International Journal of Pharmaceutics 411 (2011) 69–77

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Comparison of ion-activated *in situ* gelling systems for ocular drug delivery. Part 1: Physicochemical characterisation and *in vitro* release

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ARTICLE INFO

Article history:
Received 16 December 2010
Received in revised form 4 March 2011
Accepted 21 March 2011
Available online 29 March 2011

Keywords:
In situ gelling systems
Ocular drug delivery
Anionic polysaccharides
Gelling behaviour
Contact angle
In vitro release

ABSTRACT

Conventional eye drops can result in poor drug bioavailability due to the unique ocular anatomy and physiology. Ion-activated *in situ* gelling systems are able to crosslink with cations present in the tear fluid, therefore forming a gel on the ocular surface, which results in prolonged corneal contact time. The present study compared a number of anionic polysaccharides (gellan gum, xanthan gum, carrageenan and alginate) to an uncharged (HPMC) and a positively charged (chitosan) polymer system with emphasis on the gelling behaviour, rheological and textural properties, gel microstructure, contact angle and *in vitro* release characteristics. All systems exhibited physically entangled polymer networks that were able to disentangle upon shear stress and significantly prolonged the *in vitro* release of a model hydrophilic drug compared to a solution. While systems based on HPMC and chitosan showed no structural changes upon addition of cations, formulations based on gellan gum and carrageenan demonstrated a remarkable increase in viscosity, pseudoplasticity and hardness upon addition of Ca^{2+} and K^{+} respectively. This renders them favourable for ocular use as they would gel once in contact with the cations of the tear fluid, thus reducing nasolacrimal drainage, but would thin upon shearing, preventing ocular irritation and therefore induced lacrimation.

1. Introduction

Ophthalmic drug delivery is one of the most interesting but also one of the most challenging areas facing the pharmaceutical scientist. The unique anatomy and physiology of the eye make it difficult to achieve an effective drug concentration at the target site. The challenge remains to circumvent the protective barriers of the eye without causing significant tissue damage or increasing the risk of systemic side effects. Various delivery systems have been investigated during the past decades, pursuing two main strategies: to increase the corneal permeability and to prolong the contact time on the ocular surface (Jarvinen et al., 1995). While the former is mainly achieved by the use of prodrugs (Jarvinen and Jarvinen, 1996; Lee and Li, 1989), penetration enhancers (Kaur and Smitha, 2002; Saettone et al., 1996) and colloidal delivery systems such as nanoparticles (Diebold and Calonge, 2010; Nagarwal et al., 2009; Zimmer and Kreuter, 1995), liposomes (Meisner and Mezei, 1995) and niosomes (Kaur et al., 2004), the latter relies on rheological and mucoadhesive properties of the ophthalmic formulations.

Polymeric eye formulations can be subdivided into three groups: viscosity enhancing polymers, which simply increase the formulation's viscosity, resulting in decreased lacrimal drainage and enhanced bioavailability, mucoadhesive polymers, which interact with the ocular mucin, therefore increasing the contact time with the ocular tissues, and *in situ* gelling polymers, which undergo sol-to-gel phase transition upon exposure to the physiological conditions present in the eye. The latter are highly advantageous over preformed gels, which do not allow for accurate and reproducible administration of a drug and, after administration, often produce blurred vision, crusting of the eyelids and lacrimation. *In situ* gelling systems, on the other hand, can be easily and accurately instilled in liquid form, and are capable of prolonging the formulation's residence time on the surface of the eye due to gelling (Krauland et al., 2003).

Keeping the physiology of the ocular surface in mind, three parameters (temperature, pH and ionic strength) can generally be exploited. A number of studies have already been performed on thermosetting gels based on Pluronic® (Bromberg et al., 2006; El-Kamel, 2002; Qi et al., 2007; Wei et al., 2002) and pH sensitive formulations of chitosan and carbopol (Gupta et al., 2010; Sultana et al., 2006b), but besides a few carbomer-based artificial tear prod-

Abbreviations: HPMC, hydroxypropyl methylcellulose; KCl, potassium chloride; $CaCl_2$, calcium chloride; PHCl, pilocarpine hydrochloride; SLF, simulated lacrimal fluid.

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doi:10.1016/j.ijpharm.2011.03.042

ucts none of these formulations has made it onto the market. The majority of studies have been conducted on ion-activated *in situ* gelling systems which are able to crosslink with the cations present in the tear fluid, forming a gel on the ocular surface. They can be formulated at the optimal pH for ocular delivery using buffers, can be easily and accurately instilled at room temperature and may therefore be less irritating to the ocular tissues than *in situ* gelling systems depending on a change in pH or temperature. Gellan gum (Gelrite®) based systems have been evaluated for many drugs such as timolol maleate (Rozier et al., 1989; Shedden et al., 2001), ciprofloxacin hydrochloride (Balasubramaniam and Pandit, 2003), indomethacin (Balasubramaniam et al., 2003), pefloxacin mesylate (Sultana et al., 2006a) and gatifloxacin (Kalam et al., 2008) and have shown prolonged corneal residence time and increased ocular bioavailability of the drug. However, only a limited number of studies have been performed on xanthan gum and carrageenan systems (Albasini and Ludwig, 1995; Ceulemans et al., 2002; Verschueren et al., 1996) with a substantial lack of studies comparing the various *in situ* gelling polymer formulations. The purpose of this study was to evaluate a variety of ion-activated anionic polysaccharides in terms of their gelling behaviour, rheological and textural properties, gel microstructure, contact angle and *in vitro* release characteristics and to compare them to an uncharged as well as a positively charged polymer system.

