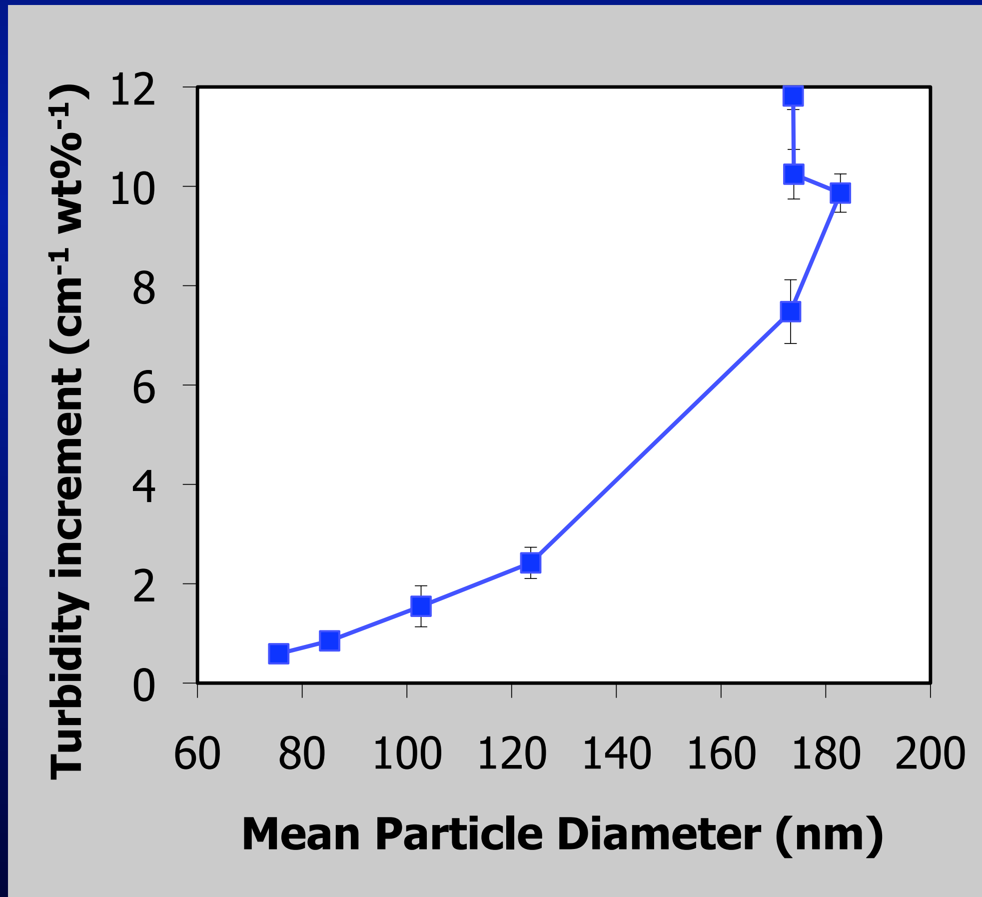
10% organic phase and 90% aqueous phase (1% WPI, pH 7)

