# A novel encapsulation method for probiotics using an interpolymer complex in supercriticial carbon dioxide

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## 1. Introduction

Evidence for the health benefits of probiotics is increasing. These benefits include protection against pathogenic bacteria, stimulation of the immune system, reduction in carcinogenesis, vitamin production and degradation of food components (Holzapfel, 1998 & 2002). These benefits make probiotics desirable ingredients in functional foods. However, their incorporation in functional foods (especially dry food formulations) is hampered by their relative instability in terms of shelf life and passage through the aggressive gastric environment upon ingestion.

Several approaches have been reported to overcome this instability, including encapsulation in hydrogels or microcapsules, freeze-drying, selection of acid-resistant strains and stress adaptation (Picot, 2004). While these approaches have been successful to varying degrees, improved methods for probiotic immobilization are still being sought.

Microencapsulation is a method routinely used in the pharmaceutical industry for protection and controlled release of drugs. However, its application in probiotics is limited due to the typical use of solvents, temperature and/or water and the presence of oxygen in the encapsulation process. Supercritical carbon dioxide ($scCO_2$) is an interesting alternative solvent – its critical point is at 31.1 ºC and 73.8 bar (Lide, 2003) and one can avoid the use of solvents, water and high temperatures.

Unfortunately $scCO_2$ is not a very good solvent for most polymers, and this severely limits its application in encapsulation of labile materials. It does however have improved compatibility with polymers as a solute, where it dissolves in polymer matrices, reduces the glass transition temperature and improves processing at reduced temperatures. Thus the PGSS (Particles from Gas-Saturated Solution) process has been applied in a number of encapsulation processes (Bahrami, 2006). The PGSS process consists of loading a reactor with a dry blend of polymer and material to be encapsulated, and subsequently pressurizing the reactor with supercritical carbon dioxide. The polymer plasticizes and the blend is mixed, typically with a helical impeller. The plasticized polymer with suspended material is then expanded into a low pressure product chamber through a nozzle. As the material is atomized through the nozzle, it solidifies due to cooling and escaping carbon dioxide.

The drawback with this process is that generally only low molar mass polymers are processable with this technique. Low molar mass polymers generally have relatively poor barrier and mechanical properties, leading to poor protection of the encapsulated material. The CSIR has developed a novel $scCO_2$-based encapsulation technology utilizing interpolymer complex formation to improve the barrier properties of the polymer system (Moolman, 2005).

The basic polymer system consists of poly(vinyl pyrrolidone) (PVP) and poly(vinyl acetate-*co*-crotonic acid) (PVAc-CA). These polymers are both liquefiable in $scCO_2$ and form an interpolymer complex through hydrogen bonding of the carbonyl groups on the PVP with the crotonic acid

groups of the PVAc-CA (Figure 1). In this paper we report some results obtained in the application of the technology in encapsulation of indomethacin and *Bifidobacterium longum* Bb-46.

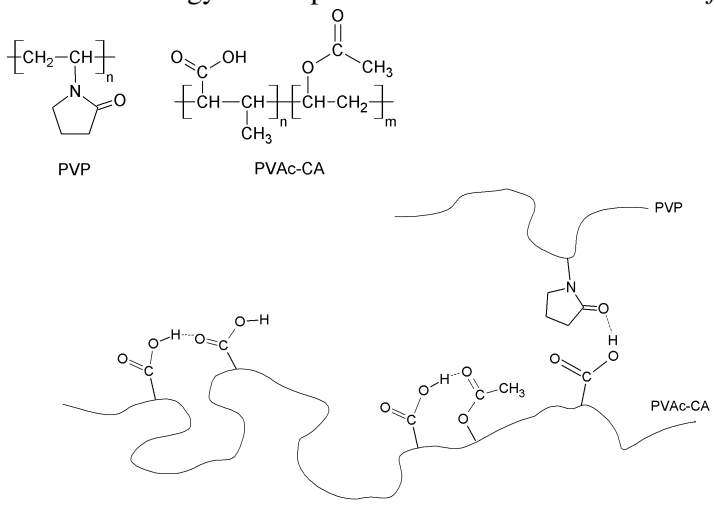



**Figure 1. Repeat units of the basic interpolymer complex system, and a schematic with some possible hydrogen bonding interactions indicated**

## 2. Materials and methods

*2.1 Encapsulation process*

All equipment is wiped with 70% ethanol in water (NCP Alcohols) using a paper towel, and allowed to dry before contact with the materials. 2g of PVP (Kollidon 12PF, mass-average molar mass 2 000 – 3 000 g/mol, BASF) was dried for 5 hours at 80 ºC and 60 mbar (absolute) in a vacuum oven (Model VO65, Vismara) and immediately placed in a desiccator to prevent moisture absorption.

