Gellan Gum Fluid Gels for Topical Administration of Diclofenac

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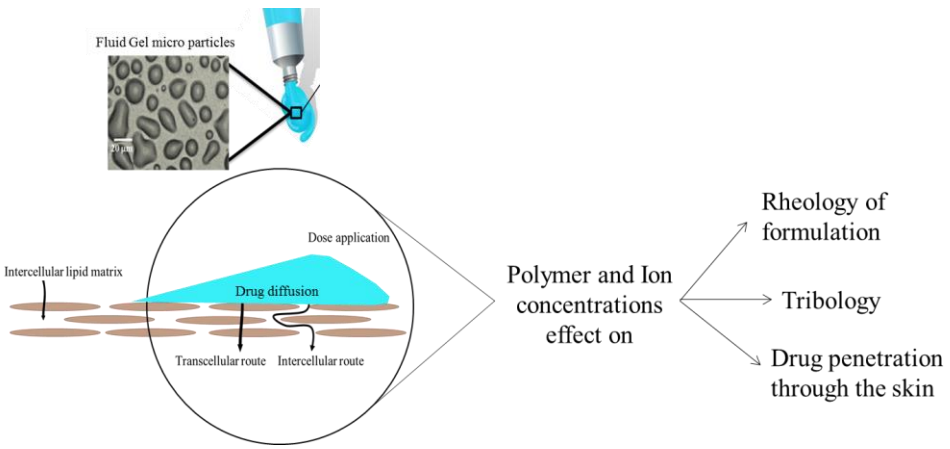
Abstract

Diclofenac topical formulations are often preferred for drug administration to patients who experience serious GIT problems. Absorption of the drug through the skin, however, can be challenging due to the natural protective feature of the stratum corneum (SC). In this article, fluid gels prepared from gellan gum were explored as a topical drug delivery vehicle. Rheological analysis of the formulations showed that it was possible to produce a topical gel with a viscosity and the mechanical strength similar to that of the commercially available Voltaren® gel using 1 % w/w of a 50:50 low acyl/high acyl (LA/HA) gellan blend. Soft-tribology was used to assess the lubrication properties of gellan fluid gels. The lubrication of the gellan gum fluid gel formulations at high rubbing speeds was similar to the lubrication of the Voltaren® gel. The use of gellan gum dramatically increased skin permeation of diclofenac when compared with the commercially available formulation and could be controlled by changing the gellan gum concentration and/or sodium ion concentration in the formulation. This study highlights the potential use of fluid gels that can be easily tuned to have physical properties suitable for topical formulations with the added advantage of increasing drug permeation.

Keywords: Gellan Gum, rheology, tribology, topical, diclofenac, skin permeation, fluid gel.

Graphical Abstract



1 Introduction

Skin is by far the largest organ of the human body making up sixteen percent of total body weight. Delivery of drugs through the skin is an attractive route for formulation development as medicines administered to the skin can exert either a local (dermal) effect or systemic (transdermal) effect. In dermal drug delivery, the drug is applied to treat skin diseases and exert their actions on the stratum corneum (SC) and those that affect the function of the epidermis and/or the dermis. In transdermal delivery, the drug is applied to the skin in order to deliver the drug into the systemic circulation. Thus, the skin serves as the site of administration, not the targeted organ. The transdermal route offers a good alternative to the oral route when oral administration of the drug causes unwanted side effects. The transdermal route is also suitable for drugs with low bioavailability due to physiological properties of the gastro-intestinal tract (GIT) (such as first pass metabolism) or physical properties of the drug (low solubility or narrow therapeutic window). It is also possible to maintain a sustained drug permeation rate by this route (Honeywell-Nguyen and Bouwstra, 2005).

Diclofenac is one such drug that has gastric side effects and a bioavailability highly affected by the physiology of GIT, with orally administered diclofenac undergoing first pass metabolism and producing considerable gastrointestinal disturbance. In order to address these two major shortcomings of the oral dosage form, different formulations have been introduced for diclofenac delivery (Warner *et al*., 1999; Giuliano and Warner, 1999). The benefits of topical drug delivery such as simple application and avoidance of the problems associated with the oral route make it useful for clinical applications (Moser, *et al*., 2001). Successful development of a topical dosage form is however, quite challenging due to the protective nature of the SC. Different methods have been assessed to enhance diclofenac permeation and to address the barrier problem of the SC; these include active methods and passive

methods (Batheja *et al*., 2011; Fang *et al*.,1999; Goh and Lane, 2014). Active methods include iontophoresis, electroporation, and microneedles while passive methods include the use of chemical penetration enhancers, supersaturated systems, prodrugs, liposomes, microemulsions and colloidal polymeric suspensions. Although active methods have shown some promise in efficiency, there are issues associated with their use such as safety and cost-effectiveness. Passive methods provide design flexibility (with formulation optimization) and the possibility of application over a larger area of skin compared with active methods. Safety issues however, can still be problematic. For example, chemical penetration enhancers have been intensively investigated over the years, but the concentrations required to improve penetration may lead to irritation or sensitization of the skin (Williams and Barry, 2012). There is therefore, a real need for alternative formulations.

Also important to the effectiveness of topical dosage forms are the perceptual attributes of the formulation. This is a major concern as these contribute substantially to whether a product is liked by patients and thus used. For instance, if the gel is unpleasant to touch, it is unlikely to be accepted by the patient even if it has potential benefits (Guest *et al*., 2013). The initial feeling of a product on the skin is likely to be dominated by the product characteristics, rather than the skin's characteristics, and driven by its bulk rheological properties and tribological properties. Tribology in particular, may be used to characterise physical perception and lubrication behaviour of materials. Tribological behaviour is usually represented by a Stribeck curve, which discribes three regimes of lubrication that can be clearly identified from the shape of the curve: Boundary lubrication, mixed regime of lubrication and hydrodynamic lubrication (De Vicente et al., 2006). Assuming the initial layer of product at application time is thick enough to provide hydrodynamic lubrication, as the rubbing action (shear) starts and the thick layer is broken down to a much thinner film, it

can be envisaged that the mixed regime is entered. Once rubbed over a large skin area, a thin surface residue may result in boundary lubrication (De Vicente *et al*., 2006).

