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Danielle L. Taylor
University Of Western Sydney

Cameron J. Ferris
University of Wollongong, cjf146@uowmail.edu.au

Alison R. Maniego
University Of Western Sydney

Patrice Castignolles
University Of Western Sydney

Marc in het Panhuis
University of Wollongong, panhuis@uow.edu.au

See next page for additional authors

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Keywords

capillary, characterization, gum, electrophoresis, gellan

Disciplines

Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

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Authors

Danielle L. Taylor, Cameron J. Ferris, Alison R. Maniego, Patrice Castignolles, Marc in het Panhuis, and Marianne Gaborieau

Characterization of Gellan Gum by Capillary Electrophoresis

Danielle L. Taylor,[A,B] *Cameron J. Ferris,*[C] *Alison R. Maniego,*[B] *Patrice Castignolles,*[B,D] *Marc in het Panhuis*[C] *and Marianne Gaborieau*[A]

[A] University of Western Sydney, Nanoscale Organisation and Dynamics Group, School of Science and Health, Parramatta campus, Locked Bag 1797, Penrith NSW 2751, Australia

[B] University of Western Sydney, School of Science and Health, Australian Centre for Separation Science (ACROSS), Parramatta campus, Locked Bag 1797, Penrith NSW 2751, Australia

[C] Soft Materials Group, School of Chemistry, ARC Centre of Excellence for Electromaterials Science, University of Wollongong, Northfields Avenue, Wollongong, NSW, Australia

[D] Corresponding author. E-mail: p.castignolles@uws.edu.au

Abstract

Gellan gums were characterized for the first time using free-solution capillary electrophoresis (CE) or CE in the critical conditions (CE-CC). CE-CC is a fast method that separates the polysaccharide. Gellan gums are shown to be heterogeneous in terms of their electrophoretic mobility at 55 °C: oligomers peak(s), broad polymer peak with random coil conformation with different degree of acylation (composition), aggregates and polymer with double-helix conformation. CE-CC is complementary with rheological analysis also performed in this work. Sonication of gellan gums is shown to decrease the viscosity of gellan gum mainly by breaking up aggregates. The effect of sonication is stronger on the high-acyl gellan gum since the latter has a far higher tendency to aggregate.

Keywords

Gellan gum, capillary electrophoresis, bacterial exopolysaccharides, separation, sonication

Introduction

Gellan gum is a natural exopolysaccharide produced by the bacterium *Sphingomonas* which is widely distributed from water to terrestrial habitats as well as in plant root systems. The rheological properties of gellan gum make it an ideal multifunctional gelling, stabilizing and suspending agent with uses in food, pharmaceutical, medical and chemical industries.[1-5] With gellan gum's increasingly widespread use its importance has become apparent with its unique properties showing advantages over other naturally occurring polysaccharides. These advantageous properties are its excellent thermal and acid stability, adjustable gel elasticity and rigidity, high transparency and good flavor release.[2]

Gellan gum is a linear anionic polysaccharide based on a tetrasaccharide repeat unit composed of two residues of D-glucose, one with up to two acyl substituents (one bearing a vicinal diol), one D-glucuronic acid and one L-rhamnose (Fig. 1). Gellan gum samples differ by the proportion of units with acyl chains attached to the glucose molecule (degree of acylation) and the distribution of these acyl chains along a polysaccharide chain. In other word, gellan gums are copolymers which composition is generally called degree of acylation. The acyl substituents dramatically affect the unique desirable properties, in particularly the rheology of the gels produced.[1,2] It is expected that the distribution of the acyl substituents also has an effect on the properties of gellan gum.[1] Currently there is no definitive method available to produce identical gellan gum samples with the same degree of acyl substituents and equal distribution of molecular weights.[2] Commercial samples are only described as exhibiting a 'high' or 'low' degree of acylation by the supplier (see Table 1). To overcome this natural variability, it is therefore important to determine the degree and distribution of degrees of acylation of a gellan gum.

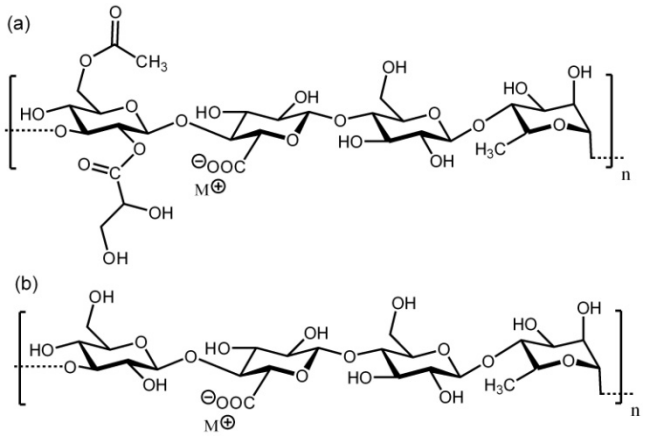


Fig. 1. Molecular structure of fully acetylated gellan gum (a, adapted from [1]) and fully deacylated gellan gum (b, adapted from [5]). The monomer unit is constituted from left to right of one D-glucose with two acyl substituents (one acetyl and one glycerate bearing a diol or glycol), one D-glucuronic acid, one D-glucose without substituents and one L-rhamnose. The gellan gums studied in this work are copolymers containing both repeating units (not necessarily forming blocks).

