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

Presented at the CSIR R&I Conference

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 $scCO_2$
  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?

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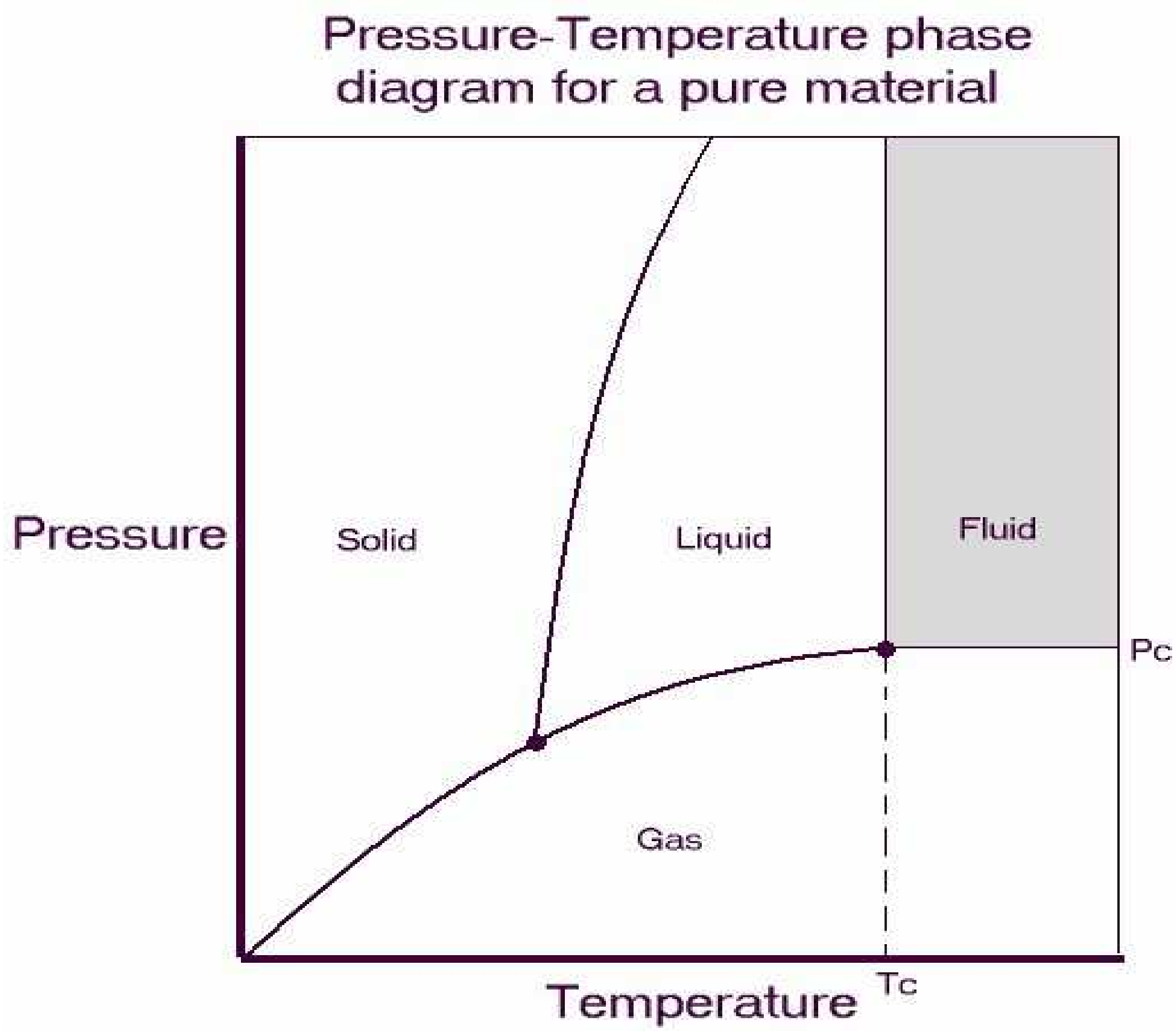
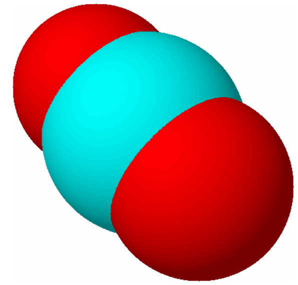

Pressure-Temperature phase diagram for a pure material
Pressure
Solid
Liquid
Fluid
Pc
Gas
Temperature
Tc

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

Material state	Diffusivity [cm^2/s]	Viscosity [Pa.s]	Density [kg/m^3]
Liquid	10^{-5}	10^{-3}	1000
Supercritical fluid	10^{-3}	10^{-5}	300
Gas	10^{-1}	10^{-5}	1

Supercritical carbon dioxide



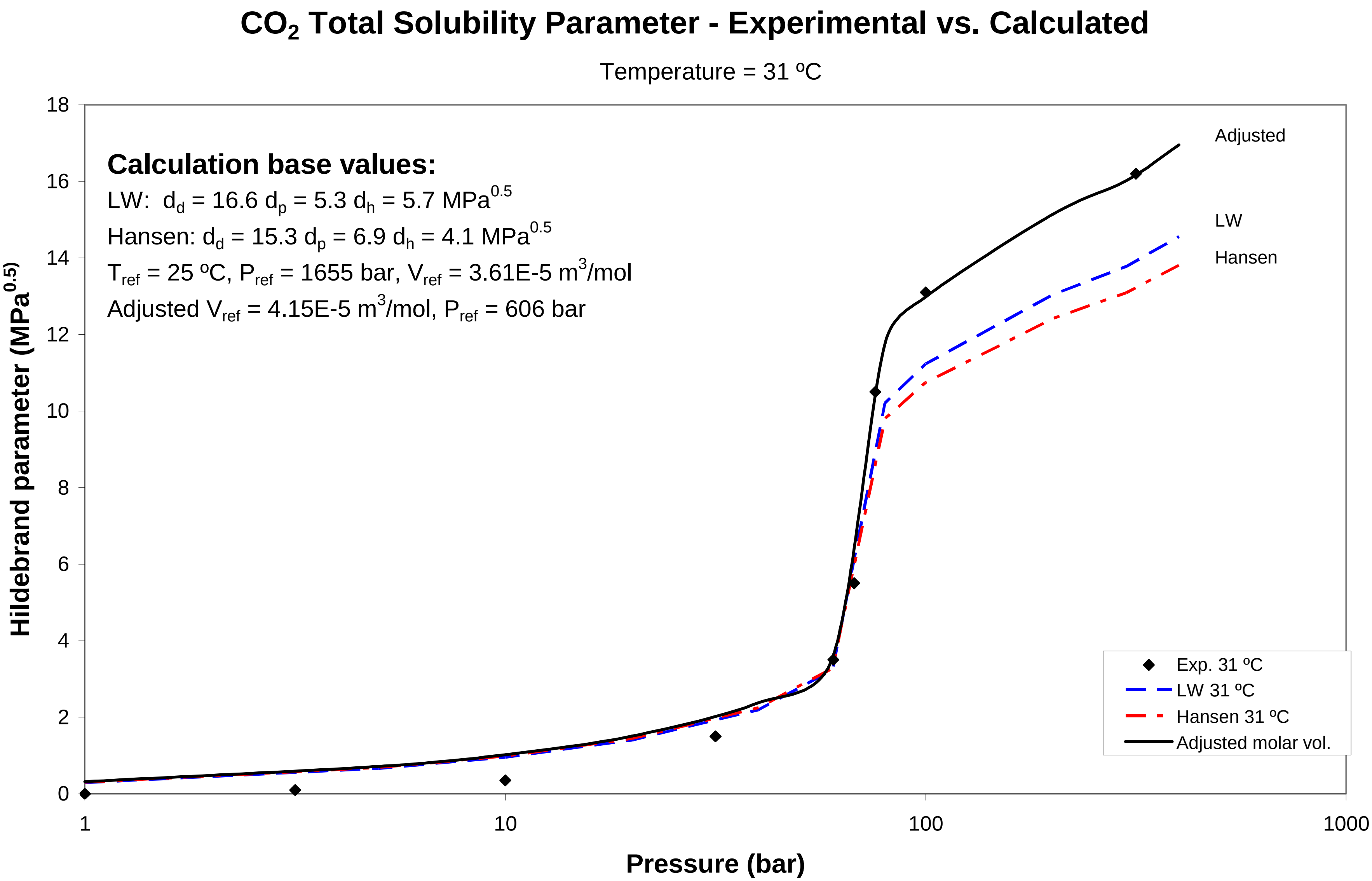
Most commonly used supercritical fluid, because:

- Non-toxic & non-flammable
- Inert – cannot be oxidised
- Relatively mild supercritical conditions (31.0 ºC, 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)



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Probiotics

What is it & why do we want to encapsulate it?

