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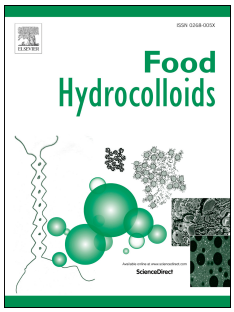

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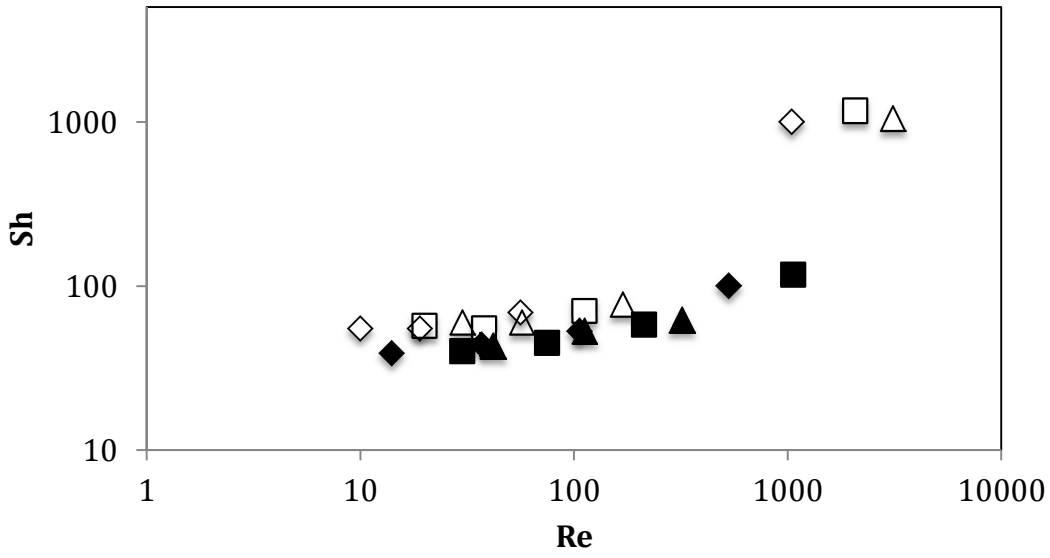

An increasing Reynolds number (i.e decreasing viscosity) results in an increase of observed mass transfer coefficient

Hydrocolloids in human digestion: Dynamic *in-vitro* assessment of the effect of food formulation on mass transfer

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Abstract

Over the last decade the effect of food formulation on digestion in healthy adults has increasingly gained interest within the scientific community. The area requires multidisciplinary skills from a wide range of fields including medical, chemical, and engineering. In this work, we aim to develop simplified *in-vitro* intestinal models to study the effect of mass transfer on food digestibility and nutrient bioaccessibility for a range of food hydrocolloids. The models developed aim to mimic intestinal motility and focus on describing phenomena occurring during digestion in the mm scale. Results indicate that hydrocolloids have a significant effect in retarding simulated glucose accessibility, and the effects are seemingly more pronounced (fivefold reduction in mass transfer and simulated glucose absorption) at viscosities around 0.01Pa s. This indicates the potential to modulate glucose availability by food formulation.

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1. Introduction

It is estimated that the food sector is currently responsible for one third (of a $15 million total market) of hydrocolloid applications worldwide [Seisun 2012]. Although primarily used as texturing agents [Dickinson 2003; 2009; Saha & Bhattacharya, 2010; Funami 2011; Ramirez, Uresti, & Velazquez, 2011], food hydrocolloids are increasingly being associated with a number of important health benefits, including glycaemic and insulinaemic control in type-2 diabetes, weight management, and cardiovascular disease prevention [Jenkins, Wolever, Leeds, Gassull, Haisman, Kilawari, Goff, Metz, & Alberti, 1978; Slavin 2005; Edwards & Garcia 2009; Dettmar, Strugala, Richardson, & 2011; Kendall, Esfahani, & Jenkins, 2010; Mills, Spyropoulos, Norton, & Bakalis, 2011; Norton, Cox, & Spyropoulos, 2011; Gidley 2013; Fiszman & Varela 2013; Bradbeer, Hancocks, Spyropoulos, & Norton, 2014]. These functionalities are typically linked with the thickening, gelling, water sequestering, and prebiotic properties of food hydrocolloids and their effect on food digestion [Doublier & Cuvelier 2006; Edwards & Garcia 2009; Douaire & Norton 2013]. A possible mechanism of action involves the resistance in mass transfer in the gut in the presence of hydrocolloids due to the increased viscosity of the digested food. This may result in slower gastric emptying and modulated nutrient absorption. However, the detailed mechanisms affecting nutrient bioaccessibility and in particular the impact of hydrocolloids on mass transfer and food digestion are currently not well understood [Gidley 2013; Fiszman & Varela 2013].

Quantifying human digestion is a challenging research area. Although the importance of "artificial digestion" has long been appreciated [Sheridan Lea 1890], it is in the last decade that there has been a significant increase in the use of *in-vitro* techniques [Guerra, Etienne-Mesmin, Livrelli, Denis, Blanquet-Diot, & Alric, 2012; Hur, Lim, Decker, & McClements, 2011; Woolnough, Morno, Brennan, & Bird, 2008]. *In-vitro* systems have been broadly classified into 'batch' and 'dynamic', depending on whether the temporal profile of *in-vivo* digestion (e.g. fluid mixing, addition of simulated gut secretions and the removal of resulting digestion products) is taken into account [Vieira,, Kirby, Ragueneau-Majlessi, Galetin, Chien, Einolf, Fahmi, Fischer, Fretland, Grime, Hall, Higgs, Plowchalk, Ridley, Seibert, Skordos, Snoeys, Venkatakrishnan, Waterhouse, Obach, Berglund, Zhang, Zhao, Reynolds, & Huang, 2013; Guerra *et al*., 2012; Thomas, Herouet-Guichenev, Ladics, Bannon, Cockburn, Crevel, Fit`Patrick, Mills, Privalle, & Vieths, 2007]. The typical 'batch' model consists of a series of vessels, each of which simulates the digestive conditions (e.g. pH, enzymes, temperature,

biosurfactants, etc.) in different regions of the gut (e.g. mouth, stomach, small intestine, and colon). Such systems have been used by Englyst, Veenstra, & Hudson [1996] to measure the rapidly available glucose in plant foods and by Oomen, Tolls, Sips, & Van den Hoop [2003] to assess the metabolism of lead into the digestive tract. Similar systems also include the multiple-step pH-stat method that simulates a four-step digestion (oral, gastric, small, and large intestinal phases) [McClements & Li, 2010] and De Boever, Deplancke & Verstraete's [2000] five-step digestive model consisting of five double-jacketed vessels. Although these models provide valuable information, they do not account for actions of the mechanical forces, flow and mixing that might have an effect on the digestion kinetics.