This article studies the application of fluid gels prepared from gellan gum as a platform to deliver diclofenac sodium to the skin. Gellan gum is a bacterial exo-polysaccharide produced by the bacterium *Sphingomonas elodea* (formally known as *Pseudomonas elodea*) and has a structure consisting of a repeating tetrasaccharide unit → 4)-L-rhamnopyranosyl-(α-1 → 3)-D-glucopyranosyl-(β-1 → 4)-D-glucuronopyranosyl-(β-1 → 4)-D-glucopyranosyl-(β-1 →. The native polymer is known commercially as high acyl gellan (HA) which contains O-5-acetyl and O-2-glyceryl groups on the (1→3)-linked glucose residue. When HA gellan is exposed to alkaline media at high temperatures, both acyl groups are hydrolysed and the deacylated form, low acyl gellan (LA), is obtained (Mao *et al*., 2000). Solutions of gellan gum undergo a sol-gel transition when cooled from > 80°C to room temperature, and in the presence of monovalent (and divalent) cations, form a strong firm gel. Applying a shear force as this gelation occurs however, results in the formation of gelled particles in the micron size range. The bulk sample is referred to as a fluid gel or sheared gel, whereby the material behaves as a pourable viscoelastic fluid whilst retaining a cross-linked gel microstructure within the particles. Fluid gels prepared from gellan gum have been previously described (Sworn *et al*., 1995; Mahdi *et al*., 2014; Mahdi *et al*., 2015) and an attractive feature of gellan gum fluid gels is that the physical properties can be easily tuned. This can be achieved by simply changing the concentration of the gellan or by changing the rate of cooling and/or shear rate during fluid gel formation (Mahdi *et al*., 2014). The mechanical properties of gellan gum fluid gels can also be changed by using blends of LA and HA gellan (Mahdi *et al*., 2015). Therefore in this study, fluid gels prepared from gellan gum blends (loaded with diclofenac), were evaluated as topical gel formulations and compared with the commercially available 1 % diclofenac sodium formulation Voltaren® (a

hydroalcoholic polyacrylic acid gel based formulation) in terms of rheological behaviour, tribological properties and permeation of the loaded diclofenac into full thickness skin.

2 Materials and Methods

2.1 Materials

HA gellan gum (Kelcogel™) was kindly donated by CP Kelco (USA). LA gellan was purchased from Sigma–Aldrich (Poole, UK). Phosphate buffer saline tablets (PBS) and diclofenac sodium were purchased from Fisher Scientific (UK). Fresh porcine ear tissue was sourced from a local abattoir. Diclofenac sodium gel (Voltaren® gel 1 %) was bought from a local pharmacy.

2.2 Methods

2.2.1 Preparation of Fluid Gel Formulations

Gellan gum fluid gels (LA, HA, gellan and 50:50 LA HA gellan blend) were prepared by adding precise amounts of HA and LA gellan to produce a 0.1 %, 0.25 %, and 1 % w/w final polymer concentration in deionised water at ~ 85 °C containing 1 % w/v diclofenac sodium. Sodium chloride (0.5 % 1 % and 2 % w/w) was added to the hot diclofenac-loaded gellan solutions at ~ 85 °C, as a crosslinking cation. The samples were 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 before use.

2.2.2 Control Formulations

Two control formulations were used in this study, diclofenac solution and Voltaren® gel. To ensure diclofenac stability was not affected by the heating process and to examine the permeability of drug excluding the effect of gellan, control solutions were prepared in the

same way the fluid gels were prepared, but without addition of gellan. Voltaren® gel was bought from a local pharmacy and used as a second reference for comparison.

2.2.3 Rheological Measurements

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

2.2.3.1 Viscosity Measurements

Viscosity measurements of all samples were taken at 32 °C using a 2 min shear ramp from 20 s^{-1} to 200 s^{-1}. The shear rate range was chosen to be within the most relevant ranges for replicating the spreading properties of pharmaceutical semisolids as previously reported by Garg *et al*., (2002).

2.2.3.2Yield Stress Determination

Stress sweeps were used to determine yield stress of the topical gel formulations to predict the stress required to initiate flow from a tube. The stress was gradually increased using small deformation oscillations from 0.1 Pa to 100 Pa at an angular frequency of 10 rad s^{-1}. All measurements were taken at 20 °C.

2.2.3.3 Frequency Sweep Measurement

The rheological behaviour of the samples was evaluated in terms of the elastic (storage) modulus (G′) and viscous (loss) modulus (G″) as a function of angular frequency (0.1-100 rad s^{-1}) to produce mechanical spectra of the samples. Measurements were taken at 20 °C and performed at 0.5 % strain (strain amplitude chosen to be within the linear viscoelastic region of the sample).

2.2.4 Tribology of Fluid Gels

The frictional properties of the produced gellan fluid gels were measured using a mini traction machine MTM2 (PCS Instruments, London). The tribometer was equipped with a stainless steel ball (3/4inch diameter) and silicone disc (46 mm diameter, 4 mm thickness) as a contact surface. A normal load (W) of 4 N and a temperature of 32 °C were used for all experiments.

Friction coefficient (μ), a dimensionless parameter is often used to represent the data and is given by

$$\mu = F/W \qquad \text{Eq. 1}$$

Where F is the tangential force and W is the applied normal load.

Stribeck curves were generated by measuring the friction upon increasing the speed (*U*) from 1 to 1000 mm s^{-1} followed by decreasing the speed from 1000 to 1 mm s^{-1} until a total of 6 runs were completed. Each test, consisting of 3 ramps up and 3 ramps down, was repeated 3 times. The error bars represent the standard deviation of the mean of 18 tests per fluid gel sample. Fresh silicone discs were cut out from the supplied sheets and were cleaned by sonicating in ethanol (5 min) and then sonicating in deionised water (5 min) and were not reused. The stainless steel ball was also cleaned in the same way.

2.2.5 Release Study

A modified USP I apparatus (baskets at a stirring rate of 100 rpm) was used to study *in vitro* drug release. Each formulation (1 mL) was placed into dialysis tubing (12,500 MWCO) then submerged (within the baskets) in small volume vessels containing 100 mL dissolution media PBS at pH 7.4 for 8 hours then recording subsequent release. The samples were then chromatographically analysed for diclofenac using a validated HPLC method (section 2.2.8).