There have been several methods used previously to characterize gellan gum, including rheology, light scattering, nuclear magnetic resonance (NMR) spectroscopy, and dichroism spectroscopy.[3-7] These methods have been used to characterize the conformational transition from a random coil to a double-helix structure (twice higher molecular weight) on cooling. In one instance, the molecular weight of the gellan gum was varied by sonication (the only information given on the sonication was the resulting intrinsic viscosity of the samples).[7] Number-average molecular weights have been measured by osmometry.[8] Weight-average molecular weights have been measured by static light scattering.[5,7-8] Molecular weight distributions have been determined by size-exclusion chromatography (SEC, also known as GPC or GFC) with state-of-the-art detection.[10-11] Preliminary injections of gellan gums showed that an aqueous LiCl eluent at 60 °C leads to repeatable results.[10] However, further work in other groups relied on different eluents ranging from salt solutions with weaker hydrogen bond breaking properties [11-12] down to pure water [2]. The quality of the separation was not assessed for example in terms of recovery or accuracy of the determined molecular weights in all these latter works despite the use of new eluents and columns. This is of importance since SEC of polysaccharides is commonly

plagued by low recovery.[13] Aggregation has been observed in aqueous KCl eluents.[12] Filtration affects the determined molecular weight values in light scattering[14] (or the determination is sometimes impossible due to filters clogging[12]) and it can also lower the recovery in chromatographic separation of gellan gum.[10] It is thus desirable to apply to gellan gums separation methods that do not require sample filtration. These are methods using no stationary phase: field-flow fractionation[15] or free-solution capillary electrophoresis (detailed later). There is no established method to determine the molecular weight distribution of gellan gums. Furthermore, the influence of a distribution of degrees of acylation has not been considered, although differences in composition could significantly alter the accuracy of the determined molecular weights.[9] The characterization of gellan gum in regard to its average degree of acylation and its distribution of degrees of acylation has not been reported.[1] The aim of this work is to separate the whole gellan gums (without filtering the solution) according to their structure.

Gellan gums are used as sieving media in gel electrophoresis.[16] Characterization of the gellan gums by electrophoresis is a lot scarcer. Free-solution capillary electrophoresis (CE) involves separation in a (fused-silica) capillary filled with a buffer solution (no stationary phase) and placed under high voltages (up to 30 kV). Free-solution CE has been applied once to the determination of enzymatically degraded gellan gums,[17] but not for its separation or structural characterization. Capillary electrophoresis is largely used for separation and characterization of a few natural polymers, DNA and proteins.[18] Applications of CE to polysaccharides are limited. Free-solution CE proved successful to separate chitosan from conjugates[19] or separate oligomers according to their molecular weight[20]. Free-solution CE exhibits a higher resolution than SEC for the separation of oligomers.[21-24] In the case of polymers, no separation by molecular weight is observed in the cases of single-stranded or double-stranded DNA, poly(styrene sulfonate) and a few other polyelectrolytes.[21,25] This corresponds to the "critical conditions" sought in liquid chromatography at the critical point of adsorption (LC-CC).[26-32] LC-CC does not separate the compound of interest by its molecular weight. Not separating by the molecular weight of one block in a copolymer, allows determining the molecular weight of the other block. In LC-

CC, critical conditions are however tedious to determine and recovery is poor.[31-32] LC-CC has never been successfully applied to polysaccharides. Free-solution CE, or CE in the critical conditions (CE-CC), was on the other hand shown to separate two polysaccharides, pectins[33] and carboxymethyl cellulose,[34] according to their composition, which is named degree of substitution. This separation has a higher selectivity at the lowest charge density, below the Manning's counter-ion condensation.[35-37] The aim of this work is to explore the potential of CE-CC for the separation of gellan gums by its composition, namely degree of acylation. In this work, the potential of CE-CC to characterize gellan gums is investigated for the first time examining the repeatability of the sample preparation and separation, and studying the effects of sonication, endotoxin removal, expected degradation stresses (mainly heat) and exceeded expiry dates. These results are compared with ones obtained by rheological analysis.

Results and Discussion

Separation of Gellan Gums by Free-Solution Capillary Electrophoresis.

Sodium borate was used as a buffer like in the literature[17] but with a higher pH (9.2 instead of 8.0) in order to increase the zeta potential of the capillary wall and thus limit potential adsorption of gellan gums to the wall. The separations were performed at 55 °C contrary to 35 °C and 20 °C in the literature[17]. At low temperature, aggregation, through double-helix formation, has been extensively observed by viscosity measurements[38] and light scattering[5]. The separations presented in this work were obtained at 55 °C to obtain random coil conformation and limited aggregation in the capillary. 4 min preconditioning with the buffer was first used as in [17] and led to some separation but with relatively poor resolution and repeatability (data not shown). An additional 1 min flush with NaOH (1 M) was added to the preconditioning (i.e. before each injection) and yielded results with a higher signal-to-noise ratio as well as repeatable separations within 10 minutes. Fast separations are important not only in terms of throughput but also to ensure no deacetylation or degradation takes place, which could be caused by long residence to times at high pH and high temperature. The UV spectra yielded by the

diode array detector are the same for the different peaks (see Fig. S12). These peaks are thus all consistent with gellan gums and not with some potential impurities such as proteins. The detection might be explained by complexation of the borate ions from the buffer by the glycol moiety present on the acetylated glucose units. Separation with ammonium acetate leads to no peaks detected in equivalent conditions (same pH, data not shown). Complexation of the borate by alcohol groups in neutral mono- and oligosaccharides have been shown to be strong enough to create an electrophoretic mobility.[39] A representative example of electropherograms is shown on Fig. 2a and 2b, more examples are shown in Supporting information (Fig. S1 to S10). Two main populations with electrophoretic mobilities around respectively 1.5 and $2.8 \cdot 10^{-8}$ $m^2.V^{-1}.s^{-1}$ are clearly separated. The second peak is broader and exhibits a shoulder showing a likely inhomogeneity in gellan gum structure. These mobilities are lower than those observed for poly(sodium acrylate)s of degree of polymerization above 10 in the same conditions (around 6 $m^2.V^{-1}.s^{-1}$, see Fig. S13 in supporting information)[23]. This is expected since the charge is due to a carboxylic acid in both cases but the charge density is lower for the gellan gum at our pH (9.2). The first peak has equivalent mobilities to tetrasaccharides in borate buffer (at lower a buffer concentration of 60 $mmol \cdot L^{-1}$ though).[39] It might thus correspond to one gellan gum monomer (which is a tetrasacharide, see Figure 1) or oligomers (see next paragraph). These separations of gellan gums were successfully reproduced with different and longer capillaries, higher voltage and higher injection volume and different operators (Figure 2c). The later parameters (series 2 to 4) resulted in a higher signal-to-noise ratio and also to a higher resolution of the peaks (the latter discussed in the next part). The separations cannot be compared with literature[17] since the latter does not indicate values of the electrophoretic mobility, only migration times.