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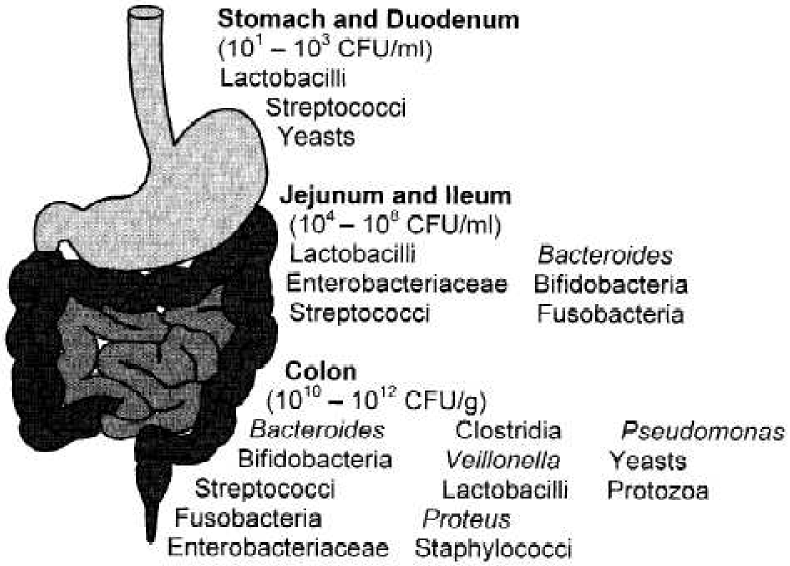
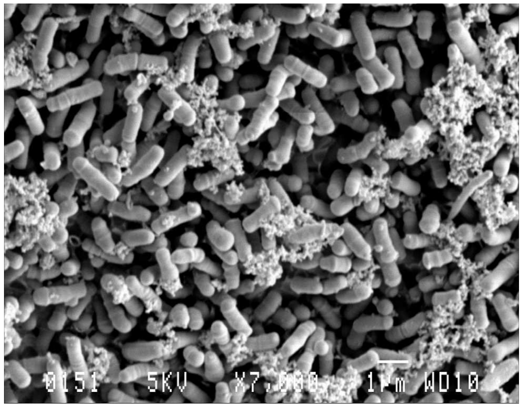
Bacteria & the human body

- 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.)



(From Holzapfel et al. 1998. Overview of gut flora and Probiotics. Int. Jnl. Food Microb. 41:85-101)

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!

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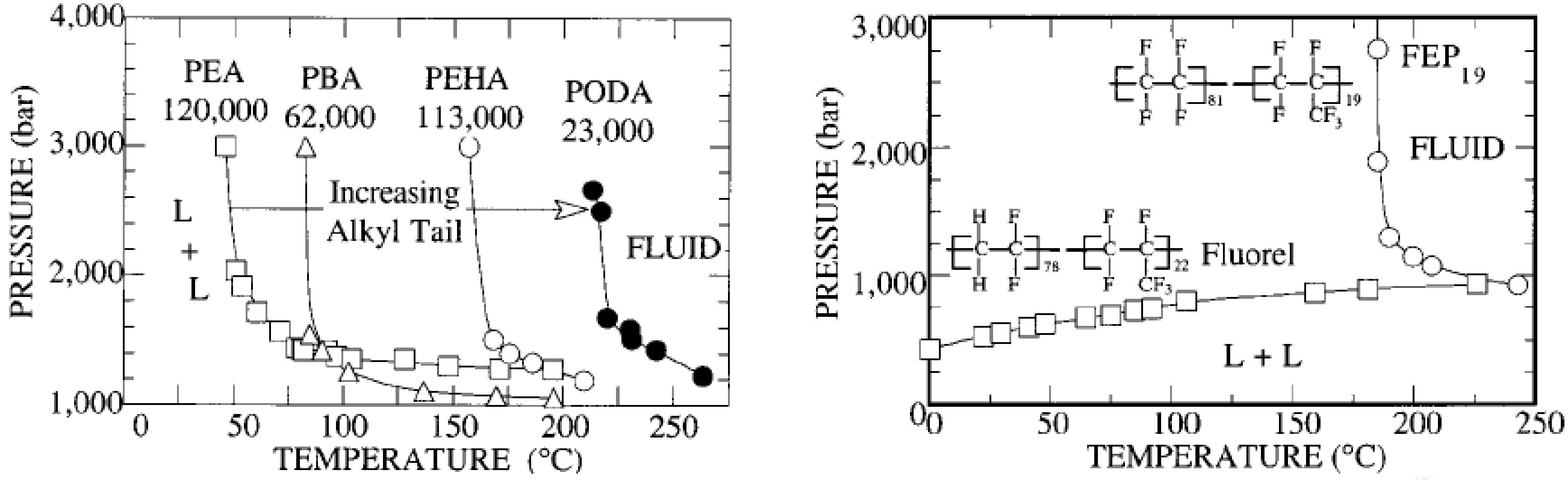
Polymers and $scCO_2$

- 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



Graphs from: Kirby CF, McHugh MA. 1999. Phase Behaviour of Polymers in Supercritical Fluid Solvents. Chem. Rev. 99:565-602.

Approaches to overcome low affinity between polymers and $scCO_2$

Approach	Elaboration	Limitations
Polymer design	Incorporation of "CO_2-philic" functional groups in new polymers	Need FDA approval for new polymers
Surfactants	The addition of CO_2-soluble surfactants	Need FDA approval for surfactants
Cosolvents	The addition of a cosolvent such as methanol or ethanol to increase the solvation power of $scCO_2$	Reintroduces requirement for use of a solvent - many actives are sensitive to solvents
Mixtures of SCFs	The use of a second supercritical fluid to enhance polymer processability	No obvious second supercritical fluid with desired combination of properties (low/no toxicity, low critical temperature & pressure, low cost, etc.)
Gas anti-solvent (GAS) technique	Use $scCO_2$ as an anti-solvent to extract the solvent from a sprayed polymer solution and thus precipitate the polymer	Reintroduces requirement for use of a solvent - many actives are sensitive to solvents
Use low molar mass and low polarity polymers	These polymers are more amenable to $scCO_2$ processing.	These polymers generally have low mechanical integrity and/or barrier properties
Use fats / waxes for encapsulation	Fats, waxes and oils are generally soluble in $scCO_2$	Limited flexibility with regards to properties

Interpolymer complexes

A potential solution!

