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Release of glucose and maltodextrin DE 2 from gellan gum gels and the impacts of gel structure

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Abstract

Structural influences of hydrocolloids gels on the release of carbohydrates was examined with a focus on structure-function relationships. This understanding will guide formulation of food gels for targeted sugar release in populations such as diabetics and athletes. Hydrocolloid gels with well characterised structures, with a focus on high acyl (HA) and low acyl (LA) gellan gum, were formulated with glucose, maltose, DE 10 maltodextrin (MD) and DE 2 MD. Gel structure did not significantly affect glucose release, but mixed gel type had a significant effect on MD availability. A DE 2 MD required amylase to release more than 10% of the carbohydrates but still had 38% retained in a gel formulated with 30% MD. Formulation with any non-melting gelling hydrocolloid decreased the amount of released MD and phase separated networks released more than interpenetrating networks. Differential scanning calorimetry was used to compare helix formation of MD gels and the number of helices was inversely correlated with carbohydrate release. These results demonstrated a range of sugar release profiles achievable from formulation from specific gelling agent structures and carbohydrates.

Keywords: sugar release, controlled release, maltodextrin, carbohydrate release, carbohydrate digestion, High acyl gellan gum

1. Introduction

Hydrocolloids can control the release, digestion, or adsorption of various nutrients ranging from starches to vitamins by controlling their network structure and response to known digestive stimuli (Norton et al., 2014; McClements, 2021). Controlling carbohydrate digestion is crucial in formulation of foods when considering specific populations which require a sustained energy release or low glycaemic index such as diabetics and athletes (Gidley, 2013; Norton et al., 2014). Carbohydrates, a major source of energy for humans, come in many sizes ranging from monomers (glucose) and dimers (maltose) and up to 30-150 saccharide units (maltodextrin (MD)) and 100-1,800 saccharide units (starches). Smaller molecules are able to diffuse through viscous solutions or gel networks (Mills et al., 2011). Larger molecules need to be broken into smaller units which can then move into the chyme where they can be absorbed (Tharakan et al., 2010; Gidley, 2013; Fabek et al., 2014). To formulate products with controlled sugar release, the relationships between carbohydrate size and hydrocolloid gel structure must be understood.

For most hydrocolloid gels, small molecules such as mono and disaccharides, salts, and artificial sweeteners, are all smaller than the pores of the gel network and are able to diffuse through the gel. Thus, the network mostly acts to prevent mixing (a faster mass transfer) and only a small deviation from diffusion coefficients has been measured (Jönsson et al., 1986; Lorén et al., 2009a; Lorén et al., 2009b). In one study, a 14% - 30% decrease in diffusion coefficient of salt was measured for hydrocolloids between 1 and 4% mass (Mills et al., 2011). Larger molecules (3 vs 8 nm) showed a greater decrease in diffusion coefficients with increasing polymer concentration (Lorén et al., 2009b). Differences between gelling agent and concentration were not significant with an exception major structural changes such as melting (Mills et al., 2011). It is well known that surface area of a gel has a large influence on the rate of release, so that brittleness or a tendency to fracture causes a quicker release (Morris, 1994; Mills et al., 2011). Texture, breakdown, and serum release of the gel

during mastication thus plays a major role in differentiation between hydrocolloids gels (Khin et al., 2021).

Release of larger carbohydrates (those not able to diffuse through the pores) is more complex. The digestive enzyme α-amylase cleaves maltose units from starch (Butterworth et al., 2011; Dhital et al., 2017) which is then small enough to diffuse out of the gel. Small amounts of glucose, maltotriose, and dextrin are also created (Butterworth et al., 2011; Dhital et al., 2017). An increased viscosity or a gel network impedes the mass transfer of the enzyme and slows the rate of digestion (Tharakan et al., 2010; Gidley, 2013; Fabek et al., 2014). Gel surface area, packing density, and subsequent entrapment impact the ability of α-amylase to reach the carbohydrate and thus the rate of digestion (Wee and Henry, 2020; McClements, 2021). In addition to the physical inhibition, chemical binding can also occur. For example, cellulose was found to inhibit α-amylase activity by binding with the enzyme (Dhital et al., 2017). Studies examining the effects of gelling hydrocolloids on the digestion of starch have typically found a decreased rate of digestion and total digestion (Butler et al., 2008; Koh et al., 2009; Sasaki and Kohyama, 2011; Ramírez et al., 2015; Zhang et al., 2018; Srikaeo and Paphonyanyong, 2020). Gelatinization, retrogradation, and steric hindrance of starch are all expected to play a role making differentiation of the separate mechanisms impossible (Zhang et al., 2018). Comparisons to MD may give insight into the contribution of network effects on retrogradation because gelatinization does not occur.

An understanding of the effects of gel structure on carbohydrate release, as a function of molecular weight (MW), is important for the formulation of products with controlled carbohydrate release. Most work has focused on either high MW carbohydrates (specifically starches) (Koh et al., 2009; Sasaki and Kohyama, 2011; Ramírez et al., 2015; Zhang et al., 2018; Srikaeo and Paphonyanyong, 2020) or very low MW, such as glucose, maltose, and sucrose (Morris, 1994; Wang et al., 2014; Yang et al., 2015; Nishinari and Fang, 2016; Khin et al., 2021), but left out intermediate MW carbohydrates like MD. The mechanism of release for the digestion of MDs has not yet been

73 determined and was thought to be slowed by incorporation of hydrocolloids. Gellan gum has been
74 shown to form an interpenetrating network with MD (Clark et al., 1999; Kanyuck et al., 2021a) and
75 high acyl (HA) gellan gum variant was capable of forming a wide variety of material properties
76 (Kanyuck et al., 2021a). Therefore, this gelling agent has considerable potential for use in
77 customizable carbohydrate release systems.

78 The objective of the present investigation was to examine the role of gelling agents on carbohydrate
79 release by comparing carbohydrates of different MW trapped within hydrocolloid gels with well-
80 characterized properties. It was hypothesized that the MW of the carbohydrate, the gel network
81 structure, and the response to environmental conditions (a stimuli-response) can predict release
82 behaviour from hydrocolloids gels. MW of a carbohydrate is known to determine the path of release
83 and digestion from gels. Although there are certainly other structural factors such as branching and
84 linkages in carbohydrates, molar mass is just as important and is sometimes overlooked (Nishinari
85 and Fang, 2021). Some gel networks display a response to stimuli such as melting, dissolution, or
86 swelling which typically have large impacts on release (McClements, 2021). After determining the
87 pathway for MD release, a structural comparison will examine the influence of mixed gel network
88 type and MD helix formation. Exploring these fundamental relationships between carbohydrate MW
89 and gelling agent structure will facilitate strategic formulation of products to achieve desired release
90 profiles.

91 **2. Materials and Methods**

92 **2.1 Materials.**

93 Both MDs were derived from potato and acquired from Avebe (Veendam, Netherlands) with a
94 dextrose equivalent (DE) of 2 (Paselli SA 2, batch H3362903) and 10 (Paselli MD 10, batch
95 H4852902). The HA (LT100) and LA (F) gellan gum were acquired from CP Kelco (Atlanta, USA). The
96 following hydrocolloids were purchased from Sigma Aldrich (St. Louis, USA): Gelatin type A with a
97 bloom strength of 300, kappa-carrageenan, iota-carrageenan, agarose type A9539, and sodium

alginate. Maltose, KCl, and $CaCl_2$ were also purchased from Sigma Aldrich. The α-amylase was from Aspergillus oryzae (10065 Sigma-Aldrich) with an activity of 32 U/mg. Glucose was purchased from Fisher Chemical (Loughborough, UK).