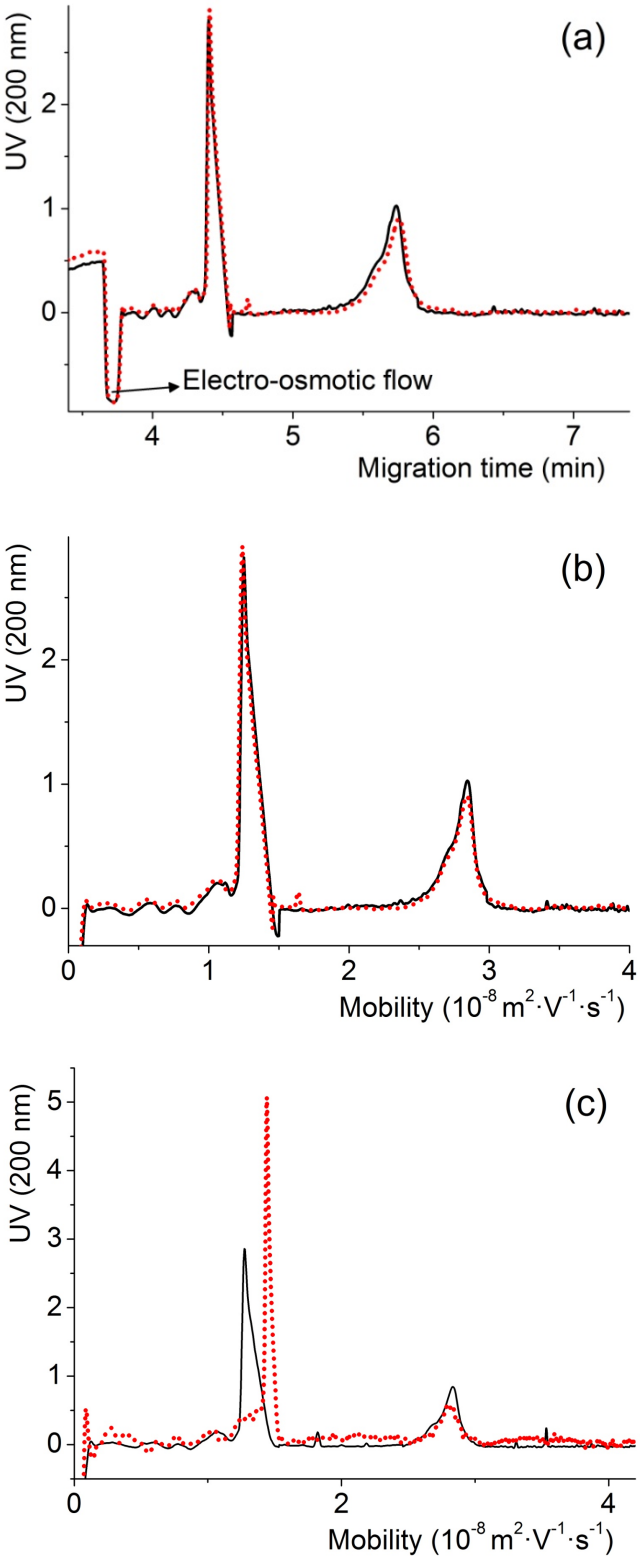


Fig. 2. Repeatability tests: (a) electropherograms of the same sample LAEFGG-Exp as a function of migration time; (b) same data as in (a) plotted as a function of electrophoretic mobility; (c) electropherograms of LAGG-Exp samples prepared at different times, under the same conditions and by

different operators. The red dotted data in (c) is from injection series 3, all others are from injections series 1.

Separation of Gellan Gum with Different Degrees of Acylation.

The potential of free-solution CEto separate gellan gum is apparent in the comparison of samples with low and high average degrees of acylation shown on Fig. 3. The low-acyl LAGG-Exp has the two relatively broad peaks described above at mobilities 1.45 and 2.8 $\times 10^{-8}$ $m^2 \cdot V^{-1} \cdot s^{-1}$ whereas the high-acyl HAGG has these two peaks but also a broad one at high mobility, 4.95×10^{-8} $m^2 \cdot V^{-1} \cdot s^{-1}$, and a number of narrow and less repeatable (see supporting information Fig. S2) peaks between the second and the third broad ones. The second broad peaks of both samples differ in shape: LAGG's peak is more symmetrical with a slight tail on the low mobility side, while HAGG's exhibits a slight tail on the high mobility side and a pronounced tail on the low mobility side. The third broad peak has a high mobility value (4.95×10^{-8} $m^2 \cdot V^{-1} \cdot s^{-1}$). Various sharp peaks, or spikes, have mobilities between 3 and 4×10^{-8} $m^2 \cdot V^{-1} \cdot s^{-1}$. These peaks significantly decrease in intensity when the sample is injected 5 days after dissolution rather than a few hours (as shown by comparison of Fig. 3 and 4). They disappear completely after sample filtration (Fig. 4). They also disappear with sonication (see last paragraph). They thus correspond to aggregates and are present mainly, if not only, in the high acyl. This is confirmed by visual observation of the clear low-acyl solution and the cloudy high-acyl gellan gum "solution" (Fig. S11). These aggregates have already been observed to lead to difficulties in light scattering and size-exclusion chromatography.[10,12,14] Capillary electrophoresis is thus complementary to these two methods which are commonly applied to polysaccharides. It could be used to monitor dissolution and is thus complementary with time-resolved solution-state NMR.[40-41]

The first peak has mobility equivalent to the tetrasaccharide monomer unit. [39] Oligomers of charged polymers have been observed to have a lower mobility than the polymer for short chains with less than 5-10 monomer units in the case of poly(styrene sulfonate)[21,42] poly(acrylic acid)[23, 24] or DNA[25].

