Bioaccessibility study of Calcium and Vitamin D3 co-microencapsulated in W/O/W double emulsions

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I. INTRODUCTION

1. Why "Bioaccessibility study"?
2. Why Calcium and Vitamin D3?
3. Why "co-microencapsulated" procedure?
4. Why W/O/W double emulsions?
1. Why "Bioaccessibility study"?

- *Bioaccessibility* ($B^*$) is the fraction of micronutrient *released* from the food matrix and *solubilized* within gastrointestinal fluids so that it can be absorbed;
- is a major factor limiting the bioavailability of micronutrients.

\[
F = F_C \cdot F_B \cdot F_A \cdot F_T
\]
2. Why Calcium and Vitamin D3?

**Calcium** is the most abundant mineral in the human body. **Calcium** is involved in many biochemical processes: nerve conductivity, muscle contraction, hormone and enzyme secretion.

**Vitamin D (VD)** was firstly identified as a vitamin (micronutrient) and now is recognized as a prohormone (precursor to seco-steroid hormone known as *calcitriol*);
3. Why ”co-microencapsulated” procedure?

- Ensure encapsulation in a single delivery system of two or more biocomponent;
- Ensure biocomponent sineering action.
- Provides biocomponent protection against physicochemical factors (temperature, oxygen, water, pH changes, light).
- Ensure the stability and controlled release of biocomponents when they pass through the gastrointestinal tract.
4. Why W/O/W double emulsions?

Double emulsions are colloidal systems that provide co-microencapsulation of lipophilic and hydrophilic components.
Scope and objectives

**Scope:** bioaccessibility study of calcium and vitamin D3 co-microencapsulated in W/O/W double emulsions.

**Objectives**

O1: Preparation of calcium and vitamin D3 double emulsions.

O2: Stabilization of double emulsions by introducing them into polymeric microspheres obtained by the ionotropic gelation process.

O3: Characterization of double emulsions and polymeric microspheres.

O4: Kinetic "in vitro" release study of calcium and vitamin D3 from polymeric microspheres.
II. METHODS AND RESULTS

O1. Preparation of $W_1/O/W_2$ double emulsions loaded with Ca and VD3

Step 1: Preparation of $W_1/O$ emulsions

**Oil phase (O)**
- Linseed oil (50, 70 % w/w)
- Span 80 (HLB=4.3) (5% w/w)
- VD3 (0.02% w/w)

UltraTurrax (6400 rpm; 5 min.)

**Internal Water phase (W1)**
- Calcium source (calcium citrate, calcium gluconate)
- Distilled water

Ultrasonication (A=50%, P=180W; 1 min.)

Macroemulsion ($W_1/O$)

Ultrafin $W_1/O$ emulsion
Step 2: Preparation of $W_1/O/ W_2$ double emulsions

External Water phase (W2)
- Tween 20 (0.5%)
- Sodium alginate 1.5% (w/w),
- Arabic gum 5% (w/w)
- Sodium azide 0.01% (w/w)
- Lactose 0.25M

W1/O emulsions

Slow stirring

600 rpm; 120 min.

Internal Aqueous phase (W1)

External aqueous phase (W2)

Oil droplet

$W_1/O/ W_2$ emulsions
O2. Stabilization of W/O/W emulsions by ionotropic gelation

Preparation of Alginate Microspheres

Alginate microspheres were prepared by dropping 50 mL W/O/W double emulsion through a syringe (needle size 22G) into 100 mL of a 0.1 M Zinc acetate solution. The microspheres obtained were kept for 2 h in 0.1 M Zinc acetate solution and stored in a 0.006 M ZnCl2 solution at 4 °C.
## Chemical composition of W/O/W double emulsions loaded with Ca and VD3