2. Materials and methods

2.1. Materials

Gellan gum (Kelcogel F) and xanthan gum (Keltrol F) were a generous gift from CP Kelco (Atlanta, GA, USA). Carrageenan (Genulacta L-100, κ-carrageenan) was a free sample from CP Kelco (Lille Skensved, Denmark). Sodium alginate (medium viscosity) was obtained from Acros Organics (Morris Plains, NJ, USA) and hydroxypropyl methylcellulose (HPMC, Methocel K4M) was purchased from Colorcon (Gao, India). Chitosan (Protasan UP CL 213) was obtained from Novamatrix (Sanvika, Norway). Salt components for construction of ternary phase diagrams and preparation of simulated lacrimal fluid (calcium chloride, magnesium chloride, sodium chloride, sodium bicarbonate and potassium chloride) were of analytical grade and were purchased from Scharlau Chemie (Barcelona, Spain). Pilocarpine hydrochloride was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Servapor dialysis tubing (regenerated cellulose, MWCO 12,000–14,000 Dalton, pore diameter 25 Å) was obtained from Serva Electrophoresis GmbH (Heidelberg, Germany). Water used for preparation of the formulations was ion exchanged, distilled and passed through a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Effect of cations on phase behaviour – construction of ternary phase diagrams

Two partial ternary phase diagrams (≤2% (w/v) of polymer and cation) were constructed for each polymer in order to investigate the effect of monovalent (K^+) and divalent (Ca^{2+}) cations on the sol-to-gel transition. Polymers were dissolved in water of 90 °C, then, while cooling down, the appropriate amount of a 1% (w/v) KCl or $CaCl_2$ solution was added and left overnight to equilibrate. Formulations were assessed the following day in terms of their visual appearance and flow (by tilting the vial to an angle of 90°), and were classified as solutions, viscous solutions or gels.

2.3. Effect of cations on rheological and textural properties

Viscosities of 0.5% (w/v) polymer formulations with cation (K^+, Ca^{2+}) concentrations ranging from 0% to 0.2% (w/v) were determined at a temperature of 32 ± 1 °C and shear rates ranging from 75 to 750 s^{-1} using a rotational Brookfield DVIII + cone and plate viscometer (Brookfield Engineering Laboratories Inc., Middleboro, MA, USA) fitted with a CP-40 spindle (48 mm diameter, 0.8° angle) and operated by Rheocalc software. A sample volume of 0.5 ml was used and measurements were performed in triplicate. Flow indices (N) were calculated according to the following equation (with F being the shear stress, G the shear rate and η' the apparent viscosity) and were plotted against the cation concentration.

$$\log \quad G = N \times \log \quad F - \log \quad \eta'$$

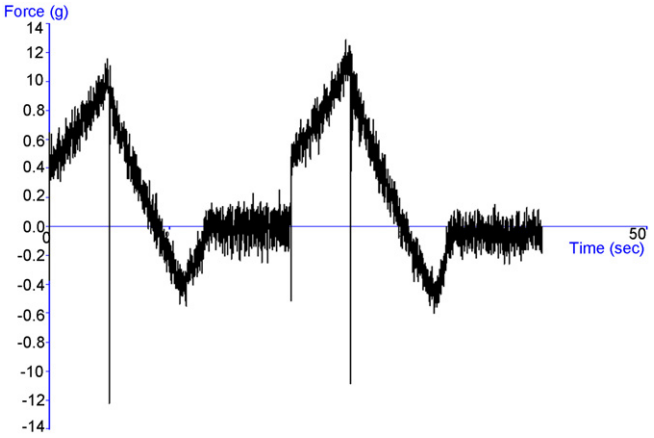

Fig. 1. Representative texture analysis profile (hardness: height of 1st peak; adhesiveness: AUC of 1st negative peak; cohesiveness: AUC of 2nd peak/AUC of 1st peak).

Changes in mechanical properties (hardness, adhesiveness and cohesiveness) of 0.5% (w/v) polymer formulations due to increasing cation (K^+, Ca^{2+}) concentrations (0–0.2% (w/v)) were measured using a TA.XT plus texture analyser (Stable Micro Systems, Godalming, England) with a load cell capacity of 5 kg. A 10 mm probe was compressed twice into each sample to a depth of 5 mm at a rate of 1 mm s^{-1} allowing a relaxation period of 30 s between compression cycles. The trigger force was set to 0.1 g. Measurements were performed in triplicate and hardness, adhesiveness and cohesiveness were determined as shown and explained in Fig. 1.

2.4. Rheology and polymer network microstructure of plain formulations

Oscillatory measurements were performed on 0.5% (w/v) polymer formulations without cations at 32 ± 0.1 °C using a Haake RS 150 Rheostress viscometer (Thermo Fisher Scientific, Karlsruhe, Germany) fitted with a cone (60 mm diameter, 0.5° angle) and plate setup. Dynamic stress sweep measurements were performed at a constant frequency of 1 $rads^{-1}$ and a range of stress amplitudes (0.001–1 Pa). A sample volume of 0.5 ml was used and measurements were performed in triplicate. The influence of shear stress on the storage (G') and loss (G'') moduli was determined and the loss tangent ($\tan\delta$) was calculated.

In addition, the gel network microstructure was visualised using SEM. Briefly, formulations were loaded into rivets and plunge frozen in liquid propane at −190 °C. Samples were transferred into the cryo chamber (Gatan, Alto 2500, UK) of the microscope (JEOL, JSM-6700F, Japan) held at a temperature of −140 °C. Samples were sublimed at −90 °C for approximately 5 min and then coated with platinum for 2 min. Samples were viewed under the microscope at −140 °C, with an accelerating voltage of 3 kV and a working distance of 6 mm.

2.5. Contact angle

Contact angle measurements were performed on a Krüss DSA 100 Drop Shape Analyzer (Krüss GmbH, Hamburg, Germany) at 32 ± 1 °C and 80% humidity. A volume of 8 μl of each formulation was dropped onto a glass slide at a rate of 60 μl min^{-1}. Five images per second were taken over a period of 20 s and images were analysed using the circle fit method. Contact angles after 10 s and the change in contact angle over the first 10 s were calculated and compared to that of an aqueous solution. All measurements were performed in triplicate.

