Development of Mucoadhesive Sprayable Gellan Gum Fluid Gels

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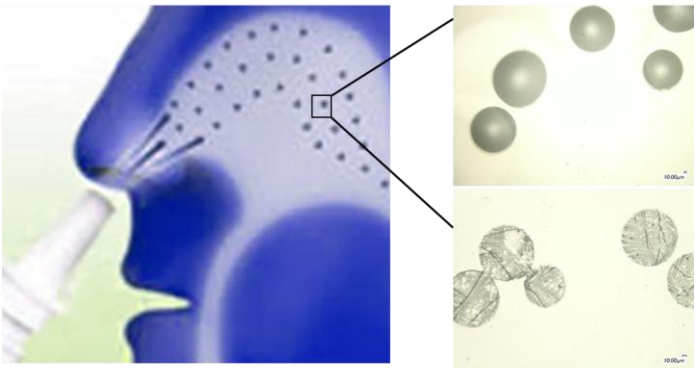
Abstract

The nasal mucosa provides a potentially good route for local and systemic drug delivery. However, the protective feature of the nasal cavity make intranasal delivery challenging. The application of mucoadhesive polymers in nasal drug delivery systems enhances the retention of the dosage form in the nasal cavity. Several groups have investigated using low acyl gellan as a drug delivery vehicle but only limited research however, has been performed on high acyl gellan for this purpose, despite its properties being more conducive to mucoadhesion. High acyl gellan produces highly elastic gels below 60 °C which make it difficult to spray using a mechanical spray device. Therefore, in this study we have tried to address this problem by making fluid gels by introducing a shear force during gelation of the gellan polymer. These fluid gel systems contain gelled micro-particles suspended in a solution of un-gelled polymer. These systems can therefore behave as pourable viscoelastic fluids. In this study we have investigated the rheological behavior and mucoadhesion of fluid gels of two different types of gellan (high and low acyl) and fluid gels prepared from blends of high and low acyl gellan at a 50:50 ratio. The results demonstrated that by preparing fluid gels of high acyl gellan, the rheological properties were sufficient to spray through a standard nasal spray device. Moreover fluid gels also significantly enhance both high acyl and low acyl gellan mucoadhesion properties.



Shear thinning Gellan Gum Fluid Gel containing Caffeine

1.0 Introduction

Liquid nasal sprays are useful dosage forms for local and systemic delivery, but often suffer from poor retention, dripping out of the nose or down the back of the throat, which leads to reduced bioavailability (Jansson et al., 2005). Many ways have been introduced to address this problem; one such way is by formulating nasal sprays that contain polymers which are mucoadhesive. These polymers possess suitable rheological properties that enable them to flow during administration and then to adhere to mucosal tissue, consequently increasing the residence time and improving bioavailability. A complete understanding of the mucoadhesion mechanism is not fully understood. It is generally accepted however, that inter-diffusion and interpenetration take place between the chains of the mucoadhesive polymer and mucus gel network, which creates sufficient contact for entanglement. Secondary chemical bonds are then formed between the polymer chains and mucin molecules (Hägerstrom et al., 2003). Several polysaccharides have been widely investigated as mucoadhesive polymers due to their intrinsic physicochemical properties that facilitate mucoadhesion such as hydrophilicity, numerous hydrogen bonding functional groups and viscoelastic properties when hydrated. Gellan gum is a bacterial exo-polysaccharide produced by the bacteria *Sphingomonas elodea* (Sworn et al., 1995; Gibson and Sanderson, 1990) and is a linear tetrasaccharide repeat unit consisting of → 4)-l-rhamnopyranosyl-(α-1 → 3)-d-glucopyranosyl-(β-1 → 4)-d-glucuronopyranosyl-(β-1 → 4)-d-glucopyranosyl-(β-1 → (Morris et al., 2012). Gellan gum is a promising polymer for use in nasal formulations because of its ability to form a gel *in situ* on exposure to physiological concentrations of cations (Mahdi et al., 2014). Typically, ion concentrations required to gel gellan are in the region of 100 mM for monovalent cations and 5 mM for divalent cation

however the strength of the gels produced depend on the concentration of gellan (Morris et al 2012). The native polymer is high acyl gellan (HA) which contains O-5-acetyl and O-2-glyceryl groups on the (1→3)-linked glucose residue (Figure 1A). When exposed to alkaline media at high temperatures, both acyl groups are hydrolyzed and the deacylated form, low acyl gellan (LA), is obtained (Figure 1B) (Mao et al., 2000). The resulting texture of HA and LA gellan gum gels are very different, and can be considered to be at the opposite ends of the textural spectrum for hydrogels, with LA gellan forming hard but brittle gels and HA gellan forming soft, elastic gels. By varying the ratio of HA:LA gellan gum, a diverse range of textures can be obtained. The properties of blends of HA and LA gellan are intermediate between that of high and low acyl gellan and it is possible to obtain textures close to those of other hydrocolloids such as xanthan gum, locust bean gum and alginate (Sworn, 2009).