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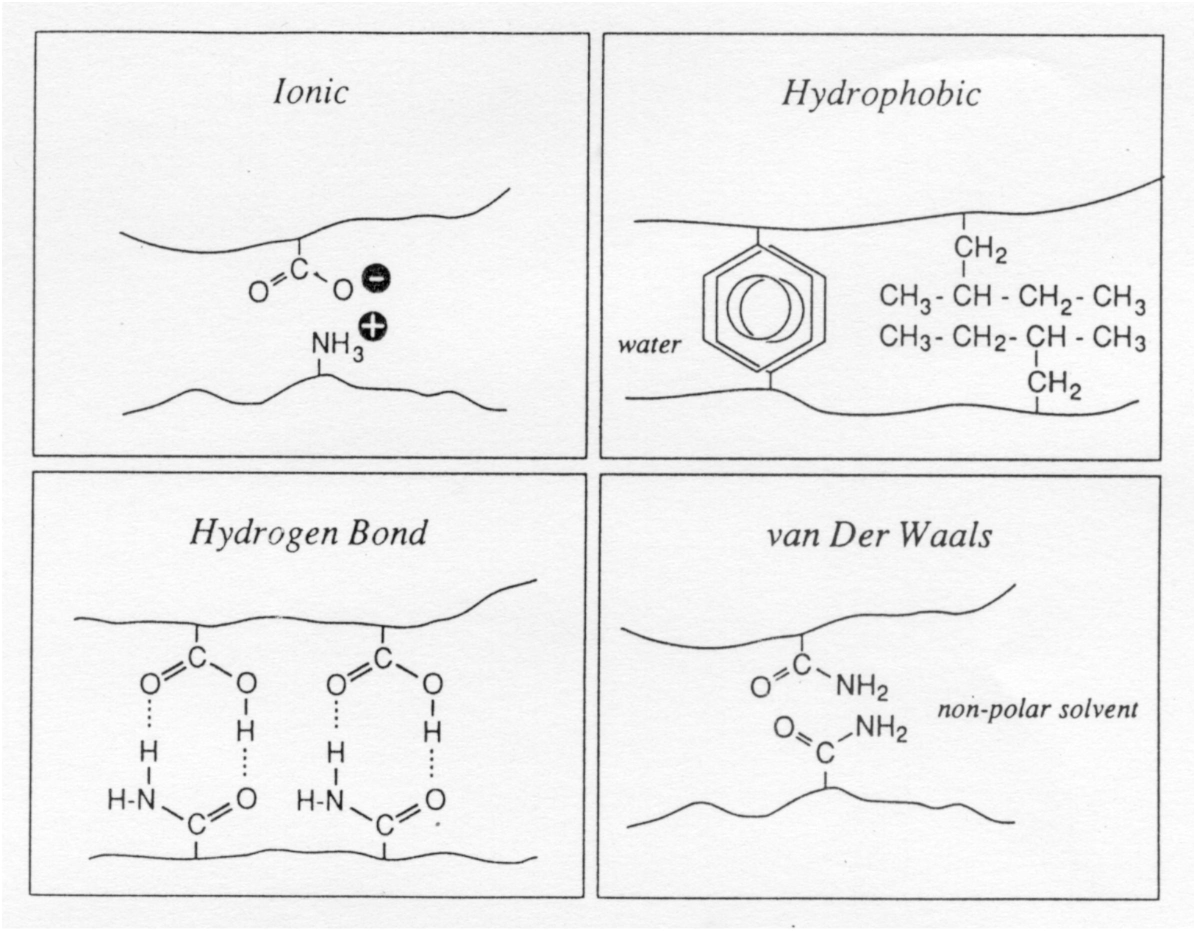
What are interpolymer complexes?

"Non-covalent interaction between two or more polymers through forces such as ionic forces, hydrophobic interactions, hydrogen bonding and Van der Waals forces"



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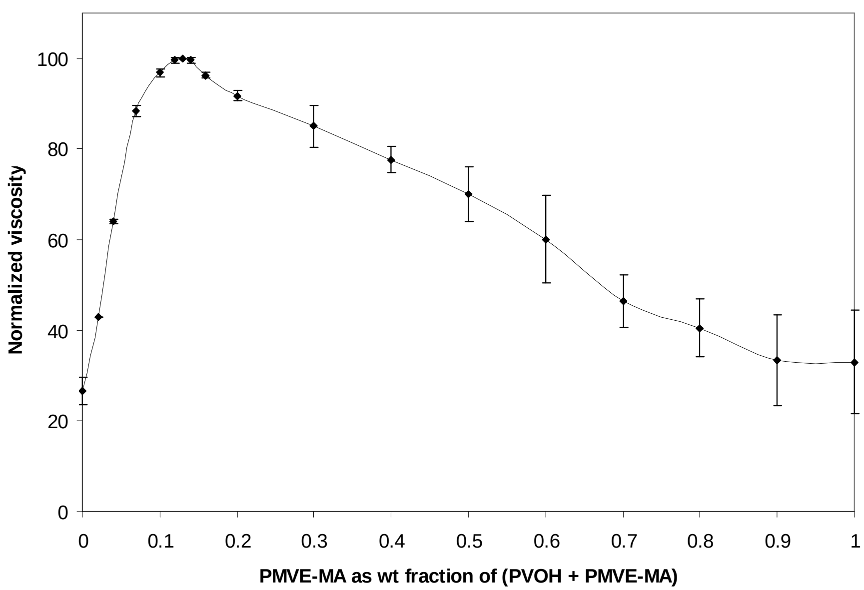
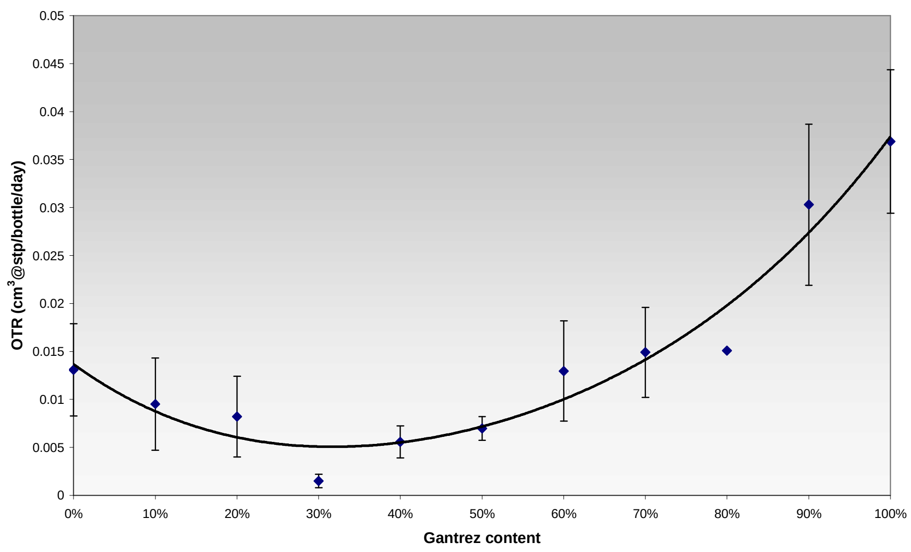
Proven, proprietary CSIR technologies based on interpolymer complexes…

- Controlled release drug delivery systems
- Barrier coating for packaging applications





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Desired properties of polymers for encapsulation system

- $scCO_2$-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?

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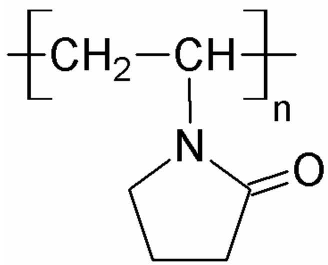
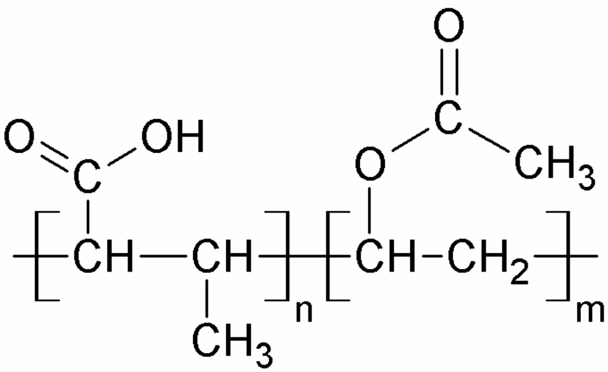
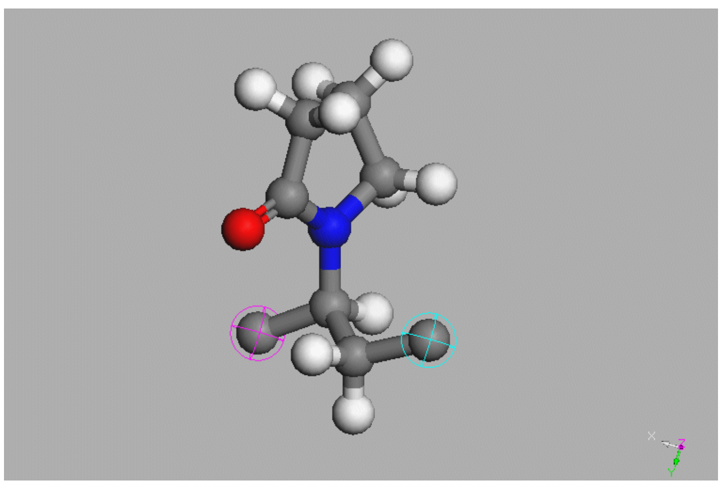
Polymer system



