Multiple emulsions as a tool to microencapsulate bioactive compounds with controlled release

Antonio Martinez-Ferez, PhD
Head of the Research Group TEP025
Department of Chemical Engineering
University of Granada
amferez@ugr.es
Sisyphus punishment and multiple emulsions

Once upon a time there was a poor shmuck named Sisyphus whose day job was pushing a rock up a hill for eternity. (\*: everybody laugh)
The beauty of multiple emulsions

Quintuple emulsion
A. R. Abate and D. A. Weitz.

Optical micrographs of monodisperse sextuple-component triple emulsions, containing one water-in-oil single emulsion and two oil-in-water-in-oil double emulsion
Georgia Institute of Technology, USA

W/O/W double emulsion
Chemical and process engineering: quo vadis?.
Jean-Claude Carpentier, 2005

How do we get to here?
• Simple emulsions.

O/W
Water is the dispersion medium and oil is the dispersed phase

W/O
Oil is the dispersion medium and water is the dispersed phase
• Multiple emulsions.

O₂ phase

O/W/O
oil-in-water-in-oil

W₂ phase

W/O/W
water-in-oil-in-water
• Schematic diagram of a W/O/W double emulsion.

W1/O/W2

W1: internal aqueous phase

O: Oil

W2: external aqueous phase

Hydrophilic emulsifiers

Lipophilic emulsifiers

W1 phase

W2 phase

O phase
- Real micrographs of a double emulsion. $W_1/O/W_2$

*W1 phase*  
*W2 phase*  
*Oil*
Multiple emulsion

Increase 3.5 times
3500 papers

Multiple emulsion & pharmaceutical

x6

Multiple emulsion & food

x6
• Double emulsions - definitions.

  – These are known as “emulsions of emulsion”.
  – These are more complex systems, as the drops of the dispersed phase contain even smaller dispersed droplets, in most cases identical with the continuous phase (but not necessarily identical), but separated physically from the continuous phase.
  – The double emulsion can be viewed as a system, in which two liquids are separated by a third liquid which is not miscible with the first two liquids.

Key factor for optimal design:

**Osmotic pressure balance**
Generic features of double emulsions

**STRUCTURE**
- Nested structures (drops in the drops)
- Two kinds of interfaces with opposite curvatures (different surfactants o/w and w/o)
- Variable phase ratios and morphologies

**ADVANTAGES**
- Complete biodegradability.
- Hydrophylic as well as hydrophobic drugs can be entrapped.
- Increase in drug dosing intervals.
- Taste masking of drugs/bioingredients.
Citotoxicity Assesment of new self-emulsifying multiple W/O/W nanoemulsions
Estelle Sigward and coworkers – Paris Descartes University

### Primary W/O nanoemulsions (PE):

<table>
<thead>
<tr>
<th>Formulation identification</th>
<th>(a)</th>
<th>(b)</th>
<th>(c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil (Medium Chain Triglycerides)</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Water</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Polysorbate 85</td>
<td>35</td>
<td>35</td>
<td>15</td>
</tr>
<tr>
<td>Surfactants = 50 %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Labrasol®</td>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cremophor® EL</td>
<td>-</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>Glycerol</td>
<td>-</td>
<td>-</td>
<td>35</td>
</tr>
</tbody>
</table>

(c) formulation: no cytotoxicity

→ great interest for:
- Parenteral route
- Oral route

### Multiple W/O/W nanoemulsions:

→ 2 volumes of water : 1 volume of PE
• Generic features of double emulsions

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**DISADVANTAGE**
- Used to have short shelf life.
Generic features of double emulsions

Compartment structure offers/allows:
- protection (chemical, sensorial) for sensitive material
- locally high concentrations at low absolute amount

Axel Syrbe, SCI Conference, 2012
• The usual two-step method for preparation of double emulsions

I. Lipophilic surfactant with a low HLB (2-8) suitable for the stabilization of such W/O system

II. Hydrophilic surfactant (HLB 6-16) to promote O/W emulsification.
One-step multiple emulsions – phase inversion

Bancroft, a normal emulsion is obtained if the phase in which the surfactant is predominately solubilized is the continuous phase of the emulsion (Y-AXIS).

