The Formation of an Interpolymer Complex in Supercritical Carbon Dioxide and its Application in the Encapsulation of Probiotics

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Materials Science and Manufacturing
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Polymers, Ceramics and Composites
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Structure of talk

- **Supercritical fluids**
  What are they & what makes them special?

- **Probiotics**
  What is it & why do we want to encapsulate it?

- **Polymers and scCO₂**
  Love/hate relationship!

- **Interpolymer complexes**
  A potential solution!

- **The CSIR technology**
  So how do we do it?

- **Results**
  (Does it actually work?!

- **Conclusions**
  Where to from here?
Supercritical fluids

What are they & what makes them special?
Pressure-Temperature phase diagram for a pure material

Pressure

Solid

Liquid

Fluid

Gas

Temperature

Pc

Tc
Comparison of typical values of physico-chemical properties for a liquid, gas and supercritical fluid

<table>
<thead>
<tr>
<th>Material state</th>
<th>Diffusivity [cm²/s]</th>
<th>Viscosity [Pa.s]</th>
<th>Density [kg/m³]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid</td>
<td>10⁻⁵</td>
<td>10⁻³</td>
<td>1000</td>
</tr>
<tr>
<td>Supercritical fluid</td>
<td>10⁻³</td>
<td>10⁻⁵</td>
<td>300</td>
</tr>
<tr>
<td>Gas</td>
<td>10⁻¹</td>
<td>10⁻⁵</td>
<td>1</td>
</tr>
</tbody>
</table>
Supercritical carbon dioxide

Most commonly used supercritical fluid, because:
• Non-toxic & non-flammable
• Inert – cannot be oxidised
• Relatively mild supercritical conditions (31.0 °C, 73.8 bar)
• Inexpensive
• Easy separation by depressurization & no residual solvent
• Environmentally benign (zero net effect – already produced by many industries, e.g. beer production)

But:
• SCF processing relatively expensive, therefore higher value add applications
(Commercially used in e.g. decaffeination, hops extraction)
**CO₂ Total Solubility Parameter - Experimental vs. Calculated**

Temperature = 31 °C

**Calculation base values:**
- **LW:** \( d_d = 16.6 \), \( d_p = 5.3 \), \( d_h = 5.7 \text{ MPa}^{0.5} \)
- **Hansen:** \( d_d = 15.3 \), \( d_p = 6.9 \), \( d_h = 4.1 \text{ MPa}^{0.5} \)

\( T_{\text{ref}} = 25 \text{ °C} \), \( P_{\text{ref}} = 1655 \text{ bar} \), \( V_{\text{ref}} = 3.61 \times 10^{-5} \text{ m}^3/\text{mol} \)

Adjusted \( V_{\text{ref}} = 4.15 \times 10^{-5} \text{ m}^3/\text{mol} \), \( P_{\text{ref}} = 606 \text{ bar} \)
Probiotics

What is it & why do we want to encapsulate it?
Bacteria & the human body

• No. of bacteria 20 times more than no. of cells!

• Large intestine – $10^{10} - 10^{11}$ bacteria / g intestinal contents; 400 – 500 species

• Up to 1.5 kg of bacteria!

• Friendly bacteria – help promote digestion, aid in absorption of nutrients

• Pathogens – responsible for digestive problems (diarrhoea, etc.)

Probiotics - definitions

• Live microbial cultures fed by mouth and surviving transit through the large intestine where they colonise the system (Frost and Sullivan, 2000; Saarela et al., 2000)

• A preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora by implantation or colonization, in a compartment of the host and by that, exert beneficial effects on host health (Schrezenmeir and de Vrese, 2001)

• Live microorganisms which when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2001).