Turbidity of Emulsions

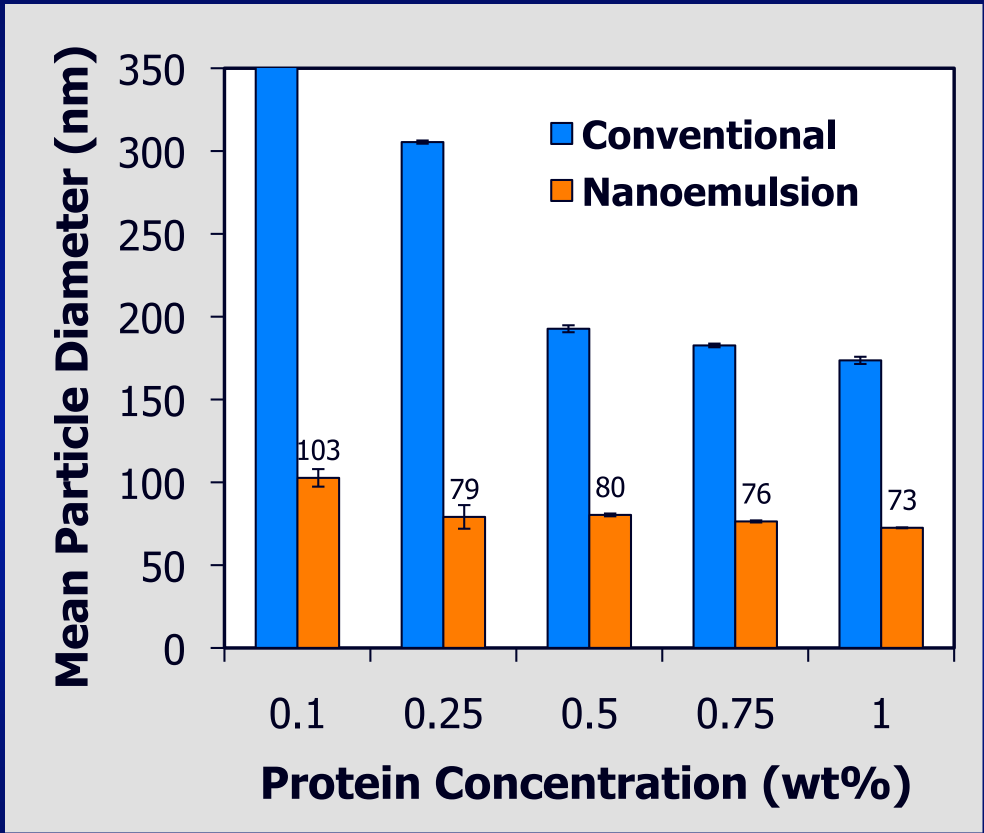
Turbidity Increment vs. Particle Size

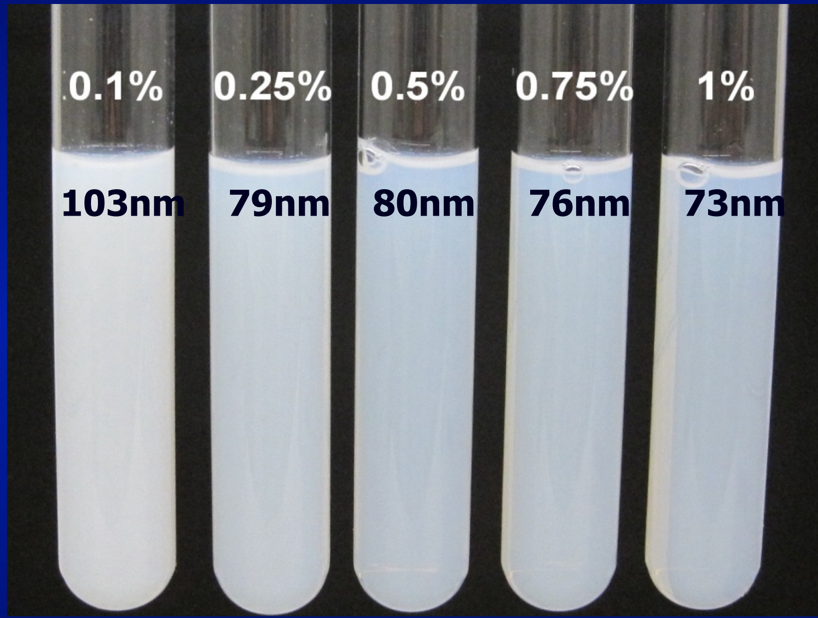






Influence of Emulsifier Concentration