2.6. In vitro release

Standard Franz diffusion cells with a 15-mm-diameter orifice (providing a diffusion area of 1.77 cm^2), thermostated by means of a water jacket connected to a VTC-220 heat circulator, were mounted to a FDC-6 transdermal diffusion cell drive console (Logan Instrument Corp., Somerset, NJ, USA). Receptor chambers (12 ml volume) were filled with simulated lacrimal fluid (SLF) (Ceulemans and Ludwig, 2002) and were constantly stirred at 600 rpm by small magnetic bars. Donor and receptor chambers were separated by a dialysis membrane, soaked in receptor medium overnight prior to the experiment. The thermostat was set to keep the membrane surface at 32 ± 1 °C in order to mimic *in vivo* conditions. A volume of 2 ml of each formulation containing 1% (w/w) pilocarpine hydrochloride was loaded into the donor compartment before occluding the chamber with parafilm. Samples of 10 ml were taken at predetermined time points and replaced with fresh receptor medium to maintain sink conditions. Triplicates were performed for each formulation and freshly collected samples were analysed spectrophotometrically at 215 nm (UV-Vis Libra S32PC Spectrophotometer, Biochrom Ltd., Cambridge, England).

3. Results and discussion

3.1. Effect of cations on phase behaviour – construction of ternary phase diagrams

Fig. 2 shows partial ternary phase diagrams for all anionic polysaccharides (≤2% (w/v) of polymer and cation). During the course of evaluation no significant microbial contamination, which could lead to disruption of the gel structure, occurred. Systems based on gellan gum were much more susceptible to Ca^{2+} than to K^+. While 0.3% (w/v) K^+ was needed to transform a 0.25% (w/v) gellan gum formulation into a gel, less than 0.025% (w/v) Ca^{2+} was required to achieve the same phase transition. This was in accordance with previous studies by Deasy and Quigley (1991), who found that divalent cations give rise to much stronger double-helical interactions at the junctional zones than two monovalent cations and gels of similar apparent viscosities can be formed with divalent cations at much lower concentrations of the polymer than are required for gelation with monovalent cations. In the case of xanthan gum, gel formation was solely dependent on the polymer concentration, with no difference between the addition of K^+ or Ca^{2+} (Fig. 2C and D). The higher gum concentration required may partly be explained by the branched nature of the polymer backbone, with trisaccharide side chains preventing helix formation at low concentrations (Zatz and Knapp, 1984). The sulphate units in carrageenan impart a strong anionic character to the polymer backbone resulting in high cation sensitivity. Depending on the type of carrageenan, and therefore the sulphate content, different gelling mechanisms have been proposed (Davidson, 1980; Morris et al., 1981; Parker et al., 1993; Thrimawithana et al., 2010). Carrageenan used in this study seemed to be mostly in the κ-form as formulations were more susceptible to monovalent K^+ than to divalent Ca^{2+} as seen by the slightly bigger gel area in Fig. 2E compared to F. κ-Carrageenan polymer networks with K^+ are much stronger than those produced by ι-carrageenan with Ca^{2+}, which is due to the fact that the negative charges of the two sulphate groups in ι-carrageenan do not allow for the helices to aggregate to the same extent as κ-carrageenan molecules, therefore developing less cohesive networks (Morris et al., 1980, 1981; Parker et al., 1993). The crosslinking in alginate is due to the stacking of guluronic acid blocks (Katchalsky et al., 1961), resulting in formation of the egg-box model described by Grant et al. (1973), and only occurred in the presence of Ca^{2+} (Fig. 2H), while addition of K^+ did not have any effect (Fig. 2G). High Ca^{2+} concentrations (≥0.25% (w/v)), however, resulted in precipitation of the polymer and loss of the gel structure.

Comparing the partial ternary phase diagrams of the different anionic polysaccharides to each other, gellan gum exhibited the largest gel area with as little as 0.1% (w/v) of the polymer needed to achieve sol-to-gel phase transition upon addition of Ca^{2+}, which makes it the most favourable polymer for ocular drug delivery. No partial phase diagrams were constructed for HPMC and chitosan, as initial experiments showed no change in phase behaviour upon addition of small amounts of cations for HPMC, while chitosan exhibited a decrease in viscosity due to electrostatic repulsion between the cationic polyamine chains and the cations present in the tear fluid, and therefore possible disentanglement of the polymer chains.

3.2. Effect of cations on rheological and textural properties

The effect of cations on rheological (Fig. 3) and textural properties (Fig. 4) of the gels was in accordance with the results obtained for the partial ternary phase diagrams. In general, pseudoplastic systems showing an increase in the flow index upon increase in the cation concentration are preferred, as they would exhibit an increase in viscosity once in contact with the ions in the tear fluid, thus prolonging the corneal residence time and reducing reflex tearing and nasolacrimal drainage. On the other hand, the pseudoplastic nature would allow the formulations to thin during blinking, rendering the formulation less irritant and easier to spread across the ocular surface. Concerning the textural profile analysis, an increase in hardness upon cation addition would relate to an increase in viscosity and is therefore preferred. Higher values for adhesiveness are advantageous, because this might translate to increased adhesion to the ocular surface and therefore reduced lacrimal drainage. As for cohesiveness, lower values are preferred, as this would allow for better spreading of the formulation during blinking.

In the case of gellan gum, a gradual change in flow index from a Newtonian ($N = 1.16 \pm 0.23$ without ions) to a pseudoplastic system ($N = 3.90 \pm 0.49$ at 0.2% (w/v)) was observed in the case of K^+. Addition of only 0.025% (w/v) of Ca^{2+} resulted in a flow index of 3.68 ± 0.50, which was significantly different ($P = 0.025$) to the one at the same monovalent cation concentration. An increased flow index with the addition of Ca^{2+} may be a result of much stronger double-helical interactions at the junctional zones and therefore a more rapid change in the rheological properties (Deasy and Quigley, 1991). Viscosities with divalent cation concentrations >0.025% (w/v) were too high to be measured with the available equipment (Fig. 3A). This was in accordance with the texture profile analysis, which showed a remarkable increase in hardness to 155 g upon addition of 0.025% (w/v) Ca^{2+} (data point not shown on plot), while also exhibiting the highest value for adhesiveness ($P = 0.001$) and the lowest for cohesiveness ($P = 0.05$) (Fig. 4). This would render gellan gum favourable for topical application to the eye. Xanthan gum formulations showed no significant difference in the