• Most commonly *Lactobacillus* and *Bifidobacterium*
Potential benefits of probiotics as supplement or in functional foods

• Prevention and treatment of diarrhoea caused by rotavirus, especially in children
• Immune system enhancement
• Reducing some allergic reactions
• Treating and preventing respiratory infections, especially in children
• Decreased faecal mutagenicity
• Decrease in the level of pathogenic bacteria
• Decreased faecal bacterial enzyme activity
• Prevention of the recurrence of superficial bladder cancer
• The restoration of the correct balance of natural microflora after stress, antibiotic treatment, alcohol use and chemotherapy
Problems with putting probiotics in food products…

- *Lactobacillus* – microaerophilic & *Bifidobacterium* – anaerobic, thus sensitive to OXYGEN…

- Short shelf-life…→ encapsulation?

- Encapsulation difficult because the bacteria also sensitive to HEAT and SOLVENTS

- Also sensitive to the aggressive GASTRIC environment

- Thus a ‘soft’ encapsulation method required – perhaps supercritical CO$_2$-based? (no solvent, low temp., no oxygen)

- Enteric release would be advantageous (i.e. protection in stomach, release in intestines)
Polymers and scCO$_2$

Love/hate relationship!
Polymers and scCO$_2$

- High-pressure CO$_2$ can depress polymer $T_g$ and/or melting point of polymers and improve processing…

- However, many polymers have insufficient solubility in or compatibility with scCO$_2$ to enable processing at mild pressures and temperatures

## Approaches to overcome low affinity between polymers and scCO₂

<table>
<thead>
<tr>
<th>Approach</th>
<th>Elaboration</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymer design</td>
<td>Incorporation of &quot;CO₂-philic&quot; functional groups in new polymers</td>
<td>Need FDA approval for new polymers</td>
</tr>
<tr>
<td>Surfactants</td>
<td>The addition of CO₂-soluble surfactants</td>
<td>Need FDA approval for surfactants</td>
</tr>
<tr>
<td>Cosolvents</td>
<td>The addition of a cosolvent such as methanol or ethanol to increase the solvation power of scCO₂</td>
<td>Reintroduces requirement for use of a solvent - many actives are sensitive to solvents</td>
</tr>
<tr>
<td>Mixtures of SCFs</td>
<td>The use of a second supercritical fluid to enhance polymer processability</td>
<td>No obvious second supercritical fluid with desired combination of properties (low/no toxicity, low critical temperature &amp; pressure, low cost, etc.)</td>
</tr>
<tr>
<td>Gas anti-solvent (GAS) technique</td>
<td>Use scCO₂ as an anti-solvent to extract the solvent from a sprayed polymer solution and thus precipitate the polymer</td>
<td>Reintroduces requirement for use of a solvent - many actives are sensitive to solvents</td>
</tr>
<tr>
<td>Use low molar mass and low polarity polymers</td>
<td>These polymers are more amenable to scCO₂ processing.</td>
<td>These polymers generally have low mechanical integrity and/or barrier properties</td>
</tr>
<tr>
<td>Use fats / waxes for encapsulation</td>
<td>Fats, waxes and oils are generally soluble in scCO₂</td>
<td>Limited flexibility with regards to properties</td>
</tr>
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</table>
Interpolymer complexes

A potential solution!
What are interpolymer complexes?

“Non-covalent interaction between two or more polymers through forces such as ionic forces, hydrophobic interactions, hydrogen bonding and Van der Waals forces”
Proven, proprietary CSIR technologies based on interpolymer complexes...

- Controlled release drug delivery systems
- Barrier coating for packaging applications
Desired properties of polymers for encapsulation system

- $\text{scCO}_2$-processable (soluble / plasticizable)
- pH-dependent release in intestinal tract
- Complementary (form interpolymer complex)
- FDA approved for food or pharma
The CSIR technology

So how do we do it?
Polymer system

Vinyl pyrrolidone repeat unit

Vinyl acetate repeat unit
SCF processing unit
SCF processing unit
Important parameters

- Temperature, pressure
- Back pressure in product chamber
- Nozzle configuration (e.g. capillary vs. orifice)
- Processing aids
- Stirrer design
Results