The Ostwald Packing concept postulates that the dispersed phase volume fraction of an emulsion cannot exceed 0.74 (X-AXIS).

SAD (Surfactant Affinity Difference) or its numerical equivalent HLD (Hydrophilic Lipophilic Difference) measures the relative affinity of the surfactant for the aqueous and oleic phase.

Abnormal emulsion formation is favored at high surfactant concentrations and/or dispersed/continuous volumetric phase ratios far from 1:1.

SAD<0 favors O/W emulsion

There is insufficient volume of water to allow O/W formation (Winsor I): abnormal emulsions (Wm/O and O/Wm/O)

SAD>0 favors W/O emulsion

There is adequate volume of water to form O/W emulsions (Winsor II)

M. Morais et al, 2009
• Parameters to take into account for successful design.

**THE OIL PHASE**

- The nature of the oil can affect the behavior of the system.
- Selection of the oil phase can affect various emulsion parameters like yield, release profile, particle size and emulsion stability.
- The release of drug from multiple emulsion is affected by the nature of the oil phase due to the difference in partition coefficient.

\[
K_{PM} = \frac{C_{P,\infty}}{C_{M,\infty}}
\]

**SURFACTANTS**

- The selection of surfactants depends upon the use of the multiple emulsion.
- Concentration of surfactant also affects the emulsion yield.
- An excess of lipophilic surfactant can cause the inversion of W/O/W emulsion to simple O/W emulsion.

Bidimensional process-composition (phase inversion) map.
Parameters to take into account for successful design.

- Rotor/stator homogenizers frequently used.
- Activation of biopolymers (CMC). Help stability.
- Generally, high agitation speed is used for primary and low speed is used for secondary emulsification.

TEMPERATURE

NATURE OF THE ENTRAPPED MATERIAL

PHASE VOLUME RATIO (Φ)
• Parameters to take into account for successful design.

Oxidative stability of W/O vs W/O/W: depending on lipid composition and antioxidant polarity

Type of antioxidant is a key factor in controlling oxidation

The rate of oxidation of α-linolenic and linoleic acid is 20 and 10-fold the value exhibited by oleic acid.

Lindseed oil: Hydrophilic antioxidant (Melissa) was more efficient in O/W emulsions, whereas the lipophilic antioxidant (BHA) was more effective in W/O/W ones.

Great stability of olive oil emulsions was observed, without noticing differences between antioxidants or type of emulsion.

C. Poyato et al, 2013
• Some examples of drop size distributions:
Mechanisms for destabilization of multiple emulsions:

- Density mismatch
- Flocculation (aggregation)
- Creaming
- Ostwald ripening
- Coalescence
- Total phase separation
• Mechanisms for destabilization of multiple emulsions (pathways):
• Complex equilibrium of multiple emulsions:

- Membrane system in dynamic equilibrium (continuous diffusion).
- Inner droplets are intrinsically unstable (Laplace pressure).
- DE “lives” from an osmotic pressure gradient that outweighs the Laplace pressure difference.

**DE samples made with opposite salt concentration gradients, 1 week @4 °C**

Axel Syrbe, SCI Conference, 2012
• Laplace pressure – Osmotic pressure: Mandatory equilibrium

\[ \Delta P = P_{\text{inside}} - P_{\text{outside}} = \gamma \left( \frac{1}{R_1} + \frac{1}{R_2} \right) \]

- Laplace pressure against the stability of emulsions (Davis, 1981) (Ostwald ripening).
- Presence of electrolytes in the inner dispersed phase: swelling until they rupture.
- Presence of electrolytes in the outer dispersed phase: shrinkage and destabilization.
- The concentration of electrolytes has to be enough to counteract the Laplace pressure but sufficiently low to avoid osmotic effects.

Stability depends on the strength of the interfacial film formed on the interface of droplets.
• Selection of components/coadjutants.