Table 1. A summary of the hydrocolloids used in formulation of the carbohydrate gels.

Gelling agent	**Source**	**Conc.**	**Gel preparation**
Agarose	Type A9539, Sigma Aldrich	2%	Powder was dispersed and heated in water at 90 °C for 30 minutes
Alginate	Sodium type, Sigma Aldrich	2%	Powder was dispersed and heated in water at 90 °C for 30 minutes and then gelled by diffusion method in a 91 mM $CaCl_2$ solution
Gelatin	Type A, Sigma Aldrich	2%	Powder was dispersed and heated in water at 50 °C for 30 minutes
High acyl gellan gum	CP Kelco LT100	0.25-3%	Powder was dispersed and heated in water at 90 °C for 2 hours
iota-carrageenan	Sigma Aldrich	2%	Powder was dispersed and heated in water at 90 °C for 30 minutes and then 2.68 mM KCl was added and immediately poured into moulds
kappa-carrageenan	Sigma Aldrich	2%	Powder was dispersed and heated in water at 90 °C for 30 minutes and then 2.68 mM KCl was added and immediately poured into moulds
Low acyl gellan gum	CP Kelco F	0.25-3%	Powder was dispersed and heated in water at 90 °C for 2 hours

2.2. Gel preparation

All gels were prepared by dispersing the hydrocolloids in heated deionized (DI) water with stirring to fully hydrate the polymers individually in stock solutions. MD and the glucose or maltose solutions were heated at 90 °C for 4 hours. Gellan gums were heated at 90 °C for 2 hours, the carrageenan, agarose, and alginate heated at 90 °C for 30 minutes, and gelatin heated at 50 °C for 30 minutes. After the individual hydrocolloids were hydrated, a hot solution (90 °C) of a stock concentration of the carbohydrate (glucose, maltose, MD DE2, or MD DE10 as indicated) was mixed with the hydrocolloids for 5 minutes to combine. These mixtures were poured into 20mm diameter cylindrical plastic moulds and set at room temperature for at least 48 hours before analysis. Samples with MD were analysed after 4 days to give sufficient time for helix aggregation (Kanyuck et al., 2019). Kappa-carrageenan and iota-carrageenan contained an added 2.68 mM KCl to reach gelation.

Sodium alginate was gelled by the diffusion method (Draget, 2009) with 91 mM $CaCl_2$ and formulated at a higher glucose concentration (40%) to account for loss during the gelation preparation.

2.3. Release measurements

The method for measuring release of carbohydrates from gels followed the procedure by Koh et al. (2009) with some modification. Gels were cut into 4 pieces of ~1 cm^3 each (5 g ± 1 g). To prevent amylase from interfering with the refractive index measurement, gel pieces were placed within a dialysis tubing membrane of molecular weight cut-off 14 kDa (MEMBRA-CEL MD44-14). Amylase isolated from Aspergillus oryzae has been found to have molecular weights of 51 kDa (sedimentation and diffusion) and 49 kDa (gel filtration) and thus is too large to cross the membrane. Membrane clips were used to seal the gel sample and 5mL of amylase solution (or water when amylase was not used) within the dialysis tubing. The sample pouch was added to a volume of 150 mL of DI water pre-warmed to 37 °C inside plastic bottles with lids. The apparatus was held in a shaker (Sciquip, Newtown, UK) at 37 °C with rotation of 200 RPM for the duration of the experiment. A schematic of the experimental setup is shown in Figure 1. At each time point, 0.5 mL from the bulk was removed for measurement by refractive index (Rudolph research J357 automatic refractometer from Hackettstown, USA) and was returned to the bulk phase. Refractive index is a measure of the relative speed of light in a solution and is linearly related to sugar concentration. Calibration curves for glucose and maltose were used to calculate the sugar concentration in each sample by the refractive index measurement.

Measurements were normalized to the 'percent of total carbohydrates released' by dividing by the amount of carbohydrate known from the sample mass. A 'total release' value was measured after 48 hours. Initial experiments showed the sugar concentration did not increase after the 48 hour time point. A 90 minute time point is compared between samples as an indication of the relative rate of digestion. This time point has also been shown to have the highest correlation with glycemic index (r=0.909) (Goñi et al., 1997).

Samples containing MD utilized a triggered release by addition of α-amylase to mimic human digestion. The enzyme α-amylase cleaves linear carbohydrate chains into maltose units (Butterworth et al., 2011). A stock solution of 100 U/mL amylase was prepared by dispersing the powder in DI water at room temperature for 30 minutes. 5 mL of the amylase solution was added to the dialysis tubing to reach an activity of 500U. The dialysis tubing was then sealed with a clip and placed into the bulk water phase at 37 °C within 5 minutes. Amylase concentrations in human saliva have wide variability based on time of day, most recent meal, and also the individual. The value of 500 U was chosen because it is within the range of human salivary enzyme activity (Mandel et al., 2010) and similar to the concentration used by Koh et al. (2009) and (Janssen et al., 2009).

It should be acknowledged that *in vitro* tests such as this can only approximate differences between samples. Amylase sourced from porcine or *Aspergillus oryzae* have shown minor differences to human amylase, but their use allows for consistent comparison between experiments. Some deviations should be expected, so for true glycemic index human tests should be used. However, true human experiments also have natural variability in oral processing, enzyme concentrations, hormones, and residence times in the stomach and intestine (Dhital et al., 2017). For comparison between different samples, the use of any of the amylases has shown to be effective.

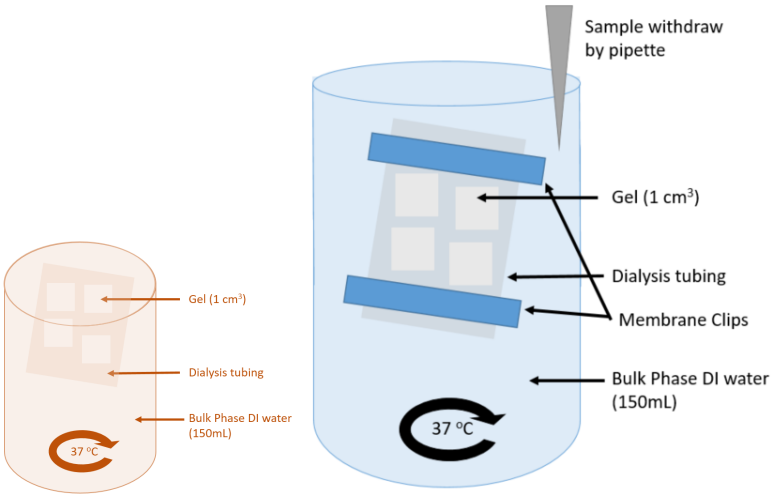


Figure 1. Diagram of release experimental setup showing the gels within dialysis tubing and inside of a larger bulk phase (150mL) which was shaken at 200 rpm at 37 °C. Method was adapted from Koh et al. (2009).

