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Fracturing Fluid Cleanup by Controlled Release of Enzymes from Polyelectrolyte Complex Nanoparticles

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Abstract

Water-based polymer gels are used widely in the oil and gas industry to viscosify fluids used in hydraulic fracturing of production wells, where they serve to increase the force applied to the rock, and improve the transport of proppants used to maintain the fracture after formation. After fracturing, the gel must be degraded to a low viscosity using enzymes or gel-breakers. Existing systems add the breaker either directly to the gelant, or encapsulated in beads that are crushed when the applied pressure is released and the fractures close. In the former case the gel may be broken prematurely, preventing efficient fracture propagation and proppant transport, whereas in the latter case, the breaker may not be uniformly distributed throughout the gel, with the result that the gel is incompletely broken and the hydraulic conductivity of the well is reduced. To obtain delayed release, combined with homogeneous distribution of enzyme throughout the gel, polyethylenimine-dextran sulfate polyelectrolyte complexes (PECs) were used to entrap pectinase. Such particles were originally developed to entrap pharmaceuticals and this group has previously demonstrated their ability to delay the release of gel crosslinking agents for oilfield applications. Degradation of both viscosity and viscoelastic moduli of borate-crosslinked guar gel by pectinase loaded in polyelectrolyte nanoparticles was delayed by up to 12 hours, compared to about two hours for equivalent systems where the pectinase was not entrapped. The combination of homogeneous mixing and delayed release of enzymes packaged in PEC nanoparticles (PECNPs) shows promise for improved cleanup after hydraulic fracturing.

Introduction

The rate of oil and gas production from a well is often limited by the hydraulic conductivity of the rock immediately surrounding the production well bore. Hydraulic fracturing increases the surface area contributing to production. In hydraulic fracturing, a viscous polymeric solution is injected onto a production well at high pressures to create and propagate a fracture and to transport propping agents (proppants).[1] A proppant such as sand is used to keep the fracture open after the injection. The fracturing fluid is usually a water-based polymer which forms filter cakes with high polymer concentration on the two faces of the fracture during the treatment.[2,3] The filter cake must be broken completely after the injection to attain high conductivity when the well is placed on production. Breakers (e.g. enzymes, oxidizers) are added to the fracturing fluid to degrade the polymer remaining in the fluid and the filter cake.

Even though fracturing jobs are shifting towards lower viscosity "slick water" treatments[4] for unconventional reservoirs, guar gum and its derivatives are still commonly used to viscosify water in fracturing fluids for treatments in conventional wells. Guar, a polysaccharide comprising a (1→4)-linked β-D-mannose backbone with (1→6)-linked α-D-galactose residues, can be gelled with borate ions complexing with the hydroxyl groups on the galactose (Figure 1). The ether bonds between the sugar units on the backbone can be degraded by enzymes which are often used as a breaker because of their low cost and because, unlike chemical breakers, they are not consumed by the reaction, and so are effective at lower concentrations.

High concentrations of enzymes can cause premature degradation of fracturing fluids. To overcome this, a delayed release or encapsulation of the breakers has been used.[1,5] A low concentration of encapsulated breakers causes the filter cake, in particular, to break nonuniformly.[2,3,6] Delayed enzyme breakers have been developed for low pH values and cannot delay the breaking time significantly. An ideal application would be to distribute high concentrations of breakers (such as enzymes) homogeneously throughout the gel and filter cake at microscopic length scales, with controlled or delayed release.

An encapsulation method originally developed for drug delivery applications,[9] polyethylenimine (PEI) – dextran sulfate (DS) polyelectrolyte complex nanoparticles (PECNPs), was developed for oilfield application and, for instance, can control the release of Cr(III) cross linker to form polyacrylamide gels for conformance control.[7-8] We hypothesize that the nanoparticle system may also be capable of entrapping and releasing pectinase in a controlled manner with the potential for application in breaking fracturing fluids.[7] To use such a carrier for breakers in fracturing fluids would require high entrapment efficiency of breaker, homogeneous distribution and flexible release time.