Bacon et al., (2000), investigated using LA gellan gum for an *in situ* intranasal formulation to deliver influenza vaccine. Jansson et al., (2005) reported that LA gellan can enhance epithelial uptake of high molecular weight fluorescein dextran. In addition, *in vivo* studies confirmed gellan gum to be nonirritant and not toxic to the epithelial tissue even for a prolonged period of time (Cao et al., 2009; Mahajan and Gattani, 2009) and these gellan formulations retained stable over 6 months (Cao et al 2009; Belgamwar et al., 2009). Recently researchers have looked to develop such dosage forms using micro-particle and liquid nasal formulations (Cao et al., 2009; Mahajan and Gattani, 2009). Although these systems have shown some promise as vehicles for nasal delivery, there are issues such as erosion and rapid clearance by microvilli. These issues could potentially overcome by using fluid gels.

Fluid gels can be formed by applying shear force to a biopolymer during a sol-gel transition, the end product is gelled particles suspended in un-gelled polymer solution. These fluid gels can

be formulated so the bulk material acts as a pourable viscoelastic fluid whilst retaining a cross-linked gel microstructure within the particles. The physical properties of fluid gels can be tuned by simply changing the concentration of the polymer or by the rate of cooling and/or shear rate during fluid gel formation (Gabriele et al 2009; Fernández Farrés et al., 2014; Mahdi et al., 2014).

In this study we have investigated the rheological behavior and mucoadhesion of fluid gels of two different types of both LA gellan and HA gellan and fluid gels prepared from blends of LA gellan and HA gellan at a 50:50 ratio. Gellan gum fluid gels of HA, LA and HA/LA blends loaded with a model drug (caffeine) were investigated as a mucoadhesive nasal spray formulation and compared with *in situ* gelling gellan solutions. The rheological properties and *in vitro* measurements of retention time on mucosal tissue were investigated.

2.0 Materials and Methods

2.1. Materials

High acyl gellan gum (Kelcogel^{TM}) was kindly donated by CP Kelco (USA). Low acyl gellan and caffeine were purchased from Sigma Scientific (UK). Phosphate buffer saline (PBS) was purchased from Fisher Scientific (UK). Fresh porcine mucosal tissue was donated from a local abattoir.

2.2 Preparation of fluid gel formulation

Gellan solutions were prepared by adding precise amounts of high and low acyl gellan gum to produce a 0.25% w/w final polymer concentration to deionised water at 85°C containing 2 mg/mL caffeine. This was allowed to quiescently cool to room temperature prior to use.

To prepare the fluid gels, sodium chloride (0.1% 0.5% and 1% w/w) was added to the hot caffeine-loaded gellan solutions, as crosslinking cations (as described above) then loaded on to a Bohlin Gemini Nano HR rheometer and allowed to cool at 2 °C min^{-1} to 20 °C whilst being sheared at a shear rate of 500 s^{-1} using a 55 mm cone and plate geometry. Once cooled, the fluid gels were recovered and stored at room temperature prior to use.

2.3. Rheological measurements

All rheological measurements were performed using a Bohlin Gemini Nano HR rheometer (Malvern Instruments, Worcestershire, UK) fitted with a 55 mm cone and plate geometry.

2.3.1 Viscosity Measurements

Viscosity measurements of all samples made were taken at 20 °C across shear rates ranging from 1 s^{-1} - 1000 s^{-1}.

2.3.2 Yield stress determination

Stress sweep rheological studies were used to determine yield stress of different gel formulations to predict the stress required to initiate flow. The stress was gradually increased from 0.1 Pa to 100 Pa at 10 rad s^{-1} angular frequency. All measurements were taken at 20 °C.

2.3.3 Frequency sweep measurement

The rheological behavior of the samples was evaluated in terms of the elastic (storage) modulus (G') and the viscous (loss) modulus (G") as a function of angular frequency (0.1–100 rad s^{-1} angular frequency) to produce mechanical spectra of the samples. Measurements were taken at 20 °C and performed at 1 % strain (strain amplitude chosen was within the linear viscoelastic region of the sample).

2.4. Microscopy Method

Samples were imaged using an optical microscope (Keyence VHX digital microscope RZ x 250- x1500 real zoom lens, Milton Keynes, UK). Samples were prepared for imaging by spraying the samples on microscope slide from a nasal spray pump then examined under the microscope.