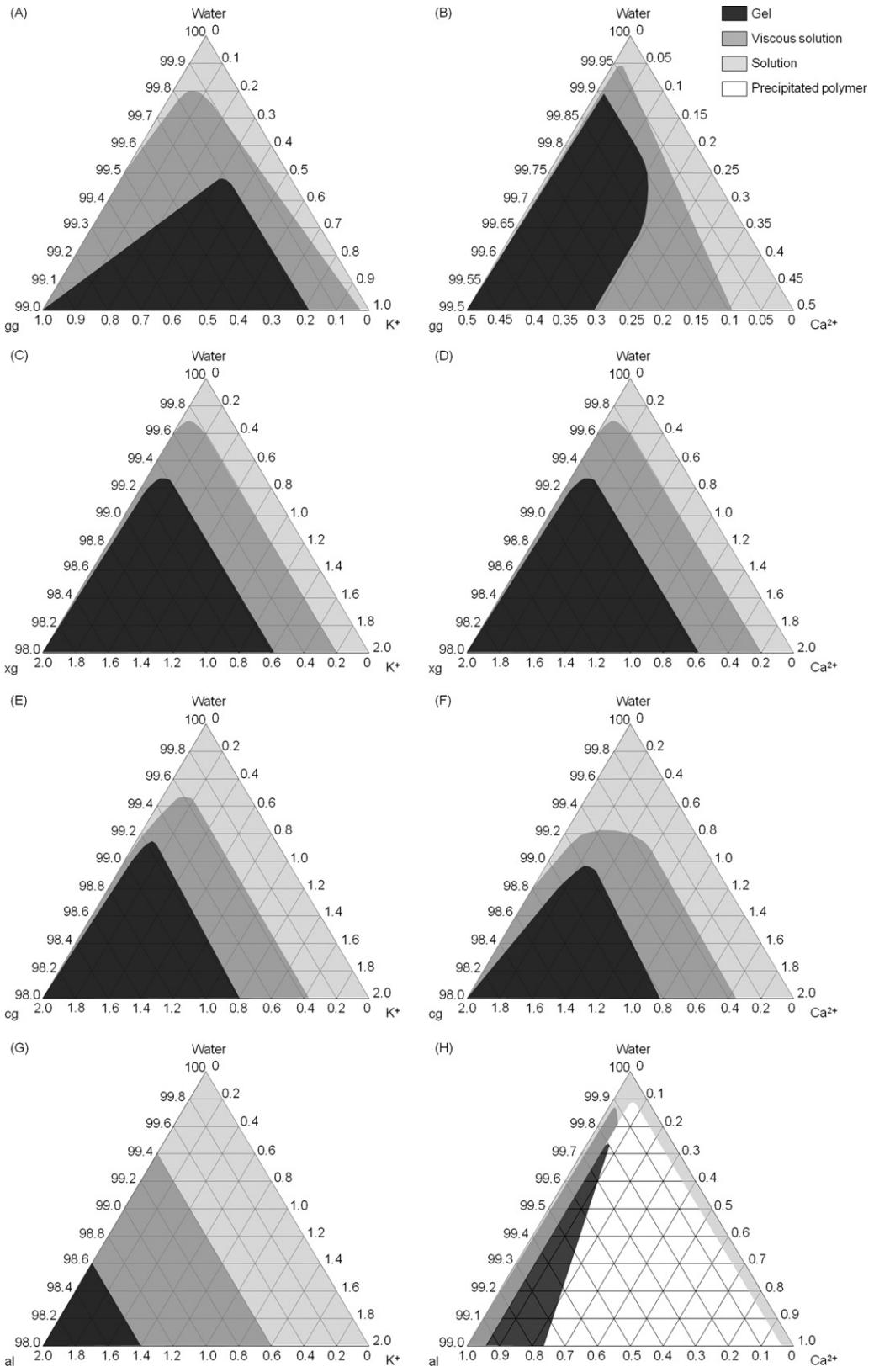

Fig. 2. Partial ternary phase diagrams showing the effect of K^+ and Ca^{2+} on the sol-to-gel transition of gellan gum (gg) (A and B), xanthan gum (xg) (C and D), carrageenan (cg) (E and F) and alginate (al) (G and H).

flow indices between mono- and divalent cations ($P = 0.731$), but exhibited a significant increase in pseudoplasticity upon increasing cation concentrations ($P = 0.001$). In addition, there was no significant change in the hardness or cohesiveness of the formulations, only adhesiveness values showed a significant increase upon Ca^{2+} addition ($P = 0.003$). Unlike gellan gum, carrageenan formulations exhibited stronger interactions with K^+, with the flow index of 7.82 ± 0.11 at 0.2% (w/v) being significantly different ($P \leq 0.001$) to