We present a proof of concept study for the application of PEC nanoparticles to entrap and release enzyme breakers for fracturing fluids.[10] Positively charged PEC nanoparticles were made by varying the total concentration and charge of a polycation (polyethylenimine) and a polyanion (dextran sulfate).[8] An enzyme used in the petroleum industry to break the fracturing and drilling fluids was added to the nanoparticles either before or after the addition of dextran sulfate (DS) and was entrapped in the PEC nanoparticles. Entrapment efficiency (EE) was calculated for the nanoparticles loaded with enzymes using a viscometric assay and confirmed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Nanoparticles were then used to degrade borate-cross-linked guar solutions at 25 °C. Different delay times were observed in the activity of enzymes for different polyethylenimine (PEI): dextran sulfate (DS) ratios. Retardation of enzyme activity was observed by measuring the viscosity of the gelled guar and by monitoring the viscoelastic moduli of the gel over time. Delay times were compared with equivalent systems using unentrapped enzymes. Note that the pectinase used in this study is active in environments with maximum pH of 10 at 25 °C and pH of 6 at 60 °C. Temperature and pH-tolerant enzymes are typically used in the field, however a more sensitive enzyme was selected to test the hypothesis that entrapment would protect the enzyme from extremes of environmental conditions.

Materials and Methods

Materials: Chemicals were used as supplied: Sodium hydroxide, 1N hydrochloric acid, dextran sulfate (M_w=500 kDa), polyethylenimine (M_w=25kDa), sodium thiosulfate pentahydrate, potassium chloride and sodium hydrate (Fisher Scientific Pittsburgh, PA), guar and pectinase from *Aspergillus aculaceatus* (Sigma-Aldrich, St. Louis, MO), hydroxypropyl guar (HPG) gum blend (Jaguar® 415, Rhodia, Paris, France), sodium borate (J.T. Baker Chemical Co., Phillipsburg, NJ), NuPAGE 4-12% Bis-Tris Gel (1.5 mL×10 lanes), NuPAGE MES SDS running buffer (20×), NuPAGE LDS sample buffer (4×), MARK12 unstained buffer (1×) – (Invitrogen, Carlsbad, CA).

Preparation of Polyelectrolyte Complex Nanoparticles: Nanoparticles were made using different ratios of PEI:DS using the method presented previously.[8] In a typical

formulation, 1 mL of a 1% w/w aqueous solution of DS (pH=7.8) was added dropwise to 2 mL of a 1% w/w aqueous solution of PEI (pH adjusted to 8 using 4N HCl) while stirring. The solution was then stirred for 20 minutes at 600 rpm unless otherwise indicated. Pectinase was added dropwise as 0.1 mL of a 25% w/w pectinase solution either before or after the DS (Table 1). Pectinase-loaded nanoparticles were used as a breaker in polymer systems at a final concentration of 0.1% w/w pectinase.

Table 1 Nanoparticle systems differed in the ratio of PEI, DS, and pectinase and order of addition.

System	1% w/w PEI(aq),mL	1% w/w DS(aq), mL	25% w/w Pectinase(aq), mL	Order of Addition
A	2.0	1.0	0.10	PEI, DS, Pectinase
A´	2.0	1.0	0.10	PEI, Pectinase, DS
B	3.0	1.0	0.10	PEI, DS, Pectinase
B´	3.0	1.0	0.10	PEI, Pectinase, DS
C	4.0	1.0	0.10	PEI, DS, Pectinase
C´	4.0	1.0	0.10	PEI, Pectinase, DS
D	2.0	1.0	0.07	PEI, DS, Pectinase
E	3.0	1.0	0.06	PEI, DS, Pectinase

Size and Zeta Potential Measurement of Polyelectrolyte Complex Nanoparticles: A ZetaPALS zeta potential analyzer (Brookhaven Instruments Corp., Long Island, NY) was used to measure the mean particle size of nanoparticles. Samples of nanoparticles were diluted approximately 40× by volume with deionized water. Recorded data were the average of three measurements by detecting light scattering at a 90° angle. The zeta potential was also measured by phase analysis light scattering using the same instrument. Samples were diluted approximately 20× with 1.0 mM KCl solution. Three measurements were averaged for each sample. Zeta potential was estimated using the Smoluchowski approximation from the previously measured hydrodynamic diameter and the electrophoretic mobility of the nanoparticles.