The presence of oligomers in gellan gums has never been mentioned in the literature. To confirm the presence of oligomers, we performed a pressure mobilization as performed by Cottet's group for Taylor Dispersion Analysis.[43] They were able to monitor mixtures of monomer and polymer by pressure mobilization due to the large differences in their diffusion coefficient.[44] Pressure mobilization was also applied to our gellan gums (Fig. 5). It is clear that the peak is the sum of narrow and broad peaks corresponding respectively to fast diffusing and slow diffusing species. This confirms the presence of monomer or oligomers. Oligomers have never been reported in Kelcogel gellan gums, but no conditions separating oligomers (such as column with small pore size in SEC) are reported to have been tested. The lowest mobility peak may thus correspond to oligomers.

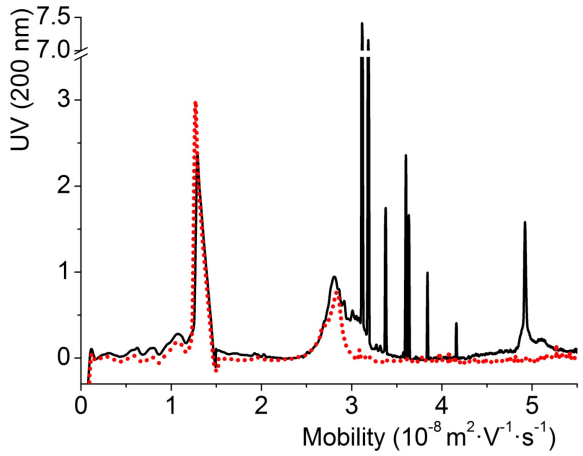


Fig. 3. Electropherograms of low-acyl gellan gum, LAGG-Exp (dotted red line), and high-acyl gellan gum, HAGG (solid black line) after a few hours dissolution. Both are from injection series 1.

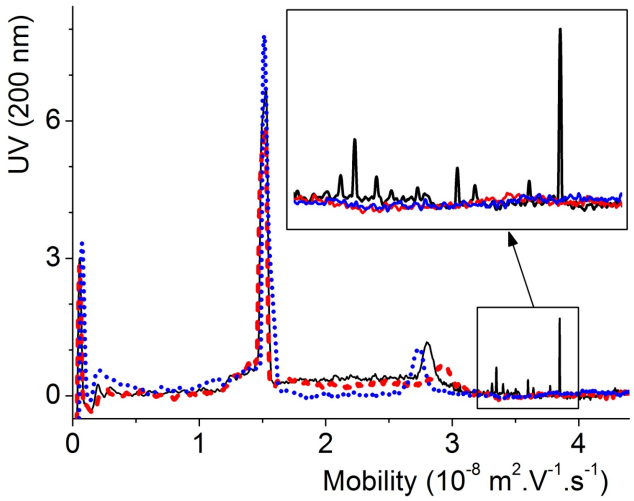


Fig. 4. Electropherograms of sample HAGG-Exp after 5 days dissolution (injection series 3): non-filtered (solid black line), filtered on 0.45 µm (dashed red line) or 0.22 µm porosity filter (dotted blue line). Insert shows a zoom on the aggregates region.

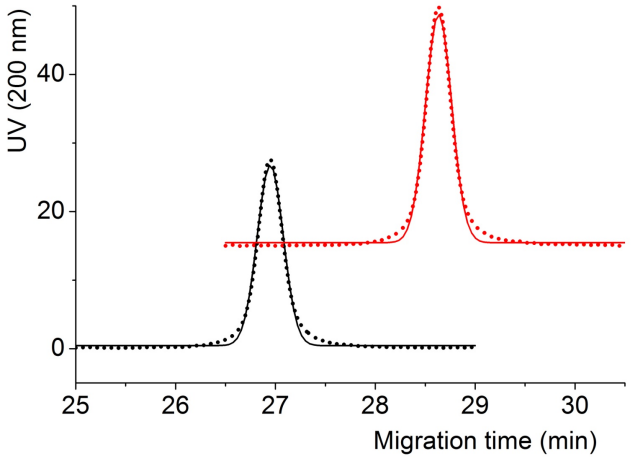


Fig. 5. Pressure mobilization of HAGG (with repeat experiment): experimental signals (dotted lines) with Gaussian fit (solid lines). Experimental conditions are the same as Series 3 except that mobilization pressure was applied at 50 mbar instead of electric field and injection time was only 5 s.

The relative area of the oligomer peak is compared to the area of the whole electropherogram (Fig. 6). The ratios of the area of the first peak to the total area are repeatable (see Fig. 6 and supporting information). The first peak is relatively more intense for the low-acyl gellan gum than for the high acyl (compare 3rd bar to 6th and 7th bars in Fig. 6). The low-acyl gellan gums contain more oligomers(s) likely due to some degradation during the deacylation process.

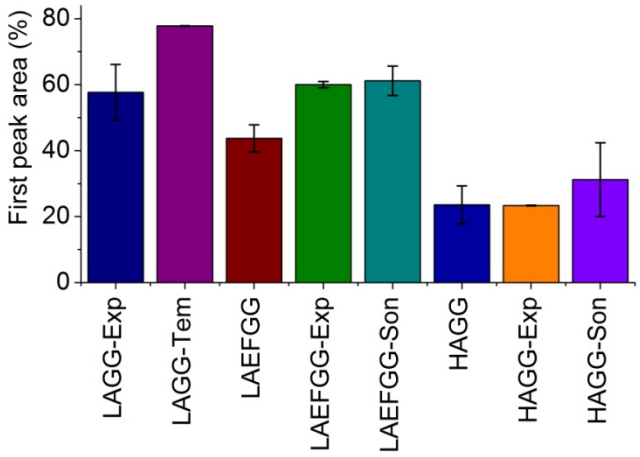


Fig.6. Ratio of the area of the lowest mobility (first) peak to the total area of the electrophoretic mobility plot for 8 different gellan gums samples. The error bars are calculated from the standard deviation or the maximum deviation to the average (see Supporting information).