Stability (shelflife)

Controlled release

Encapsulation/protection efficiency

System properties

Organoleptic characteristics

Example: Na reduction

Healthy/sens

Salt perception > really present

Implications

Osmotic pressure regulators, Hydrocolloids, Thickening/gelling agents, Chelating agents (to decrease minerals release), etc

Fat (calorie reduction)

Healthy fats

Improved protection against oxidation (hydrophilic/hydrophobic antioxidants)

Flavor distribution, Control over the exposure time of W2 onto taste buds, reduction of salt content strategy, etc
• Selection of components/coadjutants: NaCMC and Cellulose microcrystalline.

➢ Activation with rotor/stator homogenisation / not simple agitation.

«W/o/w emulsion technology offers a sensory perception closer to full fat than the classical thickener approach»
- Applications already published.

<table>
<thead>
<tr>
<th>Main purpose</th>
<th>Content of inner aqueous solution (W₁)</th>
<th>Lipid oil (O)</th>
<th>Lipophilic emulsifier (% in oil phase)</th>
<th>Relation W₁/O (%)</th>
<th>Hydrophilic emulsifier (% in external aqueous solution, W₂)</th>
<th>Ratio (%) W₁/O and W₂</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formation of multiple emulsion</td>
<td>NaCl (0.1M), gelatine (5%), Poly R-478 (0.5%)</td>
<td>Sunflower oil</td>
<td>PGPR (4%)</td>
<td>20/80</td>
<td>WPI</td>
<td>15/85</td>
<td>Scherze et al., 2005</td>
</tr>
<tr>
<td>Encapsulation of Mg</td>
<td>MgCl₂ (0.1M), lactose 0.02M-0.2M, SC (1.9%)</td>
<td>Olive oil, MTC</td>
<td>PGPR (5%)</td>
<td>40/60</td>
<td>SC (1.9-12.0%), lactose (lactose 0.20 or 0.3 M)</td>
<td>10/90</td>
<td>Bonet et al., 2010a</td>
</tr>
<tr>
<td>Encapsulation of ascorbic acid</td>
<td>Ascorbic acid (30%), gelatin gum (0.5%), Panodor SDK (8%)</td>
<td>Chia oil</td>
<td>6% (1 part of Panodor SDK + 4 parts of PGPR)</td>
<td>20/80</td>
<td>Mesquite gum, maltodextrin, WPC</td>
<td>20/80</td>
<td>Carrillo-Navas et al., 2012</td>
</tr>
<tr>
<td>Encapsulation of ferrous bisglycinate</td>
<td>Ferrous bisglycinate (30%)</td>
<td>Mineral oil (food grade)</td>
<td>5% of PGPR/Panodor SDK (6:4)</td>
<td>50/50</td>
<td>WPC: polysaccharides (gum arabic or mesquite gum or low methoxyl peptin) 2:1</td>
<td>20/80</td>
<td>Jiménez-Alvarado et al., 2009</td>
</tr>
</tbody>
</table>

- Minerals: Ca, Fe, Mg.
- Carotenoids.
- Vitamins: hidrophilic W/O/W (C, B₁, B₁₂, etc.); lipophilic O/W/O (A, D, E, etc.)
- Resveratrol.
- Microorganisms: Lactobacillus acidophilus, L. rhamnosus.
- Lactoferrin.
- Aminoacids
- Lipids (O/W/O)
Entrapment of ferrous bisglycinate in a multiple emulsion

- ferrous deficiency is the most common nutritional deficiency in both the industrialized and developing worlds, affecting mostly infants, children, and women of childbearing age.

**Ferrous bisglycinate:**
- food fortificant / absorption in humans not limited by action of phytates or polyphenols;
- interacts with other food components changing the taste of foods;
- it is easily oxidized.

Double emulsions:

- W1/O emulsion: 0.5 dispersed phase mass fraction.
- WS surfactant: esters of monoglycerides and diglycerides of diacetyl tartaric acid
- OS surfactant: esters of polyglycerol and polyricinoleate fatty acids.
- 2-phase method.
- W1/O/W2 emulsion: 0.2 dispersed phase mass fraction
- W2: Protein:polysaccharide complexation (WPC-MG-AG-LMP)
- pH of W/O/W was adjusted where the formation of Pr:Ps complexes was maximized to allow the formation of a biopolymeric complex at the outer oil–water interface.