2.5. Preparation of mucosal membrane for retention studies

The outer muscle layers of fresh porcine esophageal tissue were removed. The internal tissue was then cut into 2 x 4 cm longitudinal sections and stored at -20 °C until required. The tissue was allowed to defrost at room temperature before it was used. The tissue section was not washed prior to use as this process may have affected the surface properties and hence the adhesive interaction as described by Batchelor et al., 2002. The tissue section was discarded however, if residual surface debris was evident.

2.6. Retention time measurements

Drug retention time in simulated nasal conditions (pH 7.4, 34 °C) was studied using a bespoke mucoadhesion apparatus (Figure 2). A sample of defrosted mucosal tissue (as prepared in section 2.5) was secured to the apparatus and the caffeine-loaded formulations (100 µl) were sprayed from a nasal spray device onto the tissue. PBS was then perfused over the mucosal membrane at a rate of 1 ml/min. The PBS perfusate was collected at time points up to 60 min and caffeine content was measured using a RP-HPLC with UV detection at 272 nm. Drug retention on the surface was calculated using equation 1

$$\frac{[C]-[CP]}{[C]} \times 100 \qquad [1]$$

Where [C] is the concentration of caffeine sprayed onto the tissue and [CP] is the concentration of caffeine detected in the PBS perfusate.

2.7. HPLC method

Reverse-phase high performance liquid chromatography analysis of the caffeine was performed following the method of Maleque and Chowdhury, (2012). Briefly, 100 µl of the prepared samples were injected on to a C18 L1, pH resistant (4.5 mm x 150 nm: 3.5µm) column. Isocratic elution of the mobile phase with a composition of methanol/water (40 : 60) (v/v) was used with a flow rate of 0.5 ml/min and a run time of 7 min. The caffeine was detected at a retention time of 5 min using a UV detector at a wavelength of 272 nm.

2.8. Statistical Analysis

Statistical significance ($P < 0.05$) between test groups was determined by one-way analysis of variance (ANOVA) and Tukey post-hoc test using Primer of Biostatistics version 4.

3.0 Results

Fluid gels were prepared using a rheometer in order to have control of cooling and shear rate and the ability to characterize the viscosity during formation of the fluid gels. Figure 3 shows cooling profile of a 0.25% w/w HA, LA and 50:50 blend of gellan gum over range of ion concentrations. There was a general trend that showed HA decreased in viscosity with an increase in ion concentration whereas the viscosity of LA increased with increasing ion concentration. As shown in Figure 3A, in the absence of added ions, the HA and the blend showed an increase in viscosity beginning at approximately 65°C which corresponded with the onset of ordering of HA, whereas no clear viscosity increase was detected for LA gellan. When increasing concentrations of NaCl were added (0.1%, 0.5% and 1% w/w), the temperature at the

onset of viscosity increase in HA and the blend shifted to increasingly higher temperatures. Moreover, the LA gellan also showed an increase in viscosity and temperature of onset when NaCl was added, which would be expected with increasing NaCl concentration (Fig 3B-D). For the blend two transitions were evident, one corresponding to the HA ordering and one corresponding to the LA gelation. The result indicates that the sodium chloride has a potential effect on the viscosities of the fluid gel; onset of gelation of HA and the 50:50 blend increased from ~65°C for the gellan solutions without sodium ions to ~78, 85 and 89°C at 0.1%, 0.5% and 1% w/w NaCl respectively. The onset of gelation of LA changed from a slight increase in viscosity for the LA gellan to a clear sharp transition about ~35°C at 0.1% w/w NaCl. The onset of gelation of LA increased further with increasing NaCl concentration to ~43°C and 46°C at 0.5% and 1% w/ NaCl respectively. Furthermore, the final viscosity of LA fluid gel increased from ~0.006 Pas in the absence of NaCl, to ~0.020 Pas at 0.5% w/w NaCl, whereas, the final viscosity of HA fluid gel decreased from ~0.045 Pas without NaCl to a similar level as the LA at 0.5% w/w NaCl. Interestingly, the final viscosity of blend fluid gel stayed the same at all the salt concentrations tested. The viscosity profile of a 0.25% w/w HA, LA and blend solutions without salt and for 0.5% w/w NaCl are shown in figure 4A and were all found to have a shear thinning viscosity profile. Figure 4B shows the viscosity of the HA, LA and 50:50 blend fluid gel formulations with 0.5% NaCl and the comparative uncross-linked solutions at 500 s^{-1}. The HA fluid gel sample with 0.5% NaCl exhibited a viscosity profile that was most similar to the 50:50 blend fluid gel and 50:50 without NaCl. For this reason, 0.5% NaCl was used to prepare the fluid gels in all further experiments. The effect of 0.5% NaCl on the rheological properties of the fluid gels was further investigated using small deformation rheological measurements. Figure 5 shows LA and the blended fluid gel produced at 0.25% w/w gellan and 0.5% w/w NaCl generally

