DEVELOPMENT OF GELLAN GUM FLUID GEL AS MODIFIED RELEASE DRUG DELIVERY SYSTEMS

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Abstract

Gelation of polysaccharides under shear conditions results in the formation of a weak gel which is able to resist elastic mechanical deformation at small strains but will flow if subjected to higher strains. The resulting material, described in the literature as a fluid gel or a sheared gel, consists of gelled microparticles which can be formulated to collectively act in bulk, as pourable viscoelastic fluids whilst retaining true gel characteristics at the micro/nano level. The tuneable behaviour of these fluid gel systems makes them potentially useful in pharmaceutical applications. Fluid gels prepared from gellan gum are particularly attractive, due to its sensitivity to physiological fluids, unique rheological and physical properties, and current regulatory approval for use as a food additive and pharmaceutical excipient. Therefore, the aim of the present study was to investigate gellan gum fluid gels as a new modified release drug delivery platform. The formation and production of fluid gels using low acyl (LA) gellan, high acyl (HA) gellan and LA HA gellan blends was investigated and applied in three different dosage forms; a modified release oral liquid, a mucoadhesive nasal spray and a topical formulation.

A modified release oral liquid was designed using a fluid gel prepared from LA gellan gum. It was demonstrated that 0.75 % w/w LA gellan gum fluid gel, containing ibuprofen as the drug, could be formulated to have a similar viscosity profile as a marketed oral ibuprofen liquid. Furthermore, due to the acid insolubility of gels prepared from LA gellan, no ibuprofen was released in stimulated gastric fluid. Subsequent release at pH 7.4 however, was affected by the duration of exposure and strength of the acidic pH used and a linear relationship between onset of release and the preceding duration of acid exposure was observed. Delayed release was a result of increasing gel stiffness, a consequence of the acidity of the initial release media and exposure time. A much faster release rate was measured when exposure time in acid was 10 min compared with 60 min. This study highlights the potential to design fluid gels that are tuned to have a specified stiffness at a particular pH and exposure time allowing the intelligent design oral liquids with specific modified release behaviour.

The second part of this study was to prepare mucoadhesive nasal drug delivery systems to enhance the retention of the nasal spray dosage form in the nasal cavity. Several groups have investigated using LA gellan solution as a drug delivery vehicle but only limited research however, has been performed on HA gellan for this purpose, despite its properties being more conducive to mucoadhesion. HA gellan (even with low concentration 0.25 % w/w) produces highly elastic gels below 60 °C which make it difficult to spray using a mechanical spray device. To address this problem, fluid gels were prepared as these systems can behave as sprayable viscoelastic fluids. In this study the rheological behaviour was investigated and the mucoadhesion behaviour of fluid gels prepared from the two different types of gellan (HA and LA) and fluid gels prepared from a blend of LA HA gellan. The results demonstrated that by preparing fluid gels from a blend of LA HA gellan, the rheological properties were sufficient to spray through a standard nasal spray device. Moreover, the fluid gels significantly enhanced both HA and LA gellan mucoadhesion properties.

In the final part of this thesis the topical application of gellan fluid gels was explored. A range of gellan fluid gel formulations were prepared containing diclofenac sodium for topical application. The rheological results showed that it was possible to produce a topical formulation with a viscosity and the mechanical strength similar to that of the commercially available Voltaren® gel using 1 % w/w of a 50:50 LA HA gellan blend. The permeation results highlighted that the penetration of diclofenac through procaine tissue is significantly increased by increasing gellan concentration and decreasing sodium ion concentration in the formulation.

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This thesis is dedicated to

My Parents,

My Sisters,

My Brother Ali,

I will always Love you

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Chapter 1

General Introduction

CHAPTER 1 GENERAL INTRODUCTION

1.1 Introduction

This chapter will discuss the general use of polysaccharides in pharmaceutics, providing a brief introduction on applications in current drug delivery systems. The physical and chemical properties of gel forming polysaccharides are a particular focus, in relation to use as physiologically responsive excipients and drug delivery vehicles in both standard and modified release systems. Despite the potential benefits of immediate release dosage forms, they do have some critical drawbacks in particularly related with the stability of drug molecules within the digestive system and their subsequent bioavailability. This often results in a requirement of frequent dosing intervals that can reduce patient compliance due to multiple dosing regimens (Tao and Desai, 2003; Builders and Attama, 2011).

To overcome these challenges many researchers pointed to a modified release dosage form using polysaccharides with desirable functional properties. The physicochemical and biodiversity of these polymers coupled with their easy modification have potentially resulted in the availability of a large array of functional polymers that are useful for modified drug delivery applications.This chapter will also introduce the concept of polysaccharide "fluid gels" (or sheared gels) as a potential platform technology for controlled release applications, which is the major focus of this thesis.

Almost all therapeutic products include excipients within the formulation. In fact, the total amount of some excipients used is greater than the amount of active material used in the formulation (Bhattacharyya *et al*., 2006). Historically, excipients have been defined as inactive ingredients within medicines. More recently, however, excipients are considered not as inactive, but to have an important impact on quality, safety, and efficacy of dosage forms (Koo,

2011). Furthermore, careful selection of excipients can have a greater impact on the functionality of the dosage form by controlling or even targeting drug release at the desired site of uptake (Karolewicz, 2015).

Polysaccharides are one class of materials that are frequently used as excipients. This is due to the large variety of polysaccharides available, which can have a wide diversity of chemical compositions and physical behaviour. Traditionally, polysaccharides were used as fillers, binders, and disintegrants in tablets or as thickeners in oral liquids, but are now finding ever increasing, more sophisticated, defined functional roles to create novel dosage forms (Karolewicz, 2015). These include, modulating solubility and bioavailability (Khadka *et al*., 2014), enhancing permeability (Fasano, 1998; Thanou *et al*., 2001; Kawabata *et al*., 2011), improving stability, maintaining pH and osmolarity of liquid formulations, acting as antioxidants, emulsifying agents, modulating the immunogenic response of active ingredients (Aleeva *et al*., 2009; Karolewicz, 2015) and controlling drug release (Pal, *et al*., 2013).

1.2 Polysaccharides

Polysaccharides are biological macromolecules composed of repeated monosaccharide sub-units that are connected to each other by *O*-glycosodic bonds. They occur widely in nature in animals, plants and microorganisms and have diverse biological functions such as energy sources, structural support, physical protection, lubrication and maintaining hydration in tissues (Izydorczyk, 2005). These diverse biological functions have inspired a wide variety of industrial applications that include: as a nutritional component and/or to create texture in foods, scaffolds for tissue engineering, encapsulation of drugs, cosmetics and personal care products, and as wound healing dressings (Malafaya *et al.*, 2007; Builders and Attama, 2011).

The wide range of industrial applications is a result of the chemical and structural variety of polysaccharides that have their own unique physicochemical properties and therefore

functional versatility (Cunha and Grenha, 2016). This functional versatility has led to many different types of polysaccharides being utilised in pharmaceutical formulations as functional excipients in a range of dosage forms. Furthermore, polysaccharides are renewable resources that are relatively cheap compared with their synthetic counterparts, which make them particularly desirable for use in many applications (Poli *et al*., 2010; Malafaya *et al.*, 2007).

The biological origin of polysaccharides, does however, impart a susceptibility to biological variation. This can be minimised by using refined processing techniques to optimise batch-to-batch consistency (Kontogiorgos, 2014). Despite the potential for batch-to-batch variations biopolymers are attractive materials for use in designing dosage forms and have shown wider therapeutic applications as biomedical materials because of their biocompatibility and biodegradability (Builders and Attama, 2011).

1.2.1 Polysaccharide Structure

There are many different types of polysaccharides that are produced by the linking together of simple monosaccharides through *O*-glycosodic bonds. This involves a condensation reaction between the OH group at C-1 of a hemiacetal on one residue and one of the -OH groups of the adjacent residue, with the elimination of water (Figure 1.1) (Pérez and Kouwijzer, 1999). The orientation of O-1 can be axial (α) or equatorial (β) and bond to the next residue at any of the other -OH groups at C-2, C-3, C-4 or C-6. This results in eight possible bonding arrangements making it possible for several different polysaccharides to be assembled from the same monosaccharide unit (Rees, 1975; Pérez and Kouwijzer, 1999; Rees, 2012).

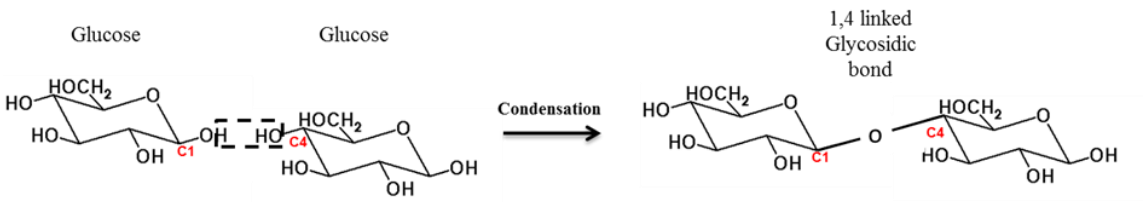

Figure 1.1 Formation of a simple glycosidic bond between monosaccharide units (adapted from Rees, 1975).

The physical behaviour and functional properties of polysaccharides are determined by the structure of the monosaccharide units in the polymer chain, orientation of the glycosidic bonds and number of individual monosaccharides that make up the polymer i.e molecular weight (Boddohi and Kipper, 2010).

Polysaccharides that consist of a single type of monosaccharide are called homopolysaccharides. Cellulose, amylose and dextran for example are all linear polysaccharides that consist of repeating glucose units. Homopolysaccharides can also be branched, having a repeating backbone with small side chains, which can also be branched (amylopectin and glycogen). Homopolysaccharides are classified according to the constituent monosaccharide in the polymer chain. Cellulose amylose, amylopectin, dextran and glycogen are all homo-polysaccharides made from glucose and are classified as glucans. Homopolysaccharides consisting of only mannose would be classified as mannans, and those consisting of only galactose would be galactans etc (McNaught, 1997).

When polysaccharides are composed of two or more different types of monosaccharide units, they are described as heteropolysaccharides. These can be linear or branched and usually contain a regular repeating sequence (McNaught, 1997; Robyt, 1998; Kontogiorgos, 2014). This type of structure is found in the seaweed polysaccharides, carrageenan (Cunha and Grenha, 2016) and agarose, which are both linear heteropolysaccharides with a disaccharide repeating sequence. Block wise structures can also occur as in alginate whereby the linear chain

consists of two different monosaccharide units arranged as homopolymeric blocks of each single monosaccharide and heteropolymeric sequences of both monosaccharides, effectively having homopolymeric regions and heteropolymeric regions within the same molecule (Gacesa and Russell, 1990; Izydorczyk *et al*., 2005). Other more complicated heteropolysaccharides are produced by bacteria such as xanthan, which consists of a repeating unit of five sugar residues and gellan gum, which has a tetrasaccharide repeating unit (gellan gum structure is discussed in detail in Chapter 3). There are also polysaccharides that have no repeating units that can also be densely branched creating complex structures like those within some exudate gums such as gum arabic.

1.2.2 Ordered and Disordered Conformation

Like most polymers, polysaccharides can exist in both ordered and disordered conformations. When hydrated disordered conformation is more common as there is usually more flexibility in the glycosidic bonds. In the disordered form, polysaccharide chains are often termed random coils (Wang and Cui, 2005). The relative orientation of the monosaccharides across the glycosidic linkage and the subsequent intra-molecular forces determine the overall shape of the polysaccharide chain. This can therefore, give rise to a range of chain shapes, which influence the physical behaviour (Wang and Cui, 2005; Kontogiorgos, 2014).

Generally, there are three major ordered orientations that polysaccharides adopt as a result of how the monosaccharide units are linked (Rees, 1975; Morris and Walsh, 1982; Wang and Cui, 2005). When two equatorial linkages join the monosaccharide units together diagonally across the saccharide ring, long extended structures occur which is termed flattened ribbon geometry (Figure 1.2). Here, the monosaccharide residues are parallel and only partially out of alignment from one another. In the disordered state, this geometry generally produces

random coils with large dimensions. Hydroxypropylmethylcellulose (HPMC) is an example of a polysaccharide used in pharmaceuticals that adopts this geometry (Wang and Cui, 2005).

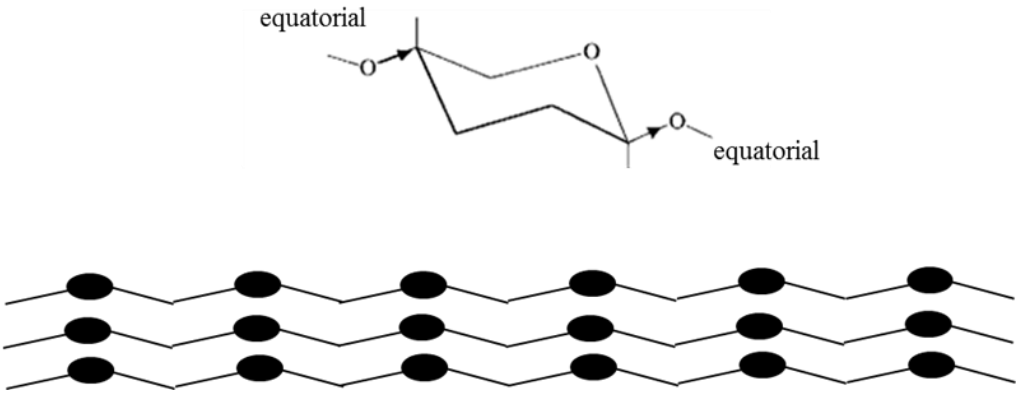

Figure 1.2 Flattened ribbon geometry (adapted from Wang and Cui, 2005).

If the glycosidic bond is via two axial linkages at C→1 and C→4, buckled ribbon geometry occurs. In this case, the linking bonds are also parallel, but are a full width of the individual monosaccharide offset from one another (Figure 1.3) (Wang and Cui, 2005). An example of this kind of geometry in a pharmaceutical polysaccharide is the poly-L-guluronate sequences of alginate. This conformation introduces large cavities to adjacent chains, which are of sufficient size to incorporate metal ions which in charged polysaccharides, such as alginate, can reduce electrostatic forces of repulsion between chains and stabilise the ordered structure. The random coils in the disordered state are usually more compact.

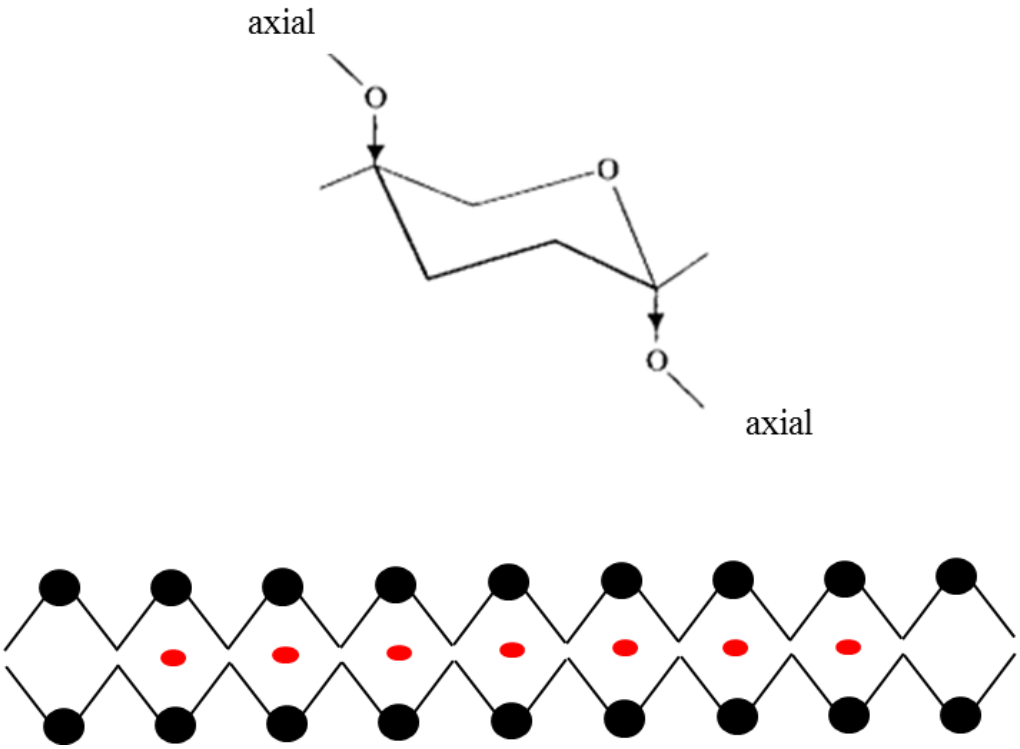

Figure 1.3 Buckled ribbon geometry with metal ions in the cavities between the chains (adapted from Wang and Cui, 2005).

In cases where the linkages are axial-equatorial the bonds between the monosaccharides are not parallel. This is the case in amylose (a component of starch), which is 1→4 axial-equatorial linked, and causes the formation of a helix (Figure 1.4) (Wang and Cui, 2005). In addition, 1→3 diequatoral linkage geometry also can causes helical conformation in the ordered state. In heteropolysaccharides where both ribbon-forming and helix-forming linkages are present, the conformational twist due to the axial-equatorial bonding or 1→3 diequatorial bonding produces an overall helical structure. This conformation is adopted by agarose, carrageenan and importantly for the context of this work, gellan gum. In the disordered form, these chains exist as extremely compact random coils.

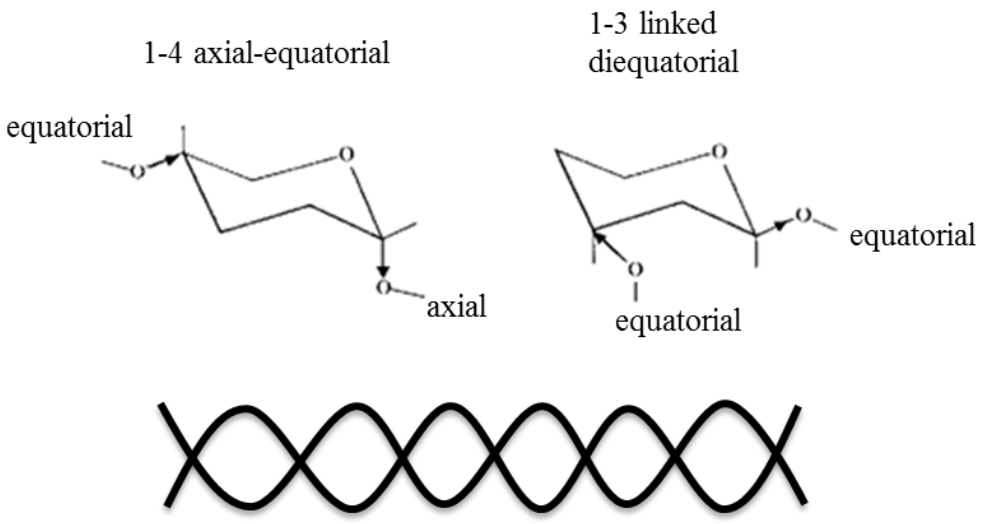

Figure 1.4 Helical geometry (adapted from Wang and Cui, 2005).

Although random coils are energetically favourable for polysaccharides in the hydrated state, conformational ordering can still occur but requires energy from interactions between the chains. These interactions include hydrogen bonding between monosaccharide residues on adjacent chains, electrostatic interactions and van der Waals forces of attraction. These non-covalent interactions are not sufficiently strong enough to stabilise adjacent chains from single monosaccharide residue and require approximately twenty participating residues to maintain an ordered conformation (McNaught, 1997). This explains why very short chain polysaccharides cannot form stable ordered structures in the hydrated form.

1.2.3 Polysaccharide Gels

One of the most useful properties of polysaccharides is that they can form firm gels at relatively low concentrations typically between 0.5 – 2.0 % w/w. This has led to multiple applications in the food, biomedical and pharmaceutical industries. The term gel, however, means different things to different industries, highlighted perfectly by Lloyd, (1926) with the definition ‘the colloidal condition, the gel, is one that is easier to recognise than define’. Indeed, within the cosmetic industry there are products such as “hair gel” and “shower gel” and in the pharmaceutical industry “topical gels”. The rheological definition of a gel is “a swollen

polymeric system showing no steady-state flow" which clearly does not describe cosmetic and pharmaceutical gel products. These systems are in fact viscous polymer solutions formed by polymer entanglement (Figure 1.5A) rather than a true gel network (Figure 1.5B) following the rheological definition (Clark and Ross-Murphy, 2009).

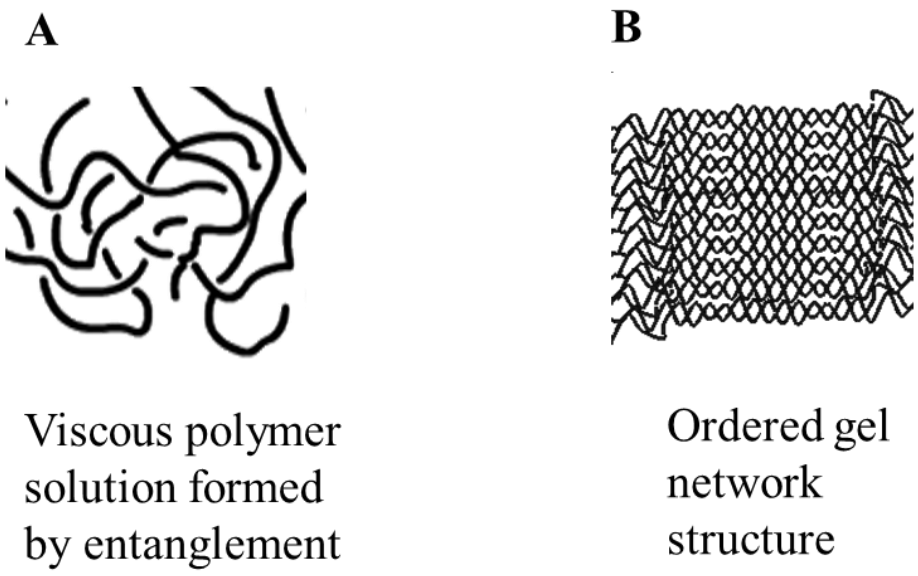

Figure 1.5 Macromolecular differences between A) viscous polymer solutions formed by entanglement and B) an ordered gel network structure.

The formation of solid gel structures is the result of association of individual polymer chains forming a three dimensional network with the pores filled with water that prevent the polymer network collapsing into a compact mass (Solari, 1994). In synthetic polymers, the network structures are often held together by strong covalent chemical crosslinks. In polysaccharides, however, the associations are held together by weaker physical interactions along specific regions of the polymer chains that form ordered junction zones (Figure 1.6) (Pérez and Kouwijzer, 1999). These junction zones in polysaccharide gels are stabilised by a large number non-covalent interactions (of the kind discussed in section 1.2.2) which include hydrogen bonding, van der Waals forces, dipolar interactions, hydrophobic interactions and

anion-cation interactions (Clark and Ross-Murphy, 1987). There are also solubilising regions of the polymer network, which prevents precipitation of the polymers.

The extent of intermolecular association impacts upon the mechanical behaviour of gel networks i.e., when most of the polymer chains participate in the junction zones the resulting gel is likely to be strong and brittle, whereas, in networks where solubilising regions dominate weaker gels are produced that are often less rigid. The variable nature of these physical interactions, enable polysaccharide solutions to form gels as a result of changes to the local environment i.e., change in conditions such as temperature, ionic strength or pH. Moreover, the fact that these physical interactions are relatively weak, the gels can also melt or dissolve in response to the local environment, which provides opportunities for designing systems with physiologically responsive functionality. This has driven the wider application of polysaccharides within the pharmaceutical industry. Indeed, the different origin and nature of polysaccharides and the presence of specific functional groups in their molecular chains gives them diverse functional and physicochemical characteristics and allows considerable versatility in potential uses, especially in formulation and drug delivery applications.

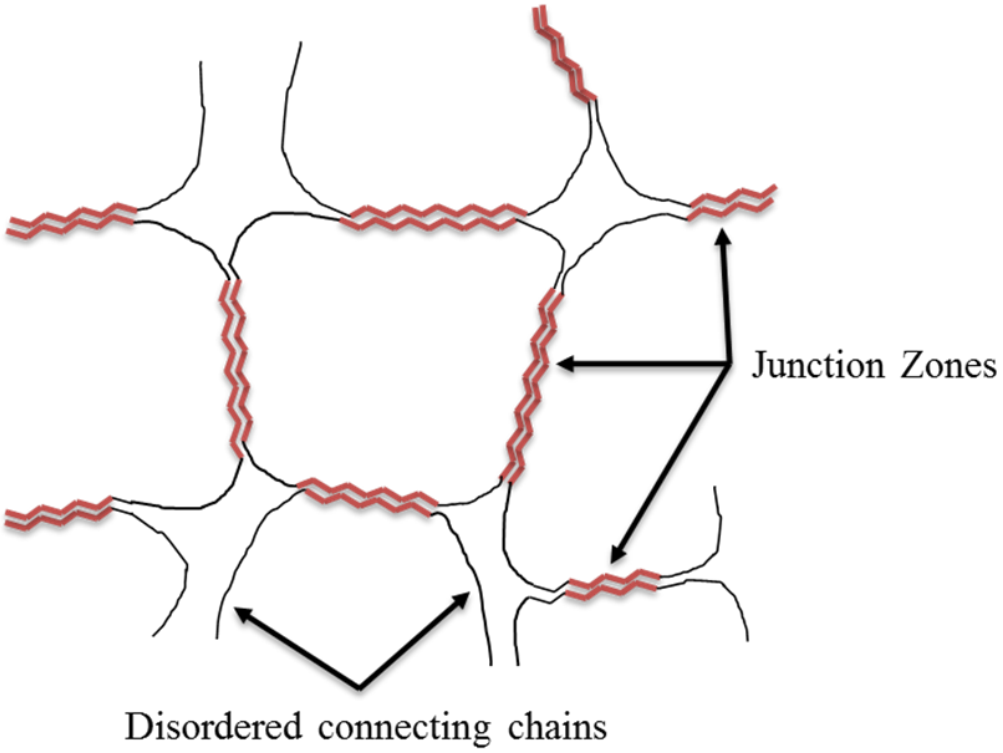

Figure 1.6 Schematic representation of a polysaccharide gel network showing ordered junction zones and disordered connecting chains.

1.3 Pharmaceutical Applications of Polysaccharides

Polysaccharides are used in a range of solid, liquid and semi-solid dosage forms (Beneke, *et al*., 2009). Many of the polysaccharides (and semisynthetic polysaccharides) that are used in pharmaceutical formulations are employed to improve the general properties of the existing dosage form. For example, microcrystalline cellulose is used in immediate release (IR) tablets as a binder, materials such as sodium starch glycolate or crosscarmellose sodium (cross-linked carboxymethyl cellulose) are also added to tablet formulations as superdisintergrants (super swelling materials which can swell to between 8-12 times of their original size on contact with water) disrupting the integrity of the tablet and resulting in disintegration (Grover and Smith, 2009). They are also used in tablets as coating materials to improve the aesthetics of the product, making the formulations more acceptable to the consumer.

1.3.1 Modified Release Formulations

Modified release formulations have superior drug delivery control that is more convenient to the patient than immediate release formulations. Furthermore, modifying drug release can be used to improve the stability, safety, efficacy and therapeutic profile of a drug (Builders and Attama, 2011). Several dosage forms have been developed as modified release systems which include modified release tablets (Gohel and Panchal, 2002), capsules (Smith *et al.*, 2010) and oral liquids (Miyazaki *et al*., 2003). Modified drug delivery systems are generally divided into four categories delayed release, sustained release, site specific release and receptor targeted (Figure 1.7) (Builders and Attama, 2011). In this thesis, the work is focused on delayed and sustained (extended) release systems.

When used in modified drug delivery systems, polysaccharides have demonstrated potential in altering the pharmacokinetics of drugs, which can improve bioavailability. The popularity of polysaccharides over synthetic polymers continues to increase. Apart from their relative non-toxicity and potential biodegradability, polysaccharides have also shown superiority in terms of being more economical, readily available having flexible structural makeup that allows easy modification (Builders and Attama, 2011).

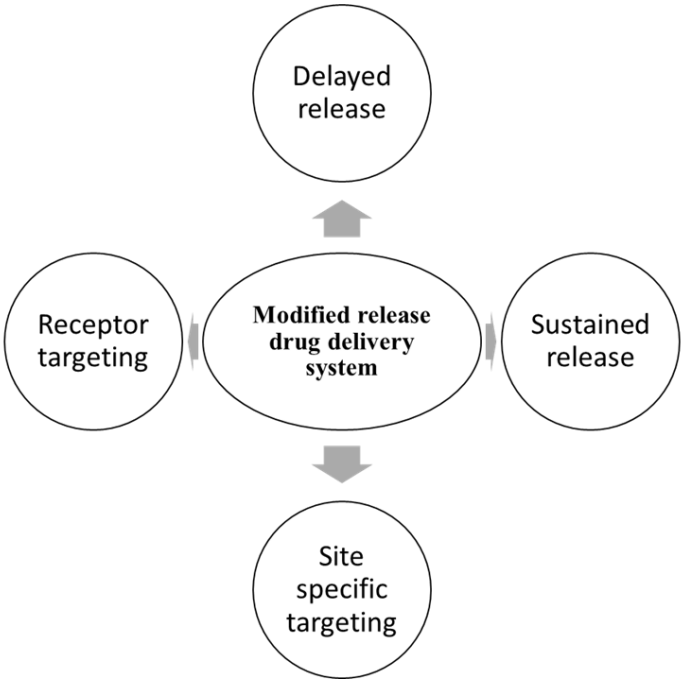

Figure 1.7 Four categories of modified release drug delivery systems.

1.3.1.1 Delayed Release

Delayed release drug delivery systems are designed to release the drugs at time other than administration time. Oral delayed release systems are often used to protect gastric mucosa from irritant bioactive materials and/or to protect certain drugs from low pH of gastric juice e.g. enteric-coated systems (Gruber *et al*., 1987). Furthermore, delayed release systems are often employed in cases where it may be beneficial to target the drug to a specific site along gastrointestinal tract (GIT) such as targeting the drug to the colon. The mechanism of the delay of drug release can be based on time or environmental changes such as pH. Delay in onset of release is followed by either immediate or sustained release of drug (Builders and Attama, 2011).

1.3.1.2 Sustained Release

Sustained release drug delivery systems (SR) are able to maintain the rate of release of the drug for extended periods (Builders and Attama, 2011). The role of ideal drug delivery system is to provide optimum concentration of a drug at the optimum time interval and at the

desired site of action to maintain a therapeutic range of drug in the blood plasma. The issue with immediate release systems is that once the drug has been fully metabolised the therapeutic effect is lost and a repeat dose becomes necessary (Grover and Smith, 2009). This can cause drug plasma concentrations to fluctuate, which could result in significant side effects due to plasma concentrations reaching toxic levels at certain points of the treatment course. This situation can be avoided by using sustained release drug delivery systems, as the drug concentration in the plasma remains within the therapeutic level (Figure 1.8).

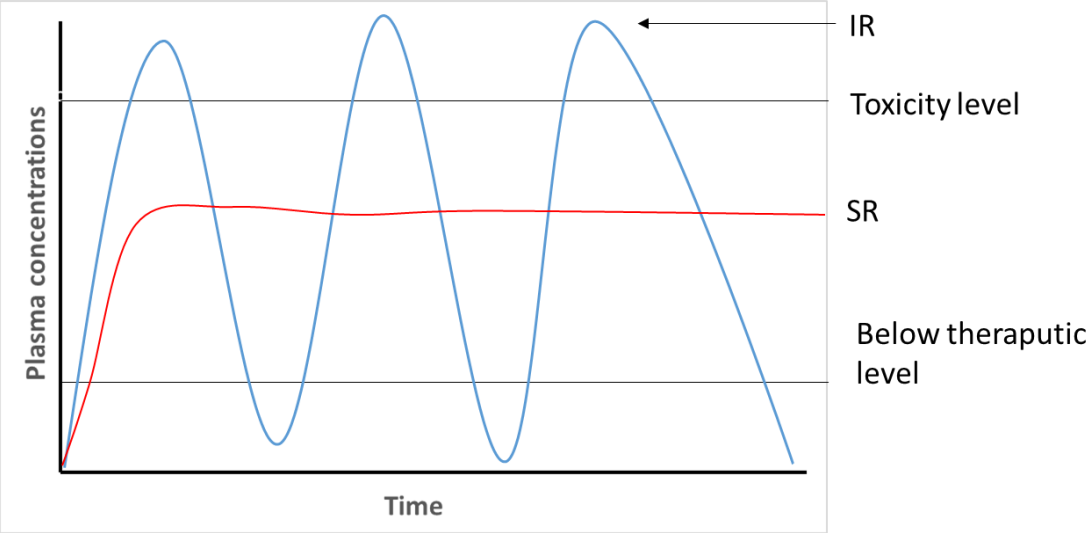

Figure 1.8 Simplified curves showing variation in drug plasma concentrations vs. Time following administration of IR and SR formulations.

1.3.1.3 Polysaccharides in Modified Release Systems

Applying different formulation technologies and taking into account the wide variety of physicochemical and functional properties, polysaccharides are particularly useful in the design of modified release drug delivery systems (Toa and Desai, 2003; Builders and Attama, 2011). Indeed, by understanding and evaluating the physicochemical properties of the vast selection of available polysaccharides, the relevance and functionality can be identified and incorporated into the delivery system. This has guided researchers to use these materials in modified release delivery systems. This approach is applied in sustained release matrix tablet

systems for example, where hydroxypropyl methylcellulose (HPMC) is used. When sustained release tablets formulated with HPMC, encounters the aqueous environment of the stomach, hydration of the HPMC occurs forming a hydrated polymeric layer surrounding the dosage form, which creates a barrier to diffusion of the drug. This is often referred to a gel layer but this should not be confused with a true gel structure containing ordered junction zones as discussed in section 1.2.3., but is the result of polymer entanglement as the HPMC becomes hydrated (Clark and Ross-Murphy, 2009; Builders and Attama, 2011).

The release of drugs from polysaccharide based sustained release tablets can be described as a complex interaction between the rate and extent of the swelling (hydration), diffusion rate of the drug through the hydrated polymeric layer and erosion or dissolution of the hydrated polysaccharides in to the release media (Harland *et al*., 1988, Peppas and Sahlin, 1989; Reynolds *et al*., 1998; Munday and Cox, 2000, Ghori *et al*., 2014 and Nep *et al*., 2015).

Increasing the proportion of polysaccharides in the formulation generally reduces the diffusion of the drug and delays the erosion of the tablet matrix due to an increased swelling volume resulting in a larger hydrated layer and hence larger diffusional path length. The rate of polysaccharide hydration and dissolution can be increased by using lower molecular weight polymers, which reduces the viscosity of the hydrated layer that subsequently increases drug release rate. Polyanionic polysaccharides such as xanthan gum can also be used to sustain drug release. In these systems, however, rate of release can be further dependent on the ionic strength of the release media, as this will affect the hydration and dissolution of the negatively charged polysaccharide (Talukdar and Kinget, 1995).

Although tablets are widely accepted as the dosage form of choice, for many patients they are inappropriate. This has resulted in the development of alternative dosage forms with sustained release behaviour. Drug release from other polysaccharide-based dosage forms is

also a controlled to some extent by the swelling, diffusion and erosion of the polysaccharide component. Such dosage forms include films (Remuñán-López *et al*., 1998), gel beads (Babu *et al*., 2010), microparticles (Dalmoro *et al*., 2010), nanoparticles (Pandey *et al*., 2005) as well as viscous liquid formulations for oral and topical delivery (Cuna *et al*., 2000; Chamarthy and Pinal, 2008).

1.4 Polysaccharides as Physiologically Responsive Excipients

The two major ways polysaccharides respond to the physiological environment are by interacting with the surfaces of tissues (bioadhesion) and by undergoing changes in mechanical behaviour on contact with physiological fluids (sol to gel or gel to sol transition). Both can be used to modify drug release and can occur on their own or synergistically.

1.4.1 Bioadhesion

Bioadhesion (and mucoadhesion) is the process whereby synthetic and natural macromolecules adhere to mucosal surfaces in the body (Woodley, 2001). This is an advantageous property of certain polysaccharides that enables them to adhere to biological tissue for an extended period. This process is often used within pharmaceutical formulations to increase the residence time of drugs at the site of absorption, and increase drug uptake, subsequently, increasing bioavailability (Woodley, 2001; Peppas, 2004).

Mucoadhesive polymers are often characterised by certain specific intrinsic properties that have been related to the muco/bioadhesive behaviour. These properties include the presence of strong hydrogen bond forming functional groups such as carboxylate and hydroxyl groups, presence of a charged groups, high molecular weight, high viscosity, high hydration capacity, chain flexibility and high surface energy that favours spreading onto the mucus (Grover and Smith, 2009). Many polysaccharides employed for drug delivery applications have

potential mucoadhesiveness since most of them show high intrinsic conformity to the above listed properties (Grover and Smith, 2009; Builders and Attama, 2011).

Many polysaccharide-based mucoadhesive polymers show a high degree of interaction with mucus-membranes. The mucoadhesion is the result of a combination of surface and diffusional phenomena that contribute to the formation of adequately strong interchain bridges between the polymer and the biological medium (Peppas, 2004). Mucoadhesive polysaccharides have been used for oral (Remuñán-López *et al*., 1998), nasal (Cao *et al*., 2009), oesophageal (Batchelor *et al*., 2002), transdermal and ocular delivery using a range of polysaccharides that include cellulose ethers (methylcellulose, ethylcellulose, hydroxypropylmethyl cellulose, hydroxy ethyl cellulose and hydroxyl propyl cellulose), chitosan and its derivatives, alginate, gellan gum and some hemicelluloses (Le Bourlais *et al*., 1998; .Lee *et al*., 2000).

1.4.2 In Situ Sol-Gel Transitions

As discussed in section 1.2.3 some polysaccharides have the ability to form firm gels at relatively low concentrations whereby the polymer chains are held together by association of chain segments through long conformationally ordered junction zones, creating an expansive three-dimensional network. In some anionic polysaccharides the polymer chains can be held together by addition of metal ions such as K^+, Na^+, Ca^{2+} and Mg^{2+} (or H^+) which supress the repulsive charge on the polysaccharide resulting in an ordered structure. This has been exploited in several pharmaceutical products whereby the formulations are delivered as liquids and then on contact with the ions in physiological fluid, undergo a rapid sol-gel transition *in situ* to enhance the therapeutic effect. The most successful of such products is Gaviscon®, which is used to treat heartburn and has been commercially available for 40 years. Gaviscon® contains sodium alginate in the formulation which is swallowed as an oral liquid (tablet formulations are also available) (Grover and Smith, 2009). As the formulation reaches the

stomach, the sodium alginate undergoes a rapid sol-gel transition on contact with the H^+ in the stomach acid, which results in the formation of a gel raft on the surface of the gastric fluid, which helps prevent gastric reflux (Liu *et al*., 2003).

Another commercially successful example of *in situ* cross-linking on contact with physiological fluid is in Timoptol-XE®. This is a sustained release ophthalmic formulation for the treatment of glaucoma containing the active ingredient timolol maleate (Grover and Smith, 2009; Shedden *et al*., 2001). Here, gellan gum is used in the formulation and utilises the salts present in lacrimal fluid as the cross-linking ions for *in situ* gelation increasing retention time of the drug at the site of action (the mechanism of gellan gum gelation is discussed in detail in chapter 3). This formulation is easily dispensed in the form of drops due to the relatively low viscosity of gellan gum in solution. On contact with the surface of the eye, the formulation provokes lacrimal fluid secretion effectively delivering more cross-linking ions to the gellan gum causing the gel to increase in strength. The gel then controls the release by providing a diffusional barrier for the drug, which is subsequently released more gradually than in immediate release formulations of timolol eye drops (Grover and Smith, 2009).

The rich ion sources of physiological fluids (e.g. nasal fluid, lacrimal fluid, saliva and GIT fluid) offers an excellent opportunity to develop other *in situ* gelling drug delivery systems. In addition, the highly tuneable and multifunctional nature of polysaccharides provides scope for intelligently designing formulations for different target sites.

1.5 Fluid Gels

One simple way of dramatically changing the physical properties of polysaccharide hydrogels without changing the chemical properties at the molecular level, is by applying shear force during the gelation process (usually by cooling of the polysaccharide solution) which produces ‘fluid gels’(sometimes referred to as ‘sheared gels’). This process creates gelled

particles, that are in the μm size scale (depending on the strength of the shear force experienced) rather than producing a singular bulk gel that occurs when left to gel quiescently (Figure1.9).

The concept of producing fluid gels by applying shear to a gelling biopolymer was first proposed in the patent literature in the 1990s by (Brown *et al.,* 1990; Kawachi *et al.,* 1993) as a method to produce liquid like behaviour in biopolymers, that formed a bulk gel when cooled under quiescent conditions (Figure 1.10). This allowed the material to be easily pumped or transported as a 'liquid' and could be returned to its original bulk gel form by simply heating above the gel melting temperature and then being allowed to cool quiescently (Morris *et al*., 2012). It was also noted that these fluid gel particles exhibited similarity to fat droplets, which prompted investigations for further applications within the food industry (Farrés *et al*., 2013; Farrés *et al*., 2014).

The mechanism behind how fluid gels form was proposed by Norton *et al.,* (1999) who describe the process as a nucleation and growth mechanism with the size of the particles growing to equilibrium permitted by the given shear environment. As fluid gel formation occurs during cooling of a gel-forming polysaccharide, there is a characteristic increase in viscosity as the molecular ordering begins and ordered domains begin to aggregate. This molecular ordering has been described as a phase separation event via spinodal decomposition and therefore under shear conditions there are many potential gel nucleation sites, which are separated from one another by the applied shear. This limits the molecular ordering to occur within distinct particles (Cassin *et al*., 2000; Norton *et al*., 2000). The mechanism of growth is not fully understood but has been proposed to occur through recruitment of polymer chains from the surrounding ungelled matrix (de Carvalho and Djabourov 1997) or by a process where the gel particles are physically forced to aggregate as a result of the shear flow (Norton *et al.,* 1999).

As the gel nuclei begin to grow there is a characteristic increase in viscosity, which peaks at the point where all gel nucleation sites have grown to the shear rate dependent limit and therefore reached the maximum permitted volume fraction. At this point further ordering only takes place within the formed particles. At the surface of the newly formed particles are disordered polymer chains that have been described as 'hairy' chains and these hairy regions at the surface facilitate particle-particle interactions manifested by an increase in viscosity. As the further ordering continues within the particles these hairy chains begin to order within the gel particle resulting in a smoother surface and consequently reduce particle-particle interactions and subsequent reduction in viscosity (Figure 1.11) (Norton *et al.*, 1999).

Once formed, fluid gels exist as a suspension of microgel particles dispersed in a non-gelled continuous medium which can be formulated to have a various structural properties (Cassin *et al*., 2000 and Norton *et al*., 2000).