2.4. Modelling of glucose release

Glucose concentrations measured after 24 hours had reached the predicted value (with ± 5% error) and were thus normalized to account for natural sample variability with the equation:

$$\text{Released Glucose} = M_t/M_\infty \quad \text{(Eq 1)}$$

Where M_t is the measured concentration at time 't' and M_∞ the final maximum concentration. The only sample with greater than 5% error was sodium alginate which was expected to lose some glucose during the gelation methodology (diffusion of calcium ions into the alginate solution along with diffusion of glucose out). The collected data was then fitted to a power law model and the Peppas-Sahlin Model. As the models are not able to account for the lowering difference in concentration gradient over time, data was only fitted below 60 % of the release (the model assumes steady-state). These were then compared to a COMSOL fit of Fickian diffusion within the gel which was able to account for changing concentrations.

Power Law Model: A simple exponential model was fit using Microsoft Excel:

$$M_t/M_\infty = k*t^n \quad \text{(Eq 2)}$$

Where 'k' is the rate constant specific to the gel formula and 'n' the diffusional exponent (Siepmann and Peppas, 2011).

Peppas-Sahlin Model: Release curves were also fit to the model proposed by Peppas and Sahlin (1989). An equation with the following form was fit to the release profiles:

$$M_t/M_\infty = k_1*t^{0.45} + k_2*t^{0.9} \quad \text{(Eq 3)}$$

Where k is the rate constant where $k_1*t^{0.45}$ represents the Fickian diffusion and $k_2*t^{0.9}$ the case II transport contributions for a cylindrical shape (Peppas and Sahlin, 1989; Siepmann and Peppas, 2011). Fickian diffusional describes the release of an active caused by a concentration gradient while the case II transport mechanism is dictated by a transition of the polymer which changes the release

rate of the active (Peppas and Sahlin, 1989; Siepmann and Peppas, 2011). Similar to the single exponential model, the model is only valid for the initial 60% of glucose release to avoid the effects of lowering differences in concentration gradients. The biexponential regression was fit using SigmaPlot (Version 12.5 SYSTAT Software, USA). Proportional contributions were calculated using the equations proposed by Peppas and Sahlin (1989). In summary, the percent contribution was calculated by the ratio of each coefficient for each time point.

COMSOL Model: The engineering software COMSOL (COMSOL Inc. Burlington, MA, USA), was used to predict diffusion of glucose using the experimental dimensions and concentration gradients. The flux of glucose from within the gel (into the water) was calculated by Ficks' law of diffusion using the dimensions of the objects (shown in Figure 1) and an initial concentration of 2.38 M (2381 moles/m^3) in the gel and 0 in the water. Gels were surrounded by a water region of 150 mL with a diffusivity of (1 m^3/s) meaning practically that mixing was instantaneous. A thin mesh was drawn around the gel to ensure release only occurred at the surface of the gel and diffusion was modelled to the edges of the gel. The model was fit for a single cube of gel (1 mL) with the measured values adjusted by a factor of 0.25 for simplicity. A diffusion coefficient of glucose in water was 6.0 x 10^{-10} m^2/sec was obtained from literature (Stein and Litman, 2014). The model accounted for changes in flux with the changing concentration gradients (which the other models do not).

2.5. Swelling

Swelling of gellan gum gels was measured by increases in mass after soaking in aqueous solutions. Gels were cut into ~20 mm height pieces from the cylindrical moulds and the mass weighed to 7.5 ± 1 g. The gel was then placed into 150 mL of DI water at room temperature. At each time point, the gel was removed using a strainer, patted dry to remove surface water, and weighed. The amount of swelling was determined from the ratio of initial mass to final mass by the equation:

$$\text{Swelling Ratio} = M/M_0 \qquad \text{(Eq 4)}$$

where M is the measured sample mass after swelling and M_0 is the initial mass.

2.6. DSC

Gelation of maltodextrin was studied by measuring the enthalpy and entropy using a µDSC3 evo (Setaram Instrumentation, France). Samples were added in the sol phase (hot) to the sample vessels and held for 4 days at room temperature prior to analysis to allow sufficient gelation of the MD component (Kanyuck et al., 2019). A heating and cooling cycle began with a hold at 5 °C for 10 minutes and then increased at 1°C/min up to 95 °C. After a 10 minute hold at 95°C, the temperature was cooled at 1°C/min down to 5 °C.

2.7. Statistical Analysis

All samples were measured in at least triplicate and data are presented as means ± standard deviation. Release curves were repeated four times for each sample. Error bars show one standard deviation above and below the mean value. On the bar charts, different letters suggest significantly different mean values. A t-test with a p-value of 0.05 was used to determine which samples were significantly different.

3. Results and Discussion

Of the many factors to consider in predicting the release, the carbohydrate MW is of crucial importance (Nishinari and Fang, 2021). To examine this effect, carbohydrates of varying molecular weight (MW) were compared by release profiles from HA gellan gel (Figure 2A) and LA gellan gum (Figure 2C). Small molecules were represented by glucose (180 Da) and maltose (342 Da) and showed complete release from the gel within 48 hours. The rate of release was slower for maltose because it is a larger molecule than glucose. Larger molecules are expected to have slower diffusion coefficients due to the greater hydrodynamic radius (Nishinari and Fang, 2021). MDs are known to contain a wide range of different molecular sizes with a bimodal distribution and the distribution of the DE 2 MD is centred at 10,000 Da and 492,000 Da (Loret et al., 2004). Both MDs used (DE 2 and

DE 10) led to a slower and incomplete release of carbohydrates from the gels. Molecules smaller than the pores are able to diffuse out of the gel network while larger molecules are trapped (Lin and Metters, 2006; McClements, 2017). The small amount of carbohydrates measured without addition of an enzyme (10% and 17% for DE 2 and 10, respectively) reflect the proportion of molecules which were small enough to diffuse out of the gellan gum gel network. Addition of amylase, the enzyme which cleaves maltose units from a larger carbohydrate chain, considerably increased the amount (to 44% and 63% for DE 2 and 10, respectively). Based on the work of (Dhital et al., 2017), amylase was thought to enter the gel network and break the MD into maltose molecules which were then small enough to diffuse out of the gel. Starch, a much larger carbohydrate, is well known to need this enzyme to break into saccharides that can be released from a gel (Koh et al., 2009; Butterworth et al., 2011; Dhital et al., 2017).

