Abstract

The composition of the gut microbiota is important for the health and disease state of the human host. This has generated an interest in the intake of probiotics, which has been associated with many different health benefits. Delivering viable probiotics to the gastrointestinal tract (GIT) can be a challenge as they are sensitive to the low pH and bile concentrations found *in vivo*. To overcome this, probiotics may be formulated into capsules or tablets, which can be coated to provide gastric protection and delayed release in the GIT. However, these delivery systems are not suitable for people having difficulties swallowing, including children and the elderly. Hence, there is a need for "easy-to-swallow" gastro-resistant, delayed release systems for probiotics.

The objective of this current PhD thesis was to develop "easy-to-swallow" delayed release delivery systems protecting freeze-dried probiotic powder against low pH in the stomach and high bile acid concentrations in the proximal part of the small intestine. One of the main challenges was to maintain probiotic viability during processing, as freeze-dried probiotics are sensitive to both heat and moisture. The first part of the thesis focuses on the development of probiotic pellets for delayed release to the lower GIT. To aid the development process, a three-step *in vitro* model simulating the conditions in the human gastric, duodenum/jejunum and ileum compartments was developed. The delayed release system was evaluated using riboflavin as a marker for coating integrity during simulated GIT transit. As the *in vitro* model was to simulate the conditions in the upper and the lower small intestine, in two individual steps, the decreased bile concentrations in the lower small intestine had to be mimicked. To simulate this, the bile acid sequestrant cholestyramine was used, which resulted in bile acid concentrations being reduced to physiologically relevant levels, when simulating the ileum area. Granulation, extrusion and spheronization was applied to develop probiotic pellets, in the size range of 1000-1500 µm, using freeze-dried *L. reuteri* as a model probiotic and crosslinking of alginate and Ca^{2+} in the pellet matrix. Viable probiotic pellets were produced, however, to achieve long-term stability, drying of the pellets was necessary. Freeze-drying the pellets lowered the level of free available water (a_w), without having impact on viability. To achieve delayed release, the probiotic pellets where fluid bed coated with the pH-sensitive polymers Eudragit S100 and Eudragit FS30D. When evaluating the delivery system in the three-step *in vitro* model, the results showed that the pH-sensitive polymers inhibited release of riboflavin in ileal conditions. The second part of the thesis focuses on the development of a coated probiotic

granulate, in the size range of 500 µm. The fatty alcohol cetostearyl alcohol was combined with selected plasticizers, to find a functional coating, which could be applied to the freeze-dried probiotic powder using hot-melt fluid bed coating. Using this technique was an advantage, as no solvent was needed during the coating process, and hereby the time the probiotics were exposed to heat and moisture could be minimized. The combinations of cetostearyl alcohol, olive oil and beeswax were found promising and were applied to a *L. acidophilus* strain and a *B. longum* strain used a model probiotics. The coated probiotic granulate was evaluated in the "The Smallest Intestine" (TSI) *in vitro* model, simulating the conditions in the human stomach, duodenum, jejunum and ileum, using physiologically relevant media. Viability in and release from the coated probiotic granulate was evaluated during simulated GIT transit. The cetostearyl alcohol coating with olive oil in the ratio 95:5 (w/w), showed gastric protection, as no release of probiotics were detected after simulation of the stomach and furthermore resulted in significantly higher viability, after simulated GIT transit, compared to the uncoated probiotic powder. Release of riboflavin from the coated probiotic granulates was furthermore evaluated, to compare the TSI *in vitro* model to the three-step *in vitro* model, and this showed a good correlation between the two models.

The cetostearyl alcohol and olive oil (95:5) (w/w) coated probiotic granulate, showing the best gastric protection *in vitro*, was evaluated in a blinded-cross over study in eight human volunteers. The efficacy of the coating was evaluated by adding caffeine to the probiotic powder prior to coating and using it as a tracer for coating integrity in the GIT. The caffeine absorbed from the GIT was quantified in saliva samples collected at selected time points, after oral ingestion of the granulate. The results showed delayed absorption of caffeine from the coated probiotic granulate with a T_{max} at 2.8 h, compared to a T_{max} at 2.0 h, for the uncoated probiotic powder. Based on the size of the coated probiotic granulate and the theoretical gastric emptying time in fasted state, it is believed that the coating provided gastric protection and that release and absorption of caffeine occurred in the small intestine. The coated probiotic granulate was furthermore studied in a dissolution model, using media simulating the stomach and small intestine. In agreement with the clinical study, the *in vitro* study also showed delayed release of caffeine from the coated probiotic granulate. In conclusion, two different probiotic delivery systems were developed in this thesis. Both systems show potential for optimizing GIT delivery of viable probiotics.