The highest mobility peak appears only for the high acyl gellan gums. This peak is of higher intensity when potassium borate is used as buffer instead of sodium borate (Fig. 7b). Injections in potassium borate are repeatable (see Fig. S9 and Fig. S10). The low-acyl gellan gum in potassium borate exhibits slightly different electrophoretic mobilities but no significant change in terms of relative quantities. The change of mobility is expected since the different counter-ion is going to change both electrostatic and hydrodynamic friction (the latter through viscosity change). The same differences are observed with the high-acyl, but the high mobility peak is also more intense. Potassium has been observed to lead to a higher proportion of double-helix conformation.[6] The highest mobility peak, around 5×10^{-8} $m^2 \cdot V^{-1} \cdot s^{-1}$ may thus correspond to double-helix conformation. CE has thus potential to study the conformation of gellan gums.

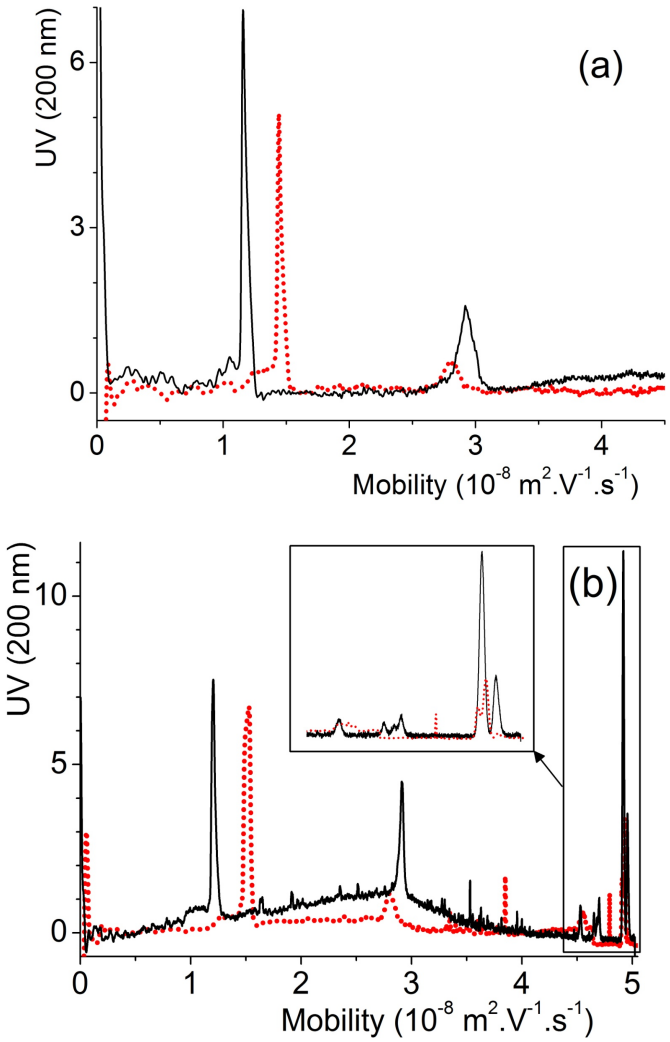


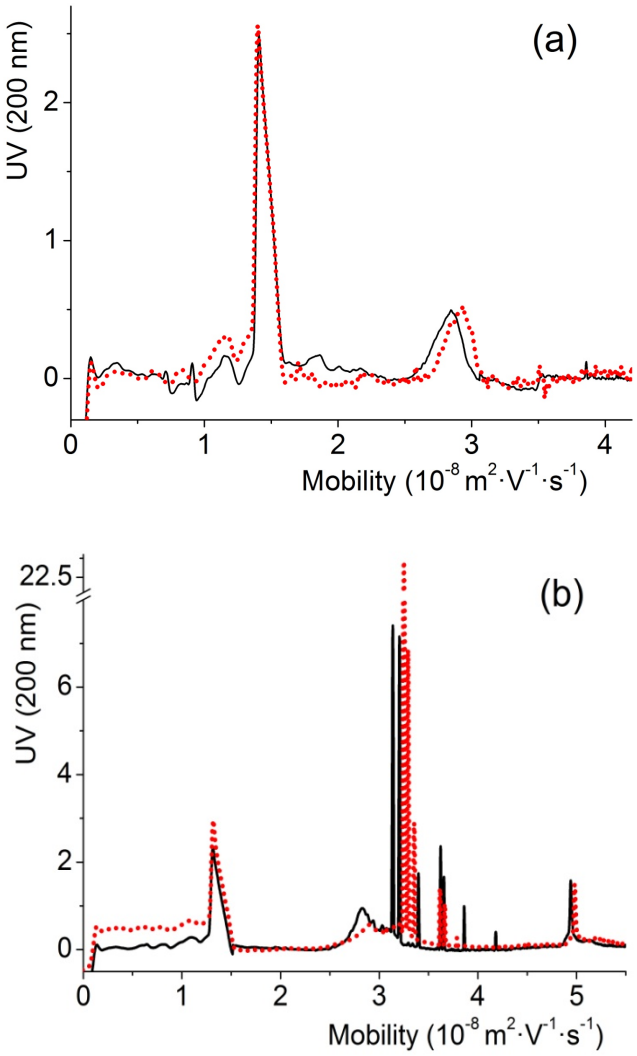
Fig. 7. Electropherograms (series 3) of sample LAGG-Exp (a) and HAGG (b): in potassium borate buffer 150 mM (solid black line), in sodium borate buffer 200 mM (dotted red line).