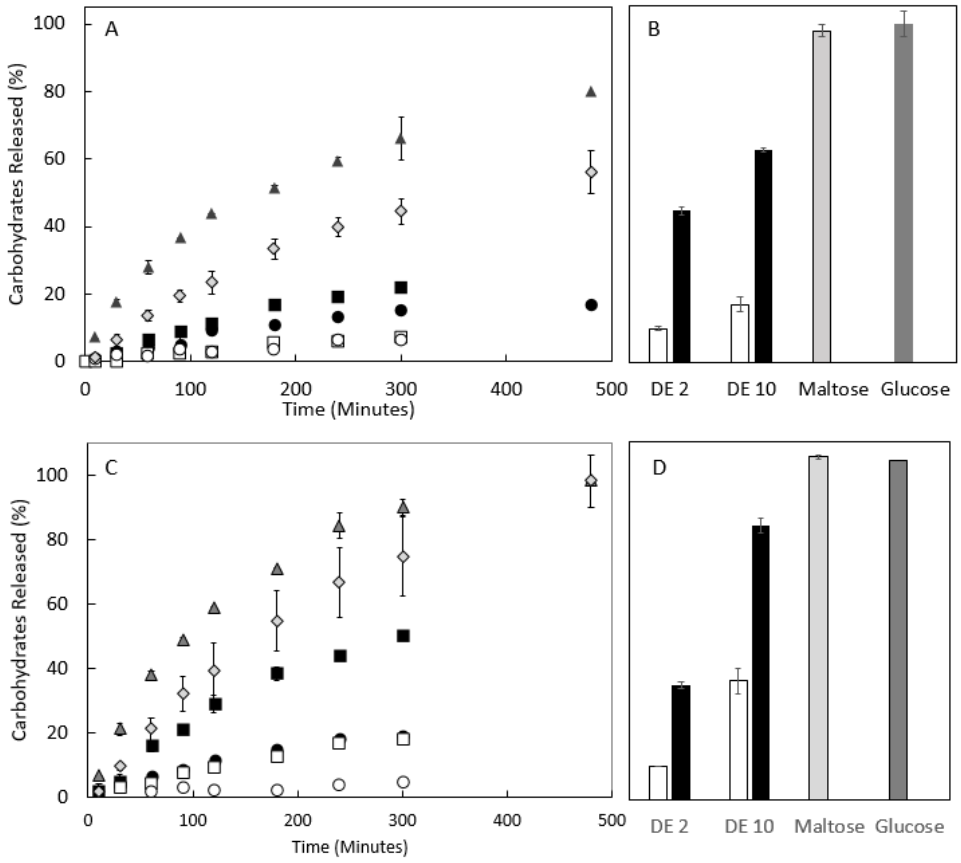


Figure 2. Release of carbohydrates from 1% HA gellan (A) and 2% LA gellan (C) gels formulated with 30% glucose (▲), maltose (◊), and with MD DE 2 (●) and DE 10 (■) with amylase (black) and without (white). Total release from the gels at 48 hours shown for 1% HA gellan (B) and 2% LA gellan (D).

Two clear pathways of release were established based on the size of the carbohydrate; diffusion based release of the small molecules, and an amylase-triggered release for large molecules that cannot diffuse out of the gel network. The following work will be split into subsequent sections to examine effects of gel network type on the release of small (3.1) and medium sized aggregated (3.3) carbohydrates. Glucose was selected to be representative of small carbohydrates and DE 2 MD chosen for the enzyme-triggered release.

3.1. Diffusion based release of small MW carbohydrates

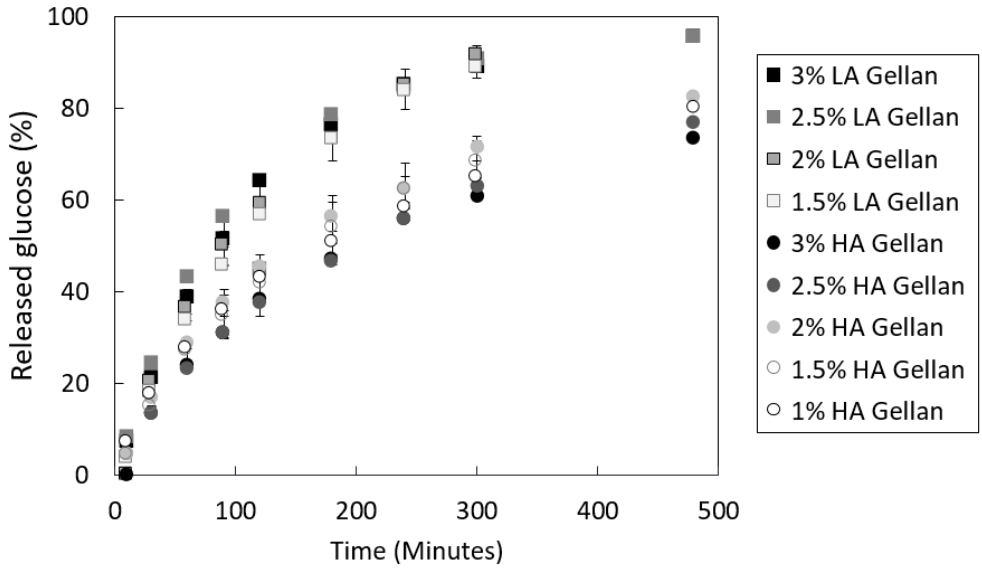
Glucose was chosen as a model for small molecule carbohydrates and the release from different gel structures (polymer types and concentration) were compared. All samples reached 100 ± 5% after 24 hours and are shown normalized in the graph to decrease the impact of variability in the gel formulation. Changes in gellan concentration led to significant differences in the release speed ($p < 0.05$), however the differences of a few percentage points had minimal practical differences (Figure 3). Increases in concentration of polymer are known to decrease the release rates of small molecules, but this is typically quite a small shift (10-20%). This trend was observed for sucrose from agar gels (Wang et al., 2014; Yang et al., 2015), salt from LA gellan and gelatin (Mills et al., 2011), and dendrimers of 3 and 8 nm from kappa-carrageenan (Lorén et al., 2009b). Higher polymer concentrations are expected to decrease the pore size within gels and provides a greater physical barrier. For glucose this is minimally important because the pores are already much larger and the hydrocolloid such a small proportion of the mass (Mills et al., 2011). Larger actives (3 and 8 nm) showed progressively a greater slowing from a kappa-carrageenan gel network (Lorén et al., 2009b).

A comparison of LA and HA however shows a difference between these two polymer types, irrespective of the concentration (Figure 3). Both HA and LA gellan gum form physical gels by double helix formation upon cold-setting and do not melt at 37 °C or below (Morris et al., 2012). Removal of acyl groups for the LA gellan yields a completely different gel texture than HA gellan due to differences in helix aggregation which may have been a factor. The surface area for each gel was

matched in these experiments (controlled in the sample preparation). After 24 hours, all of the glucose (within a reasonable standard deviation of +/- 5%) had been released which suggested there was no significant binding between HA gellan and glucose to cause the lower diffusion rates and therefore the difference appeared to be kinetic in nature.

Mathematical modelling of release profiles has become a popular method for understanding the mechanisms of release from gels (Lin and Metters, 2006). Comparison of models for HA and LA gellan gum release were used to elucidate the origin or mechanism of the difference. Quality of fit for the models is shown in Figure 4 and the equations displayed in Table 2. The commonly used Peppas-Sahlin equation (Eq 3) models the release of an active as a summation of the Fickian diffusion ($k_1*t^{0.45}$) and case II release ($k_2*t^{0.9}$). As the model cannot account for changes in concentration, analysis should only be conducted on the initial 60% of the release profile. This was reflected in the curves of Figure 4 which end at the 60% release point.

