In Vitro Dynamic Model of the Stomach and Small Intestine for Liquid Foods with Rheological Monitoring – First Prototype

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ABSTRACT

The present work presents a dynamic *in vitro* apparatus that aims to mimic the digestive process occurring in the gastro intestinal track (GI). The system is able to conduct viscosity measurements and to control the shear conditions. This is a work in progress and a preliminary assessment of the current status of the apparatus is presented in this article.

INTRODUCTION

The interest for a better understanding of how foods affect human health is growing. To deepen the understanding of the relations between food and health, more knowledge about the fate of macro and micro nutrients during digestion and absorption is needed. The "gold standard" for this type of research, is *in vivo* studies with humans or animals, however, several challenges related to ethical issues, economy and infrastructure restrict their application. Scientists have developed several different static and dynamic *in vitro* models that simulate the digestive process[1].

For static systems, a consensus model[2] has recently been developed. The experimental conditions (pH, composition of simulated digestive fluids, activities of digestive enzymes, duration of gastric and duodenal phases) applied in the model described here, are based on this consensus model. When it comes to dynamic models, no consensus has yet been achieved among researchers, and the models vary greatly in their level of sophistication, experimental conditions used, on-line monitoring of relevant parameters and sampling possibilities. To our knowledge, no other dynamic model allows an in-line monitoring of rheological parameters during gastric and duodenal phases.

A rheological monitoring is needed to describe the viscous characteristics of the food mixture along the digestive process. These measurements (e.g. viscosity) can follow the structural changes in the food during digestion. Viscosity is known to be affected by molecular changes[3]. Shear rates and viscosity measurements provided by *in vitro* digestion models can describe the bio-mechanics occurring in a mimicked GI tract (e.g. peristaltic movements). By measuring viscosity, it is also possible to estimate nutrient transport across the membrane mimicking the absorption process.

Our previous article[1], summarized the main information from literature, relevant to the elaboration of a dynamic *in vitro* model of the human stomach and small intestine, identifying the need to simulate the flows and shearing of the food. The process diagram described herein is the basis for the apparatus described in the current paper.

The aim of the present work is to present the first *in vitro* dynamic prototype of the

stomach and small intestine mounted in a rotational rheometer. The rheometer will monitor the viscosity of fluids foods such as milk during the *in vitro* process. The intestinal section uses a custom designed membrane unit to simulate absorption of small molecular weight degradation components like amino acids from protein digestion. The prototype has a main chamber, a cup made of glass, where viscosity measurements take place. A titrator allows constant pH monitoring and adjustments, and a peristaltic pump transports the fluids between the parts of the system, simulating *in vivo* peristalsis[4].

PROCESS DESCRIPTION

Process modelling of the *in vitro* stomach and small intestine

The *in vitro* process has been explained previously[1] and it will be summarized in order to understand the first prototype.

In reference to Fig. *1*, the stomach is represented by the items (1) to (7) and item (13) where: item (1) a dosing mechanism for human gastric juices (HGJ) or simulated gastric juices (SGF), (2) is a rotational rheometer that aims to mix and measure viscosity of the fluid composition while the digestion process occurs, (3) represents a titrator to monitor and adjust pH with HCl depending on the buffering capacity of the food and the target pH, (4) is a peristaltic pump that mimics the shearing during peristalsis in the stomach. The peristaltic pump (4) is connected to an acid resistant hose, (5), to pump and shear the gastric mixture. Item (6) is a temperature control unit, which in this prototype is a water bad. Item (6) is meant to keep the cup (7) at 37°C. Item (13) represents the connectivity of the rheometer (2) and peristaltic pump (4) to a PC.