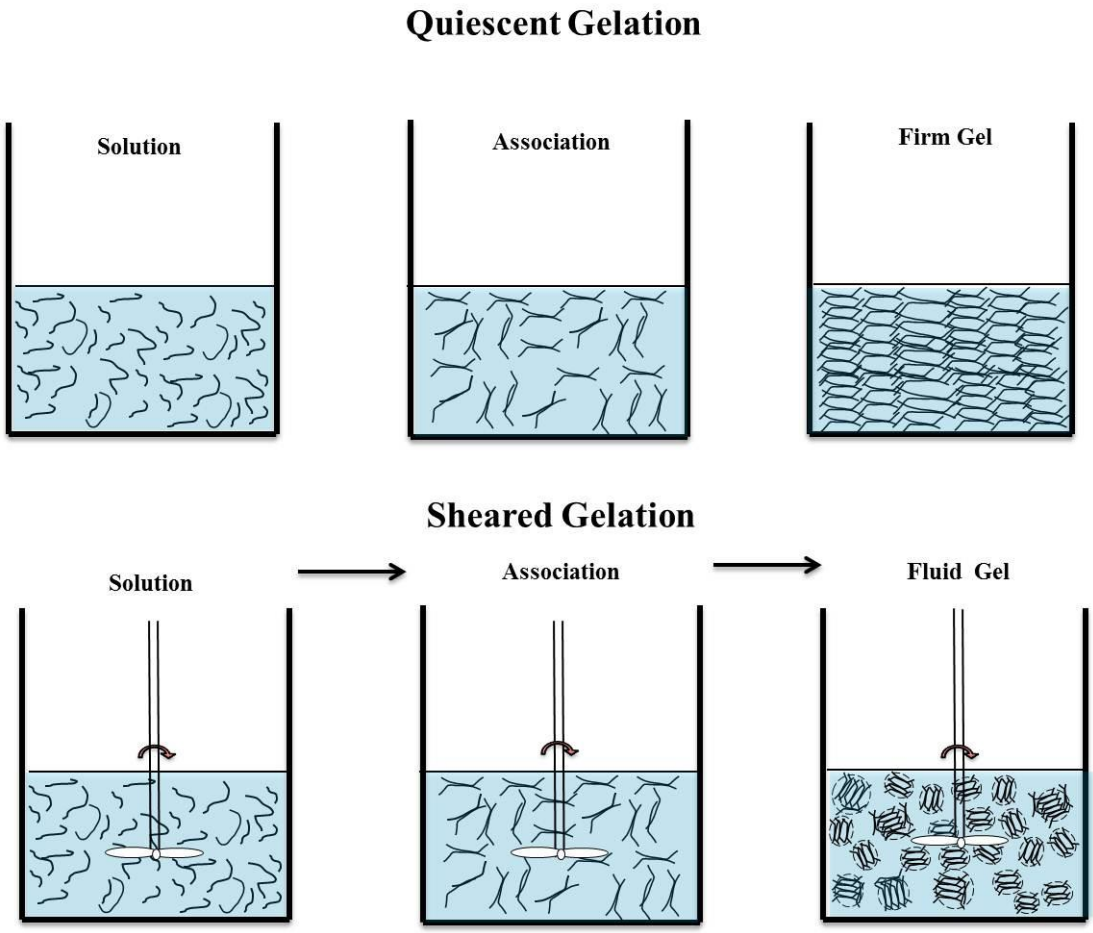

Figure 1.9 Schematic diagram of fluid gel formation by applying shear during a sol-gel transition.

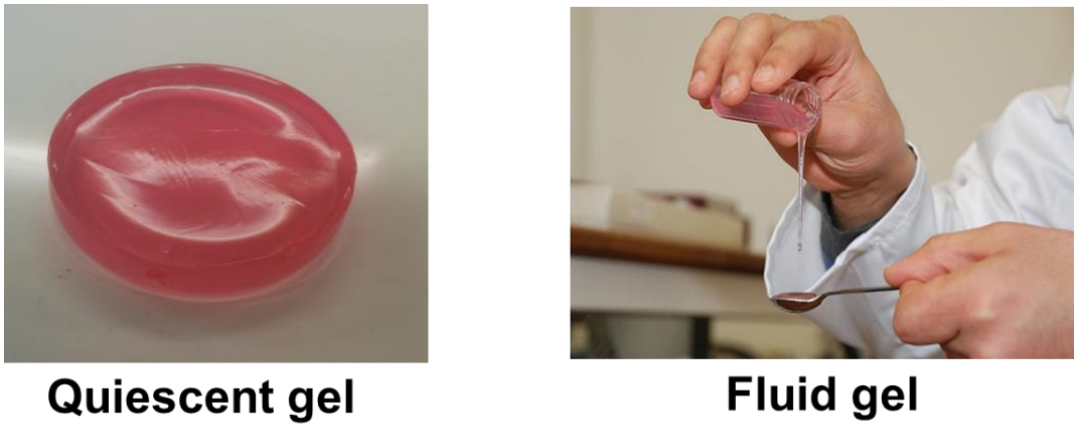

Figure 1.10 Photographs of gels produced under quiescent (left) and sheared conditions (right).

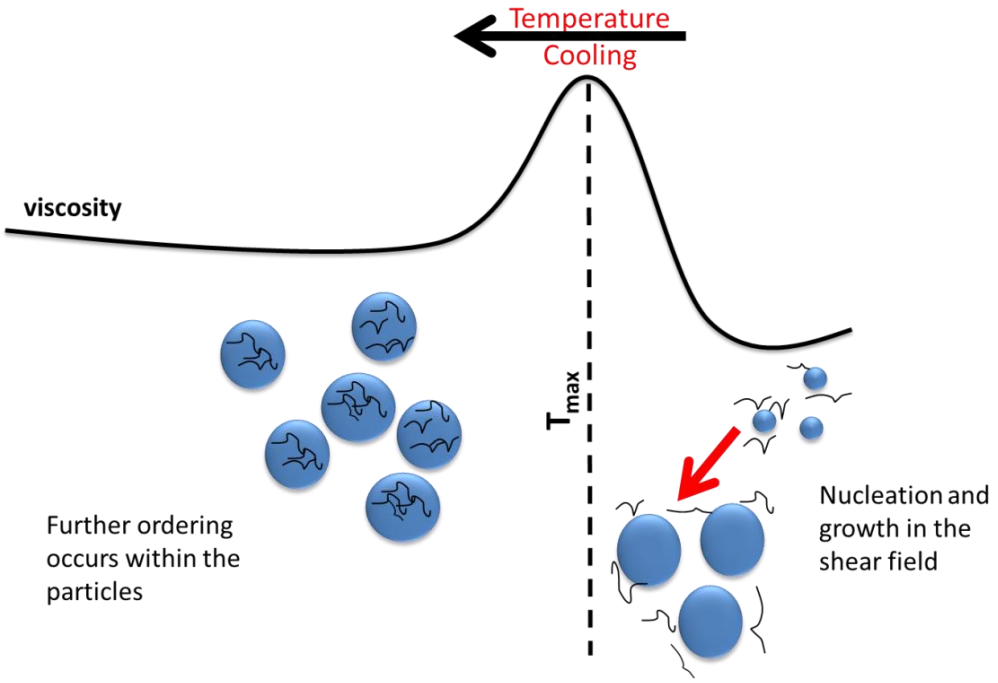

Figure 1.11 Schematic diagram of the molecular events occurring during fluid gel formation (adapted from Norton et al., 1999).

The physical behaviour of fluid gels is dependent on the microstructure of the particles and the inter-particle interactions. Both of these can be controlled by varying the process parameters such as the applied shear rate and cooling rate used during conformational ordering and subsequent gelation of the polysaccharide. By manipulating these parameters, a wide range of structures can be obtained from the same polymer. At low cooling rates, the gelation process generally occurs at a slow rate causing the applied shear rate to dominate and fine spherical-shaped particles are produced. Increasing rate of cooling however, causes larger irregular-shaped particles to be produced. This can be overcome however, by increasing the magnitude of shear rate, which results in small particles forming. Fluid gels with small and uniform particles size have a lower viscosity and elasticity compared with fluid gels with larger particles (Norton *et al*., 1998 and Gabriele *et al*., 2009).

Other ways to control fluid gel physical properties can be achieved by manipulation of polymer concentration, polymer type, and for polymers that undergo ionotropic gelation, ion species and concentration (Norton *et al*., 2000).

Fluid gels can be made from a wide range of gel forming biopolymers that include gelatin (de Carvalho and Djabourov, 1997), agar/agarose (Norton *et al.,* 1998; Norton *et al.,* 2000; Farrés and Norton, 2015) whey protein (Lazidis *et al*., 2016), alginate (Farrés *et al.,* 2013) carrageenan (Gabriele *et al*., 2009) and gellan gum (Sworn *et al.,* 1995).This wide choice of materials combined with the potential to tune the physical properties by simply altering the processing parameters offers potential to the pharmaceutical industry. This coupled with the potential for biologically responsive behaviour of the materials when exposed to different physiological fluids makes fluid gels prepared from polysaccharides particularly attractive.

In this thesis, the investigation focus on developing fluid gels from gellan gum as a drug delivery platform for several modified release drug delivery dosage forms. Gellan gum was chosen as a suitable candidate due to its particular sensitivity to pH and physiological concentrations of salts, interesting rheological properties, formation of gels that are transparent and current commercial use within pharmaceutical preparations.

1.6 Aims and Objectives

The overall aim of this study was to highlight the potential applications and limitations of gellan gum fluid gels as drug delivery systems and to provide a platform of knowledge for the investigation of other fluid gel systems for pharmaceutical use in the future. The main objectives were to evaluate the range of properties that could be produced in fluid gels produced from gellan gum, then to apply this knowledge to develop bio-responsive drug delivery systems using these fluid gels while evaluating the physical and chemical characteristics of these particular drug delivery systems.

Working towards these objectives, modified release ibuprofen oral liquid formulations were prepared from low acyl (LA) gellan fluid gels, blends of low acyl and high acyl LA HA gellan fluid gels were evaluated as an *in situ* mucoadhesive nasal spray system containing caffeine and blends of LA HA gellan gum fluid gel was also investigated to formulate a topical diclofenac formulation.

The effect of polymer concentration on rheological properties and drug release was investigated along with the effect of shear rate and cooling rate on the size of fluid gel particles. These properties relate to the ability for the formulations to be easily administered and release drug at the appropriate rate and probe the influence of the production conditions on the structural and physical behaviour of gellan gum fluid gels. The effect of physiological environmental factors such as pH, and ion concentration was also considered and how these parameters influence drug release at the desired site of delivery. Of particular interest was the potential for bioresponsive behaviour such as changes in gel strength in the GIT (oral liquid), adhesion to mucus membranes (nasal spray) and lubrication properties (topical gel).

1.7 Thesis Structure

This thesis covers the production and properties of gellan gum fluid gels and designing novel drug delivery systems from these gels to solve particular pharmaceutical problems.

- Chapter 2 (Rheology and Tribology) will provide background information to the rheological methodology presented in all the results chapters (chapter 4-6) and also includes an introduction to tribology used in chapter 6. Methodologies, which are specific to the investigated formulations, are given separately in the appropriate chapters
- Chapter 3 will provide an in depth background of gellan gum which was the main material used throughout the thesis focusing on the chemical structure and

conformational properties, the gelation mechanism and a literature review of pharmaceutical applications of gellan gum

- The results begin in Chapter 4, which discusses the results obtained from developing a modified release oral liquid formulations (published as "Evaluation of gellan gum fluid gels as modified release oral liquids" (Mahdi *et al.,* 2014)). This begins with an introduction to the recent work on modified release oral liquids and the issues with current formulations. Then information on the characterisation of the fluid gels is provided highlighting their physical properties and structure. This characterisation also includes measuring particle size, where the development of a method to control the particle size is presented. The final part of chapter 4 discusses the potential use of gellan gum fluid gels in the formulation of modified release oral liquids investigating how the physical behaviour in simulated GIT environmental conditions can influence drug release
- Chapter 5 will present the results obtained from the development of a sustained release nasal spray formulation (published as "Development of mucoadhesive sprayable gellan gum fluid gels" (Mahdi *et al*., 2015)). This chapter begins with an introduction to the challenges associated with nasal spray formulations, nasal physiology and problems with commercial nasal spray solutions. Here, rheological properties of fluid gel formulations obtained from different ratios of LA HA gellan gum blends are investigated. How this can potentially influence mucoadhesion and drug release from such formulations is then discussed.
- Chapter 6 will discuss the results of a fluid gels obtained from gellan gum LA HA blends as topical formulations (Published as gellan gum fluid gels for topical administration of diclofenac and titled in this thesis as development of gellan gum fluid gels as topical formulations). This chapter begins with an introduction to the challenges

of transdermal delivery, skin physiology and the properties of commercially available gels. Rheological properties and drug permeation through *ex vivo* skin is discussed. The lubrication properties of the gellan gum fluid gels were also studied. This was performed alongside a commercial topical gel formulation using soft tribology to provide an insight to the topical application of the gel.

- Chapter 7 will give a summary of the conclusions from this thesis together with recommendations for future work.

1.8 Publications and Presentations

Publications from this thesis are as follows

Journal Publications:

- **Mahdi, M.H**. Conway B. R. & Smith, A.M. * (2014) Gellan gum fluid gels as modified release oral liquids International Journal of Pharmaceutics 475 pp. 335-343
- **Mahdi, M.H**. Conway B. R. & Smith, A.M. * (2015) Development of Mucoadhesive Sprayable Gellan Gum Fluid Gels International Journal of Pharmaceutics
- Ghori M.U., **Mahdi, M.H**., Smith, A.M. & Conway B.R. (2015) Nasal Drug Delivery Systems: An Overview. American Journal of Pharmacological Sciences 3(5), 120-125
- ***Mahdi, M.H[1]***. *Conway B. R[1]. Mill T[2] & Smith, A.M[1]. (2016) Gellan Gum Fluid Gels for Topical Administration of Diclofenac, International Journal of Pharmaceutics in press*

Conference Presentations:

- Mahdi, M.H., Saleem, I. & Smith, A.M. (2013) Gellan gum blends as an *in situ* gelling nasal delivery system, UK PharmSci, Edinburgh, UK
- Mahdi, M.H. and Smith, A.M. (2013) Development of modified release paediatric liquids, 5th European Paediatric Formulation Initiative Conference, Barcelona, Spain

- Mahdi, M.H. & Smith, A.M. (2014) Gellan Gum Fluid Gels as Modified Release Oral Liquids, 41st Annual Meeting & Exposition of the Controlled Release Society Chicago, USA
- Mahdi, M.H. Conway B.R. & Smith, A.M. (2014) Evaluation of Gellan Gum Fluid Gels as Modified Release Oral Liquids, UK PharmSci, Hertfordshire, UK
- Mahdi, M.H. Conway B. R. & Smith, A.M. (2015) Gellan gum fluid gel as an *in situ* gelling nasal delivery system 42nd Annual Meeting & Exposition of the Controlled Release Society, Edinburgh, UK
- Mahdi, M.H. Conway B. R. & Smith, A.M. (2015) Evaluation of gellan gum fluid gels in gastric environment, UKICRS, Nottingham, UK
- Mahdi, M.H. Conway B.R. & Smith, A.M. (2015) Gellan gum fluid gels as a new nasal mucoadhesive release platform. 2nd UK Hydrocolloids Symposium, Birmingham UK
- Mahdi, M.H. Conway B.R. & Smith, A.M. (2015) Development of Gellan Gum blend Fluid Gels as Topical Formulations. American Association of Pharmaceutical Sciences Annual Meeting, Orlando, USA.

Chapter 2

Rheology and Tribology

CHAPTER 2 RHEOLOGY AND TRIBOLOGY

2.1 Introduction to Rheology

An important part of this thesis involves the rheological characterisation of gels; therefore, the basic concepts of rheology will be discussed here. First of all, a brief overview of rheology will be discussed, however since the work involved polysaccharides for pharmaceutical applications, aspects of rheology related to pharmaceutical application is covered in more detail.

The word 'rheology' is derived from the Greek words *rheo* (″to flow″) and *logos* (″science″) and was first coined in 1929 by Bingham and Reiner. Rheology can be described as the relationship between stress and strain within a material as a function of time, temperature, frequency and etc. The term 'stress' refers to the force per unit area applied on a system while 'strain' refers to the deformation as a result of the applied stress. Rheology therefore, is the scientific study of the deformation and flow properties of matter (Picout and Ross-Murphy, 2003).

Materials are classified according to observed physical behaviour i.e. liquid (viscous) or solid (elastic) with the two extremes of behaviour corresponding to a perfect (Newtonian) liquid or and a perfect (Hookean) solid. Materials such as polysaccharides having properties that are both viscous and elastic, hence, are referred to as viscoelastic (Mezger, 2006; Marriott, 2007). Since rheology gives information about the physical and mechanical properties of a sample, it is important to use rheological measurements to evaluate the viscoelastic properties of pharmaceutical products as this behaviour can have an impact on all the stages of dosage form development right through to administration. Moreover, in this thesis the changes in rheological behaviour in a physiological environment are central to the efficacy of the developed formulations.

2.2 Principles and Basic Concept of Rheology

2.2.1 Stress and Strain

Stress is, defined as the force (F) per unit area (A) on or within a material and is measured in units of pressure (Pa) (Equation 2.1).

$$\text{Stress} = \text{F}/\text{A} \qquad \text{Eq. 2.1}$$

When measuring the rheological properties of materials there are three different types of deformation that can be considered depending on the direction in which the force is applied. Compressive/elongational stress where the force is perpendicular to the area, shear deformation where the force is lateral to the area and isotropic or (bulk stress) where force is applied from all directions (Figure 2.1).

The stress causes strain and the resulting strain is dependent on type and amount of stress applied. Strain is geometric quantity and therefore is dimensionless ratio and has no units. In rheology strain is defined as fractural deformation induced by the stress and it is given by Equation 2.2.

$$\text{Strain} = \Delta l/l \qquad \text{Eq. 2.2}$$

Where Δl is the change in sample length and l is the original length of the sample prior to applying the stress. The relationship between stress and strain can be used to determine material properties and is given the term modulus. The change in strain over change in time is also an important parameter and is described as the strain rate or shear rate $(\dot{\gamma})$ and it has units of reciprocal seconds (1/s).

2.2.2 Stress-Strain Relationship (modulus) for Materials

Longitudinal/Young's modulus (E) (Figure 2.1A):

Useful for solid materials which are self-supporting with a defined shape and size. After applying a force, perpendicular to a solid material, the energy is stored as a potential energy which can be fully recovered after the applied force is released causing the material to return to its original form. A perfect example of this is elastic materials such as springs which deform to a given strain in response to a given stress and remain compressed indefinitely unless the stress is removed, at which time the material returns to its initial position. This behaviour follows Hooke's law in which stress is always directly proportional to the strain but independent of the rate of strain. Longitudinal modulus is equal to the stress divided by the strain (Equation 2.3).

$$\mathrm{E} = \frac{\text{stress}}{\text{strain}} = \sigma/\varepsilon \qquad \text{Eq. 2.3}$$

Shear modulus (G) (Figure 2.1B):

This type is more useful for non-self-supporting materials such as soft solids or viscoelastic liquids which makes it more suitable for the fluid gel systems used in the work described in this thesis. The force is applied tangentially0 and causes the material to deform through an angle θ. The shear stress is defined in Equation 2.4.

$$\sigma = \mathrm{F}/\mathrm{A} \qquad \text{Eq. 2.4}$$

Where F represents the tangential force and A is the tangential area. The resulting shear strain is given by Equation 2.5.

$$\gamma = \Delta l/l \ = \tan(\theta) = \theta \qquad \text{Eq. 2.5}$$

Where Δl is the tangential displacement, l is the sample thickness and is the θ the angle of deformation.

The shear modulus (G) is given as described in Equation 2.6.

$$G = \sigma/\gamma \qquad \text{Eq. 2.6}$$

Bulk relaxation modulus (K) (Figure 2.1C):

Bulk modulus is defined as the modulus of the volume expansion: the ratio of the isotropic stress to the relative change in volume (Equation 2.7).

$$K=\sigma_v/\varepsilon_v \qquad \text{Eq. 2.7}$$

Where σ_v represents the bulk stress and ε_v represents the volumetric strain.

Most of matter can be compressed and therefore all matter has bulk modulus. Measurements of bulk modulus in polysaccharide systems are relatively uncommon.

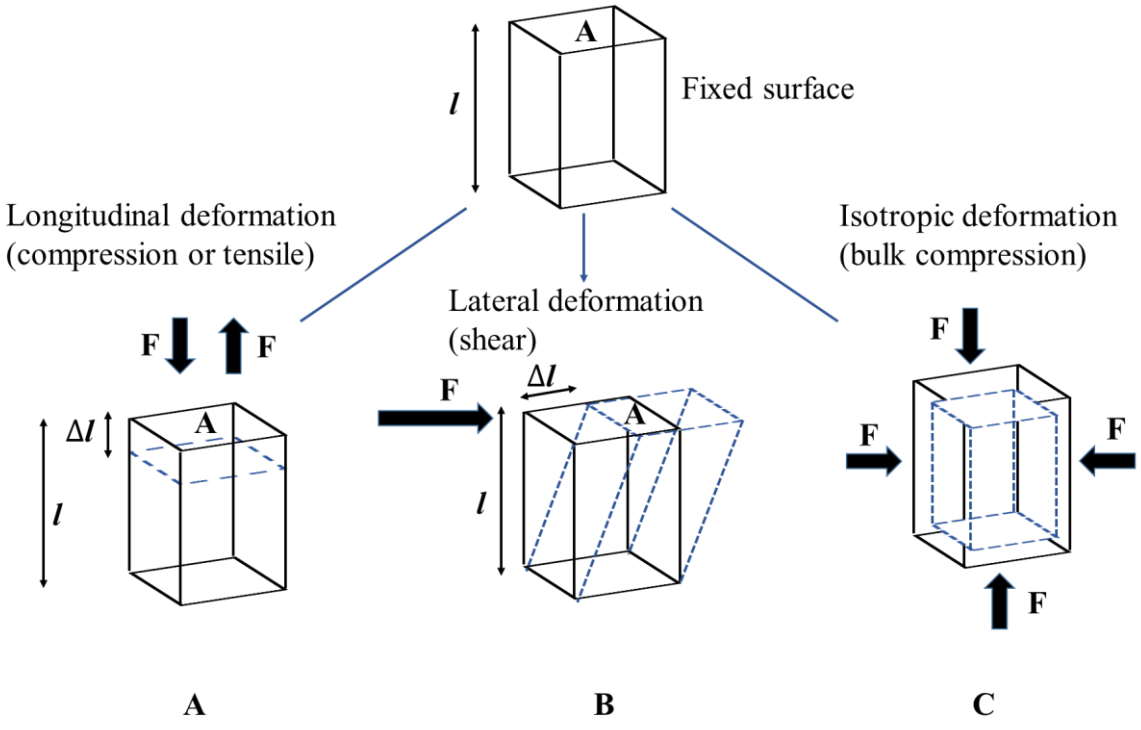

Figure 2.1 Schematic illustration of material deformation.

2.3 Rheological Measurements

2.3.1 Steady State Flow (Viscosity)

Viscosity can be described as the friction between the internal molecules of a material, i.e., its resistance to flow (Lewis, 1996). Viscosity of liquids is a very important parameter that can be used to predict the behaviour of pharmaceutical formulations during manufacturing, on application and storage, and can also have an impact in drug release.

2.3.1.1 Newtonian and Non-Newtonian systems

Viscosity was first time described by Isaac Newton who was the first that realized that the flow of certain liquids is directly proportional to the stress applied. This is true for Newtonian liquids and can be expressed as in Equation 2.8 (Marriott, 2007).

$$\text{Viscosity } (\eta) = \text{Stress } (\sigma)/\text{rate of shear } (\dot{\gamma}) \qquad \text{Eq. 2.8}$$

Such behaviour occurs in liquids such as water, oils and other solvents over a wide range of shear rates (Steffe, 1996). When materials do not follow the Newtonian law of flow the viscosities will be shear rate dependent. There are several common types of non-Newtonian flow behaviour found in pharmaceutical systems highlighted in (Figure 2.2) as a plot of shear stress vs. shear rate.

The power law is generally used for non-Newtonian materials which is given in Equation 2.9.

$$\sigma = k\,\dot{\gamma}^{n} \qquad \text{Eq. 2.9}$$

where k is the consistency index and n is the flow index. This model describes both shear thinning and shear thickening behaviour. For a shear thinning (pseudoplastic) material where the viscosity generally decreases with increasing shear rate, $n < 1$ and for a shear

thickening (dilatant) material where the viscosity increases with increasing shear rate, n > 1 therefore, when *n* = 1 the material will be Newtonian. Emulsions, suspension and topical gels are examples of non-Newtonian systems found in the pharmaceutical sector (Schramm, 2004a).

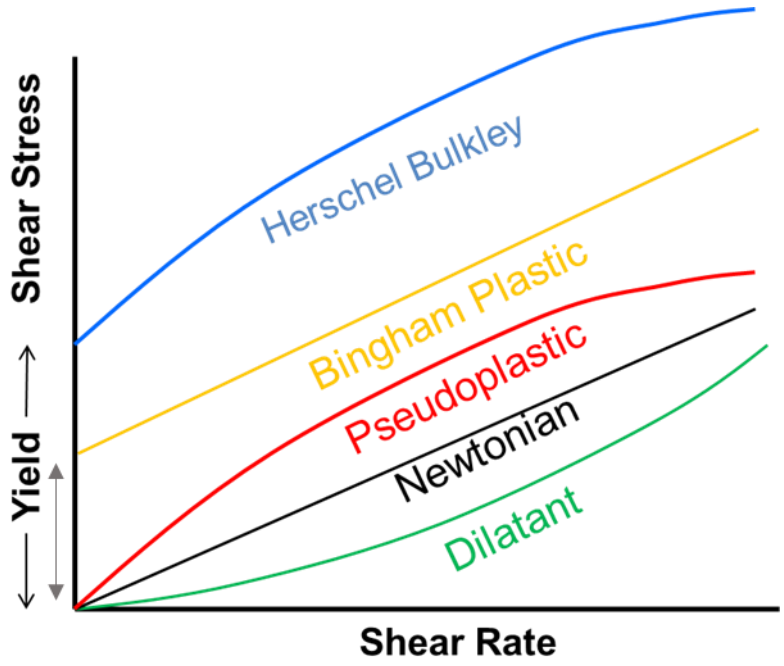

Figure 2.2 Flow curves (shear stress vs shear rate) for Newtonian and non-Newtonian flow behaviour with yield stress region shown (Adapted from Miri 2010).

For some materials, a minimum shear stress needs to be exceeded for flow to begin. This is known as the yield stress. Once the yield stress is reached, the system begins to flow either in a Newtonian manner, which is termed Bingham plastic behaviour and can be expressed as given in Equation 2.10.

$$\sigma = \sigma_o + \eta\dot{\gamma} \qquad \text{Eq. 2.10}$$

where σ_o is the yield stress, or in a pseudoplastic (shear thinning) manner (Herschel-Bulkley model behaviour) whereby the power law in Equation 2.9 can be extended to include the yield stress value as in Equation 2.11:

$$\sigma = \sigma_o + k\,\dot{\gamma}^n \qquad \text{Eq. 2.11}$$

Hydrated polysaccharides generally follow shear thinning behaviour and the degree of shear thinning depends on intrinsic molecular characteristics including conformation, molecular weight and charge for anionic polysaccharides. Factors such as concentration, temperature and pH may also affect flow properties (Koliandris, 2008). The concept of shear thinning can be explained by 'make and break' interactions between the polymer chains (Graessley, 1974). At low shear rates, dis-entanglement and re-entanglement of polymer chains occurs at the same rate which causes the solution to have Newtonian behaviour (at the yield point) because the entanglement density remains constant. As the shear rate increases however, the rate of dis-entanglement is greater than the rate of re-entanglement, reducing entanglement density which causes the viscosity measurements to decrease (Figure 2.3). This kind of behaviour is beneficial in most liquid pharmaceutical formulations as at low shear rates the viscosity is high which helps to suspend and stabilise formulations. Then by simply shaking the formulation the shear thinning behaviour causes a reduction in viscosity and subsequently facilitates dispensing by either pouring or spraying.

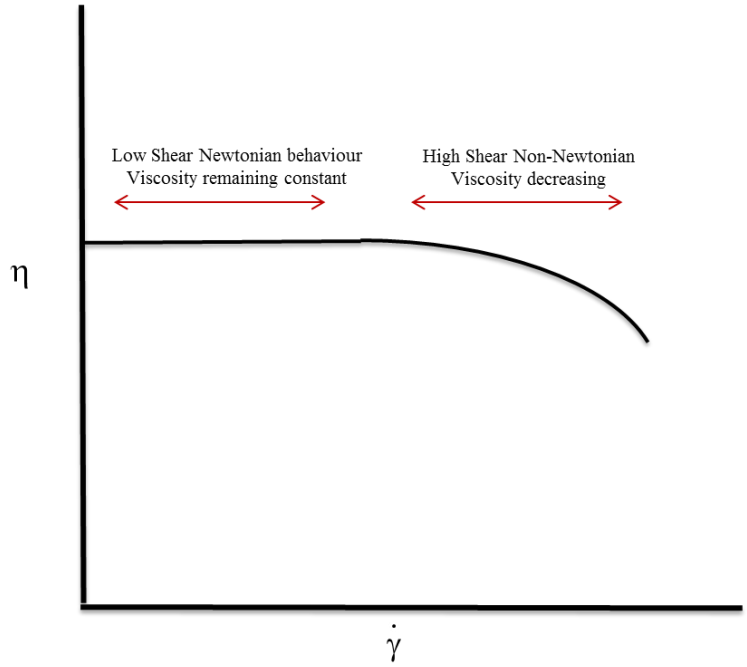

Figure 2.3 Viscosity vs shear rate for a typical shear thinning polysaccharide solution showing the shear dependent regions of Newtonian and non-Newtonian flow behaviour.

2.3.2 Oscillation and Viscoelasticity

Oscillatory measurements are widely used in characterization of viscoelastic materials where a sinusoidally oscillating stress or strain is applied to the material. In this method, both stress and strain vary cyclically with time, with sinusoidal variation being the most commonly used. This is one of the most popular methods to characterize viscoelasticity in biopolymer materials, since relative contributions of viscous and elastic response of materials can be measured and provides a valuable insight into the polymer-polymer interactions. This is particularly useful to study any transitions that occur in the system, for example aggregation phenomena, gel formation or melting.

The cycle time, or frequency of oscillation, defines the timescale of these measurements. The tests are performed by subjecting a sample to sinusoidal deformation and measuring the resulting mechanical response as a function of time, frequency of oscillation or amplitude of oscillation (Schramm, 2004b).

If for instance, a sinusoidal strain wave with a fixed low amplitude and frequency, is applied then the sinusoidal strain can be represented as described in Equation 2.12:

$$\gamma = \gamma_0 \sin \omega t \qquad \text{Eq. 2.12}$$

where γ is the instantaneous strain, γ_0 is the strain amplitude and ω the angular frequency. The resulting shear stress will be a sine wave as well, but with different amplitude and phase and can be written as described in Equation 2.13:

$$\sigma = \sigma_0 \sin (\omega t + \delta) \qquad \text{Eq. 2.13}$$

where δ is the phase angle between the strain and stress waves.

The recorded stress wave resulting from the applied strain is highly dependent on samples viscoelastic properties and is theoretically presented in (Figure 2.4).

In case of a perfect elastic solid (Hookean solid), where the stress is proportional to strain, the stress and strain waves would be completely in phase (phase angle, δ=0°). While in case of perfect fluid (Newtonain fluid), where the stress is proportional to strain rate, the resulting stress wave would be exactly 90° out of the phase with the strain wave. For a material which has an element of both liqiud-like and solid-like behaviour (viscoelastic materials), the phase angle, δ would be between 0° and 90°.

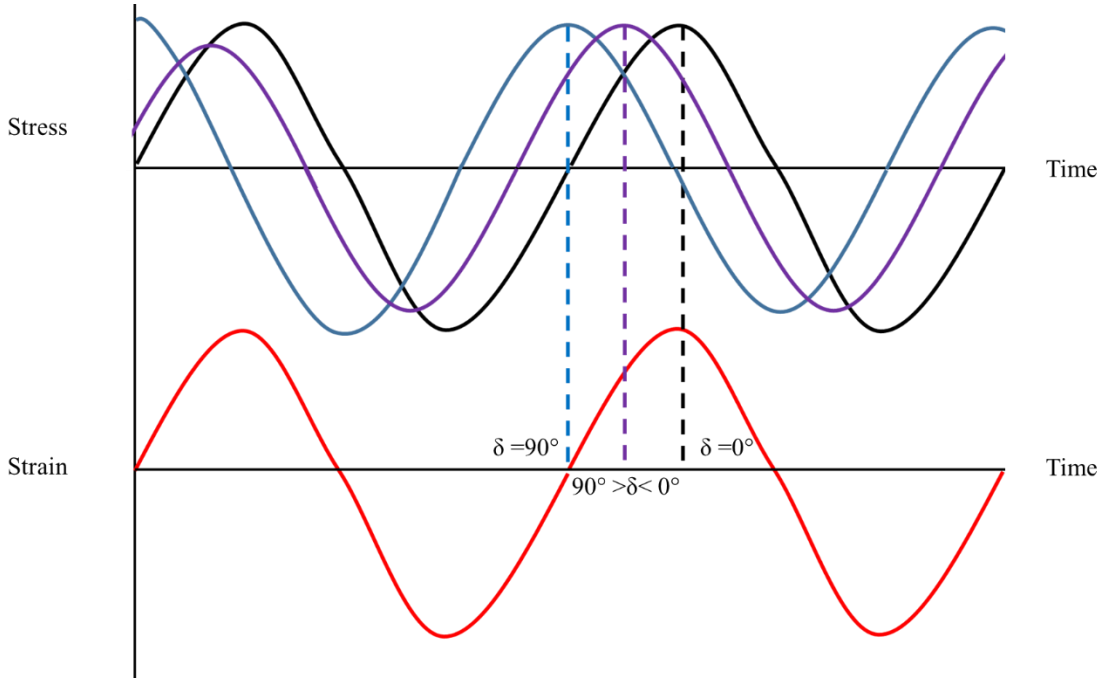

Figure 2.4 Differences in stress response for elastic, viscous and visocelastic materials under small amplitude oscillatory testing following an applied strain (red curve). Elastic solid response (black curve), viscous liquid response (blue curve) and viscoelastic response (violet curve).

In constant deformation experiments, the modulus is defined as the ratio of stress/strain, while for a dynamic sinusoidal experiments the response of viscoelastic materials is quantified by resolving in-phase and out-of-phase stress components. Resolving the in-phase and out of phase behaviour allows the determination of several important parameters for describing the viscoelastic behaviour (Schramm, 2004b).

The storage (elastic) modulus (G′) is defined as the ratio between the in-phase stress and strain, it is given by Equation 2.14:

$$G' = (\sigma_0 / \gamma_0) \cos \delta \qquad \text{Eq. 2.14}$$

G′ therefore, is measure of the energy stored in the material and recovered from it per cycle and is taken as an indication of the solid or elastic character of the material under the test.

The loss (viscous) modulus G″ is defined as the ratio between out of phase stress and strain as described in Equation 2.15.

$$G'' = (\sigma_0 / \gamma_0) \sin \delta \qquad \text{Eq. 2.15}$$

G″ therefore, is a measure of energy dissipated or lost as heat per cycle and it taken as indication of liquid or viscous character of material under the test.

The loss tangent (tan δ) is an important parameter which is useful in providing information as the material undergoes gelation or melting and it can be derived from elastic and viscous moduli as described in Equation 2.16.

$$\tan \delta = G'' / G' \qquad \text{Eq. 2.16}$$

The overall stress response to the strain, defined as the ratio of stress amplitude to strain amplitude regardless of the elastic or viscous response is described as the complex modulus G* and it is given by Equation 2.17:

$$G^* = [G'^2 + G''^2]^{1/2} \qquad \text{Eq. 2.17}$$

Complex modulus is related to another useful parameter, the complex dynamic viscosity (η*), which is a ratio of the complex modulus to the frequency of the oscillation as given in Equation 2.18:

$$\eta^* = G^*/\omega \qquad \text{Eq. 2.18}$$

Complex dynamic viscosity is often compared with steady shear viscosity to detect any sensitivity of sample structure to the large strains.

Critical to oscillatory experiments for analysis of viscoelasticity is the determination of the linear viscoelastic region (LVR). Within this region the applied stress will give a proportional strain response (Figure 2.5A). This region can be determined experimentally by gradually increasing applied amplitude of strain or stress until the deformation becomes large enough that the linear region is exceeded and modulus G′ begins to decrease as the sample begins to fail (Figure 2.5B). Once the LVR is determined for the sample a stress or strain value that sits well within the linear region is selected for all other small deformation rheological tests on that particular sample (Hyun *et al*., 2011).

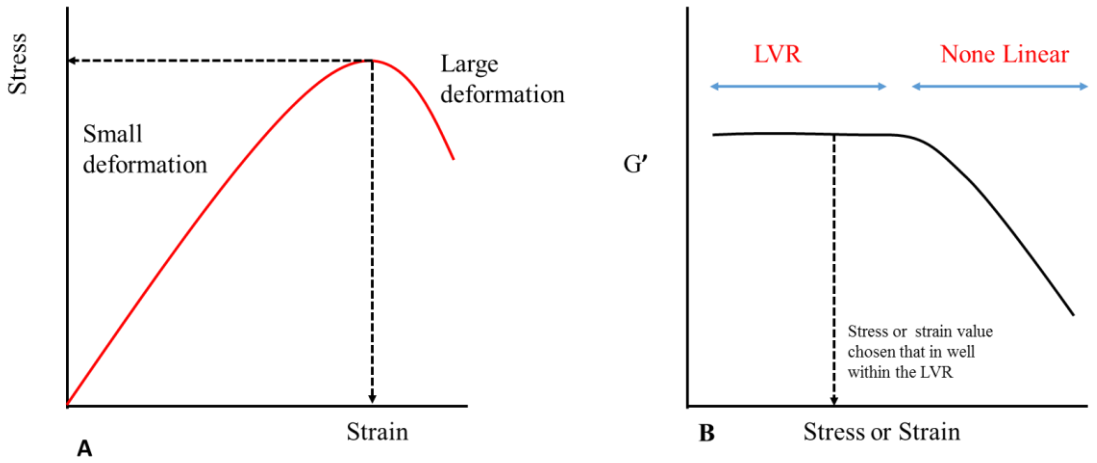

Figure 2.5 A) Illustration of the proportional behaviour of stress and strain within the linear viscoelastic region (dotted arrows indicate the point the system becomes non-linear) and B) experimental determination of the LVR required for the selection of an appropriate stress or strain to use in further viscoelastic testing.

Several recent studies have used the stress at the onset of G′ non linearity in polysaccharide systems as a measurement of yield stress (Farrés and Norton, 2015). Stress (or strain) sweep measurements therefore are a simple and convenient method of determination of

not only the LVR but the critical stress (or strain) at failure that can be related to the yield stress of the sample.

2.3.2.1 Mechanical Spectrum of Polysaccharide Solutions and Gels

Mechanical spectra profiles provide useful information on structural properties of a polysaccharide samples. They are obtained by measuring G′, G″ and η* plotted against a range of oscillation frequencies and reveals the profile of the mechanical characteristics of the samples tested. Figure 2.5 shows the mechanical spectra for four typical polysaccharide systems dilute solutions, concentrated polymer solutions, strong gels (true gels) and weak gels (including fluid gels).

Dilute polysaccharide solution (Figure 2.6 A):

This type of spectrum characterized by liquid-like behaviour with loss modulus G″ greater than G′ throughout all the frequency ranges. Both moduli increase with frequency and the complex dynamic viscosity (η*) is independent of frequency (ω). This system is experienced when the polymer concentrations are low and chains are in isolation from one another.

Concentrated polysaccharide solution (Figure 2.6 B):

This mechanical spectrum is obtained when the concentration of polysaccharide is large enough for the polymer chains to entangle with one another. At low frequency, the entangled polymer chains have enough time for the entanglements to become detached within the oscillation period. This causes the concentrated polymer solution to appear behaving as a dilute solution with G″ being dominant over G′ and η* independent of ω. As the frequency increases further the polysaccharide sample develops more elastic response with G′ becoming greater than G″ and with η* beginning to decrease steeply with frequency. This is due to the disentanglement of the chains becoming more difficult within the period of oscillation.

Weak gels (Figure 2.6 C):

The mechanical spectra of weak gels is characterized by both moduli having ω dependence with G′ > G″ and a liner decrease in η* with increasing ω. The most widely used example of this kind of behaviour is that of xanthan gum, however, the fluid gels often have similar spectra. These types of systems have free flowing solution behaviour and can be stirred or poured but have elastic behaviour in response to very small deformations. Unlike concentrated polymer solutions, weak gels are formed by tenuous association of rigid, ordered structures or by junction zones, rather than by entanglement. The mechanism of interchain associations are responsible for the strange behaviour of weak gels. In a recent review on the gelation of gellan gum the term "weak gel" was discussed with several different terminologies for this kind of behaviour to avoid confusion between with conventional gels that are "weak" i.e. having low moduli (Morris *et al.,* 2012). Other descriptions included "pourable gels" (Morris, 1991) or "structured liquids" (Ross-Murphy, 2008). Conventional gels, which are often described as "true gels", fracture in response to high stress, whereas "weak gels" respond by flowing. The work of this thesis focus on this type of gel.

True gels (Figure 2.6 D):

True gels have a distinctive mechanical spectrum with G′ substantially greater than G″ and independent of ω across a wide range of frequencies. Moreover, η^* decreases linearly as ω increases. True gels are strain independent until the strain is large enough to result in material failure.

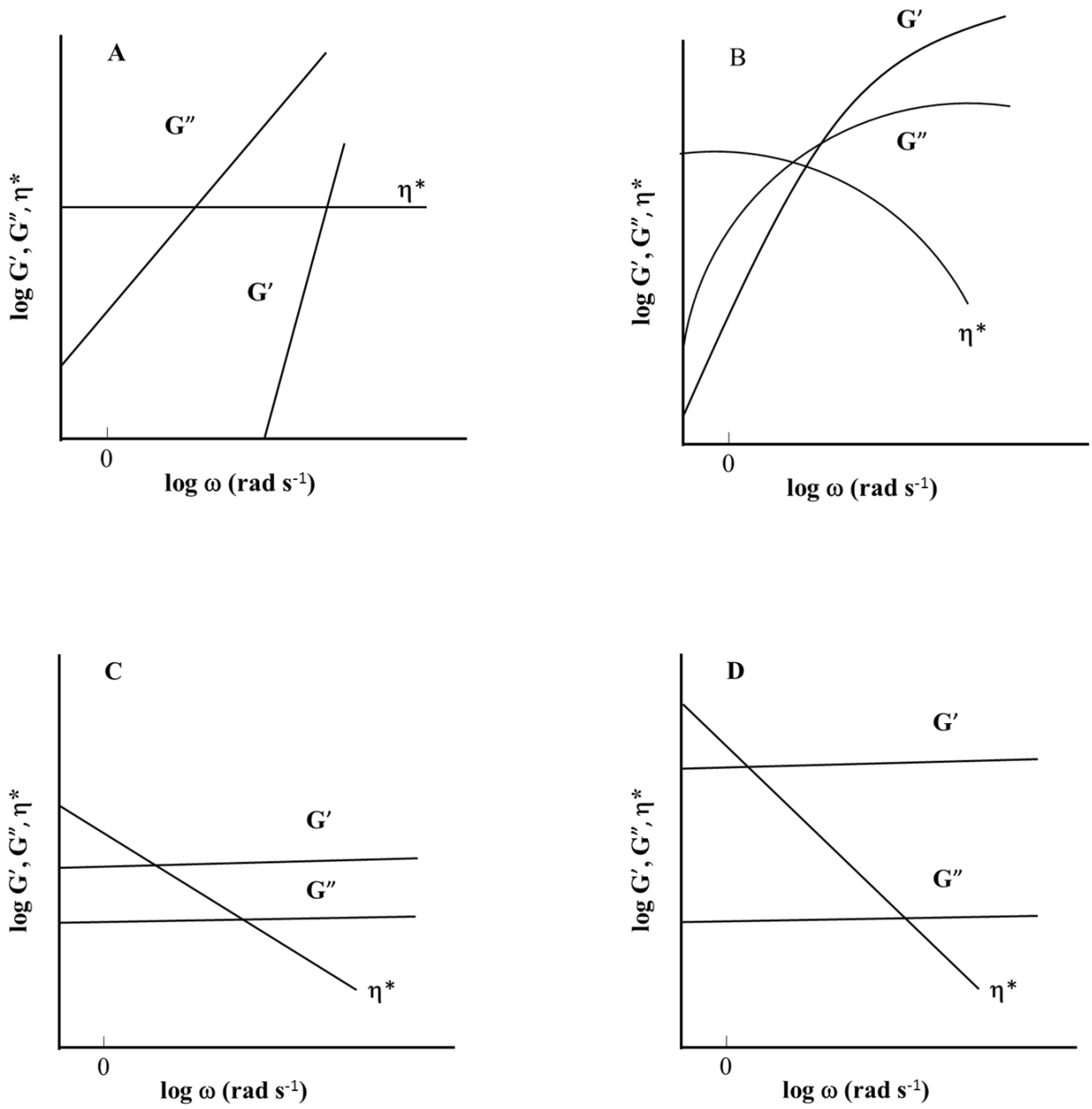

Figure 2.6 The four classes of mechanical spectra for biopolymer systems: (A) dilute solution; (B) entangled polymer solution; (C) weak gel; and (D) true gel.