A sealed packet of *B. longum* (Bb-46, Chr. Hansen) or B. lactis (Bb-12, Chr. Hansen) was removed from storage at -12 ºC and allowed to warm to room temperature while sealed. 2 g of the bacteria was then weighed off, and ground to a powder passing through a 150 µm sieve using a coffee grinder (Model CG100, Kenwood). 6 g of PVAc-CA (Vinnapas C305, mass-average molar mass 45 000 g/mol, Wacker) was then weighed off and added to the bacteria, together with any additives (e.g. glyceryl monostearate – Croda Chemicals, Synperonic PE/F68 - Uniqema) and the dried PVP. The blend was then ground and mixed for 1 minute.

The powder blend was then immediately transferred to the pre-heated 1 litre reaction chamber. The chamber was then sealed and flushed and pressurized with sterile filtered $CO_2$ (99.995% purity, Air Products) up to a pressure of 300 bar, with the temperature controlled at 40 ºC. The material was left to equilibrate for 2 hours with intermittent stirring, after which the plasticized product was sprayed through a 500 µm capillary with length 50 mm, into a 10 litre expansion chamber that was pressure-controlled at 15 bar (gauge).

*2.2 Controlled release studies*

Indomethacin (TEVA Pharmaceutical Industries Ltd) was encapsulated using the exact process as described in Section 2.1 for the bacteria. Subsequently, twelve 6 mm tablets were pressed from the product powder using a Manesty F3 tablet press (Manesty Machinery Ltd). The dissolution study was then carried out using a Hanson SR2-8 dissolution bath with 1 litre containers. Eight tablets were placed in buffer solution at pH 6.85 and four tablets in buffer solution at pH 1.2. The dissolution bath paddles rotated at 75 rpm and the bath temperature was maintained at 37 ºC.

Indomethacin concentration was determined using a Helios Alpha UV Analyzer by measuring absorbance at 320 nm after calibration with standard solutions.

*2.3 Bacteria enumeration*

1 g of B. longum (either control or encapsulated) was dissolved in 9 mL of Ringer's solution (pH 7). A series of dilutions up to 10-10 was prepared from this suspension. 0.1 mL of appropriate dilutions was pour plated onto MRS (De Man, Rogosa and Sharpe) agar (Merck, Pty.Ltd), supplemented with 0.05% cysteine hydrochloride. Each dilution was plated out in triplicate. The plates were incubated anaerobically in anaerobic jars with Anaerocult A gaspaks (Merck), at 37 ºC for 72h. To confirm anaerobic conditions inside the jars Anaerocult C test strips were included. The numbers of colonies grown were counted and from these the numbers of viable cells were calculated (cfu/g).

*2.4 Testing in simulated gastric juice (SGJ) and simulated intestinal fluid (SIF)*

Briefly, SGJ (pH 2) was prepared according to the method described by Lian et al (2003) and SIF (pH 6.8) was prepared as per US Pharmacopeia (2005). Free and encapsulated bacteria were then exposed to the SGJ for two hours, and subsequently to SIF for 8 to 24 hours.

## 3. Results and discussion

The polymer system was selected to comply with a number of requirements, including FDA approval for pharmaceutical applications for the component polymers, ability to be liquefied in $scCO_2$, ability to form an interpolymer complex and pH-responsiveness. Figure 2 shows the influence of pH on the release of indomethacin from the polymer system. Less than 5% of the drug was released at pH 1.2, while 85% was released in 24 hours at pH 6.8 (with about 50% released in the first 3 hours – the typical transit time through the small intestine (Ashford, 2002)).