Finally the peaks around 3×10^{-8} $m^2 \cdot V^{-1} \cdot s^{-1}$ may correspond to the gellan gum chains in the expected random coil conformation. This peak is broad for low acyl gellan gums and significantly broader for the high acyl gellan gums. This is attributed to separation by the degree(s) of acylation in CE-CC.

Uncontrolled Degradation of Gellan Gum.

As gellan gum is a natural exopolysaccharide produced by a bacterium and it is expected to be easily degradable in normal environmental conditions, it is usually kept at low temperatures.[1,2] Samples of gellan gum were left on a bench in summer where they experienced temperatures up to 40 ºC for about

14 days. It is expected that under these conditions the gellan gum structure would experience degradation. Fig. 8a depicts a comparison of two LAGG samples, one of which has been exposed to high temperatures. Exposure to 40 °C does not significantly change the mobilities (the second peak's electrophoretic mobility is only 2.8 % lower). The relative intensity of the first peak however significantly increases with exposure to temperature and leads to the higher ratio observed in this work (Fig. 6). Exposure to 'high' temperature thus likely leads to some degradation leading to oligomers formation.



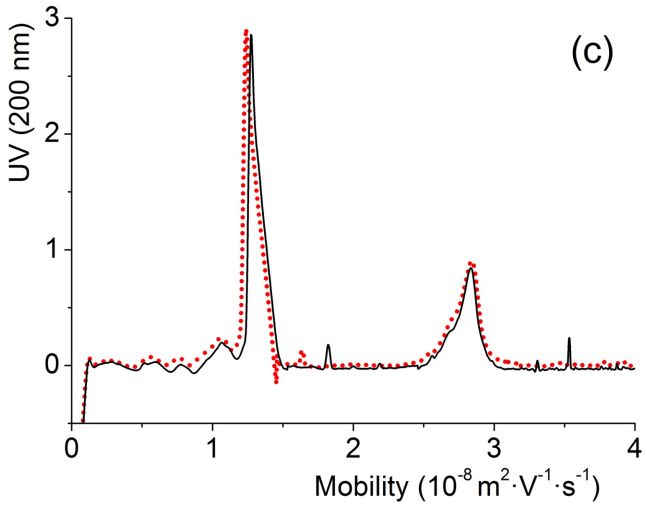


Fig. 8. Electropherograms of samples before and after potential degradation (first injection series): (a) LAGG before and after exposure to temperatures of up to 40 °C (samples LAGG-Exp and LAGG-Tem, solid black line and dotted red line, respectively), (b) HAGG before and after expiry date (samples HAGG and HAGG-Exp, solid black line and dotted red line, respectively), (c) non endotoxin-free low-acyl LAGG-Exp (black solid line) and endotoxin-free low-acyl LAEFGG-Exp (red dotted line)

Lapse of time is also expected to cause gradual natural degradation of gellan gum. Samples that have exceeded the manufacturer's expiry dates were kept in the same conditions and compared to the current gellan gum samples (Fig. 8b). No significant change is observed in terms of electrophoretic mobility (the largest shift, observed for the second peak, is of 2.1% of the mobility value and may be due to different baselines). No significant increase of the relative area of the first peak is however observed (Fig. 6). This confirms the literature where osmometry showed no change of molecular weight after two years storage of deacetylated gellan gums.[7].

Endotoxin-free (low-acyl) gellan gum LAEFGG was compared to non-endotoxin free (low-acyl) gellan gum LAGG-Exp in order to determine if the treatment done to remove the endotoxins has any effect on the gellan gum structure (Fig. 8c). LAEFGG's peaks have a similar intensity and electrophoretic mobility compared to LAGG-Exp's corresponding peak. The maximum difference is again observed on the second peak although the LAEFGG's peak has an electrophoretic mobility only 2 % lower: 2.78 compared to 2.84 × 10^{-8} $m^2 \cdot V^{-1} \cdot s^{-1}$. A comparison of the relative intensity of the first

peak in LAGG-Exp and LAEFGG-Exp (Fig. 6) shows that the treatment done to gellan gum to remove the endotoxins has no effect on its oligomers content or composition that can be observed with CE.

Sonication of Gellan Gum.

A controlled sample treatment technique that has a possible effect on the structure of gellan gum was also examined. Sonication is a useful technique that enables conducting fillers such as carbon nanotubes to be effectively dispersed within a gellan gum solution.[3] However, it has been observed that sonication has a detrimental effect on further processing of gellan gum-carbon nanotubes composites, impeding hydrogel formation[4] and fiber spinning[45]. This has been attributed to a sonication-induced reduction in molecular weight of gellan gum.[4] Fig. 9 displays the apparent viscosity of as-prepared and sonicated solutions of low-acyl and high-acyl gellan gums. An as-prepared solution of HAGG exhibits a relatively high viscosity and shear-thinning behavior, while LAGG-Exp has a lower viscosity which is independent of shear rate across the measured range. This difference is attributed to the higher degree of acylation of HAGG, with more acyl groups promoting stronger hydrogen bonding, which increases viscosity and imparts some pseudo-plasticity. Sonication significantly reduces the viscosity of both LAGG and HAGG, enforcing the conclusion that the process leads to a reduction in molecular weight. Interestingly, the reduction in viscosity is more marked for HAGG – the viscosity of both sonicated solutions is comparable and the sonicated HAGG is no longer shear-thinning.