2.4 Introduction to Tribology

Tribology is the science that focuses on the interaction between two surfaces in relative motion and encompasses the study of friction and lubrication. The word tribology is derived from the Greek ‘trivee’ meaning ‘to rub’ and ‘logos’ meaning ‘discourse’. Traditionally, tribology has long been studied for the purpose of optimising machine elements and lubrication of such systems. Recently, the use of tribology has widened to cover food and healthcare products. The most useful areas of tribology for the purpose of food and healthcare research are in the measurements of friction and lubrication (Malone *et al*., 2003). This section presents the basic principles of tribology with a focus on the areas that are relevant for topical formulations (as discussed in chapter 6 of this thesis) with the rationale of the finger and the skin being two contacting surfaces that are in relative motion when applying a topical gel.

To initiate or continue motion the required force must be greater than the frictional force. Leonardo da Vinci was first pioneer of the study of friction in 16^{th} century. Then in 1699 the first law of friction was published by Guillaume Amonton as the force of friction is proportional to the normal load, and therefore, the tribological properties are always described by the coefficient of friction (μ) described in Equation 2.19 (Amontons, 1699; Bowden and Tabor, 2001).

$$\mu = F/W \qquad \text{Eq. 2.19}$$

where F is the tangential friction force and W is the normal load, both in units of Newton (N).

Lubrication is the process or technique employed to reduce friction by separating two opposing tribo surfaces by means of a lubricant. For example, skin is susceptible to friction in everyday life situations. However, sweat or skin care products can serve as a lubricant providing protection of surfaces from skin injury (Vilhena and Ramalho, 2016). Measurements of friction are usually performed on traction machines consisting of a ball and a disk whereby

a normal force (W) is applied and the ball and disk are rotated at different speeds enabling a relative motion between the ball surface and the disk (Figure 2.7). The ball and disk velocities (V) determine the entrainment speed (*U*), which is defined in Equation 2.20 (De Vicente *et al*., 2006):

$$U = (V_{ball}+V_{disk})/2 \qquad \text{Eq. 2.20}$$

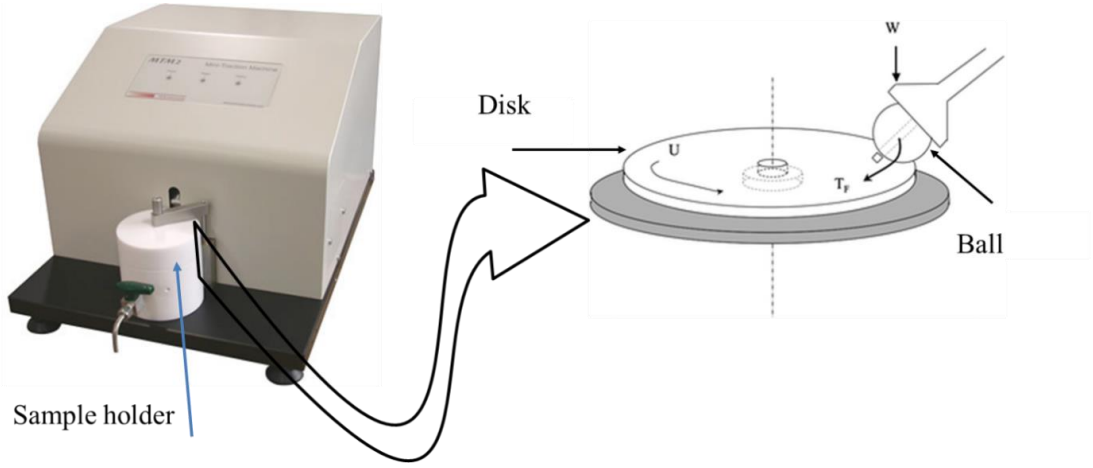

Figure 2.7 Schematic representation of a mini traction machine (adapted from De Vicente et al., 2006).

The lateral force exerted on the ball is measured through a force transducer and yields the friction coefficient (μ) (Equation 2.19). This is performed at increasing entrainment speeds to understand the mechanism of lubrication in the form of a Stribeck curve.

2.4.1 Stribeck Curve

The Stribeck curve was first proposed by Richard Stribeck in 1908 when it was noticed that the friction coefficient was not linear with entrainment velocity while studying the lubrication mechanism in metal bearings. Stribeck curves can be obtained by monitoring μ as a function of *U*, where friction initially decreases to a minimum, then followed by an increase as the gap height widens (or lubrication film thickness) as shown in Figure 2.8.

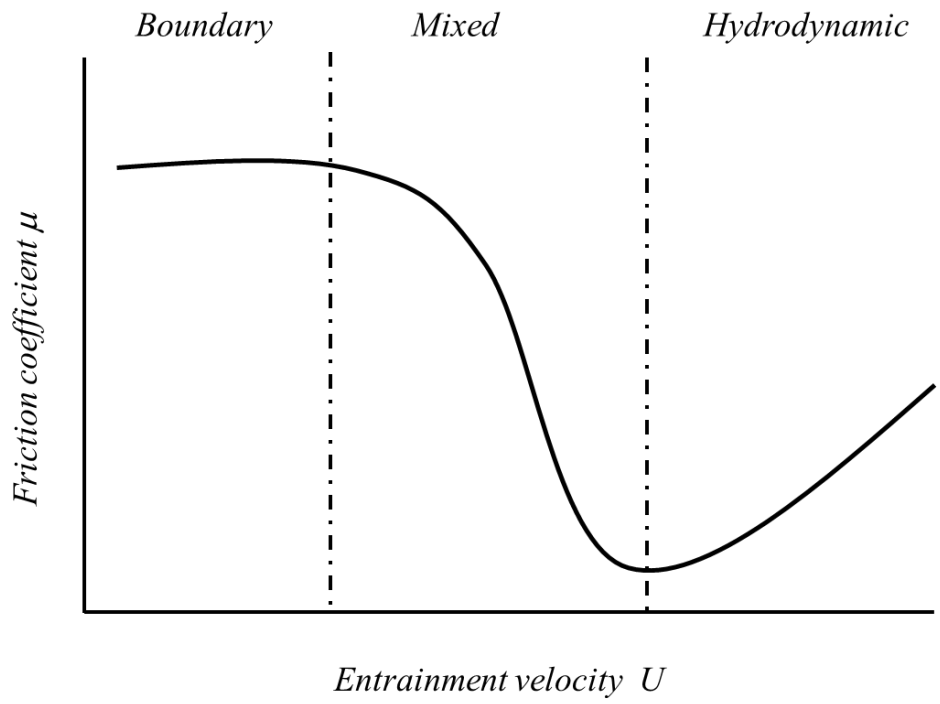

Figure 2.8 Schematic diagram of a complete idealised Stribeck curve showing three principle regimes of lubrication: boundary, mixed and hydrodynamic where the ball and disc surfaces are in full contact, partial separation and full separation, respectively (adapted from De Vicente et al., 2006).

Three regimes of lubrication can be clearly identified from the shape of the curve: Boundary lubrication, mixed regime of lubrication and hydrodynamic lubrication (De Vicente *et al.*, 2006). The relation of friction coefficient to entrainment speed is highly related to lubricant structure and properties of contacted surfaces.

In case of boundary lubrication the μ is independent of *U* and is observed at low speeds. At this stage the two surfaces are in full contact and the applied normal load is fully supported by the contact of the surfaces. In the boundary regime the bulk lubricant is excluded from the contact (Williams, 2005).

In the mixed regime of lubrication, a decrease of friction with increase in entrainment speed can be clearly identified and occurs due to surface separation as a result of increased lubricant pressure. At this stage, W is supported partially by the contact of the surfaces and

partially by lubricant pressure. As a result, both the surface and lubricant properties influence friction. As the speed increases further the contact between two surfaces decreases until full separation occurs where μ reaches a minimum (Spikes, 1997).

Hydrodynamic lubrication then observed at high entrainment velocity and occurs due to an increase in the film thickness requiring a greater volume of fluid to be sheared by the rotating surfaces. Friction in this regime is solely dependent on the lubricant rheology (de Vicente *et al*., 2005).

Malone *et al.,* (2003) were the first to correlate mixed regime of lubrication to the complex sensory oral processing by using a modified mini traction machine and a trained sensory panel. Since then, focus on the biological tribology of surfaces such as human skin, is growing (Chen and Stokes, 2012). Skin has shown frictional properties that are different from those in the mouth. This is a due to differences related to the physical attributes of the surface of the skin, such as roughness and elasticity. The average friction coefficient of the dry skin has been found to be 0.7 (Adams *et al* 2007).

Tribology is particularly relevant to topical pharmaceutical research, as the physical processes involved in rubbing the topical dosage form (e.g. cream, lotion or gel) into skin is relative motion. The skin is an elastic tissue and steel-on-steel tribometry gives high pressures (Vilhena and Ramalho, 2016; Cassin *et al*., 2001) which are not appropriate for describing the lubricating processes occurring in the skin. In addition, the wetting and adsorption properties are different from that of the skin surface. To reduce the pressure and to assemble similar physical parameters of skin surface at least one soft surface (either ball or disk) is usually required when performing tribological experiments relevant to soft biological tissues.

2.4.2 Lubrication of Biopolymers in Soft-tribological Contacts

There have been many of soft-tribology studies performed on polysaccharides in an attempt to determine their behaviour during oral consumption. A correlation between the perception of 'slipperiness' and μ of guar gum solutions in the mixed regime in a steel-silicone contact has been shown by Malone *et al*., (2003) and Cassin *et al*., (2001) reported that increasing concentrations of guar gum leads to a reduction of μ in the mixed-regime. Similar observations were noticed with xanthan gum by de Vicente *et al*., (2005). The reduction in μ in these systems was explained by a combination of viscous and hydrodynamic forces.

Tribology of fluid gels was first studied by Gabriele *et al.*, (2010) who investigated agarose fluid gels (~ 100 μm diameter particle size) using a steel-on-silicon tribopair. A conceptual model was proposed which identified three distinguishable zones represented schematically in (Figure 2.9). Zone A, at the lowest speeds, represents partial entrainment of the continuous phase in a mixed regime of lubrication; μ then increases with speed as the particles begin to be entrained forming a monolayer and the friction resulted from the rolling-sliding motion of the particles in the thin film (Zone B); on increasing speed further Zone C is reached where the bulk fluid is entrained and the mixed regime continues.

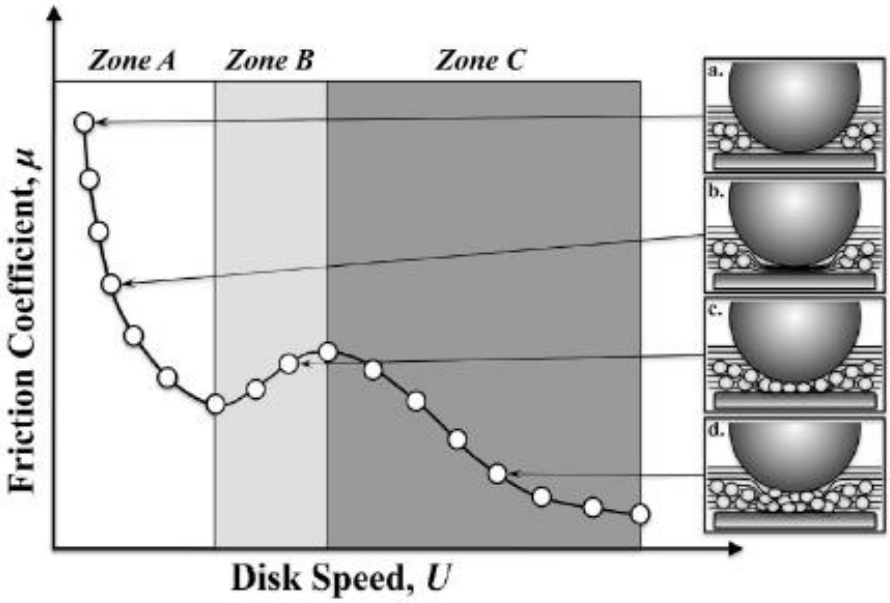

Figure 2.9 Schematic diagram for the conceptual model of fluid gel lubrication (Gabriele et al., 2010) (used with permission).

In the study by Gabriele *et al*., (2010), increasing particle size was shown to increase μ. This was similar to the findings of de Wijk and Prinz (2005) in custard systems. Gabriele *et al*., (2010) also noticed that the critical velocity at which friction starts to increase in the mixed regime, decreased with increasing agar concentration which was assigned to the elasticity of the particles, with stiffer particles requiring lower velocities for entrainment. However, it could also be argued that less stiff particles may entrain at lower velocities due to their increased deformability. An alternative hypothesis for the increase in μ is that particles build-up around the ball-on-disc contact thereby preventing the entrainment of the continuous phase.

Chapter 3

Gellan Gum

CHAPTER 3 GELLAN GUM: PHYSICOCHEMICAL PROPERTIES AND PHARMACEUTICAL APPLICATIONS

3.1 Introduction

Gellan gum is bacterial exo-polysaccharide produced by bacterium called *Sphingomonas elodea* (formally known as *Pseudomonas elodea*), and was discovered by Kaneko and Kang in 1978. In 1988 Japan approved gellan gum to be used in food and few years later in 1992 the USA food and drug administrations (FDA) also approved gellan for use as a food additive (Sworn *et al*., 1995; Kirchmajer *et al*., 2014).

Gellan gum is produced by an aerobic fermentation process. A pure culture of *S. elodea* is inoculated in a fermentation medium. The medium contains a carbon source, such as glucose, phosphate and nitrogen sources, and appropriate number of inorganic salts (Gibson and Sanderson, 1997). The viscosity of the broth increases gradually as the organism metabolises glucose and the gellan gum is secreted. Fermentation conditions such as pH, agitation and temperature are important to assure product consistency. Therefore, batch to batch variability can be reduced by strictly controlling these parameters. After fermentation, the viable cells in the viscous broth are killed by pasteurisation and the gellan is then recovered (Gibson and Sanderson, 1997; Bajaj *et al*., 2007). There are two ways to recover the gellan. Direct recovery by alcohol precipitation from the broth yields production of substituted native form known as high acyl gellan gum (HA gellan). The second way is by treatment of the broth with hot alkali prior to alcohol precipitation, which results in de-acylation reaction and yields production of unsubstituted, low acyl gellan gum (LA gellan) (Sworn *et al*., 2009). Gellan gum is commercially available in both the HA and LA form and have very different physical properties.

3.2 Gellan Gum Structure

Gellan gum is a high molecular weight polysaccharide (approximately ~5 x10^5 Da). Chemically it is a liner anionic polymer with repeating tetrasaccharide unit built up by glucose, glucuronic acid and rhamnose residues in 2:1:1 ratio: [→3)–β– D – glucose – (1→4)–β– D – glucuronic acid – (1→4)–β– D – glucose – (1→4) –β– L – rhamnose – (1→]. The native polymer is in the HA gellan form and has two acyl substituents, L-glyceryl at O (2) and acetyl at O (6), present on the 3-linked glucose (Figure 3.1) (Morris *et al*., 2012). Free carboxylate groups are present on the glucuronic acid residue in the structure, which gives gellan gum an anionic charge that is crucial to the gelation mechanism (Sworn *et al*., 2009).

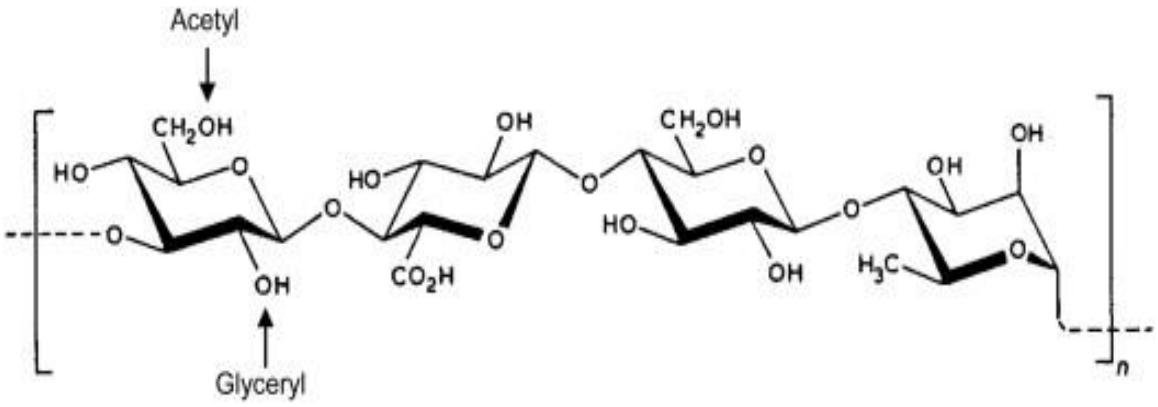

Figure 3.1 Chemical structure of gellan gum (Morris et al., 2012) (used with permission).

3.3 Gelation Mechanism

Gellan gum forms aqueous gels through a molecular ordering process whereby the polymer chains adopt double helical conformations that aggregate to form a three dimensional network. The mechanism of gelation is described by the domain model (Robinson *et al*., 1991), which assumes the formation of distinct junction zones that are connected adjacently by disordered polymer chains. This model is depicted schematically in (Figure 3.2).

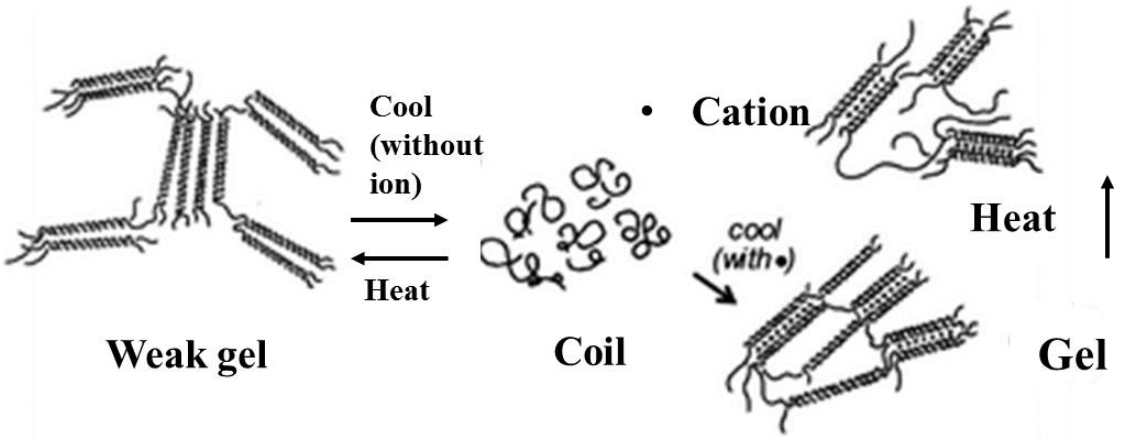

Figure 3.2 Domain Model used to describe gellan gelation (adapted from Robinson et al., 1991).

After hot dispersion (about ~85 °C) of gellan in dissolution media (normally water), gellan gum hydrates and exists in the random coil state. On cooling, gellan undergoes disorder-order (coil-helix) transition (Miyoshi *et al*., 1996), similar to that of agarose and κ-carrageenan. The solution at this stage behaves as a viscous solution (Durand *et al*., 1987). Formation of gel requires association of double helices into a stable aggregated form. Natural aggregation of gellan double helices is inhibited by electrostatic repulsion due to the presence of carboxylate groups. This negative charge can be reduced to promote aggregation either by reducing the pH of the aqueous media or by adding cations (Morris *et al*., 2012). Cations cause further reduction in repulsion by clustering around the helices and thus lowering their effective negative charge hence three dimensional structure forms by aggregation of two or more double helices (Huang *et al*., 2003; Morris *et al*., 2012).

3.3.1 Gelation of Low Acyl Gellan Gum

Low acyl gellan forms a three-dimensional network following aggregation of the gellan doubles helices, by gel promoting cations to form hard, brittle gels. Monovalent cations enhance aggregation of double helices by attaching directly to glucuronate carboxyl groups and forming stable ion pairs. Site binding is triggered initially by electrostatic attraction of

cations to the carboxylate groups of the polymer. The order of effectiveness for the gelation of LA gellan with monovalent cations was reported by (Grasdalen and Smidsrød, 1987; Milas and Rinaudo, 1996). They reported that the order of monovalent cations in promoting aggregation of gellan double helices lie in order as $Li^+ < Na^+ < K^+ < Cs^+ < H^+$ (Grasdalen and Smidsrød, 1987). This would imply that Cs^+ ions give the best geometric fit to the binding site, with progressively less efficient coordination as the size of the cation decreases.

Divalent cations can also cross link gellan gum helices however this occurs by forming direct bridges between adjacent pairs of helices rather than by simply balancing the negative charge of the carboxylate as with monovalent ions. As a consequence the concentrations of divalent ions required to crosslink gellan are much lower than monovalent ions. Moreover, divalent ions promote aggregation of gellan double helices twice as strongly as monovalent ions and the resulting gels have a greater thermal stability (Morris *et al*., 2012). The affinity of divatent ion species for gellan are thought to be similar for Group II cations Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+} although transition metal ions have been found to have an even greater affinity to gellan. (Grasdalen and Smidsrød, 1987).

In addition, gel strength increases with increasing ion concentrations for both monovalent and divalent ions which allows the mechanical properties to be tailored by addition of various concentrations and species. When gellan is dissolved in water, the only cations present in the resulting solution are those present as counter ions to the charged groups of the polymer chains. These low concentrations of cations are not sufficient to facilitate aggregation and subsequent gel formation. Therefore, gellan gum can undergo coil-helix transition but not sol-gel transition. When ion concentrations are equivalent to the number of COO^- groups then gellan undergo sol-gel transition (Matsukawa *et a*l., 1999; Miyoshi and Nishinari, 1999).

Intermolecular association of LA gellan molecules in solution results initially in formation of small, soluble clusters. On cooling further these clusters grow gradually until they become large enough to span the entire volume of the solution and form a continuous cross-linked network. The point at which cation-mediated aggregation occurs is called the "critical gel point". In LA gellan this aggregation occurs rapidly, immediately after a specific temperature has been reached. When following this using a rheometer, G′ increases steeply as intermolecular association occurs on cooling, until stabilising when the network is fully formed (Figure 3.3). The point at which the two curves (storage modulus G′ and viscous modulus G″) crossover (i.e., going from $G' < G''$ to $G' > G''$) is often taken as the sol–gel transition temperature (Dai *et al*., 2008).

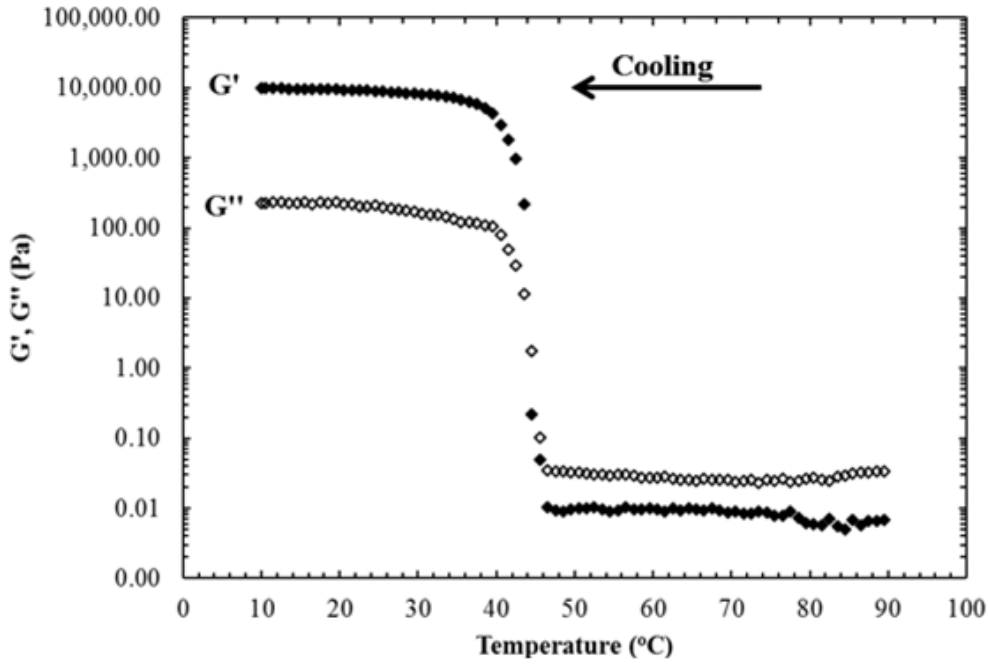

Figure 3.3 Typical rheogram showing the cation mediated gelation of LA gellan on cooling (adapted from Moxon and Smith, 2016).

3.3.2 Gelation of High Acyl Gellan

On average, there is one acetyl for every two repeating units and one glyceryl per repeating unit in HA gellan (Kuo *et al*., 1986) and these acyl groups have a profound influence on the gel characteristics. The acetyl group lies on the periphery of the double helices while the glyceryl group lie in the interior of the helix, hence exerts steric hindrance on gellan chain and modifies the underlying helix geometry by forcing the carboxylic group on the adjacent glucuronic acid residue to rotate which impacts upon the ion binding characteristics (Huang *et al*., 2003; Morris *et al*., 2012) and therefore effects the gelation mechanism. Moreover, the three oxygen atoms in the glyceryl group stabilize the double helical structure by forming new hydrogen bonds within and between the participating strands (Huang *et al*., 2003). Therefore, HA gellan helices are intrinsically more stable than the LA gellan helices.

High acyl gellan gum solutions exhibits similar coil helix transitions on cooling, however further aggregation of the helices is restricted by the presence of the acyl groups (Morris *et al*., 1996) therefore HA gellan has little capacity for cation-mediated aggregation (Morris *et al.,* 1996). It is thought that the acyl groups also inhibit end-to-end type intermolecular associations through steric hindrance, resulting in a decrease in the degree of continuity and homogeneity of the gelled system (Noda *et al.*, 2008).

Due to the increased stability of HA gellan helices, true gels can be formed without the addition of cations (Huang *et al*., 2003). Coil-helix transition of HA gellan occurs on cooling at ~ 70 $^{\circ}$C, and shows no thermal hysteresis (i.e., their setting and melting temperatures are identical) (Morris *et al*., 2012). Addition of cations does, however, result in increased gelation temperature (Huang *et al*., 2004). The concentration of HA gellan gum required to form "self-supporting gels" is > 0.2 % w/w (Sworn *et al*., 2009). From a physical behaviour point of view HA gellan generally produces gels that are softer and more elastic than those formed by LA

gellan gum. Thus, the strain before the material breaks is much greater and therefore less brittle (Morris *et al*., 2012).

Huang *et al*., (2004), studied effect of Na^+, K^+, Ca^{2+} and Mg^{2+} cations on the sol–gel transitions of HA gellan dispersions using dynamic rheological analysis. They reported that the gelling temperature of gellan increased as the cation concentrations was increased and gelation with monovalent cations of either species tested (Na^+ and K^+) exhibited similar gel properties. There was no significant difference in setting temperature between gellan samples dispersed in equivalent concentrations of Ca^{2+} and Mg^{2+} indicating that the size of cations used had no effect on the gelation (Huang *et al*., 2004).

3.3.3 Gelation of HA LA Gellan Blends

Mixtures of LA and HA gellan produce interpenetrating networks, with double helices through incorporating strands of same types only (Sworn *et al*., 2009; Morris *et al*., 2012). Blending the LA and HA gellan gives diverse range of gel textures that lie between the extreme brittleness of LA gellan and the extreme extensibility of the HA gellan (Sworn *et al*., 2009). Figure 3.4 shows schematically how mixtures of HA and LA gellan gum gels compare with other common gelling systems (Mao *et al*., 2000; Huang *et al*., 2003). By varying the ratio of LA HA gellan gum, it is possible to obtain textures close to those of other polysaccharides (Sworn *et al*., 2009).

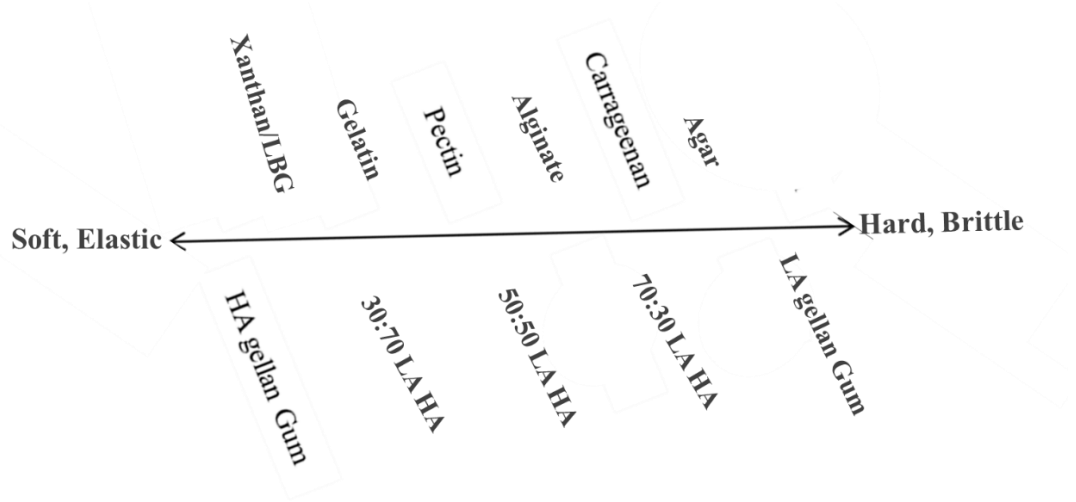

Figure 3.4 Schematic comparison of the gel texture of HA and LA gellan gum gels compared with other common gelling systems (adapted from Sworn et al., 2009).

Kasapis *et al*., (1999) studied the rheological response of the LA HA blends and found that on cooling, there were two separate gelation regions, one at high temperature (designated to HA gellan) and second at lower temperature (designated to LA gellan). It is thought that the gellan gum blends are likely to have "segregative interpenetration", i.e. double helices with the same gellan type, rather than having "associative interaction" between both polymers. In fact, segregative interactions are much more common in general and occur in virtually all biopolymer mixtures where there is no over-riding drive to heterotypic binding (Morris, 2009).

During preparation of LA HA gellan blends, at high temperatures a water-in-water emulsion is produced with HA gellan acting as the continuous phase with LA gellan dispersed through it as small liquid droplets (Morris, 2009). As the solution is cooled the HA undergoes ordering first (as it has a much higher gelation temperature compared with LA gellan) resulting in a biphasic co-gel in which LA gellan is dispersed as liquid droplets through the HA gellan network. On further cooling LA gellan then undergoes a sol-gel transition producing a gel within a gel. At this stage the polymer with stronger gel properties acts as the continuous phase

while the other will act as the dispersed phase (Mohammed *et al*., 1998). Varying the LA to HA gellan ratio therefore can produce a wide range of gel textures (Sworn et al., 2009).

The quantity of acyl groups on gellan can also be controlled as both glyceryl and acetyl groups on the HA gellan can be hydrolysed when exposed to an alkali media such as NaOH and KOH. The reaction can be expressed as in equation 3.1 (Morris *et al*., 2012).

$$R - COO - R' + Salt - OH \rightarrow R - COO - Salt + HOR' \qquad \text{Eq. 3.1}$$

Where R–COO and R′ denote, respectively, the substituent and the polymer chain. By controlling parameters of the reaction such as the temperature at which the reaction occurs, time of exposure and alkali concentrations, it is possible to produce partial acylated gellan (Morris *et al*., 2012). When the hydrolysis reaction takes place at high temperature the gellan polymer will be in disordered form. This causes the glyceryl substituents to be removed faster than acetyl groups. Hydrolysing at low temperature however, release of acetyl groups from the periphery of the double helix occurs far more rapidly than removal of glyceryl substituents embedded within the helix. Moreover, the reactions at low temperature require much longer time.

3.3.4 Effect of pH on Gelation

Gelation of gellan can also occur by reducing the pH, forming strong gels when the pH is lowered to below pK_a of carboxylic acid on the glucuronic acid residue (~ pH 3.4) (Grasdalen and Smidsrød, 1987). Low pH induces gelation by minimising the negative charge of the gellan and promotes the aggregation (Sworn *et al*., 2009; Morris *et al*., 2012). Since the carboxyl group is a weak acid, thus the degree of dissociation of carboxyl groups in aqueous systems is dominated by the dissociation constant. The lower the pH value, the smaller fraction of dissociated carboxyl groups, thus making the gellan less charged by conversion of

glucuronate carboxyl group from negatively-charged COO^- form to the uncharged COOH form. The overall network structure becomes less charged and electrostatic repulsion of the double helices is reduced (Grasdalen and Smidsrød, 1987). It is also reported that the decrease in electrostatic repulsion between the intramolecular segments may result in suppression of gellan chain expansion, making association and aggregation even easier (Horinaka *et al*., 2004).

Previous studies reported that the LA gellan is particularly acid sensitive forming strong gels at low pH (Norton *et al*., 2011), whereas HA gellan is much less sensitive (Bradbeer *et al*., 2014). Moreover, Bradbeer *et al*., (2014), investigated the effect of acidic medium on mechanical properties of LA HA gellan blends and found that post-production exposure, of blends with a high LA to HA ratio, to an acidic medium leads to an increase in total work required to fracture the gel. It was suggested that the cross-links between the LA gellan chains are reinforced by acid exposure. Increasing acid exposure for more than one hour showed no effect on mechanical properties of gels with high LA to HA ratio. In contrast, gels with a high HA proportion began to show signs of breakdown after 3 hours acid exposure, with a decrease in bulk modulus and total work to fracture. This weakening of the gel became more pronounced with increasing HA to LA ratio and reached a maximum at 0:100 LA HA ratio.

3.4 Pharmaceutical Applications

Polysaccharides in particular, find widespread use in pharmaceutical formulations as they possess flexible physicochemical properties (Prajapati *et al*., 2013). Gellan gum has received particular attention from pharmaceutical researchers because of its unique physicochemical properties. In pharmaceuticals, gellan gum is found in many dosage forms performing a variety of functions these includes: swelling agents, binders and disintegrants in tablets, shells of hard capsules, gelation agents in oral liquid formulations. The first commercial

pharmaceutical product containing gellan was the *in situ* gelling eye drop formulation containing timolol maleate (Timoptic-XE®) to treat glaucoma (Shedden *et al*., 2001; Grover and Smith, 2009). Since then, gellan has been investigated as carrier in many dosage forms for various routes of delivery.

3.4.1 Oral Drug Delivery

Drug delivery through oral cavity is the most preferred route of drug administration for both solid and liquid dosage forms. The oral route is considered as the safest and most convenient way of drug administrations. Gellan gum is a widely used food ingredient (E418) and is generally regarded as safe to consume. Using gellan gum in oral pharmaceutical formulations has mainly been used to try to deliver or retain the active pharmaceutical ingredients (API) at specific sites of the gastric intestinal tract (GIT). This section describes gellan gum used in oral dosage forms.

3.4.1.1 Tablets

There are several different types of tablet formulations where gellan gum has been investigated for various functionalities.

3.4.1.1.1 Swelling Agent

Size-increasing tablets and floating tablets are commonly used approaches to achieve prolonged residence time in stomach. Transit time for normal tablets in the stomach is very short therefore a conventional tablet will rapidly pass to the small intestine. Tablets however, can be retained in stomach for a prolonged period of time, if the size of tablet is bigger than the dimension of the pyloric sphincter. This can be in the order of 5 cm length or a diameter of 3 cm (Klausner *et al*., 2003). Difficulty swallowing such large tablets was an obstacle for these formulations. This problem can be potentially addressed by using a polymer which can swell rapidly *in situ*. Indeed, many hydrophilic polysaccharides have been investigated to be used in

formulations of *in situ* swelling tablets due to their ability to absorb large amounts of water and rapidly swell. Using these highly swelling polymers, tablets can be formed at a standard size suitable for swallowing and can swell and increase in size in the stomach thus, prolonging residence time. Gromova *et al*., (2007) formulated acyclovir gastro-retentive tablets based on a mixture of guar gum and LA gellan (Figure 3.5). This formulation utilised the synergistic swelling effect of guar and gellan and results showed that the degree of swelling was enough to retain the formulation in the stomach for an increased period of time. The tablet was found to swell more than two-fold during the first 60 minute of dissolution, which was enough for gastro-retentive effect.

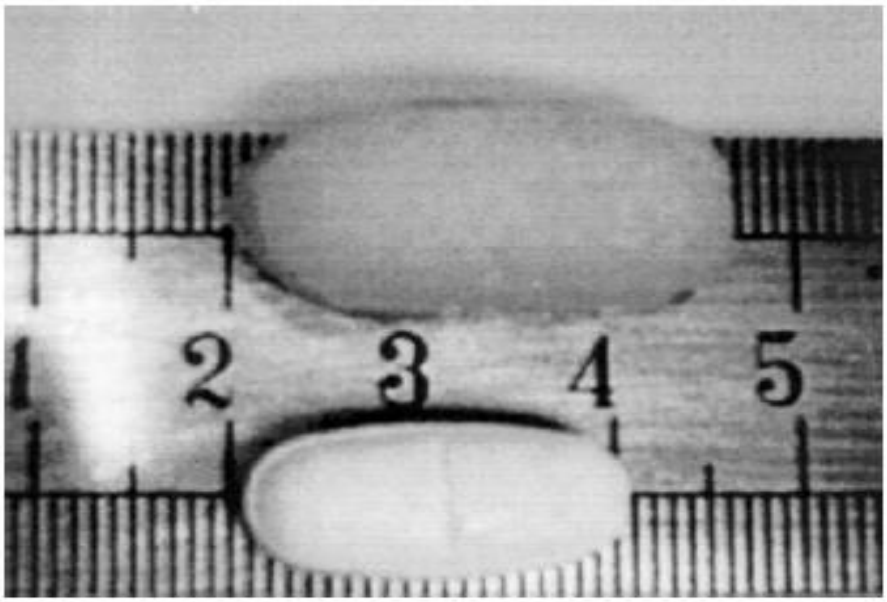

Figure 3.5 Relative dimensions of gastro-retentive acyclovir tablets (500 mg) before and after the dissolution test (Gromova et al., 2007) (used with permission).

El-Zahaby *et al*., (2014) studied the effect of cross linking cations (Ca^{2+} and Al^{3+}) on *in situ* swelling tables prepared from LA gellan. The results of the study indicated that the addition of cations slow down the release of API drastically. In addition, type of ions had a significant effect on the drug release. Surprisingly, they found that divalent ions (Ca^{2+}) sustained release of API more than the trivalent ion tested (Al^{3+}). This was explained by an

increased capacity for water uptake of gellan-calcium compared with that of gellan-aluminium causing a greater swelling effect. A greater swelling capacity results in an increased diffusion path length, which in turn increases diffusion time and thus a slower drug release (El-Zahaby *et al*., 2014). The angle of repose, bulk and tap density of gellan gum and gellan-Ca^{2+} were also investigated and the results showed that gellan has excellent flow with good compressibility (El-Zahaby *et al*., 2014).

3.4.1.1.2 Disintegrant

Disintegrants are agents added to tablets and some encapsulated formulations to promote the break-up of compacted powder when exposed to aqueous media. The use of gellan gum as a disintegrant was first investigated by Antony and Sanghavi, (1997) and later by Emeje *et al*., (2010) who compared it with standard disintegrants (maize starch and sodium starch glycolate). Different gellan concentrations 0.2 %, 5 %, 10 % and 15 % w/w and different modes of incorporation of the gellan were investigated and they found that the gellan was as most effective as a disintegrator when used extra-granularly at 0.2 % w/w. This formulation had a disintegration time in 0.1 N HCl of 12.9 min (Emeje *et al*., 2010). The disintegration time for 5 %, 10 % and 15 % w/w, however, were slower 90, 100 and 119 min respectively. Hence gellan gum at these higher concentrations was not useful for immediate release dosage forms as disintegration time is much to slow. In another study, 4 % w/w gellan gum showed the shortest disintegration compared with other disintegrants used in sublingual tablets (Prajapati *et al*., 2014). These results are not in good agreement with Emeje *et al*., (2010) and this may be due to deionised water being used as the disintegration media in Prajapati study rather than 0.1 N HCl. This ignores the fact that gellan is an ion active polymer and it is strongly affected by the pH and the ions present in the dissolution media. In both studies the results showed that the disintegration time increases by increasing gellan concentration which could be useful to design sustained release formulations.

3.4.1.1.3 *Binding* Agent

Some polymers such as gellan gum have both disintegrant and binding properties when used in different concentrations. Binders are usually used when making granules for formulating tablets or capsule fillings. Selecting the appropriate binding agents for granulation can be important in preparing tablets with specific mechanical strength and drug release profiles.

The adhesion properties of LA gellan and its binding capacity has previously been investigated (Ike-Nor *et al*., 2006; Franklin-Ude *et al*., 2008). Gellan when used at concentrations of 20-30% showed comparable binding capacity to acacia gum and gelatin and a stronger binding capacity compared with maize starch (Franklin-Ude *et al*., 2008). The gellan formulations however had the longest disintegration time which was in good agreement with (Emeje *et al*., 2010), who previously reported using gellan at high concentration leads to an increase in disintegration time. This increased binding capacity and subsequent increased disintegration time can be utilised to prolong the release of drug. This effect was reported by Franklin-Ude *et al*., (2007) when using 20 % to 30 % of gellan gum to produce granules which sustained drug release for up to 8 hours (Franklin-Ude *et al*., 2007). This is due to the binding and swelling capacity of gellan gum and shows excellent potential as a controlled release matrix.

3.4.1.2 Capsules

Capsules are solid dosage forms, the word capsule comes from Latin capsula which means a small box. Capsules are superior to tablets when problems arise such as compressibility, solubility and bitter taste problems. There are two types of capsules hard and soft capsules. Hard capsules consist of two hard shells (cap and body). Gelatin is widely used to make hard shell capsules but due to its animal origin, there was a demand to find alternative

non animal source. Recently, a great interest has been given to HPMC as an alternative to gelatin to formulate hard shell capsule as HPMC is promising from a manufacturing, regulatory, dietary and religious perspective (Missaghi and Fassihi, 2006). Shells formed by HPMC, however, are weaker than gelatine-shells (Al-Tabakha, 2010) and often require a gelling agent in the formulation to maintain the capsule shape. To overcome this problem, Ku *et al*., (2010) used HPMC blended with gellan gum to support the shell wall (Ku *et al*., 2010), which resulted in less brittle capsule compared with HPMC alone and observed slower *in vitro* disintegration and dissolution. The slower disintegration and dissolution was likely due to the insolubility of gellan in the gastric acid.