<table>
<thead>
<tr>
<th>Compounds</th>
<th>DE I</th>
<th>DE II</th>
<th>DE III</th>
<th>DE IV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oil phase</strong></td>
<td></td>
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<tr>
<td>Linseed oil (g)</td>
<td>50</td>
<td>70</td>
<td>50</td>
<td>70</td>
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<tr>
<td>Span 80 (g)</td>
<td>5</td>
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<tr>
<td>VD3 (g)</td>
<td>0.02</td>
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<td>0.02</td>
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<tr>
<td><strong>Internal aqueous phase (W₁)</strong></td>
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<tr>
<td>Ca(2+) (g)</td>
<td></td>
<td>1.2(citrate)</td>
<td>1.2(citrate)</td>
<td>1.2(gluconate)</td>
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<tr>
<td>Distilled water (g) (add 100)</td>
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<tr>
<td><strong>External aqueous phase (W₂)</strong></td>
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<tr>
<td>Gum arabic (g)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
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<tr>
<td>Tween 20 (g)</td>
<td>0.5</td>
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<tr>
<td>Sodium alginat (g)</td>
<td>1.5</td>
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<tr>
<td>Sodium azide (g)</td>
<td>0.01</td>
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<tr>
<td>Lactose 0.25M (g)</td>
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<td>2</td>
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<tr>
<td>Distilled water (g) (add 100)</td>
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</tbody>
</table>
O3. Characterization of double emulsions (W / O / W) and polymeric microspheres

Morphology and particle size determination

a) Morphology

(W/O/W)
Double emulsion II

(Alginate Microspheres)

b) Particle size determination

- W/O/W double emulsions: volume mean diameter was \( d = 21.5\pm0.12 \, \mu m \).
- Alginate microspheres: volume mean diameter was: \( d = 0.89\pm0.21 \, mm \).
Efficiency of the encapsulation

The efficiency of the encapsulation (EE%) has been calculated with the relation:

\[ EE(\%) = \frac{M_{\text{Encapsulated}}}{M_0} \cdot 100 \]

- The encapsulation efficiency for W/O/W double emulsions is higher than for microspheres.
- Higher solubility of calcium citrate induces a loss of Ca ions in the aqueous solution.
- The vitamin D3 encapsulation efficiency increases with increasing oil content in the oil phase.
O4. Dynamic "in vitro" digestion model

- **Simulated saliva fluid (SSF)**
  - pH 6.8
  - Buffer solution
  - Salts
  - Mucin (3%wt)
  - Sample (Double emulsion/Microspheres)
  - Stirring 2 min (37°C)

- **Simulated gastric fluid (SGF)**
  - pH 2.5
  - NaCl
  - HCl
  - Pepsin
  - Shaking 2h (37°C)

- **Simulated intestinal fluid (SIF)**
  - pH 7
  - Ionic salts
  - Phosphate buffer
  - Porcine lipase
  - Stirring 2h (37°C)

- **Bolus sample**
  - W/O/W Double emulsions (confocal fluorescence microscopy)

- **Chyme sample**
  - Alginate microspheres (electronic microscopy)
Release of Ca and VD3 from W/O/W double emulsions and from alginate microspheres

- Kinetic curves of VD3 and Ca released from free double emulsions (DE) and entrapped in alginate microspheres (MS):
  - In double emulsions, the release rate of Ca is higher than the release rate of VD3;
  - In microspheres, the release rate of Ca is lower than in double emulsions due to Ca retention by alginat.

FFAs released under simulated small intestinal conditions (calculated by pH stat method) : DE-double emulsions; MS-alginate microspheres
Four W/O/W double emulsions loaded with Calcium and Vitamin D3 were prepared;

W/O/W double emulsions loaded with Calcium and Vitamin D3 were stabilized by incorporating them into sodium alginate microspheres;

The calcium encapsulation efficiency was less than of vitamin D3;

Digestion of W/O/W double emulsions and alginate microspheres was studied using dinamic ”in vitro” digestion model;

The release rate of free fatty acids obtained by enzymatic hydrolysis of glycerides was measured;

The percentage of free fatty acids released increases with the increase in the oil content of the W/O/W double emulsions;

The release rate of free fatty acids from the microspheres is lower than from double emulsions due to limited lipase access to the oil phase.
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