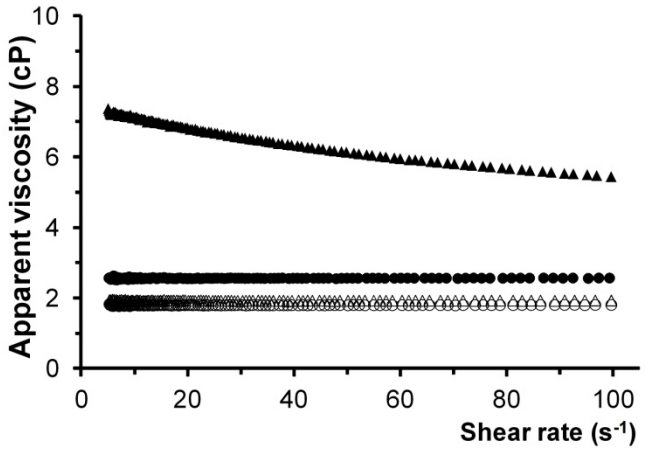
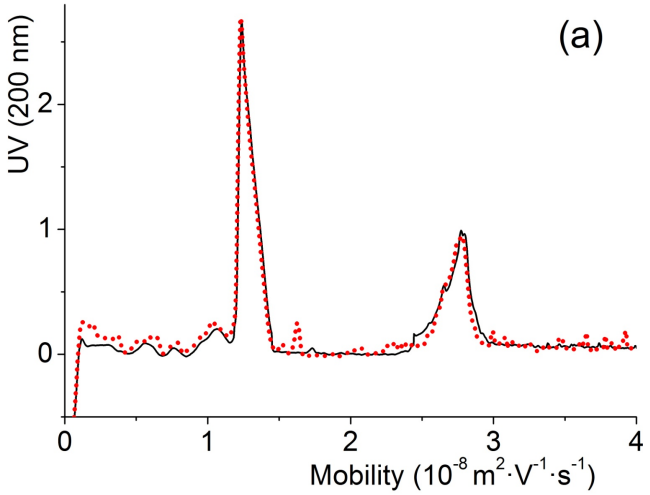


Fig. 9. The apparent viscosity of as-prepared (full symbols) and sonicated (empty symbols) solutions of 0.5 g·L^{-1} of LAGG (circles) and HAGG (triangles) in water.

To investigate this further, the structure of sonicated gellan gum solutions was measured using CE (Fig. 10). The effect of sonication on the gellan gum structure of LAFEGG is illustrated in Fig. 10a. Both peaks of each injection appear very similar. However, the relative intensity of the first peak significantly increases with sonication of sample LAEFGG (Fig. 6), suggesting that sonication leads to some degradation into oligomers in the low-acyl gellan gum. The effect of sonication on the structure of HAGG is shown in Fig. 10b. The spikes consisting in aggregates disappear after sonication. This indicates that the stronger decrease in viscosity for the high acyl than the low acyl is not due so much to a decrease of the molecular weight of gellan gum chains but rather to a decrease in apparent molecular weight, or in other words an aggregate dissolution. The relative intensity of the first peak does not increase after sonication for the high-acyl gellan gum, indicating the absence of significant degradation of the polymer chain themselves into oligomers. The stronger decrease of viscosity in HAGG than in LAGG may be due mainly to dissolution of aggregates of high-acyl gellan gums chains rather than deacylation.



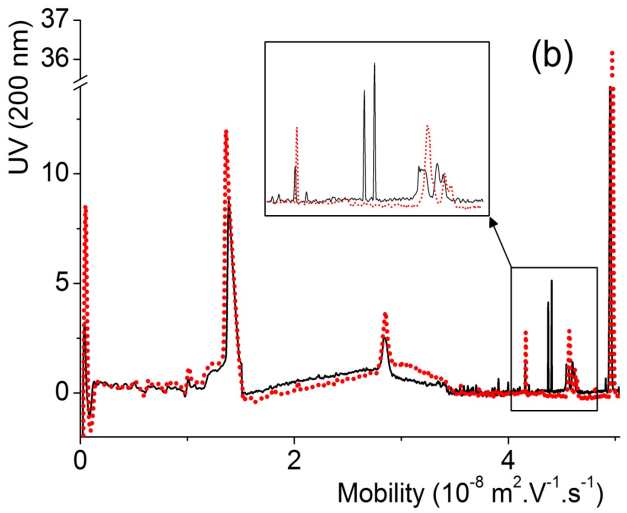


Fig. 10. Comparison of gellan gum before and after sonication (solid black line and dotted red line, respectively): (a) LAEFGG (samples LAEFGG and LAEFGG-Son, first injections series), (b) HAGG (samples HAGG and HAGG-Son, third injections series).

Conclusion

Capillary electrophoresis in the critical conditions (CE-CC) was applied for the first time for the characterization of gellan gum. The method is fast (less than 10 min) and was validated in terms of sample preparation and repeatability. The proof-of-principle was established for the successful separation of gellan gum in regards to its degree of polymerization in the case of oligomers and its degree of acylation for polymers, as well as the conformation (random coil vs double helix). The separations are unaffected by the presence of microgels, as there is no stationary phase or light scattering detection. The microgels can be observed and slow dissolution monitored. Dilute solutions of high-acyl gellan gums contain aggregates that slowly dissolved. Five days are the recommended dissolution time, although it does not provide yet full dissolution, The effect of sonication, endotoxin removal, expected degradation stresses (mainly heat) and exceeded expiry dates on gellan gum were tested. Exposure to high temperatures leads to some degradation of the gellan gum into oligomers, contrary to endotoxin removal treatment. Sonication mainly changes the high-acyl chains through the dissolution of microgels/aggregates. The detection of aggregates initially in high-acyl gellan gum but not in low-acyl ones explains a stronger viscosity decrease for the high-acyl gellan gum.

The CE-CC methods developed in this work proved a valuable tool to determine differences in composition of gellan gums samples. It is complementary to rheology. CE-CC could be applied to a wider variety of gellan gum samples with applications in food or tissue engineering. In particular, it will be valuable for the characterization of the natural variability of gellan gum.

Experimental

Materials.