exhibit greater G' (~10 Pa compared with un-crosslinked gel ranging from ~0.1 and 1 Pa for LA and blend respectively). The HA however exhibits almost same profile in both fluid gel and HA without NaCl having a G′ of ~10 Pa. Furthermore, G′ was slightly greater than G″ across the range of frequencies measured which indicates typical 'weak gel' rheological behavior. To evaluate sprayability through the nasal spray device, stress sweep rheological measurements were performed to determine the yield stress. Figure 6 shows the effect of adding NaCl on yield stress after formulation of fluid gels of HA, LA and the 50:50 blend (figure 6A) compared with the yield stress of the gellan solutions without addition of NaCl (figure 6B). The stress required to yield the fluid gel formulations were 1.07 Pa 1.2 Pa and 5.7 Pa, for the LA, 50:50 blend and HA respectively, which was significantly less than the corresponding solutions without NaCl (figure 6B). The distribution of caffeine in the sprayed droplets is shown in the microscopy images in figure 7. These images reveal that caffeine was suspended in a uniform distribution in the nasal spray drops within the sprayed HA fluid gel samples (figure 7A) whereas un-crosslinked HA gellan shows caffeine accumulated in the core of the droplet (figure 7B). To investigate the mucoadhesion properties of gellan blends, the release of caffeine from 0.25% LA, HA and blend (fluid gel and un-crosslinked gellan) at different ratios were studied and are shown in Figure 8. Pure LA fluid gel gellan shows almost 96% of drug released after 1 h; whereas pure HA fluid gel shows only 50 % drug release at the same time point with the 50:50 blend of these two polymers releasing 65 % after 1h. Un-crosslinked gellan samples however, present large difference in drug release between HA, LA and the 50:50 blend. Pure LA gellan releases almost 100% of drug after 10 min; whereas pure HA shows only 6% drug release at the same time point with the 50:50 blend of these two polymers releasing 70% after 10 min.

4.0 Discussion

There are two main prerequisites for *in situ* gelling nasal spray systems: optimum viscosity and gelling capacity. The viscosity is a critical factor as the formulation should be at a enough low viscosity to be easily dispensed from the nasal spray device. It should then undergo a rapid sol–gel transition due to the physiological environment of the target site, which in the case of gellan, is due to ionic interactions with the ions in nasal fluid. Also the viscosity needs to be sufficient to facilitate adherence to the mucus membrane and prevent the formulation draining out of the nose or dropping to back of the throat. Moreover, the formed gel should preserve its integrity to facilitate sustained release of drugs locally, for a prolonged period of time without quickly dissolving or eroding. Previously *in situ* gelling nasal spray formulations have been investigated using LA gellan gum (as the *in situ* gelling agent) suspended in xanthan gum (used to reach to the optimum viscosity) (Cao et al., 2009). Here we have investigated the potential use of fluid gels prepared from LA, HA and 50:50 blend of LA and HA gellan gum as a mucoadhesive system for nasal spray formulations. The preparation of fluid gels is a simple process, producing gelled particles that are dispersed in an un-gelled medium. Producing fluid gels using a rheometer allows the cooling rate and the shear rate to be accurately controlled and the characteristic change in viscosity monitored. When the gellan gum fluid gels were formed with 0.1%, 0.5% and 1% w/w NaCl, the onset of gelation of HA and blend increased (Figure 3B-D) compared with when no ions are added (Fig 3A), which can be explained by promoting aggregation of double helix with sodium chloride (Mahdi et al., 2014; Morris et al., 2012). The LA sample containing 0.1% w/w NaCl exhibited a clear transition (figure 3B) because the concentration at this level was sufficient to allow the crosslinking between two or more double

helixes (Morris et al., 2012). Sanderson et al. (1988) reported intermediate textural properties between high and low acyl gellan gels when combining low acyl gellan with high acyl gellan to form a mixed gel. This is in good agreement with the present study, as the blend exhibited two transitions that are characteristic of the individual components (figure 3). Once manufactured, the bulk fluid gels containing caffeine showed shear thinning behavior suitable for spraying through nasal spray device (figure 4). Interestingly, HA viscosity dramatically decreased in presence of NaCl; this is thought to be due to the competitive inhibition by negatively charged glycerate group binding to some of the Na^+ ions resulting in a stereochemical change that leads to the loss in the inter or intra-chain hydrogen bonds (Huang et al., 2003). For LA gellan, the absence of glycerate group facilitates binding of the Na^+ ions to the carboxylate group in the β - glucuronate residue, thus reducing the repulsive electrostatic force on the gellan helicies, promoting aggregation and development of a three dimensional network. There was no significant difference in viscosity of 50:50 blend of HA and LA fluid gels prepared with and without 0.5% NaCl, due to the balance between the HA properties and the LA properties present in the mixture.