2.2.6 Permeation Experiments

Whole porcine ears, obtained from a local abattoir, were used for the permeation studies. The pinna (ear flap) was removed carefully and separated from cartilage and subcutaneous fat. The entire skin thickness (1.2 to 2.0 mm) was used. The tissue was then cut into 4 × 4 cm longitudinal sections and stored at -20 °C until required. The tissue was allowed to defrost at room temperature in PBS for 30 min before being mounted on a Franz diffusion cell (epidermis on the top face) and equilibrated for a further 30 min before addition of 0.5 mL of sample to the donor compartment. The diffusion area was 3.8 cm^2 and the receiver compartment volume was 30 mL. The environmental parameters selected were previously used by Sintov and Botner, (2006). Briefly, the porcine skin surface was maintained at 32 °C and PBS (pH 7.4) was used in the receiver compartment. Samples (0.5 mL) were withdrawn from the receiver solution at predetermined time intervals, and the volume of the receiver chamber maintained by replacing the withdrawn sample with fresh buffer solution at each time point. The samples were then chromatographically analysed for diclofenac using a validated HPLC method (section 2.2.8).

To determine the stready state flux, the cumulative amount permeated per unit area was plotted against time and the flux calculated from the slope of the linear section of the graph. The values reported are the mean of triplicate measurements of diclofenac permeation.

2.2.8 Diclofenac HPLC assay

The HPLC method for diclofenac sodium analysis was based on the method reported by Khan et al., (2016) with some modification. Chromatographic separation was performed on a Shimadzu HPLC system equipped with a SPD-20 AV Prominence UV/VIS detector, a LC 20 AT pump, and SIL-20A Prominence auto sampler. The data acquisition was carried out on a LC solution software integrator. The separation was performed using C18 L1, pH resistant (4.5 mm x 150 nm: 3.5μm) column (Waters, UK).The mobile phase composition was acetonitrile/water (60:40) (v/v). Stock solutions of diclofenac sodium were prepared by dissolving 100 mg in 100 mL of the mobile phase. The mobile phase was filtered (0.22 μm filter) and degassed prior to use. Standard solutions were prepared in concentrations between 0.5 μg mL^{-1} and 200 μg mL^{-1} by diluting the stock solution with the mobile phase and were analysed in triplicate. Calibration standards were prepared by plotting the area under the curve (AUC) *versus* concentration. The limit of detection (LOD) and the limit of quantification (LOQ) were determined as 0.045 and 2.3 μg/mL respectively.

2.2.9 Statistical Analysis

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

3 Results

3.1 Rheological Characterisation of Gellan gum Fluid Gels

Stress sweep rheological measurements were used to evaluate the critical stress required by the formulation to yield, and are shown in Figure 1. Voltaren® gel was used as a reference throughout all experiments. All gellan samples reported in Figure 1A were composed of 1 % w/w of gellan and crosslinked with 0.5 % NaCl. The samples had similar

values of storage modulus (G′), however, the stress required to yield was noticably different. The sample made of LA gellan showed the lowest yield stress while, the sample made with HA gellan, had the highest yield stress, even greater than that of the Voltaren® gel (**Figure 1A**). Interestingly, the 50:50 LA HA gellan blend had a yield stress that was intermediate to that of LA gellan and HA gellan and was similar to that of Voltaren® gel. Therefore, this formulation was used for further investigations. The critical stress required to yield for 1 % w/w 50:50 LA HA gellan blend samples decreased with increasing NaCl concentrations above 0.5 % (**Figure 1B**), and with decreasing total polymer concentration (**Figure 1C**). In general, the formulation with 1 % w/w 50:50 LA HA gellan blend fluid gel with 0.5 % w/w NaCl exhibited similar elasticity and yield stress to that of the proprietary Voltaren® gel (**Figure 1**). This formulation was therefore used for further rheological investigations.

To predict the spreading characteristics of gellan gum fluid gel formulations, measurements of viscosity as a function of shear rate were performed. Both 1 % w/w 50:50 LA HA gellan blend fluid gel and Voltaren® gel had shear thinning viscosity profiles (**Figure 2**). Dynamic small deformation oscillatory measurements of G′ and G″ (**Figure 3**) highlight the viscoelasticity of the 1 % w/w 50:50 gellan blend fluid gel and Voltaren® gel, with G′ slightly greater than G″ across a range of frequencies typical of ‘weak gel’ rheological behaviour.

3.2 Lubrication Properties of Gellan Gum Fluid Gels

Characterisation of the lubrication behaviour of both the gellan fluid gels and the marketed Voltaren® gel were performed using soft tribology. The low contact pressures provided by the deformable surfaces were used in this study to mimic the skin, thus yielding frictional data with strong correlations to in-skin sensory attributes. Stribeck curves of several gellan gum formulations were compared with commercial Voltaren® gel (**Figure 4**).

The LA gellan gum fluid gel showed a slight increase in the friction and then a decrease in friction followed by a plateau in the boundary regime until it reached critical speed in the mixed regime after which the friction coefficient began to increase again. On increasing the speed further (hydrodynamic regime), the Stribeck curve began to decrease again. HA gellan sample showed a continuous decrease in friction coefficient with no clear peak observed. The friction coefficient of the 50:50 LA HA gellan blend fluid gel sample showed equivalent values to that of HA gellan at low speeds and equivalent to that of LA gellan at high speeds (**Figure 4A**).

The Stribeck curve of the Voltaren® gel showed that friction was steady at the beginning then decreased slightly with increasing disk speed in the mixed lubrication regime before it increased again in hydrodynamic regime at higher speeds, indicating a classical friction tribology curve. The friction coefficient (μ), values had the following trend LA > 50:50 gellan LA HA blend > HA > Voltaren® gel. Moreover, the speed at which onset of the mixed regime peak began earlier at speed of approximately 13 mm s^{-1} for the LA gellan formulation compared with the 50:50 LA HA gellan blend fluid gel formulation at about 24 mm s^{-1} (**Figure 4A**). The μ of fluid gel samples decreased on decreasing gellan concentrations as shown in **Figure 4B**.

3.3 Effect of Gellan Gum on Release and Penetration of Diclofenac

The permeation flux of diclofenac sodium was measured *ex-vivo* through porcine ear skin. The permeability of diclofenac from the formulations with different gellan gum fluid gel concentrations and Voltaren® gel across pig ear skin is shown in **Figure 5** while fluxes are summarized in **Table 1**.

The cumulative amounts of diclofenac sodium in the receiver chamber of the Franz cell following the application of gellan gum fluid gel formulations to the porcine skin tissue, were significantly higher ($p < 0.05$) than those obtained by application of the Voltaren® formulation and an aqueous solution of diclofenac, which was used as a control (**Figure 5**).