For the subsequent duodenal digestion and absorption, the process is replicated by the items (1) to (4), and (6) to (13). Item (1) is a pipette used to add human duodenal juice (HDJ) or simulated intestinal fluid (SIF). The rheometer (2) is used to provide a continuous mixing of the fluids while monitoring the viscosity of the digesta, the titrator (3) is used to gradually increase pH (7-8) to mimic the duodenal conditions by either sodium hydroxide (NaOH) or sodium bicarbonate ($NaHCO_3$). The peristaltic pump (4) is connected to a hose (8) to pump continuously the intestinal fluids towards the porous media (e.g. dialysis membrane, (9)). The membrane (9) is located at the inside of a custom made glass tube (10), further referred as membrane separation unit. This unit resembles a tube-tube heat exchanger where the inner pipe is replaced by a cylindrical membrane. Through the external tube, a solution flows in counter flow, this liquid is further referred as buffer. The reservoir for the buffer is represented by item (12) and it is pumped through a peristaltic pump (11) to allow circulation during the intestinal process.

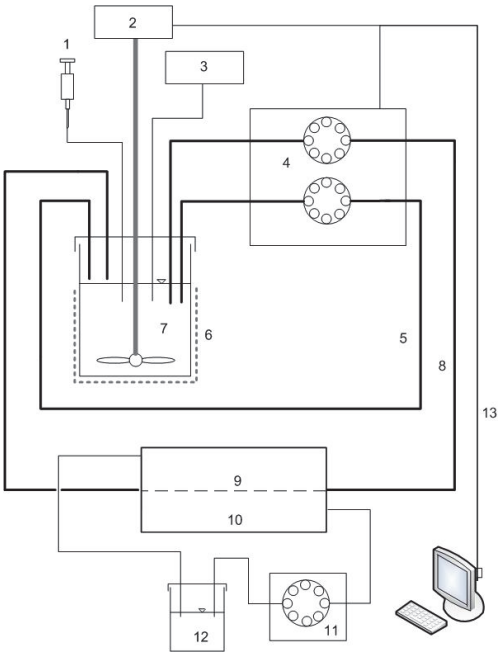

Figure 1. *In vitro* process diagram.

DESCRIPTION OF THE FIRST PROTOTYPE AND PROCESS STEPS

The first prototype is assembled in a rotational rheometer (Paar Physica UDS200). A stirrer type of probe is 3D printed, this manufacturing technique allow the researchers to easily perform custom design improvements to the probe and thus to increase performance depending on the food viscosity and agglomeration characteristics during the digestive process. By 3D printing is also possible create and work with disposable probes.

The stirrer design is meant to mix the components while measures viscosity, this is particularly challenging during the first steps when small volumes (~7 ml), see step 1 and 2 in Table *1*, are hold in the feed cup (see Fig. 3).

If using the prototype for steps 1 and 2, the mixer design is important. The wetted area of the mixer probe can be visualized from Fig. 2. The liquid volumes are drawn in the figure where the ~7 ml is represented by the volumes 1 and 2 together. In practice, during step 1 and 2, the probe can be moved down to decrease the gap probe-cup and thus to increase the probe wetted area.

Table 1. The digestion process step by step using saliva, gastric and duodenal juices.

Step	Added component	Vol [ml]	Total Vol [ml]	pH	Step Time [min]
1*	Milk	3.63	3.63	6-7	0
2*	Saliva	3.63	7.25	6-7	0
3**	HGJ^{+} or SGF^{++}	7.25	14.5	2-4	30
4***	HDJ^{+++} or SIF^{++++}	14.5	29	6-7	60

* It also can be mixed and measured outside the apparatus
** Milk, Saliva plus HGJ or SGF are mixed and measured in the cup
*** Added to the mix in the cup
$^{+}$ Human gastric juices, $^{++}$ Simulated gastric fluids
$^{+++}$ Human duodenal juices, $^{++++}$ Simulated intestinal fluids