Fig. 2. Zeta potential in function of pH for biopolymers solutions: (○) WPC; (●) GA; (▲) MC; (●) LMP.

Jimenez-Alvarado et al, 2009
• Entrapment of ferrous bisglycinate in a multiple emulsion

Change in the amount of Fe\(^{2+}\) and Fe\(^{3+}\) across the time and encapsulation yield for the whole (W\(_1\)/O/W\(_2\))\(_{1,2,3}\) multiple emulsions, and loss of total iron in W\(_1\) after 480 h of storage time.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>(W(_1)/O/W(<em>2))(</em>{1,2,3})</th>
<th>(W(_1)/O/W(<em>2))(</em>{2})</th>
<th>(W(_1)/O/W(<em>2))(</em>{3})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>60.38 ± 0.51</td>
<td>39.62 ± 0.51</td>
<td>80.88 ± 0.65</td>
</tr>
<tr>
<td>48</td>
<td>59.12 ± 0.70</td>
<td>40.88 ± 0.70</td>
<td>87.85 ± 0.52</td>
</tr>
<tr>
<td>72</td>
<td>53.85 ± 0.42</td>
<td>46.15 ± 0.42</td>
<td>85.72 ± 0.21</td>
</tr>
<tr>
<td>96</td>
<td>53.28 ± 0.31</td>
<td>46.72 ± 0.31</td>
<td>85.72 ± 0.15</td>
</tr>
<tr>
<td>144</td>
<td>50.73 ± 0.25</td>
<td>48.27 ± 0.25</td>
<td>84.06 ± 0.38</td>
</tr>
<tr>
<td>168</td>
<td>49.22 ± 0.29</td>
<td>50.78 ± 0.29</td>
<td>81.37 ± 0.27</td>
</tr>
<tr>
<td>192</td>
<td>55.00 ± 0.53</td>
<td>53.20 ± 0.53</td>
<td>81.97 ± 0.39</td>
</tr>
<tr>
<td>240</td>
<td>55.34 ± 1.55</td>
<td>44.66 ± 1.55</td>
<td>77.52 ± 0.54</td>
</tr>
<tr>
<td>336</td>
<td>42.99 ± 0.57</td>
<td>57.01 ± 0.57</td>
<td>69.77 ± 0.75</td>
</tr>
<tr>
<td>480</td>
<td>40.58 ± 0.26</td>
<td>59.42 ± 0.26</td>
<td>71.25 ± 0.58</td>
</tr>
</tbody>
</table>

EY (%) | 73.84 ± 1.51
Loss of total iron in W\(_1\) (%) | 36.0 ± 0.53

Higuchi’s model: \[ [\text{Fe}_{\text{W}_2}] = K_H t^{1/2} \quad K_H: \text{release rate constant} \]

1. Initial stage characterized by a steep gradient induced by the relatively high initial total iron concentration in W\(_1\) vs W\(_2\).
2. Gradient drastically diminished, probably because concentrations in total iron between W\(_1\) and W\(_2\) tended to equilibrium.

Release kinetics of ferrous bisglycinate from W\(_1\) to W\(_2\) is highly dependent on the length of the diffusion pathways, i.e. the thicker the Pr:Ps complex interfacial membrane thickness in the (W\(_1\)/O/W\(_2\)) emulsions, the slower the release kinetics.

Jimenez-Alvarado et al, 2009
Multiple emulsions as solid particles/microcapsules.

Atomization of emulsions in a controlled manner is a growing research field for food engineers.

Capability to carry both polar and non-polar components

Conclusions. The interfacial properties of microencapsulated lecithin-chitosan multilayer emulsion droplets remain intact upon reconstitution into an aqueous system.
Multiple emulsions as solid particles/microcapsules.

- Lactobacillus acidophilus
- Microencapsulation (WPI+CMC+pectin +GMO/GMP)
- GIT simulation (Gastric and Intestinal fluids)

Spray-drying
Niro Mobile-Minor (Niro Atomizer)
• Multiple emulsions as solid particles/microcapsules.