The flux values indicate that the permeation of diclofenac decreased with decreasing gellan concentrations. Also noticed was that the penetration of the drug released from control formulation was faster than commercial Voltaren® gel with fluxes of 210.24 μg cm^{-2} h^{-1} and 116.99 μg cm^{-2} h^{-1} respectively (**Table 1**). To investigate whether the difference in drug permeation from the different formulations was a result of a more rapid drug release from the vehicles due to negative-negative repulsion force between the drug and the polymer, drug release was determined separately. **Figure 6** shows the *in vitro* diclofenac release and *ex-vivo* diclofenac permeation for 1 % 50:50 LA HA gellan blend fluid gel and the Voltaren® gel formulation, expressed as % of the finite dose in the donor. The results show that there was no significant difference ($p > 0.05$) in release between fluid gel and Voltaren® gel and drug was completely released from both formulations after 8 hours.

Thus, the question arises as to whether the affinity of the anionic charge of gellan gum for Na^+ has a crucial role in increasing drug permeation, due to promotion of dissociation of Na^+ counter ions from the carboxylic acid group on the diclofenac and hence increasing

quantity of unionized drug. To address this question, the 50:50 LA HA gellan blend fluid gel, at a concentration of 1 %, was formulated with increasing NaCl concentrations to provide an increasing reservoir of Na^+ for the gellan to bind and consequently discouraging dissociation of the Na-diclofenac. The results highlighted that the diclofenac permeation was strongly affected by NaCl concentrations (**Figure 7**).

There was no significant difference ($p > 0.05$) in permeation between formulations with no NaCl and 0.5 % NaCl. Increasing NaCl concentration further however, led to slower diclofenac penetration. Interestingly, permeation of diclofenac from the gellan formulation containing 2 % NaCl was almost similar to that of the Voltaren® gel (**Figure 7**). To analyse how the drug escaped from formulation vehicle and was deposited in the different compartments (receiver, membrane and donor) of the Franz cell, the percentage of diclofenac in each compartment was plotted vs. time. The results showed that the drug migration rate from the vehicle to the membrane is much higher for gellan formulation (**Figure 8A**) compared with Voltaren® gel (**Figure 8B**). In the case of the gellan formulation after four hours ~90 % of the drug passed out the formulations towards the skin, while only ~20 % of the drug migrated into skin after the same time for the Voltaren® gel (**Figure 8A and B**). The results for the 50:50 LA HA gellan fluid gel formulations highlighted that the percentage of drug increased sharply and accumulated in the skin tissue until a maximum was reached (approximately ~70 %) after four hours. The drug percentage in the skin then began to decrease gradually until the entire drug had penetrated into the receiver (**Figure 8A**). The Voltaren® gel, however, behaved differently as the drug concentration in the skin reached a maximum of ~20 % of the drug at four hours and then plateaued until the end of the test (Figure 8B).

4 Discussion

The use of fluid gels as a platform technology for pharmaceutical formulations has great potential due to the tuneable mechanical properties and their ease of manufacture. In previous studies, the potential use of gellan gum fluid gels in pharmaceutical formulations was presented; these have included an oral liquid formulation (Mahdi *et al*., 2014) and a nasal spray (Mahdi *et al*., 2015). Here, the investigation focused on the potential use of gellan gum fluid gels for topical formulations. It has previously been reported that stress sweeps can reflect the gel strength and yield stress of hydrated polysaccharides (Farrés and Norton, 2015). Through the yield stress measurements, the relative forces required to dispense from a tube can be predicted, thus, ensuring that the formulation will not leak easily. The value of G′ gives an indication of gel stiffness (Huang *et al*., 2004) while; the yield stress value provides an indication of particle-particle interaction. Therefore, the reduction in G′ in HA gellan gum formulations can be explained by softer particles being produced and the reduction in the yield stress value for LA gellan indicates a reduction in the degree of particle-particle interactions (**Figure 1A**) (Farrés and Norton, 2015). The 50:50 LA HA gellan blend fluid gel had an elastic modulus and yield stress value approximately between that of the LA gellan and the HA gellan sample (**Figure. 1A**) as previously reported with 0.25 % gellan fluid gel formulations (Mahdi *et al*., 2015). Interestingly, the 50:50 LA HA gellan blend fluid gel exhibited similar yield stress with slightly greater stiffness to that of the Voltaren®gel (**Figure 1A**). Therefore, a similar force would be required to squeeze such a formulation from a tube during application. For this reason, the 50:50 LA HA gellan blend fluid gel formulation was used further to study the effect of NaCl and gellan concentration on the gel strength and particle-particle interaction (**Figure 1B and C** respectively).

Ions can promote aggregation of polymer chains in charged polysaccharides such as gellan and this usually leads to increased gel strength. The increase in gel strength usually

continues with increasing ion concentrations until maximum gel strength is reached. At higher ion concentrations excessive aggregation can occur, leading to collapse of the gel structure and ultimately, precipitation of the polymer (Morris *et al*., 2012). This phenomenon was apparent in the results shown in **Figure 1B** as there was significant increase in G′ in the 1 % w/w 50:50 LA HA fluid gel formulation from 85 Pa to about 400 Pa with increasing the ion concentration to 0.5 % w/w, suggesting that the Na^+ at this level was ≤ the maximum gel strength. By increasing the NaCl concentration further to 1 %, and 2 % w/w the value of G′ reduced to approximately 110 Pa and 66 Pa respectively, indicating that the ion concentration for maximum gel strength was exceeded, causing a reduction in gel strength.

The viscoelastic properties (e.g. elastic modulus) and particle size of fluid gel can be changed by changing polymer concentrations (Norton *et al.,* 1999; Mahdi *et al.,* 2014). The results in **Figure 1C** are in good agreement with the previously reported data as they show that G′ decreased with decreasing gellan concentrations. The reduction in yield stress with decrease in gellan concentration could be explained by smaller particles being formed which then resulted in a decline in particle-particle interactions and hence, less stress required for yielding (**Figure 1C**).

Rheological testing can provide a useful prediction to evaluate the spreadability of a topical gel preparations, (Garg *et al*., 2002). Viscosity measurements at different shear rates from 20 to 200 s^{-1} have previously been shown to be a suitable range to model the spreading behaviour of topical formulations (Boylan, 1966). The viscosity profiles for 1 % w/w 50:50 LA HA gellan blend fluid gel and Voltaren® gel exhibit shear thinning behaviour; the gellan gum formulation however, shear thined to a slightly greater extent than Voltaren® gel, indicating potential advantageous spreadability of the gellan formulation (**Figure. 2**).