Separation of Nanoparticles: Samples of the enzyme-loaded nanoparticles were centrifuged at 14000 *g* for 1.5 hour at 4 °C. Supernatants were then separated from the nanoparticles for activity measurements.

Determination of Enzyme Activity for Nanoparticles and their Supernatants: 2.0 g of either 5000 ppm guar or HPG solution was mixed with 0.5 g of the diluted nanoparticles or supernatants at room temperature. After mixing, 1.5 mL of the solution was placed between the plates of a parallel plate Bohlin CS rheometer (Malvern Instruments, Malvern, England) and the viscosity of the solution was measured over time at 25 °C. The time for the viscosity of the solution to fall to 50% of its initial value ($t_{1/2}$, hours) was used to calculate the activity of the nanoparticles after the method reported by Bell et al. (1955).[11] Activity was defined as the reciprocal of $t_{1/2}$. Activity of the free enzyme was also calculated using the same method. Entrapment efficiency based on enzyme activity, EE_A, was calculated using the activities of the supernatant and free enzyme as in Eq.1.

$$EE_A = \frac{A_E - A_S}{A_E} \times 100\% \quad \text{.....Eq.1}$$

Where A_E is the activity of the equivalent enzyme concentration added to the nanoparticles and A_S is the activity of the supernatant.

Determination of Enzyme Concentration using SDS-PAGE: SDS-PAGE analysis was performed using a NuPAGE® kit, following the manufacturer's instructions. SDS-PAGE is a type of gel electrophoresis technique which uses the sodium dodecyl sulfate to separate proteins by size, independent of their net charge. This technique works by binding of the negatively-charged SDS molecule to hydrophobic side chains of a protein. On average, one SDS molecule binds to every two residues of a typical protein, giving it a large overall negative charge proportional to the length of protein.[12] Samples including several concentrations of pectinase and supernatants were first diluted (30 μL of samples + 10 μL of buffer). One lane was loaded with a mixture of protein markers of known molecular weights in order to calibrate the gel and determine the weight of unknown proteins. After the electrophoresis, the gel was stained, destained and optically measured. The area under the intensity-distance curve was measured for different intensity peaks.

Calibration curves of peak area against enzyme concentration were generated to determine the concentration of enzyme in the supernatants. The entrapment of enzyme in the nanoparticles based on concentration, EE_C, was calculated using Eq.2.

$$EE_C = \frac{C_E - C_S}{C_E} \times 100\% \quad \text{.....Eq.2}$$

Where C_E is the enzyme concentration added to the nanoparticle system and C_S is the concentration of enzyme in the supernatant.

Preparation of Guar and HPG Solutions: Sufficient mass of polymer to create a 5000 ppm guar or HPG solution was added slowly to the shoulder of a vortex of a vigorously (800 rpm) stirred solution of 2% KCl and 1.35 g/L of sodium thiosulfate in a 1-liter beaker. The solution was stirred for 5 minutes after the addition of guar. Then the stirring rate was reduced to 600 rpm for another hour and allowed to become hydrated for another 24 h at 200 rpm. Guar solutions were centrifuged at 9600 *g* for 1.5 hours at 25 °C in order to separate the residue.[14]

Preparation of Borate-Crosslinked Guar/HPG: 24 mL of the 5000 ppm HPG or guar was mixed with 7.5 mL of diluted enzyme-loaded nanoparticles or an aqueous solution with equivalent concentration of pectinase (final concentration = 0.1% w/w pectinase). 7.5 mL of a 1000 ppm borax aqueous solution was added to the mixture and the pH was adjusted to 8.8 using 0.1 M NaOH. Gel samples were incubated on a table shaker (LAB-LINE 3520 JR, Melrose, IL) at 150 rpm and 25 °C.