Models with dynamic elements are typically application specific, and may include oral, gastric, or intestinal digestion. Oral digestion is complex and difficult to mimic [Le Reverend, Gouseti, & Bakalis, 2013]. Many investigators simplify this step and use commercial meat mincers to simulate oral processing [Bornhorst & Singh 2013; Hoebler, Lecannu, Belleville, Deneaux, Popineau, & Barry, 2002]. Others have developed models to study the effect of chewing [Salles, Tarrega, Mielle, Paratray, Gorria, Liaboeuf, & Liodenot, 2007], tongue action [Benjamin, Silcock, Beauchamp, Buettner, & Everett, 2012; Benjamin, Silcock, Kieser, Waddell, Swain, & Everett, 2012], shearing [Lvova, Denis, Barra, Mielle, Salles, Vergoignan, Di Natale, Paolesse, Temple-Boyer, & Feron, 2012] and compression [De Loubens, Panouille, Saint-Eve, Deleris, Trelea, & Souchon, 2011; Mills *et al*., 2011] on oral digestion.

Dynamic gastric digestion models typically consider mechanical mixing of the bolus alongside choosing the required physiological conditions (pH, mixing and flow, enzyme concentrations, etc.). In the model of Kong and Singh [2008] mixing is achieved by the motion of small plastic beads, which provide the required mechanical stresses on the food samples. In Chen, Gaikwad, Holmes, Murray, Povey, Wang, & Zhag's [2011] model, mixing is generated using a spherical probe with controlled vertical movement, positioned in the axial centre of a jacketed vessel. The Dynamic Gastric Model (DGM), an apparatus that simulates gastric digestion using a conical flexible walled vessel and a cylinder that processes the food at representative shear rates, has recently been developed at the Institute of Food Research in Norwich UK [Lo Curto, Pitino, Mandalari, Daintry, Fauls, & Wickham, 2011; Mercuri, Lo Curto, Wickham, Craig, & Barker, 2008; Vardakou, Mercuri, Barker, Craig, Faulks, & Wickham, 2011; Wickham & Faulks 2012]. The DGM replicates the physical mixing, transit and breakdown forces in the stomach, as well as the relevant physiological conditions (pH gradient and enzymes).

Intestinal models in which mixing conditions (segmentation and peristalsis) are an integral part of the process are scarce in the literature. One such model has been reported by Tharakan, Rayment, Fryer, & Norton [2007] and Tharakan, Norton, Fryer, & Bakalis [2010], where segmentation is simulated by squeezing the flexible dialysis tube used to represent the gut wall with the aid of two pneumatically controlled rubber cuffs. In this model, flow conditions have shown to significantly affect simulated absorption rates of chemicals in water as well as in guar gum solutions. The flow characteristics of a shear thinning fluid during simulated peristaltic motion (squeezing of an elastic tube) have been experimentally investigated by Nahar, Jeelani, & Windhab [2012].

In the mid-1990s, TNO in the Netherlands introduced TNO intestinal model (TIM), a computer-controlled *in-vitro* digestive system, which represents the different sections of the digestive tract (stomach, duodenum, jejunum, ileum, and colon) using different compartments [Blanquet, Marol-Bonnin, Beyssac, Pompon, Renead, & Alric, 2001; Marteau, Minekus, Havenaar, & Huis in't Veld, 1997; Minekus, Marteau, Havenaar, & Huisintveld,, 1995; Minekus, Smeets-Peeters, Bernalier, Marol-Bonnin, Havenaar, Marteau, Alric, Fonty, & Huis in't Veld,, 1999]. Each compartment is equipped with a flexible membrane where simulated digestion takes place, and two outer glass jackets that allow for both temperature and pressure control. Today, two TIM models exist: TIM1 (stomach & small intestine) [Minekus *et al.*1995; Marteau *et al.* 1997] and TIM2 (large intestine) [Minekus *et al.* 1999; Blanquet *et al.* 2001].

Models with both 'batch' and 'dynamic' elements have also been described in the literature. For example, 'batch' gastric digestion has been combined with dialysis membranes in cell wells [Argyri, Birba, Miller, Komaitis, & Kapsokefalou, 2009; Argyri, Theophanidi, Kapna, Staikidou, Pounis, Komaitis, Georgiou, & Kapsokefalou, 2011] or dialysis bags [Bouayed, Deuber, Hoffmann, & Bohn, 2012] to simulate absorption of chemicals through the small intestinal wall. In some other systems, peristaltic pumps have been used to control flow of digested foods and related secretions for adults [Mainville, Arcand, & Farnworth, 2005; Savalle, Miranda, & Pelissier, 1989] and infants [Menard, Cattenoz, Guillemin, Souchon, Deglaire, Dupont, & Picque, 2014].