The Stribeck curve of LA gellan fluid gel followed behaviour slightly different from what had been previously reported by Gabriele *et al*., (2010). They described the lubrication

mechanism in three distinctive behaviours with increasing speed (boundary, mixed and hydrodynamic). They assumed that in both the boundary and mixed regime the gel particles are excluded from the gap, and the thin film thickness is responsible for lubrication under these conditions.

In gellan gum fluid gel systems, however, the Stribeck curve showed a small peak at low speeds (boundary regime) suggesting that the smaller fraction of fluid gel particles, rather than being excluded at low speeds, are entrained between the tribo-surfaces thereby providing friction between the two rotating surfaces together with the ungelled medium (**Figure 9A-B**). On increasing the speed further, rearrangement of the entrained particles occurs and gap size increases as the un-gelled medium forms a layer over entrained particles, providing lubrication by preventing contact between the two rotating surfaces (**Figure 9B-C**). This causes the friction coefficient to decrease slightly and plateau. As the rotation speed is increased further, the larger fluid gel particles are gradually entrained within the gap and this results in an increase in friction coefficient and a second peak appears (**Figure 9D**) (Farrés and Norton, 2015). According to De Wijk and Prinz, (2005), at this stage only the gel micro particles are responsible for separating the gap which results in a higher friction coefficient. At rotation speeds of ~ 150 mm s^{-1} and higher, more fluid gel particles become entrained between the ball and the disk. Multilayers of gel particles then cause the two surfaces to be further apart, therefore friction coefficient begins to reduce again (**Figure 9E**) (Gabriele *et al*., 2010).

The Stribeck curve of HA gellan fluid gel, however, suggests a different mechanism. There is no peak observed at mixed-hydrodynamic regime (Figure 4A), and this is thought to be due to the elastic properties of gel particles which makes it able to deform to resist the external applied force (**Figure 10**). Therefore less frictional force is measured. The 50:50 LA HA gellan blend fluid gel formulation showed intermediate behaviour between the two

mechanisms. The peak in mixed regime is less pronounced (**Figure 4A**). This can be explained by softer and less brittle gel particles formed compared with the LA gellan system (Huang *et al*., 2003). The onset of the peak in the mixed regime occurs at higher speeds compared with the curve obtained from the LA gellan fluid gel samples, this could suggest that there is a greater fraction of larger gel particles formed in 50:50 LA HA gellan blend fluid gel. This explanation has been reported previously for alginate fluid gels (Farrés *et al.*, 2013; Farrés and Norton, 2015).

As discussed earlier, by decreasing gellan gum concentration, smaller gel particles are produced that have a reduced yield stress due to a reduction in particle-particle interactions. This could explain the lower friction coefficient values obtained from formulations containing lower gellan concentrations (**Figure. 4B**), whereby the fluid gel particles are responsible for the friction at all rotation speeds.

The permeation study and flux values (**Figure 5; Table 1**) highlighted that diclofenac permeation is higher in the case of gellan gum fluid gel formulations compared with Voltaren® gel. This could be explained by changing the degree of ionization of diclofenac due to competitive interaction between carboxylic acid groups of both gellan and diclofenac for the sodium ions. The degree of ionization of drugs has an effect on drug partitioning into the skin (Kalaria *et al*., 2012). The free acid of diclofenac can permeate the skin faster than diclofenac salts (Minghetti *et al*., 2006). At high gellan gum concentrations more carboxylic acid groups are available to bind with sodium ions hence, more unionized diclofenac will be available to penetrate the skin. This could explain the difference in drug permeation with different concentrations of gellan (**Figure 5**).

It seems that the mechanism by which hydrophobic drugs such as diclofenac penetrate the skin depends on drug-vehicle interaction (Sintov and Botner, 2006). Therefore, the permeation of the drug from the aqueous control was higher compared with the Voltaren®

gel. This could be explained by lipophilic excipients that are in the Voltaren® gel formulation, inhibiting drug penetration (Goh and Lane, 2014) or the gel controlling drug release.

Figure 6 illustrates that the release of diclofenac from both Voltaren® gel and gellan formulations follow the same trend and the drug leaves the vehicle and becomes available to the skin at the same rate. Thus, indicating that the difference in penetration was likely due to an increased degree of drug ionization and not a result of electrostatic repulsion force between drug and anionic gellan polymer. This is further supported by the data presented in **Figure 7**. The addition of sodium ions to the system causes an increase in the amount of sodium available to the gellan and therefore, the dissociation of Na-diclofenac will be less likely to occur.

The results obtained shown in **Figure 8A** indicated that the diclofenac released from 50:50 LA HA gellan blend formulation had higher deposition within the skin compared with the drug released from Voltaren® gel (**Figure 8B**). In fact, after 4 hours the entire diclofenac drug load had migrated from gellan vehichle (the donor chamber) to the skin and a depot or reservoir of the drug was formed within the skin. It is thought that the deposition and accumulation of the dug in this layer determined the rate of permeation controlling the release from skin to the receiver in a controlled manner.

5 Conclusion

In this study, it has been demonstrated that gellan gum fluid gel blends have the potential to be formulated with a similar rheological and mechanical profile to that of a marketed formulation (Voltaren® gel). The stress required for the gel to flow depends on LA:HA ratio, overall concentration of both polysaccharides and ion concentration. It was shown that gel particles have an effect on lubrication properties of the formulations and therefore the potential to improve tactile perception. Furthermore, penetration of diclofenac

across the skin barrier was dependent on the vehicle-drug interactions and could be controlled by simple addition of counter ions. The relatively simple formulation and manufacturing process, tuneable physical behaviour and increased drug permeation, highlight the potential of gellan gum fluid gels as an alcohol-free alternative to current commercially available topical diclofenac formulations.

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Table 1 Flux (J) values for fluid gel formulations 50:50 LA HA gellan blend fluid gel cross linked with 0.5 % NaCl at different concentrations of gellan compared with those of the control and proprietary formulation.