Measurement of Viscosity and Viscoelastic Moduli

Guar/HPG Solution: A Bohlin CS10 rheometer was used to measure the viscosity of guar and HPG solutions. All the viscometric assays were performed under a shear rate of

90 s^{-1}, except for the shear sensitivity analysis, in which viscosities were also measured at 180 and 270 s^{-1}.

Gelled Guar: A digital cone-and-plate viscometer (DVII+ Pro, SP-40 0.8° cone, Brookfield Engineering, Middleboro, MA) was used to monitor the viscosity of the gelled guar at 0.6 rpm (4.5 s^{-1}) versus time. This technique is convenient due to the closure surrounding the platens that retain the gel in place, and is a method of choice in industry. It is included here to allow comparison to existing data. However, viscosity is not strictly a property of gels and so in addition, a Bohlin CS10 rheometer was used to measure the elastic (G′) and viscous (G″) moduli for 30 mL gel samples over time at a frequency of 1 Hz, strain of 0.1 Pa, and initial stress of 0.1 Pa in "auto-stress" mode. The double-gap configuration of Couette geometry was used to measure G′ and G″.

Results and Discussion

Preparation and Characterization of Nanoparticles

Preparation of Pectinase-Loaded PEC Nanoparticles: Formulations varied in PEI:DS ratio and in the order of addition as shown in Table 1. Repeatability of the formulation procedure was demonstrated by preparing 10 samples under identical conditions and measuring their size over time (Table 2). Size and zeta potential were measured periodically to demonstrate the stability of the nanoparticles over time (Table 3). The size and charge of the nanoparticles decreased with increasing PEI:DS ratio.

Nanoparticles were diluted to reach a specified enzyme concentration. In addition, 1 mL samples of undiluted nanoparticles were centrifuged and the supernatants were diluted by the same dilution factor (8× for Systems *A* and *A*′; 6× for Systems *B* and *B*′). Viscometric assay was performed for nanoparticle suspensions, supernatants and enzymes with concentrations equivalent to those of diluted nanoparticles. Table 4 shows EE of nanoparticles with different ratios of PEI:DS loaded with pectinase. Nanoparticle systems with a 2:1 PEI:DS ratio (Systems *A* and *A*′) showed the highest EE of the systems prepared using 0.1 mL of pectinase. Entrapment efficiency (EE_A) of the nanoparticles decreased with increasing PEI:DS ratio in the range studied. Nanoparticles which were prepared by adding pectinase to the PEI before addition of DS (Systems *A*′ and *B*′) showed higher EE_A comparing to the nanoparticles prepared by addition of pectinase to pre-formed PEI-DS nanoparticles (Systems *A* and *B*).

To increase EE_A, the amount of pectinase added to the nanoparticles was reduced from 0.1 mL to 0.07 mL and 0.06 mL of 25% w/w pectinase for A and B nanoparticles. Nanoparticles prepared with less pectinase (Systems *D* and *E*) showed improved EE_A s of 88% and 81% respectively.

Table 2 Mean diameter vs. time for 10 samples of nanoparticles A

Time, h	Mean Diameter, nm	Standard Deviation
0	461	10.2
1	473	13.8
2	463	16.9
5	473	16.1
13	472	12.4
24	460	15.1

Table 3 Mean particle diameter and zeta potential (ζ) vs. time for nanoparticles, pH=8.7

Nanoparticle System	8 hours		32 hours	
	Diameter, nm	ζ, mV	Diameter, nm	ζ, mV
A	433	29.4	408	35.0
A′	435	36.3	424	34.9
B	370	28.3	362	29.6
B′	313	28.2	292	24.3
C	250	27.4	238	17.5
C′	239	18.7	235	10.4

Table 4 Activity and entrapment efficiency of pectinase-loaded nanoparticles and their supernatants using viscometric assay (T=25 °C, pH=8.8). Centrifuged 5000 ppm guar prepared in de-ionized water was used for this assay.