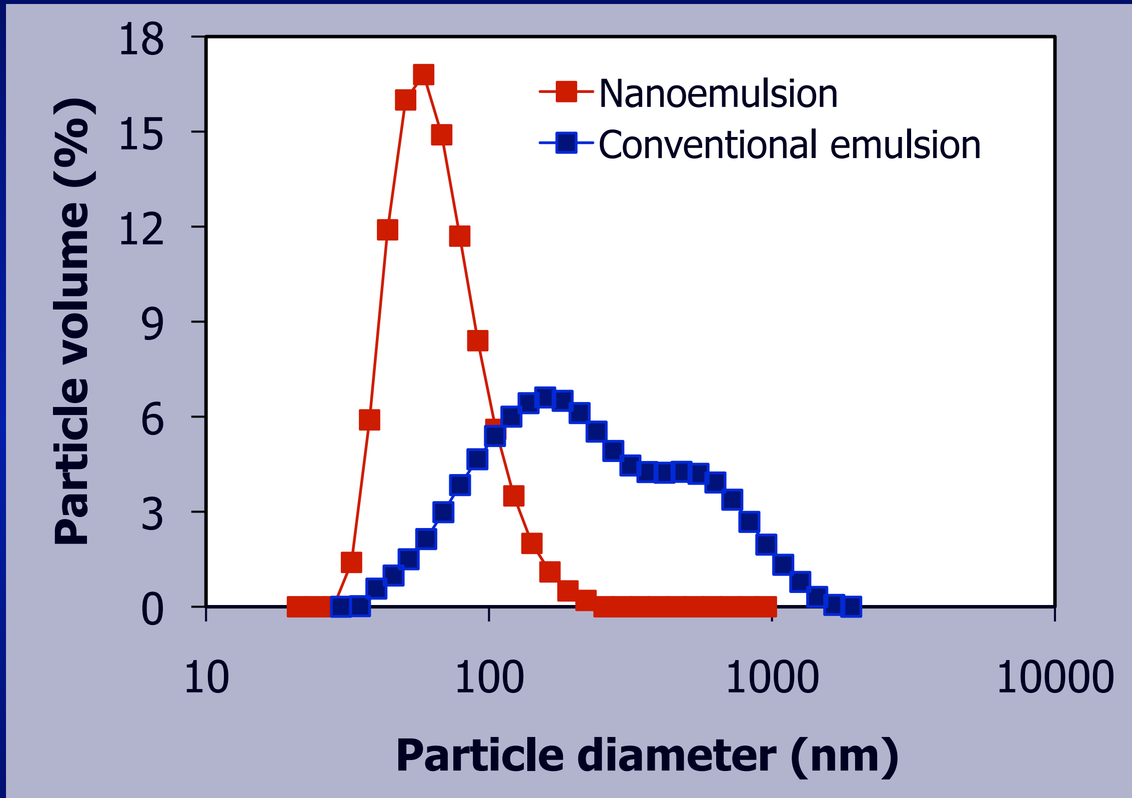


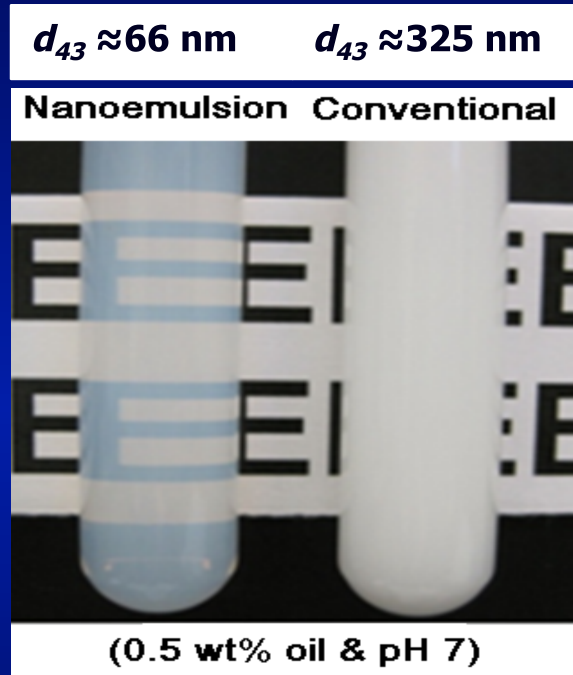


Nanoemulsions with 0.5 wt% oil

10% organic phase (5:95 = oil : solvent)
90% aqueous phase with WPI concentrations (0.1-1%)