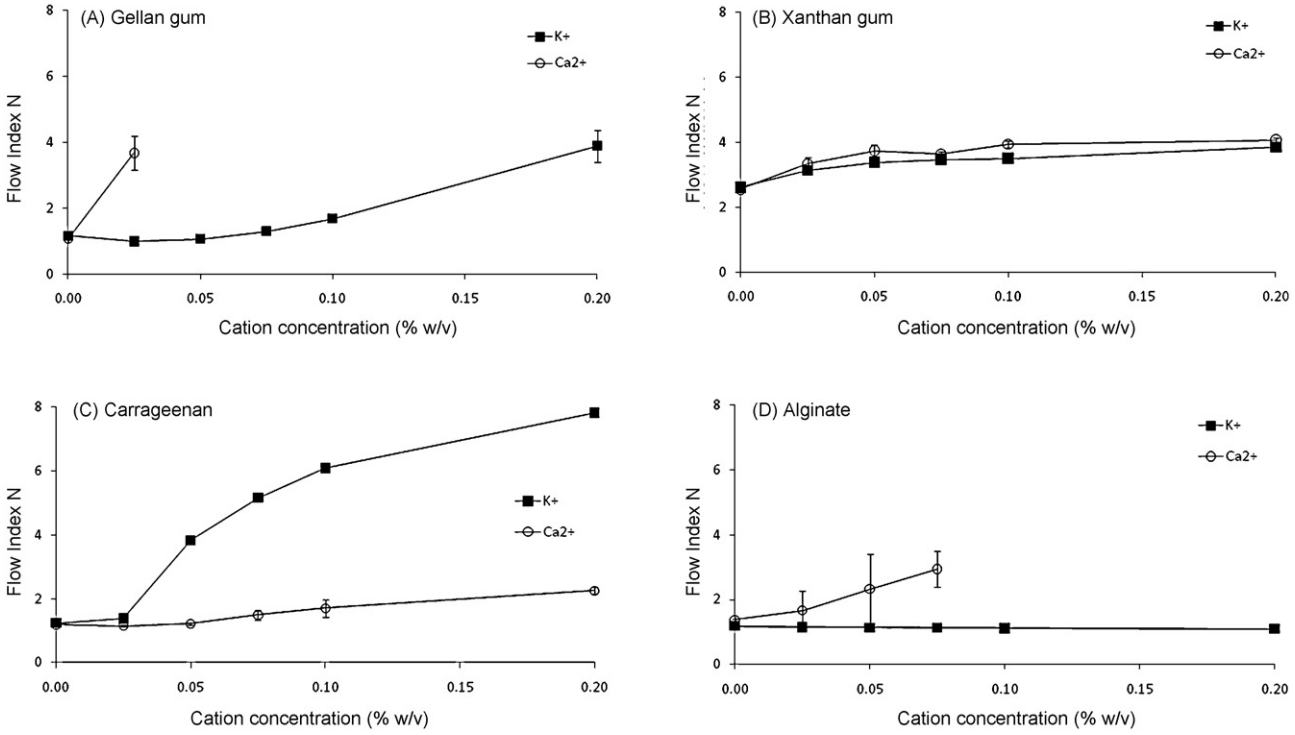

Fig. 3. Flow indices for 0.5% (w/v) anionic polymer formulations with increasing amounts of K^+ and Ca^{2+} (data points represent mean values ± SD, $n = 3$).

the one with the same Ca^{2+} amount ($N = 2.26 \pm 0.10$). This was in agreement with the textural properties, with carrageenan-K^+ formulations showing a significant difference in hardness ($P \leq 0.001$), adhesiveness ($P = 0.001$) and cohesiveness ($P \leq 0.001$) compared to the same formulations with Ca^{2+}, rendering carrageenan promising for topical ocular use. As for alginate, no change in flow index was seen upon addition of monovalent K^+, while addition of Ca^{2+} resulted in crosslinking of the guluronic acid blocks and therefore a significantly increased flow index ($P = 0.001$). Flow indices for divalent cation concentrations ≥0.1% (w/v) could not be determined as the viscosities of the formulations were too high to be measured with the available viscometer. However, the effect of higher Ca^{2+} concentrations was visible in the texture analysis profiles, which showed a significant increase in the hardness ($P = 0.038$). Only a slight increase ($P = 0.044$) was seen for adhesiveness of the gel upon addition of Ca^{2+} while there were no changes in the cohesiveness for any of the gels. Formulations based on HPMC and chitosan respectively did not exhibit any significant change in the rheological or textural properties upon addition of mono- or divalent cations (results not shown), which may have been a result of the non-ionic and cationic nature of the polymer chain backbone.

3.3. Rheology and polymer network microstructure of plain formulations

Stress sweep profiles were used to investigate the influence of the stress on the dynamic moduli (G', G''), whose magnitude is a qualitative indication of the structure present in the sample. Secondary bonding (hydrogen bonding, hydrophobic, electrostatic and Van-der-Waals interactions) was found in all formulations at rest, with $G' > G''$ at very low shear stresses (≤0.05 Pa) (data not shown). Calculating the loss tangent ($\tan\delta$), a ratio of the vis-

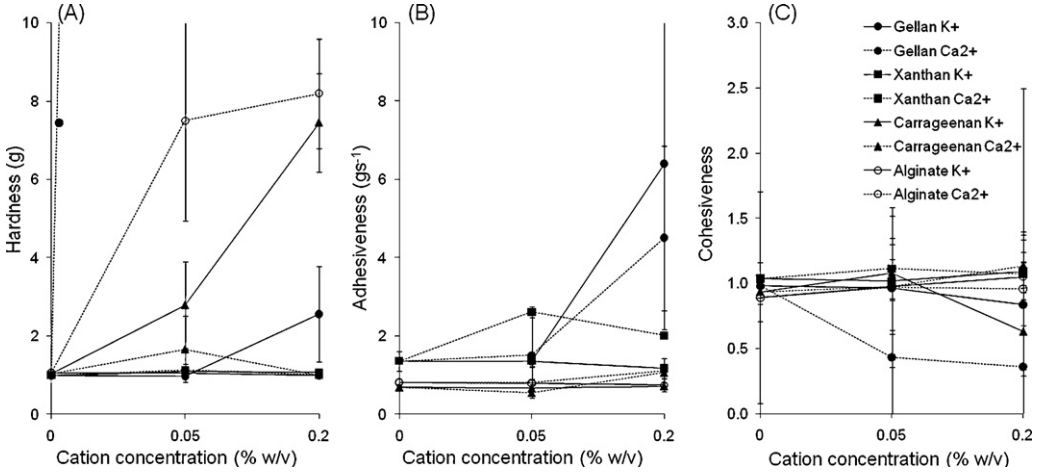

Fig. 4. Effect of K^+ and Ca^{2+} on the textural properties of 0.5% (w/v) anionic polymer systems (data points represent mean values ± SD, $n = 3$).

Table 1
Loss tangent ($\tan\delta$) at the beginning and the end of the measurement as well as the shear stress applied to achieve a cross-over of G' and G'' ($\tan\delta = 1$) (results represent mean values ± SD, $n = 3$).