Comparing the importance of each coefficient (k_1 for the Fickian contribution and k_2 for relaxational case II contribution) was used to give evidence of the type of release (Siepmann and Peppas, 2011). According to this model, the Fickian or case II contribution can be modelled over time to show any changes in type of release. Relative contributions of each type, and how that shifts over the release profile, are shown in (Supplemental Figure 1). For LA gellan gum, the release was suggested to be largely case II driven (Supplemental Figure 1) which could also be predicted from the diffusional exponent (n) value of the single power exponent of 0.92 which is near to that of 'pure relaxation' of a 0.9 value. Alternatively, the single n for HA gellan (0.76) was between that of Fickian and case II and was reflected in the relative greater Fickian contribution (Supplemental Figure 1). The Peppas-Sahlin model suggested the HA gellan release profile was more similar to a Fickian release while the LA gellan was predominately dictated by case II release. A greater similarity of HA gellan gum to Fickian diffusion may suggest that the LA gellan gum network relaxes to increase the release rate but was not sufficient to fully explain the difference between HA and LA gellan gum.



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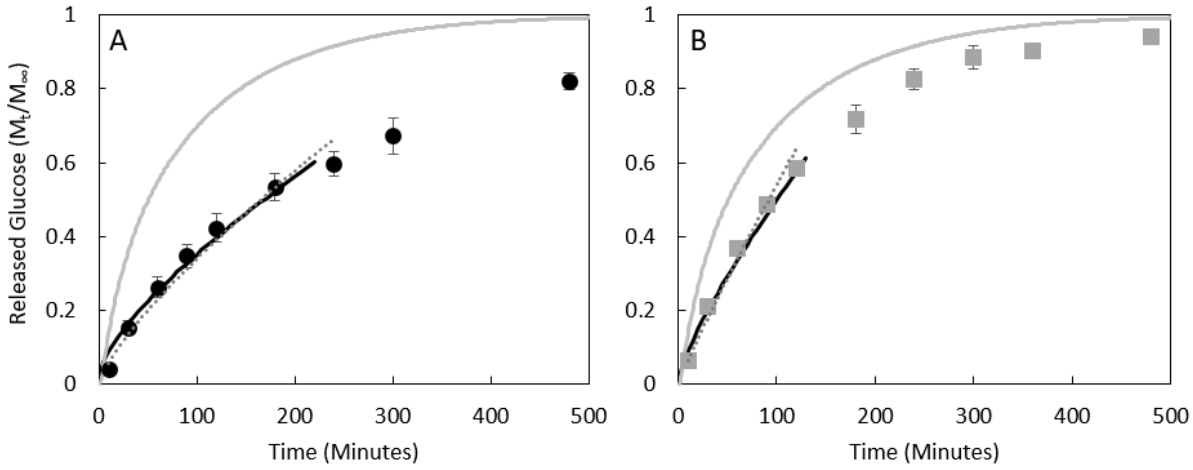
296 **Figure 3**. Concentration dependence of release profiles from LA gellan (squares) and HA gellan
297 (circles) formulated with 30% glucose.



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299 **Figure 4**. Modelling glucose release for 2% HA gellan (A) and LA gellan (B) comparing the Peppas-
300 Sahlin Model (solid black line), single exponential (dashed grey line), and COMSOL mass transfer
301 model (grey line). Equations are shown in Table 2.

302 **Table 2**. Equations modelled to the release of glucose from high acyl (HA) and low acyl (LA) gellan
303 gum.

Model	HA Gellan	LA Gellan
Single Exponential	$M_t/M_\infty = 0.010*t^{0.76}$ $R^2 = 0.98$	$M_t/M_\infty = 0.008*t^{0.92}$ $R^2 = 0.99$
Peppas-Sahlin	$M_t/M_\infty = 0.028*t^{0.45} + 0.002*t^{0.9}$	$M_t/M_\infty = 0.017*t^{0.45} + 0.007*t^{0.9}$

304

Using a chemical engineering modelling software (COMSOL) and considering dimensions, initial concentrations, and changes during release a curve from 'pure diffusion' through the gel can be predicted for this specific system. The software was able to account for non-steady state behaviour and used the literature diffusion coefficient (D) of 6.0×10^{10} m^2/s (Stein and Litman, 2014). Higher similarity was observed between the expected pure diffusion and LA gellan, while the release from HA gellan was clearly slower. A marginal slower release from a hydrogel was expected due to the steric obstacle of the network by 14-30% (Mills et al., 2011). A shift farther from pure diffusion for the HA gellan gel suggested a stimuli-driven change to the gel was responsible for the slower release behaviour.

With evidence from modelling that LA gellan was closer to a 'typical' diffusion pattern, glucose release from other gelling agents were compared to give context to the different gel network structures. Release profiles from gelatin, alginate, and kappa-carrageenan are compared to that of HA and LA gellan gum in Figure 5. Release from alginate and kappa-carrageenan were similar to LA gellan gum. An alginate gel network is held together by chemical crosslinks (calcium bridges) between chains (Draget, 2009) while kappa-carrageenan forms a gel network through potassium induced aggregation of double helices (Morris et al., 1980). These three different gel structures did not appear to affect the release of glucose. At the measurement temperature (37 °C) the gelatin network melted and caused a quicker release profile than any of the other gelling agents. The other gelling agents did not melt. In comparison, the behaviour of HA gellan was unprecedently slower than any of the other gelling agents. Recent work has shown that swelling of HA gellan was responsible for the slower release of glucose compared to LA gellan (Kanyuck et al., 2021b). This stimuli-driven swelling was proposed to be the cause of the slower release from HA gellan gum and will be discussed in the following section (3.2). For diffusion-based release, network structure of the hydrocolloid was not important and differences were only observed from stimuli-driven changes to the gel, specifically melting and swelling.

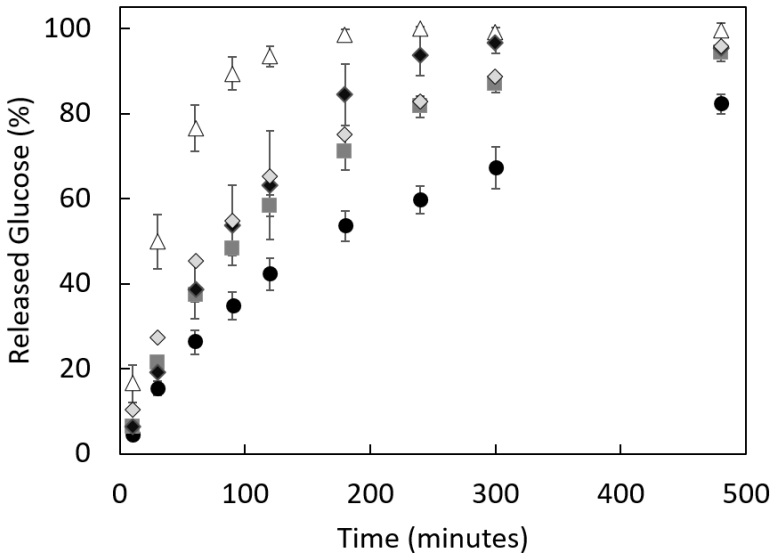

Figure 5. Release of glucose from 2% HA gellan gum (●), LA gellan gum (■), alginate (◆), kappa-carrageenan (◆), and gelatin (△) at 37 °C.