Comparison between nanoemulsion and conventional emulsion

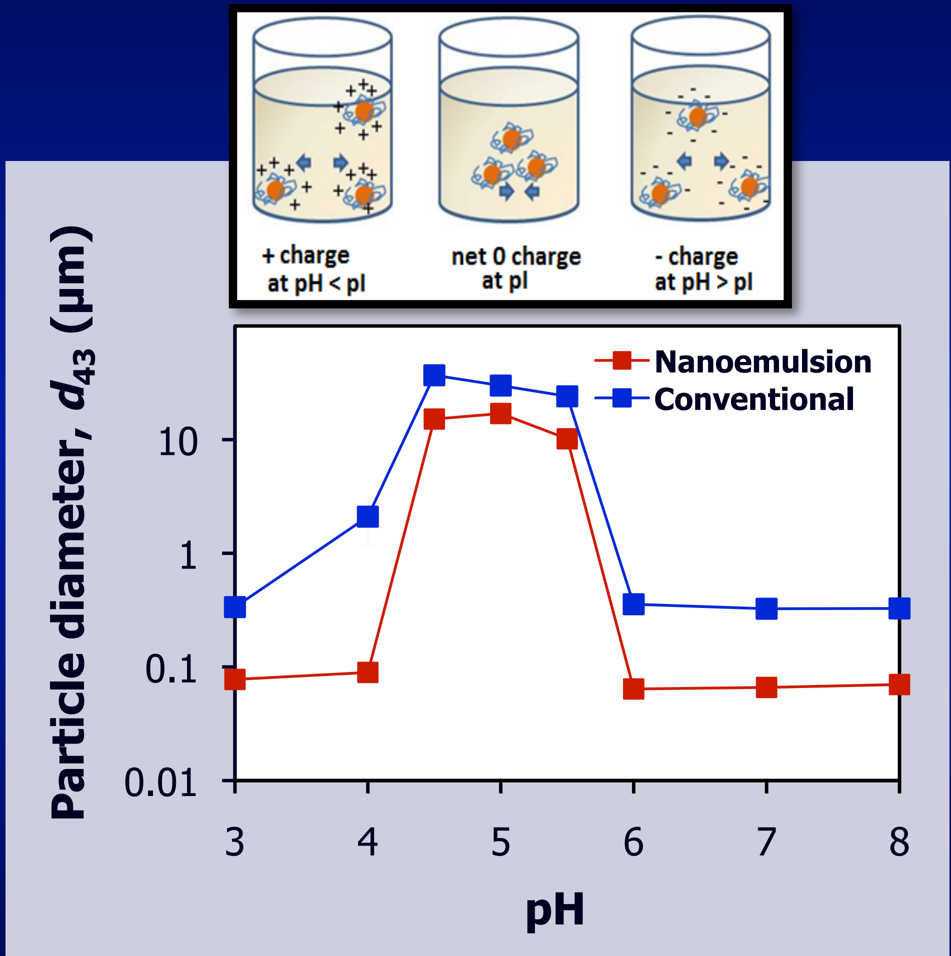


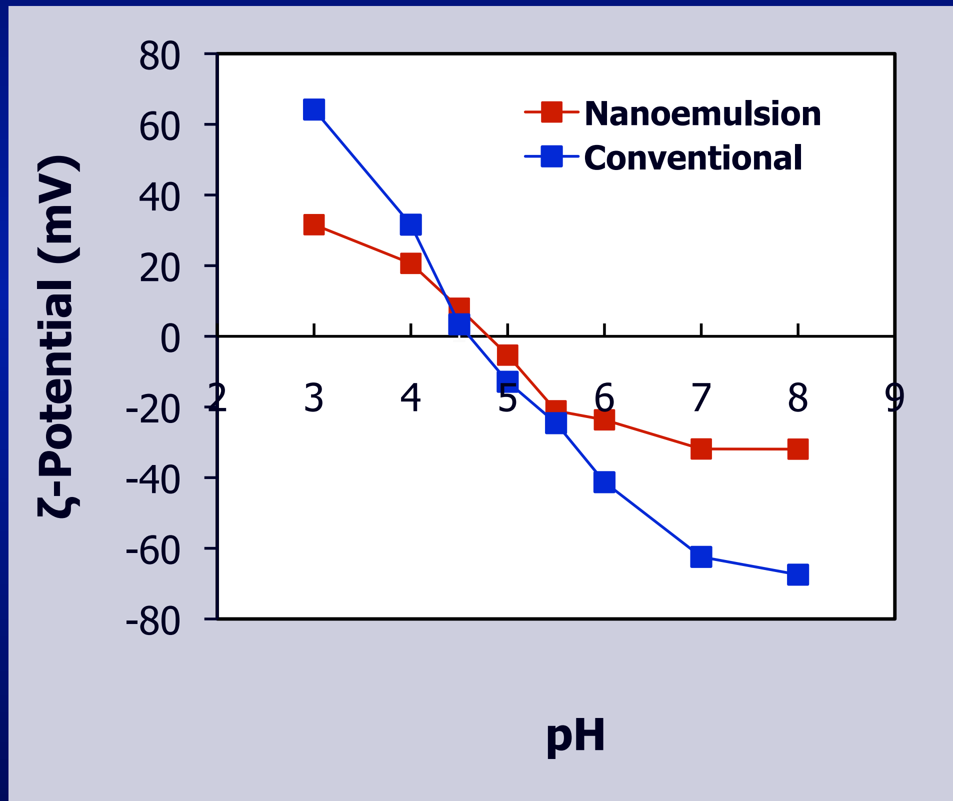


Nanoemulsion
10% organic phase (0.5:9.5 = oil solvent)
90% aqueous phase (1% WPI)

Conventional emulsion
10% oil
90% aqueous phase (1% WPI)

Effect of pH on the particle size and zeta potential of nanoemulsions and conventional emulsions