Formulation	$\tan\delta$ at 0.05 Pa	$\tan\delta$ at 1 Pa	Crossover shear stress in Pa for $\tan\delta = 1$
Gellan gum	0.595 ± 0.153	4.489 ± 0.061	0.130 ± 0.045
Xanthan gum	0.615 ± 0.098	8.676 ± 0.043	0.144 ± 0.052
Carrageenan	0.608 ± 0.182	6.526 ± 0.119	0.205 ± 0.091
Alginate	0.904 ± 0.131	16.897 ± 1.013	0.119 ± 0.040
HPMC	0.483 ± 0.120	12.373 ± 1.048	0.243 ± 0.070
Chitosan	0.827 ± 0.173	13.130 ± 1.783	0.143 ± 0.041

cous to the elastic properties in the sample, at a shear stress of 0.5 Pa, all formulations exhibited $\tan\delta$ values less than 1 confirming a high degree of elasticity (due to secondary bonding) in the sample at rest (Table 1). Secondary bonds, however, were easily destroyed upon an increase in the shear stress, which was seen by a cross-over of G' and G'' (data plots not shown). This would result in a decrease in viscosity upon blinking, resulting in reduced reflex tearing and therefore prolonged corneal residence time. The cross-over at $\tan\delta = 1$ occurred in the order of alginate < gellan gum < chitosan < xanthan gum < carrageenan < HPMC. Formulations based on gellan gum, xanthan gum and carrageenan exhibited comparable amounts of elasticity at rest ($\tan\delta$ values at 0.05 Pa around 0.600) as well as similar amounts of shear stress applied before destruction of the secondary bonds occurred. This

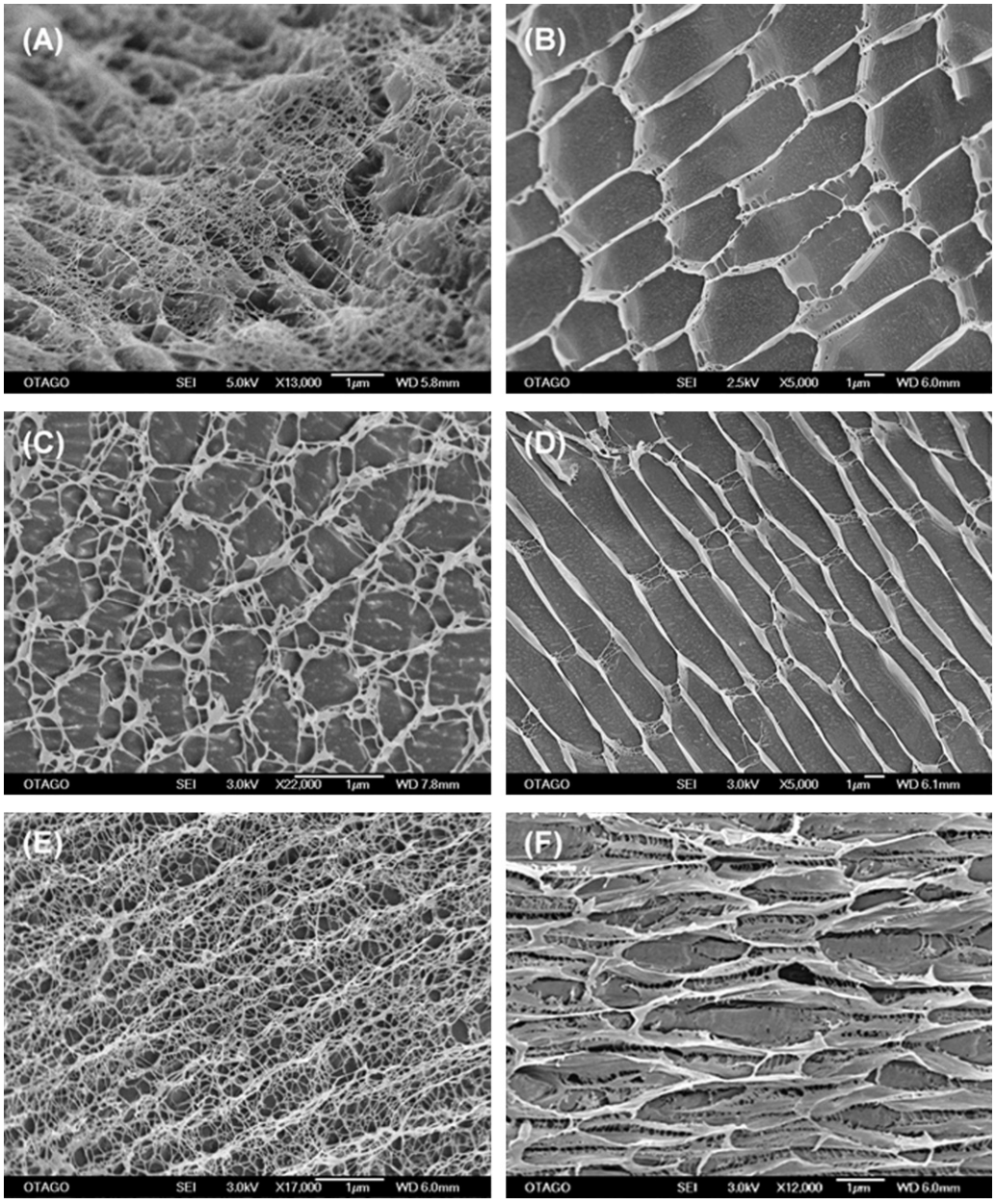

Fig. 5. Cryo SEM micrographs for 0.5% (w/v) formulations of gellan gum (A), xanthan gum (B), carrageenan (C), alginate (D), HPMC (E) and chitosan (F) (scale bar = 1 μm).

Table 2
Contact angle measurements (results represent mean values ± SD, $n = 3$).