The ability to support the structure of HPMC capsules and slow disintegration time in acid was investigated by Smith *et al.,* (2010), who developed a capsule shell formulation using various ratio of HPMC, sodium alginate and gellan gum to design two-piece hard capsule for post-gastric delivery. The resultant capsule parameters such as thickness and water content found to be comparable to commercial gelatin capsules. These capsules were shown to open after only 5 min in phosphate buffer (pH 6.8) after being submerged in 0.1 M HCl (pH 1.2) for 2 h, highlighting the potential for delayed release capsule formulations using gellan gum.

HA and LA gellan gum blends have also been used in formulations to replace gelatin in soft capsule formulations. Different ratios of blended LA HA gellan were mixed with carrageenan and mannan gums to produce soft capsules. (Winston *et al*., 1994).

3.4.1.3 Oral Liquids

Different polysaccharides are used in the formulation of oral liquid dosage forms. These polysaccharides are usually used to thicken the formulation, to facilitate suspension of drugs, aid dispensing of the liquid and improve mouth feel. Recently the acid gelling properties of gellan gum has been identified by researchers as a good candidate for an *in situ* gelling oral

liquid due to the low pH of the stomach. Rajinikanth and Mishra, (2008) have described the main prerequisites for oral liquid *in situ* gelling systems, these are optimum viscosity for ease of swallowing and mouth feel, gelling cappacity and a rapid sol-gel transition due to ionic interactions. All of these prerequisites can be suitably addressed using gellan gum.

Indeed, previous studies (Miyazaki *et al*., 1999; Miyazaki *et al*., 2001; Kubo *et al*., 2003; Rajinikanth *et al*., 2007; Rajinikanth and Mishra, 2008) reported that gellan has the potential to be used for oral-liquid *in situ* gelling systems containing complexed calcium ions. Once the formulation is administered, the calcium ions are released due to low pH of stomach, resulting in crosslinking of the gellan *in situ*. Miyazaki *et al*., (1999) reported sustained release of theophylline from an *in situ* gelling formulation and the bioavailability was increased 3 fold in an animal model, following administration of LA gellan oral liquid formulations.

This *in situ* gelling system using LA gellan has been further investigated by Rajinikanth *et al*., (2007) and Rajinikanth and Mishra, (2008) to treat stomach ulcers caused by *H. pylori* using clarithromycin and amoxicillin, respectively. In these works, the formulations showed better anti *H. pylori* activity than conventional suspensions. The formulations form a strong gel in the stomach acid and are buoyant on the gastric fluid causing an increase of gastric residence time. Therefore, a lower dose of clarithromycin and amoxicillin are required in the formulation and dosing frequency can be reduced, which can improve patient compliance and lead to more successful treatment of *H. pylori.*

3.4.1.4 Gellan Beads

Gellan beads can be easily prepared using an external ionotropic-gelation method. Beads are formed by the dropwise addition of aqueous solution of gellan gum into aqueous solution of crosslinking ions or vice versa (Tripathi *et al*., 2012). This simple technique can be used to entrap drugs by simply dissolving or dispersing the drug in the polymer solution or in

the crosslinking ions. The gellan gum gels almost instantaneously at the external surface when the droplets come into contact with the crosslinking ions creating a gel coat (Osmelak *et al*., 2014). This is due to the rapid gelation kinetics of gellan. Using this technique, the crosslinking ions must diffuse through the gellan gum structure to crosslink the central regions of the bead. In addition the un-crosslinked gellan will also migrate towards the crosslinking ions at the surface subsequently producing a gel that has a higher concentration of gellan at the surface than at the centre of the structure (Figure 3.6). Time exposed to the crosslinking ion source therefore, also impacts on the strength of the gellan beads and homogeneity. The longer the beads are submerged in the crosslinking ion solution the more time there is for diffusion of the ions to occur and increase the crosslinking density of the gellan beads.

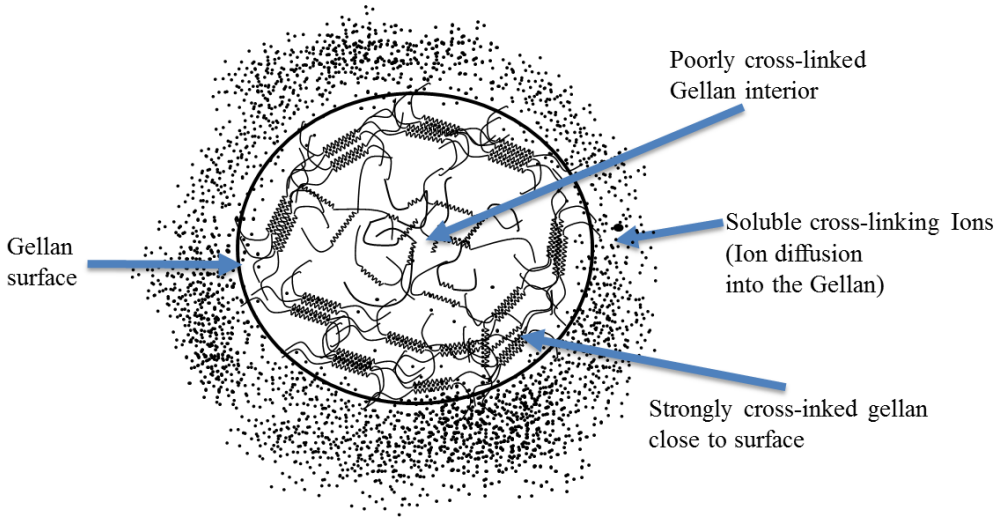

Figure 3.6 Mechanism of Gellan Gum Bead formation using external ionotropic gelation.

Once the beads are prepared they are usually washed with water and dried prior to use. The properties of formed beads can be controlled by different factors that includes polymer concentration, polymer molecular weight, ion concentration and species, aperture size, pH of the crosslink media, and drying parameters (Narkar *et al*., 2010; Verma and Pandit, 2011). In low pH media gellan beads are stable and swell rapidly but in high pH media beads can

dissolve. Therefore, any encapsulated drug releases faster in intestinal conditions compared with the acidic gastric conditions (Babu *et al*., 2010). LA gellan gum has been shown useful in the preparation of sustained release bead systems, either alone (Kedzierewicz *et al*., 1999; Emeje *et al*., 2009; Babu *et al*., 2010), or by blending with clay (Santucci *et al*., 1996) or with other polymers such as chitosan (Dixit *et al*., 2011; Verma *et al*., 2012; Yang *et al*., 2013), sodium alginate (Srinatha and Pandit, 2008; Totosaus *et al*., 2013; Prajapati *et al*., 2013b) and hydroxypropyl methylcellulose (Ariza *et al*., 2013) to deliver API to specific target sites (stomach, intestine and colon). Thus by appropriate manipulation of above parameters, it is possible to obtain beads with different functionality.

Narkar *et al*., (2010) used chitosan coated gellan gum to formulate mucoadhesive beads containing amoxicillin trihydrate to treat *H. pylori*. The *in vitro* release study illustrated that the drug released in controlled manner for up to 7 hours. Additionally, chitosan contributed to greater mucoadhesive behaviour. Ahuja *et al*., (2010) showed that sustained release from beads can be obtained by combining of gellan gum with gum Cordia which is strongly mucoadhesive. The experiments revealed that release of metformin HCl was prolonged up to 24 h (Osmelak *et al*., 2014).

Many APIs used to treat *H. pylori* infections have been encapsulated within gellan beads that float on the surface of the stomach to increase retention time, such as acetohydroxamic acid (Rajinikanth, 2007), clarithromycin (Rajinikanth and Mishra, 2009), ofloxacin (Kabbur *et al*., 2011), amoxicillin (Tripathi *et al*., 2012) and rifabutin (Verma and Pandit, 2011). The release of the drug from floating gellan bead systems can be sustained up to 10 h depending on formulation parameters (Tripathi *et al*., 2012). Gellan gum formulations blended with other polysaccharides have also been investigated and shown a beneficial effect for sustained release. Kabbure *et al*., (2011) compared beads formulated from low methoxy

pectin (LMP) alone and a blend of LMP and gellan gum. The results showed that beads formulated with gellan demonstrated sustained release of ofloxacin for more than 8 h.

3.4.2 Intranasal Drug Delivery

Nasal cavity is used mainly for local treatment of nasal congestion, infections or allergic symptoms (Illum, 2003). However, intranasal route may be suitable to achieve the systemic action, especially for drugs that are quickly metabolized or not efficiently absorbed in the GI tract (Jansson *et al*., 2005). LA gellan gum has generated interest as a promising polymer for use in nasal formulations because of its ability to form strong clear gels *in situ* at physiological conditions. *In situ* forming polymer formulations are drug delivery systems that are in solution (Cao *et al.*, 2009) or in dry microparticle (Mahajan and Gattani, 2009a) form before administration, but once administered, undergo gelation in response to the physiological environment. *In vivo* studies have confirmed gellan gum to be non-irritant and non-toxic to the epithelial tissue even when applied for a prolonged period of time (Cao *et al*., 2009; Mahajan and Gattani, 2009a), in addition to remaining stable for over 6 months.

LA gellan has been used to formulate a metoclopramide dry powder nasal spray in the form of micro-particles manufactured by spray drying. This formulation is based on *in situ* transition from a dry powder to a swollen gel as the particles delivered in dry solid state. (Mahajan and Gattani, 2009a). Following administration, the LA gellan micro-particles were shown to swell and adhere to nasal mucosa. Release of the metoclopramide was moderately sustained with no observed lag time.

More widely investigated LA gellan *in situ* gelation formulations are those based on a sol-gel transition with the gellan solution sprayed out from a nasal spray device in the liquid state with the drug dispersed or dissolved within the gellan. This was first described by Bacon *et al*., (2000) who reported that to achieve a reproducible dispensing volume from a nasal spray device requires a low viscosity formulation but viscous enough to aid adhesion on application

site. LA gellan is a particularly good candidate vehicle for nasal sprays as it exhibits shear thinning behaviour which facilitates the dispensing through spray devices and rapidly thickens and forms a gel on contact with the physiological concentrations of cations. Various beneficial properties have been reported when using LA gellan as an *in situ* gelling system for the delivery of a range of drugs via the nasal route (Table 3.1).

Table 3.1 Shows investigated *in situ* gellan formulation.

Gellan Formulations	Active Pharmaceutical Ingredients	Target of Action	Reference
***In situ* gelling – Liquid**	Carvedilol	Systemic	(Saindane *et al*., 2013)
***In situ* gelling – Liquid**	Mometasone furoate	Local	(Cao *et al*., 2009)
***In situ* gelling – Liquid**	Sumatriptan succinate	Systemic	(Galgatte *et al*., 2014)
***In situ* gelling – Liquid**	Gastrodin	Systemic	(Cai *et al*., 2011)
***In situ* gelling – Liquid**	Scopolamine hydrobromide	Systemic	(Cao *et al*., 2007)
***In situ* gelling – Liquid**	Huperzine A	Systemic	(Tao *et al*., 2006)
***In situ gelling* – Liquid**	Influenza vaccine	Systemic	(Bacon *et al*., 2000)
***In situ* gelling – Microparticle**	Metoclopromide hydrochloride	Systemic	(Mahajan and Gattani, 2009a)
***In situ* gelling – Microparticle**	Ondansetron	Systemic	(Mahajan and Gattani, 2009b)

Bacon *et al*., (2000) reported a moderate enhancement to the local and serum antibody response in mice after gellan nasal administration of influenza vaccine. A few years later Jansson *et al*., (2005) reported that a LA gellan based *in situ* gelling intranasal formulation enhanced epithelial uptake of high molecular weight fluorescein dextran. Compared with oral and subcutaneous formulations, gellan based nasal spray formulations of scopolamine hydrobromide showed superior action in significantly minimising motion sickness symptoms (Cao *et al*., 2007). Saindane *et al*., (2013) reported that LA gellan base nasal spray enhanced bioavailability and improved therapeutic efficacy of carvedilol. They also demonstrated that the carvedilol was released in sustained manner but was dependent on the concentration of gellan used. A concentration of 1 % w/w LA gellan had zero order release indicating concentration independent drug release while, 0.5 % and 0.25 % w/w LA gellan showed first order release indicating concentration dependent drug release. This was explained by the gellan gel eroding more rapidly at low concentrations, in contrast to the higher concentrations of LA gellan which tend to form stronger gels that are less likely to erode.

3.4.3 Ocular Drug Delivery

The eye is a complex site of application because of its unique anatomic and physiological structure. There are challenges to deliver drugs to the eye such as restricted precorneal permeability and rapid clearance of the dose by the action of both the lacrimal fluid and blinking, which results in a short precorneal contact time. Therefore, only a small amount of the medication is delivered and maintained in the place of action (Gurtler *et al*., 1995). This subsequently leads to poor bioavailability (Osmelak *et al*., 2014). Due to these limitations, *in situ* gelling systems with the prolonged drug release are particularly useful as the elastic properties of gels can resist ocular drainage. Indeed, the application of *in situ* gel formulations has been shown to sharply increase precorneal residence time for up to several hours. This was first exploited by Pramoda *et al.*, (1979) who used the pH dependent sol-gel transition of

xanthan locust bean gum mixtures. The formulation exists as a liquid at low pH (~pH 3.5) and then undergos gelation at the physiological pH of the eye (~pH 7). This approach increased residence time and improved bioavailability, however, application of acidic formulation to the eye could be problematic and uncomfortable to the patient (Chastaing *et al*., 2001). A more patient friendly approach to deliver the drugs to the eye via *in situ* gelling systems is to use LA gellan, which utilises the ionic composition of lacrimal fluid to induce gelation. Indeed, ophthalmic formulations are the most frequently encountered examples of LA gellan in current commercial pharmaceutical use (Grover and Smith, 2009). Lacrimal fluid consists of a complex mixture of proteins, vitamins, sugars and lipids but importantly also contains a range of electrolytes that include Ca^{2+}, Na^{+} and K^{+} at concentrations sufficient to crosslink gellan gum. In addition, the gels formed by LA gellan in particular are optically transparent which has obvious benefits for ocular application. It is well tolerated and can be used without the risk of any toxic effects (Rozier *et al*., 1989). One such example that is currently on the market is Timoptic XE® which uses LA gellan-based formulation for the sustained release of timolol maleate. In comparison with the standard timolol solution, the gellan containing *in situ* gelling formulation enhances the bioavailability of the drug by three- to four-fold when applied to the cornea (Rozier *et al*., 1989).

Table 3.2 Ionic content of tear fluid (adapted from Grover and Smith, 2009).

Electrolyte	Concentration mMol/L
Calcium	0.57
Sodium	~140
Potassium	15 - 29
Chloride	120 - 135
Bicarbonate	26

Gan *et al*., (2009) designed a microemulsion *in situ* electrolyte triggered gelling system for ophthalmic delivery of a lipophilic drug (cyclosporine A). Cyclosporine A loaded microemulsion was prepared using castor oil, solutol HS 15 (surfactant), glycerol and water. This microemulsion was then dispersed in a LA gellan solution to form the final microemulsion *in situ* electrolyte-triggered gelling system. *In vivo* studies revealed that the area under the curve $AUC_{0\ -\ 32h}$ of corneal cyclosporine A for the microemulsion LA gellan system was approximately 3-fold greater than for a cyclosporine A emulsion.

The rate of the gel formation after drop installation in the eye is very important, as a solution or weak gel will also be prone to elimination by the fluid of the eyes. Rate of gelation depend on the rate of electrolyte binding by the gel, which is dependent on osmotic gradient across the surface of the gel. Osmolality of the solution therefore, might have an influence on the rate of the sol-gel transition and subsequent efficacy of the formulation, since the precorneal residence time of the gels depend on rheological properties. Carlfors *et al*., (1998) investigated

contact time of gellan formulations at concentrations ranging from 0.4 - 1.2 % w/w, with glycerol added at varying concentrations 0 – 4 % w/w to obtain a formulation with different osmolality. Irrespective of gellan concentration, contact time decreased with increasing osmolality. At 1.5 % w/w glycerol, contact time increased with increasing gellan concentrations. In the absence of glycerol, however, contact time was independent on gellan concentrations (Carlfors *et al*., 1998).

3.4.4 Topical Drug Delivery

In the past, many of natural materials such as honey pastes, plant fibers, and animal fats were used to cover wounds and burns in order to prevent infection and to enhance healing. Nowadays, biopolymers play a significant role in modern wound dressings. Amin *et al*., (2012) investigated developing a wound dressing using polyelectrolyte complex of chitosan-LA gellan containing levofloxacin and titanium dioxide (TiO_2). In this system, the LA gellan composite supported the growth of fibroblasts (L929) cells and had excellent antibacterial properties. These results were in good agreement with (Lee *et al*., 2010), as they also have shown that gellan films have similar behaviour on L929 cells (Amin *et al*., 2012). Gellan-silver wound dressing films have also been designed to provide similar antimicrobial activity of topical silver, with advantages of a sustained silver release and a reduced number of dressing changes (Cencetti *et al*., 2012).

A new hydrogel based on gellan gum and sulphated hyaluronic acid was recently introduced by Cencetti *et al*., (2011) to prevent post-surgical adhesion. The characterization of the new material demonstrated that the gellan, due to its high-viscosity, could effectively act as a barrier with a long *in situ* residence time. The material showed good stability for 12 months at room temperature. Formulation with 2 % w/w gellan showed high elastic modulus values which facilitates a long residence time (Cencetti *et al*., 2011). The transparent gels that LA-

gellan form are another advantage of topical application especially when used as a wound healing material as it possible to view the underlying wound without removal of the dressing (Figure 3.7).

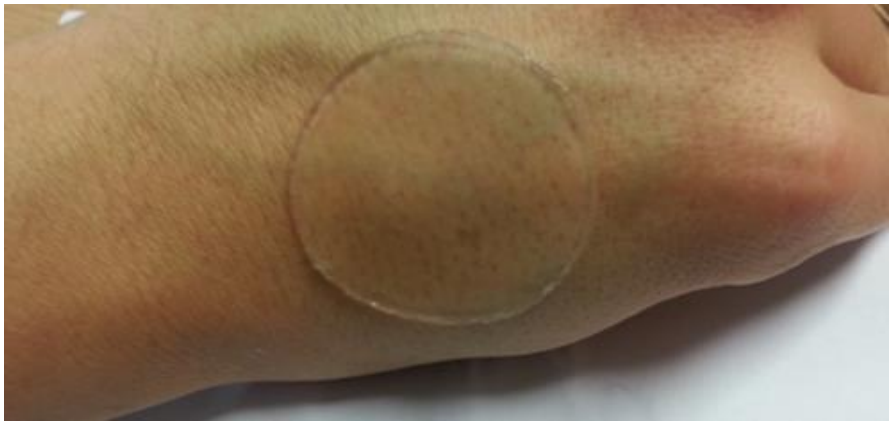

Figure 3.7 1 % w/w LA gellan hydrogel highlighting the optically transparent properties when applied to the skin (adapted from Smith et al., 2016).

3.5 Gellan Gum Fluid Gels

As discussed in section 3.3, on cooling a solution of gellan gum in presence of monovalent or divalent cations (LA gellan), under quiescent conditions results in the formation of a three dimensional structured gel network. By applying a shear force during this gelation process, however, it is possible to form 'fluid gels', which are suspensions of gelled particles dispersed in a non-gelled continuous medium (as described in Chapter 1 section 1.5).

Typically, ion concentrations required to gel gellan are in the region of ~100 mM for monovalent cations and ~5 mM for divalent cations, however, the strength of the gels produced depend on the concentration of gellan (Morris *et al.,* 2012). Fluid gels have the ability to flow freely, whilst having mechanical spectra similar to those of typical gel networks (Bradbeer *et al*., 2014). Fluid gels are able to self-structure but they are unable to fully support themselves therefore have been termed weak gels. The main different between true gels and fluid gels is that true gel respond to external force by fracturing which cannot be reversed whilst a fluid gel

responds to external force by flowing and restructuring again to the same initial status when the applied stress is removed. Therefore, conventional gels that show ‘weak’ gel properties in that they have low moduli are not classified as “fluid gels” (Morris *et al*., 2012). Mechanical spectra of “weak gels” normally differs from those of true gels in having greater frequency-dependence of G′ and G″ and smaller separation between the two moduli.

There has been an intensive research focus on formation and evaluation of gellan gum fluid gels. Sworn *et al*., (1995), studied the properties of gellan gum gels prepared under shear force (fluid gels) and gels prepared under quiescent condition (true gels). A fixed concentration of gellan (0.125 % w/v) was incorporated in solutions of NaCl (ranging from 0-18 %) or $CaCl_2$ (ranging from 0 to 0.7 %) measured at 25 °C. The onset temperature for gelation on cooling, as determined by measurements of η, increased with increasing ionic concentration to a maximum with both calcium and sodium ions. The maximum transition temperature observed with Na^+ (55.1 °C) was greater than the maximum observed with Ca^{2+} (48 °C). The fluid gels produced, had a viscosity when measured at 25 °C that follows the same trends as those of gel strength in the quiescent gels, relative to the ion concentrations used. In other words, the stronger the gel prepared under quiescent conditions, the higher the viscosity of the same system when prepared as a fluid gel (Sworn *et al*., 1995). By controlling the concentration of LA gellan and the ions used in the gelation it is possible for the fluid gels to be prepared as homogeneous structured liquids at a specific range of concentration, especially in combination with sodium ions. For example, a smooth fluid gel capable of suspending herbs was produced for the food industry by using 1 % NaCl with as little as 0.03 % gellan gum. At high concentrations, however, i.e., those used to create very strong quiescent gellan gels, the fluid gels produced have cloudy or grainy appearance described as the 'crushed ice' effect (Sworn *et al*., 1995).

Caggioni *et al*., (2007), have used video imaging to study the internal rheology of both sheared and un-sheared gellan gel structures. They found that the microgel particles created as a result of cooling under shear have identical internal structures as the continuous gel networks obtained on quiescent cooling. The failure of gellan gum "fluid gel" networks formed under shear have been recently investigated by Garcia, *et al*., (2011). LA gellan solutions (0.025 – 0.25 wt.%) were used alongside a fixed NaCl concentration (0.22 M) at 80 °C, and the sol-gel transition temperature on cooling was recorded at ~ 41 °C. "Fluid gels" were then formed at 700 rpm shear rate at 41 °C for 1 min. Fluorescently labelling the gellan using fluoresceinamine enabled the observation of irregularly-shaped particles (0.1 – 1 mm dimension at 0.25 wt.% gellan) which were roughly 10 times larger than those reported by Caggioni *et al*., (2007) where vigorous and prolonged shearing was performed.

Garcia *et al*., (2011) also reported that the mechanical spectra of the fluid gels were comparable to the quiescent gel mechanical spectrum, with G′ being approximately an order of magnitude greater than G″, and with both moduli displaying slight increases with increasing angular frequency (ω) (Morris *et al*., 2012). Furthermore, Sworn *et al*., (1995) stated that the mechanical spectra for a 0.125 % gellan fluid gel with 6 % NaCl exhibit typical gel-like response at all frequencies tested (0.01- 10 Hz). Gellan gum fluid gels exhibit pseudoplastic flow behaviour when subjected to steady state shear, similar to that of entangled polysaccharide solutions. Particle-particle interactions between gellan micro-particles, is strong enough therefore, to require a high stress to yield flow (Sworn *et al*., 1995). This rheological behaviour would account for the remarkable suspending power of gellan gum fluid gels, which is evident at much lower concentrations than with other polysaccharides.

These functional mechanical properties coupled with the sensitivity of gellan to physiological concentrations of ions and the potential for tuneable and bioresponsive behaviour

make fluid gels prepared from gellan an attractive material to investigate potential applications as various dosage forms. The following chapters propose the potential use of gellan gum fluid gels as an oral sustained release liquid, mucoadhesive nasal spray and a topical gel.

Chapter 4

Evaluation of LA gellan gum fluid gels as modified release oral liquids

Results presented in this chapter are published in the International Journal of Pharmaceutics

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CHAPTER 4 EVALUATION OF LA GELLAN GUM FLUID GELS AS MODIFIED RELEASE ORAL LIQUIDS

4.1 Introduction

There is an ever increasing demand for the development of age-appropriate dosage forms, especially for paediatric patients and older adults who have difficulties in swallowing tablets. This is most apparent in modified release formulations where the functional excipients responsible for controlling drug release can become ineffective due to manipulation prior to administration to children. Even over the counter, antipyretic formulations have an increased risk of side effects in children. Worryingly, there are very few oral modified-release drug delivery platforms suitable for administration to paediatrics.

Generally, for children and other patient groups who find swallowing is difficult, syrup-based oral liquids are the preferred dosage form. However, formulating these dosage forms to have modified release properties can be challenging. Recently researchers have looked to develop such dosage forms using enteric coated microparticles (Dalmoro *et al*., 2010) and ion exchange resins (Cuna *et al*., 2000) however these systems are often costly, suffer from poor mouth feel and are only suitable for use with specific drugs. There is therefore a real need for alternative formulations. A potential route to achieve modified release in oral liquids is by using polysaccharide solutions which undergo a sol-gel transition on exposure to stomach acid. Indeed, several authors have evaluated the oral sustained delivery of drugs such as theophylline, ambroxol, paracetamol and cimetidine from various *in situ* gelling polysaccharides which have included xyloglucan (Miyazaki *et al*., 2003; Itoh *et al*., 2008; Itoh *et al*., 2010), pectin (Kubo *et al*., 2004; Miyazaki *et al*., 2005; Kubo *et al*., 2005; Itoh *et al*., 2008), and sodium alginate (Kubo *et al*., 2003; Itoh *et al*., 2010). Although these systems have

shown some promise as drug delivery vehicles, there are issues associated with their use such as leaching of water soluble drugs and lengthy gastric retention due to large bulk gel formation *in situ* (Kubo *et al*., 2003).These issues could potentially be overcome by using LA gellan gum fluid gels. The ability of LA gellan gum to form acid-insoluble gels renders it a particularly attractive candidate for developing oral bioresponsive drug delivery systems.

LA Gellan gum has previously been investigated in the form of gastro-retentive controlled release (Babu *et al*., 2010), enteric release (Smith *et al*., 2010) and as floating *in situ* gelling systems (Rajinikanth and Mishra, 2008). Furthermore, oral sustained delivery using gellan solutions (which formed acid gels in the stomach) has also been explored and drug bioavailability released from these gels formed *in situ* was similar to that of a commercially available suspension (Kubo *et al*., 2003).

Unlike tablet or capsule formulations, there is no standard technique for measuring the dissolution properties of oral liquids. Biopharmaceutical measurements of such formulations are usually performed using modified USP dissolution apparatus which can lead to high variability. This is a particularly important issue when designing medicines for children as extrapolating adult biopharmaceutical measurements is difficult due to the difference in gastrointestinal physiology in paediatric patients (Batchelor *et al*., 2013). Moreover, large variations in physiology within paediatric populations are also evident from birth through to adolescence (Bowles *et al*., 2010) which further complicates the design of suitable biopharmaceutical methodologies. There are several physiological factors such as gastric transit time and gastric pH variation that could have an effect on bioresposnsive formulations, consequently, effecting drug release and subsequent absorption. Therefore, it is necessary to understand the physiology of gastrointestinal tract (GI) and how this may impact on the behaviour of LA gellan fluid gels during passage through GI tract (Charman *et al*., 1997; Pang, 2003).

This chapter provides an overview of the anatomy and physiology of GI tract and the physical, chemical and pharmacological properties of ibuprofen (which is the drug used in this investigation). LA gellan gum fluid gel formulations loaded with ibuprofen, are then evaluated as a modified release oral liquid. The fluid gel formulations were investigated over a range of pH and acid exposure times to evaluate how variations in gastric physiology may impact on the mechanical properties of these physiologically responsive fluid gels, and how this relates to release behaviour.

4.2 Anatomy and Physiology of Gastrointestinal Tract

The gastrointestinal tract (GI) provides the pathway for both digested food and digested medication through the body. From the anatomical point of view, the GI is a muscular tube approximately 6 m long starting from the oral cavity and terminating at the anus (Ashford, 2007). The wall of GI tract is similar throughout the tract and is lined by a mucous membrane. Most parts of the mucous membrane are covered by a viscoelastic thin a layer of mucus. This layer is continuously replaced with freshly secreted mucus that acts as a protective layer and mechanical barrier. The GI tract is anatomically subdivided into different sections and subsections. The four parts of GI tract are oesophagus, stomach, small intestine and large intestine or colon (Dressman *et al*., 1990; Ashford, 2007) (Figure 4.1). The average transit time from the mouth to the anus is approximately 24 h.

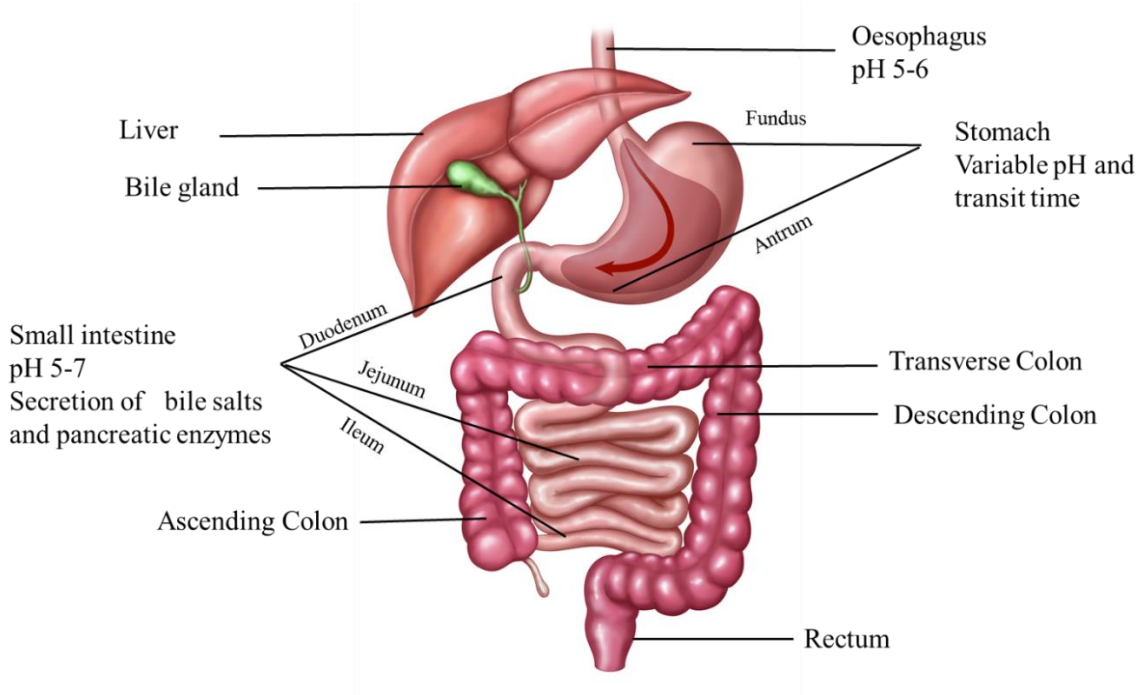

Figure 4.1 Illustration of the main anatomical regions of the GI tract (adapted from Ashford, 2007).

4.2.1 Oesophagus

The oeophagus is a thick muscular layer about 250 mm long and 20 mm in diameter, connecting oral cavity with the stomach. It is vertical in orientation with a few curves along its path. It passes down through the neck along a central path in front of the trachea and is connected to the stomach at cardiac orifice. The oesophagus lumen has pH ranging from 5 to 6. Following oral ingestion, material travels down the oesophagus (few seconds transit time) to the stomach (Patti *et al*., 1997; Ashford, 2007).

4.2.2 Stomach

The stomach is defined as the dilated part of GIT, located between the oesophagus and small intestine. It is about 200 mm in length and has a surface area of 0.2 m^2 (Minami and McCallum, 1984). The stomach has two major functions, act as temporary storage for ingested materials and to decrease the size of ingested solids to a uniform mass by acid – enzyme digestion (Hoichman *et al*., 2004). Also, the stomach has a protective role, reducing the risk of

noxious agents reaching the intestine (Ashford, 2007). The stomach produces gastric secretions when food is ingested. These gastric secretions consist of hydrochloric acid (HCl) (~0.1 M), pepsin (secreted in the form of the precursor pepsinogen), gastric lipase, gastrin and mucus, which together, make up the main components of gastric fluid.

The volume of gastric fluid in the stomach ranges from ~50 mL in the fasted state to ~1500 mL in fed state (Bannister, 1995; Waugh *et al*., 2001). The pH of the stomach varies and is dependaent on the amount of HCl excreted, which in turn, depends on whether the person is in the fasted state or fed state. Under fasted conditions, the pH of gastric fluid is usually between 1 and 3. In the fed state, however, the gastric fluid is buffered to a less acidic pH ranging from 3 to 7 (Ashford, 2007). The fed state gastric fluid pH eventually returns to fasted state values after approximately 2 to 3 hours. There are also variations in gastric pH between age groups, for instance, the pediatric gastric pH value is neutral at birth and it may not reach comparable adult values until two years (Bowles *et al*., 2010; Batchelor *et al*., 2013).

The movement of materials from the stomach to the small intestine is controlled by pyloric sphincter which relaxes to release the stomach contents into the small intestine (Deshpande *et al.*, 1996). The gastric transition is controlled by the migrating motor complex (MMC) and digestive motility pattern. The MMC are cyclic waves of electrical activity that triggers the recurring motility pattern of peristalsis that occurs in the smooth muscle tissue of the stomach and small intestine (Deloose *et al*., 2012). Starting at the lower oesophagus the MMC occurs in the fasted state and spreads over the whole stomach and small intestine ending at the terminal region of the ileum. MMC cycle can be divided into 4 phases, starting with phase I (basal phase) which is a prolonged period of approximately 45 min to one hour. Phase II (pre-burst phase) lasts for approximately 40 to 60 min. During phase I and II the stomach exhibits low mechanical activity compared with the most active Phase III (burst phase) (Minami and McCallum, 1984). Phase III only lasts for approximately 4 to 6 min and consists

of intense regular contractions originating from the duodenum or antrum and that migrates distally, which helps to empty the stomach contents into the small intestine (Soppimath *et al*., 2001). The cycle is completed in Phase IV, which lasts for a maximum of 5 min as the activity declines between Phase III and Phase I of consecutive cycles. A complete cycle lasts between 90 to 120 min. In the fed state, however, the MMC is interrupted by the digestive motility cycle, which starts within 5 to 10 minutes after food ingestion. The muscle contractions during this phase exhibit relatively low mechanical activity and can lasts for up to 4 hours (Pawar *et al*., 2011).

Gastric emptying of pharmaceutical dosage forms depends therefore, on which cycle is active at the time of drug is administered. In the fed state, after the comminution of food to small sizes (between 1 and 2 mm) the residence time depends on the type of material consumed. Liquids and small particles will be easily transferred into the small intestine while solids and larger particles are released much more slowly (Conway, 2005). The gastric emptying time of pharmaceuticals can therefore range from 5 to 120 min (Ashford, 2007). There are also variations in gastric emptying times between age groups. This is most dramatically highlighted in neonates who have a much slower and more irregular gastric emptying time compared with that of older children and adults (Bartelink *et al.*, 2006; Bowles *et al*., 2010; Batchelor *et al*., 2013; Bonner *et al*., 2015).

4.2.3 Small Intestine

The small intestine begins at the pyloric sphincter of the stomach and stretches to the ileocecal junction where it attaches to the large intestine. It is approximately 4 to 5 m in length and it is divided into three sections, with each section varying in length (Ashford, 2007). The duodenum which contributes about 250 mm of the length, the jejunum which is approximately 2 m in length and the ileum, the final part of the small intestine, which contributes approximately 3 m of the length (Barrett *et al*., 2010).

The fluid within the small intestinal consists of pancreatic juice (containing several digestive enzymes), bicarbonate and bile secretions which collectively are responsible for elevation and fluctuation of pH values in the intestinal fluid which are usually between 6 and 7.4. These differences in pH values provides a variable environment for drug formulations (Ashford, 2007; Ibekwe *et al*., 2008) which need to be considered when designing dosage forms. The main functions of the small intestine are digestion and absorption of nutrients. Most drugs are absorbed in the small intestine because of the large surface area which is ~ 200 m^2. This large surface area is due to the presence of villi and microvilli which increases the absorptive area manyfold (Pang, 2003). The wall of the small intestine has a rich network of both blood and lymphatic vessels which also facilitate absorption. Once absorbed, nutrients and drugs are carried via the hepatic portal vein to the liver and then to the systematic circulation. Therefore, drugs absorbed from the small intestine are susceptible to first pass metabolism in the liver (Ashford, 2007). Transit times through the small intestine have been shown to vary considerably lasting from 1 to 6 hours, with the average 3 hours (McConnell *et al*., 2008). The small intestine transit time is controlled by propulsive movements of peristalsis which unlike the stomach does not discriminate between solid and liquid contents of the intestine therefore does not discriminate between liquid and solid dosage forms. The transit time within the small intestine is particularly important as this is the main site of drug uptake from orally administered medicines and can therefore impact on bioavailability especially in controlled release systems or with drugs that dissolve slowly in intestinal fluid (Ashford, 2007).

4.2.4 Large Intestine

The large intestine is the terminal part of GI tract and consists of the caecum, the appendix, the colon and the rectum. The diameter of the large intestine is twice the diameter of the small intestine and it makes up 25% of the total length of the GI tract (Ashford, 2007; Ibekwe *et al*., 2008). The main function of the large intestine is the formation of feces. The

absorption of ions and water, the secretion of mucus and the extensive metabolic action of microorganisms that are present in the large intestine are all involved in the production of feces which is stored until it is eliminated by the process of defecation (Macfarlane and Macfarlane, 1997; Ashford, 2007).

There are a huge number of different bacteria types are colonized inside the colon. This huge bacterial mass is responsible for metabolic activity that includes hydrolysis of fatty acid esters and enzymatic degradation of certain polysaccharides. This process can cause the pH of the colon to reduce to between pH 6 and pH 6.5 (Ibekwe *et al*., 2008). The colonic transit of drugs can range from 2 to 48 hours depending on the type of the dosage form, eating habits, frequency of defecation and disease state (Ashford, 2007).

4.3 Ibuprofen

Ibuprofen is an over the counter antipyretic and analgesic drug. It belongs to the family of nonsteroidal anti-inflammatory drugs (NSAIDs). Scientifically, ibuprofen is known as (RS)-2-(4-Isobutylphenyl) propionic acid with molecular formula is $C_{13}H_{18}O_2$ (Potthast *et al*., 2005) (Figure 4.2). Ibuprofen was launched in the UK in 1969 and was the first member of the propionic acid derivatives to be introduced as an alternative to Aspirin. It is a non-selective inhibitor of cyclo-oxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) which prevents the synthesis of prostaglandins, which play an important role in the development of inflammation, fever and pain. It has been reported that the anti inflammatory properties of ibuprofen is weaker than those of some other NSAIDs (Bushra and Aslam, 2010) but remains one of the most comminly used.

Ibuprofen is a white to off-white crystalline powder, with a melting point of approximately 74 °C. It is practically insoluble in water (< 0.1 mg mL^{-1}), but it is readily soluble in organic solvents and in aqueous alkaline solutions. Ibuprofen has a pK_a of 4.9 and an n-

octanol/water partition coefficient of 11.7 at pH 7.4 (Potthast *et al*., 2005). Ibuprofen is usually marketed as tablets with a potency of 200, 400 and 800 mg or in liquid formulations with the potency of 100 mg/5mL (e.g. Calprofen®). It is used to treat moderate to mild pain related to dysmenorrhea, headache, postoperative dental pain, soft tissue disorder management of spondylitis, rheumatoid arthritis and osteoarthritis.

As with other NSAID, ibuprofen with high and frequent dose can cause gastrointestinal damage, ranging from minor damage such as dyspepsia to severe damages such as ulceration and GI bleeding. The mechanism of NSAIDs induced GI damage is not entirely understood, but it can be divided into a direct topical damage and indirect systemic action. For topical damage, there are two proposed theories, disruption of the epithelial barrier which allows the back-diffusion of acid into the mucosa caused by the "trapped ion" effect where ionised NSAIDs accumulate in gastric epithelial cells, a reduction in the hydrophobicity of the mucosal surface and the uncoupling of oxidative phosphorylation (Becker *et al.,* 2004). Another mechanism has been suggested whereby NSAIDs reduce mucus and bicarbonate production impacting upon the decreasing the effectiveness of the juxtamucosal pH gradient in protecting the gastric epithelium (Allen *et al*.,1993; Phillipson *et al*., 2002 Baumgartner, *et al*., 2004; Wallace, 2008).

NSAIDs can also induce gastric ulceration by suppression of gastric prostaglandin synthesis and rendering the gastric mucosa more susceptible to the damaging effects of stomach secretions (acid, pepsin) including, in some cases, the NSAID itself (Wallace, 2008). One of the techniques used to try to prevent NSAIDs related GI upset is by using modified release drug delivery systems such as enteric coated tablets. There are limited options however, for liquid based controlled release NSAIDs formulations. The aim of the work in this chapter was to evaluate the potential of LA gellan gum fluid gels as a modified release oral liquid formulation for ibuprofen.

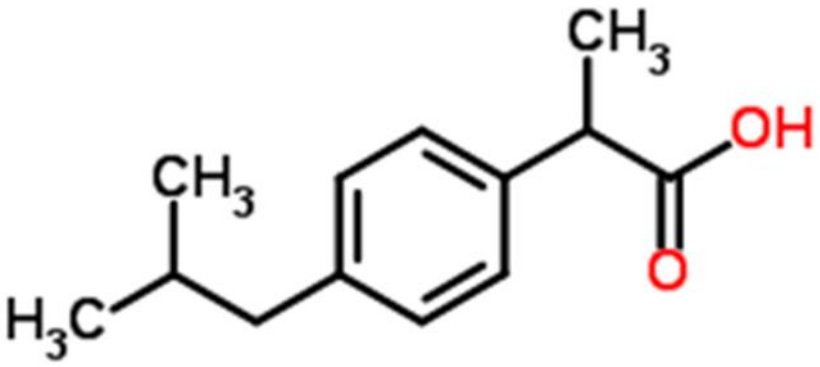

Figure 4.2 Structural formula of ibuprofen (adapted from Bushra and Aslam, 2010).

4.4 Materials and Methods

4.4.1 Materials

LA gellan gum (Kelcogel^{TM}) was kindly donated by CP Kelco (USA). Ibuprofen powder (Ibuprofen 38) was obtained from BASF (Germany). All other materials were obtained from Sigma–Aldrich (Poole, UK).

4.4.2 Methods

4.4.2.1 Preparation of Fluid Gels

Fluid gels were prepared by adding LA gellan at concentrations from 0.1 to 1 % w/w to deionised water at 85 °C while stirring. Once fully dissolved, the solutions were allowed to cool to ~60 °C then a paediatric dose of ibuprofen (20 mg mL^{-1}) was added and the pH was adjusted to 7.4 using 1 M NaOH. Solutions were then cooled further at 2 °C min^{-1} whilst being sheared using Bohlin Gemini Nano HR rheometer at a shear rate of 500 s^{-1}. To evaluate the potential of varying the particle size during formulation, fluid gels were prepared with changes to the processing conditions. To investigate the effect of cooling rate, 0.75 % w/w LA gellan gum fluid gels were prepared as described above at a fixed shear rate of 500 s^{-1} with cooling

rates of 0.5 °C min^{-1}, 2 °C min^{-1} and 10 °C min^{-1}. Similarly, to investigate the effect of shear rate, 0.75 % w/w LA gellan gum fluid gels were prepared at a fixed cooling rate of 2 °C min^{-1} using shear rates of 100 s^{-1}, 500 s^{-1} and 1000 s^{-1}.

4.4.2.2 Preparation of Control Formulations

4.4.2.2.1. Viscosity Test Controls

To ensure the LA gellan fluid gel formulations had a suitable viscosity profile a marketed paediatric ibuprofen suspension was used as a standard comparison and is referred to as C1.

