Output 5.1

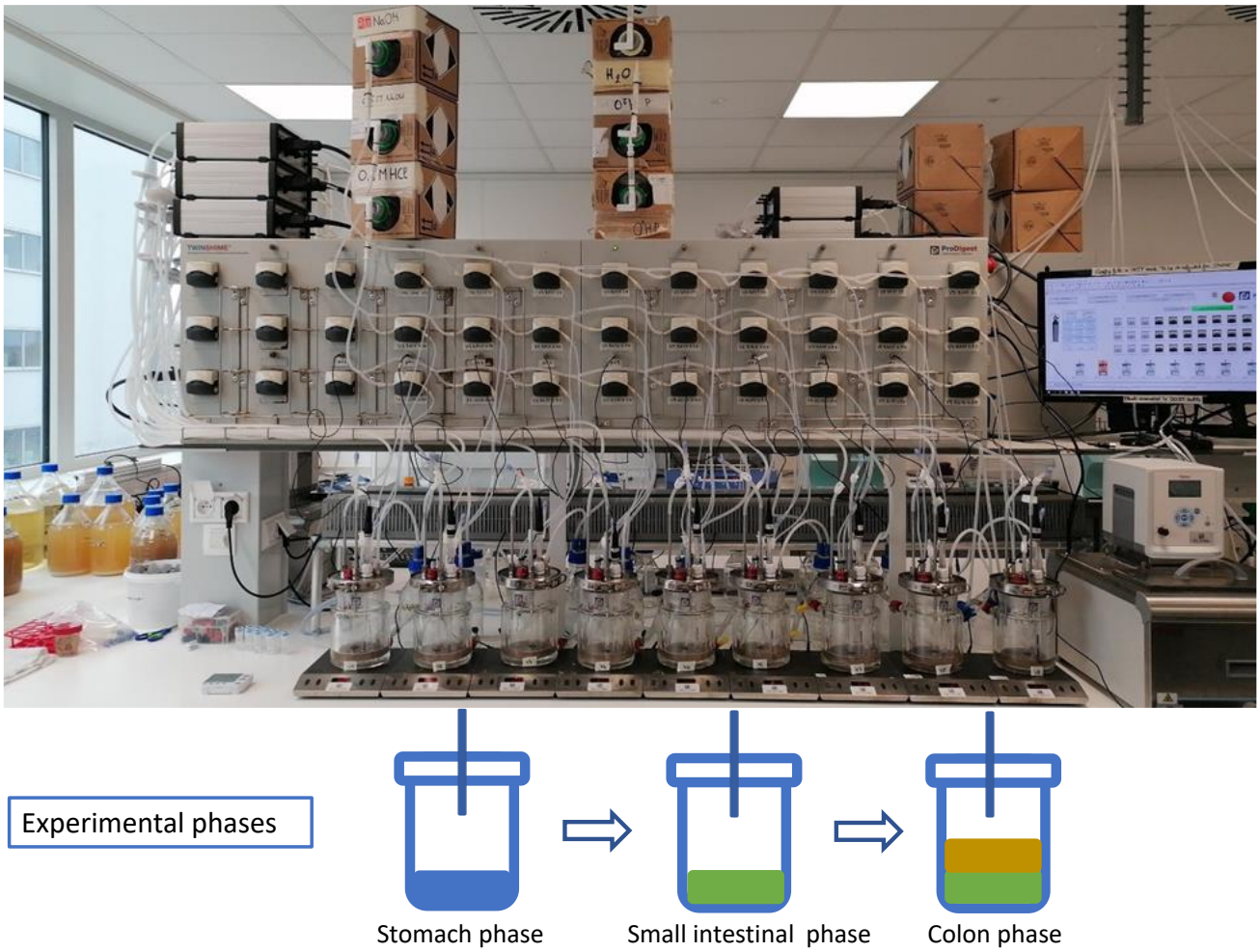
"an *in vitro* release test method simulating the conditions in the gastrointestinal tract of a patient"

Project No.	2S07-033
Acronym	Site Drug
Title	Site-Specific Drug Delivery
Start date of Project	1st January 2020
Duration of the Project	39 months
Output Number	O 5.1
Submission Date	30th June 2023

This project has received funding from the Interreg 2 Seas programme 2014-2020 funded by the European Regional Development Fund under subsidy contract No 2S07-033.

"An *in vitro* release test method simulating the conditions of the gastrointestinal tract of a patient"

The semi-dynamic SHIME® model (Upper GIT) for gastrointestinal release testing



ProDigest's semi-dynamic SHIME® in vitro model for studying the release of a test product from targeted release dosage forms during transit through the complete gastrointestinal tract.

Report detailing the design of the test method and operation mode

ProDigest is specialized in the development and offering of a variety of *in vitro* gastrointestinal tract models for feed, food, and pharma industries. The *in vitro* tool box consists of static *in vitro* models, for high-throughput testing of several separate parameters and products, and semi-dynamic and dynamic *in vitro* models that are more complex and that simulate the influence of several parameters on the test products at the same time.