Nanoparticle system	Activity of nanoparticle suspension, 1/h	Activity of supernatant, 1/h	EE_A, %
B	1.24	1.11	60
A	0.87	1.00	65
B′	0.91	0.75	73
A′	0.68	0.70	75
D	1.75	0.86	88
E	1.33	1.00	81

SDS-PAGE was used to measure the molecular weight of the pectinase and to compare PEI, DS, pectinase and supernatants from centrifuged nanoparticle samples. (Figure 2, lane contents reported in Table 5). A marker containing proteins of a range of known molecular weight was run in Lane 1. Lane 5 shows the molecular weight distribution of pectinase. Distinct peaks were seen at 116 kDa and 66 kDa. PEI travelled (Lane 2) off the end of the gel suggesting a low MW. Lane 10 is the supernatant of the 2:1:0.1 nanoparticles (PECNP system A), in which low concentrations of pectinase were observed. Note that Lane 3 shows no peaks since the NuPAGE 4-12% Bis-Tris Gel accepts proteins with maximum M_W of 200 kDa while M_W of DS is 500 kDa.

Table 5 Samples in SDS-PAGE lanes

Lane	1 MW Marker	2 Diluted PEI	3 Diluted DS	4 BSA	5 Pectinase	6 Diluted PEI + pectinase	7 Diluted DS + pectinase	8 Diluted pectinase	9 50:50 PEI:pectinase	10 Supernatant (A)
MW marker	3.1	—	—	—	—	—	—	—	—	—
25% w/w pectinase	—	—	—	—	3.1	0.1	0.1	0.1	1.55	0.1
1% w/w PEI	—	2.0	—	—	—	2.0	—	—	1.55	2.0
1% w/w DS	—	—	1.0	—	—	—	1.0	—	—	1.0
BSA	—	—	—	3.1	—	—	—	—	—	—
D.I. Water	—	1.1	2.1	—	—	1.0	2.0	3.0	—	—

SDS-PAGE was also performed for different pectinase concentrations (gel not shown) and the area of the most distinct absorbance peak (~ 116 kDa) of pectinase was plotted versus pectinase concentration to yield a calibration curve. The area under the intensity-distance peak for the supernatant of the nanoparticle systems was correlated to the concentration of enzyme.

The concentration of pectinase in the supernatant, measured by SDS-PAGE, was used to calculate EE_C of the nanoparticles from Eq.2. Calculated entrapment efficiencies were verified by measuring the activity of the enzyme in the supernatant (Eq.1). Entrapment efficiencies determined from concentration were slightly higher than those calculated from enzyme activity.

Table 6 Entrapment efficiencies and enzyme concentrations for different supernatants using the first intensity peak

Polyelectrolyte Complex Nanoparticle System	Pectinase concentration in nanoparticle suspension, % w/w	Pectinase concentration in supernatant, % w/w	Entrapment efficiency, %
A	0.81	0.22	72
B	0.61	0.23	62

Effect of Shear during the Preparation of Pectinase-Loaded Nanoparticles: Batches of nanoparticle system *A* were prepared while stirring at 300, 600 and 900 rpm at 25 °C and pH 8.8. There was no relationship between preparation shear and size, zeta potential or entrapment efficiency of the nanoparticles within this range (data not shown).

Effect of Applied Shear on Activity of Nanoparticles: Viscometric assays were performed on samples from the nanoparticle system *A*, prepared while stirring at 600 rpm. Activity of the nanoparticles was determined from the time required for viscosity of

a 5000 ppm HPG solution in 2% KCl to reach 50% of its starting value. Figure 3 shows that when the shear applied during the experiment increased, the activity of the nanoparticles decreased. However, the activity of the pectinase also decreased with the same trend, indicating that any additional release of pectinase from the nanoparticles caused by increase in the shear is insignificant. Shear rates were chosen to be broadly representative of the shear rates encountered by the gel in the field.[1]

Characterization of Borate Cross-Linked Guar/HPG Gels Degraded by Pectinase-loaded Nanoparticles

A 5000 ppm solution of guar in 2% KCl was used to prepare borate cross-linked gels. Diluted nanoparticles with different ratios of PEI:DS were mixed with the guar before addition of borate. Concentrations of guar, borate and enzyme were the same in all the preparations. The resulting gels were incubated on a table shaker at 150 rpm and 25 °C and samples were removed at intervals for viscosity measurement at a shear rate of 4.5 s^{-1}. Figure 4 shows the viscosity of gelled guar containing pectinase entrapped nano particles using different ratios of PEI:DS. Nanoparticles with pectinase added before addition of DS (Systems *A′* and *B′*) showed later degradation time compared to the nanoparticles where pectinase was added after addition of DS *(*Systems *A* and *B).*