4.4.2.2.2 Dissolution Test Controls

To ensure ibuprofen could be fully dissolved in the dissolution media (PBS pH 7.4) at the formulated dose following 20 min exposure to acid at pH 1.2 (and that any delayed release was not an effect of the pK_a of the ibuprofen), control solutions were prepared by adding the drug (20 mg mL^{-1}) to deionized water at ~60 °C which was cooled to room temperature and the pH adjusted to 7.4 using 1 M NaOH (referred to as C2).

To ensure the same grade of ibuprofen was used in all dissolution experiments formulations based upon standard ibuprofen suspensions were prepared as a control by adding 0.3 % w/w xanthan gum and 0.2 % w/w sorbitol to deionized water / glycerol 50:50 at 85 °C while stirring (to prevent any interference with UV analysis no preservatives, colouring agents or flavours were added). Once fully dissolved, the solution was allowed to cool to ~60 °C then a paediatric dose of ibuprofen (20 mg mL^{-1}) was added. The suspension was then cooled to room temperature and referred to as C3.

4.4.2.3 Viscosity Measurements

Viscosity measurements of all samples were performed at 25 °C using a Bohlin Gemini Nano HR rheometer using a 55 mm parallel plate geometry and across a shear rate range of 1 s^{-1} to 1000 s^{-1}.

4.4.2.4 Microscopy

Fluid gel samples were imaged using an optical microscope (Keyence VHX digital microscope RZ x 250 - x2500 real zoom lens in high dynamic range). Samples were prepared for imaging by dispersing the fluid gel samples in 10 mL of 50 mM $CaCl_2$. The suspension was then centrifuged at 13000 rpm and the pellet was then examined under the microscope. $CaCl_2$ was used as the diluent during the processing of the sample prevent aggregation of the gel particles during the centrifugation step.

4.4.2.5 Dissolution Studies

A modified USP I apparatus (baskets at a stirring rate of 100 rpm) was used to study *in vitro* drug release. Each formulation (5 mL) was placed into dialysis tubing (12500 MWCO) then submerged (within the baskets) in small volume vessels containing 200 mL dissolution media at pH values of 1.2, 2, 3, 4, 5, and 7.4 for 20 min. The media was subsequently changed to pH 7.4 phosphate buffered saline (sodium chloride 137 mM, potassium chloride 2.7 mM, disodium hydrogen phosphate 10 mM and potassium dihydrogen phosphate 2.0 mM). All buffers used were prepared at the same ionic strength and pH 7.4 was used to represent the highest pH the formulations may encounter during intestinal transit (terminal ilium).

To understand how release in simulated intestinal conditions was affected by residence time in acidic media, samples were also exposed to pH 1.2 and pH 2 environments for time periods increasing from 5 min to 120 min before changing the media to pH 7.4 and recording the subsequent release.

4.4.2.6 Determination of Ibuprofen Content by Ultraviolet (UV) Spectroscopy

Ibuprofen standards were prepared at concentrations ranging from 10 to 1000 μg mL^{-1} and measured using a UV spectrophotometer at a wavelength of 254 nm to generate calibration curves which were plotted at all pH values tested. The concentration of ibuprofen released from the sample was determined from the corresponding calibration curves. All experiments were carried out in triplicate. A typical calibration curve for ibuprofen dissolved in phosphate buffer saline (PBS) pH 7.4 is shown in Figure 4.3.

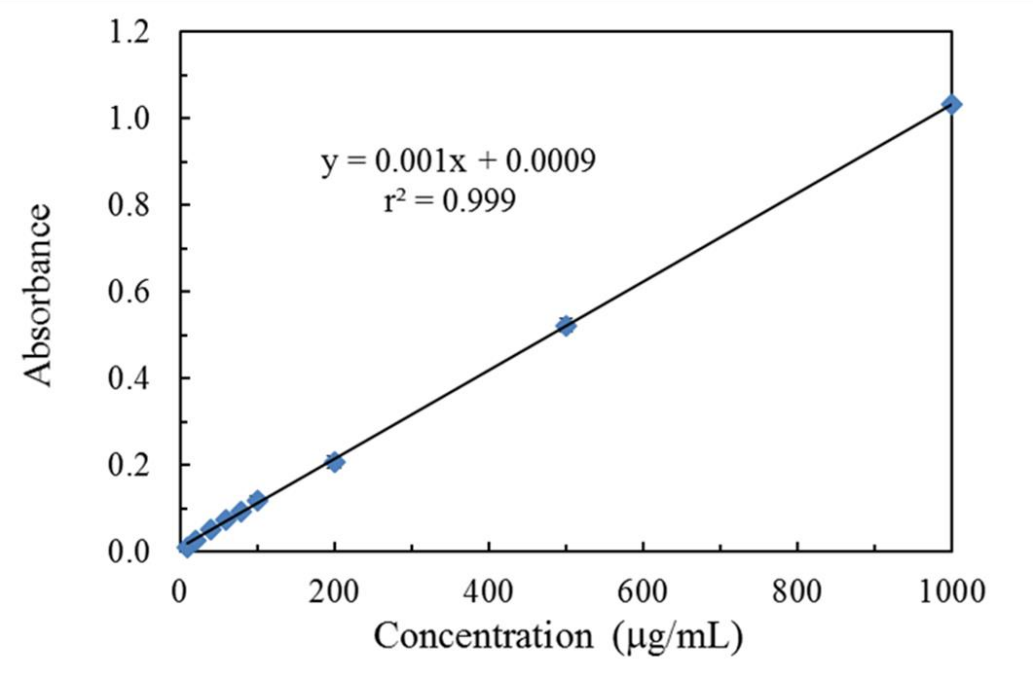

Figure 4.3 Mean calibration curve for ibuprofen preparation in PBS measured at λ 254 nm. Values represent mean ± SD (n=3).

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. The linearity is determined by the correlation coefficient r^2.

Limit of detection (LOD) and limit of quantification (LOQ) are important parameters that are used to discribe the smallest concentrations of a sample that can be reliably measured by an analytical procedure. LOD is defined as minimal concentration of analyte that can be

detected with a certain degree of confidence and LOQ is defined as the minimal concentration that can be measured with acceptable accuracy. LOD and LOQ are quantified by using equations 4.1 and 4.2 respectively:

$$\mathrm{LOD} = 3.3\,\sigma/S \qquad \text{Eq. 4.1}$$

$$\mathrm{LOQ} = 10\,\sigma/S \qquad \text{Eq. 4.2}$$

where σ is the standard deviation of Y-intercept and S is the slope of the calibration curve.

The accuracy of an analytical method is defined as the degree of closeness between the accepted true value in the analyte samples and the actual result obtained from the experiment. Accuracy can be measured by analysing samples with known concentrations and comparing the measurement values with real value. Accuracy studies were evaluated at 50 %, 100 % and 150 % of the assay concentration in triplicate. For all samples, it was found that the relative standard deviation (RSD) < 1 % which is accepted as a satisfactory value for RSD.

The precision of an analytical method is defined as the measure of how close the data values are to each other from the number of measurements under the same analytical conditions. The intra- and inter-day repeatability was determined through analysis of the samples on three different days in triplicate. Like accuracy, for precision the RSD for all samples were within the satisfactory range (RSD < 1 %). The UV method validation for the ibuprofen assay presented in table 4.1.

Table 4.1 UV method validation for ibuprofen assay.

Wavelength	254 nm
Correlation coefficient (r^2)	0.99
LOQ	6.00 μg/mL
LOD	1.98 μg/mL
Precision and accuracy	RSD < 1 %

4.4.2.7 Rheological Measurements

The following rheological measurements were performed to investigate how gel stiffness changes during the *in vitro* dissolution tests and were used to facilitate correlation of stiffness (G′) to drug release.

To understand how elastic modus (G′) was affected by residence time in acidic media, 5 mL of the formulation was placed into a dialysis tube (12500 MWCO) then submerged in 200 mL 0.1 M HCl at pH 1.2 for time periods increasing from 5 to 120 min before loading the sample on the rheometer.

To study the impact the change of dissolution media (to PBS pH 7.4) has on the stiffness of the gel following exposure to acid, another set of samples was also exposed to pH 1.2 for 10 and 60 min (batch A and B respectively). The medium was then changed to pH 7.4 for a period of time ranging from 30 to 600 min for batch A and 60 to 1200 min for batch B, prior to loading on the rheometer.

All rheological measurements were carried out using a Bohlin Gemini Nano HR rheometer. Oscillation mode was used to determine viscoelasticity of the gel. Mechanical spectra were obtained by taking measurements of the elastic (storage) modulus (G′), viscous

(loss) modulus (G″) and complex dynamic viscosity (η*). The measurements were recorded at 10 rad s^{-1} angular frequency and 0.5 % strain using a 55 mm parallel-plate geometry with a 0.5 mm gap. The strain amplitude chosen was within the linear viscoelastic region of the samples determined by amplitude sweeps. All measurements were taken at 37 °C.

4.4.2.8 Statistical Analysis

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

4.5 Results

4.5.1 Rheological Measurements

LA gellan fluid gels loaded with 20 mg mL^{-1} ibuprofen were formed using a rheometer which enabled charcaterisation during manufacture with real-time measurements of the changes in viscosity that occur during formation. Figure 4.4 shows the relative viscosity vs. temperature of a 0.1 %, 0.375 %, 0.5 %, 0.75 % and 1 % w/w LA gellan fluid gel during manufacture. As the temperature is decreased there is an increase in viscosity that occurs at the onset of gelation of the LA gellan, a maximal viscosity is then reached which is the temperature beyond which no further gel particles are formed (T_{max}), followed by a plateau in viscosity as the formed particles are smoothed. The results indicate that the viscosity of the fluid gels are concentration dependant; onset of gelation increases from ~40 °C for 0.1 % LA gellan to ~45 °C for 1 % LA gellan. Furthermore, the final viscosity (at 500 s^{-1} and 20 °C) of the fluid gels increases with increasing concentration from ~0.01 Pas for 0.1 % w/w LA gellan up to ~0.1 Pas for 1 % w/w LA gellan.

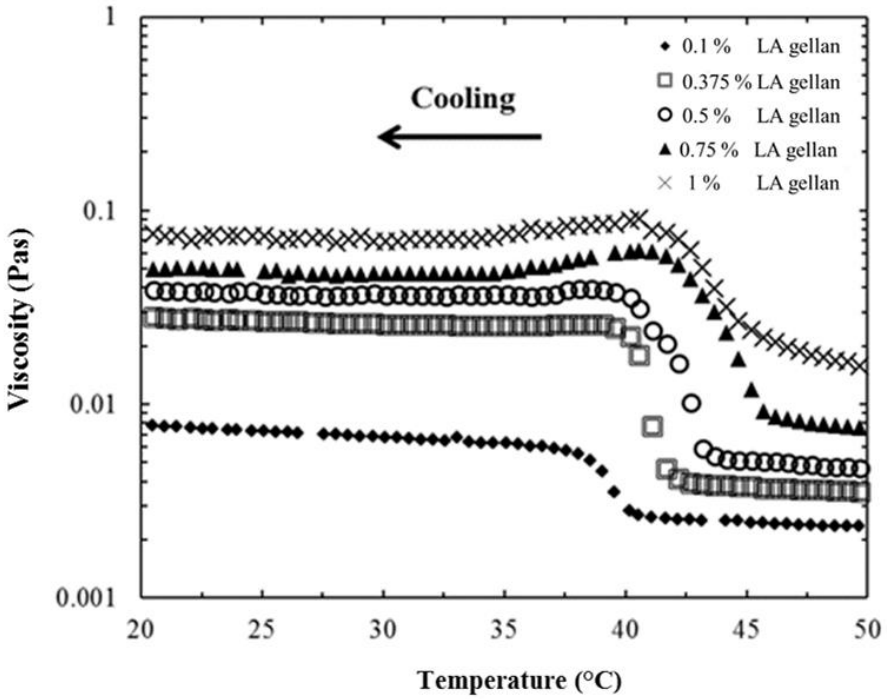

Figure 4.4 Viscosity of LA gellan during fluid gel formation (cooling at 2 °C min^{-1} at a shear rate of 500 s^{-1}) for 0.1 % w/w (filled diamonds) 0.375 % w/w (open squares) 0.5 % w/w (open circles) 0.75 % w/w (filled triangles) and 1 % (black crosses) w/w LA gellan loaded with 20 mg mL^{-1} ibuprofen (Mahdi et al., 2014) (used with permission).

To evaluate the potential of LA gellan fluid gels as an oral liquid, samples were tested and compared with a proprietary ibuprofen suspension. The viscosity profiles of LA gellan fluid gel formulations (0.1-1 % w/w) are shown in (Figure 4.5A) and have a shear thinning viscosity profile. The 0.75 % fluid gel sample exhibited a viscosity profile that was most similar to that of a standard ibuprofen paediatric suspension. In addition, the yield stress was sufficient to allow inversion of the fluid gel sample without any flow however following mild shaking of the sample it is easily poured on a dispensing spoon as illustrated in (Figure 4.6). This formulation was therefore used in further investigations. Dynamic small deformation oscillatory measurements of G′ and G″ (Figure 4.5B) highlight the viscoelasticity of the 0.75 % w/w fluid gel with G′ slightly greater than G″ across a range of frequencies typical of 'weak gel' rheological behaviour.

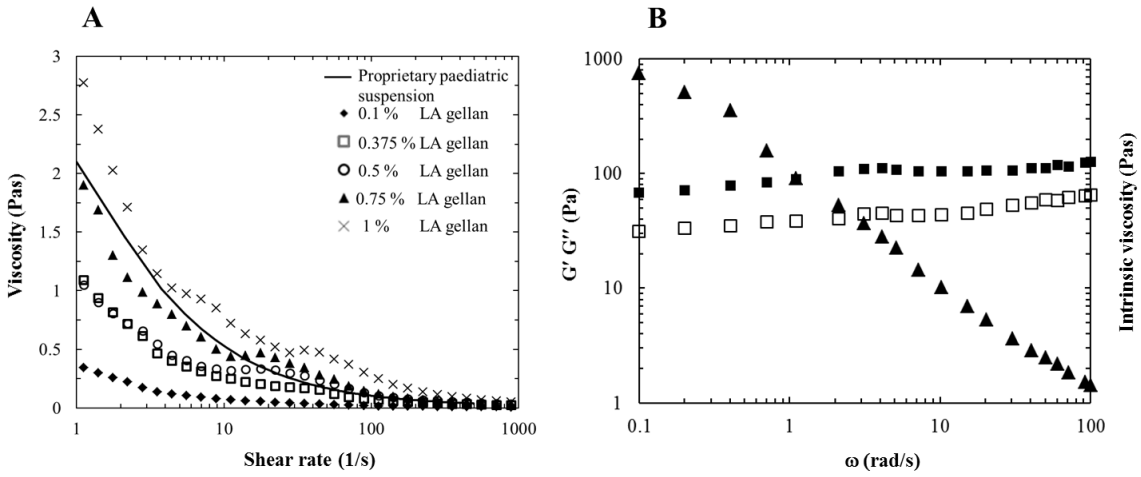

Figure 4.5 A) Viscosity vs. shear rate at 25 °C for 0.1 % w/w (filled diamonds) 0.375 % w/w (open squares) 0.5 % w/w (open circles) 0.75 % w/w (filled triangles) and 1 % w/w (black crosses) LA gellan loaded with 20 mg mL^{-1} ibuprofen. Black line indicates a proprietary ibuprofen paediatric suspension. B) Mechanical spectrum (0.5 % strain; 37 °C) of a 0.75 % w/w LA gellan fluid gel loaded with 20 mg mL^{-1} ibuprofen showing variation of G′ (filled squares), G″ (open squares) and η* (filled triangles) with angular frequency (Mahdi et al., 2014) (used with permission).

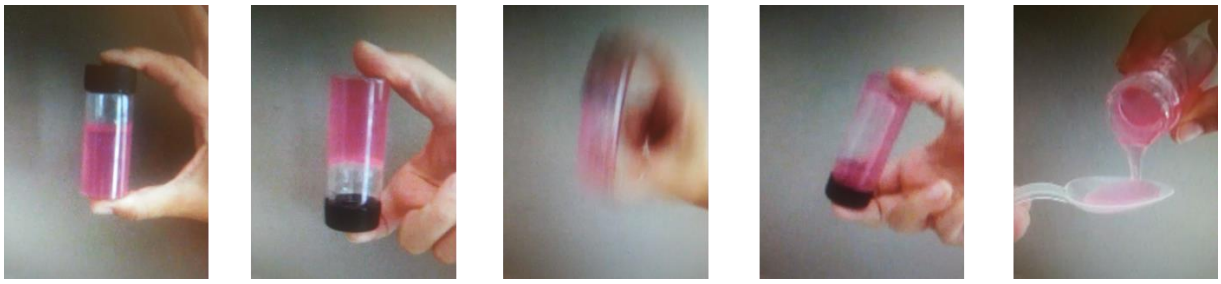

Figure 4.6 Images illustrating the shear thinning behaviour of an ibuprofen loaded fluid gel sample with the ability to invert without any flow (Mahdi et al., 2014) (used with permission).

Figure 4.7 shows the effect of cooling rate on the viscosity during formation of a 0.75 % w/w fluid gel at a fixed shear rate of 500 s^{-1} (Figure 4.7A) and the effect of shear rate at a fixed cooling rate of 2 °C min^{-1} (Figure 4.7B). The viscosity of the fluid gels during formation increased with increasing cooling rates and viscosity decreased when shear rate was increased.

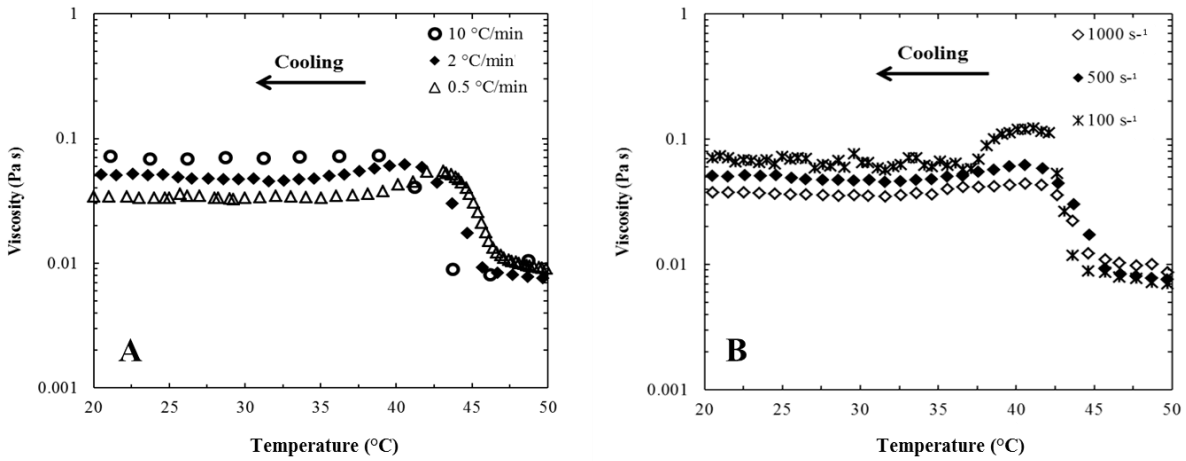

Figure 4.7 Viscosity of 0.75 % w/w LA gellan loaded with 20 mg mL^{-1} ibuprofen during fluid gel formation using (A) different cooling rates; 10 °C min^{-1} (open circles), 2 °C min^{-1} (filled diamonds), 0.5 °C min^{-1} (open triangles) at a shear rate of 500 s^{-1} and (B) different shear rates cooling at 2 °C min^{-1}; 1000 s^{-1} (open diamonds), 500 s^{-1} (filled diamonds), 100 s^{-1} (black crosses) (Mahdi et al., 2014) (used with permission).

4.5.2 Effect of LA Gellan Gum Concentration

Microscopy images in (Figure 4.8) reveal particle sizes of the fluid gels are highly dependent on concentration. At 0.1 % LA gellan the particles were in the region of 1-5 μm and were generally spherical in shape (Figure 4.8A). As the concentrations increased to 0.5 % w/w the particles had a larger, binomial size distribution with a population of micron sized particles (similar to 0.1 % w/w) and a population and a population in the region of 10-20 μm (Figure 4.8B). At 0.75 % w/w the particles appear less polydisperse than at 0.5 % and more spherical with the majority of the population in the region of 20 μm (Figure 4.8C). When the concentration is increased further to 1 % w/w the particles were much larger and irregular in shape (Figure 4.8D).

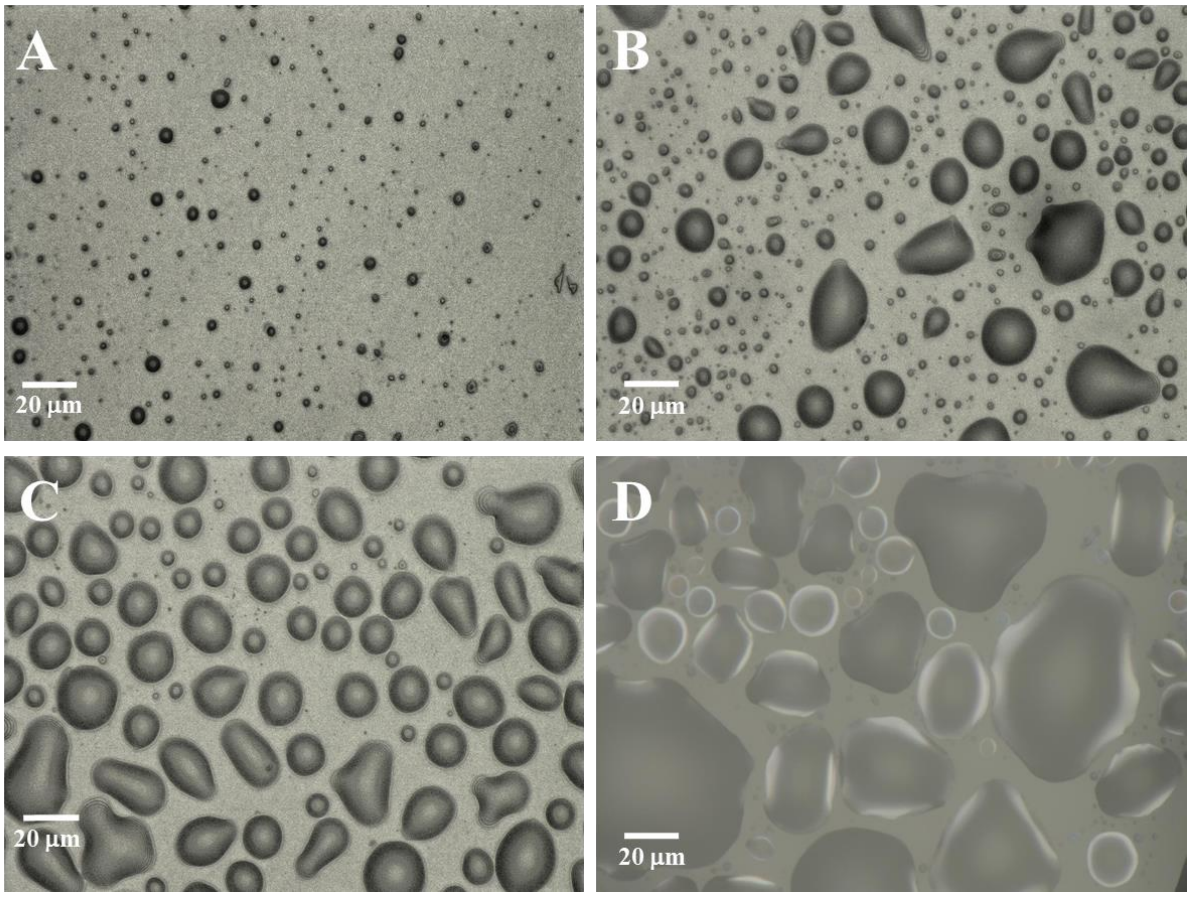

Figure 4.8 Light microscopy images of LA gellan fluid gels prepared at different concentrations loaded with 20 mg mL^{-1} ibuprofen A) 0.1 % w/w, B) 0.5 % w/w, C) 0.75 % w/w and D) 1 % w/w (Mahdi et al., 2014) (used with permission).

4.5.3 Effect of Cooling Rate and Shear Rate

Figure 4.9 shows the effect of increasing cooling rates on the particle size of 0.75 % w/w at fixed shear rate of 500 s^{-1} (Figure 4.9A-C) and the effect of increasing shear rates on the particle size of same concentration of LA gellan at fixed cooling rate of 2 °C min^{-1} (Figure 4.9D-F). These micrographs indicate that a smaller particle size can be obtained by decreasing cooling rate and increasing the shear rate when forming the fluid gels.

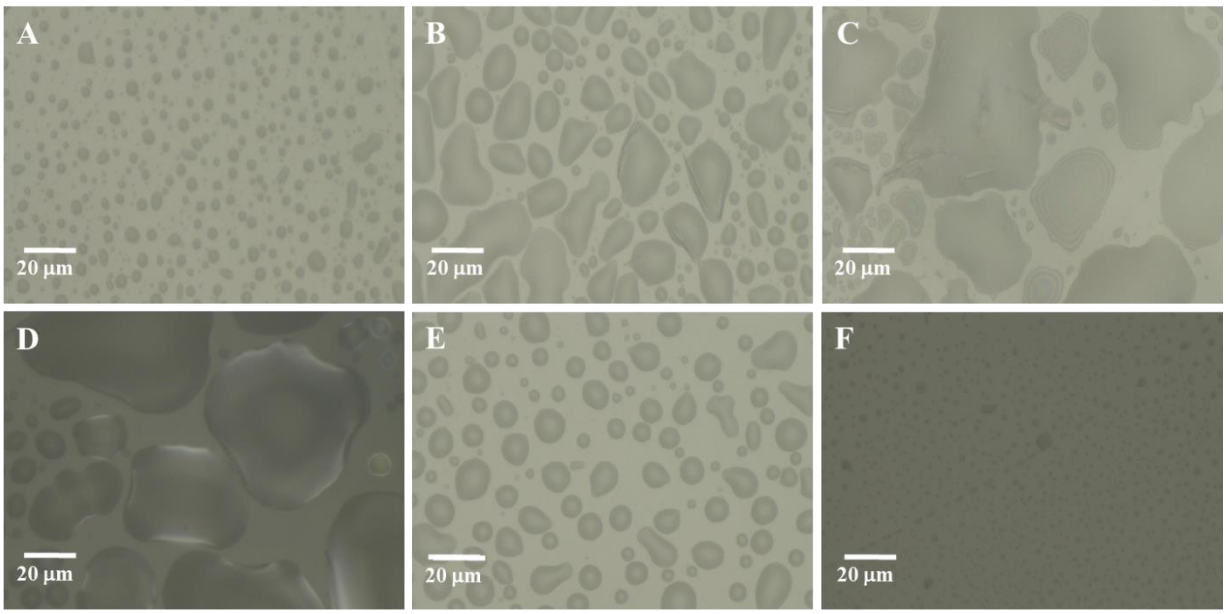

Figure 4.9 Light microscopy images of 0.75 % w/w LA gellan loaded with 20 mg mL^{-1} ibuprofen prepared at a shear rate of 500 s^{-1} using different cooling rates (A-C); A) 0.5 °C min^{-1}, B) 2 °C min^{-1} and C) 10 °C min^{-1} and different shear rates cooling at 2 °C min^{-1} (D-F); D)100 s^{-1}, E) 500 s^{-1} and F) 1000 s^{-1} (Mahdi et al., 2014) (used with permission).

4.5.4 Dissolution Behaviour

To investigate the effects of exposure to low pH had on the fluid gels a 5 mL sample of each was dispensed into 0.1 M HCl at pH 1.2. A proprietary ibuprofen suspension (C1) was also used for comparison. The proprietary formulation formed a cloudy dispersion in the acid which is attributed to the poor solubility of ibuprofen at low pH. The LA gellan fluid gel on the other hand formed an acid gel with the ibuprofen remaining associated with the gellan. This remained as large aggregated gel pieces for over 6 hours (Figure 4.10). This was supported by dissolution experiments which showed no ibuprofen was released at pH 1.2 and in Figure 4.11 where ibuprofen crystals can be seen to remain entrapped within the fluid gel particles.

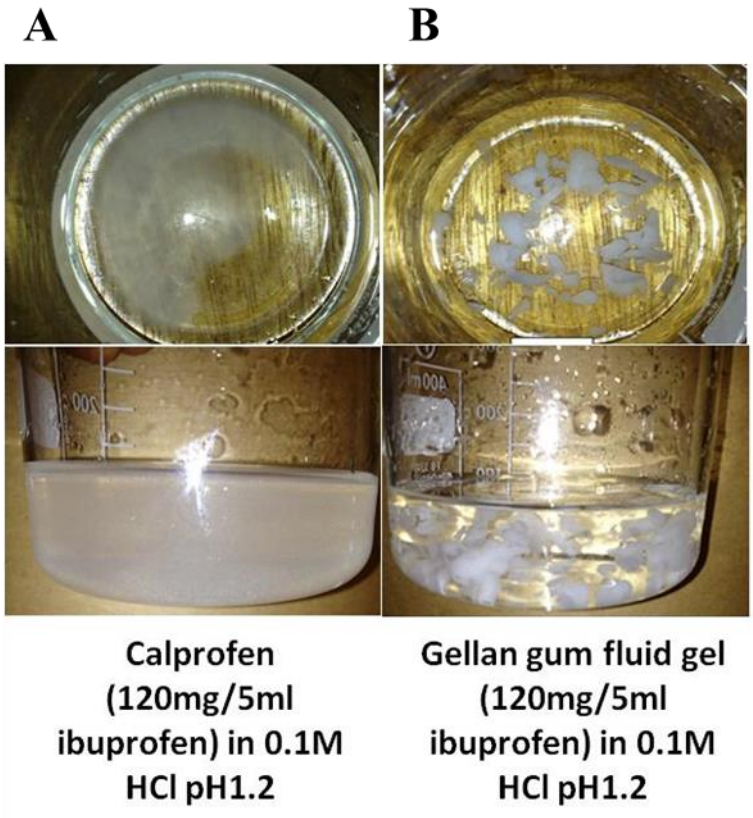

Figure 4.10 Appearance of A) proprietary ibuprofen paediatric suspension and B) ibuprofen loaded 0.75 % gellan fluid gel following incubation in 0.1M HCl at pH 1.2 for 6 hours.

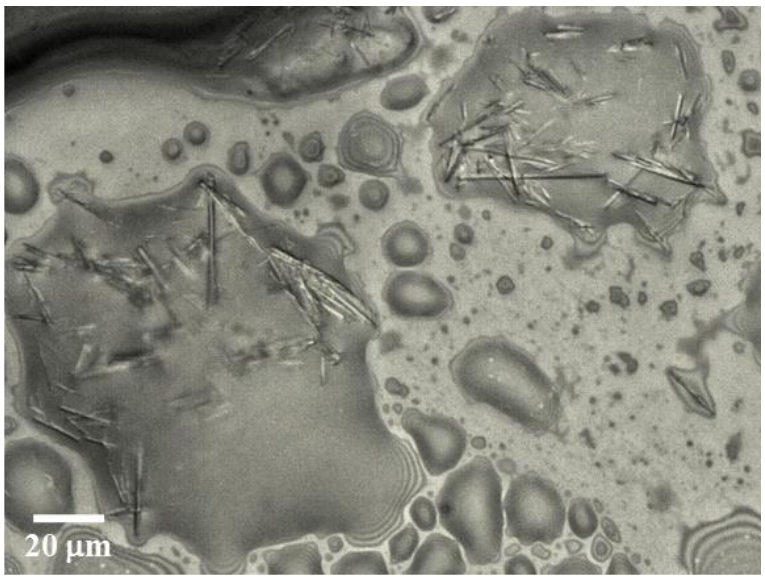

Figure 4.11 Light microscopy images of LA gellan fluid showing crystallised ibuprofen entrapped within gel particles (adapted from Mahdi et al., 2014).

Figure 4.12 illustrates the *in vitro* release of ibuprofen from different LA gellan fluid gel concentrations ranging from 0.0 % (ibuprofen alone) to 0.75 % w/w determined at pH 1.2 then the release media was changed after 20 minutes to PBS pH 7.4 . The results show that there was a small quantity of ibuprofen released in acidic media for the gels containing lower concentrations of LA gellan and control formulations (C2 and C3). At 0.75 % w/w however, there was no release in acid medium and subsequent release was retarded in PBS for 30 min.

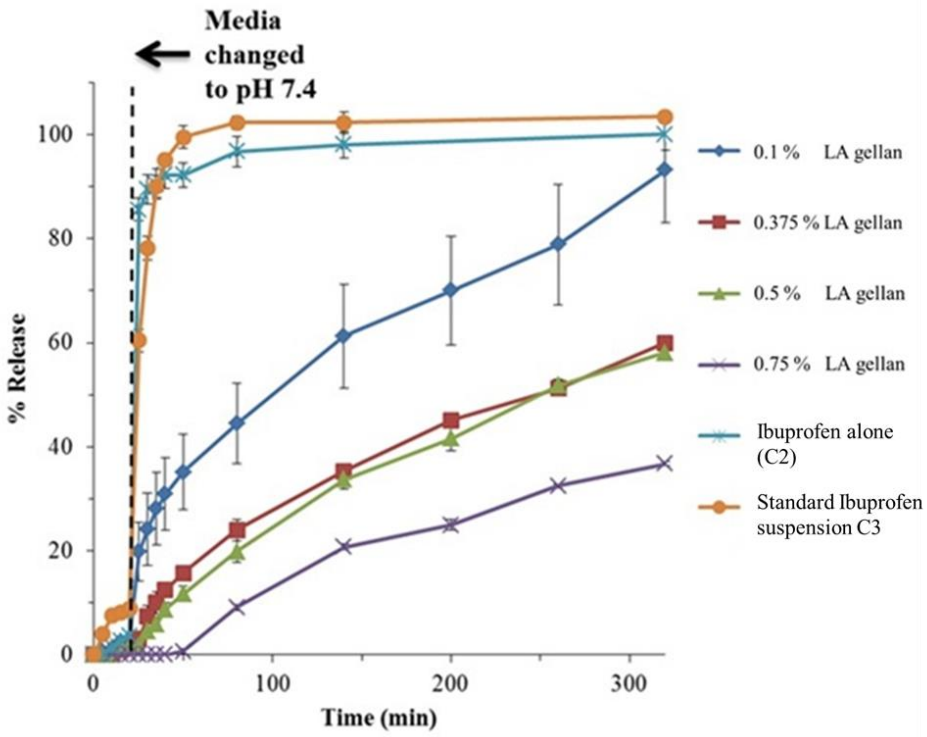

Figure 4.12 Cumulative % release of ibuprofen from fluid gels prepared at different concentrations of LA gellan compared with a standard ibuprofen suspension. Dotted line indicates the point the media was changed from 0.1 M HCl at pH 1.2 to PBS at pH 7.4. Values are represented as mean ± SD (n=3) (Mahdi et al., 2014) (used with permission).

To account for the wide variation in stomach pH found in paediatric patients, release characteristics were determined *in vitro* at different pH values (1.2, 2, 3, 4, 5 and 7.4) then the release medium was changed after 20 minutes to PBS pH 7.4. Figure 4.13 highlights that the release of ibuprofen from the LA gellan fluid gel was strongly affected by pH of the dissolution media. There was no significant difference ($p > 0.05$) in release between samples initially immersed in pH 7.4, pH 5 and pH 4. At pH 3 however, subsequent release of ibuprofen was

retarded. The retardation of release became progressively more pronounced as the pH was dropped further, to the point where exposure to pH 1.2 for just 20 min delayed the onset of drug release for a further 60 min when transferred to pH 7.4.

The duration of exposure to acidic pH was shown to dramatically affect the lag time to onset of release following transfer to pH 7.4. Figure 4.14 illustrates the linear relationship between onset of release in pH 7.4 and the preceding exposure time at pH 1.2 and pH 2. The onset of release in pH 7.4 was shown to be dramatically affected by the acidity of the initial dissolution medium taking almost 3 hours after exposure to pH 1.2 (for 2 h) compared with 30 min to onset of release following exposure to pH 2 (for 2 h). This lag time was shown to be dependent on gel stiffness.

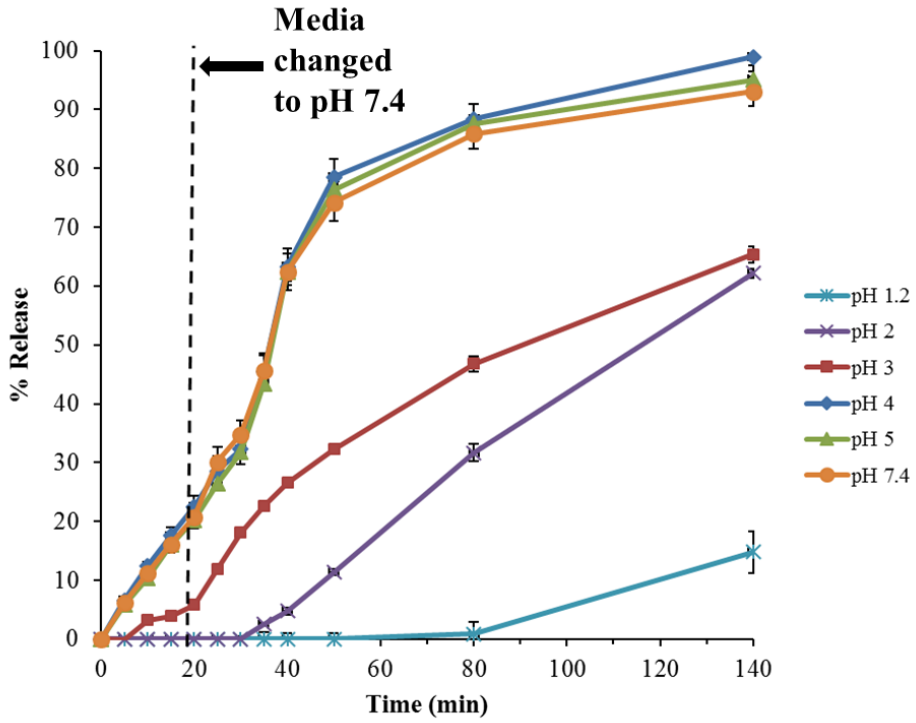

Figure 4.13 Cumulative % release of ibuprofen from 0.75 % w/w LA gellan fluid gel loaded with 20 mg mL^{-1} ibuprofen exposed to different acidic pH values for a period of 20 min. Dotted line indicates the point the media was changed to PBS at pH 7.4. Values are represented as mean ± SD (n=3) (Mahdi et al., 2014) (used with permission).

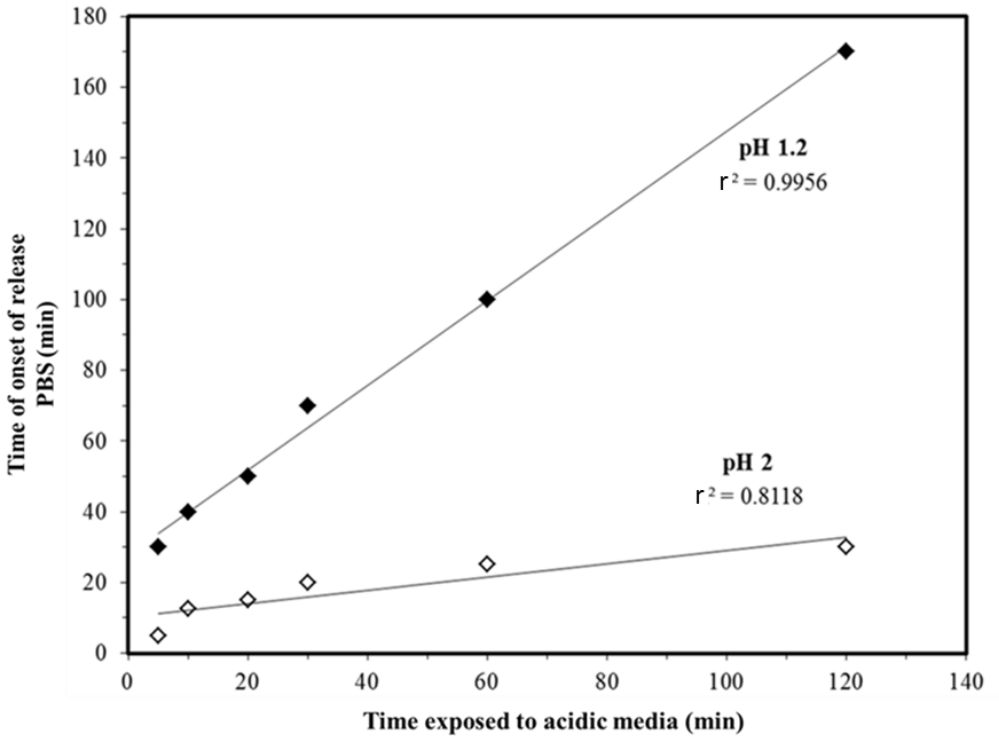

Figure 4.14 Relationship between onset of release at pH 7.4 and preceding exposure time in simulated gastric fluid at pH 1.2 (filled diamonds) and pH 2 (open diamonds) (Mahdi et al., 2014) (used with permission).

Figure 4.15 shows onset of release time rises exponentially with increase in G′, which in turn, is dependent on exposure time to pH 1.2 as highlighted in Figure 4.16. Interestingly, when the fluid gel was transferred to PBS pH 7.4 following 10 min in pH 1.2, the gel stiffness continued to increase, albeit at a slower rate, until a plateau was reached (following 90 minutes in pH 7.4) where G′ was approximately 1200 Pa. When the gel was exposed to pH 1.2 for 60 minutes the stiffness was almost an order of magnitude greater than after 10 minutes exposure. However following transfer to PBS pH 7.4 the stiffness gradually decreased over a period of 180 min to the plateau where G′ is approximately 1200 Pa.

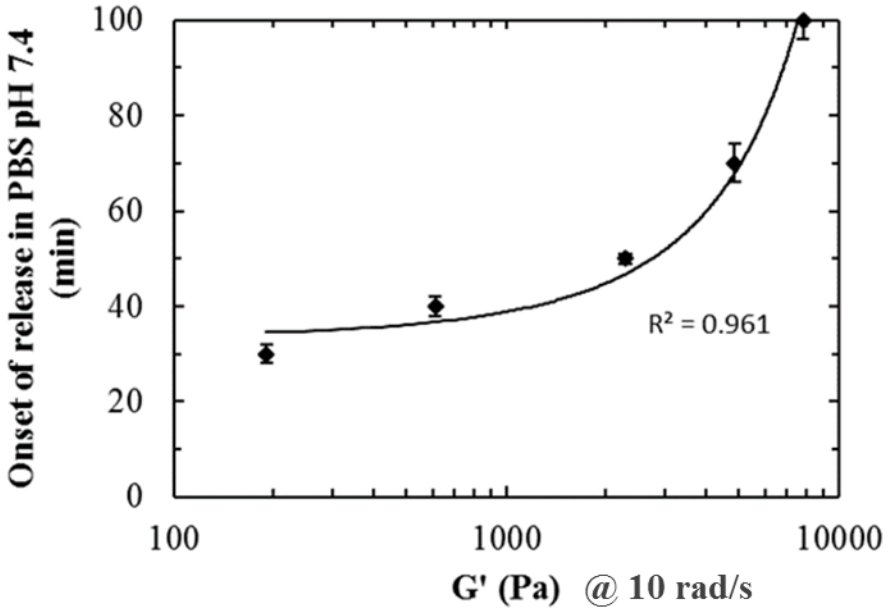

Figure 4.15 Exponential relationship between the onset of release in PBS pH 7.4 as a function of gel stiffness (G′) (Mahdi et al., 2014) (used with permission).