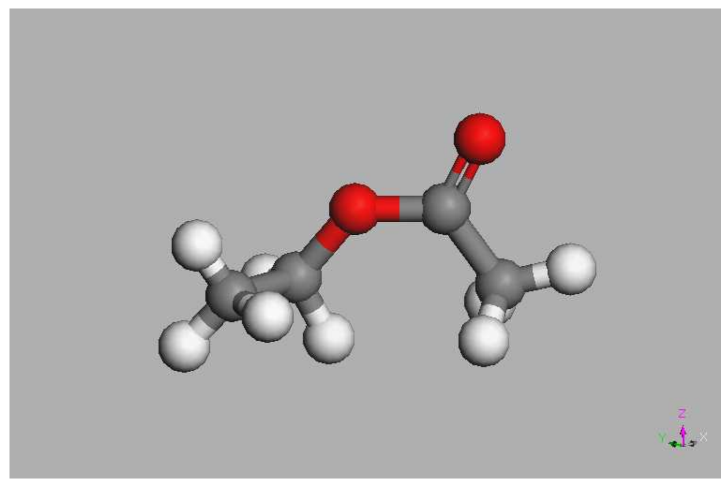
PVP



PVAc-CA

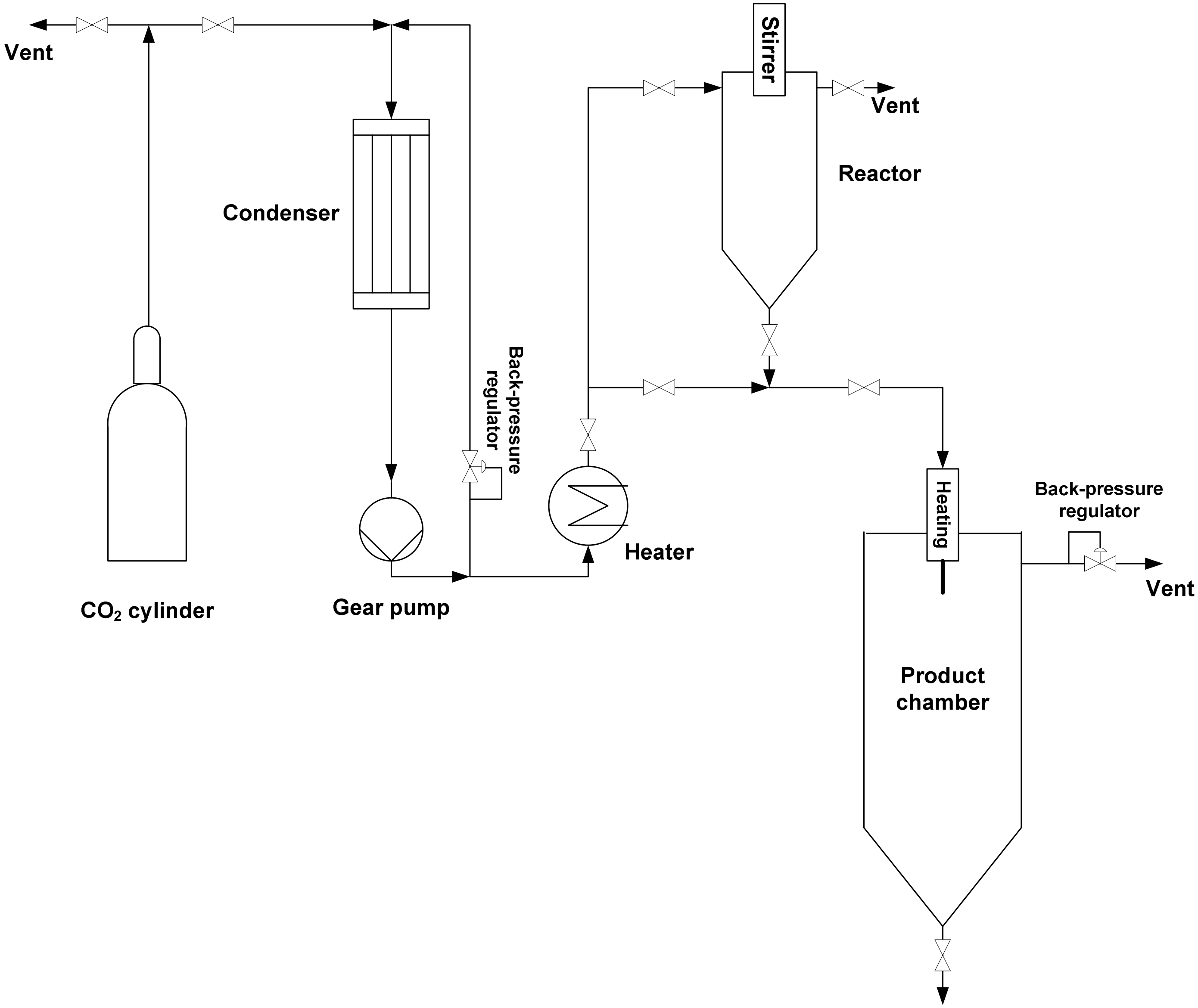


Vinyl pyrrolidone repeat unit



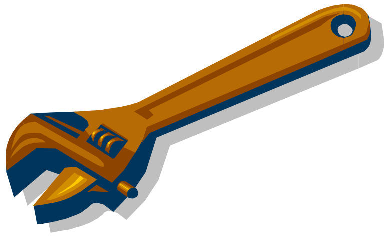
Vinyl acetate repeat unit

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?!)

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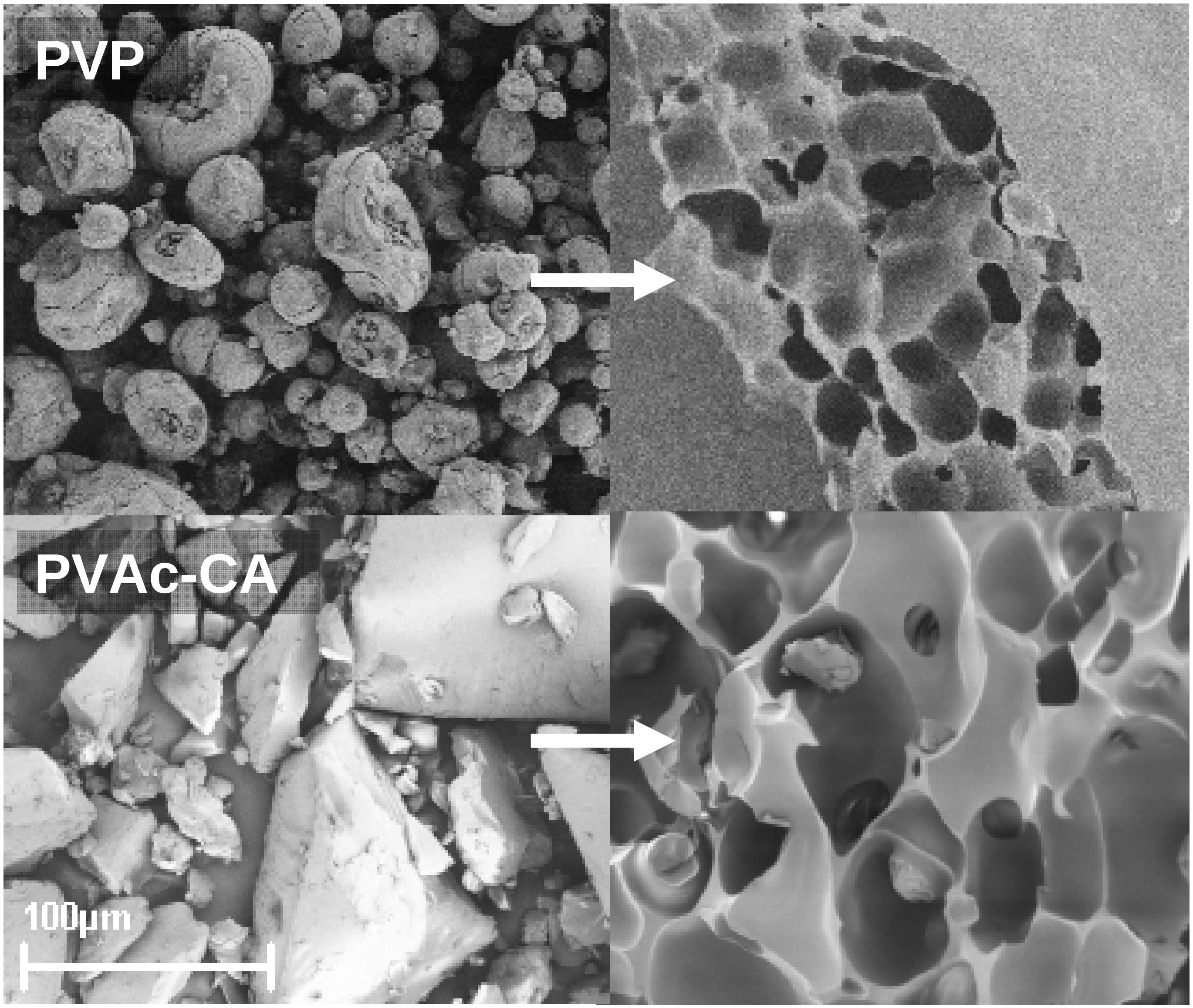
Plasticization of PVP and PVAc-CA