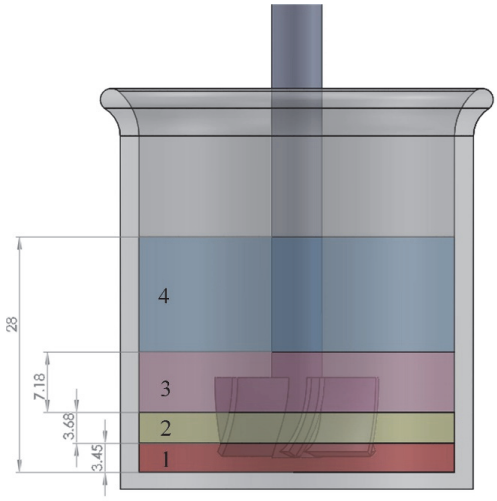

Figure 2. Feed cup and stirrer type probe for the digestive process. Volumes used during the different steps are indicated by numbers: (1) Saliva, (2) milk, (3) HGJ or SGF and (4) HDJ or SIF. The sum of all volumes (1-4) is 29 ml. Dimensions are in millimetres.

Setting a defined gap and a defined wetted area during step 1 and 2 is important as it is needed for the calibration process to obtain viscosity from torque and rotational speed given by the rheometer. Shear stress depends on the wetted area of the shaft. The calibration procedure will be described later in the article.

When adding HGJ or SGF, step 3 in Table *1*, casein in milk starts coagulating. If this process occur, the user can adjust the vertical position and shear from the mixer probe to split the coagulate in smaller sizes. As seen from Fig. 2, the stirrer probe is completely submerged in the feed volume (~14-15 ml, volume 1, 2 and 3 together) during step 3. The complete submersion of the tip of the pH probe, also referred as pH electrode, is critical in this step to perform correct measurements. The first prototype with the pH probe from the titrator is drawn in Fig. 3.

During the simulation of intestinal digestion and subsequent absorption process (step 4 in Table *1*), the volume is increased by the addition of HDJ or SIF (~29 ml), a first stage in this step will be the mixing of the fluids using the stirrer probe. Once well mixed, the fluids can be moved by the peristaltic pump to the membrane separation unit (see Fig. 3) to start the diffusion of nutrients through the membrane. This process will reduce the volume in the cup to an equivalent to volumes 1, 2 and 3 together (ref. Fig. 2).

During all stages, it is important to have enough space in the feed cup to place the hoses described on Fig. *1*, items (5) and (8). Hoses are placed in the annular gap between the tip of the mixer propeller and the side walls of the cup, a top view picture of assembled hoses to the cup can be seen on Fig. 4.

Fig. 5 presents a detail view of the membrane unit presented in Fig. 3. The unit is made in glass to allow the visualization of the digestive process throughout the testing time.

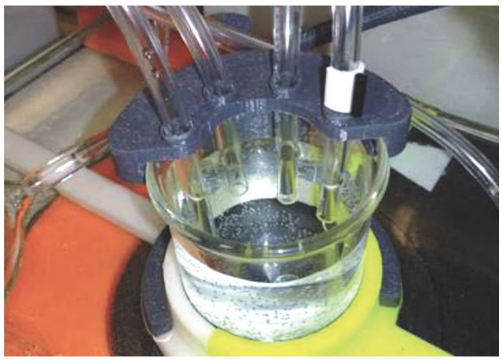

Figure 4. Hoses connected to the feed cup.

Fig. 6 shows the assembly of a membrane to the feed circuit. Rubber rings are used to seal the membrane to the glass tube. During this step, 4, as seen on Table 1, the pH should neutralize, so the use of rubber rings is not a problem.

The flow rates of the feed and buffer should be adjusted to prevent an inflation or collapse of the membrane. Since the membrane unit is made of glass, the shape of the membrane can be easily follow while adjusting the flow rates in the peristaltic pump.