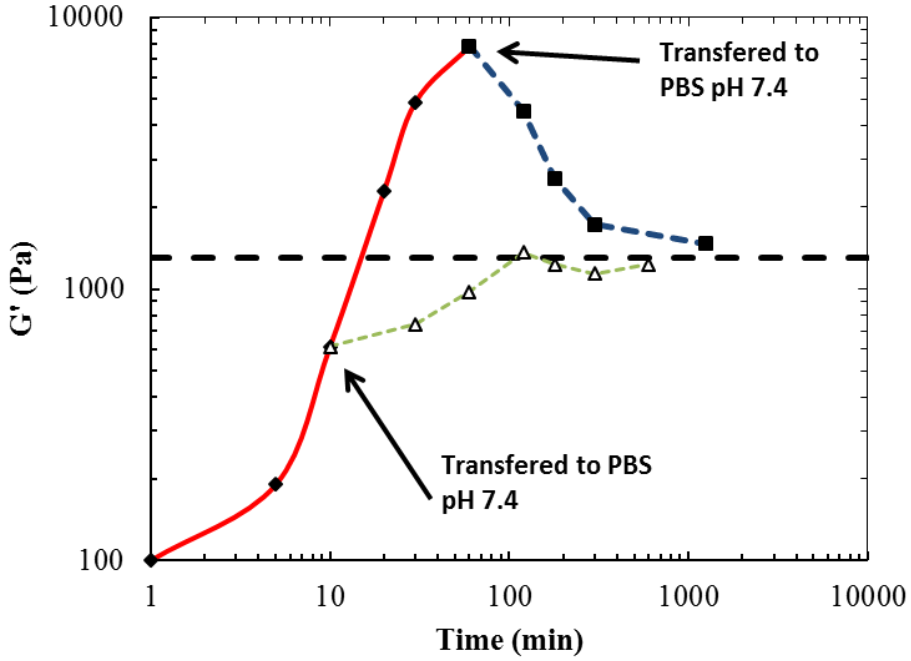

Figure 4.16 Effect of time exposed to pH 1.2 on gel stiffness (G′) and subsequent stiffness on transfer to pH 7.4. The red line (filled diamonds) indicates the stiffness of the gel when exposed to pH 1.2 (0.5 % strain; 37 °C at 10 rad s^{-1}). The green dashed line (open triangles) represents the stiffness of the gel in PBS at pH 7.4 following 10 min exposure to pH 1.2. The blue dashed line (filled squares) represents the stiffness of the gel in PBS at pH 7.4 following 60 min exposure to pH 1.2 (Mahdi et al., 2014) (used with permission).

The relationship between gel stiffness and release in pH 7.4 is highlighted in Figure 4.17. Following 60 min exposure to 0.1 M HCl the gel stiffness was 8000 Pa which gradually decreased on transfer to PBS. No released drug was detected until the stiffness of the gel had reduced to ~2000 Pa (which took 2 hours), following which zero order release 0.15 mg min^{-1} was apparent (Figure 4.17A). When the sample was exposed to pH 1.2 for 10 min, the gel stiffness was only ~600 Pa and gradually increased to ~1300 Pa on transfer to PBS pH 7.4. In this system the zero order drug release occurred within 40 minutes and at an increased rate of 0.44 mg min^{-1} (Figure 4.17B). After this time, the gel disintegrated and was no longer included in the study. These results highlight that increased gel stiffness can reduce the release rate.

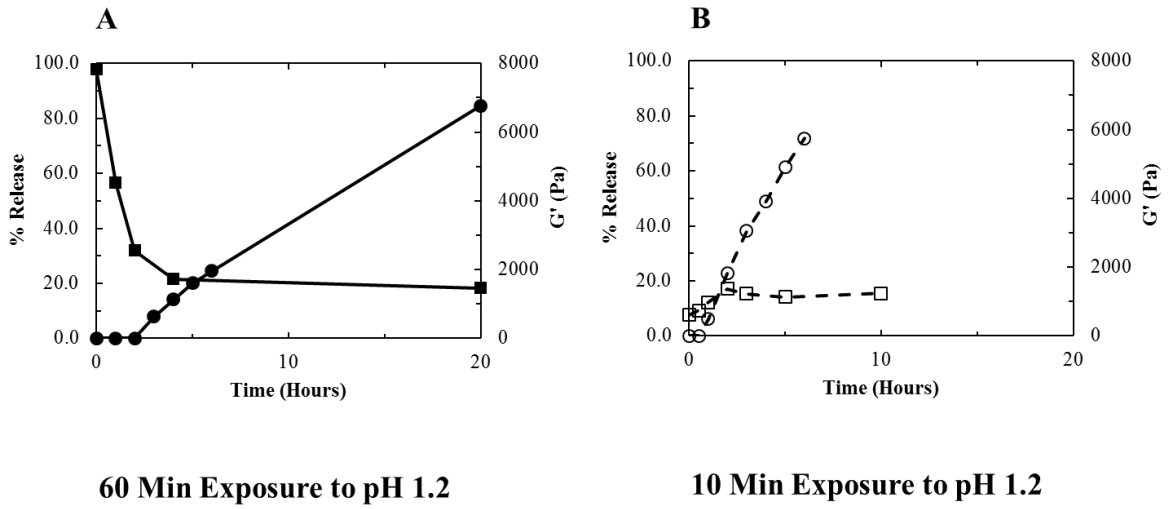

Figure 4.17 Cumulative % release (primary vertical axis) and gel stiffness (G′) (secondary vertical axis) versus time following A) 60 min exposure to pH 1.2 and B) 10 min exposure to pH 1.2 (Mahdi et al., 2014) (used with permission).

4.6 Discussion

The use of fluid gels as a platform technology for pharmaceutical formulations has great potential due to the tuneable mechanical properties and ease of manufacture. It has been previously shown that fluid gels can be prepared with many different biopolymers including gelatin (de Carvalho and Djabourov, 1997), agarose (Norton *et al*., 1998), κ-carrageenan (Garrec and Norton, 2012; Gabriele *et al*., 2009) and LA gellan (Sworn *et al*., 1995). Most of these investigations have been focused towards applications in foods to improve stability and improve texture. Here we have investigated the potential of LA gellan fluid gels as a modified release oral drug delivery system.

The preparation of fluid gels is a simple process, producing gelled particles that are dispersed in an un-gelled medium. Production using a rheometer allows the cooling rate and the shear rate to be accurately controlled and the characteristic change in viscosity monitored (the process however, is easily carried out on a larger scale using application of shear). When the LA gellan fluid gels were formed containing ibuprofen, the onset of ordering increased with increasing gellan concentration (Figure 4.4) which can be explained by the consequential increase in concentration of the counterions to the charged group of the polymer promoting aggregation (Morris, *et al*., 2012). Interestingly, this onset of ordering occurs at a slightly lower temperature that has been previously reported for LA gellan fluid gels without a drug load (Sworn *et al*., 1995). This is thought to be due to the competitive inhibition by the negatively charged ibuprofen binding some of the Na^+ ions (introduced during pH adjustment with NaOH) reducing the overall ionic strength of the bulk, consequently reducing the viscosity and gelation temperature.

Once manufactured, the bulk fluid gels containing ibuprofen showed shear thinning behaviour similar to that of a proprietary paediatric oral ibuprofen suspension with the 0.75 %

w/w fluid gel having the closest match (Figure 4.5). However, unlike the proprietary paediatric oral ibuprofen, at very low shear rates the viscosity was sufficient for the preparation to be inverted without any steady state flow as illustrated in Figure 4.6. This is due to the weak gel properties of the ibuprofen LA gellan fluid gel (Figure 4.5B) which are thought to be a result of gel particle – gel particle interactions (Garrec *et al*., 2013) which give a larger yield stress to the fluid gels compared with the proprietary formulation.

Oral liquid 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 efficiently during product storage, while not impacting on the ease of administration. Furthermore, producing oral liquid formulations with modified release properties would provide an alternative dosage form for paediatric patients in particular. The physical properties of LA gellan 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 (Figure 4.7). This has previously been demonstrated in food based applications with agarose and carrageenan fluid gels (Norton *et al*., 1998; Gabriele *et al.,* 2009). This allows the particle size to be controlled as shown in Figures 4.8 and 4.9.

LA gellan has previously shown promise as a sustained release oral liquid which undergoes *in situ* physical crosslinking (gelation) within the gastric fluid (Miyazaki *et al*., 1999). In the present study, the LA gellan fluid gel oral liquid was formulated to have a physically cross-linked microstructure prior to exposure to an acidic gastric environment. When the fluid gel comes into contact with the low pH an acid gel is formed from the previously un-gelled LA gellan in the continuous phase, effectively immobilising the pre-gelled particles. This system was shown to prevent the dispersion of ibuprofen in the gastric fluid as occurred with a proprietary oral liquid and the drug remained associated with the LA gellan for over 6 hours at pH 1.2 (Figure 4.10).

A problem often associated with hydrogel drug delivery systems is drug leaching through the pores of the gel. In this system however, the poor solubility of ibuprofen resulted in precipitation within the gel when exposed to 0.1M HCl pH 1.2, illustrated by the opaque nature of the gel (Figure 4.10) with the precipitated drug particles remaining entrapped within gel particles as illustrated in Figure 4.11.

To develop a modified release oral liquid designed particularly for children it is vitally important to take into account paediatric gastrointestinal physiology when designing *in vitro* biopharmaceutical tests. Variables such as stomach acid volume, gastric pH and small intestinal transit time, which are important for drug release, are well documented (Bowles *et al*., 2010). For example, in paediatric patients the age at which gastric acid secretion reaches adult values is often quoted as 6 months, however in reality the pH remains variable and the time that intragastric pH is maintained below pH 2 increases as a function of age. Nagita *et al*., (1996) reported that gastric acidity rapidly increased from infancy to 3 years of age and then slowly increased and attained adult levels (< pH 2 for 65 % of a 24 h period) by adolescence (age 14).

In vitro release data shown in Figure 4.12 reveal that even at concentrations as low as 0.1 % w/w, LA gellan fluid gels have the ability to retard the release of ibuprofen following 20 min exposure to 0.1 M HCl pH 1.2 compared with the control formulations (C2 and C3). There is however, still some ibuprofen (approximately 5 %) released while exposed to pH 1.2. Increasing LA gellan concentration further slows release and at 0.75 % w/w, no ibuprofen was measured during acid exposure. Moreover, when the medium was changed to pH 7.4, there was a lag time of a further 30 min before onset of release.

The effects of varying acidic pH on the subsequent release of ibuprofen from the 0.75 % w/w LA gellan fluid gel following transfer to pH 7.4 was also evaluated. It was found that the release of ibuprofen from a 0.75 % w/w LA gellan fluid gel was strongly affected by the

pH of the dissolution media (Figure 4.13). There was no significant difference ($p > 0.05$) in release between samples that were initially exposed to pH 4, pH 5, and pH 7.4. However, as the pH was decreased below the pK_a of the carboxyl group of the LA gellan (~3.4), an acid gel was formed, preventing the dissolution of the gel, thus retarding ibuprofen release. This lag time became progressively more pronounced as the pH was dropped further and the acid gel strengthened; exposure to pH 1.2 for just 20 min prevented the onset of release for a further 60 min following transfer to pH 7.4. Moreover, there was a linear relationship between onset of release in pH 7.4 and the preceding exposure time at pH 1.2 for up to 120 min (Figure 4.14). A linear relationship was also found following exposure to pH 2 although the effect was substantially less pronounced. This was thought to be due to fewer H^+ ions present and an increase in ionised carboxylate groups on the gellan at pH 2 compared with pH 1.2. This will result in formation of a weaker acid gel with an associated increase in hydration and dissolution of the ibuprofen when transferred to pH 7.4. Indeed, the stiffness of the gel had an exponential relationship with onset of release in pH 7.4 media (Figure 4.15). Furthermore, the stiffness of the LA gellan was dependent on the duration of exposure to acidic pH which has also recently been reported by Bradbeer *et al*., (2014).

Interestingly, regardless of the duration of acid exposure, the stiffness of LA gellan fluid gels eventually plateaued at approximately 1200 Pa when transferred to pH 7.4 (Figure 4.16). Subsequently, the gel stiffness as a function of exposure time relates to *in vitro* release. When the gel was exposed to pH 1.2 for 60 min, G′ was approximately 8000 Pa and release was retarded, probably due to the time required for ion exchange to occur between the H^+ cross-linked gel and the phosphate buffer. This exchange gradually reduces the gel strength until drug release is enabled. Moreover, the diffusion of the phosphate buffer into the gel also increases the solubility of the ibuprofen by increasing the pH within the gel. This is thought to have facilitated drug diffusion into the surrounding release medium increasing release rate as

highlighted, with a much faster release rate of 0.44 mg min^{-1} when the exposure time in acid was only 10 min compared with 0.15 mg min^{-1} following 60 min exposure (Figure 4.17). This dependence on acidic residence time and the strength of acidic pH may be problematic in determining reproducible pharmacokinetics between patients where gastro intestinal physiology can vary. Therefore, strategies to overcome this issue would need to be addressed if such a carrier was to be used in clinical practice. However, by understanding the three way relationship between acid exposure time, gel stiffness and onset of release, there is potential for controlling release behaviour by tuning the fluid gels to have a specified stiffness at a particular pH and duration of exposure. Furthermore, producing formulations using this relatively simple method is particularly attractive and by careful design of processing parameters, the size, shape, viscoelasticity and behaviour in physiological fluids of the microgel particles can be manipulated to suit the application. This could open the door to multiple applications of fluid gel systems in pharmaceutical technology in addition to use as modified release oral liquids.

4.7 Conclusion

In this study we have demonstrated that LA gellan fluid gels have the potential to be formulated with a similar viscosity profile to that of a marketed paediatric oral liquid with a yield stress sufficient that the sample can be inverted without any immediate flow but shear thins sufficiently by shaking, to be poured onto a dispensing spoon. Furthermore, we have shown that it is possible to modify the release of ibuprofen from LA gellan fluid gels, providing a simple and effective technology in formulating modified release oral liquids. The release behaviour of ibuprofen from LA gellan fluid gels in a simulated intestinal pH environment was dependent on the stiffness of the gel following exposure to simulated gastric pH media. The stiffness, and hence drug release, could be controlled with exposure time and acidity of the simulated gastric pH environment. This work highlights the potential application of LA gellan

fluid gels as modified release oral liquids while at the same time, illustrates the importance of understanding how subtle differences in patient physiology could impact on drug release from such formulations. A realization of this is very important especially when designing medicines for children.

Chapter 5

Development of Mucoadhesive Sprayable Gellan Gum Fluid Gels

Aspects of the introduction in this chapter are published in the American Journal of Pharmacological Sciences and the results are published in the International Journal of Pharmaceutics

*Ghori, M. U., **Mahdi, M. H**., Smith, A. M., and Conway, B. R., 2015. "Nasal drug delivery systems: An Overview". American Journal of Pharmacological Sciences, Vol. 3, no. 5 pp. 110-119.*

***Mahdi, M.H**., Conway, B.R. and Smith, A.M. (2015), "Development of mucoadhesive sprayable gellan gum fluid gels", International journal of pharmaceutics, vol. 488, no. 1-2, pp. 12-19.*

CHAPTER 5 DEVELOPMENT OF MUCOADHESIVE SPRAYABLE GELLAN GUM FLUID GELS

5.1 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). A way to address this problem is by formulating nasal sprays that contain polymers that 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 entirely available. It is generally accepted, however, that inter-diffusion and interpenetration takes 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 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 promising polymer for use in nasal formulations because of its ability to form a gel *in situ* on exposure to physiological concentrations of cations. This helps to retain drugs on mucosal surfaces for a prolonged time and is currently used for this purpose in ocular formulations of timolol maleate as discussed in section 1.4.2.

Indeed, Mahajan and Gattani, (2009a) developed a nasal spray from solid microparticles using LA gellan gum prepared by spray drying technique, while, Cao *et al*., (2009) developed liquid nasal formulations using LA gellan solution. 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 be overcome by using fluid gels (Figure 5.1).

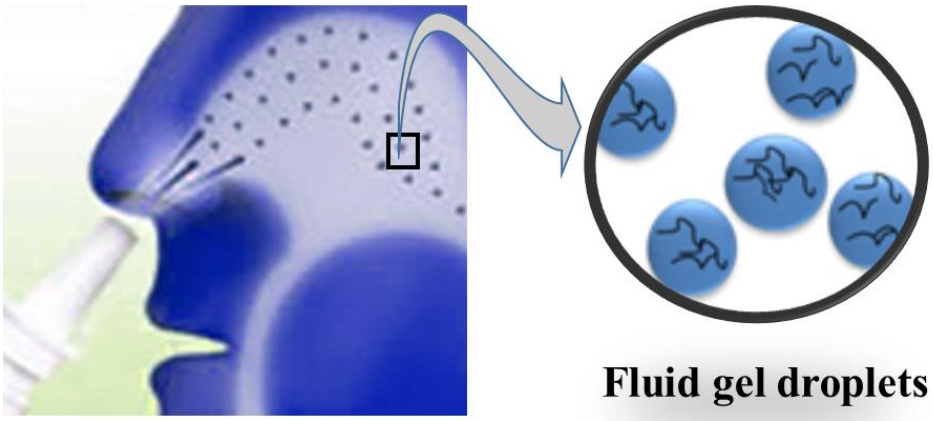

Figure 5.1 Illustration showing gellan gum fluid gel droplet deposition.

This chapter provides an overview of the anatomy and physiology of the nasal cavity and the physical, chemical and pharmacological properties of caffeine (which used as a model drug in this investigation). LA gellan, HA gellan and a 50:50 LA HA gellan blend fluid gel formulations (loaded with caffeine), were then evaluated as mucoadhesive nasal sprays and compared with gellan solutions that gel *in situ*. The rheological properties and *in vitro* measurements of retention time on mucosal tissue were also investigated.

5.2 Anatomy and Physiology of Nose

The nose is the primary entrance to the respiratory tract, allowing air to enter into the body for respiration. The nasal cavity is 120-140 mm deep, runs from the nasal vestibule to the nasopharynx and further leading to the trachea and oesophagus. The nasal cavity is split into two symmetrical halves by a cartilaginous wall called the nasal septum (Harkema *et al*., 2006; Chaturvedi *et al*., 2011). The nose has a surface area of around 160 cm^2 and a total volume of up to 20 mL (Taylor, 2007). In addition to the breathing and olfactory functions of the nose, it also provides protective systems by warming, filtering and humidifying of the inhaled air into the lungs (Ghori *et al*.,2015). It is the primary organ for filtering out particles in the inspired air, and it also provides a first-line immunological defence as it brings the inspired air into contact with the mucous-coated membrane. The nose has three main regions: vestibular, turbinate and olfactory regions (Figure 5.2).

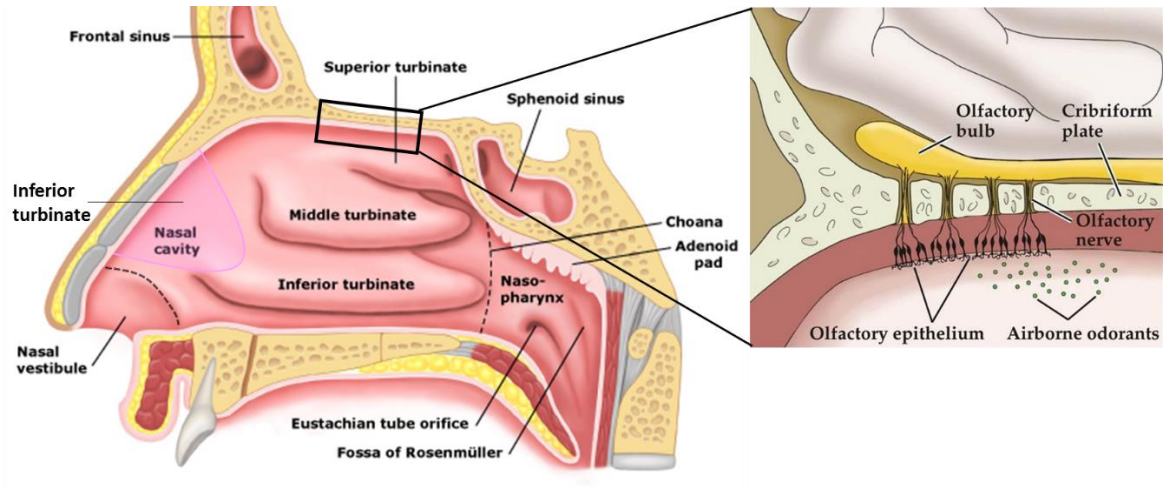

Figure 5.2 Illustration of the nasal cavity anatomy (adappted from Arora et al., 2002).

The vestibular region is the anterior part of the nose, and it is the narrowest part of the nasal cavity. The vibrissae cover most of this area which renders it capable of filtering out airborne particles with an aerodynamic particle size larger than 10 μm that may be inhaled with air. In the vestibular region, the surface lining changes from skin, at the first part of the passage,

to a stratified squamous epithelium (Chien *et al*., 1989; Chaturvedi *et al*., 2011) which are keratinised with sebaceous glands. Therefore, it can withstand the harmful effects of any inhaled toxic materials and is very resistant to dehydration. However, it allows only limited permeation of substances and is therefore not a preferred site for the administration and absorption of drugs (Taylor, 2007).

The turbinate region is a significant vascular part of the nose and constitutes approximately 80 to 90 % of the total area of the nasal cavity. This region is divided into three main sections; the superior turbinate, the middle turbinate and the inferior turbinate. The inferior and middle region of turbinate are large, and they nearly cover the entire length of the main nasal passage. The superior turbinate is much smaller compared with the inferior and middle turbinate, being only about half the length of the latter two regions (Figure 5.2). The turbinate region is lined with a pseudostratified columnar epithelium. It is composed of the mucus-secreting cells, ciliated cells, nonciliated cells and basal cells (Figure 5.3). Each ciliated and non-ciliated cell is covered with approximately 300 non-motile microvilli, which are responsible for increasing the surface area, thus, this is the region where the drug absorption is optimal (Chien *et al*., 1989).

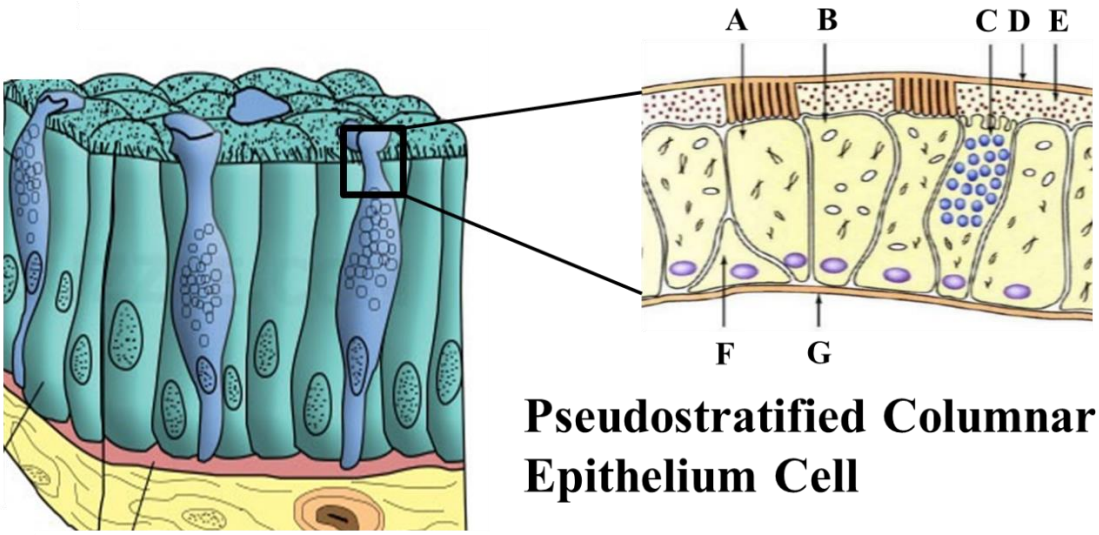

Figure 5.3 Cell types of the nasal epithelium with covering mucous layer showing ciliated cells (A), non-ciliated cells (B), goblet cells (C), mucous gel-layer (D), sol layer (E), basal cells (F) and basement membrane (G) (Ugwoke et al., 2005; Ghori et al.,2015).

There are approximately 100 motile cilia covering each ciliated cell which are responsible for mucus transport, so mucociliary clearance prevails. Once drugs (as particles or in solution) find their way to the mucociliary area, they are cleared from the nasal cavity and then have limited access to the absorption site (Prajapati *et al*., 2015).

The olfactory region is an area comprising about 8 % of the total surface area of the nasal epithelium and is made of a non-ciliated, pseudostratified columnar epithelium. It is important for transporting drugs to the brain and cerebrospinal fluid (CSF). There is a mucus layer of 5 μm in thickness covering the epithelium cells which traps unwanted particles (Illium, 2012 and Ghori *et al*., 2015).

5.3 Nasal Mucociliary Clearance

5.3.1 Cilia

Cilia are a small hair-like motile projections extending from the epithelium cells with each cilia measuring between 5 to 10 μm in length and from 0.1 to 0.3 μm in width (Taylor,

2007). The number of cilia is about 100 per ciliated cell. The function of these cilia is to facilitate mucus movement towards nasopharynx by regular waves a beats with an average frequency of 10 Hz. The cilia beats have three phases, an effective stroke, during which the cilia are extended maximally, the rest phase, in which they are parallel to the cell surface, and the recovery stroke (Marttine *et al*., 1998).

The ciliary beat frequency can be increased or decreased by alteration one or more of the following factors: physical factors (such as temperature, humidity and air flow), pharmacological factors (such as intracellular $Ca^{2+}$and cAMP levels, and extracellular ATP), mechanical and chemical factors (such as viscosity of a formulation and bioadhesion) as well as to the disease state of the nose (Marttine *et al*., 1998; Gizurarson, 2015).

For instance, the ciliary beat frequency of human nasal cells *in vitro* move faster with rising temperature, from 5 to 20 °C. Between 20 and 45 °C, it was found to stabilize at around 8–11 Hz, or around 14 Hz between 32 and 37 °C (Clary-Meinesz *et al.*, 1992; Marttin *et al*., 1998).

5.3.2 Mucus

A thin layer of viscoelastic fluid (mucus) covers most of the respiratory part of the nasal cavity. The mucus possesses some physiological functions including the enzymatic and physical protection of the mucosa and the transportation of particulate matter due to its adhesive nature. The nasal mucus is secreted mainly by submucosal glands. These glands are composed of both mucous cells and serous cells (Marttin *et al*., 1998). The viscoelastic fluid is split into two mucus layers, the periciliary layer which is produced by serous cells, composed of a low viscosity fluid with a length slightly shorter than cilia and the ciliary layer which produced by mucous cells, composed of more viscous (gel-like) layer which covers the periciliary layer. The mucus is also secreted by goblet cells which secrete mucous granules by

exocytosis. Once excreted, granules hydrate and swell resulting in the formation of a viscous gel-like material that exhibits non-Newtonian behaviour (Marttin *et al.*, 1998; Bansil and Turner, 2006; Cone, 2009). The volume of nasal secretion ranges from 1.5 - 2 L per day and the pH of the nasal secretions ranges from 5.0 to 6.5 (Washington *et al*., 2000; Taylor, 2007). The low vscosity fluid layer helps cilia during its recovery phase to return back to initial position and to intiate new effective stroke, while, the viscous layer helps forgien material to stick on it to move it to the GIT (Marttin *et al*., 1998).

The mucus secretion is a complex mixture of many substances. Water composes 90-95 % of the mucus. The remainder of the mucus secreations consist of ions (such as potassium, sodium, phosphate, magnesium, chloride and bicarbonate), (Bansil and Turner, 2006; Burke, 2014), < 1 % of lipids (such as fatty acid phospholipid, cholestrol), 2 % mucin and 1 % proteins (which serve as defensive agents and consist of immunoglobulins and albumins, lysosymes and lactoferrins) (Kaliner *et al*., 1984; Bansil and Turner, 2006).

The main macromolecule in mucus secretion is the mucin, and the physiochemical properties of the mucus is likely to correlate with physiochemical properties of the mucin. Mucin is a high molecular weight glycoprotein ranging from 5×10^5 up to 2×10^7 g mol^{-1}, crosslinked with disulphate bridges, physical entanglement and ionic bonds (Bansil and Turner, 2006; Abodinar *et al*., 2016). Mucin is responsible for viscoelastic properties of mucus and it is composed of 20 % protein, and 80 % carbohydrates, namely N-acetylgalactosamine, N-acetylglucosamine, fucose, galactose, and sialic acid (N-acetylneuraminic acid) and traces of mannose and sulphate. Sialic acid (N-acetylneuraminic acid, Neu5Ac) and sulphate (SO_4^{2-}) gives the mucin polymer negative charge, with a ζ-potential of ~ −4.4 to −10 mV at nasal pH (5.0 - 6.5) (Abodinar *et al*., 2016).

Central protein segments of mucin are composed of repeats units that are rich in serine, threonine, and proline. In addition, it has been shown that the oligosaccharide chains link to the hydroxyl side chains of serine and threonines of the protein core via *O*-glycosidic covalent bonds and arranged in a “bottle brush” shape about the protein core (Bansil and Turner, 2006; Yuba and Kono, 2014).

5.3.3 Mucociliary Clearance

Mucociliary clearance is non-selective defence system used to remove and prevent foreign substances from reaching the lower airways. Inhaled foreign particles and drugs are entrapped by the sticky mucus layer while ciliated cells provide the driving force to transfer them to the nasopharynx and ultimately to the GI tract. The cilia push the mucus towards the nasopharynx by frequently beating. The beat cycle begins, when the cilium is extended during the effective stroke phase, the tip of the cilia penetrates the mucus and transfers kinetic energy which induces slow movement of mucus. The tip then continues to the rest phase downwards into the periciliary fluid followed by the recovery phase which returns the cilia to the start position before the commencement of a new cycle (Figure 5.4) (Guirao and Joanny, 2007; Gizurarson, 2015)

Mucociliary clearance is therefore a combination of mucus entrapment and cilia mediated clearance. Therefore, mucociliary clearance depends upon factors that affect mucus production and beat frequency coordination of cilia. Average mucociliary transit time in humans has been reported to be between 12 to 15 min (Marttin *et al*., 1998).

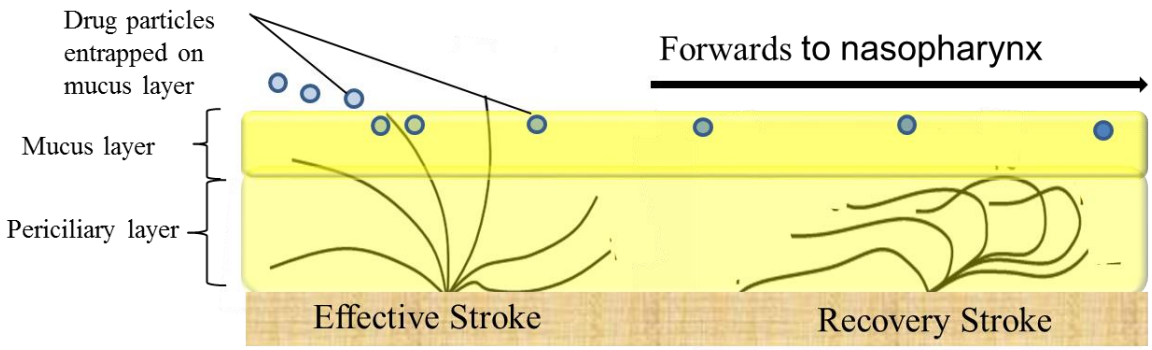

Figure 5.4 Airway mucus secretion.

5.4 Caffeine

Caffeine is the most widely consumed psychoactive drug. Caffeine (1,3,7-trimethylxanthine) is a purine heterocyclic alkaloid with molecular formula $C_8H_{10}N_4O_2$ (Figure 5.5). Caffeine is a natural product that can be extracted from several plants sources such as coffee and tea. Caffeine has pharmacological effects on the body that include enhancing alertness, relaxing bronchial smooth muscle, diuretic effects, stimulation of cardiac muscle and increase in metabolic rate. These stimulatory effects of caffeine are induced by blocking A_1 and A_2 subtypes of the adenosine receptors. Adenosine is an endogenous neuromodulator and it is responsible for inhibitory effects on the nervous system. Caffeine therefore acts as an antagonist of the adenosine receptors reducing the inhibitory effects of adenosine at dopamine receptor thus, increased dopamine release and stimulating the central nervous system (CNS) (Solinas *et al*., 2002).

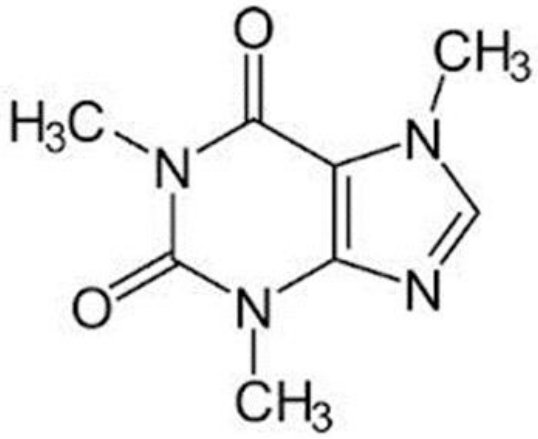

Figure 5.5 Caffeine structure (adapted from Agyemang-Yeboah and Oppong, 2013).

Caffeine at low and moderate doses has a positive pharmacological effect on human body. At high doses (more than 1.5 g), however, caffeine can lead to serious health problems such as elevation in blood pressure, and anxiety and caffeine withdrawal syndrome (consisting of fatigue and sedation) (Meredith *et al*., 2013). Caffeine is highly soluble in water. It is available as a white powder that is odourless with a bitter taste, it has a molecular weight of 194.2 g mol^{-1} with a pK_a 10.4 (Agyemang-Yeboah and Oppong, 2013).

Caffeine alone is not considered as an analgesic but in doses of 65 mg or higher it can be used as an analgesic adjuvant. Painkillers such as aspirin and paracetamol exhibit greater analgesic activity in combination with caffeine (Derry *et al*., 2012). This has been demonstrated by Palmer *et al*., (2010) who reported the benefit of paracetamol and caffeine combination over paracetamol alone formulations and that paracetamol/caffeine at a dose of 1000 mg/130 mg is efficient and safe for use in acute management of pain.

5.5 Materials and Methods

5.5.1 Materials

HA gellan (Kelcogel^{TM}) was kindly donated by CP Kelco (USA). LA gellan and caffeine were purchased from Sigma–Aldrich (Poole, UK). Phosphate buffer saline (PBS) was purchased from Fisher Scientific (UK). Fresh porcine mucosal tissue was donated from a local abattoir.

5.5.2 Methods

5.5.2.1 Preparation of Fluid Gel Formulations

Gellan gum fluid gels (LA, HA and LA HA blends) were prepared by adding precise amounts of HA and LA gellan to produce a 0.25 % w/w final polymer concentration in deionised water at 85 °C containing 2 mg mL^{-1} caffeine; sodium chloride (0.1 % 0.5 % and 1 % w/w) was added to the hot caffeine-loaded gellan solutions at ~ 85 °C, as crosslinking cations, 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.

5.5.2.2 Preparation of Control Formulations (uncross-linked gellan)

Gellan solutions (LA, HA and LA HA blends) were prepared by adding precise amounts of HA and LA gellan to produce a 0.25 % w/w final polymer concentration in deionised water at 85 °C containing 2 mg mL^{-1} caffeine. This was allowed to quiescently cool to room temperature prior to use.

5.5.2.3 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 and a Peltier thermal control system.

5.5.2.2.1. Viscosity Measurements

Viscosity measurements of all samples were taken at 20 °C across shear rates ranging from 1 s^{-1} - 1000 s^{-1} for 5 min.

5.5.2.2.2. Yield Stress Determination

Stress sweeps 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.

5.5.2.2.3. Frequency Sweep Measurements

The rheological behaviour 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 determined from amplitude sweeps).

5.5.2.4 Microscopy

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.

5.5.2.5 Preparation of Mucosal Membrane for Retention Studies

The outer muscle layers of fresh porcine oesophageal 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 if residual surface debris was evident.

5.5.2.6 Retention Time Measurements

Drug retention time in phosphate buffer saline (pH 7.4, 34 °C) was studied using a bespoke mucoadhesion apparatus (Figure 5.6).

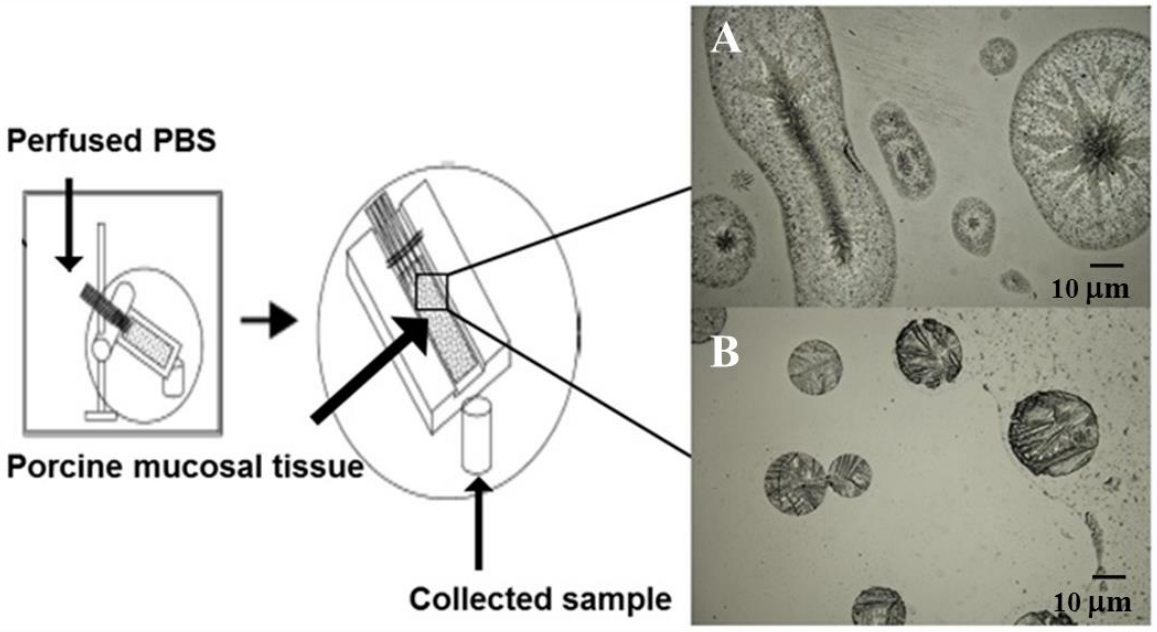

Figure 5.6 Schematic representation of the model retention apparatus (adapted from Batchelor et al., 2002), A) gellan solution droplet B) gellan fluid gel droplets.

A sample of defrosted mucosal tissue (as prepared in section 5.5.2.5) was placed on the apparatus and the caffeine-loaded formulations (~100 µL) were sprayed from a mechanical nasal spray device onto the tissue. PBS was then perfused over the mucosal membrane at a rate

of 1 mL min^{-1}. 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 5.1.

$$\frac{[C]-[CP]}{[C]} \times 100 \qquad \text{Eq. 5.1}$$

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

5.5.2.7 Caffeine Assay

Chromatographic separation was performed on a Shimadzu 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). Isocratic elution of the mobile phase with a composition of methanol/water (40: 60) (v/v) was used. HPLC conditions for the caffeine assay are presented in Table 5.1.

Stock solutions of caffeine were prepared by dissolving 100 mg in 100 mL phosphate buffer saline pH 7.4. Standard solutions were prepared in concentrations between 2 μg mL^{-1} and 20 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) against the concentrations. Precision and linearity over the concentration range were assessed. Precision was calculated from the relative standard deviation (% RSD < 1) of the standard curve and linearity was assessed by the linear regression with r^2 of 0.999. All compounds were identified by comparison of retention times obtained from the sample and standard solutions. LOD and LOQ were calculated as in section 4.4.2.6. HPLC validation method for caffeine assay presented in Table 5.2.

Sample chromatogram of caffeine is shown in Figure 5.7; typical calibration curve constructed from peak area against concentration is shown in Figure 5.8. All chemicals used in this assay were of analytical grade; solvents were HPLC grade and were used as received without further treatment. HPLC grade water was utilized in the preparation of the mobile phases. The mobile phase was filtered and degassed by sonication using an ultrasonic bath (Fisher Scientific). All samples were analysed immediately.

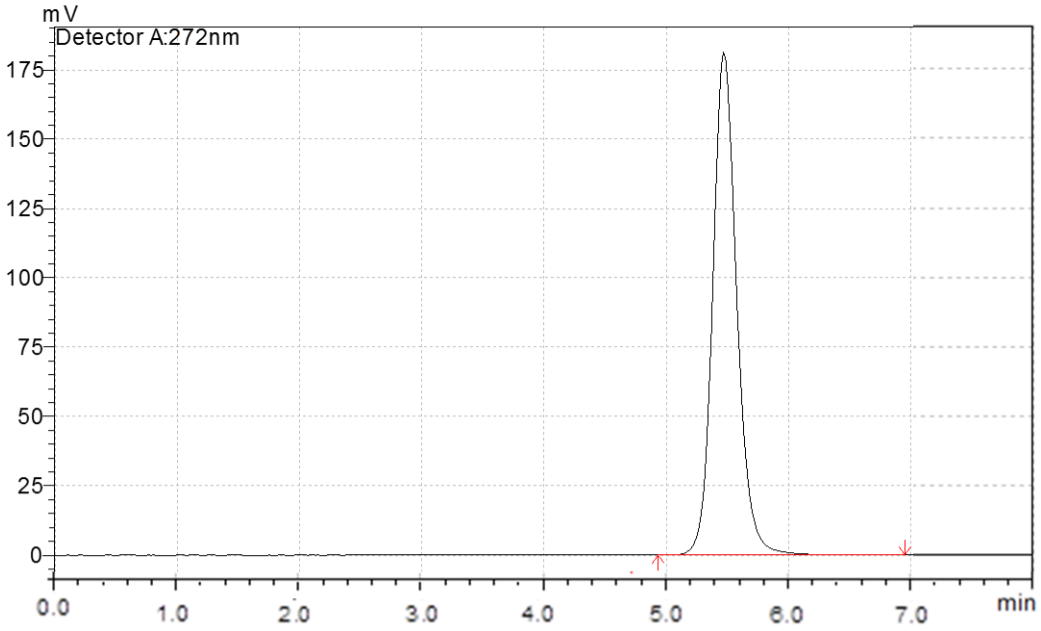

Figure 5.7 Typical chromatogram of caffeine detected at 272 nm.

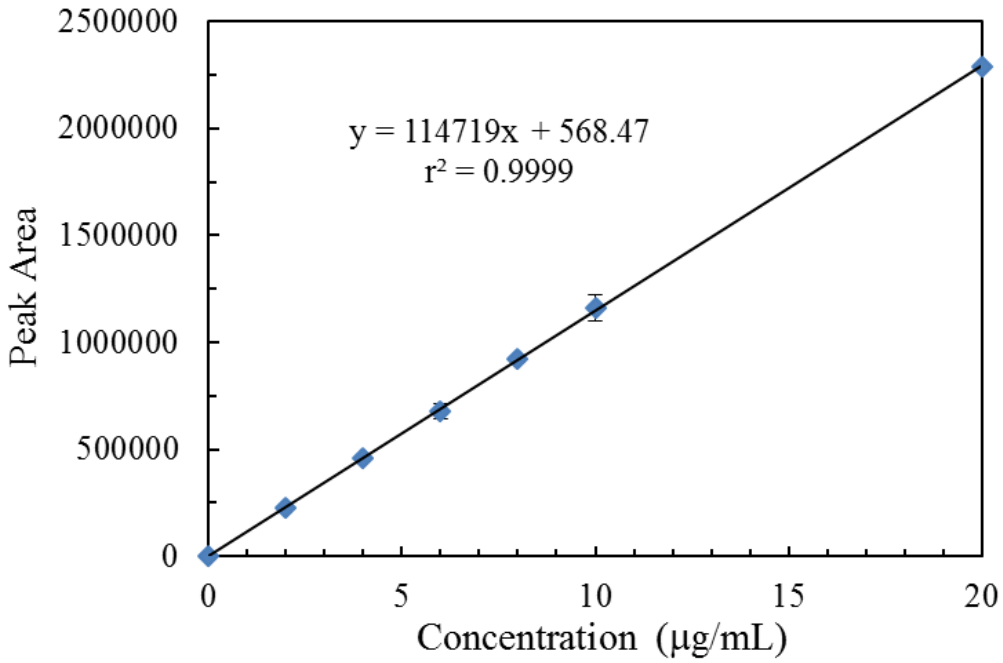

Figure 5.8 Mean calibration curve for caffeine measured at λ=272 nm. Values represent mean ± SD (n=3).