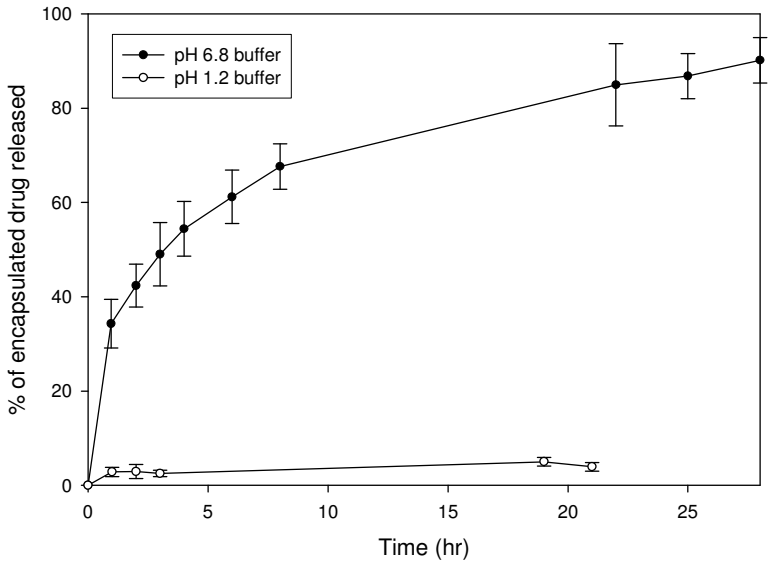
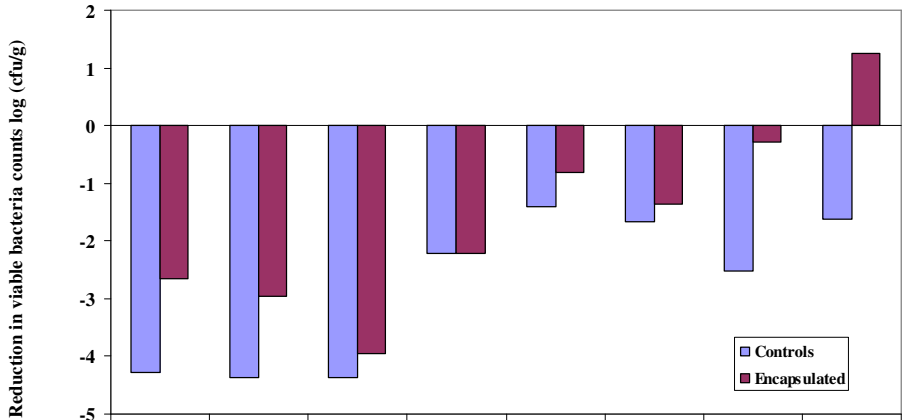



**Figure 2. pH-dependant release of indomethacin from PVP:PVAc-CA particles**

Figure 3 shows the log decrease in counts from beginning to end of testing in the gastrointestinal environment for a number of different formulations. For the normal system, the typical improvement was around 1.5 log units for the encapsulated system vs. the controls. Reduction in product chamber pressure or addition of a plasticizer (Synperonic PE/F68 – a triblock copolymer of ethylene oxide and propylene oxide) resulted in poorer performance of the encapsulated system. High levels of GMS (glyceryl monostearate) yielded improved survival of the encapsulated system,

probably due to the good moisture and oxygen barrier properties of GMS. Substituting beeswax for PVAc-CA resulted in numbers for the encapsulated system increasing from initial counts – this may have been due to incomplete release during initial counts.




| | Normal system | Normal system | Normal system + Synperonic | Normal system - lower press. | Normal system + GMS (10%) | Normal + GMS + gelatin | Normal system + GMS (60%) | PVP + beeswax |
|---|---|---|---|---|---|---|---|---|
| Controls | -4.3 | -4.4 | -4.4 | -2.2 | -1.4 | -1.7 | -2.5 | -1.6 |
| Encapsulated | -2.7 | -3.0 | -3.9 | -2.2 | -0.8 | -1.4 | -0.3 | 1.3 |

**Figure 3. Change in bacteria counts after two hours in SGJ and 6 hours in SIF.**

## Conclusions

The CSIR has developed a novel encapsulation technology for labile actives based on interpolymer complex formation in $scCO_2$. The polymer system exhibits pH-dependent release, with almost no release at pH 1.2 and 85% release over 24 hours at pH 6.8. This should provide protection to encapsulated materials from the aggressive gastric environment. Encapsulated *B. longum* showed improved survival through a simulated gastrointestinal environment compared to free bacteria. The technology has promise for food and pharmaceutical applications.

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