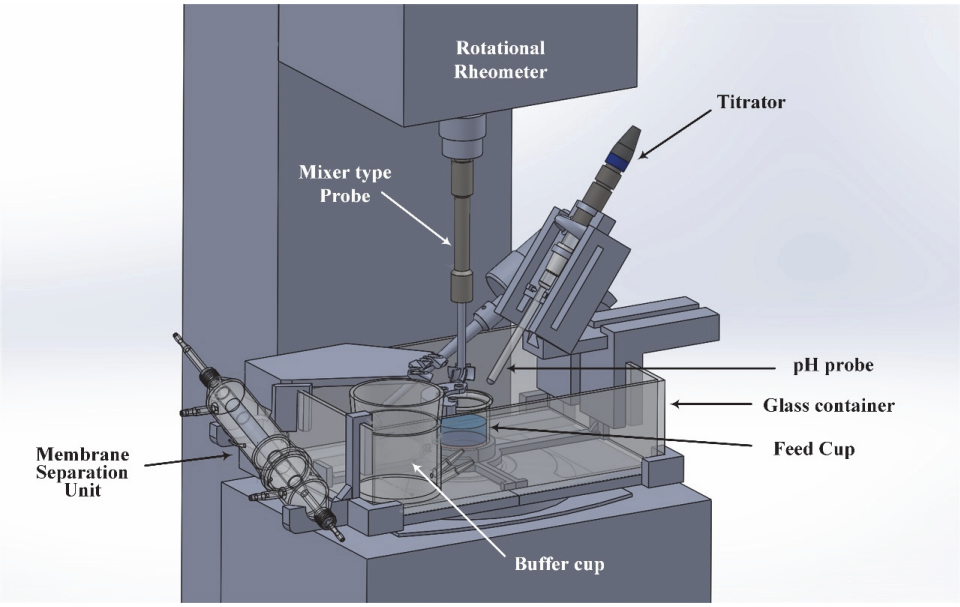

Figure 1. Main components of the *in vitro* system mounted in a Paar Physica UDS200 rheometer.

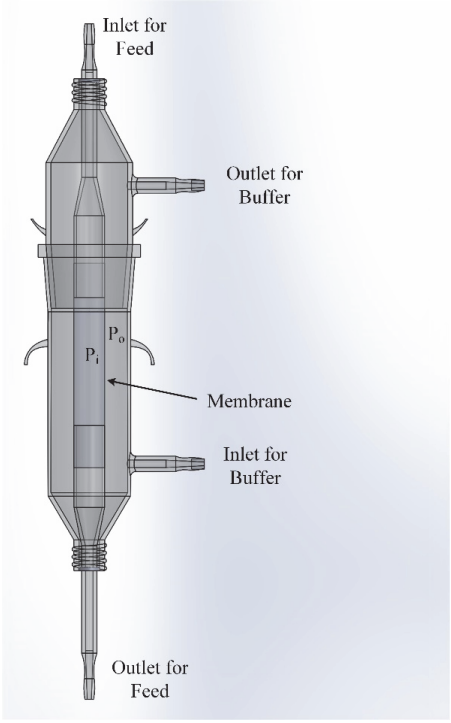

Figure 5. Membrane separation unit simulating the small intestine. The unit is made in glass. P_i is the pressure at the inside of the membrane (pressure of feed) and P_o is the pressure at the outside surface of the cylindrical membrane (pressure of buffer).

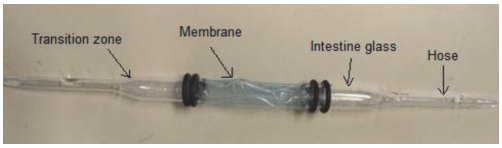

Figure 6. Assembly of the membrane to the feed circuit.

Temperature is kept at 37°C in the system by immersing the parts having the largest volumes, the cup and the buffer reservoir, in the glass container shown in Fig. 3. The glass container holding the assembly is sitting in the Peltier plate of the rheometer. The heat transfer from the Peltier plate to the glass container can be facilitated by placing a thin film of a thermo-conductive paste.

The prototype is intended to provide good repeatability. The system is sheared by the mixer probe connected to the rheometer and by the peristaltic pump. The working parameters are set by the users on the instruments (e.g. rheometer, peristaltic pump, titrator, etc.) without direct contact with the process.

Concerning assembling the *in vitro* system, all parts have dedicated fittings to increase repeatability.

CALIBRATION FOR VISCOSITY MEASUREMENTS

The mixer probe inside the cup (Searle type of rheometry system) has a complex geometry. This characteristic forms complex flows in the feed cup which creates difficulties to analytically determine an average shear stress and an average shear rate by means of torque and rotational speed. However, from the behaviour or relation between torque and rotational speed, it is possible to predict viscosity throughout a calibration experiment using a Newtonian standard with known viscosities at different temperatures.