Gellan gum fluid gel formulations exhibit typical weak gel properties with G′ slightly higher than G″ (figure 5), furthermore the G′ and G″ for samples with NaCl have greater values. This has previously been demonstrated by Huang et al., (2003) and Huang et al., (2004). This weak gel rheological behavior causes these formulations to be more stable at low shear rates with sufficient viscosity to allow the samples to be inverted without any steady state flow as a result of particle-particle interactions (Garrec et al., 2013). Nasal spray formulations with relatively high values of zero shear viscosity that rapidly shear thin to enable dispensing would be greatly beneficial by suspending the drug more effectively on the shelf while not impacting the ease of

administration. Furthermore, stress sweep measurements were used to determine the yield stress and to gain an understanding of the strength of particle-particle interactions. The HA with no ions has a higher yield stress value compared with the 50:50 HA/LA blend (figure 6B) and for this reason this HA gellan was poorly dispensed from the nasal spray, whereas the 50:50 blend could be dispensed without any problems.

The mucoadhesive properties shown in figure 8 highlight that the HA containing formulations significantly slowed down the caffeine release (detected in the PBS perfusate), indirectly indicating that the gel remains adhered to the mucosal membrane for an increased time period. This is thought to be due to the greater elasticity and viscosity of HA promoting physical interations with mucins on the surface of the mucosa (Mao et al., 2000). Most of the HA (80%) formulation remained on the mucosal membrane for over 1 h when applied in the un crosslinked form compared with LA gellan which was 100% detached from the membrane in less than 10 min. This is thought to be due to the strong *in situ* gelation of LA on contact with the ions on the mucosal surface. LA favours self-association rather than interactions with the mucins in the mucosal membrane. In addition LA gellan is prone to syneresis which could also contribute to the poor adhesion to the mucosal surface. HA gellan therefore appeared to be an excellent candidate for retaining the formulation at the site of action, however, the relatively high viscosity (figure 3B), elasticity and yield stress (figure 6B) hindered the administration from the nasal spray device. By formulating the HA gellan as a fluid gel (containing 0.5% NaCl) the viscosity and yield stress were reduced to a level similar to LA gellan fluid gel (containing 0.5% NaCl) (figure 6A), which is easily administered, while maintaining ~70% of the mucosal retention of the uncrosslinked HA (figure 8). This bulk rheology was also shown to be tunable by creating

HA/LA blends with rheological properties (figure 5) and mucoadhesive properties (figure 8) intermediate to those of 100% HA and 100% LA. Another attractive feature of the fluid gel formulation is presented in figure 7 where microscopy has shown that the drug (caffeine) was uniformly distributed throughout the gelled micro-particles of the fluid gel, whereas, when the formulation is in the uncrosslinked form the drug accumulated at the center of the dispensed droplet which is likely to influence stability, dissolution and uptake. The relatively simple process for creating fluid gels provides an attractive route to tune the bulk rheology of HA gellan to that which is applicable to liquid formulations while maintaining the elastic gel properties at the micro level. For these sprayable fluid gels to realize their potential, however, the biopharmaceutics of the formulations should be fully investigated.

Conclusion

In this study we have demonstrated that a mucoadhesive gelling nasal spray has the potential to be formulated using gellan gum fluid gels with a viscosity sufficient to spray out from the device and with elasticity great enough to adhere to the mucosal membrane. Furthermore, we have shown that it is possible to modify the physical behavior of the formulation by modifying the LA/HA ratio. Increasing HA gellan content in the fluid gel formulations increases the adherence time on mucosal surfaces. This work highlights the potential of using HA gellan gum in nasal spray formulations, providing a simple and effective technology to retain drugs at the site uptake.

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Figure Captions

Figure 1

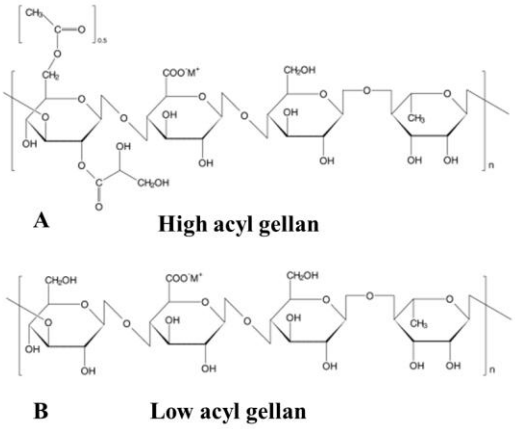


Figure 1 Chemical structure of gellan gum A) High acyl gellan gum B) Low acyl gellan