Formulations	**J ($mg/cm^2.h^{-1}$)**
1% LA HA gellan blend fluid gel	427.68
0.25% LA HA gellan blend fluid gel	364.77
0.1% LA HA gellan blend fluid gel	238.06
Control solution	210.24
Voltaren® gel	116.99

Figures

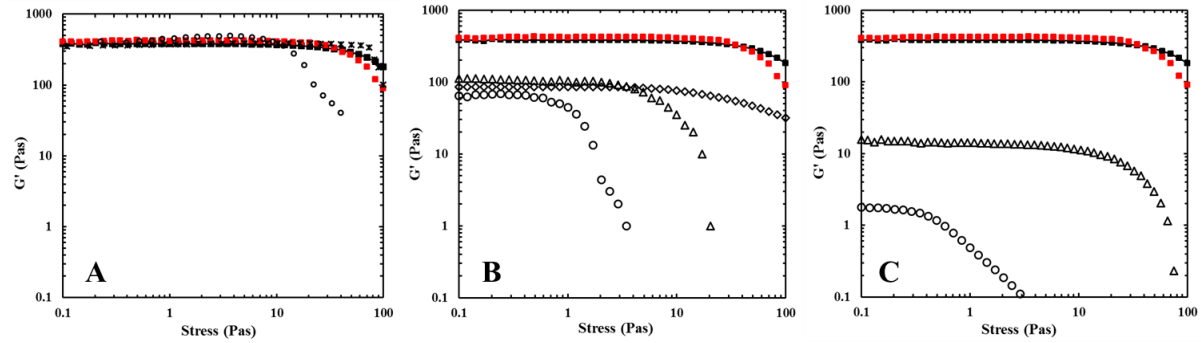

Figure 1(A) Stress sweep for 1 % gellan fluid gels crosslinked with 0.5 % NaCl as a function of HA LA ratio (LA gellan open circles, HA gellan black stars and 50:50 LA HA gellan blend filled red diamonds) (B) stress sweep for 1 % w/w 50:50 LA HA gellan blend fluid gels crosslinked with 0% (open diamonds), 0.5 % (filled red diamonds), 1 % (open triangles) and 2 % (open circles). (C) Stress sweep for 0.1 % (open circles), 0.25 % (open triangles) and 1 % (filled red diamonds) w/w 50:50 gellan LA HA blend fuid gels at 0.5 % w/w NaCl. Voltaren® gel 1 % presented in all three graphs as open circle. Voltaren® gel 1 % diclofenac sodium stress sweep presented in all three graphs as filled squares.

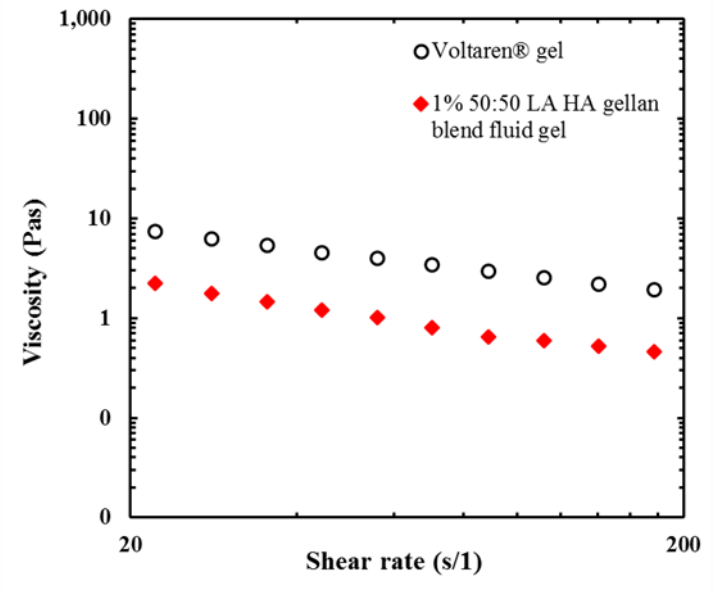

Figure 2 Viscosity vs. shear rate at 32 °C for 1 % w/w (filled-red diamonds) LA HA gellan blend fluid gels at 0.5 % w/w NaCl and for Voltaren® gel 1 % diclofenac sodium (open circles).

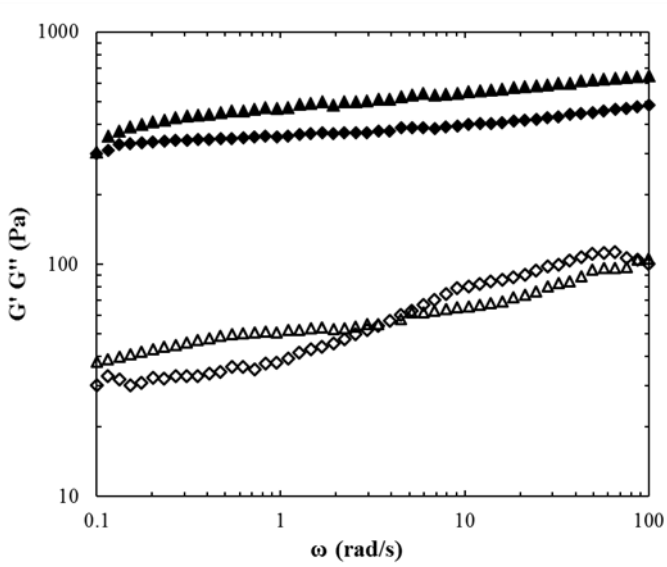

Figure 3 Mechanical spectrum (0.5 % strain; 32 °C) of a 1 % 50:50 LA HA gellan blend loaded with 1 % diclofenac sodium showing variation of G′ (filled triangles), G″ (open triangles) and of Voltaren® gel 1 % diclofenac sodium G′ (filled diamonds), G″ (open diamonds).

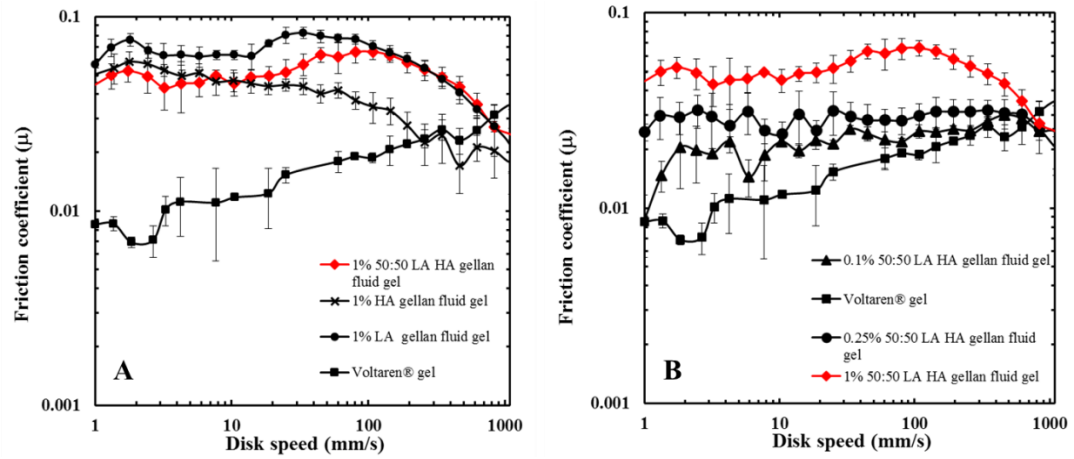

Figure 4 (A) Stribeck curves for 1% gellan fluid gels crosslinked with 0.5 % NaCl as function of LA:HA ratio (LA filled circles, HA black stars and 50:50 LA HA blend filled red diamonds), (B) Stribeck curves for 0.1 % (filled triangles), 0.25 % (filled circles) and 1 % (filled red diamonds) w/w 50:50 LA HA gellan blend fuid gels at 0.5 % w/w NaCl. Stribeck curves for Voltaren® gel 1 % diclofenac sodium presented both figures as filled squares.