Boric acid and sodium hydroxide pellets (Sigma) were used as received to prepare a 200 mM sodium borate buffer at pH 9.2 (NB200). Boric acid (BDH AnalaR) and potassium hydroxide flakes (Unilab) were also used as received to prepare a 150 mM potassium borate buffer at pH 9.2 (KB150). Ammonium acetate (Scharlau) and a 28% ammonia solution (Fronine Pty LTD) were used as received to prepare a 75 mM ammonium acetate buffer at pH 9.2 (AA75). Gellan gum samples produced by fermentation of a pure culture of *Sphingomonas elodea* were a gift from CP Kelco. All samples were prepared with gellan gum powder added to hot, approximately 80 ºC, Milli-Q water at 1 $g{\cdot}L^{-1}$, and stirred for 1 h. This yielded a clear solution for low-acyl gellan gum, but a whitish suspension for high-acyl gellan gum (see Fig. S11 in supporting information). All low-acyl gellan gum samples where dissolved in Milli-Q water, while the high-acyl samples were dissolved in 1/20 v/v NaOH and NB200 solutions in Milli-Q water, and treated at 60 ºC for 1 h. The samples were not filtered except if stated otherwise. Millipore filters were either 0.45 μm (Millex HV, PVDF membrane) or 0.22 μm (Millex GP, PES membrane). The high-acyl gellan gum samples used in sonication treatment were dissolved in Milli-Q water (1 $g{\cdot}L^{-1}$ and 0.5 $g{\cdot}L^{-1}$). Due to gellan gum's gelling properties and thermal instability, samples are required to be kept at low temperatures in order to preserve the gellan gum and prevent dissociation. Samples were stored in a fridge unless otherwise specified. The different gellan gum samples are described in Table 1. The low-acyl gellan gum LAGG is (almost) fully deacetylated as shown by the weak acetyl signal at 2 ppm in ^{1}H NMR[46] (Fig. S14). Unfortunately, NMR and other common analytical techniques are unable to yield information on the glycerate group content. The ^{1}H

NMR spectrum also shows no significant impurity such as residues from bacteria or from the fermentation medium.

Table 1. Gellan gum samples.

LA and HA stand for low and high degrees of acylation, respectively; EF stands for Endotoxin-free.

Sample	Description	Treatment	Catalog name	Lot number	Expiry
LAGG-Exp	LA	Expired	KELCOGEL-F	7C9228A	26/3/08
LAGG-Tem	LA	Kept at up to 40 ºC	KELCOGEL-F	7C9228A	26/3/08
LAEFGG	LA, EF		GELZAN CM	9K6968A	20/11/11
LAEFGG-Son	LA, EF	Sonicated	GELZAN CM	9K6968A	20/11/11
LAEFGG-Exp	LA, EF	Expired	GELZAN CM	7K1383A	19/11/08
HAGG	HA		KELCOGEL-LT100	9K6878A	5/11/11
HAGG-Son	HA	Sonicated	KELCOGEL-LT100	9K6878A	5/11/11
HAGG-Exp	HA	Expired	KELCOGEL-LT100	814441A	29/9/09

Methods.

The capillary electrophoresis experiment was carried out using an Agilent CE 7100 (Agilent Technologies). The capillaries used were 50 μm ID fused silica capillaries (Polymicro, USA). High-sensitivity ("bubble") capillaries were not needed contrary to the literature[17] and only regular capillaries were used. The pretreatment of the capillary included an initial 10 min flush with 1 M NaOH, 5 min flush with 0.1 M NaOH and a final 10 min pretreatment flush with Milli-Q water. This wash was also used after the series in order to clean the capillary. For the latter series of injections extra wash sequences were added after every two or three injections and after every high-acyl gellan gum sample injection. These extra wash sequences were the same as the initial pretreatment flush sequences. The standards used to validate the capillary and act as a reference for the gellan gum sample injections were a model oligoacrylate, AA4, and acetone.[23-24] Both standards and all samples were injected into a 200 mM sodium borate buffer of (NB200). The capillary post-treatment was a flush for 10 min with NaOH and then again with Milli-Q water. Four series of injections were completed, each with different parameters, in sodium borate buffer 200 mM unless otherwise specified. The set conditions for both

injection series are illustrated in Table 2. All data was recorded at 200 nm and treated using Origin 7 plotting intensity versus mobility. Electrophoretic mobility was calculated.[23]

Table 2. Experimental conditions for the four injection series

Parameter	Series 1	Series 2	Series 3	Series 4
Capillary total length L (m)	0.645	1.20	1.00	1.20
Capillary detection length l (m)	0.56	1.115	0.915	1.115
Voltage (kV)	20	25	30	20
Temperature (ºC)	55	55	55	30
Injection Pressure (mbar)	30	30	30	30
Injection Time (s)	10	20	10	20

Sonication of samples was performed using a Branson digital probe sonifier. 10 mL of 0.5 $g \cdot L^{-1}$ samples were subjected to 5 min of pulsed sonication (0.5 seconds on/off) at 30 % amplitude. The apparent viscosity of as-prepared and sonicated samples was measured at 25 °C on an AR-G2 controlled stress rheometer (TA Instuments) using a 60 mm (2°) cone and plate geometry. Shear rate was ramped from 5 to 100 s^{-1} over a measurement period of 5 min.

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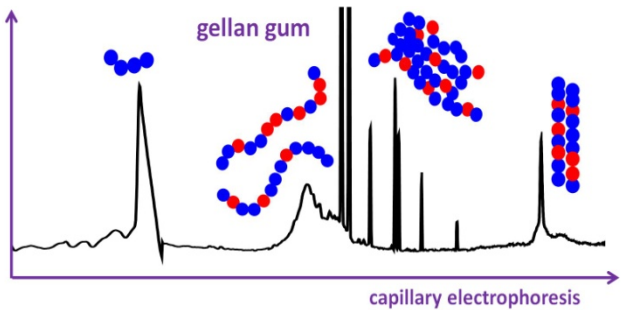

Gellan Gum is a food thickener and emulsifier, and it has potential in tissue engineering. Free-solution capillary electrophoresis reveals the presence of oligomers and also separates it according to composition and conformation, even in the presence of aggregates. It provides a tool to assess natural variability, dissolution, ageing and sonication.