Formulation	Contact angle after 10 s (°)	Change over the first 10 s (%)
Gellan gum	50.10 ± 0.97	−9.42 ± 0.72
Xanthan gum	47.20 ± 1.33	−12.36 ± 0.56
Carrageenan	46.24 ± 6.07	−14.75 ± 1.54
Alginate	45.62 ± 2.86	−9.05 ± 2.95
HPMC	46.16 ± 2.46	−17.49 ± 3.25
Chitosan	47.66 ± 0.82	−1.08 ± 0.16
Solution	63.30 ± 0.82	−12.07 ± 1.98

may be attributed to the similar molecular configuration and polymer concentration of these formulations, also seen in the cryo SEM micrographs (Fig. 5) All formulations exhibited tan δ values greater than 1 upon an increase of the shear stress (>0.25 Pa), leaving viscous physically entangled polymer solutions ($G' \leq G''$) which seem to disentangle even further at higher shear stress. This behaviour is advantageous as polymer networks would disentangle upon blinking, making the formulations less irritant and easier to spread across the corneal surface.

Besides the difference in polymer conformation shown by oscillation viscometry, cryo SEM micrographs revealed differences in the gels' microstructure (Fig. 5). Formulations based on xanthan gum, carrageenan and alginate exhibited a similar sized, highly porous honeycomb structure, with the combs of xanthan gum and alginate being of a much more uniform size and arrangement than the ones in the carrageenan sample. Similar results were previously reported by Bertram and Bodmeier (2006), who studied the characteristics of *in situ* gelling nasal inserts based on these polymers and demonstrated a sponge-like structure of the inserts at a polymer concentration of 2% (w/w). Formulations based on gellan gum and HPMC revealed less porous spiderweb-like polymer networks, which are likely to retard the diffusion of the drug out of the dense matrix. Chitosan exhibited a sponge-like structure with fine polymer combs connecting the almost parallel polymer sheets. Pores in the polymer networks are generally an indication of the possible routes for the drug to diffuse out of the matrix (Nerurkar et al., 2005) and different microstructures of the formulations are likely to influence the drug release characteristics.

3.4. Contact angle

Contact angle measurements evaluate the angle between a liquid droplet and the surface over which it spreads and therefore determine how well the formulations may spread on the ocular surface. Results revealed a significant difference ($P < 0.005$) between the aqueous solution and the individual polymer formulations, which showed lower contact angle values and would therefore exhibit better spreading on the ocular surface. However, no significant differences ($P > 0.005$) were detectable between the different *in situ* gelling systems (Table 2). This suggests that the contact angle is more dependent on the concentration of the polymer present rather than the polymer structure itself. In general, addition of a polymer would reduce the contact angle, most likely due to a reduction in surface tension, and would allow better spreading of the formulation across the corneal surface. In terms of the change in contact angle over the first 10 s, anionic polymer systems seem to exhibit similar properties to the aqueous solution, while the HPMC formulation showed the most pronounced change, therefore exhibiting the best spreading ability over time. The chitosan system on the other hand revealed only a minor change in contact angle over the first 10 s, suggesting that this formulation would not spread any further over time. This could be attributed to the cationic nature of the polymer backbone and possible charge–charge interactions with the surface.

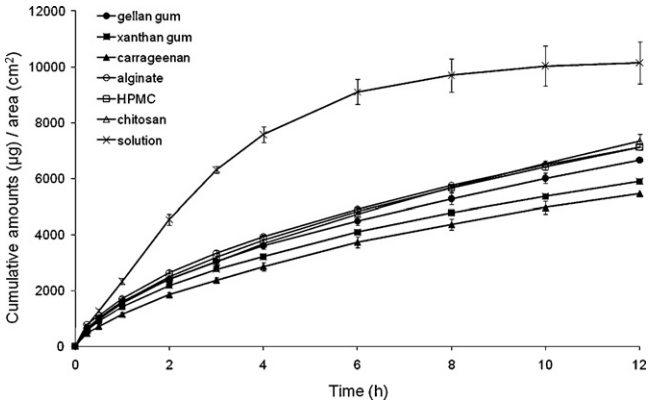

Fig. 6. Cumulative amounts of PHCl released versus time (data points represent mean values ± SD, $n = 3$).

3.5. In vitro release

Drug release from all polymer systems was much slower than that from an aqueous pilocarpine solution with no polymer excipients (Fig. 6) and was in accordance with the results obtained in Figs. 2–4. The influence of viscosity on the diffusion of the drug can be described by the Stokes–Einstein equation (Sinko and Martin, 2006), which demonstrates that an increased viscosity of the formulation results in slower diffusion of the drug across the gel matrix and into the receptor medium. This was previously reported for poloxamer formulations, where the viscosity of the gel represented the main factor controlling the drug diffusion (Pandit and Wang, 1998; Ricci et al., 2005). Moreover, Jones et al. (1996) reported that the drug release characteristics of various topical gels containing hydroxyethyl cellulose, polyvinyl pyrrolidone and polycarbophil mainly depended on the viscosity of the formulation.

In order to objectively quantify this phenomenon and compare the different formulations to each other, the percentage of PHCl released was plotted versus time (graphs not shown) and the area under the 'percentage-PHCl-released-versus-time' curve ($AUC_{0-12\,h}$) as well as the percentage of drug released after 2 and 12 h were determined (Table 3). There was a more than two-fold increase in the $AUC_{0-12\,h}$ and the percentage of drug released after 2 h, when comparing the solution to the carrageenan and xanthan gum formulations, and while less than 60% PHCl was released from all anionic polysaccharide systems after 12 h, more than 86% of the drug was released from the solution at the end of the study. A one-way ANOVA followed by Tukey's pairwise comparison revealed that all polymer formulations were significantly different ($P < 0.01$) from the solution in terms of these three parameters (Table 4). Moreover, carrageenan showed the slowest release characteristics, with $AUC_{0-12\,h}$, $PHCl_{2\,h}$ and $PHCl_{12\,h}$ values not only being significantly different from the solution, but also from all other polymer systems apart from xanthan gum (Table 4).

In addition, data was fitted to the model proposed by Higuchi (1961) by plotting the cumulative amounts PHCl released versus the square root of time (plots not shown) and release rate constants were calculated from the slope of the linear regression equations (Table 3). A linear relationship with good correlation coefficients ($R^2 \geq 0.9966$) was obtained indicating matrix order release kinetics. The drug release mechanism could therefore be described by the diffusion of the cations present in the receptor medium into the polymer systems, helix formation and therefore gelation of the anionic polymer chains, followed by diffusion of the drug through the resultant gel matrix. This assumption was confirmed by measuring the viscosity of the formulations in the donor compartment after the release study (data not shown), with gellan gum, xanthan gum and carrageenan formulations

Table 3
Area under the 'percentage-drug-released-versus-time' curve ($AUC_{0-12\,h}$), percentages PHCl released after 2 h ($PHCl_{2\,h}$) and 12 h ($PHCl_{12\,h}$) and rate constants (k) for the release of PHCl from the different formulations according to the Higuchi model (results represent mean values ± SD, $n = 3$).