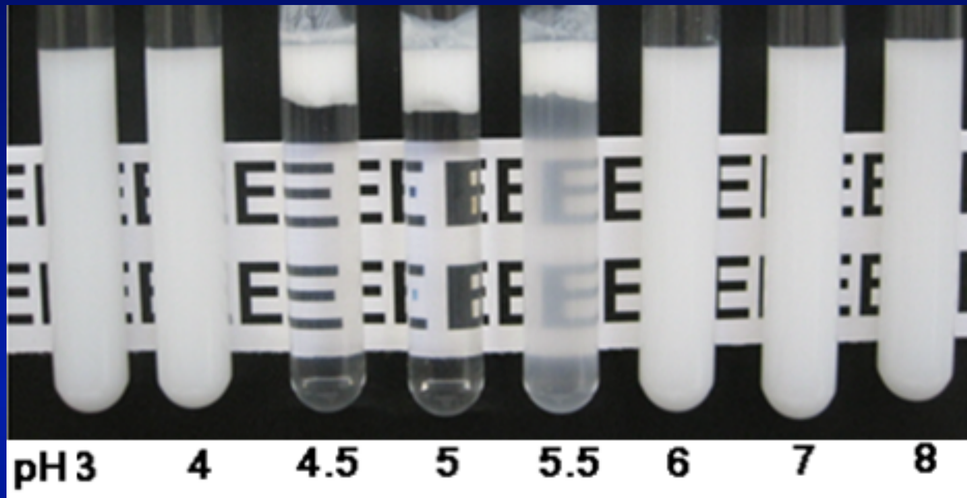




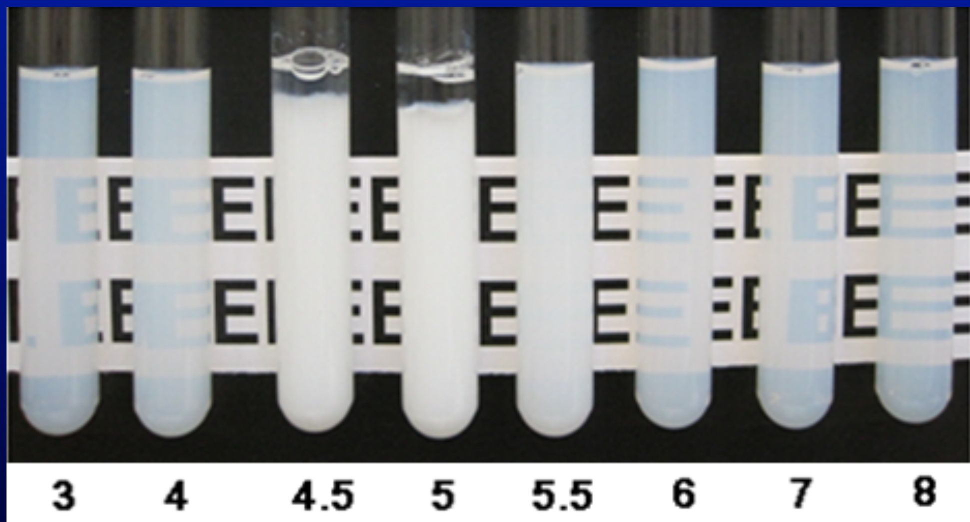
Nanoemulsions (0.5% oil and 0.9% WPI)
Conventional emulsions (0.5% oil and 0.045% WPI)