Overall, there is evidence that the dynamic nature of human digestion is important in determining digestibility of foods. In particular, flow and mixing in the gut may significantly affect digestive processes, however the link between mass transfer and food digestion is still a

largely unexplored area. In this framework, we have developed *in-vitro* models that simulate gut wall contractions with the aim to investigate the effect of gut motility on the accessibility of glucose from model solutions, using a range of food hydrocolloids (guar gum, CMC, pectin). We have analysed our data using engineering principles and dimensionless numbers that characterise the flow (Reynolds number) and mass transfer (Sherwood number) in the gut. We have found that irrespective of the hydrocolloid used or the segmentation patterns applied, the relationship between Reynolds and Sherwood numbers of all investigated digestive conditions and for all model chyme solutions superimposes to a single line. As Reynolds number increased and the flow became less laminar, mass transfer was enhanced. The transition of flow regime was observed at solutions with viscosities of the order of 0.1Pa s, which correlates well with results reported by Tharakan *et al.* [2010]. This viscosity value is within the range of luminal viscosities reported from animal studies [Ellis, Roberts, Low, & Morgan, 1995]. Systems with lower viscosities (higher Reynolds number) showed enhanced mass transfer levels. It is noted that guar gum is a commonly used, relatively inexpensive ($0.83/lb; $1.83/kg [Seisun 2012]) and highly acceptable by consumers [Varela & Fiszman 2013] hydrocolloid, which has been shown to reduce postprandial blood glucose levels *in-vivo* [Jenkins et al. 1978].

2. MATERIALS AND METHODS

2.1 Sample preparation

Model 1% wt/vol (55mM) glucose (D-(+)-glucose by Sigma-Aldrich, UK) solutions of different viscosities were used in this study to evaluate the effect of mass transfer in simulated glucose absorption. This concentration approximates the glucose content of a cup of coffee with half a sachet of sugar added and it is 10 times higher than the homeostatic blood glucose levels. Viscosity was adjusted by addition of different hydrocolloids (guar gum, pectin, carboxymethyl cellulose (CMC)). Distilled water was used in all experiments. Guar gum (105008, ICN Biomedicals, USA for the SIM experiments and Sigma-Aldrich, UK for the DDuo experiments) and pectin (degree of esterification7680) by Fluka, UK were added slowly into stirred glucose solutions and heated to 80^{o}C for 5min. CMC (Sigma-Aldrich, UK, C5013) was also added slowly into stirred glucose solutions but was more moderately heated (60^{o}C for 10min). Mixtures were left to fully hydrate overnight at room temperature under mixing with

an overhead stirrer and were further used within 24h. Viscosity was measured using rotational rheometer with cone/plate geometry prior to the experiments (Figure 1).

2.2 *In-vitro* Models: SIM and DDuo

2.2.1 Model description

The Small Intestinal Model (SIM) used in this work has been developed at the School of Chemical Engineering, University of Birmingham and has been described in detail elsewhere [Tharakan 2008; Jaime Fonseca 2011]. The model (schematic of Figure 2) consists of an inner dialysis tube (Spectre/Por 7®, MWCO 8kDa) that represents the intestinal lumen (diameter of 32mm, characteristic of the average adult human small intestine [Schmutz, Le Pennec, Dede, & Perdriel,, 2005]), and an outer, concentric, impermeable silicone tube (Flexible Hose supplies, UK, 50mm diameter, 3mm thickness) that borders the outer (recipient) zone. Large pore size (8kDa) was selected to minimise the resistance of mass transfer incurred by the membrane. In a typical experiment, chyme enters from one end of the lumen (feed) and may recirculate with the aid of a peristaltic pump. The recipient fluid (initially distilled water) is also re-circulating and passes through a collection jar, which allows sampling as required. Gut motility is simulated by the pneumatically controlled inflating-deflating motion of two rubber cuffs. Cuff inflation causes squeezing of the tubes, which simulates gut wall contractions. Deflation releases the squeezing pressure and allows the tubes to retrieve their initial cylindrical shape. In the present work, 1% wt/vol (55mM) glucose solutions with or without the addition of hydrocolloids (guar gum, CMC, pectin) were used as model 'chyme' systems and the glucose collected in the recipient zone was measured (DNS method, section 2.3) over time.

A second, improved *in-vitro* model (Dynamic Duodenum, DDuo) has recently been developed and initial results are also presented here. The new model implements a more automated and flexible design, with the aim to allow for a more systematic investigation of the effect of peristaltic and segmentation motions on digestion. The DDuo (schematic of Figure 3) uses the same twin tube concept as the SIM, where the small active chemical passes through the pores of a dialysis membrane from the chyme (lumen) to the recipient zone. A fixed secretions port designed for injection of intestinal secretions (such as pancreatic and hepatic fluids) is located 100mm away from the feeding end. This is representative of the average distance between the pylorous and the emptying of the pancreatic duct (at the major duodenal papilla) in humans [Kong, Kim, Hyun, Cho, Keum, Jeen, Lee, Chun, Um, Lee, Choi, Kim, Ryu, & Hyun, 2006].

Segmentation and peristaltic motions are achieved by squeezing of the membrane at 8 independently controlled segmenting positions.

It is noted that the models have been specifically designed for studying engineering aspects (mass transfer) of human digestion, which is scarce in the open literature. So far, the effects of other physiological conditions, such as nutrient transportation through the gut membrane or feedback mechanisms, are not represented.

2.2.2. Methods

Unless otherwise stated, the two cuffs of the SIM operated in sequence (one after the other), in cycles of 6s (2s inflation time, 2s deflation time, 2s delay time), performing 10 cycles per minute (cpm) in total. The effect of mixing (segmentation / no segmentation) on simulated glucose absorption was studied for the systems detailed Table 1 (zero-shear viscosity also shown). The ends of the dialysis tubing were closed and no chyme recirculation occurred (closed configuration). Experiments were conducted in triplicates and the average with error bars is shown in the graphs.

The effect of segmentation frequency on simulated glucose absorption was studied for the systems detailed in Table 2 using the open configuration, where chyme re-circulated at 1.6×10^{-4} $m^3 s^{-1}$ with the aid of a peristaltic pump. Cuffs operated at cycles of 3s, 6s, and 9s with equal inflation, deflation, delay intervals of 1s (20cpm), 2s (10cpm) and 3s (5cpm), respectively. Glucose increase in the recipient zone was determined using the DNS method, described in section 2.3. Experiments were conducted in triplicates and the average with error bars is shown in the graphs.