(Does it actually work?!)
Plasticization of PVP and PVAc-CA

PVP

PVAc-CA

2000 – 3000 g/mol

45 000 g/mol
Formation of interpolymer complex in scCO$_2$

24 h, 30 °C, 60% RH

**Physical Blend**

**SC-CO$_2$ Complexed**

Moisture absorption, 72 hours, 60% RH, 30 °C

- PVP:PVAc-CA
- PVAc:PVAc-CA
- PEO-PPO-PEO:PVAc-CA

Dry blend

scCO$_2$-processed

Carbonyl absorption band

acetate absorption band overlapping with two carbonyl stretching modes of the free and self-associated carboxylic acid groups
Product particles containing *B. lactis*

- **Particle size (µm)**
  - Normalized volume distribution
  - Sauter mean diameter 6.9 µm
  - Sauter mean diameter 168 µm

- **Survival of *B. longum* through encapsulation process**
  - Control SCF-encapsulated Spray-dried
  - Viable cell count (cfu/g)
    - Control: 3.0E+12
    - SCF-encapsulated: 3.0E+12
    - Spray-dried: 9.3E+10

- PVP:PVAc-CA 0.25:0.75
  - GMS:PVP:PVAc-CA 0.75:0.0625:0.1875
pH-dependent release of indomethacin
Dissolution/swelling of encapsulated *B. longum*
Shelf-life improvement

Bb12 (B. lactis) survival after 7 weeks

RH = Humidity cabinet, 30 °C, 60% RH  
RT = Room temperature, dessicated  
4 ºC = 4 ºC, dessicated

Sample / conditions

- Bb12 - RT
- PL3/129 - RT
- Bb12 - 4 ºC
- PL3/129 - 4 ºC
- Bb12 - RH
- PL3/129 - RH

B. bifidum in alginate-polylysine, 4ºC*

SGJ & SIF results – vs time

Viable cells (cfu/g)

Before experiment

Simulated gastric juice
(HCl, pepsin, saline, pH 2)

Simulated intestinal fluid
(KH$_2$PO$_4$, NaOH, pancreatin, pH 6.8)

Time (h)


Bb-46 control
Encapsulated - pellet
Encapsulated - supernatant
Encapsulated - total
Summary of *B. longum* results - final counts

Counts after exposure to simulated gastric juice and simulated intestinal fluid

<table>
<thead>
<tr>
<th>Condition</th>
<th>Controls (cfu/g)</th>
<th>Encapsulated (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal system</td>
<td>2.25E+08</td>
<td>3.29E+09</td>
</tr>
<tr>
<td>Normal system + Pluronic</td>
<td>1.16E+07</td>
<td>1.35E+08</td>
</tr>
<tr>
<td>Normal system - low press.</td>
<td>1.16E+07</td>
<td>1.52E+09</td>
</tr>
<tr>
<td>Normal system + GMS</td>
<td>1.21E+08</td>
<td>9.45E+08</td>
</tr>
<tr>
<td>Normal + GMS + gelatin</td>
<td>1.07E+10</td>
<td>1.52E+12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.05E+08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.20E+09</td>
</tr>
</tbody>
</table>
Conclusions

Where to from here?
Conclusions

• Can form interpolymer complexes in scCO$_2$
• Can process these interpolymer complexes in scCO$_2$, without complexation inhibitor
• Can successfully encapsulate drugs and bacteria using PVP:PVAc-CA and the PGSS system, with minimal damage to bacteria
• PVP:PVAc-CA interpolymer complex insoluble in acidic environments, but swells & releases in alkaline environments
• SCF-encapsulated *B. longum* has improved survival through gastric environment compared to controls
Further work

• Investigate other applications (e.g. oral vaccines)
• Improve shelf-life performance / testing methodology
• Shelf-life trials with subsequent SGJ-SIF testing
• Trials on SHIME system (Gent University – Belgium)
• Atomistic simulation to investigate optimum stoichiometric ratios
• Culture *B. bifidum* and determine effect of SCF-based encapsulation
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  - Philip Labuschagne, Dr. Thilo van der Merwe (CSIR)
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