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).

Continuous Phase

Dispersed Phase

oil

water

surfactant
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

<table>
<thead>
<tr>
<th>Emulsion type</th>
<th>Diameter range</th>
<th>Thermodynamic stability</th>
<th>Surface-to-mass ratio (m²/g)</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macroemulsion</td>
<td>0.1-100 µm</td>
<td>Unstable</td>
<td>0.07 – 70</td>
<td>Turbid/ opaque</td>
</tr>
<tr>
<td>Nanoemulsion</td>
<td>20-100 nm</td>
<td>Unstable</td>
<td>70 – 330</td>
<td>Transparent</td>
</tr>
<tr>
<td>Microemulsion</td>
<td>5-50 nm</td>
<td>Stable</td>
<td>130 -1300</td>
<td>Transparent</td>
</tr>
</tbody>
</table>

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

Homogenization
## Types of Emulsifiers

<table>
<thead>
<tr>
<th>Natural (macromolecules)</th>
<th>Synthetic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phospholipids</strong></td>
<td>• Mono-diglycerides</td>
</tr>
<tr>
<td>• Lecithin from soy bean and egg yolk</td>
<td>• Mono-diglycerides derivatives: DATEM, CITREM, LACTEM, etc</td>
</tr>
<tr>
<td><strong>Proteins</strong></td>
<td>• Propylene Glycol Esters (PGE)</td>
</tr>
<tr>
<td>• Milk proteins (caseins, whey proteins, ( \beta )-lg, lactoferrin, etc), soy proteins, egg white proteins</td>
<td>• Sorbitan esters (Spans)</td>
</tr>
<tr>
<td><strong>Hydrocolloids</strong></td>
<td>• Ethoxylated sorbitan esters (Tweens)</td>
</tr>
<tr>
<td>• Gum Arabic</td>
<td>• Polyglycerol esters</td>
</tr>
<tr>
<td>• Chemically modified hydrocolloids (e.g. pectin, cellulose)</td>
<td>• Sucrose esters</td>
</tr>
</tbody>
</table>
Emulsion Stability: Instability Mechanisms

- **Creaming**: Low density oil droplets
  - Prevented by reducing droplet size

- **Flocculation**: Weak interface, Attractive forces
  - Prevented by electrostatic & steric stabilization

- **Kinetically stable emulsion**

- **Coalescence or Ostwald ripening**
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.
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

<table>
<thead>
<tr>
<th>High energy method</th>
<th>Low energy method</th>
</tr>
</thead>
</table>
| 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°C for 15 min/reduced pressure
Homogenization
12,000 psi
for 4 cycles

Solvent

Organic phase
(oil + solvent)

Emulsification
& Evaporation

Conventional emulsion
without addition of solvent

Aqueous phase
(WPI)

Evaporation
50°C for 15 min

Nanoemulsion
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°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

Corn Oil : Solvent in Organic Phase
- 5:95
- 20:80
- 50:50
- 100:0

Mean Particle Diameter (nm)

Corn oil in Diluted Emulsion (%)

Turbidity (cm$^{-1}$)

Turbidity increment (cm$^{-1}$ wt%$^{-1}$)
Influence of Emulsifier Concentration

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

103nm    79nm    80nm     76nm     73nm

Mean Particle Diameter (nm)

Protein Concentration (wt%)

Conventional
Nanoemulsion

Nanoemulsions with 0.5 wt% oil

0.1%    0.25%    0.5%    0.75%    1%

103nm    79nm    80nm    76nm    73nm
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)

Particle diameter (nm)

- $d_{43} \approx 66 \text{ nm}$
- $d_{43} \approx 325 \text{ nm}$

(0.5 wt% oil & pH 7)
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

![Graph showing the effect of NaCl concentration on particle volume and diameter for both nanoemulsions and conventional emulsions.](image-url)
Formation of TBARS in emulsions containing 0.5% menhaden oil during storage at 37°C
In vitro Digestibility of Emulsified Lipids in Simulated Intestinal Fluid

Free fatty acids hydrolyzed from oil droplets from emulsions

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**Graph Description:**

- **Y-axis:** FFA released (%) 0 to 90
- **X-axis:** Digestion time (min) 0 to 20
- **Graph Legend:**
  - Red squares: Nanoemulsion
  - Blue squares: Conventional emulsion

Graph shows a comparison between nanoemulsion and conventional emulsion in terms of free fatty acid release over time.
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