Tablet 5.1 HPLC conditions for caffeine assay.

Wavelength	272 nm
Injection volume	100 µm
Flow rate	0.5 mL.min^{-1}
Run time	7 min

Tablet 5.2 HPLC method validation for caffeine assay.

Peak tailing factor $\{\boldsymbol{Tf} = \frac{\boldsymbol{a+b}}{\boldsymbol{2a}}\}$	1.2
Retention time	5.2 min
Limit of quantification $\{\boldsymbol{LOQ} = \boldsymbol{10}\frac{\boldsymbol{\sigma}}{\boldsymbol{S}}\}$	0.045 μg/mL
Limit of detection $\{\boldsymbol{LOD} = \boldsymbol{3.3}\frac{\boldsymbol{\sigma}}{\boldsymbol{S}}\}$	0.016 μg/mL
Precision and accuracy	RSD < 1 %

5.5.2.8 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.

5.6 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 5.9 shows cooling profile of a 0.25 % w/w HA, LA and 50:50 blend of gellan over range of NaCl concentrations. There was a general trend that showed HA gellan decreased in viscosity with an increase in ion concentration whereas the viscosity of LA gellan increased with increasing ion concentration. As shown in Figure 5.9A, in the absence of added ions, the HA gellan and the blend showed an increase in viscosity beginning at approximately 65 °C which corresponded with the onset of ordering of HA gellan, 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 gellan 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 (Figure 5.9B-D). For the blend two transitions were evident, one corresponding to the HA gellan ordering and one corresponding to the LA gelation.

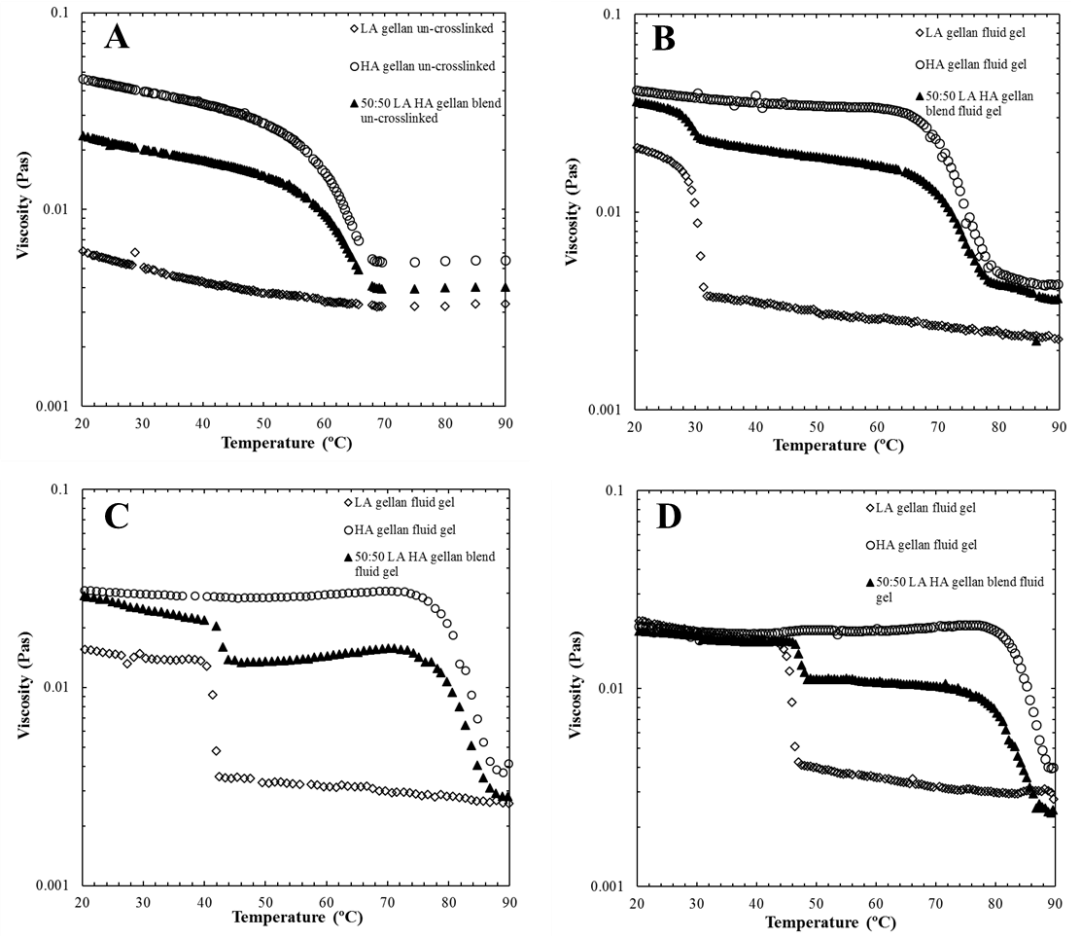

Figure 5.9 Viscosity of gellan gum during fluid gel formation at 0.25 % w/w gellan (cooling at 2 °C min^{-1} at a shear rate of 500 s^{-1}) for 0.0 % (A), 0.1 % (B), 0.5 % (C) and 1 % (D) w/w NaCl loaded with 2 mg mL^{-1} caffeine (Mahdi et al., 2015) (used with permission).

The results indicate that the NaCl has a potential effect on the viscosities of the fluid gel. The onset of gelation for HA gellan and the 50:50 LA HA gellan 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 gellan 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 gellan 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 gellan 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 gellan fluid gel decreased from ~0.045 Pas without NaCl to a similar level as the LA gellan at 0.5 % w/w NaCl.

Interestingly, the final viscosity of the LA HA gellan blend fluid gel stayed the same at all the salt concentrations tested. The viscosity profile of a 0.25 % w/w HA gellan, LA gellan and blend solutions without salt and for 0.5 % w/w NaCl are shown in Figure 5.10A and were all found to have a shear thinning viscosity profile. Figure 5.10B shows the viscosity of the HA gellan, LA gellan and 50:50 LA HA gellan blend fluid gel formulations with 0.5 % NaCl and the comparative un-crosslinked solutions at 500 s^{-1}. The HA gellan fluid gel sample with 0.5 % NaCl exhibited a viscosity profile that was most similar to the 50:50 LA HA gellan blend fluid gel and 50:50 LA HA gellan without NaCl (un-crosslinked). 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.

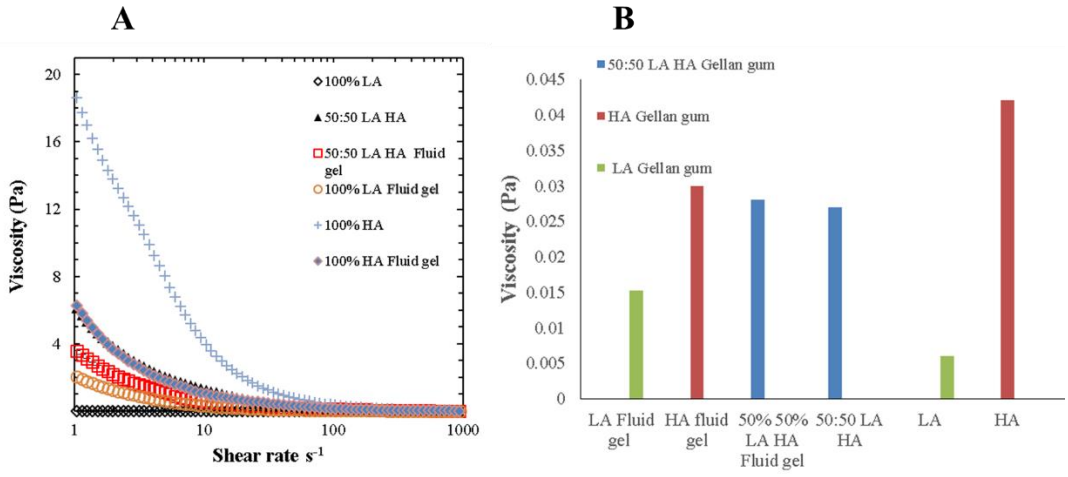

Figure 5.10 Viscosity vs. shear rate at 20 °C for 0.25 %w/w gellan at 0.5 % NaCl fluid gel and for un-crosslinked gel, B) Viscosity measurements at 20 °C at a shear rate of 500 s^{-1} of gellan blends containing 2 mg mL^{-1} caffeine (Mahdi et al., 2015) (used with permission).

LA gellan and the 50:50 blend fluid gel produced at 0.25 % w/w gellan and 0.5 % w/w NaCl, generally exhibited greater G′ (~10 Pa) compared with the un-crosslinked samples that ranged from ~0.1 and 1 Pa for LA gellan and the 50:50 blend respectively. The HA gellan however, exhibits almost the same profile in both the fluid gel and the HA gellan 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 behaviour (Figure 5.11).

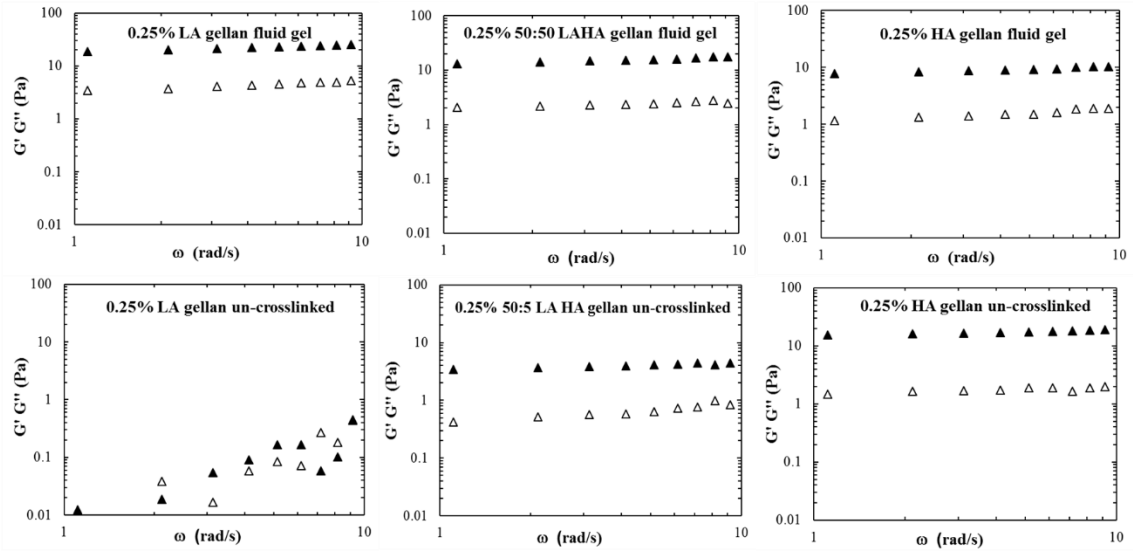

Figure 5.11 Mechanical spectram (1 % strain; 20 °C) of a 0.25 % gellan gum loaded with 2 mg mL^{-1} caffeine showing variation of G′ (filled triangles), G″ (open triangles) (Mahdi et al., 2015) (used with permission).

To evaluate sprayability through the nasal spray device, stress sweep rheological measurements were performed to determine the yield stress. The stress required to yield the fluid gel formulations were 1.1 Pa, 1.2 Pa and 5.7 Pa, for the LA gellan, 50:50 LA HA gellan blend and HA gellan respectively (Figure 5.12A), which was significantly less than the corresponding solutions without NaCl (Figure 5.12B).

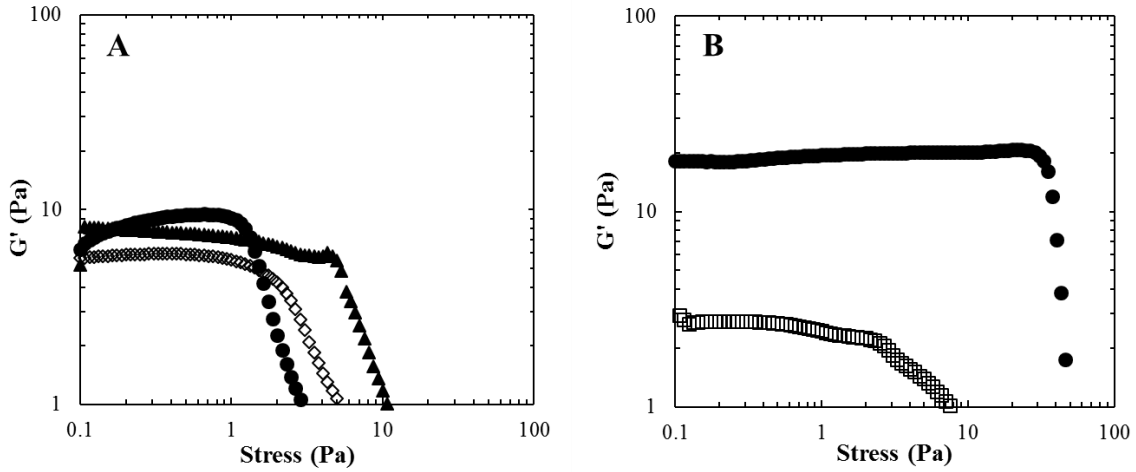

Figure 5.12 A) Stress sweep for 0.25 % gellan fluid gels crosslinked with 0.5 % NaCl as function of HA LA ratio (LA gellan filled circles, HA gellan filled triangles and 50:50 LA HA gellan blend open diamonds), B) Stress sweep for 0.25 % un-crosslinked gellan for HA gellan(filled circles) and 50:50 LA HA gellan blend (open squares) (Mahdi et al., 2015) (used with permission).

To investigate the retention properties of the formulations on the mucosal surface, the release of caffeine from 0.25 % LA gellan, HA gellan and the 50:50 LA HA gellan blend (fluid gel and un-crosslinked gellan) were studied (Figure 5.13). The LA gellan fluid gel formulation released almost 96 % of after 1 h, whereas the HA gellan fluid gel had only 50 % drug release at the same time point with the 50:50 LA HA gellan blend releasing 65 % after 1 h. Un-crosslinked gellan samples however, present large difference in drug release between HA gellan, LA gellan and the 50:50 LA HA gellan blend. LA gellan released almost 100 % of drug after 10 min, whereas the HA gellan only released 6 % of the drug, release at the same time point with the 50:50 LA HA gellan blend was 70 %.

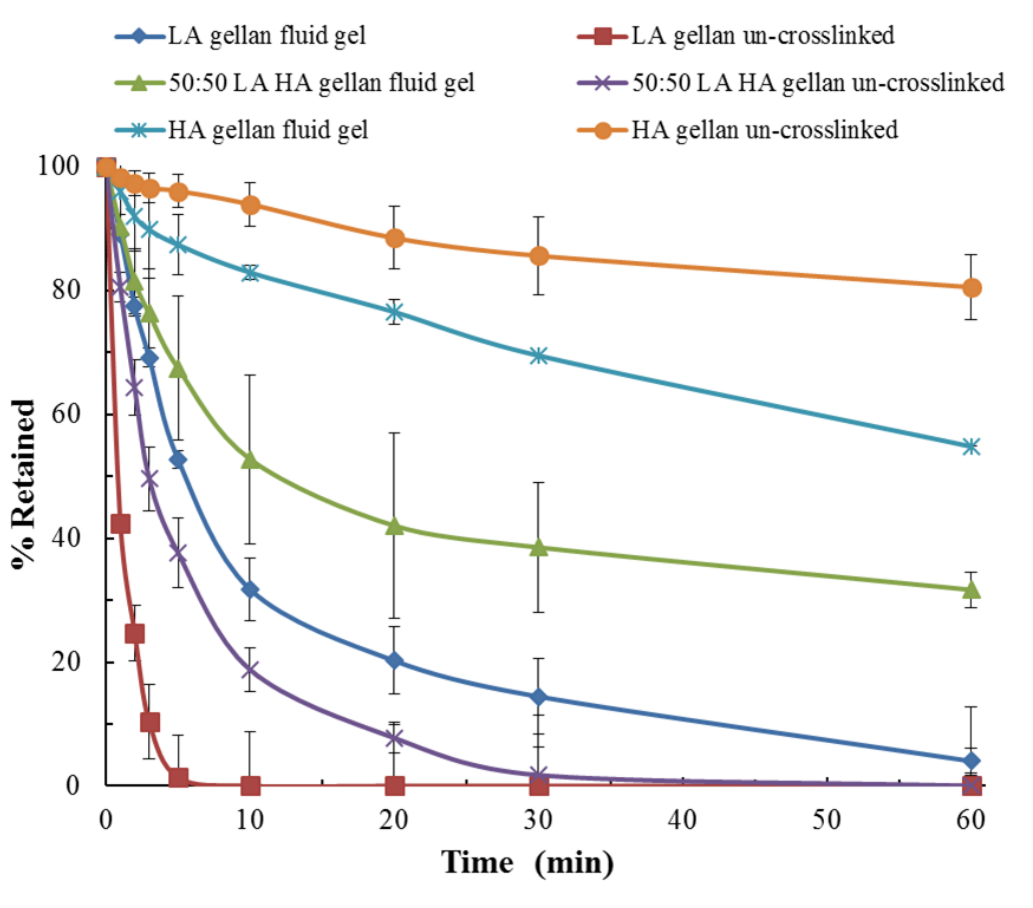

Figure 5.13 Cumulative % caffeine retained on the mucosal membrane after 60 min (Mahdi et al., 2015) (used with permission).

5.7 Discussion

There are two main prerequisites for *in situ* gelling of nasal spray systems: optimum viscosity and gelling capacity. The viscosity is a critical factor as the formulation should be at a low enough 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 cations in the 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 the 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 (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 gellan, HA gellan and a 50:50 LA HA gellan blend, as mucoadhesive systems 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 gellan and 50:50 LA HA gellan blend increased (Figure 5.9B-D) compared with when no ions were added (Figure 5.9A) which can be explained by promoting ordering via suppression of the repulsive negative charge between gellan chains by the addition of NaCl (Morris *et al*., 2012). The LA gellan sample containing 0.1 % w/w NaCl exhibited a clear transition (Figure 5.9B) because the concentration at this level was sufficient to allow the crosslinking between two or more LA gellan helices (Morris *et al*., 2012). Sanderson *et al*., (1988) reported intermediate textural properties between HA and LA gellan gels when combining LA gellan with HA gellan to form a mixed gel. This is in good agreement with the present study, as the 50:50 LA HA gellan blend exhibited two transitions that were characteristic of the individual components (Figure 5.9) (Sworn et al., 1995).

Once manufactured, the bulk fluid gels containing caffeine showed shear thinning behaviour suitable for spraying through nasal spray device (Figure 5.10). 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 groups facilitates binding of the Na^+ ions to the carboxylate group of the β-glucuronate residues in the gellan chains, thus reducing the repulsive electrostatic force on the gellan helices, promoting aggregation and development of a three dimensional network. There was no significant difference in viscosity of 50:50 LA HA gellan blend 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 5.11), 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 behaviour 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. Moreover, stress sweep measurements were used to determine the yield stress and to gain an understanding of the strength of particle-particle interactions. The HA gellan with no NaCl added had a higher yield stress value compared with the 50:50 LA HA gellan blend (Figure 5.12B) and for this reason this HA gellan was poorly dispensed from the nasal spray, whereas the 50:50 LA HA gellan blend could be dispensed without any problems.

It was shown that the HA gellan 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 and therefore inferring mucoadhesive properties (Figure 5.14). This is thought to be due to the greater elasticity and viscosity of HA gellan, promoting physical interactions with mucins on the surface of the mucosa (Mao *et al*., 2000). Most of the caffeine (80 %) in the HA gellan 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 gellan on contact with the ions on the mucosal surface. LA gellan 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 5.9B), elasticity and yield stress (Figure 5.12B) 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 the LA gellan fluid gel (containing 0.5 % NaCl) (Figure 5.12A), which is easily administered, while maintaining ~70 % of the mucosal retention of the uncrosslinked HA gellan (Figure 5.14). This bulk rheology was also shown to be tuneable by creating a 50:50 HA LA gellan blend with rheological properties (Figure 5.11) and mucoadhesive properties (Figure 5.14) intermediate to those of 100 % HA gellan and 100 % LA gellan.

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.

5.8 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 behaviour of the formulation by creating a LA HA gellan blend. 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 of uptake.

Chapter 6

Development of Gellan Gum Fluid Gels as Topical Formulations

Results presented in this chapter are published in the International Journal of Pharmaceutics

Mahdi, M.H[1]. Conway B. R[1]. Mill T[2] & Smith, A.M[1]. (2016) Gellan Gum Fluid Gels for Topical Administration of Diclofenac, International Journal of Pharmaceutics in press

CHAPTER 6 DEVELOPMENT OF GELLAN GUM FLUID GELS AS TOPICAL FORMULATIONS

6.1 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 systematic (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 get the drug into the systemic circulation. Thus, the skin serves as the site of administration, and not as the targeted organ. The transdermal route offers a good alternative to the oral route when oral administration of the drug causes serious side effects. The transdermal route is also suitable for drugs with low bioavailability due to physiological properties of GIT (such as first pass metabolism) or physical properties of the drug (such as a drugs with low solubility and drugs with a 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. It has been reported that orally administered diclofenac undergoes first pass metabolism and produces considerable gastrointestinal disturbance. In order to overcome these two major shortcomings of the oral dosage form, different transdermal formulations have been introduced for diclofenac delivery (Warner *et al*., 1999). The benefits of transdermal drug delivery such as simple applications and avoidance 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; that include active methods and passive methods (Batheja *et al*., 2011; Fang *et al*.,1999). Active methods include iontophoresis, electroporation, and microneedles (Batheja *et al*., 2011; Goh and Lane, 2014). 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 often lead to irritation or sensitization of the skin (Williams and Barry, 2012). There is therefore, a real need for alternative formulations.

Equally important to the effectiveness of topical dosage forms, the perceptual attributes of the topical formulation is very important for patients as these contribute substantially to whether a product is liked by patients and thus used. For instance, if the gel appearance is unpleasant to touch, its 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, assuming the initial layer of product at application time is thick enough to provide boundary lubrication. However, as the rubbing action (shear) starts, 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, thin surface residue may control the hydrodynamic lubrication (De Vicente *et al*., 2006).

This chapter provides an overview of the anatomy and physiology of the skin, drug penetration through the skin and pharmacological properties of diclofenac (which is the drug used in this study). The experimental part of this chapter discusses the application of gellan gum fluid gel as a platform to deliver diclofenac sodium to the skin. LA gellan, HA gellan and a 50:50 LA HA gellan blend fluid gel formulations (loaded with diclofenac), were then evaluated as a topical gel formulation and compared with the commercially available Voltaren® gel. Rheology measurements were used to characterize the flow properties of the formulations and to indirectly predict the squeezability and spreadability of the fluid gels. The lubrication properties of the gellan fluid gel formulations were also examined to assess potential for easy application on the skin.

6.2. Anatomy and Physiology of the Skin

6.2 Skin Structure and Function

The skin is a very complicated vital organ that covers the outer layer of the body and plays a critical role in insulation and protection from the external environment. The skin comprises mainly of three layers, the epidermis, the dermis and the subcutaneous tissue (Moser *et al*., 2001). These layers are in fact sub-divided into sub-layers in which thickness and cell morphology differ (Figure 6.1).

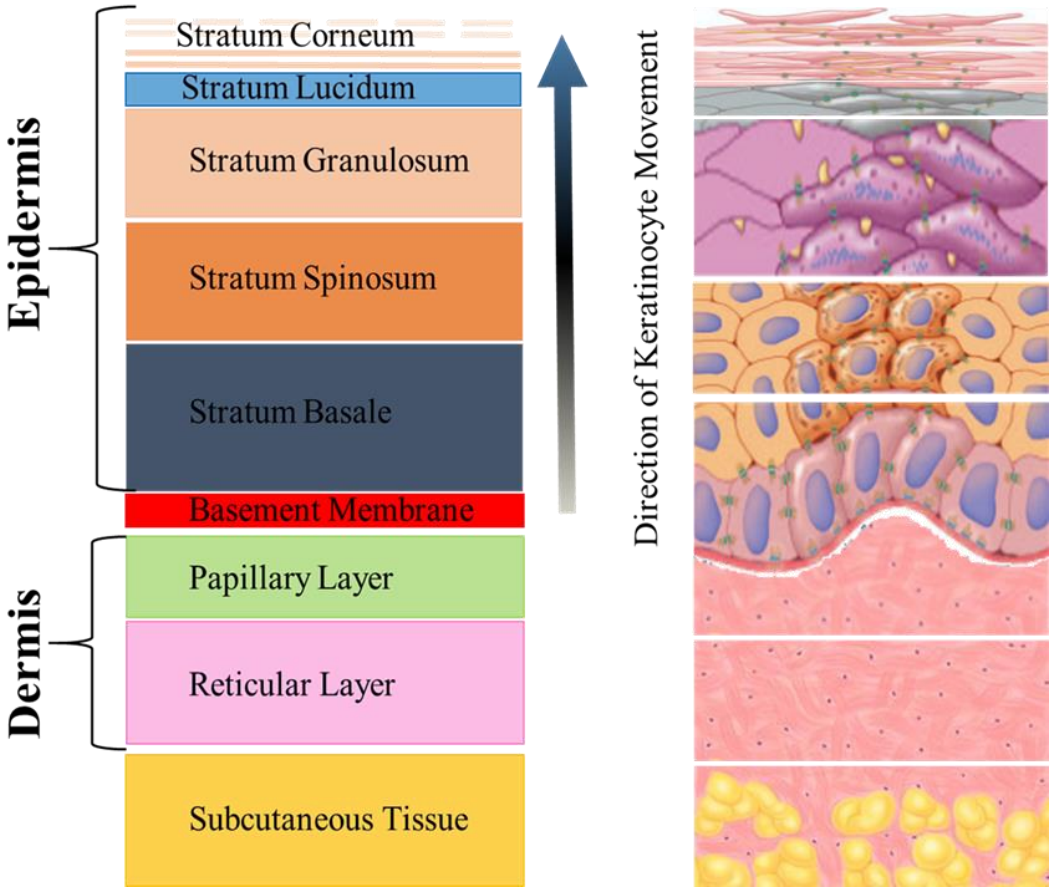

Figure 6.1 Schematic diagram of the layers of the skin (adapted from Seeley et al., 2004).

Within the skin, there are other tissues including those of vascular and nervous origin. The skin also has a number of appendages including about 2 to 5 million eccrine sweat glands that produce sweat, which consists of mainly water and NaCl and has a pH ranging from 4 to 6.8. Their main function is to aid thermoregulation (Barry, 2007). When body temperature increases thermoreceptir neurones within the hypothalamus causes the rate of sweat production to increase and once on the surface of the skin, it evaporates causing the body to cool. (Seeley *et al.*, 2004). The hairy skin, however, contains follicles, sebaceous glands and apocrine sweat glands. The apocrine sweat glands found in some areas such as the armpit produce an oily odorless secretion, which can be metabolized by bacteria to produce the characteristic body smell (Figure 6.2).

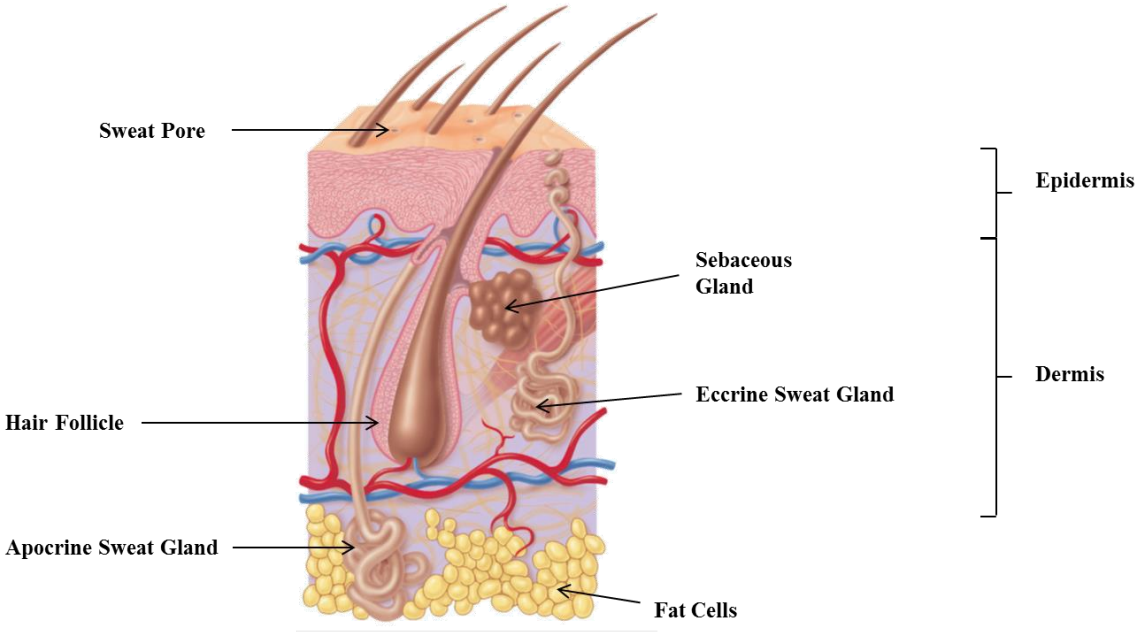

Figure 6.2 Illustration of skin structure and skin appendages (adapted from Seeley et al., 2004).

The dermis and epidermis adhere to the basement membrane which, consists of multiple extracellular matrix components that include collagen fibres, secreted by basal keratinocytes and dermal fibroblasts (Kanitakis, 2001). This junction is also responsible for metabolite exchange between the two layers and the interactive signalling between the dermis and epidermis (Burkhard and Ruppert, 1981; Kanitakis, 2001).

6.2.1 Epidermis

The epidermis is of ectodermal origin and mainly consists of keratinocytes, which differentiate as they move up through the skin towards the outer layer of the epidermis where they become flattened cornified corneocytes (Figure 6.1). The epidermis is composed of five distinguishable sublayers, stratum basal, stratum spinous, stratum granulosum, stratum lucidum and SC (Figure 6.1). The basal layer is rich in stem cell, which produces keratinocytes. Each layer of the epidermis can be clearly recognized by the morphology and differentiation state of the keratinocytes (Franssen *et al*., 2004).

The SC is the main protective layer composed of dead cells of a multilayer of keratin-rich corneocytes and an intercellular matrix of a unique composition of lipids (Wickett and Visscher, 2006). The lipids make the SC impermeable to water, and therefore, humans survive in a nonaqueous environment without dehydration. The SC layer varies in thickness, ranging from a very thick layer of the palm and sole (~80 μm) to very thin layer of the eyelid (~6 μm) (Barry, 2007).

6.2.2 Dermis

The dermis (also called the corium) is localised between epidermis from the top and subcutaneous layer from the bottom. The dermis has a thickness around 3-5 mm, and is of mesodermal origin. The dermis is subdivided into two layers, the papillary layer and the reticular layer, and both layers contain elastic connective tissue consisting of fibrous proteins that include elastin, reticulin and collagen. As well as the tissue and proteins, the dermis layers contain nerve endings, hair follicles, sweat glands and sebaceous glands, blood and lymphatic vessels all embedded in gelatinous mucopolysaccharides (Figure 6.2) (Wilkes *et al*., 1973).

The main dermis layer functions are to provide flexibility and mechanical support to the skin (Kanitakis, 2001; Bouwstra *et al*., 2003). The presence of the vasculature in the dermis is an important in controlling body temperature, delivering nutrients and removal of small waste molecules with the lymphatic system responsible for the elimination of larger molecules and immunological response (Cross and Roberts, 1993). Sebum produced by the sebaceous gland maintains skin hydration and flexibility.

6.2.3 Subcutaneous Tissue

The subcutaneous tissue (hypodermis) is the inner layer of the skin, and it is located between dermis and muscles or bone. The thickness varies and depending on age, sex and anatomical site. The subcutaneous tissue is mainly composed of adipose tissue, providing a

thermal barrier to the body and serving as a shock absorber to protect against mechanical stress (Avram *et al*., 2005; Kolarsick *et al.*, 2011).

6.3 Drug Penetration Through the Skin

For successful topical delivery of drugs, active pharmaceutical ingredients need to penetrate or cross the skin layers to exert their functions. The SC however, represents the main barrier to drug penetration due to its unique structure and its lipid compositions, having a "brick-and-mortar" shape (Figure 6.3) (Barry, 2007).

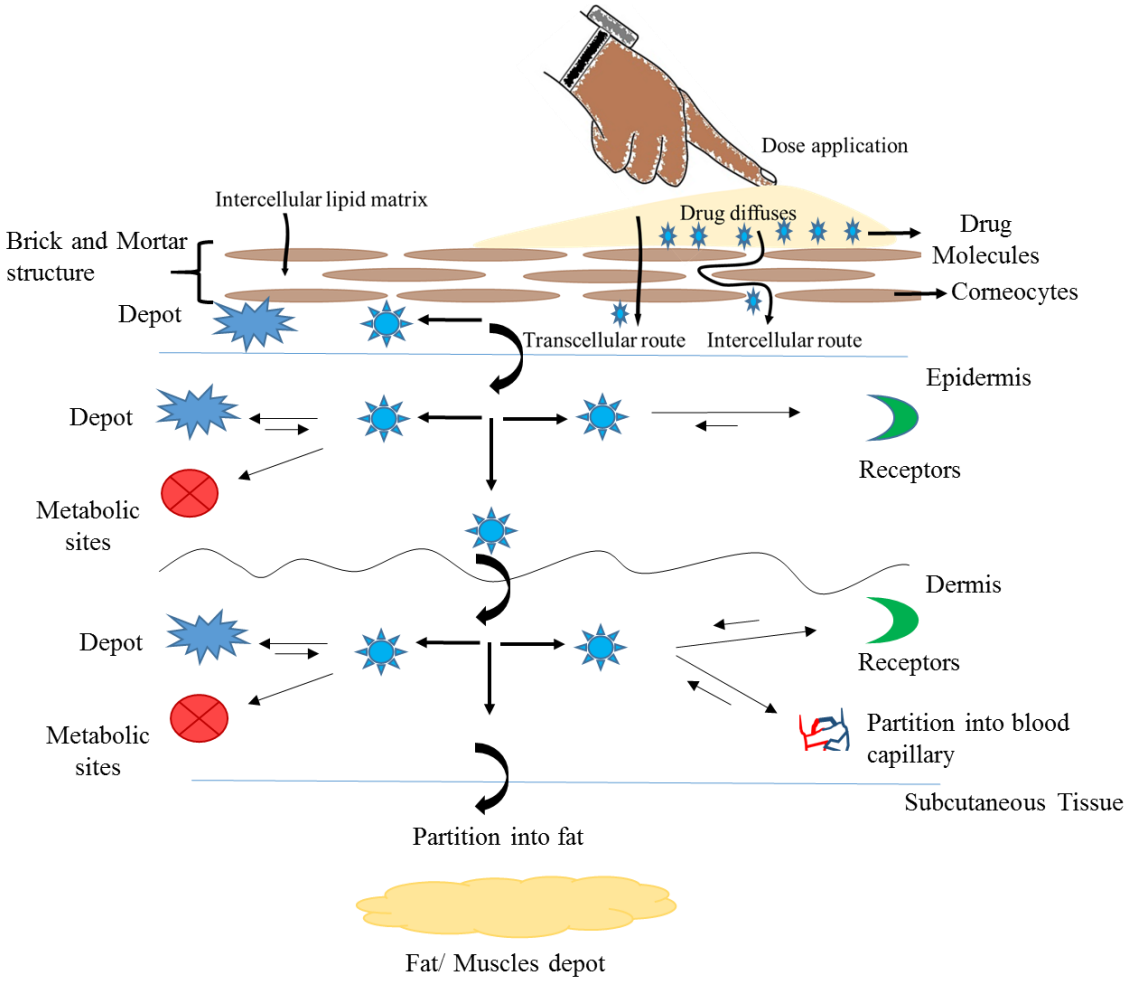

Figure 6.3 Schematic diagram illustrating the stages in drug delivery after topical/transdermal application.

Drug molecules can penetrate through the skin by passive diffusion. Passive diffusion is a transport mechanism where the drug is transported according to its concentration gradient, namely the transport from high concentration area to low concentration area, and no energy is needed to trigger such reaction. Following dose application drugs diffuse from the drug delivery vehicle towards the skin and partition onto the surface to pass through the SC. There are two main pathways for the drug to permeate through, the intercellular lipid route between the corneocytes and the transcellular route crossing through the corneocytes (Figure 6.3). A small amount of drug can also penetrate the skin through hair follicles and sweat gland ducts, which serve as shunt pathways through the intact epidermis. At the SC layer, some drug molecules may bind to a depot site while the rest of the drug penetrates further down the epidermis.

The drug then passes into the dermis, where some of the drug partitions into the capillary walls and then into the systematic circulation. The remainder of the diffused drug partitions into the subcutaneous fat and muscles layers forming another drug depot (Figure 6.3). This penetration process can be affcected by many factors including biological factors such as skin condition, skin age, blood flow, regional skin site and physiochemical factors such as drug concentration, diffusion coefficient and molecular size.

6.4 Diclofenac Sodium

Diclofenac is a commonly used, highly effective non-steroidal anti-inflammatory agent (NSAID). It is used in the treatment of acute conditions of inflammation and pain, musculoskeletal disorders, arthritis, and dysmenorrhea (Warner *et al*., 1999). Scientifically diclofenac is known as 2-(2-(2,6-dichlorophenylamino)phenyl) acetic acid and commercially as Voltarol® and Voltaren® with a molecular formula of $C_{14}H_{11}Cl_2NO_2$ (Figure 6.4) and a molecular weight of 296.14 g mol^{-1} (Hadgraft *et al*., 2000).

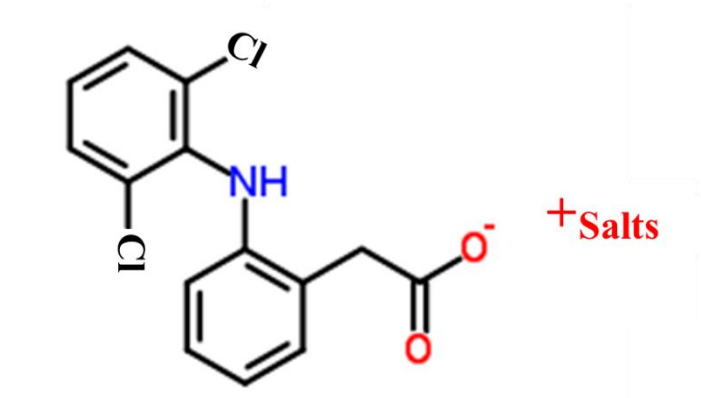

Figure 6.4 Structural formula of diclofenac (adapted from Altman et al., 2015).

Diclofenac has been available in oral formulations since 1983 and two years later topical formulations were introduced (Kienzler *et al*., 2010). Diclofenac is a white to off-white crystalline powder, with a melting point of approximately 157 °C. It is practically insoluble in water (12 μg mL^{-1}), but it is readily soluble in organic solvents and aqueous alkaline solutions and has a pK_a of 4.18 (Hadgraft *et al*., 2000).

The salt forms of the drug have higher aqueous solubility than the free acid. Therefore, the salt forms often are preferable in commercial formulations. Topical formulations of diclofenac are available in several different salt forms. These include diclofenac sodium 1 % gel, diclofenac diethylamine gel 1.16 %, diclofenac spray 4 % gel, diclofenac DMSO lotion, and diclofenac epolamine patch (Nair and Taylor-Gjevre, 2010; Fini *et al*., 2012). Similar to other NSAID drugs, diclofenac inhibits prostaglandin production, which plays a significant role in the causation of inflammation, pain and fever. However, diclofenac inhibits cyclooxygenase (COX-2) enzyme with greater potency than it does (COX-1) (Altman *et al*.,2015).

In this study, we have used diclofenac sodium as the model drug formulated in gellan gum fluid gel formulations for topical application.

6.5 Materials and Methods

6.5.1 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 donated from a local abattoir. Diclofenac sodium gel (Voltaren® gel 1 %) was bought from a local pharmacy.

6.5.2 Methods

6.5.2.1 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 % of diclofenac sodium. Sodium chloride (0.5 % 1 % and 2 % w/w) was added to the hot diclofenac-loaded gellan solutions at ~ 85 °C, as crosslinking cations. 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.

6.5.2.2 Control Formulations

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

6.5.2.3 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.

6.5.2.4 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).

6.5.2.5 Yield 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.

6.5.2.6 Frequency Sweep Measurement

The rheological behaviour 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}) to produce mechanical spectra of the samples. Measurements were taken at 20 °C and performed at 0.5 % strain (strain amplitude chosen was within the linear viscoelastic region of the sample).

6.5.2.7 Tribology of Fluid Gels

The frictional properties of the produced gellan fluid gels were measured using a mini traction machine MTM2. 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, a temperature of 32 °C where used for all experiments.

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

$$\mu = F/W \qquad \text{Eq. 6.1}$$

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.

6.5.2.8 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.

6.5.2.9 Preparation of Skin Membranes

Whole porcine ears, obtained from local abattoir, were used for the permeation studies. The pinna (ear flap) was removed carefully and separated from cartilage. 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 before it was used (the tissue section was discarded if residual surface debris was evident).

6.5.2.10 Ex vivo Permeation Study

The permeability of diclofenac through pig skin was determined *in vitro* using a Franz diffusion cell system (Figure 6.6). 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 receiver solution temperature was maintained at 32 °C and stirred by an externally driven, Teflon-coated magnetic bar. Phosphate buffer saline pH 7.4 was used in the receiver compartment. The tissue prepared as described in section 6.5.2.9 was defrosted and soaked in PBS for 30 min before being mounted on the Franz cell (epidermis on the top face). The system was left for another 30 min to equilibrate then 0.5 mL of samples were added on top of the skin. 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 addition of PBS to the receiver compartment was performed with great care to avoid trapping air beneath the dermis. The samples were then chromatographically analysed for diclofenac using HPLC.

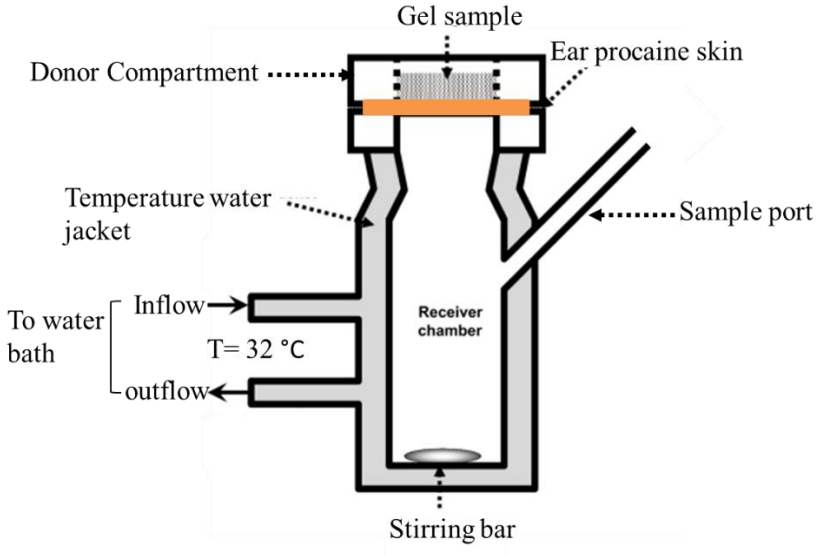

Figure 6.5 Illustration of a Franz diffusion cell.