2000 – 3000 g/mol

45 000 g/mol

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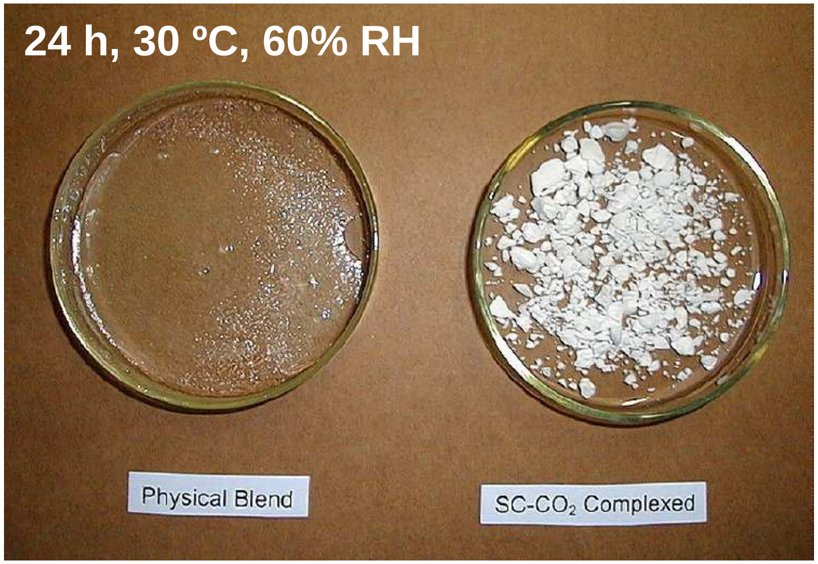
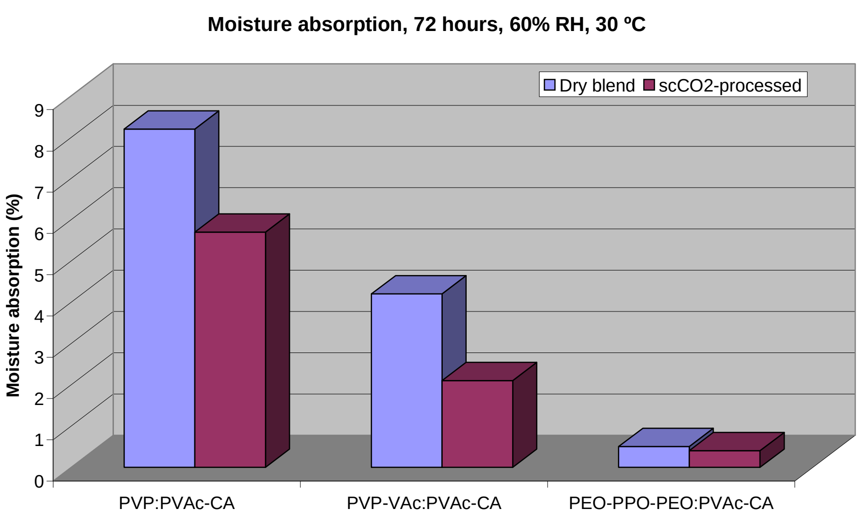
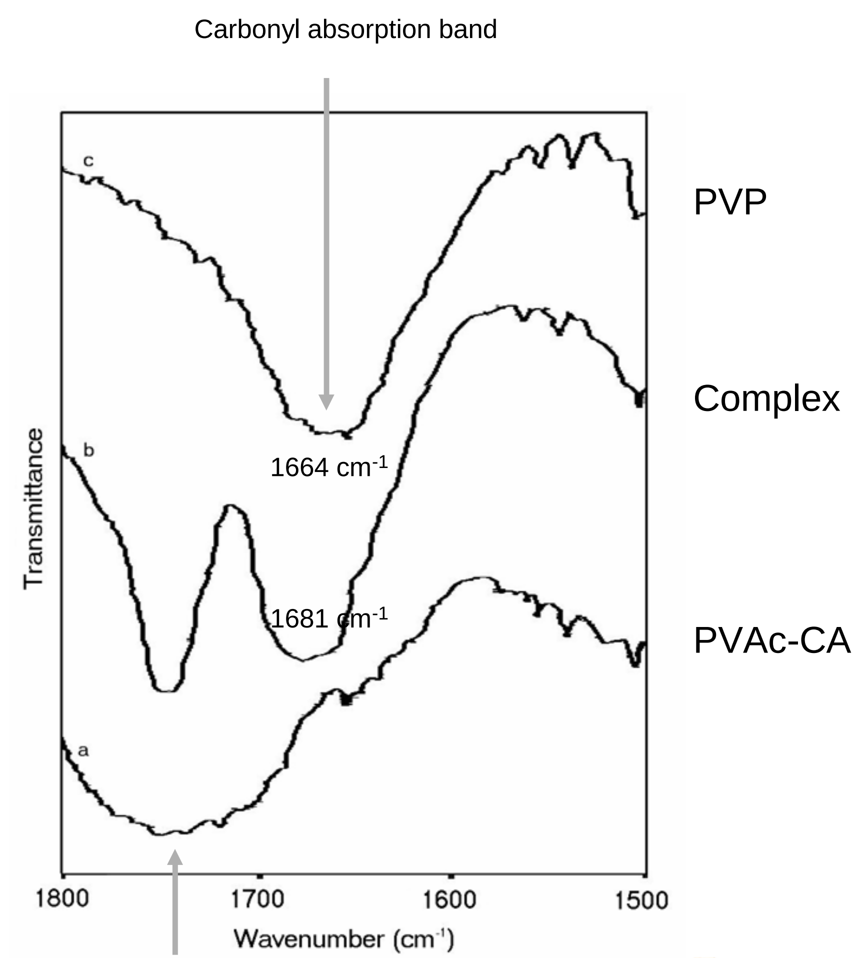
Formation of interpolymer complex in $scCO_2$







acetate absorption band overlapping with two carbonyl stretching modes of the free and self-associated carboxylic acid groups