The most appropriate calibration procedure for viscoelastic measurements in addition to future viscosity monitoring, is through the determination of a coaxial cylindrical analogue. This can be done by determining an analogue radius. The analogue radius should represent the position of the average shear rate. Also, the surface of the analogue cylinder and torque should represent the average shear stress. This calibration procedure has been successfully used in other systems, like screw probes[5] and in probes with paddles[6] when a linear relation between torque and speed occur with Newtonian fluids.

The calibration procedure must be done for the different fluid levels, when the paddles of the mixer are partially cover by the fluid (steps shown in Table 1). Placing the hoses in the annular gap during the

calibration procedure will also increase the accuracy of future predictions.

PRELIMINARY ASESSMENT OF THE MEMBRANE SEPARATION UNIT

A number of tests need to be done to determine the reliability of the prototype, some of them have been done until this development stage and are presented in this work. Those tests are referred as preliminary.

One of the most challenging parts to simulate is the membrane separation unit, at this stage, the preliminary tests focussing on this unit are presented.

Methodology to assess the membrane separation unit

Permeability and diffusivity are common membrane characterizations. To estimate permeability, the system needs to be calibrated in the rheometer as viscosity is needed (in Darcy's law). However, at this stage, diffusivity can be determined in the system without viscosity measurements by measuring the concentrations of a substance on both sides of the membrane (in Fick's law).

Before conducting diffusion tests through the membrane, the membrane system need to be tested for leakages. For this reason a substance with strong color, unable to diffuse through the membrane, easy to detect and quantify, is added. The substance chosen was Evans blue and it circulated through the system for one hour. Repeated preliminary static and dynamic tests showed that Evans blue adsorbed to the membrane and did not diffuse. This was also later confirmed by direct contact with the suppliers (Spectrum Labs).

Once the reliability of the membrane unit was achieved (Fig. *6*), a dynamic test was done to assess the membrane separation capabilities. L-Tryptophan is chosen for this test. L-Tryptophan is an aromatic amino acid present in milk proteins and it was chosen as it is easy to quantify based on its ability to absorb light at 220 and 280 nm. L-Tryptophan also has good solubility in water.

For the diffusivity calculations, the following equation derived from Fick's law can be used[7, 8]:

$$j_A = -D_A \frac{dc_A}{dl} \quad (1)$$

Where j_A is the molar flux of component A (L-Tryptophan), D_A is the diffusion coefficient, c_A is the gradient of feed concentration and l is the distance in the direction of diffusion. For this case l is the thickness of the membrane.

Eq. (1) can be expressed as follows:

$$D_A = \frac{j_A \cdot l}{c_1 - c_2} \quad (2)$$

Where c_1 is the concentration of L-Tryptophan in the feed and c_2 is the concentration L-Tryptophan in the buffer.

Two membranes of different pore sizes were chosen, 3.5 kDa and 8 kDa.

According to the producer (Spectrum Labs), l for 3.5 kDa membrane is 48.5 µm and l for 8 kDa membrane is 64.2 µm.

The concentrations c_1 and c_2 were measured by spectrophotometry at 220 nm using 1 mL quartz cuvettes.

Concerning the experimental design for the membrane tests, since it was no previous information about D_A for L-Tryptophan for the two membranes, it was not possible to calculate the molar flux to the buffer beforehand. If the molar flux is known beforehand, it is possible to calculate the amount of buffer volume needed to be in the detection range of the spectrophotometer (Shimadzu UV-160A) at 10 minutes. As consequence, two tests with different buffer volumes were conducted when using the membrane with the smallest pore size (referred as 3.5 kDa).

The same volume of the feed (29 ml, Step 4 in Table *1*) is chosen for all tests and the same initial concentration of L-Tryptophan (0.73 µM).