6.5.2.11 Diclofenac Assay

Chromatographic separation was performed on a Shimadzu System (See section 5.5.2.7). The mobile phase composition was acetonitrile/water (60:40) (v/v). HPLC conditions for the diclofenac sodium assay are presented in Table 6.1.

Stock solutions of diclofenac sodium were prepared by dissolving 100 mg in 100 mL of the mobile phase. Standard solutions were prepared at 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) against concentration. Precision and linearity over the concentration range were assessed. Precision was calculated from the relative standard deviation (% RSD < 1) of the standard curve and linearity was assessed by the linear regression with $r^2 = 0.999$. All compounds were identified by comparison of retention times obtained from the sample and standard solutions. LOD and LOQ were calculated as in section 4.4.2.6. HPLC method validation for the diclofenac assay is presented in Table 6.2.

A sample chromatogram of diclofenac is shown in Figure 6.6, and a typical calibration curve constructed from peak area against concentration is shown in Figure 6.7. All chemicals used in this assay were of analytical grade; solvents were HPLC grade and were used as received without further treatment. HPLC grade water was utilized in the preparation of the mobile phases. The mobile phase was filtered and degassed by sonication using an ultrasonic bath (Fisher Scientific). All samples were analysed immediately.

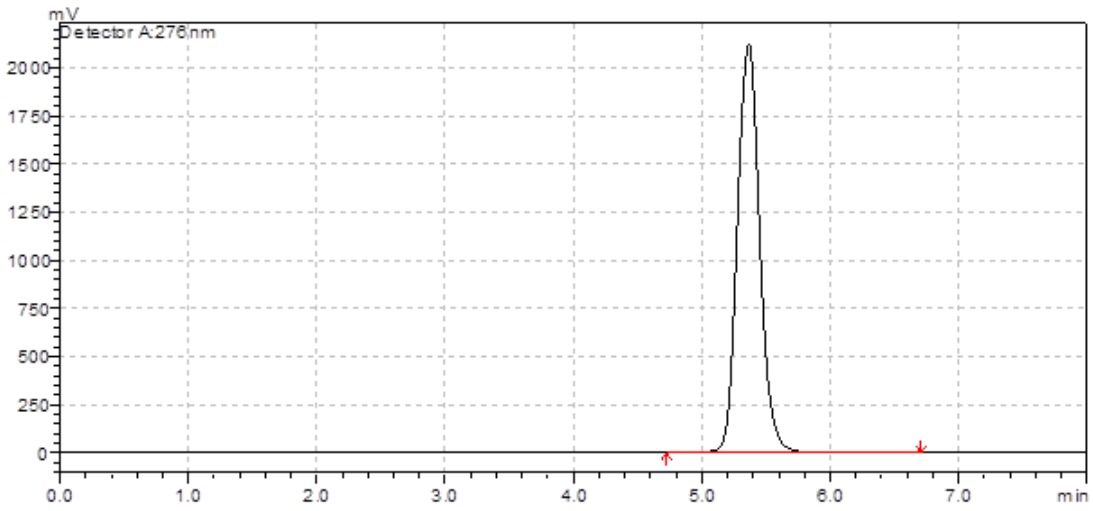

Figure 6.6 Typical chromatogram of diclofenac detected at 276 nm.

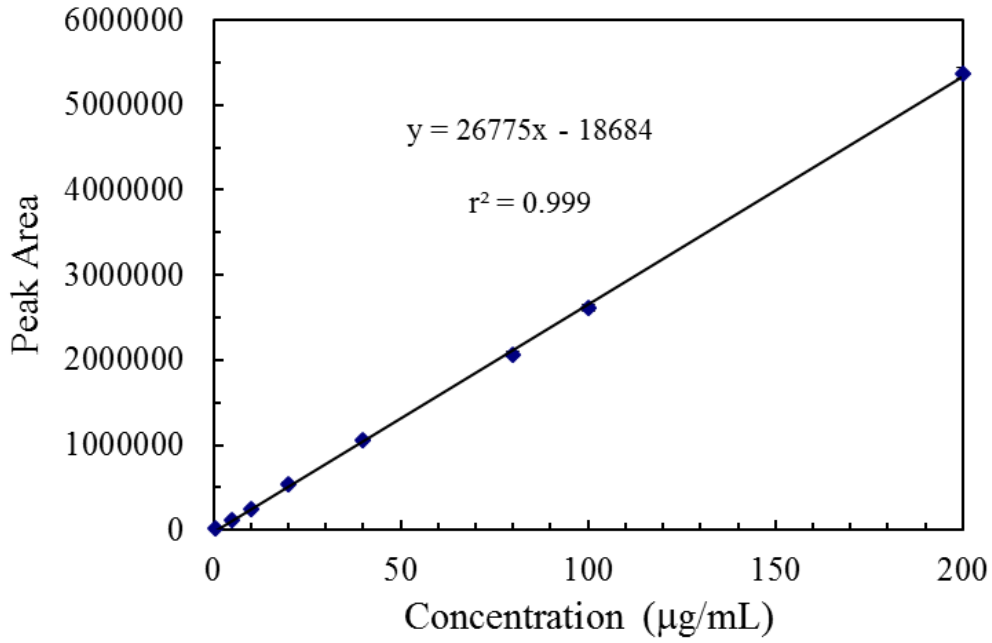

Figure 6.7 Mean calibration curve for diclofenac measured at λ 276 nm. Values represent mean ± SD (n=3).

Tabel 6.1 HPLC conditions for diclofenac assay.

Wavelength	276 nm
Injection volume	10 μm
Flow rate	0.75 mL.min^{-1}
Run time	8 min

Tabel 6.2 HPLC method validation for diclofenac assay.

Peak tailing factor $\{\boldsymbol{Tf = \frac{a+b}{2a}}\}$	1.3
Retention time	5.6 min
Limit of quantification $\{\boldsymbol{LOQ = 10\frac{\sigma}{S}}\}$	0.045 μg/mL
Limit of detection $\{\boldsymbol{LOD = 3.3\frac{\sigma}{S}}\}$	2.3 μg/mL
Precision and accuracy	RSD < 1 %

6.5.2.12 Calculation of Flux Values

To determine the flux values, the mass of substance that crossed the membrane per unit area was plotted against time. The flux can then be calculated from the slope of the linear section of the curve.

6.5.2.13 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.

6.6 Results

6.6.1 Rheological Characterisation of Gellan gum Fluid Gels

To evaluate the particle-particle interaction of gellan gum fluid gels, the stress required by the formulation to yield was measured and are shown in Figure 6.8. Voltaren® gel was used as a reference throughout all experiments. All gellan samples reported in Figure 6.8A were composed of 1 % w/w of gellan and crosslinked with 0.5 % NaCl. The samples had similar elasticity, however, the stress required to yield was 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 6.8A). 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 6.8 B), and with decreasing total polymer concentration (Figure 6.8 C).

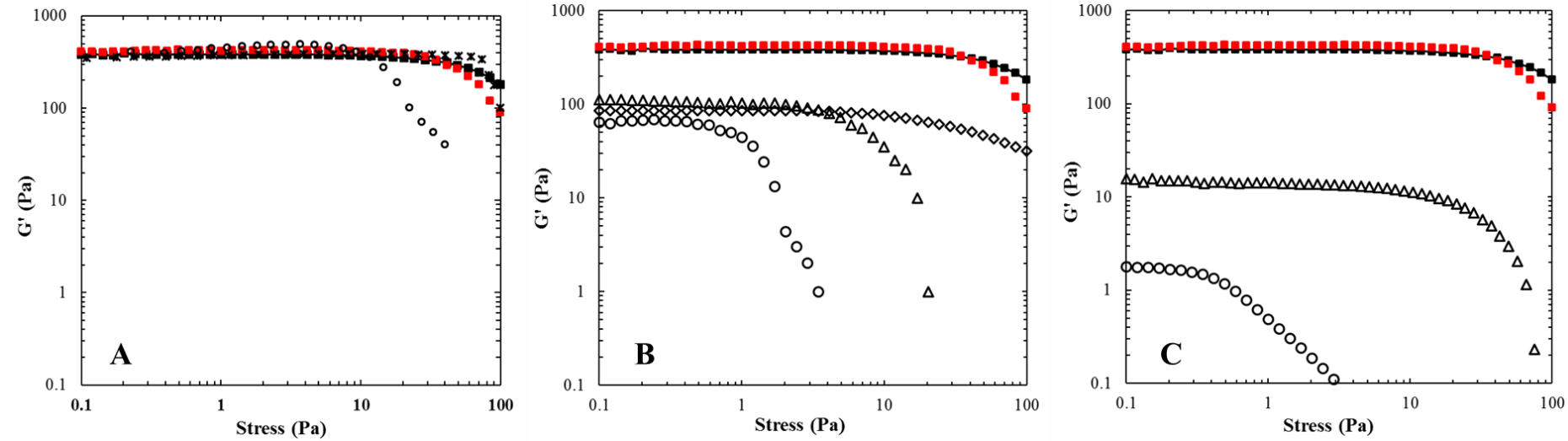

Figure 6.8 (A) Stress sweep for 1 % gellan fluid gels crosslinked with 0.5 % NaCl as function of HA LA ratio (LA gellan open circles, HA gellan black stars and 50:50 LA HA gellan blend filled red diamonds) (B) stress sweep for 1 % w/w 50:50 LA HA gellan blend fluid gels crosslinked with 0% (open diamonds), 0.5 % (filled red diamonds), 1 % (open triangles) and 2 % (open circles). (C) Stress sweep for 0.1 % (open circle), 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.

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 6.8). 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. Viscosity profiles of both 1 % w/w 50:50 LA HA gellan blend fluid gel and Voltaren® gel had shear thinning viscosity profiles (Figure 6.9).

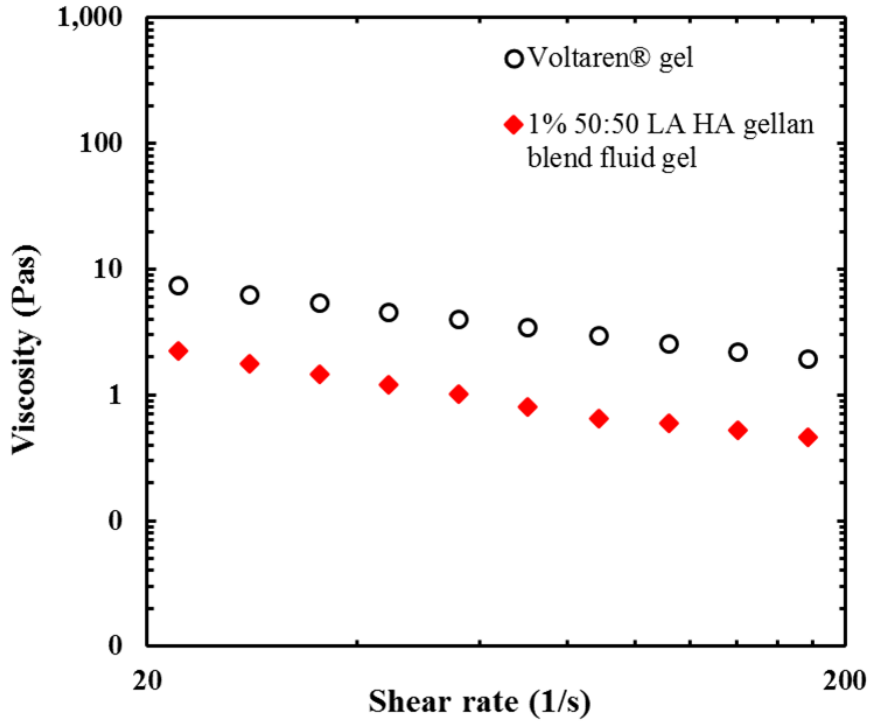

Figure 6.9 Viscosity vs. shear rate at 32 °C for 1 % w/w (filled-red diamonds) LA HA gellan blend fluid gels at 0.5 % w/w NaCl and for Voltaren® gel 1 % diclofenac sodium (open circles).

Dynamic small deformation oscillatory measurements of G′ and G″ (Figure 6.10) 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. Moreover, G′ value obtained by gellan blend fluid gel was slightly higher.

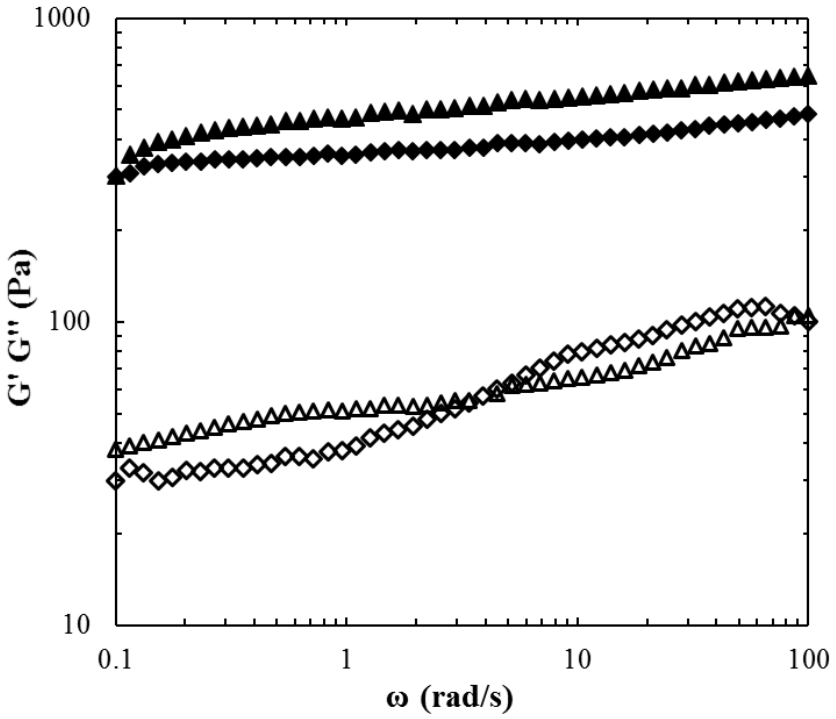

Figure 6.10 Mechanical spectrum (0.5 % strain; 32 °C) of a 1 % 50:50 LA HA gellan blend loaded with 1 % diclofenac sodium showing variation of G′ (filled triangles), G″ (open triangles) and of Voltaren® gel 1 % diclofenac sodium G′ (filled diamonds), G″ (open diamonds).

6.6.2 Lubrication Properties of Gellan Gum Fluid Gels

Characterisation of the lubrication behaviour of both the gellan fluid gels and the marketed Voltaren® gel were been performed by the means of 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 6.11).

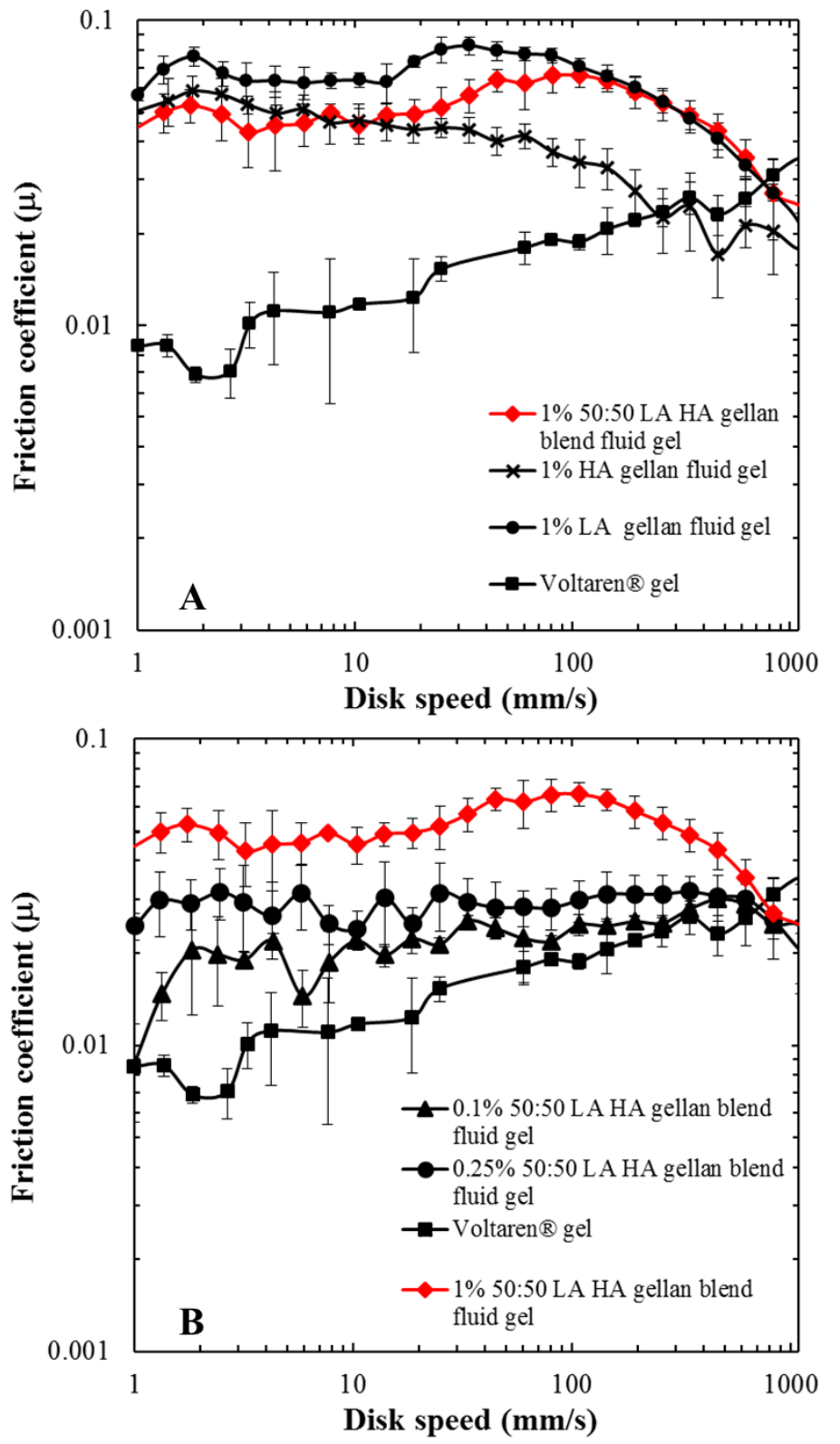

Figure 6.11 (A) Stribeck curves for 1% gellan fluid gels crosslinked with 0.5 % NaCl as function of LA:HA ratio (LA filled circles, HA black stars and 50:50 LA HA blend filled red diamonds), (B) Stribeck curves for 0.1 % (filled triangles), 0.25 % (filled circles) and 1 % (filled red diamonds) w/w 50:50 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.

The LA gellan gum fluid gel showed a slight increase in the friction and then a decrease in friction followed by a plateau in boundary regime until it reached critical speed in the mixed regime after which the friction coefficient began to increase again. 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 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 speed (Figure 6.11A).

The Stribeck curve of the Voltaren® gel showed that friction was steady at the beginning then slightly decreased 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 6.11A). The μ of fluids gel samples decreased on decreasing gellan concentrations as shown in Figure 6.11B.

6.6.3 Effect of Gellan Gum on Release and Penetration of Diclofenac

The transdermal 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 6.12 while penetration fluxes are summarized in Table 6.3.

Table 6.3 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 (μg.h/cm^2)**
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	210.24
Voltaren® gel	116.99

The cumulative amount of diclofenac sodium found 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 (control), which was used as a control (Figure 6.12). The flux values indicate that the penetration 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 flux value 210.24 μg cm^{-2} h^{-1} and 116.99 μg cm^{-2} h^{-1} respectively (Table 6.3).

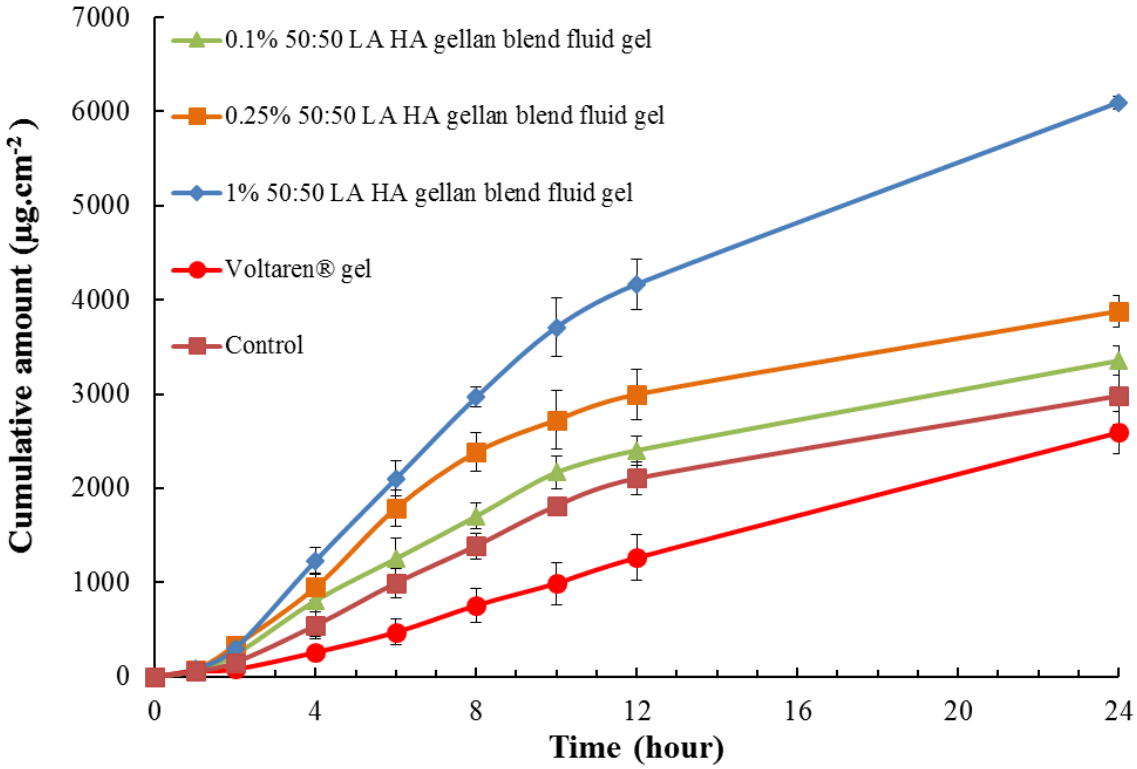

Figure 6.12 Cumulative amount µg.cm^{-2} of diclofenac sodium permeated from 50:50 LA HA gellan blend fluid gel formulations prepared at different concentrations compared with Voltaren® gel. Values are represented as mean ± SD.

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, or whether it was is related to enhanced degree ionization of the drug, experiments on drug release and permeation were performed. Figure 6.13 shows the *in vitro* diclofenac release and *ex-vivo* diclofenac permeation study for 1 % 50:50 LA HA gellan blend fluid gel and the Voltaren® gel formulation. The results show that there was no significant difference ($p > 0.05$) in release between fluid gel and Voltaren® gel and the drug reached 100 % release after 8 hours form both formulations.

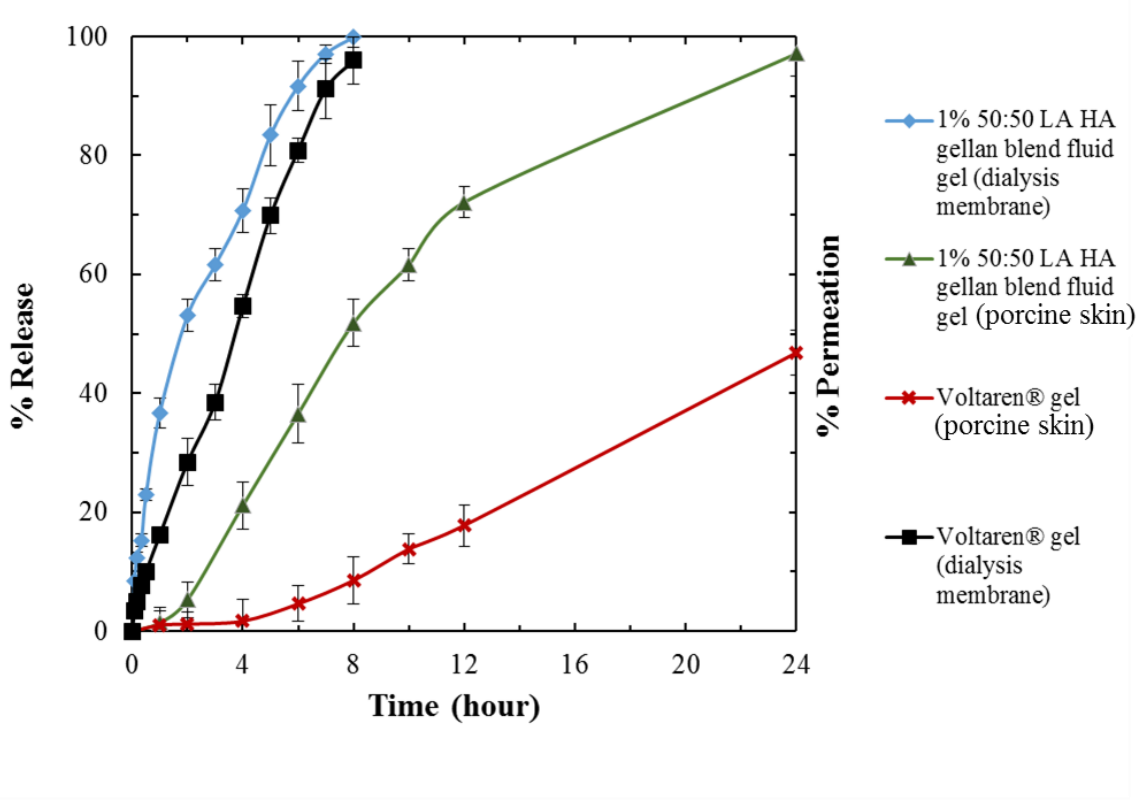

Figure 6.13 Cumulative % release of diclofenac from 1 % w/w 50:50 LA HA gellan blend fluid gel (blue diamonds) and from Voltaren® gel (black squares). Cumulative % permeation of diclofenac from 1 % w/w 50:50 LA HA gellan blend fluid gel (green triangles) and from Voltaren® gel (red crosses) (n=3).

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 6.14). 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 released from the gellan formulation containing 2 % NaCl was almost similar to that of the Voltaren® gel (Figure 6.14).

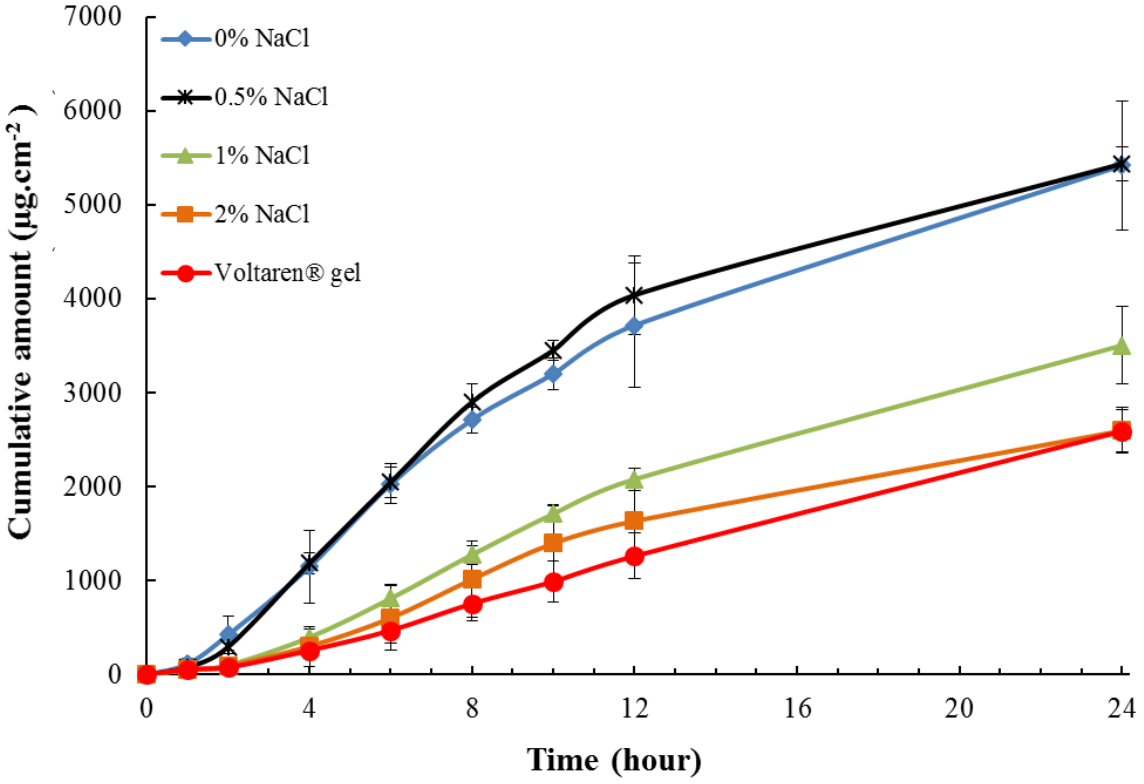

Figure 6.14 Cumulative amount µg.cm^{-2} of diclofenac sodium permeated from 1 % w/w 50:50 LA HA gellan fluid gels prepared at different NaCl concentrations compared with Voltaren® gel. Values are represented as mean ± SD (n=3).

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 6.15A) compared with Voltaren® gel (Figure 6.15B). 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 6.15A 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 penetrated into the receiver (Figure 6.15A). 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 plataued until the end of the test (Figure 6.15B).

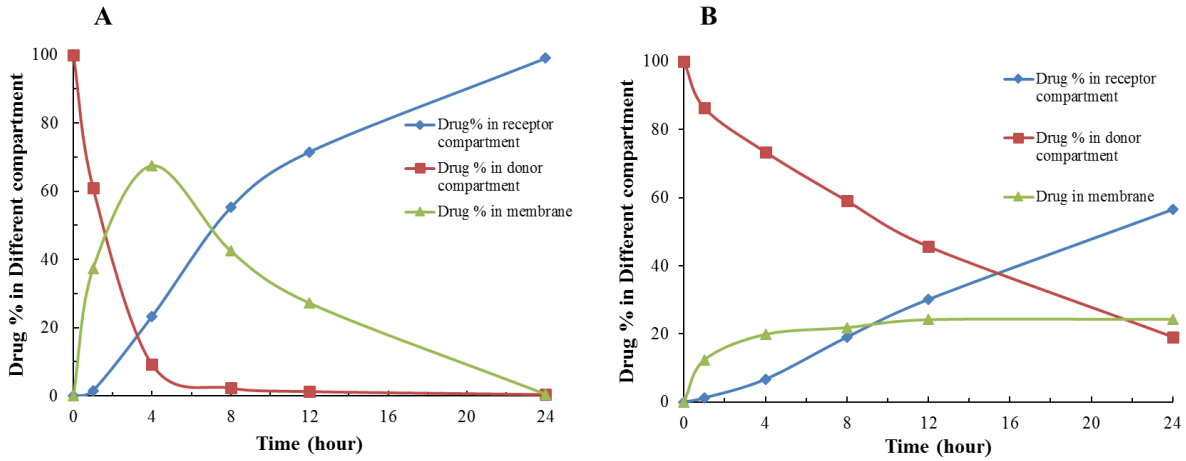

Figure 6.15 Drug % profile in different compartments of the Franz cell, donor (red line), membrane (green line) and receiver (blue line) for A) 1 % gellan 50:50 LA HA gellan blend fluid gel and B) Voltaren® gel.

6.7 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 the previous chapters, the potential use of gellan gum fluid gels in pharmaceutical formulations was presented, these have included an oral liquid formulation (Chapter 4) and a nasal spray (Chapter 5). Here, the investigation focused on the potential of use 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 the strength 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 6.8A) (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. 6.8A) as previously reported with 0.25 % gellan fluid gel formulations in Chapter 5. 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 6.8A). 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 on the gel strength and particle-particle interaction (Figure 6.8B).

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 a maximum value is reached. At higher ion concentrations excessive aggregation can occur, leading to collapse of gel structure and ultimately, precipitation of the polymer (Morris *et al*., 2012). This phenomenon was apparent in the results shown in (Figure 6.8B) 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 less than 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 ion concentration for maximum gel strength was exceeded, causing a reduction in gel strength.

The viscoelastic properties (e.g. elastic modulus) and particle size (as shown in Chapter 4 Figure 4.8) of fluid gel can be changed by changing polymer concentrations (Norton *et al*., 1999). The results in Figure 6.8C are in good agreement with the previously reported data as it shows 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 6.8C).

To evaluate the spreadability of a topical gel preparations, rheological testing can provide a useful prediction (Garg *et al*., 2002). Viscosity measurements at different shear rates from 20 to 200 s^{-1} have previously been shown to be in 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 adventageous spreadability of the gellan formulation (Figure. 6.9).

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 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 6.16A-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 6.16B-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 6.16D) (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 6.16E) (Gabriele *et al*., 2010).

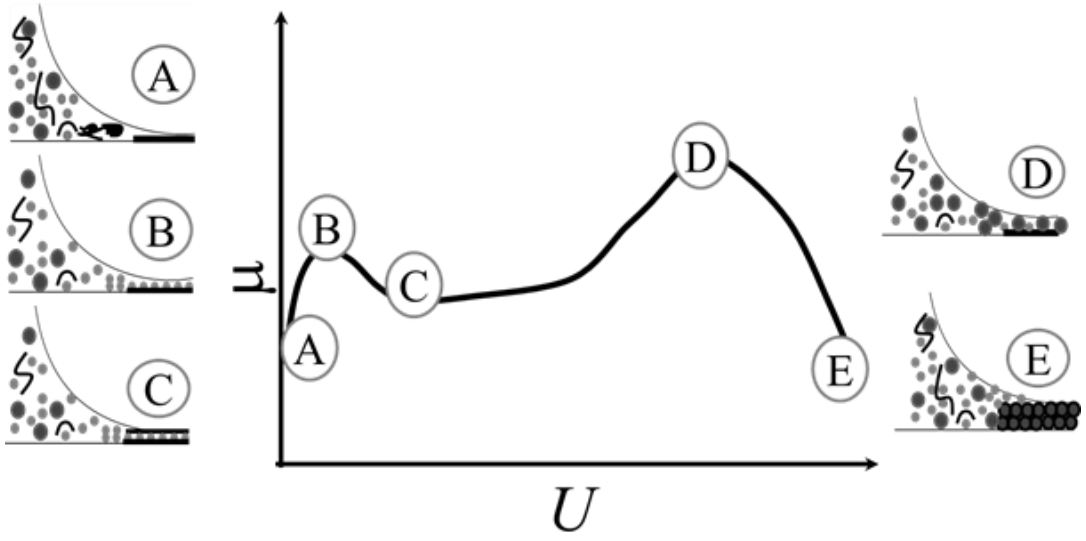

Figure 6.16 Schematic representation of the proposed mechanism of gellan fluid gel lubrication. (A-B) represents the boundary regime. (C-D) represents the mixed regime and (E) represents the hydrodynamic regime.

The Stribeck curve of HA gellan fluid gel, however suggests a different mechanism. There is no peak observed at mixed-hydrodynamic regime (Figure 6.11A), 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 6.17). 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 6.11A). 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. 6.11B), whereby the fluid gel particles are responsible for the friction at all rotation speeds.

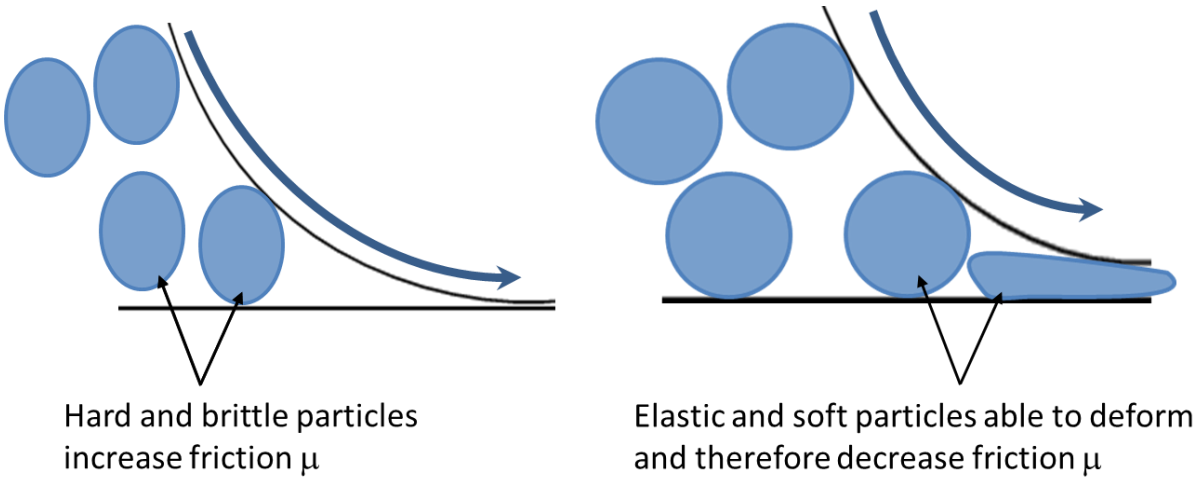

Figure 6.17 Illustrates the two different behaviours of gellan particles A) hard and brittle (LA gellan particles) and B) Soft and elastic particles (50:50 LA HA gellan and HA gellan particles).

The permeation study and flux values (Figure 6.12; Table 6.3) highlighted that the amount of the diclofenac penetrated through the porcine skin 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 differences in drug permeation measured from gellan gum fluid gel formulations prepared with different concentrations of gellan (Figure 6.12).

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 through the skin (Goh and Lane, 2014).

Figure 6.13 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, confirming that the different penetration obtained is due to different drug ionization and not because of electrostatic repulsion force between drug and anionic gellan polymer. This is further supported by the data presented in Figure 6.14. The addition of sodium ions to the system causes an increase in the amount of sodium available to the gellan and the dissociation of Na-diclofenac will be inhibited. This therefore, reduces the ionized/unionized ratio and as a consequence, reduces the tendency for the drug to penetrate through the skin.

The results obtained (Figure 6.15A) indicated that the diclofenac released from 50:50 LA HA gellan blend formulation has higher deposition within the skin compared with the one released from Voltaren® gel (Figure 6.15B). In fact, after 4 hours the entire diclofenac 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 was likely that the deposition in this layer determined the rate of permeation of diclofenac. Thus, the drug release from skin to the receiver then occurred in a controlled manner. This finding can promote the use 50:50 LA HA gellan fluid gel as a novel platform for such topical formulations.

6.8 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 commercial formulation (Voltaren® gel). The stress required for the gel to flow

depends on LA:HA ratio, overall concentration of both polysaccharides, and ion concentration. This work is also believed to be the first to report the tribological properties of gellan gum fluid gels. It was shown that gel particles have an effect on lubrication properties of the formulations and therefore may improve tactile perception. Furthermore, penetration of diclofenac across the skin barrier depends on the vehicle-drug interactions and can be controlled by simple addition of counter ions.

Chapter 7

General Conclusion and Future Recommendations

CHAPTER 7 GENERAL CONCLUSIONS AND FUTURE Recommendations

The purpose of this research was to investigate a potential new drug delivery platform using bio-responsive fluid gel technology. Different gellan gum fluid gel preparations were produced that had various functional behaviours that were applied to develop pharmaceutical formulations. In particular, the gellan gum fluid gels were investigated for potential use as three different bioresponsive dosage forms (modified release oral liquid formulation, mucoadhesive nasal gel spray and a rapid permeation topical gel formulation). The following sections summarise the main conclusions made for each of the experimental results chapters.

Fluid gels were produced by applying a shear force during gelation process (on cooling). Modifying the process parameters such as cooling rate and shear rate during fluid gel production were shown to effect gel particle size and therefore had an impact on the rheological behaviour. Gellan fluid gel particle size can also be controlled by altering both the gellan and/or crosslinking ion concentrations. This enables the microstructure and bulk rheological properties of the fluid gels to be tuned towards particular applications.

- **Effect of Acid Sensitive LA Gellan Fluid Gels on the Release Behaviour of Ibuprofen in Oral Liquid**

Chapter 4 highlighted the potential application of LA gellan gum fluid gels as a modified release oral liquid. Rheological properties of the fluid gels were optimised to behave similar to that of a marketed paediatric suspension but with a higher viscosity at low shear rates, that rapidly shear thins on shaking, to allow easy pouring on to a dispensing spoon. The release of ibuprofen from LA gellan gum fluid gels was also modified by altering LA gellan concentrations. This was shown by the variation in the ibuprofen release in PBS pH 7.4 at

different LA gellan concentrations. Also, it was found that variation in gastric pH exposure time, and pH value, had an impact on the release. Lower pH values and longer gastric pH exposure times, resulted in a greater delay in the release of ibuprofen in PBS pH 7.4.

There is further work that could be done to further develop this formulation that includes optimisation of the particle size and studying the effect these different particle sizes on the drug release behaviour. Furthermore, an investigation on the release of drug in different PBS pH values to represent the other parts of GI tract would also provide important data on the potential variations in release profiles. Ultimately, magnetic resonance imaging (MRI) or gamma scintigraphy in an animal model would be useful technique to have a genuine insight to the behaviour of the fluid gel once ingested.

- **Performance of LA, HA and LA HA blend Gellan Fluid Gels as Mucoadhesive Nasal Spray Formulations**

Chapter 5 highlighted the potential of gellan fluid gels as a sprayable mucoadhesive nasal spray. HA gellan was shown to be much more mucoadhesive to procaine mucosal tissue than LA gellan. The increased viscosity of the HA gellan however, prevented the formulation being sprayed. To overcome this fluid gels were produced from a blend of LA gellan with HA gellan which reduced the viscosity sufficiently to allow the formulation to be sprayed without dramatically reducing the mucoadhesive functionality. This work elegantly illustrated the tuneability of the physical properties gellan gum fluid gels by simply blending LA and HA gellan. Future studies on these blended formulations could be applied to other anatomical regions that are traditionally difficult to target such as the throat or the oesophagus.

- **Effect of LA, HA and LA HA Blend Gellan Fluid Gels on Diclofenac Penetration and Tribological Behaviour in Topical Formulations**

Chapter 6 focussed on diclofenac topical formulations that were prepared using LA gellan HA gellan and a 50:50 LA HA blend fluid gel. These formulations had an excellent match in rheological behaviour with that of the commercially marketed Voltaren® gel. The gelled particles in gellan fluid gels however, increased the friction compared with commercial Voltaren® gel when measured using soft tribology. Particle characteristics (such as size and elasticity) had a direct effect on lubrication behaviour of gellan fluid gels. Interestingly, the increasing gellan concentration in the formulations enhanced diclofenac penetration through skin which was attributed to an increase in dissociation of the sodium counter ion from the diclofenac.

For future work it would be interesting to investigate other anionic gelling polysaccharides having the same negative group of gellan (carboxylic group) such as alginate and pectin or even other anionic gelling polysaccharides with a different negatively charged group such as carrageenan.

Overall this work has demonstrated the wide applicability of gellan fluid gels as a platform technology for modified release dosage forms. The ease of manufacture and ability to tailor both physical properties and bioresponsive behaviour will no doubt prompt further research on gellan gum fluid gels for pharmaceutical applications.

Chapter 8

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CHAPTER 8 REFERENCES

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