Initial experiments with the DDuo were performed using 1% w/w glucose solutions with or without addition of 1% guar gum as model chyme systems. Unless otherwise stated, segmentation occurred at 4 positions (blue arrows in Figure 3), alternating (with the black arrows in Figure 3) every 10s. Although further work is required for conclusions to be reached, initial results are included here to indicate the potential of the new model and how it compares with the SIM.

2.3 Sample analysis: DNS

Samples from the recipient side were analysed using the dinitrosalicylic acid (DNS) method for reducing sugars [Jaime-Fonseca, 2011; Miller 1959]. Equal volumes (1mL) of sample (or

water as reference system) and DNS reagent (0.1% dinitrosalicylic acid; 30% w/w potassium sodium tartrate; 0.4M NaOH) were added in a test tube, mixed, and placed in boiling water for 5min. The resultant products were immediately cooled to room temperature and measured spectrophotomercially at 540nm.

2.4. Data analysis

2.4.1 Mass Transfer Coefficients

Mass transfer coefficients were determined as described previously [Tharakan *et al.*, 2007; Tharakan *et al.*, 2010; Jaime-Fonseca, 2011]. A typical graph of glucose absorption in the recipient zone over time is shown in Figure 4 and is used to estimate mass transfer in the model gut. The molar flux across the membrane is calculated using equations 1 and 2. The overall mass transfer coefficient ($K_{overall}$) is then obtained from equation 3.

$$A = 2 \cdot \pi \cdot r \cdot L \quad (1)$$

$$M_T = \frac{mol_{glucose}}{A \cdot t} \quad (2)$$

$$K_{overall} = \frac{M}{\Delta C} \quad (3)$$

where r is the membrane radius (m), L is the length (m), A is the total absorbing surface area (m^2), $mol_{glucose}$ is the glucose in the recipient side (mol) , M_T the total molar flux (mol $m^{-2}s^{-1}$), ΔC is the concentration difference (mol m^{-3}) between the two sides of the membrane (taken as the initial concentration difference of 0.055M, assumed to change insignificantly within the experimental time), and $K_{overall}$ is the overall mass transfer coefficient (m s^{-1}).

Detection of a glucose molecule requires transportation from the lumen to the dialysis membrane, passing through the membrane, and transfer to the recipient fluid. This three-stage process is characterised by the luminal mass transfer coefficient, (K_{lumen}, m s^{-1}), diffusion (described by coefficient $D_{membrane}$, m^2 s^{-1}) through the membrane of thickness $Z_{membrane}$ (m), and the recipient side's mass transfer coefficient (K_{rec}, m s^{-1}). Equation 4 gives the relationship between the local and overall transfer coefficients (K_{system} is the combined mass transfer through the membrane and the recipient zone, m s^{-1}).

$$\frac{1}{K_{overall}} = \frac{1}{K_{lumen}} + \frac{Z_{membrane}}{D_{membrane}} + \frac{1}{K_{rec}} = \frac{1}{K_{lumen}} + \frac{1}{K_{system}} \quad (4)$$

To determine K_{lumen} of the investigated chyme samples, it is first necessary to estimate K_{system}, which is assumed constant for all the experiments. This was achieved from experiments that minimise resistance to mass transfer at the lumen side (maximise K_{lumen}), so that $1/K_{lumen}$ would be much smaller than $1/K_{System}$. To minimise resistance in the luminal side, an increasing flow rate was applied in the inner tube, which was filled with 1% glucose in water until no significant increase in $K_{overall}$ was observed. This value (estimated at 5.3×10^{-7} m s^{-1}, Tharakan, 2008) was taken as K_{system}.

2.4.2 Reynolds and Sherwood numbers

The dimensionless Reynolds (Re) and Sherwood (Sh) numbers were estimated from equations 5 and 6, to further characterise mass transfer and study the relative importance of convective and diffusive processes in the model gut.

$$Re = \frac{\rho u(2r)}{\mu} \quad (5)$$

$$Sh = \frac{K_{lumen} r}{D_{glucose}} \quad (6)$$

where ρ is the density of the fluid (kg m^{-3}), u is the velocity of the fluid (m s^{-1}), r is the radius of the membrane (m), μ is the viscosity of the solution (Pa s), $D_{glucose}$ is the diffusion coefficient of glucose (6.9×10^{-10} m^2 s^{-1}). The velocity value used for u was estimated as follows. Each cuff contraction was assumed to displace fluid of volume equal to the volume of a cylinder with diameter 2r (the diameter of the membrane) and length L_{cuff}, the length of each rubber cuff. This was divided by the inflation time to calculate the volumetric flow rate, which was then divided with the cross sectional area of the membrane to obtain the velocity value.

3. RESULTS

3.1 Mass transfer in the SIM

Simulated glucose absorption from 1% glucose in aqueous, guar gum (0.1%), and CMC (0.1% and 0.5%) solutions with and without segmentation showed linear curves of the shape of figure 4 without any plateaus (no lag time or converge limit, data not shown). The relevant overall mass transfer coefficients were calculated from equation 3, and results are shown in Figure 5 as a function of zero-shear viscosities. Figure 5 demonstrates increased glucose

absorption with application of segmentation movements, which can be attributed to enhanced mass transfer to the membrane wall due to the squeezing motions of the cuffs. The effect was more profound for the aqueous solution, where application of segmentation resulted in 30% increase in mass transfer coefficient. More viscous solutions of 0.1% guar gum and 0.1% CMC solutions showed maximum 20% increase in $K_{overall}$ on application of squeezing movements. This is in good agreement with Tharakan et al. [2007; 2010], who reported reduced effect of squeezing on mass transfer as viscosity increased. Figure 5 also indicates maximum overall mass transfer coefficient for the lowest viscosity fluid on application of segmentation movements, suggesting that at low viscosities there is minimal resistance to mass transfer. Increasing chyme viscosity (above 2mPa s) resulted in reduction of mass transfer (by 15% and 90% as viscosity increased from 1 to 20 and 200Pa s, respectively). Interestingly, at 0.5% CMC (200mPa s zero viscosity), glucose transport to the recipient zone was practically inhibited without segmentation within the timescale of the experiments.