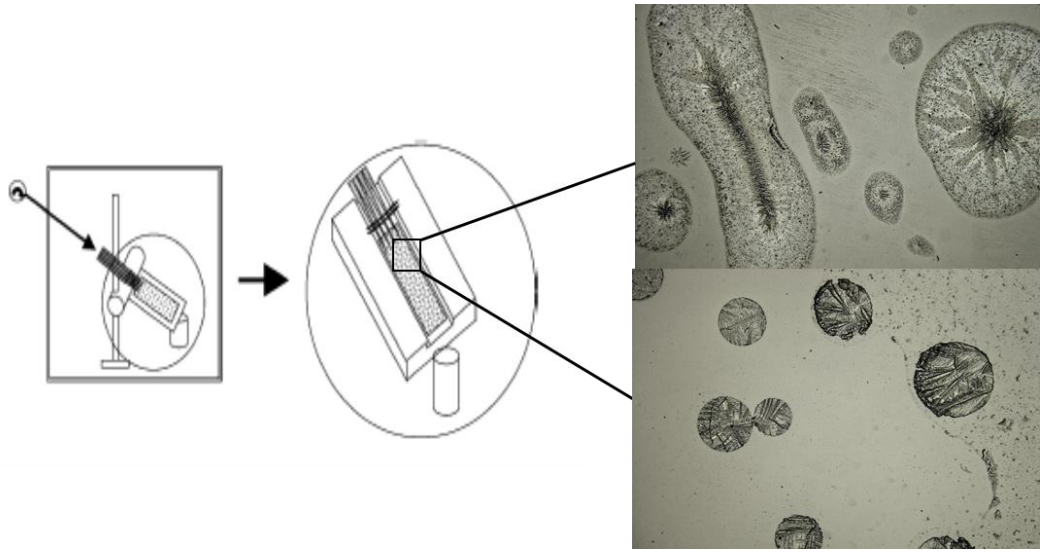


Figure 2 Schematic representation of the retention model apparatus (adapted from Batchelor et al., 2002)

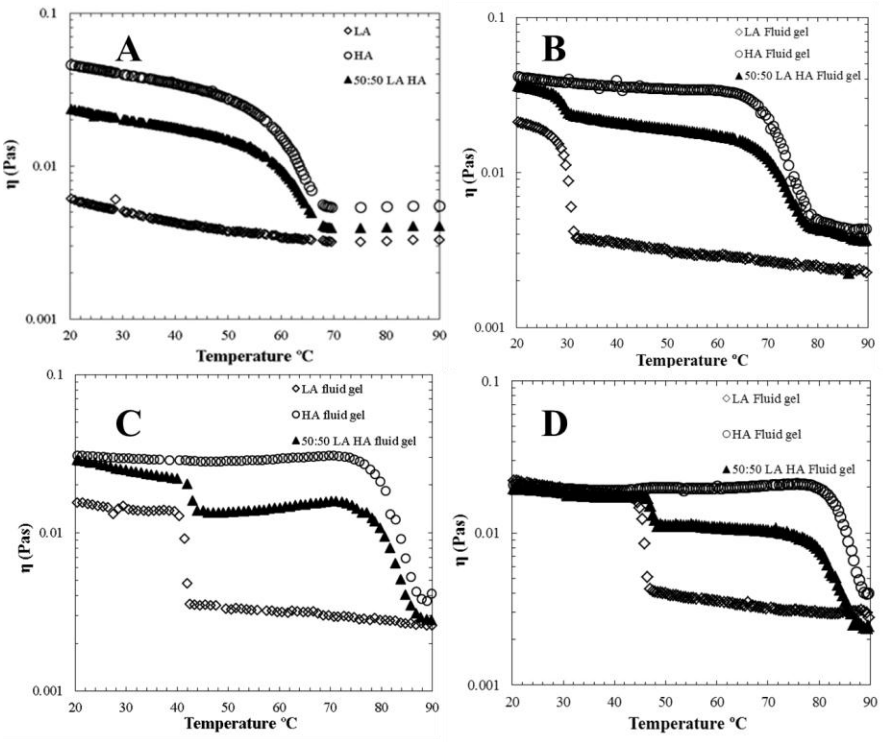


Figure 3 Viscosity of gellan gum during fluid gel formation at 0.25% w/w gellan gum (cooling at 2°C /min at a shear rate of 500 s^{-1}) for 0.0% A), 0.1% B), 0.5% C) and 1% D) w/v NaCl loaded with 2 mg/mL caffeine.