Encapsulating material characteristics:

- **proteccion** against external environmental conditions
- protection against internal gastrointestinal conditions
- specific release in the colon

- Complete release
- Film forming capacity
- Protection
- Disperse / emulsify bioactive
- High conc. handling

- Goats milk oligosaccharides
- Whey proteins
- CMC
- Gums
- Pectin
- Pectinase activity in the colon
Multiple emulsions as solid particles/microcapsules.

**Encapsulating material characteristics:**

- protección against external environmental conditions
- protection against internal gastrointestinal conditions
- specific release in the colon

Coacervation
- Low protection
- Low payload

Liposomes
- Low cost
- High yield
- Easy scale-up

Spray drying

Whey proteins
- CMC
- Gums

Goat milk oligosaccharides

Pectin
- Pectinase activity in the colon
- Multiple emulsions as solid particles/microcapsules.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Spray – drying (%)</th>
<th>GIT simulation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>With goat milk oligosaccharides (GMO)</td>
<td>91.0</td>
<td>82.2</td>
</tr>
<tr>
<td>Without goat milk oligosaccharides (GMP)</td>
<td>80.0</td>
<td>64.3</td>
</tr>
</tbody>
</table>

Developing a simulator of the human gastrointestinal tract using automated chemical reactors
Multiple emulsions as solid particles/microcapsules – GIT simulation.

1. Stomach + postprandium
2. Small intestine + absorption
3. Large intestine + colon

- Stable
- Controlled instability

- Matrix compatibility
  - Optical
  - Rheological
  - Stability
  - Flavor

- Processing
  - Heating
  - Cooling
  - Drying
  - Shearing

- Storage
  - Temperature
  - Mechanical stress
  - Light
  - Oxygen
  - Time

- Consumption
  - Appearance
  - Texture
  - Taste and Aroma
  - Convenience

- Ingestion/Digestion
  - Digestion
  - Absorption
  - Toxicity
• Multiple emulsions as edible films.

Double emulsions:
• Coadjutants: WPI – CMC – LMP.
• pH: where the maximum stoichiometric difference of the electrostatic charges between protein (Pr) and poly-saccharide (Ps).
• Water-soluble surfactant: esters of monoglycerides and diglycerides of diacetyl tartaric acid
• Oil- soluble surfactant: esters of polyglycerol and polyricinoleate fatty acids.

Edible films:
• Within 6 h of preparation, double emulsion was spread on glass plates.
• Drying at 25°C in a convective oven for 5 hours.

Microstructure consisting of closely packed elongated double emulsion droplets into a biopolymers mainframe.

Greater degree of cross-linking between biopolymers when emulsion droplets were smaller, resulting in a more closed film mainframe (LMP-WPI film).

Fig. 1. Changes in the apparent viscosity a function of shear rate for the double emulsions.
Multiple emulsions as edible films.

Double emulsions:
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- pH: where the maximum stoichiometric difference of the electrostatic charges between protein (Pr) and poly-saccharide (Ps).
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- 2-phase method.

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Table: Mechanical properties and water vapour permeability (WVP) of the edible films.

<table>
<thead>
<tr>
<th>Film code</th>
<th>Tensile strength (MPa)</th>
<th>% Elongation</th>
<th>Young’s Modulus (MPa)</th>
<th>WVP (g/m² h kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F_{CMC-WPI}</td>
<td>0.93 ± 0.11</td>
<td>6.11 ± 0.87</td>
<td>48.76 ± 4.90</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>F_{LMP-WPI}</td>
<td>1.49 ± 0.17</td>
<td>2.15 ± 0.43</td>
<td>119.74 ± 48.61</td>
<td>1.6 ± 0.1</td>
</tr>
</tbody>
</table>

Mechanical properties comparable with those exhibited by hydrophilic films, but with water vapour permeability comparable with those displayed by hydrophobic films.

Possibility for incorporating antimicrobials, nutraceuticals, probiotics, etc, providing the basis for designing a new generation of edible films with enhanced functional properties.
Thank you for your attention

The struggle itself towards the heights is enough to fill a man's heart. One must imagine Sisyphus happy.

A Life Without Challenges Is A Life Not Worth Living

Agradecimientos:
Universidad de Málaga. Campus de Excelencia Internacional Andalucía Tech por la ayuda recibida para el desarrollo de Conferencias Científicas.