Photographs of nanoemulsions and conventional emulsions at different pH levels

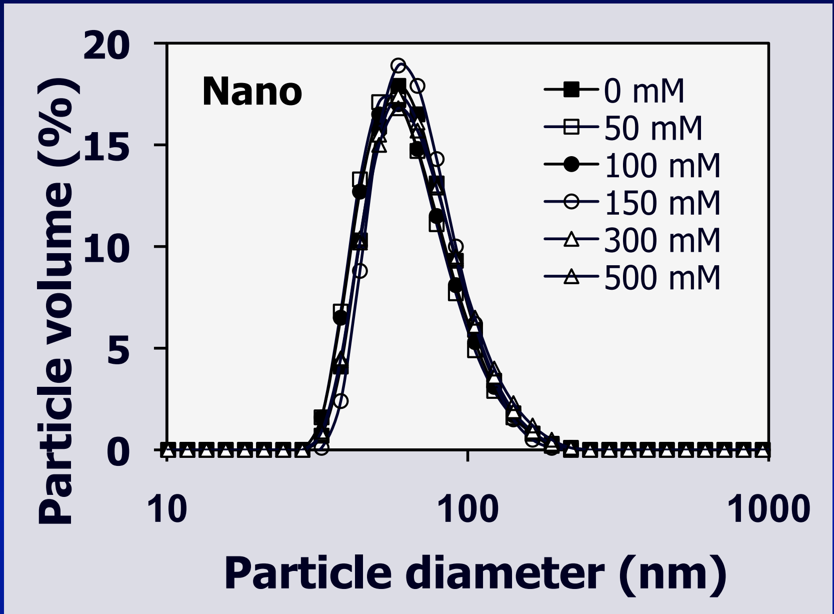
Conventional emulsions

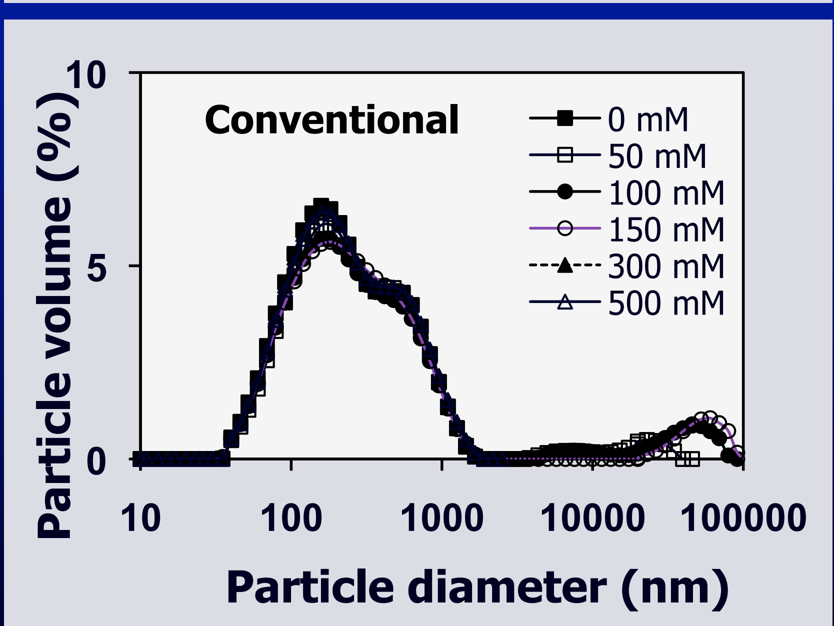


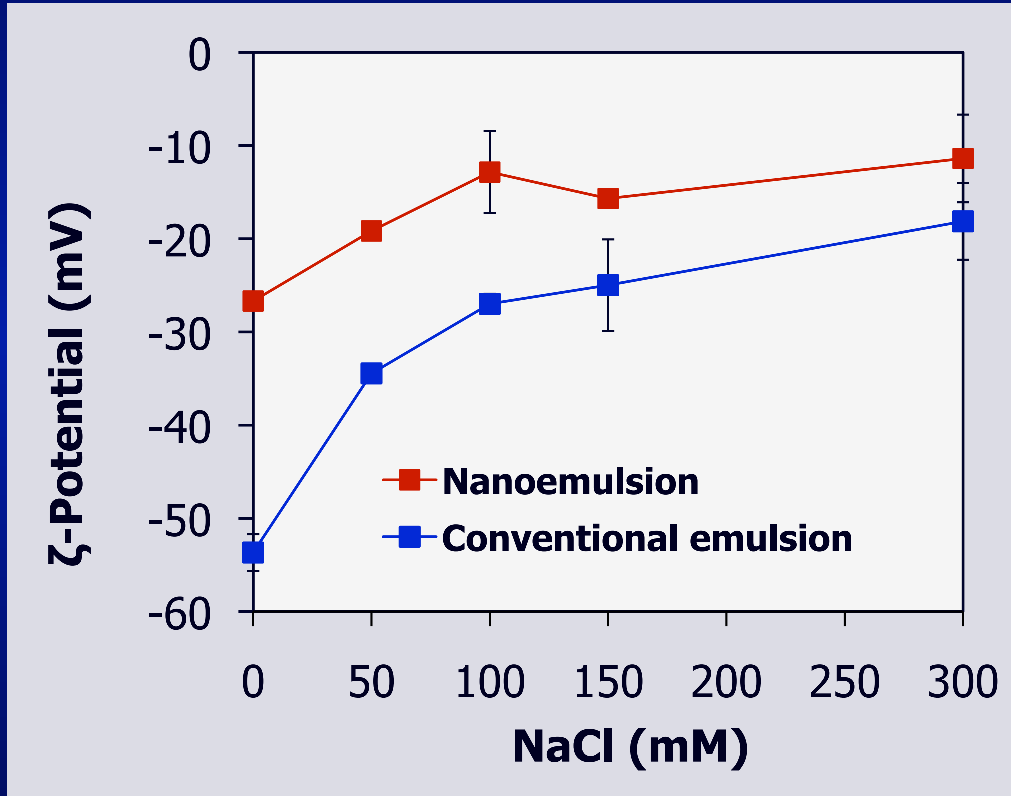
Nanoemulsions



Effect of ionic strength (NaCl) on the stability of nanoemulsions and conventional emulsions