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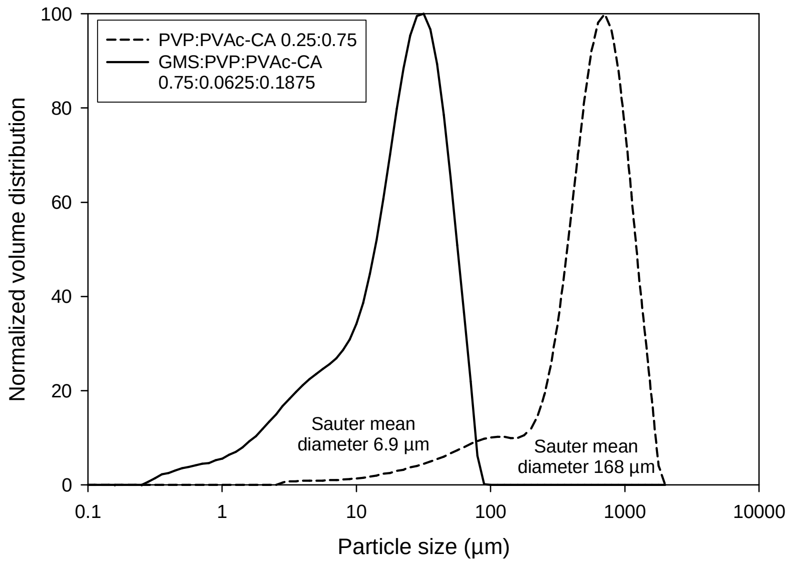
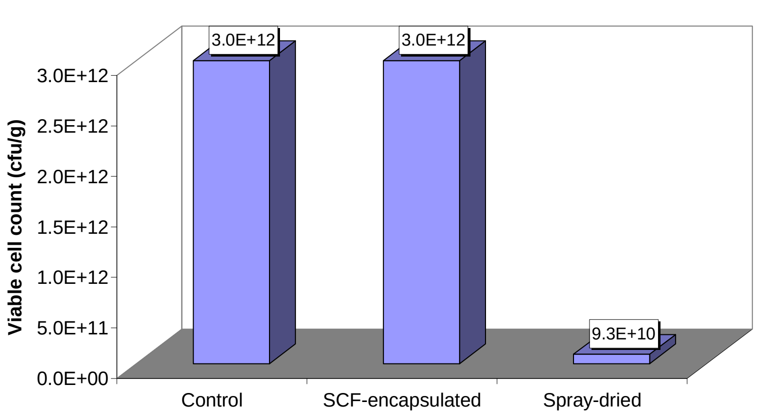
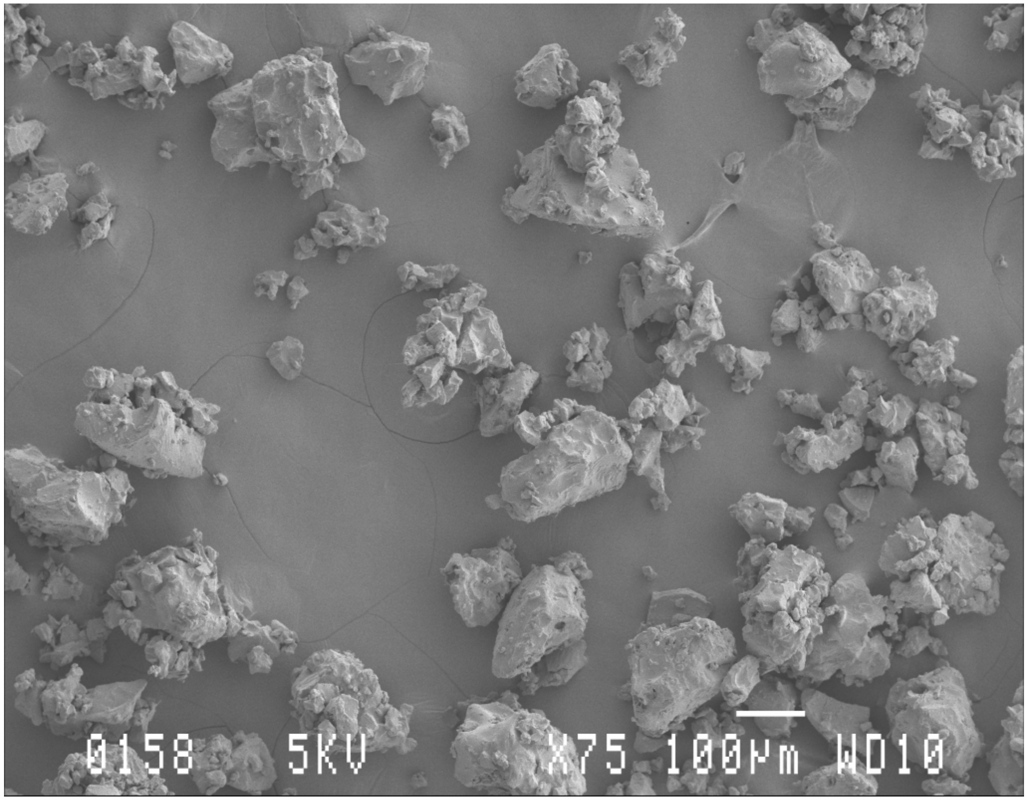
Product particles containing *B. lactis*