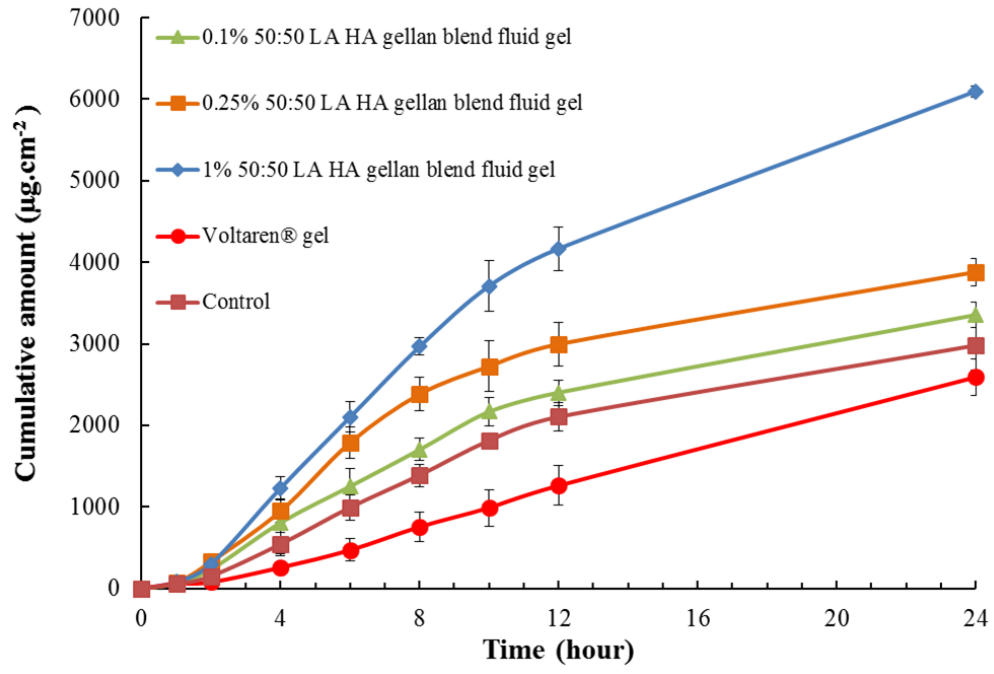

Figure 5 Cumulative amount µg.cm^{-2} of diclofenac sodium permeated from 50:50 LA HA gellan blend fluid gel formulations prepared at different concentrations compared with Voltaren® gel. Values are represented as mean ± SD.

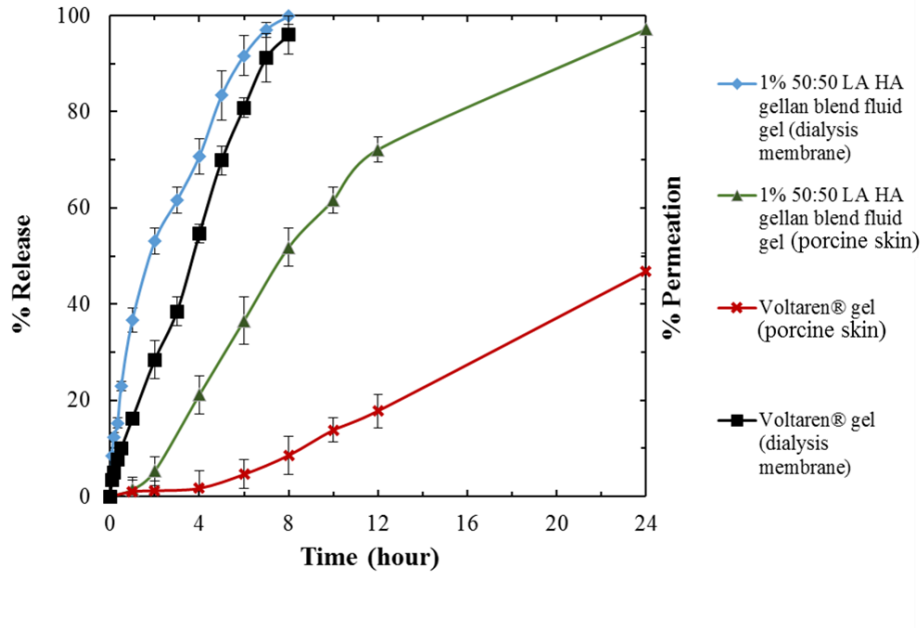

Figure 6 Cumulative % release of diclofenac from 1 % w/w 50:50 LA HA gellan blend fluid gel (blue diamonds) and from Voltaren® gel (black squares) and cumulative % permeation of diclofenac from 1 % w/w 50:50 LA HA gellan blend fluid gel (green triangles) and from Voltaren® gel (red crosses) (n=3).