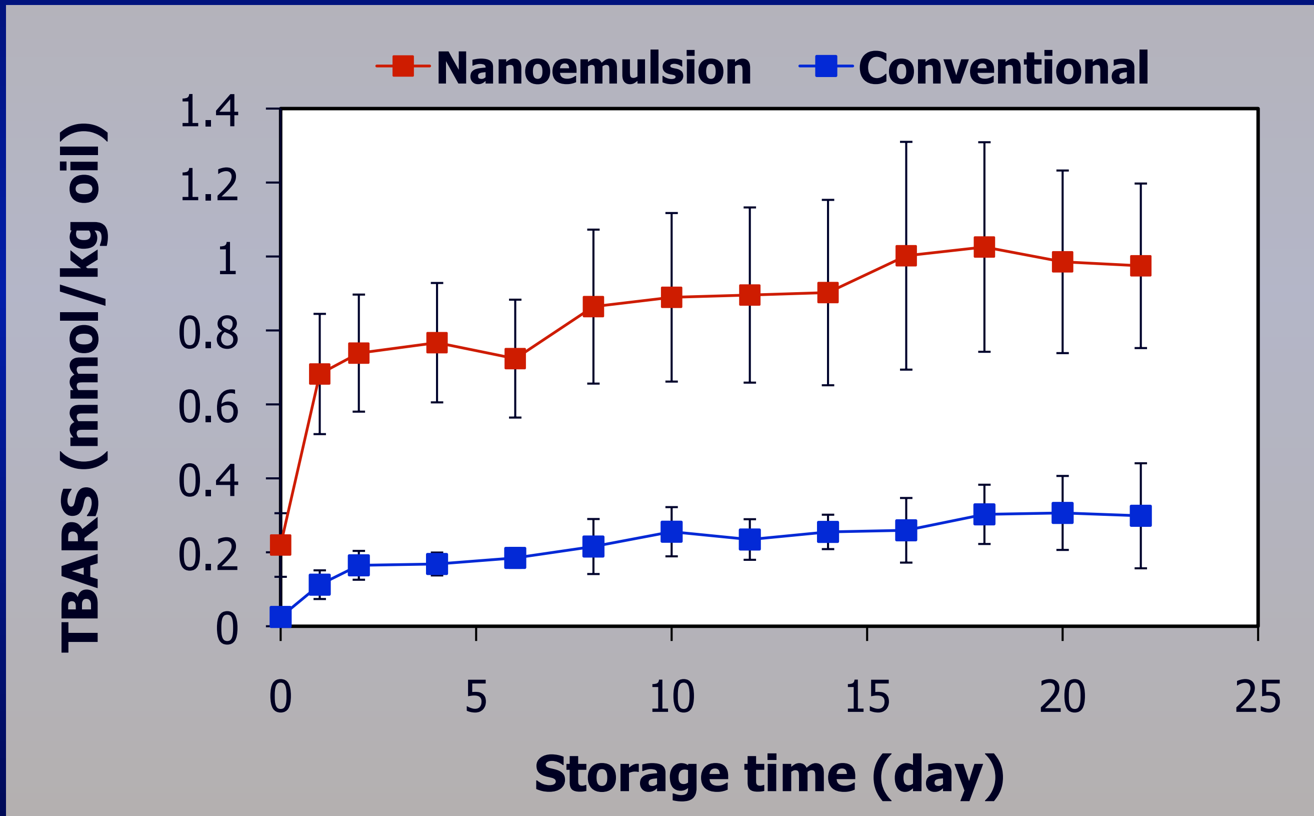






Oxidative Stability of Emulsions

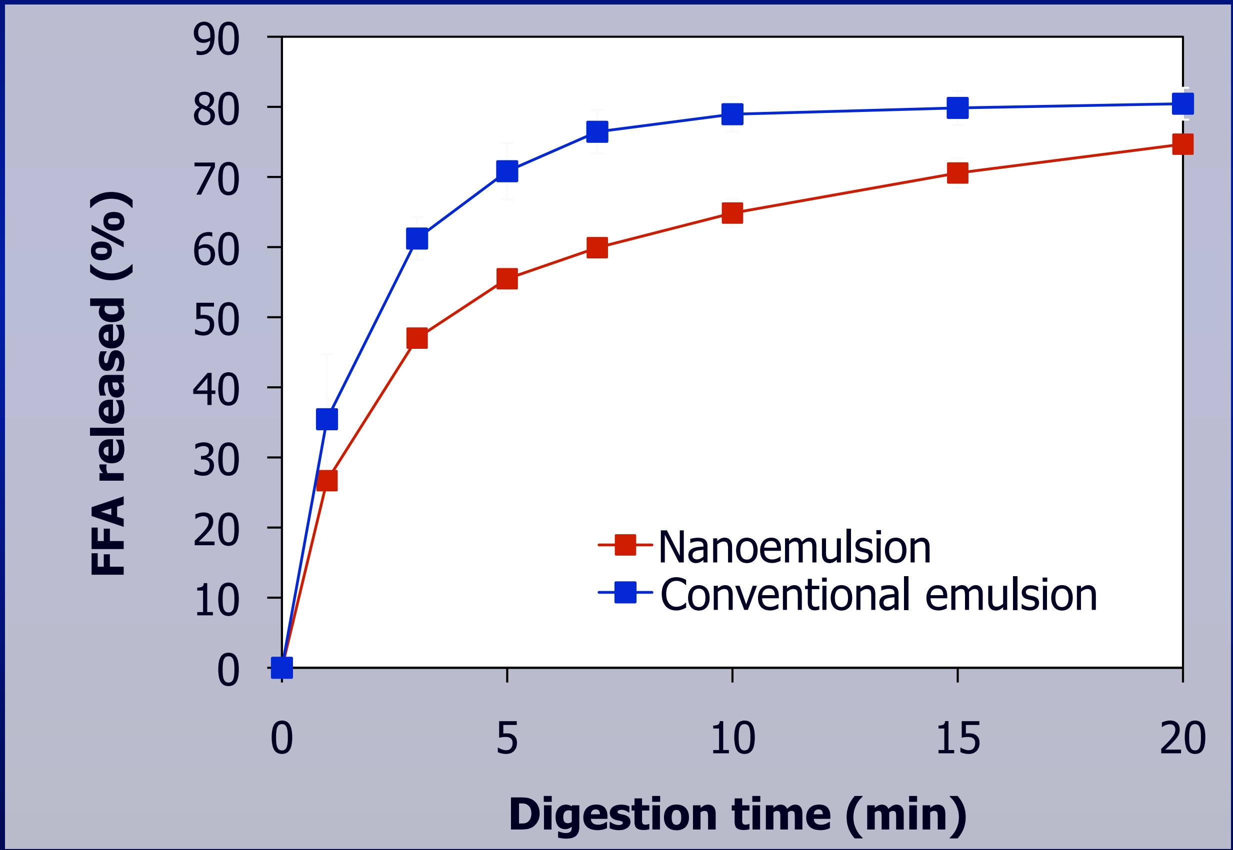
Formation of TBARS in emulsions containing 0.5% menhaden oil during storage at 37^{o}C



TBARS: Thiobarbituric acid reactive substances

In vitro Digestibility of Emulsified Lipids in Simulated Intestinal Fluid

Free fatty acids hydrolyzed from oil droplets from emulsions



Conclusions

- Nanoemulsions smaller than 75 nm can be produced by a combined method of emulsification and solvent evaporation.
- The physicochemical properties of nanoemulsions and conventional emulsions are very different.
  - Nanoemulsions are more stable than conventional emulsions.
- This study has important implications for the development of natural nanoemulsions suitable for the food application.
  - Delivery of functional lipophilic substances
- A major limitation of this method is that a large amount of organic solvent is required to prepare emulsions.

Further studies & Research collaboration

1. Characterization of interfacial layers (e.g. structure and surface load)
2. Separation and concentration of nanoemulsion oil droplets
3. Long-term storage stability
4. Digestion behaviour and oxidative stability of nanoemulsions prepared with different polymers
5. Fabrication of the physicochemical properties of nanoemulsions by depositing different polymers onto the surface droplets
6. Encapsulation of various types of lipophilic components into nanoemulsions
7. Application of nanoemulsion technique for formation of nanoparticles

Thank you