Formulation	$AUC_{0-12\,h}$	$PHCl_{2\,h}$ (%)	$PHCl_{12\,h}$ (%)	k ($\mu g\,cm^{-2}\,h^{-0.5}$)
Gellan gum	431.72 ± 13.50	20.35 ± 0.73	55.57 ± 1.56	2025.6 ± 57.6
Xanthan gum	387.90 ± 5.15	18.60 ± 0.21	50.16 ± 0.61	1813.2 ± 27.6
Carrageenan	351.40 ± 13.95	15.73 ± 0.62	46.52 ± 1.91	1726.2 ± 72.1
Alginate	468.13 ± 6.56	22.39 ± 0.72	60.57 ± 0.54	2172.6 ± 19.9
HPMC	458.89 ± 13.91	21.10 ± 1.07	60.59 ± 1.09	2222.6 ± 36.1
Chitosan	457.34 ± 5.58	20.69 ± 0.18	62.43 ± 1.97	2266.7 ± 60.1
Solution	785.19 ± 40.72	38.55 ± 1.70	86.21 ± 6.39	–

Table 4
Tukey's pairwise comparison of the area under the curve ($AUC_{0-12\,h}$) and the percentage PHCl released after 2 h *($PHCl_{2\,h}$)* and 12 h **($PHCl_{12\,h}$)**; S = significant difference ($P \leq 0.01$), NS = no significant difference ($P > 0.01$).

	Solution	Gellan gum	Xanthan gum	Carrageenan	Alginate	HPMC
Gellan gum	S; *S*; **S**	–	–	–	–	–
Xanthan gum	S; *S*; **S**	NS; *NS*; **NS**	–	–	–	–
Carrageenan	S; *S*; **S**	S; *S*; **S**	NS; *NS*; **NS**	–	–	–
Alginate	S; *S*; **S**	NS; *NS*; **NS**	S; *S*; **S**	S; *S*; **S**	–	–
HPMC	S; *S*; **S**	NS; *NS*; **NS**	S; *NS*; **S**	S; *S*; **S**	NS; *NS*; **NS**	–
Chitosan	S; *S*; **S**	NS; *NS*; **NS**	S; *NS*; **S**	S; *S*; **S**	NS; *NS*; **NS**	S; *NS*; **S**

exhibiting a substantial increase in viscosity. The viscosity of the HMPC system did not change over the course of the experiment, while signs of precipitation were visible in the alginate and chitosan formulations, resulting in decreased viscosities. The measured viscosities correlated well with the results obtained from the partial ternary phase diagrams (Fig. 2) and the calculated release rate constants (Table 3). Release rate constants were found to be in the order of chitosan > HPMC > alginate > gellan gum > xanthan gum > carrageenan, with the release rates of carrageenan ($P < 0.005$), xanthan gum ($P < 0.005$) and gellan gum ($P < 0.05$) being significantly different from those of alginate, HPMC and chitosan. This could be explained by the fact that besides the slow diffusion of the drug through the gel matrix, there might also be ionic interactions between the anionic polymer chains of gellan gum, xanthan gum and carrageenan and the positively charged PHCl, slowing down the release of the drug even further. This would not be the case with non-ionic HPMC, while there might have even been repulsion between the positively charged amino groups of the chitosan backbone and the drug, facilitating the diffusion of the drug through the chitosan gel matrix as seen by the comparably high value for the chitosan release rate constant ($k = 2266.7\,\mu g\,cm^{-2}\,h^{-0.5}$). The relatively high k-value for the alginate formulation ($k = 2172.6\,\mu g\,cm^{-2}\,h^{-0.5}$) may be explained by that fact that it is unaffected by addition of monovalent cations, which might also result in less interaction with PHCl, exhibiting only one positive charge. This is the opposite for the carrageenan system, whose phase behaviour is more affected by the addition of monovalent cations, which may cause stronger ionic interactions between the negatively charged polysaccharide chains and the positively charged drug, explaining the slow release rate of PHCl from this system ($k = 1726.2\,\mu g\,cm^{-2}\,h^{-0.5}$). Another possible explanation is based on the fact that alginate formulations precipitate upon addition of high cation concentrations (Fig. 2H). Due to maintenance of sink conditions in the receptor compartment, high concentration of cations were always present and may have resulted in precipitation of alginate close to the membrane/gel interface, facilitating the release of the drug present in that layer.

4. Conclusion

All systems exhibited physically entangled polymer networks, which renders them favourable for ocular use as they can easily disentangle upon shear stress associated with blinking, hence preventing induced lacrimation, which is usually provoked by more viscous systems. Formulations based on gellan gum and carrageenan exhibited the most favourable characteristics in terms of phase transition, rheological and textural properties, as their viscosity remarkably increased upon contact with cations of the tear fluid, thus prolonging corneal residence time and reducing nasolacrimal drainage. These systems should therefore be further investigated and compared for their *in vivo* performance.

Acknowledgements

The authors would like to thank Marian Kelly, Sonalee Ghosal, Debby Chen, Hwiju Park and Tzu-Hong Chang from the School of Pharmacy, University of Auckland, for their support with the rheological and textural analysis, Liz Girvan from the Department of Anatomy and Structural Biology, University of Otago, for her assistance with the cryo SEM imaging and the R&D team at Kettenbach GmbH & Co. KG for the use of the oscillation viscometer and drop shape analyser. Thanks also to the University of Auckland for the financial support in form of an international doctoral scholarship for Ilva Dana Rupenthal and CP Kelco for kindly donating samples of gellan gum, xanthan gum and carrageenan polymers.

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