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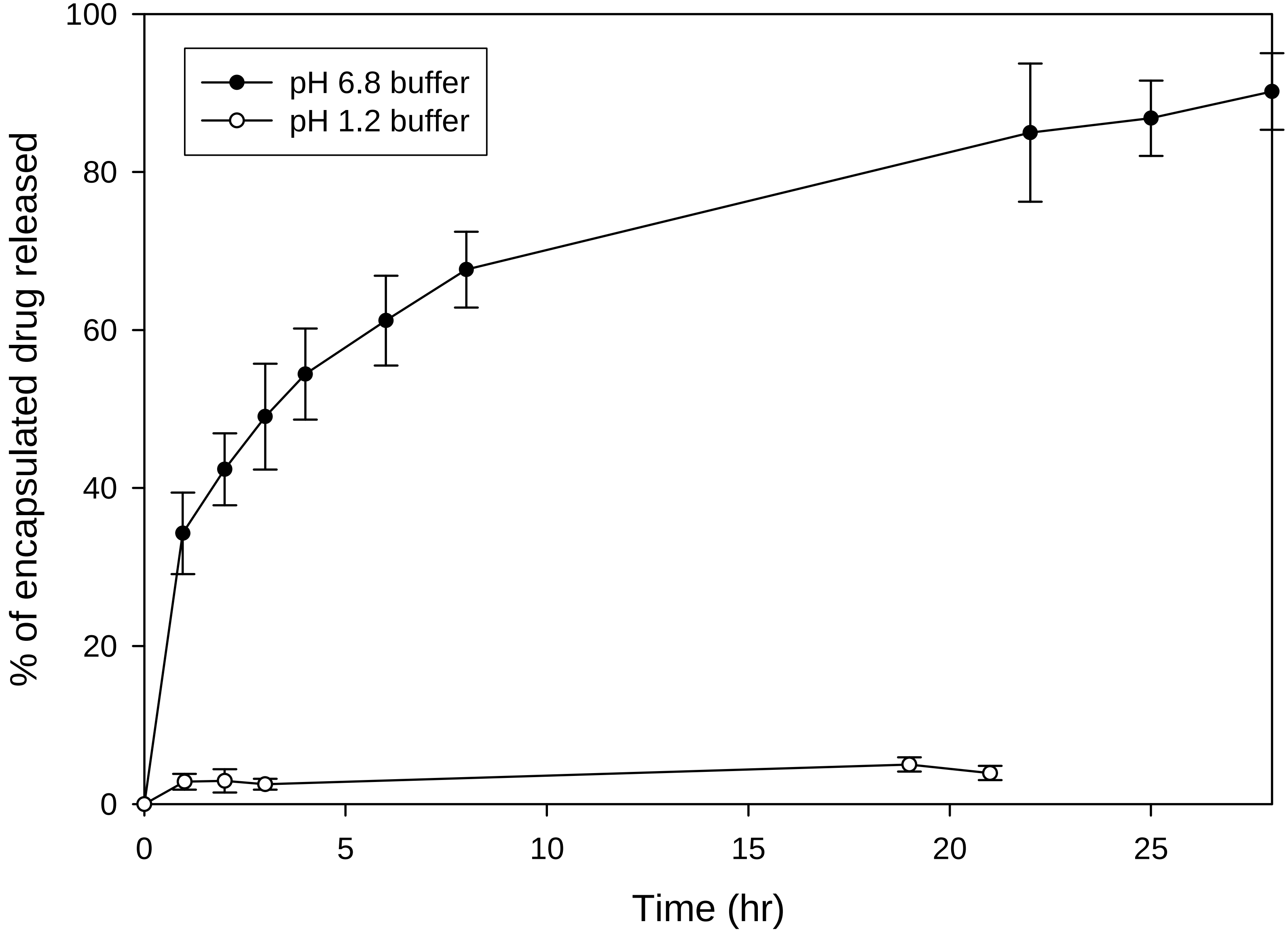
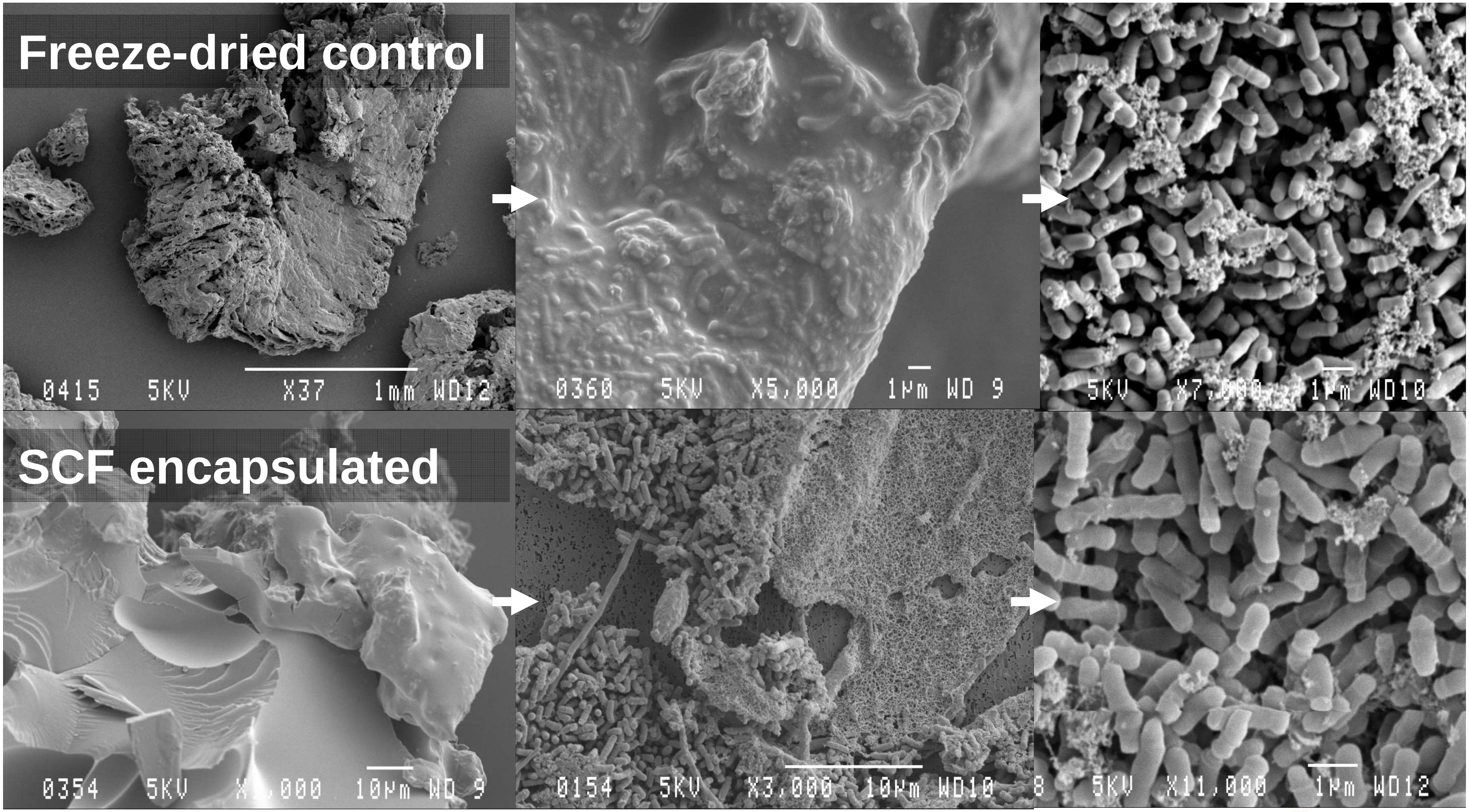
pH-dependent release of indomethacin