3.2. Impacts of stimuli-driven structural changes of gels on release

Melting: Stimuli from the environment which cause structural changes to a hydrocolloid gel, such as swelling, dissolution, and erosion can modify the release profile (McClements, 2017). The quicker release of glucose from gelatin was hypothesized to have been caused by the melting at the analysis temperature of 37 °C. The experimental procedure was repeated at 25 °C which is below the melting temperature of gelatin. When gelatin did not melt, the release was similar to LA gellan gum (Supplemental Figure 2). Thus the inherent structure of gelatin did not distinguish from the other gels but instead the temperature-driven structural change. Previous work has also confirmed that environmental temperatures which cause melting of a gelatin gel showed much faster release of salt than release at a temperature that did not cause melting (Mills et al., 2011). Melting of hydrocolloids gels was a stimuli-driven structural change that impacts release from gelatin.

Swelling: In many cases, swelling of a polymer increases the release rate of a small molecule due to the increased pore size of the hydrocolloid (McClements, 2017). In the case of HA gellan, swelling actually slowed the release of glucose (Kanyuck et al., 2021b) and has the potential to impact larger carbohydrates. Swelling kinetics of HA gellan is shown in Figure 6 for formulations with glucose,

maltose, DE 10 MD, and DE 2 MD. There was significantly less swelling with inclusion of glucose or DE 10 MD, but the mass had still doubled after 180 minutes. The aggregates formed by the DE 10 MD decreased the swelling more than glucose or maltose (Figure 6). Very clearly the network formed by DE 2 MD inhibited the swelling of the mixed gel. This MD (DE 2) is known to form large and bulky aggregates within the HA gellan gum network (Kanyuck et al., 2021a). Not surprisingly, these appeared to have prevented much of the typical swelling for HA gellan. Slower release of glucose, maltose, and DE 10 MD was subsequently suspected for HA gellan due to a decreased mass transfer caused by swelling (Kanyuck et al., 2021b). Swelling of a gel causes a greater volume and larger dimensions, and subsequently the slower release was thought to have been caused by a lower effective concentration inside the gel and a greater distance for the active to travel (Kanyuck et al., 2021b). This effect was observed by comparing HA gellan and LA gellan gum release (Figure 2). A slower release from these carbohydrate sources (glucose, maltose, and DE 10 MD) was measured for HA gellan and emphasised the importance of this stimuli-response. Just as the environmental temperature dictated melting of gelatin, the osmotic environment dictated HA gellan swelling (Kanyuck et al., 2021b). These stimuli-responsive changes were shown to be crucial for predicting release profiles and specific conditions were of critical importance.

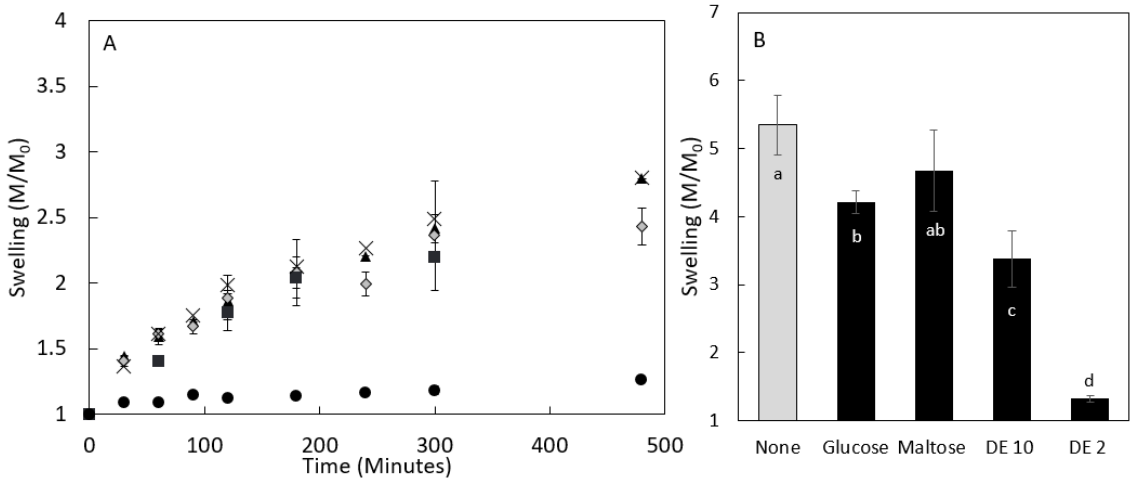

Figure 6. Swelling of 1% HA gellan during the timeframe of release experiments (X) compared to formulations with 30% glucose (▲), maltose (◊), and with MD DE 2 (●) and DE 10 (■). Part B displays the swelling after 48 hours.

3.3. Amylase-triggered release of MD

Addition of the digestive enzyme amylase was essential for the release of MD from gellan gum gels. Only 10% of the DE 2 MD chains were small enough to diffuse out of the 1% HA gellan gum gel, while addition of the hydrolysing enzyme allowed 44% of the carbohydrates to be release from the gel (Figure 2). Similarly for 1% LA gellan gum, 40% was released with amylase but only 10% without. The ability of amylase to enter the gel network and reach the MD to begin cleavage was of chief importance (Dhital et al., 2017). However, even with addition of amylase more than half of the carbohydrate was resistant in the experiment. Aggregates of MD were hypothesized to be the source of enzyme resistance and will be explored. Impacts of hydrocolloid gel structure on the availability of these MD aggregates will then be explored with amylase-triggered release.

3.3.1. MD aggregation

Self-aggregation of MD was hypothesized to play a role in the carbohydrate availability. This MD (DE 2) is well characterized in literature and known to form aggregates of double helix that form a gel at high enough concentrations (15-20%) by connection of these dense aggregates (Kasapis et al., 1993a; Loret et al., 2004; Kanyuck et al., 2019). Holding temperature during gelation is known to affect the size and enthalpy of the aggregates formed (Kanyuck et al., 2019). Exploiting that knowledge, the impacts of MD aggregation on availability for amylase cleavage were determined by varying the gelation temperature. Higher temperatures formed fewer aggregates but at a higher entropy which was thought to be from the participation of longer chains in aggregate formation and connectivity (Kanyuck et al., 2019). Release of 30% and 40% MD gels formed at different temperatures is shown in Figure 7. For both concentrations of MD, lower carbohydrate release was measured for gels formed at lower temperatures. Correlation between enthalpy (Kanyuck et al., 2019) and carbohydrates released (R^2 = 0.82) suggested the helices contributed to the enzyme resistance. Structural composition was the same between gels (linkages and branch points) and all release experiments were conducted at the same temperature (37 °C) so the differences showed

how aggregation impacted the accessibility of carbohydrates to amylase. Aggregation of MD was thought to function similarly to retrograded starch. Recrystallization and retrogradation of starch resulted in amorphous structures with inhibited enzyme affinity because of the irregular structure (Gidley et al., 1995; Butterworth et al., 2011; Dhital et al., 2017).