These results correlate well with estimated $K_{overall}$ from *in-vivo* data of human volunteers who consumed an oral glucose dose (250mL of 10% by weight glucose drink) with or without 3.6% wt/vol guar gum [Blackburn, Redfem, Jarjis, Holgate, Hanning, Scarpello, Johnson, & Read, 1984]. Although glucose and guar gum concentrations were different to those used in the present work, it is encouraging to notice that both the present and the *in-vivo* data resulted in $K_{overall}$ of the same order of magnitude (for aqueous solutions 5.35×10^{-7} and 5.47×10^{-7} m/s, respectively) and that addition of the hydrocolloid prompted reduction of $K_{overall}$ (from 5.47×10^{-7} to 2.91×10^{-7} m/s). The effect of guar gum in reducing postprandial glucose levels was attributed to the inhibiting action on fluid convection by the intestinal motility due to increased chyme viscosity.

Figure 6 shows the effect of segmentation frequency on mass transfer for guar gum and pectin solutions. For all investigated conditions, increasing the viscosity resulted in a decrease in mass transfer. Guar gum and pectin systems showed similar trends: an approximately threefold reduction in $K_{overall}$ was observed as zero shear viscosity increased from 0.02 Pa s to 1.2 Pa s in systems containing guar gum (0.25% and 0.63%, respectively) and from 0.05Pa s to 1.9Pa s in pectin systems (10% and 30%, respectively). For the same systems, the effect of segmentation frequency was found marginal and similar overall mass transfer coefficients were estimated for all investigated protocols. Further increase in guar gum concentration (to

0.75%) had an insignificant effect on mass transfer, in agreement with previous work reported by Tharakan *et al.* [2007; 2010].

Interestingly, increased frequency of segmentation contractions (i.e. faster squeezing of the membrane) is expected to result in increased mixing and therefore higher mass transfer coefficients. It may also further enhance mass transfer by decreasing the "unstirred water" layer adjacent to the gut wall, which further obstructs molecular diffusion and nutrient absorption [Doublier & Couvelier 2006]. Similar conclusions would be made according to the 'surface-renewal' theory [Cussler 2000]. However, frequency of contractions did not have a significant effect on the estimated $K_{overall}$ for both guar gum and pectin solutions in all investigated concentrations. It is possible that the time scale of the perturbations induced by the squeezing motions of the cuffs is smaller than the relaxation time of the system under investigation. Any changes in the squeezing frequency would then be expected to have marginal effect on mass transfer. This has been identified as a possible limitation of the SIM and it has been addressed in the next generation (DDuo).

Overall figures 5 and 6 demonstrate the potential of both food formulation and segmentation in controlling digestion processes. From those results one could conclude that the effect of formulation on food digestibility is complex and rheological variables other than viscosity may play an important role in determining nutrient bioaccessibility. In addition, food formulation is believed to further impact *in-vivo* segmentation patterns (e.g. liquid foods are said to stimulate deep contractions while highly viscous foods are generally associated with shallow muscle movements) [Jaime-Fonseca, 2011].

Figure 7 shows the Reynolds and Sherwood numbers, calculated from equations (5) and (6). As a general trend, convection becomes increasingly more important than diffusion (i.e. Sh number increases) as Re number increases above 100. This indicates that higher Re enhances convective mass transfer. Interestingly, a notable "step" towards convective processes appears in Re numbers in the region of 1000 (low viscosity fluids, of about 20mPa s) for the guar gum solutions. This could be the result of a change of the flow regime from laminar to transitional-turbulent, resulting in increased mixing and mass transfer. At Re numbers below 100, the flow becomes fully laminar and an increase of Re does not result in a significant increase of Sh (i.e. convection is not enhanced). The different segmentation patterns appeared to influence the relationship between Sh and Re only marginally.

3.2 Mass transfer in DDuo

Having established that both formulation and mixing conditions are significant in determining mass transfer and nutrient bioaccessibility in the gut, a new model was built with improved functionality and automation, as discussed in section 2.2.1. The new model aims at addressing the limitations observed in the SIM and offers flexibility in reproducing gut motility: there are 8 segmentation positions (i.e. squeezing of the porous membrane), each of which is only 1cm long (with respect to the 12cm long cuffs of SIM). The segmentation points can be controlled separately, so that each moves at the required time and rate.

Initial data obtained with the DDuo are shown in Figures 8-10. Figure 8 shows the effect of mixing conditions on glucose absorption from 1% glucose in aqueous and 1% guar gum solutions. Mixing was induced by squeezing at alternating positions at either 4 locations (gray/black arrows in Figure 3) or 1 location (positions 2 and 6 in Figure 3). The results are comparable to those obtained from the SIM model. When mixing was reduced to one segmenting point, a delay of 10min was observed for both water and guar gum solutions, before determining glucose in the recipient zone. These results indicate that the way intestinal motility is reproduced in the *in-vitro* models could affect the observed mass transfer coefficient. The results from DDuo indicate that increasing the number of segmentation points can result in a change of accessible glucose indicating an increase of mixing.

In Figure 9 the estimated overall mass transfer coefficients are shown for different segmentation points. Results indicate that at 1 segmentation point (i.e. lower mixing) mass transfer was reduced by 25% and 45% for aqueous and guar gum systems, respectively. In addition, the effect of the number of segmentation points was more profound at higher viscosity mixing (40% reduction of $K_{overall}$ for the 1% guar gum) when compared to low viscosity (only 15% reduction on water).

Figure 10 shows the effect of mixing frequency (at 4 segmentation points) on $K_{overall}$ from1% glucose in aqueous and 1% guar gum systems. Results indicate that under investigated conditions, increased segmentation frequency appears to enhance mass transfer. On all occasions, the lower viscosity fluid resulted in higher (up to 30%) mass transfer. However, at 12cpm it appears that the difference between the aqueous and viscous systems was marginal (<10%), indicating a nearly homogeneous mixing. Overall, Figures 8 - 10 demonstrate the flexibility of DDuo and its potential as a more adaptable tool to understand the effect of

intestinal motility on glucose bioaccessibility. Further work is required to obtain an understanding of the detailed effect of gut motility on mass transfer and food digestibility.