Dissolution/swelling of encapsulated *B. longum*



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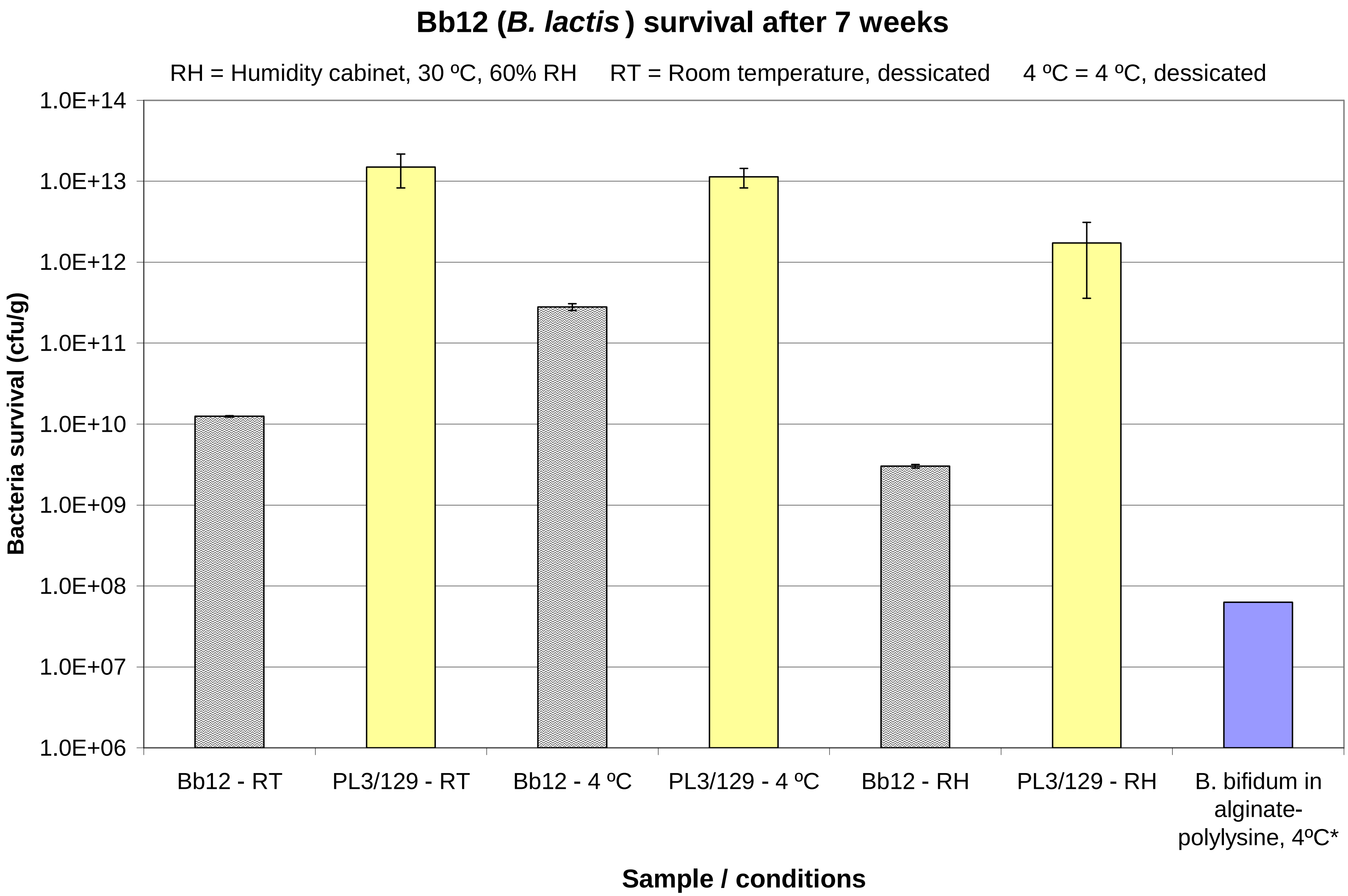
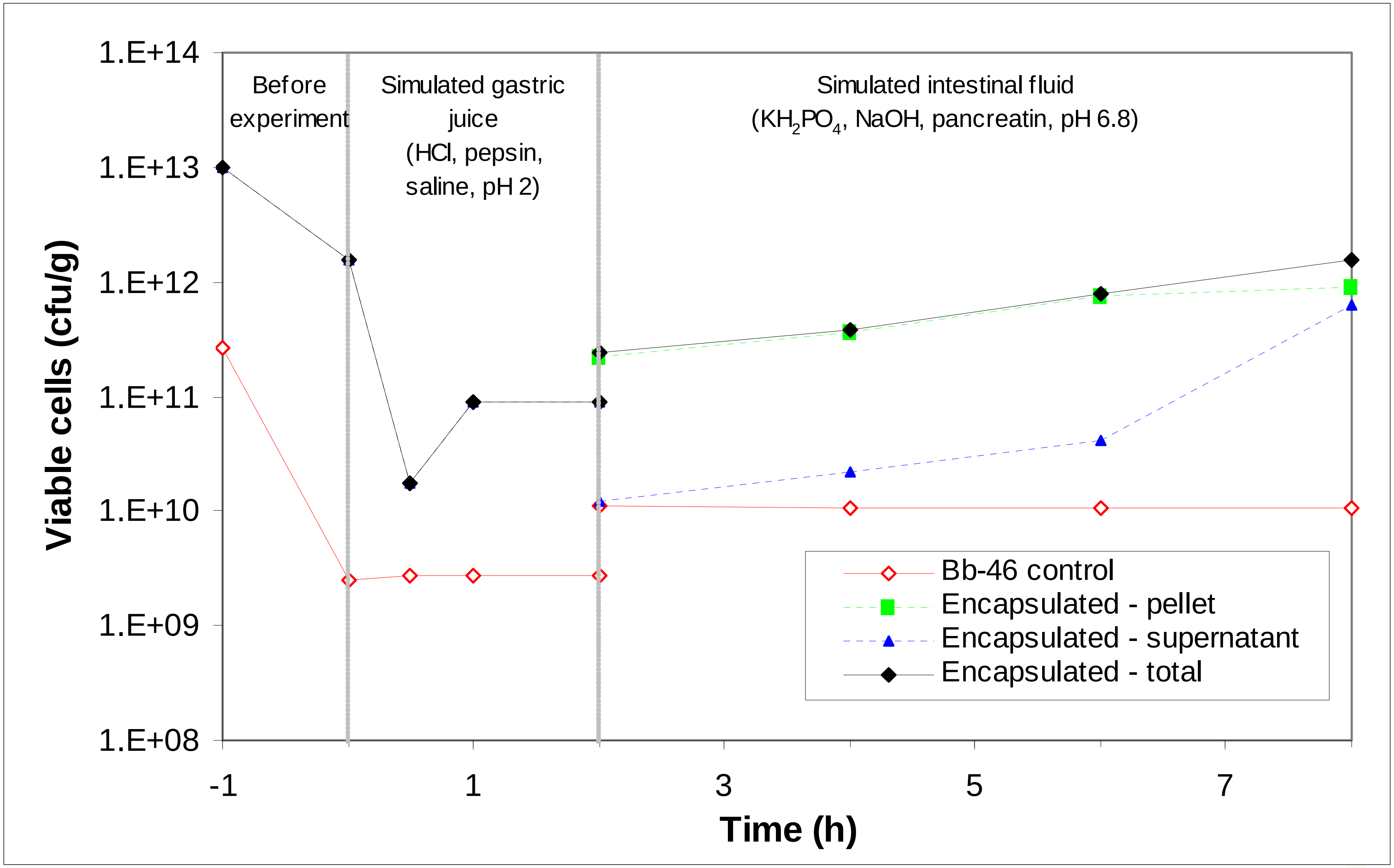
Shelf-life improvement



*Cui et al. 2000. Survival and stability of bifidobacteria loaded in alginate poly-l-lysine microparticles. Internat. J. of Pharmaceutics 210:51-59

SGJ & SIF results – vs time



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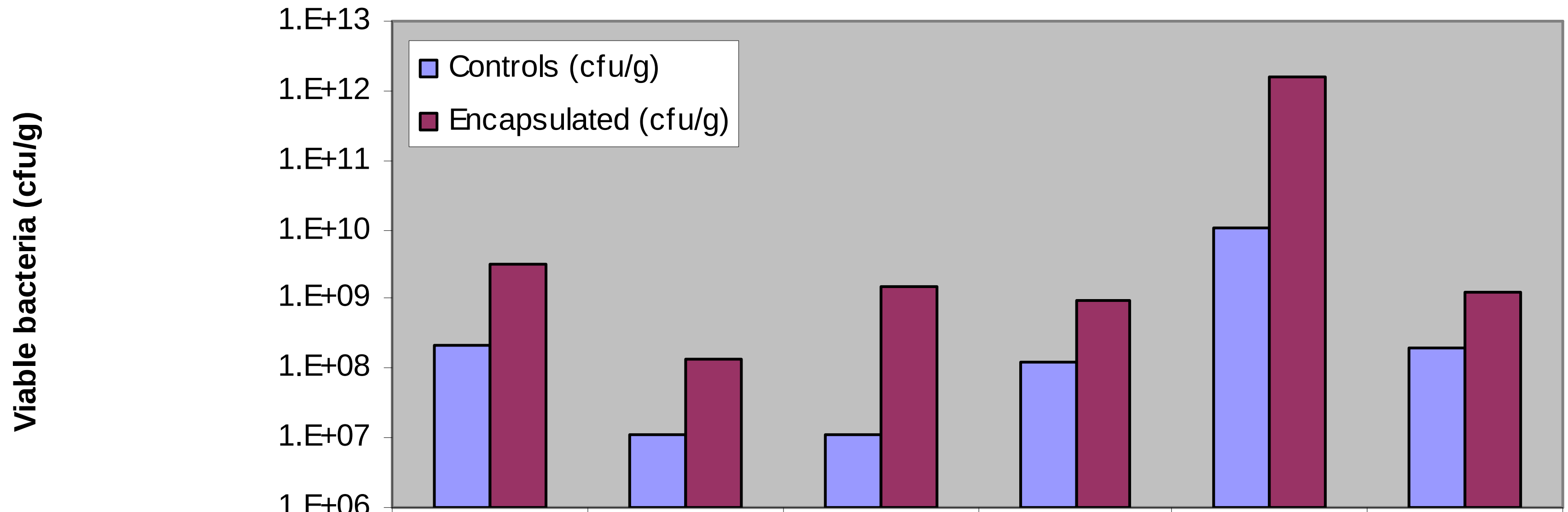
Summary of *B. longum* results - final counts

Counts after exposure to simulated gastric juice and simulated intestinal fluid



	Normal system	Normal system + Pluronic	Normal system	Normal system - low er press.	Normal system + GMS	Normal + GMS + gelatin
Controls (cfu/g)	2.25E+08	1.16E+07	1.16E+07	1.21E+08	1.07E+10	2.05E+08
Encapsulated (cfu/g)	3.29E+09	1.35E+08	1.52E+09	9.45E+08	1.52E+12	1.20E+09

Conclusions

Where to from here?

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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

Acknowledgements

- Project team:
  - Philip Labuschagne, Dr. Thilo van der Merwe (CSIR)
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- IDC (joint investment)
- DST (funded first supercritical fluid reactor system)

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