An aggregation effect was also seen in mixed gels of MD with HA gellan gum. Higher gelling temperatures resulted in greater percentages of released carbohydrates (Figure 7). The presence of the HA gellan gum network also decreased the amount of available carbohydrates, and at lower temperatures there was a greater inhibitory effect. At 60 °C the HA gellan network decreased availability by 11% while at 5 °C the difference was 24%. Based on the 90-minute values which were lower than either concentration of MD alone, the HA gellan network slowed amylase diffusion through the gel irrespective of the gelling temperature. At lower temperatures the gel network was more inhibitory and possibly due to a greater steric inhibition from more MD aggregates. The structure of this mixed gel consists of MD aggregates within pores of the HA gellan network (Kanyuck et al., 2021a). More MD aggregates would add considerable bulk within the HA gellan network that appeared to have blocked and prevented amylase from reaching as many aggregates in the mixed gel. These factors emphasise the contributions of MD aggregation and a gel network exclusion effect in the release of carbohydrates from mixed gel formulations.

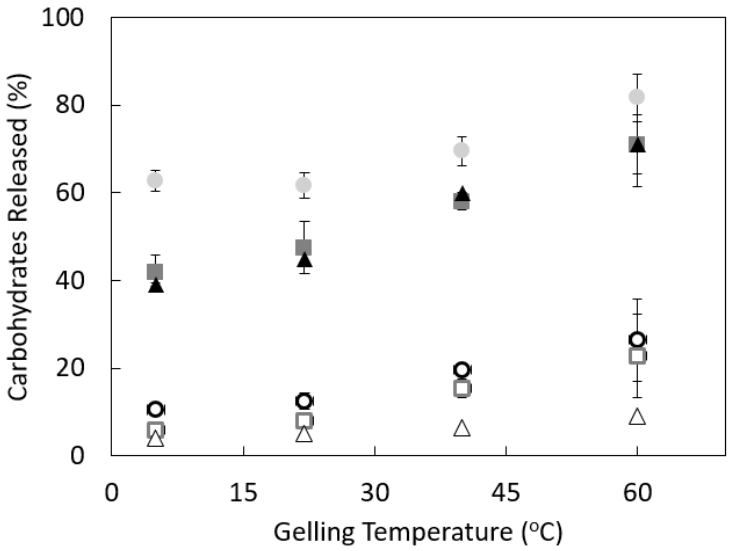

Figure 7. Carbohydrate release by amylase hydrolysis compared by gelling temperature for DE 2 MD at 30% (●), 40% (■), and 30% with 1% HA gellan gum (▲). Percentage released at 90 minutes shown by open symbols and final release shown by filled symbols. Samples were held at the indicated gelling temperature for 4 days prior to measurements and release experiments were all conducted at the same temperature (37 °C).

3.3.2. Gelling agents

MD gels were formulated with various gelling hydrocolloids to examine their structural effects on MD availability. Different concentrations of HA and LA gellan gum at a constant 30% MD and the release profiles are shown in Figure 8. Addition of either HA or LA gellan gum decreased the speed of carbohydrate release as well as the total availability compared to a MD-only gel. Higher concentrations also decreased the total carbohydrate availability (Figure 8B). Unlike the similarity observed for glucose release, a smaller pore size from greater concentrations of polymer decreased the speed of release. The slower release from HA gellan gum was likely due to swelling from the greater distance amylase needed to travel into the gel (Figure 6). Additionally, a lower amount of total carbohydrate was released with higher concentrations of gellan gum. Even at concentrations below gelation of gellan (0.5% for LA and 0.25% for HA) the gel network caused a decrease in availability compared to MD alone (Figure 8). Higher concentrations of gelling agents produce gels

433 which have a higher modulus, more helices, and a smaller pore size (Djabourov et al., 2013). As
434 shown previously (section 3.1), this change in gel network density had no significant effect on the
435 diffusional release of glucose because it was much smaller than the pores. However, the behaviour
436 of MD was different. Release was likely prevented because of entrapment of MD aggregates within
437 these pores and a network density that limited the accessibility of amylase to reach all parts of the
438 gel. For gels that slow the release, typically the polymer slows the movement of critical lyzing
439 enzymes into the gel (McClements and Xiao, 2014). A comparison to other gelling agents with
440 differing network types and structural arrangements, was thought to also have an impact on MD
441 availability.

442 The structural influence of gelling agents was hypothesized to be based on the type of mixed gel
443 network. Both HA and LA gellan gum are known to form interpenetrating polymer networks (IPNs)
444 with MD and the structures have been described as MD aggregates within pores of the gellan
445 network (Clark et al., 1999; Kanyuck et al., 2021a). Phase separated networks are known to form
446 with gelatin (Kasapis et al., 1993a), agarose (Loret et al., 2005), and carrageenan (Wang and Ziegler,
447 2009; Gładkowska-Balewicz, 2017). Additionally, gelatin melted at the measurement temperature
448 (37 °C). These network characteristics will be compared to explain the structural influences of the
449 gels on release behaviour. MD with an IPN (HA and LA gellan gum) resulted in the slowest release
450 and the lowest total release (Figure 9). Non-melting phase-separated gels (k-carrageenan, i-
451 carrageenan, and agarose) resulted in greater total release than the IPNs but less than MD alone.
452 The phase-separated melting gel (gelatin) released more than with no gelling agent.

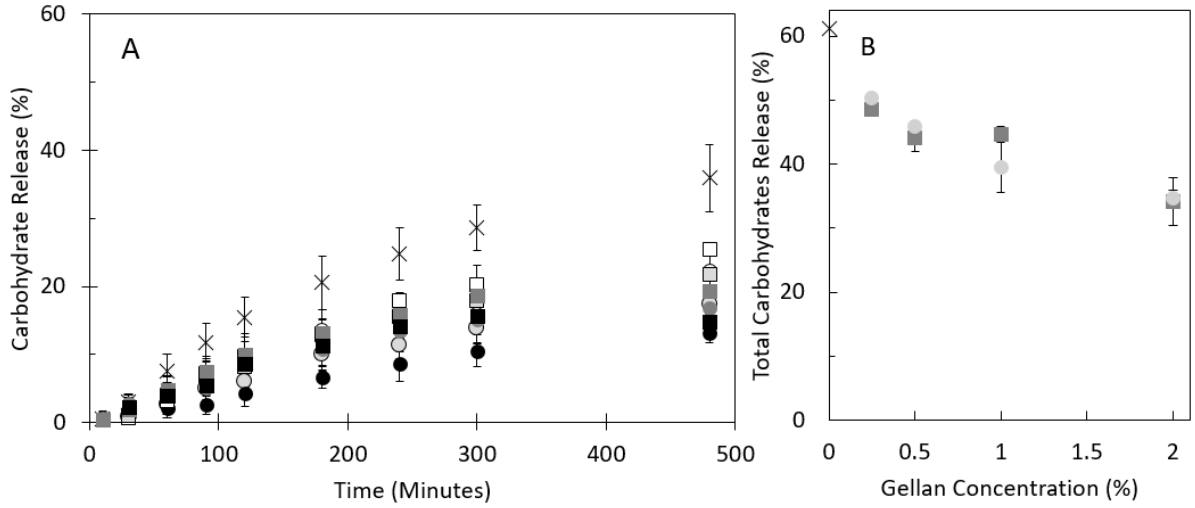

Figure 8. Release profiles (A) of 30% DE 2 MD without any gelling agent (X), HA gellan gum at 0.25% (○), 0.5% (●), 1% (●), and 2% (●), and with LA gellan gum at 0.25% (□). 0.5% (■), 1% (■), and 2% (■). Part B displays the total release after 48 hours for only MD (X) and each concentration of HA gellan (●) and LA gellan (■).