4. CONCLUSIONS

There is a growing interest in controlling the nutritional values of foods using hydrocolloids. A mechanism of slowing glucose bioaccessibility has been attributed to reduction in mass transfer through the gastrointestinal tract. This work presents *in-vitro* digestion studies using novel models with the ability to simulate intestinal motility, and illustrates the importance of mass transfer on simulated glucose absorption by using a range of food hydrocolloids. The models simulate flow and mixing in the gut. Addition of guar gum, CMC, and pectin showed reduction of glucose bioaccessibility by up to 30% compared with aqueous solutions *in-vitro*. Further work is required to understand if this reduction of mass transfer could result in/explain the significant delay of *in-vivo* post-prandial blood glucose observed by the addition of hydrocolloids. Overall, obtained results indicate that the effects of hydrocolloids on simulated digestibility are complex and for investigated hydrocolloid systems/conditions, increasing viscosity appeared to reduce mass transfer coefficients. This implies the potential of designing healthier foods by engineering the viscosity of the digested food.

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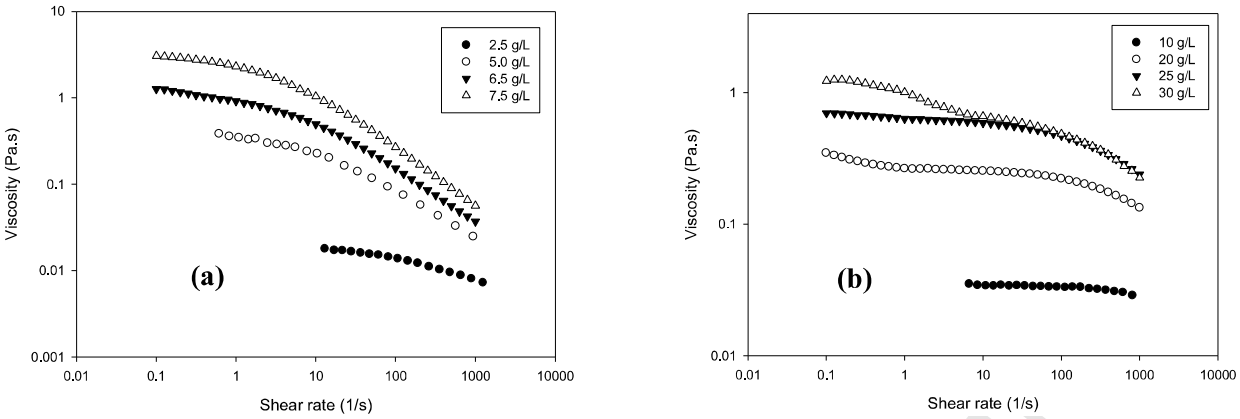

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590 Figure 1: Shear viscosity of solutions (with concentrations) used in the experiments: (a) guar
591 gum; (b) pectin.

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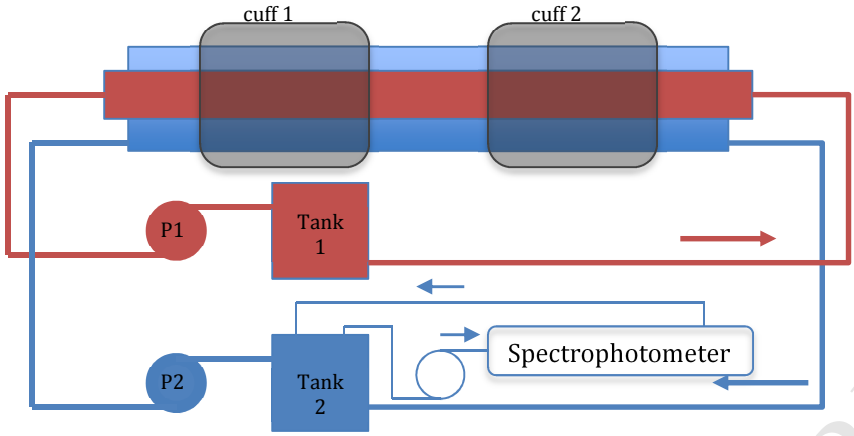


Figure 2: Schematic drawing of Small Intestinal Model (SIM). The investigated (red colour) and recipient (blue colour, initially water) fluids recirculate in the luminal and recipient sides of the model respectively, using peristaltic pumps P1 and P2. Segmentation is mimicked by squeezing the tubes radially, using two pneumatically controlled rubber cuffs (cuff 1 and cuff 2). The active compound passes through the porous inner membrane from the luminal to the recipient side, where it is quantified spectrophotometrically.

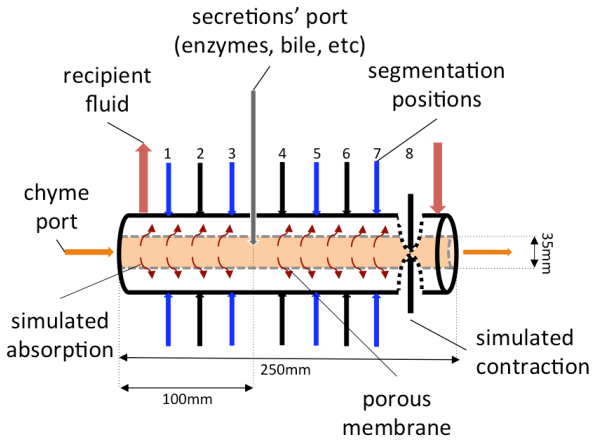
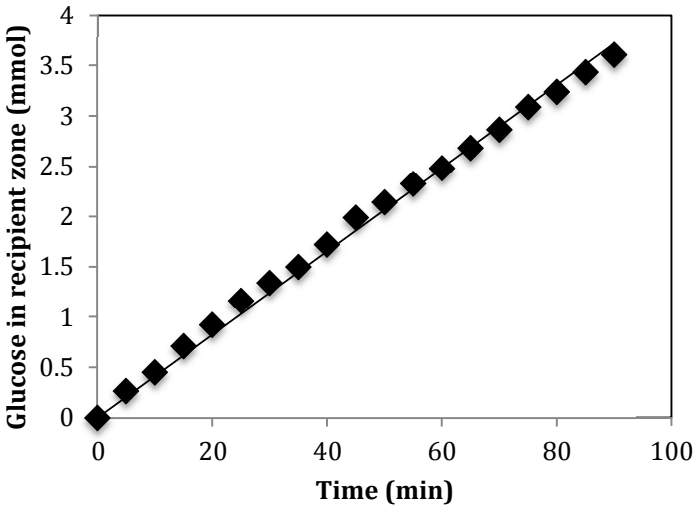