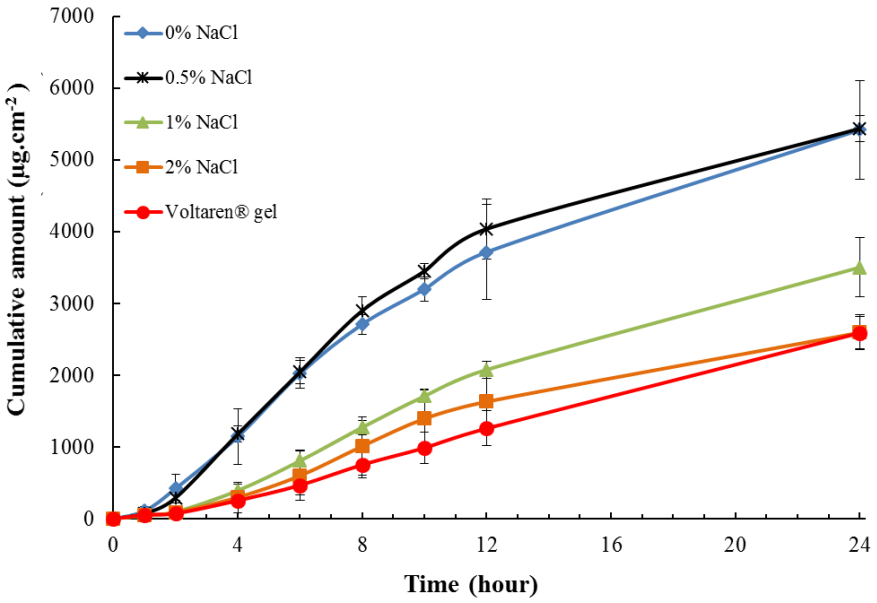

Figure 7 Cumulative amount µg.cm^{-2} of diclofenac sodium permeated from 1 % w/w 50:50 LA HA gellan fluid gels prepared at different NaCl concentrations compared with Voltaren® gel. Values are represented as mean ± SD (n=3).

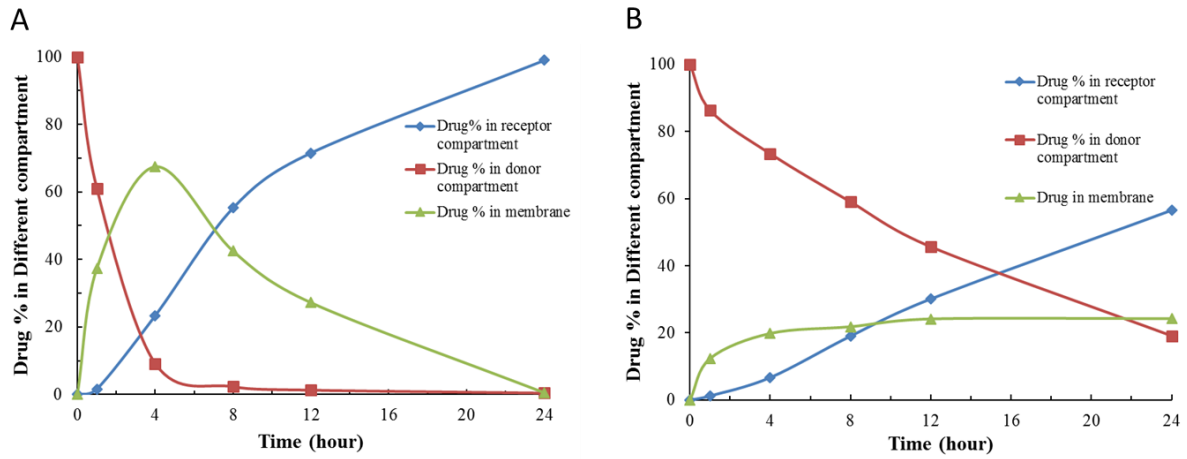

Figure 8 Drug % profile in different compartments of the Franz cell, donor (red line), membrane (green line) and receiver (blue line) for A) 1 % gellan 50:50 LA HA gellan blend fluid gel and B) Voltaren® gel.

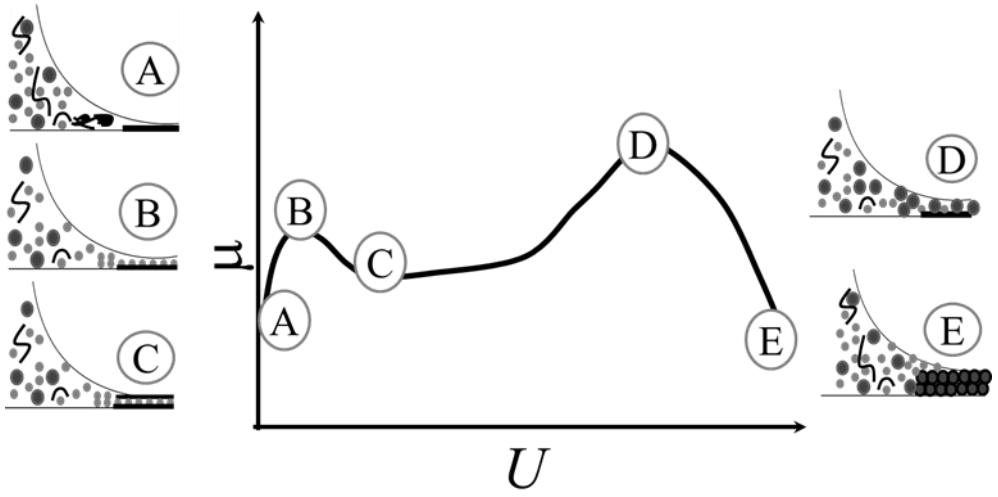

Figure 9 Schematic representation of the proposed mechanism of fluid gel lubrication. (A-B) represents the boundary regime. (C-D) represents the mixed regime and (E) represents the hydrodynamic regime.

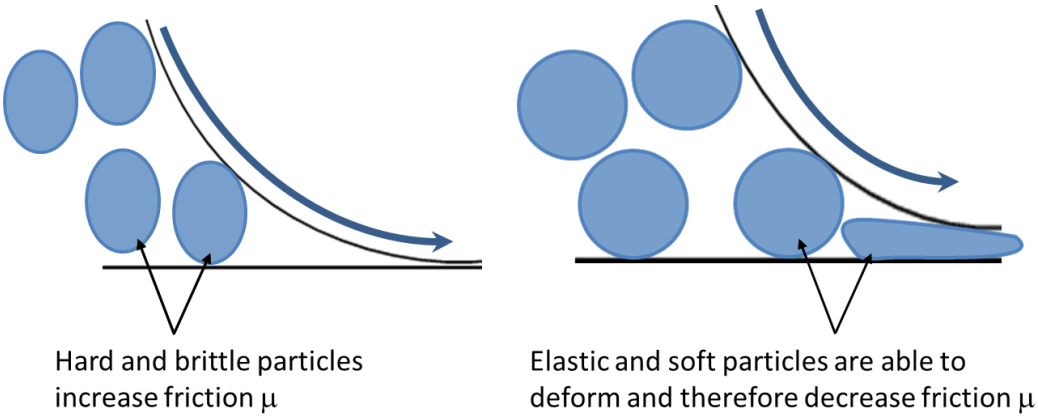

Figure 10 Illustrates the two different behaviour of gellan particles A) hard and brittle (LA gellan particles) and B) Soft and elastic particles (50:50 LA HA gellan and HA gellan particles).