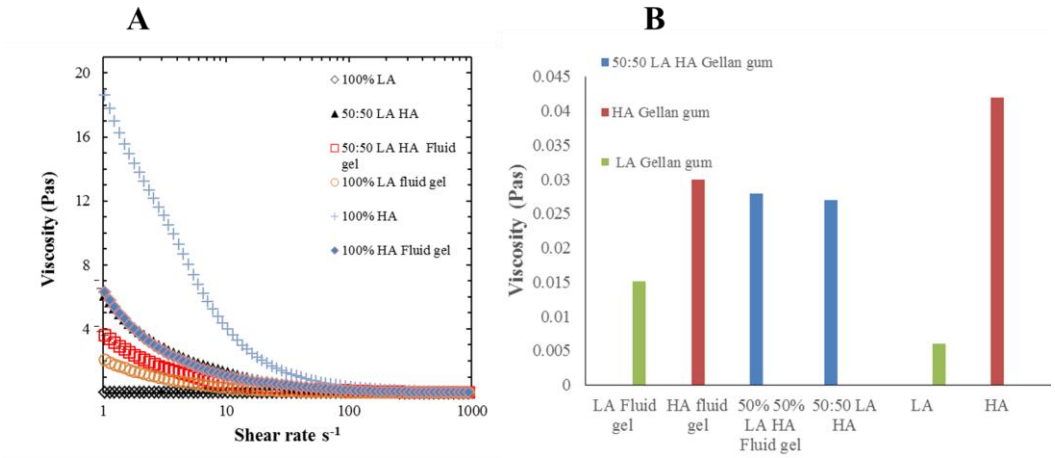


Figure 4 A) Viscosity vs. shear rate at 20°C for 0.25%w/w gellan at 0.5% NaCl fluid gel and for un-crosslinked gel, B) Viscosity measurements at 20 °C at a shear rate of 500s^{-1} of gellan gum blends containing 2 mg/mL caffeine.

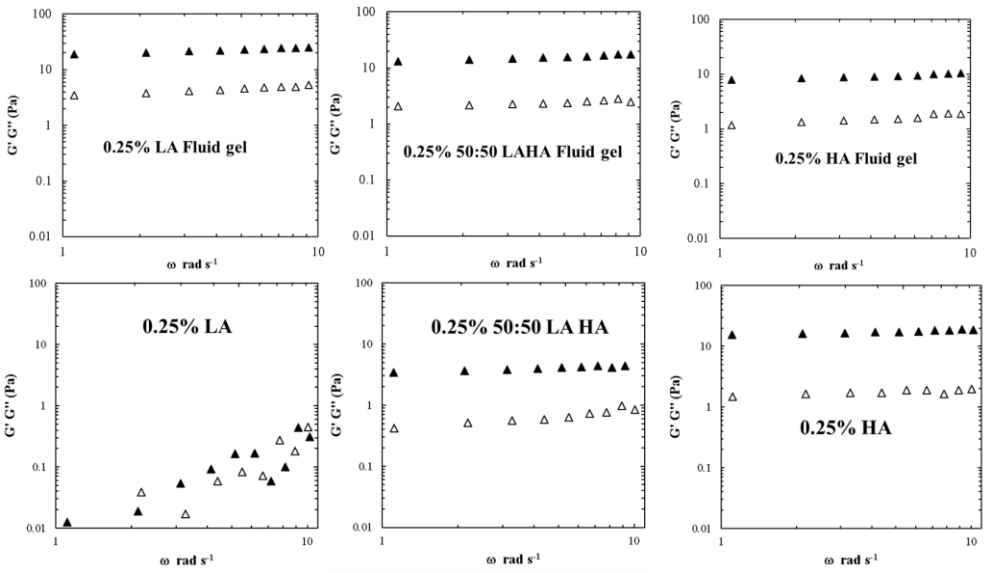


Figure 5 Mechanical spectrum (1% strain; 20 °C) of a 0.25% gellan gum loaded with 2 mg/mL caffeine showing variation of G' (filled triangles), G" (open triangles).