Figure 3: Schematic of Dynamic Duodenal Model (DDuo). The investigated fluid (orange coloured here for clarity) enters the luminal side of a porous membrane used to simulate intestinal wall. The recipient side is bordered by a non-permeable silicone tube. Enzymes and other secretions are injected through the secretions port, located at 100mm distance from the chyme entrance to represent physiological conditions. Segmentation and peristaltic movements are simulated by applying pressure at the membrane at 8 possible positions. Motion can be controlled independently.



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624 Figure 4: Typical plot of absorbed glucose in the recipient zone versus time (from 1% aqueous
625 glucose solution).
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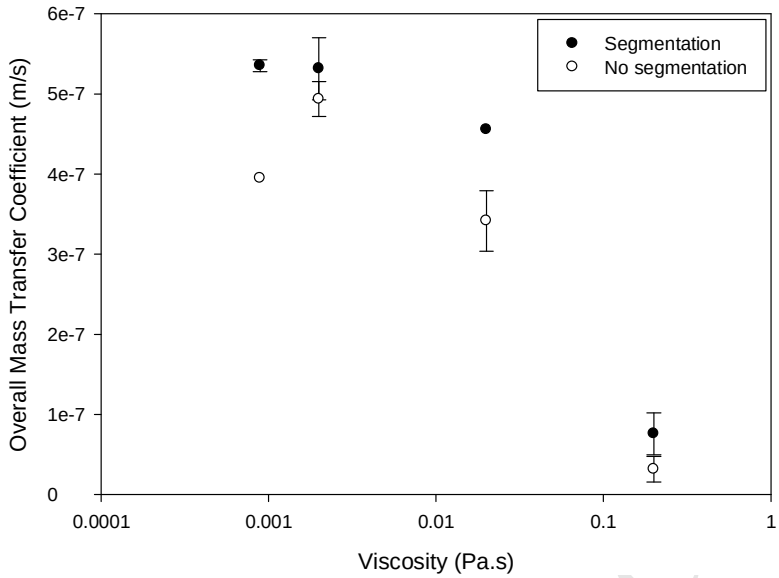
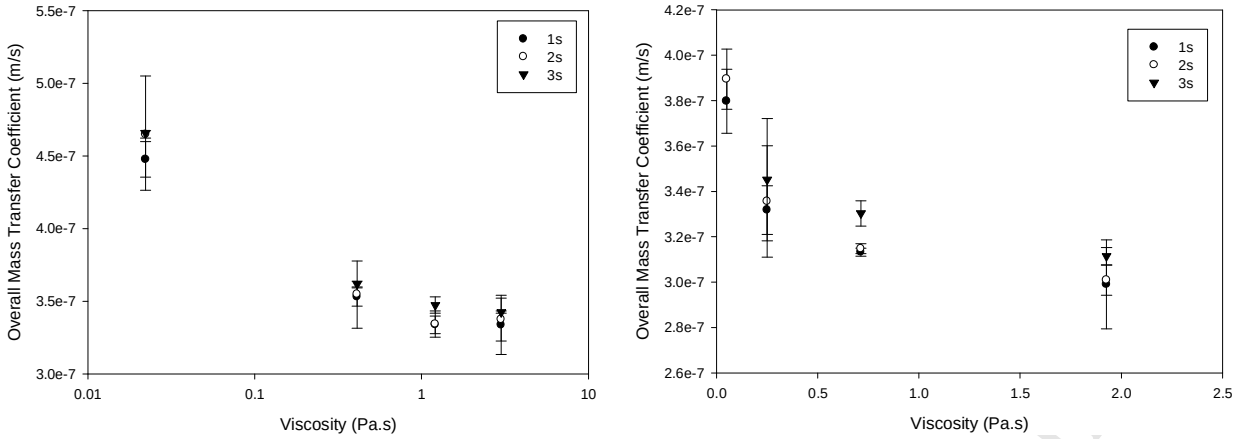


Figure 5: Overall Mass Transfer Coefficient with and without segmentation for systems of different zero-shear viscosities.



(a) (b)

631 Figure 6: Effect of segmentation frequency on overall mass transfer rate from 1% glucose in
632 (a) guar gum; (b) pectin solutions of different zero-shear viscosities.
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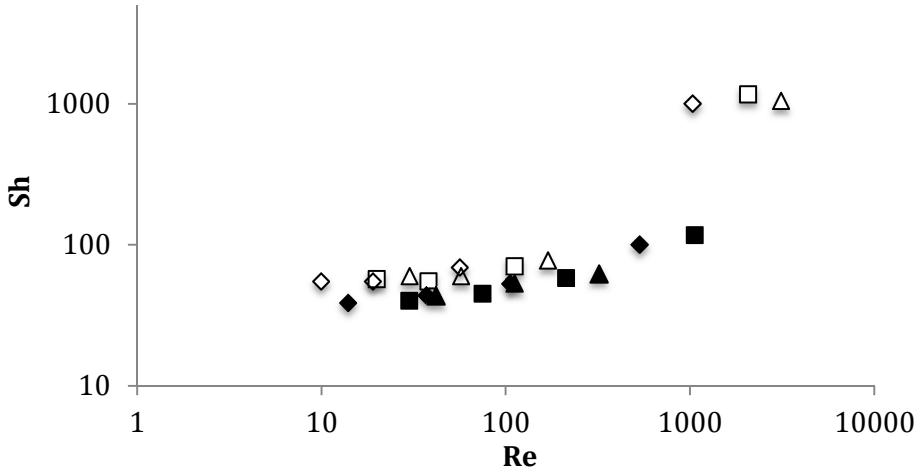

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638 Figure 7: Correlation between Sherwood (Sh) and Reynolds (Re) numbers for guar gum
639 (white symbols) and pectin (black symbols) solutions at high (1s, rhombus), medium (2s,
640 squares), and low (3s, triangles) mixing.