During the Site Drug project the overall objective was to develop innovative drug products for targeted drug delivery. The Université de Lille and the University College London were responsible for the development of innovative drug delivery systems for colon-targeting for treatment of patients suffering from inflammatory bowel disease (IBD). Different concepts were developed by these consortium partners. ProDigest was responsible to test the gastrointestinal performance of the innovative drug products *in vitro* to select the most promising drug delivery systems that could be transferred into clinical trials.

To do so, ProDigest used two *in vitro* models of its technology portfolio namely the semi-dynamic SHIME® model and the batch model of the colon

The semi-dynamic SHIME® model (Upper GIT) is a fully automated computer-controlled *in vitro* model that simulates the passage of a dosage form through the complete gastrointestinal tract. Upon dosing, this model sequentially simulates the passage of the dosage form through the stomach, the small intestine, and the colon. Biorelevant media compositions, volumes, and transit times are used during each phase of the experiment and the pH is automatically controlled to simulate the physiological pH changes that occur throughout the gastrointestinal tract. Furthermore, during the colonic phase of the experiments a colonic microbiota of a specific donor is added to the system in order to study the bidirectional interplay between the colonic microbiota and the dosage form. Multiple samples can be taken from the system during each experimental phase thereby allowing to study the targeted release characteristics of dosage forms. The biorelevant simulation of the stomach, small intestinal, and colonic transit time, pH, and colonic microbiota composition allows to accurately study the performance of colon-targeting release systems since their targeted release characteristics are mainly time-, pH-, and/or, microbiota-dependent. Finally, this model offers superior bio relevance since it can simulate the gastrointestinal physiology and colonic microbiota activity and composition under certain disease conditions. This is especially relevant for colon-targeting release systems for the treatment of IBD since this disease is characterized by an altered gastrointestinal physiology and colonic microbiota composition as compared to healthy individuals.

The batch model of the colon is an *in vitro* model that specifically simulates the physiology and the microbiota composition and activity present in the colon. This static high-throughput model is ideally suited to test the degradation of polysaccharides, that can be used for microbiota-depending colon-targeting drug delivery systems, by the colonic microbiota. Furthermore, the bidirectional interplay between the API and the colonic microbiota can be studied. Upon release of the drug from its formulation inside the colon the drug can be subjected to colonic microbiota metabolism which could potentially result in the activation, inactivation, or toxification of the drug. Next to this, the drug itself could have a negative effect on the overall colonic microbiota composition and functionality. All these aspects can be studied using this *in vitro* model by sampling in function of time and offline analysis of the drug concentration, microbiota composition, and microbiota metabolite production. Finally, coupling of the batch model with cell assays allows to test the effect of the dosage form on gut barrier integrity and immunomodulation in a leaky gut model.

Report documenting the usefulness and superiority of the test method.

In vivo-predictive *in vitro* models simulating the complete gastrointestinal tract are valuable tools to evaluate the gastrointestinal behavior of oral dosage forms thereby accelerating the formulation development process and de-risking drug development projects. Pre-clinical animal models suffer from ethical constraints and are characterized by a different gastrointestinal physiology and microbiota as compared to human individuals. In most cases, the obtained results only relate to the input and final output resulting in a black box effect. Furthermore, real-life formulations can not be tested using these models

Hence, the combination of biorelevant media with biorelevant *in vitro* technologies offers a great advantage. The use of *in vitro* models allows to look inside the gastrointestinal tract making it possible to obtain mechanistic insights into formulation performance and the impact on the microbiota composition and activity. Furthermore, flexible *in vitro* models can be adapted in such a way that they simulate the gastrointestinal physiology and colonic microbiota of certain disease conditions. This especially relevant when the disease itself has an impact on the gastrointestinal tract.

"An *in vitro* release test method simulating the conditions of the gastrointestinal tract of a patient"

During this project the use of two *in vitro* models of the gastrointestinal tract allowed to mechanistically study the gastrointestinal behavior of innovate drug products for colon-targeting delivery under IBD conditions thereby accelerating the formulation development process of two of the consortium partners.

The use of the semi-dynamic SHIME® model allowed to study the colon-targeting release characteristics of different polymer/polysaccharide-based drug delivery systems. The accurate simulation of the stomach, small intestinal, colonic transit time and pH and colonic microbiota present under IBD conditions generated clear insights in which formulations resulted in early drug release in the small intestine and which formulations had superior colon-targeting characteristics. Furthermore, the use of fecal samples from different IBD patients as a source of microbiota for the colonic simulation allowed to study inter-individual differences in the degradation of the polysaccharide-containing coating of the formulation.

Next to this, the static batch model allowed to study the colon-targeting release characteristics of co-polymers. Mechanistic insights were generated about the capacity of the colonic microbiota to degrade the co-polymers and the final impact on the colonic microbiota composition and activity and cell barrier integrity and immunomodulation. Finally, the impact of multiple drugs on the colonic microbiota composition and activity was tested using the *in vitro* models.

In conclusion, the use of advanced *in vitro* models of the gastrointestinal tract generated mechanistic insights on the colon-targeting release characteristics of novel drug delivery systems and the final bidirectional interplay between the API and the colonic microbiota upon release.