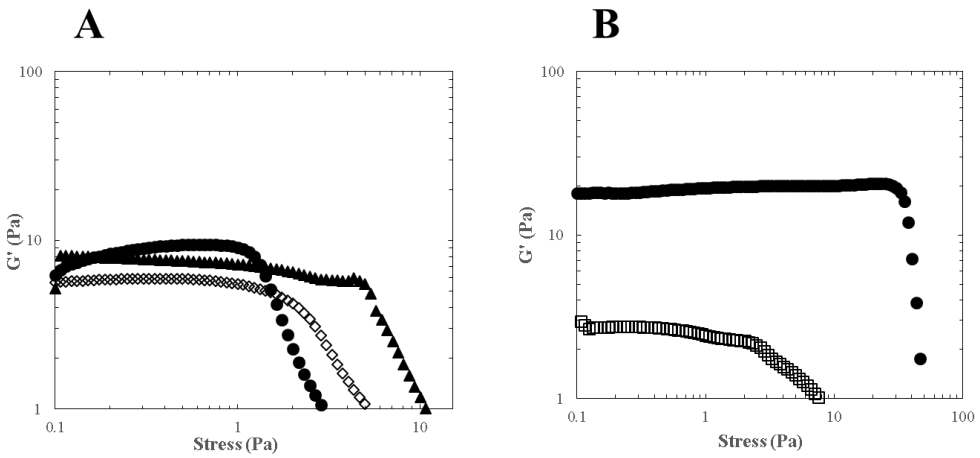
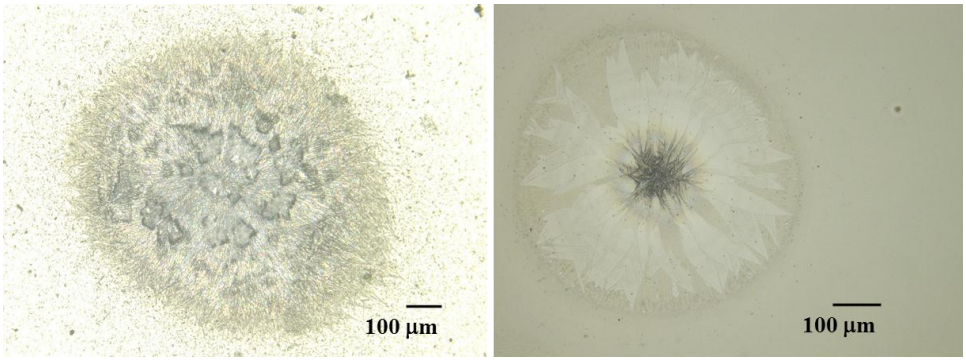
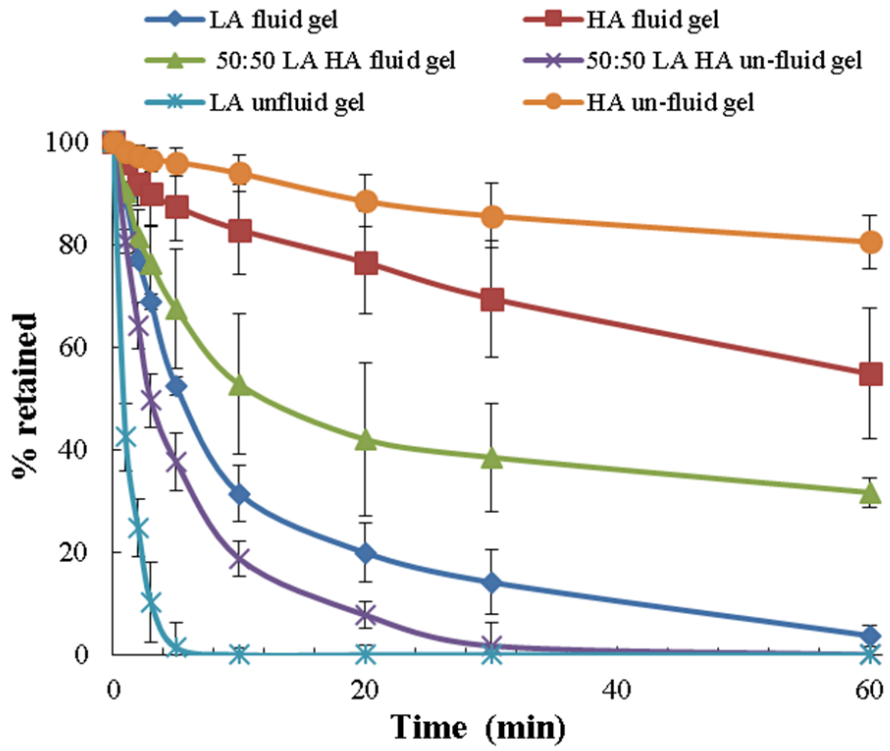


Figure 6 A) Stress sweep for 0.25% gellan fluid gels crosslinked with 0.5% NaCl as function of HA:LA ratio (pure LA filled circles, pure HA filled triangles and 50:50 blend open diamonds B) Stress sweep for 0.25% un-crosslinked gellan for HA (filled circles) and 50:50 blend (open squares).

400 

401 **Figure 7** Light microscopy images of gellan gum loaded with 2 mg/mL caffeine A) Cross-linked

402 HA B) un-crosslinked HA.



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404 **Figure 8** Cumulative % caffeine retained on the mucosal membrane after 60 min

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