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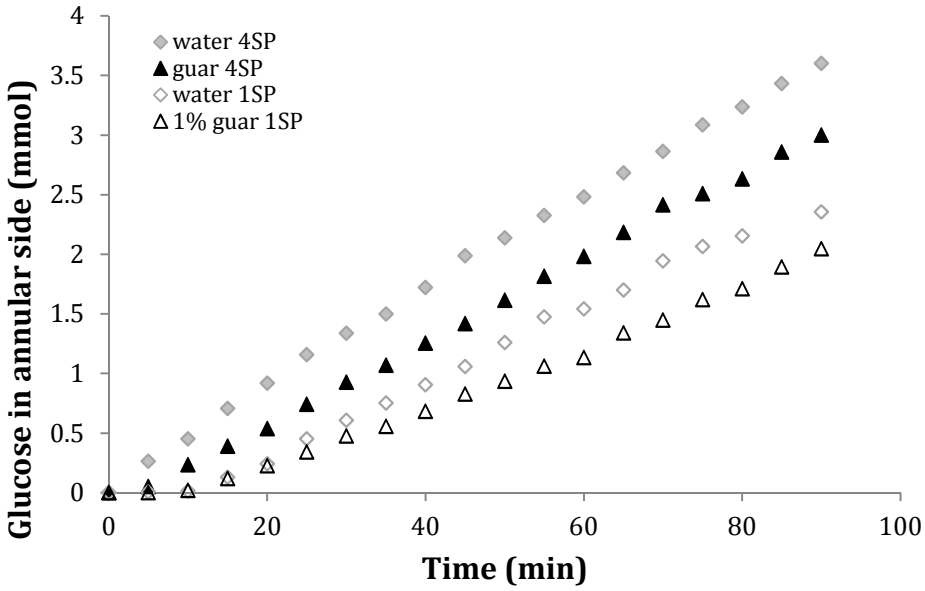

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645 Figure 8: Simulated glucose absorption at high (4 segmenting positions) and low (1
646 segmenting position) mixing for 1% glucose in aqueous or 1% guar gum solutions, using
647 Dynamic Duodenal model (DDuo).

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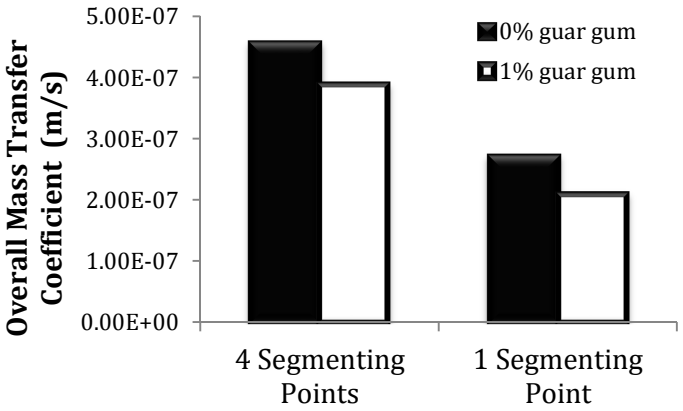

Figure 9: Overall mass transfer rates associated with the conditions of Figure 9 (initial lag time not considered in the calculations)

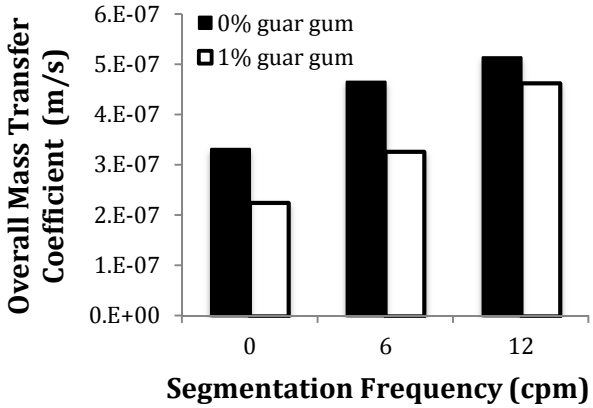

Figure 10: Overall mass transfer coefficient in Dynamic Duodenal model (DDuo) for 1% glucose in aqueous and 1% guar gum solutions at different segmentation frequencies (0, 6, and 12cpm)

Table 1: Hydrocolloid systems and zero-shear viscosities studied with and without segmentation movements in the Simulated Intestinal Model (SIM) and their respective viscosities.

System	η_0 *(mPa s)*
aqueous	1.0 ± 0.2
Guar gum 0.1%	2.0 ± 0.4
CMC 0.1%	20.0 ± 0.2
CMC 0.5%	200.0 ± 0.1

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682 Table 2: Hydrocolloid systems and zero-shear viscosities studied under different

683 segmentation patterns in the Simulated Intestinal Model (SIM) (as described in section 2.2.2).

System	*Concentration (g/L)*	η_0 *(Pa s)*
Guar gum	2.50	0.0222 ± 0.0018
	5.00	0.4108 ± 0.0296
	6.25	1.2090 ± 0.0961
	7.50	3.192 ± 0.1982
Pectin	10	0.0498 ± 0.0217
	20	0.2530 ± 0.0770
	25	0.7133 ± 0.0607
	30	1.9265 ± 0.1039

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Please find below 5 brief bullet points to convey the core findings of the work.

- Food formulation impacts mass transfer in simulated *in-vitro* model gut
- Flow regime affects mass transfer independently of formulation
- As flow becomes less laminar mass transfer increases in the model gut
- At increased mass transfer simulated glucose absorption is increased
- Preliminary data with improved *in-vitro* model agree with previous observations