Results and Discussions for the tests using the membrane separation unit

The results in Fig. *7* shows a gradual increase of L-Tryptophan in the buffer volume throughout the 60 min test, this clear trend can be used as an indication that no sudden leakage occurred while running the test. As seen on Fig. *7*, L-Tryptophan started to be detected only after 30 minutes when using 150 ml of buffer volume. This is due to the detection limits of the spectrophotometer.

It is observed from Fig. *7* that the concentration of L-Tryptophan in the 100 ml buffer volume resulted to be higher than the concentration in the 150 ml buffer volume when using the 3.5 kDa membrane. This expected result indicates that the apparatus works as intended.

The results shown in Fig. 8 can be taken as an evidence of a reasonably good repeatability of the diffusion process when using the 8 kDa membrane with 150 ml buffer volume. This test was done twice, the system was completely disassembled and cleaned, and the membrane replaced, before performing the second test.

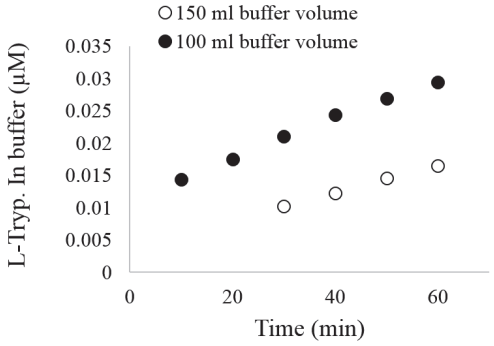

Figure 7. Concentration of L-Tryptophan in the buffer volume when using a 3.5 kDa membrane. L-Tryptophan was only detected after 30 min

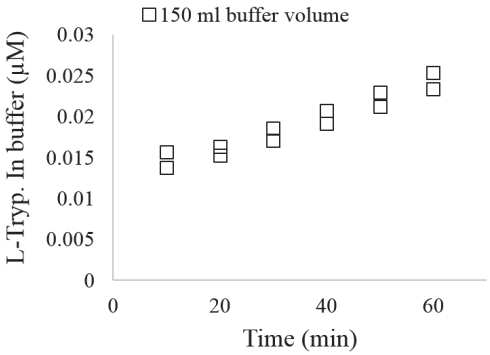

Figure 8. Concentration of L-Tryptophan in the buffer volume when using a 8 kDa membrane. The points represent two independent tests using the same settings.

The results from the estimation of D_A on Fig. *9* shows a gradual decrease in diffusivity along the testing time for all tests until at about 40-50 minutes when the curve start presenting an asymptotic direction which indicates that from approximately 40-50 minutes, the molar flux is relatively constant.

A further investigation to the cause of the changes in diffusivity along time should be carried, along with evaluating the use of this membrane to conduct *in vitro* tests.

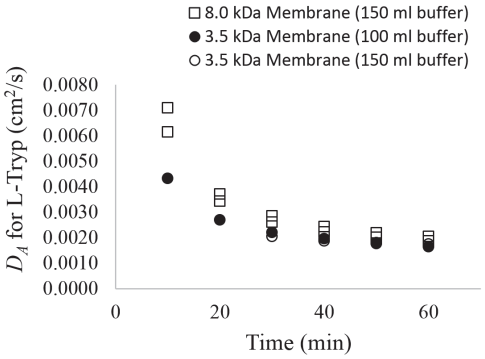

Figure 9. Diffusion coefficient (D_A) for 8 and 3.5 kDa membrane when using L-Tryptophan.

FINAL REMARKS

This work shows that the future use of the *in vitro* model presented in this article is feasible, however many tests will need to be performed in order to indicate its capabilities and limitations. Major challenges can be faced if the *in vitro* model is intended to simulate all physic-chemical processes occurring in *in vivo* systems. The monitoring of viscosity and the possibility to control the shear conditions during the *in vitro* process, is with no doubt, a step forward.

The preliminary assessment of the model presented in this work indicates that the system can be used to provide information concerning the digestion of liquid foods under controlled shear conditions. This is an advantage over most *in vitro* models currently in use, as these do not have in-line viscosity measurement or control over the shear conditions during the process.

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