For the IPNs, the arrangement of MD aggregates within the pores of the network reasonably could have inhibited amylase movement. Additionally, a more heterogeneous arrangement of the aggregates and inhibition of the formation of large aggregates (Clark et al., 1999; Kanyuck et al., 2021a) could have caused the lower release. Phase separated networks (all characterized as MD continuous at these concentrations) have gelling agent rich domains dispersed amongst a continuous MD phase where the gelling agents is not present (Kasapis 1993, Loret 2005). Separation into these domains means the gelling agent would have had less potential to sterically block amylase movement through the gel. Consequently, the release from phase separated gels was higher than IPNs gels (Figure 9). Clustering by network type confirmed structure was an important factor for comparing carbohydrate availability. In other work, hydrocolloids have been shown to decrease the amylase digestion of retrograded starch, but differences between gelling agents has largely been nominal (no intrinsic ordering or grouping) and the association with mixed gel network type may carry over to starch applications.

Although gelatin forms a phase separated structure, the network also melted at the analysis temperature and was thought to be the cause of the difference from the other phase separated

networks. This was confirmed by repeating the release measurement at 25 °C where the profile was less than MD alone and no longer significantly different from the other phase separated network (Supplemental Figure 3). Similarity to the other phase separated networks demonstrated the importance of melting in increasing the total amount released. Melting of a gel network would suggest it was no longer able to slow amylase from entering gel, but the greater total release from gelatin could not be explained as simply.

Fractionation (self-separation) of MD within phase separated biopolymer systems have been observed for agarose (Loret et al., 2005) and gelatin (Kasapis et al., 1993b). One phase contained the larger molecular weight fraction of MD and the other phase the gelling agent mixed with a fraction of the smaller molecular weight MD chains. It was thought that phase separation may have changed the structure of helices to increase the availability of carbohydrates (Kasapis et al., 1993b). Any changes in the MD aggregation and distribution from the gelling agent could have contributed to the enzyme accessibility. DSC was used to measure the melting temperatures and enthalpy of MD to detect any changes in the aggregation behaviour with gelatin (Table 3) with thermographs shown in Supplemental Figure 4. From Table 3 the network enthalpy of a mixed gel with gelatin was not significantly different than summation of the individual gels. Prior DSC analysis of mixed gels of gelatin and MD was not able to achieve this resolution (Kasapis et al., 1993a). Enthalpy of MD with HA gellan was demonstrated to be not significantly different than alone (Kanyuck et al., 2021a). No change in enthalpy or melting temperature (Table 3) suggested any fractionation of MD did not change the helix or aggregate formation. It was possible that the smaller MW chains that separate do not participate in helix formation and thus the change was not detectable. From these results, mixed gels did not cause a measureable change in the helix formation. This suggests the greater percentage of carbohydrates released from gelatin was caused by an organisational difference. Melting of gelatin regions may have allowed the amylase increased accessibility through the MD continuous network by liquefying the gelatin phase regions. These structural differences of network type with MD were shown to be predictive of the amount of carbohydrate release.

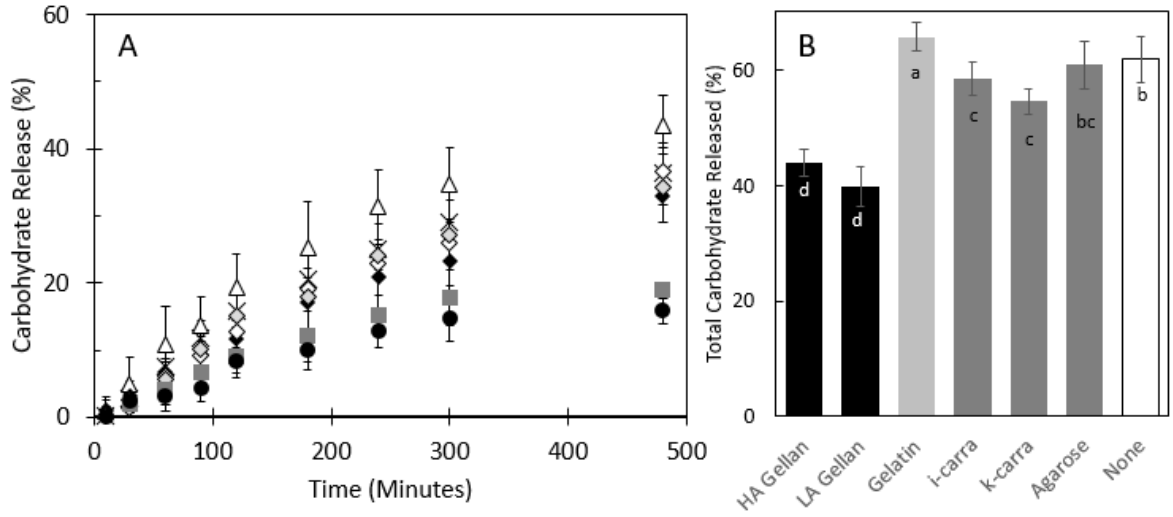

Figure 9. Release profiles from mixed gels of 30% MD comparing gelling agents forming and IPN (HA gellan (●) and LA gellan (■)) and phase separated networks (i-carrageenan (◊), k-carrageenan (♦), agarose (♦), and gelatin (△)) and no gelling agent (X). Gelatin melted at the release temperature. Total release (B) is shown for IPN (black), phase separated networks (grey), and no gelling agent (white).

Table 3. Peak melting temperatures and enthalpy from DSC heating thermographs for MD and gelatin independently and the mixed gel of both hydrocolloids. Curves are shown in Supplemental Figure 3.

		Gelatin		MD	
	Total Enthalpy (J/g)	Peak (°C)	Enthalpy (J/g)	Peak (°C)	Enthalpy (J/g)
MD (30%)	3.18 ± 0.2			70 ± 2	3.18 ± 0.2
Gelatin (2%)	0.59 ± 0.2	33 ± 0.3	0.59 ± 0.2		
Summation	*3.77 ± 0.2*				
MD (30%) with Gelatin (2%)	3.60 ± 0.3	33 ± 0.3		71 ± 1	

4. Conclusion

Carbohydrate size and a hydrocolloid's response to stimuli were shown to be important for all types of release. Gel structure, specifically the network type, was influential for the larger aggregate-forming MD but not for glucose. Glucose offered a simplified system to compare the effects of responses to stimuli (melting and swelling) of hydrocolloid gels on release. The structuring of MD introduced dependencies on amylase accessibility, self-aggregation, and the microstructure of the system. Interestingly, because MD does not undergo gelatinization, the results may provide an

indication of the effects of starch retrogradation within hydrocolloid networks on the carbohydrate availability. The complex findings from this simplified two polymer system is another demonstration of the complexity of food digestion when dealing with multiple ingredients. The work presented here provides a framework for formulating and processing to achieve specific carbohydrate release profiles from gels. Future work, including evaluation of digestion characteristics in humans, is encouraged.

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CRediT author statement.

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