While viscosity is the most commonly measured property during field application, it is difficult to measure viscosity of a gel. In order to further characterize the degradation process, a rheometric study was performed. Pectinase-loaded nanoparticles (final pectinase concentration 0.02 %) with 2:1 ratios of PEI:DS (Systems *A* and *A′*) were mixed with 5000 ppm HPG solution in 2% KCl. Gels were shaken on a table shaker at 150 rpm and 25 °C; samples were removed at intervals and viscoelastic moduli were measured using a Bohlin rheometer. A frequency sweep was performed at different times and viscous and elastic moduli were plotted versus time for a frequency of 1 Hz (Figure 5). Delay in degradation of both moduli was observed. Both G′ and G″ reached values lower than those of an equivalent HPG solution in 10 h, indicating a significant delay in activity of enzyme. The point at which plots of G′ and G″ cross (i.e. the time at which the elastic modulus becomes smaller than the viscous modulus) was shifted towards longer times for the systems degraded with nanoparticles compared to the systems degraded with enzyme represents a delay in transition from gel to solution. Faster decline of G′ compared to G″ is typical for enzymatic degradation of guar solutions since the gel structure is attacked first, followed by the degradation of the guar back bone .[13]

Note that viscosity measurements (Figure 4) showed that gels containing PEC nanoparticles reach the same viscosity values as those degraded using unentrapped pectinase given enough time. The decreasing rate of viscoelastic moduli confirms this result.

Injection of higher enzyme concentrations has the potential to break fracturing fluids and their filter cake more efficiently.[1-3,6] Breakers are required to break the fracturing fluid and formed filter cakes typically in a 6-24 hour period of time depending on the size of the fracturing job and depth of the reservoir. However, if efficient fracturing and proppant transport are to be achieved, premature degradation of fracturing fluids during

the injection time must be avoided. Reversible trapping of enzymes in polyelectrolyte complex nanoparticles by a combination of transient electrostatic binding and steric interactions (electrosteric interactions)[10,15] delays the activity of the enzyme. This may allow the injection of higher enzyme concentrations.

In summary, polyelectrolyte nanoparticles made with PEI:DS ratio of 2:1 (Systems *A* and *A′*) showed good EE for pectinase. They were stable over time and did not degrade with shear in the range studied. Size and EE of the nanoparticles were not strongly correlated with preparation shear. Viscometric assays were performed on the diluted nanoparticles, diluted supernatants and equivalent concentrations of enzyme. EE was calculated after measuring the activity of the supernatant. SDS-PAGE was applied to measure the concentration of free pectinase in the supernatant. EE values measured using viscometric assay are consistent with the SDS-PAGE data. Viscosity of gelled guar was monitored under 4.5 s^{-1} shear rate in the presence of enzyme-loaded nanoparticles. Nanoparticles with 2:1 ratio of PEI:DS (Systems *A* and *A′*) showed the best controlled release of enzyme over time.

Conclusions

Nanoparticle-entrapped pectinase were able to completely break borate-crosslinked guar and HPG gels, with the breaking being delayed significantly compared to unentrapped enzymes at the same concentration. The nanoparticles exhibited high enzyme entrapment efficiency, and were largely insensitive to the shear forces likely to be encountered in the field. The delayed release of the enzyme allows the loaded particles to be mixed with the gelant before gelation occurs. This, along with the small size of the particles means that the enzyme is distributed homogeneously through the gel, which may result in a more complete breakage of the gel and hence higher post-treatment hydraulic fracture conductivity.[16] Polyelectrolyte complex entrapment of gel-breaking enzymes shows promise for improving the performance of hydraulic fracturing treatments in conventional oil and gas reservoirs, but further investigation of enzymes with different pH and temperature optima is required to identify the range of conditions in which the method may be applied.

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Figure Legends

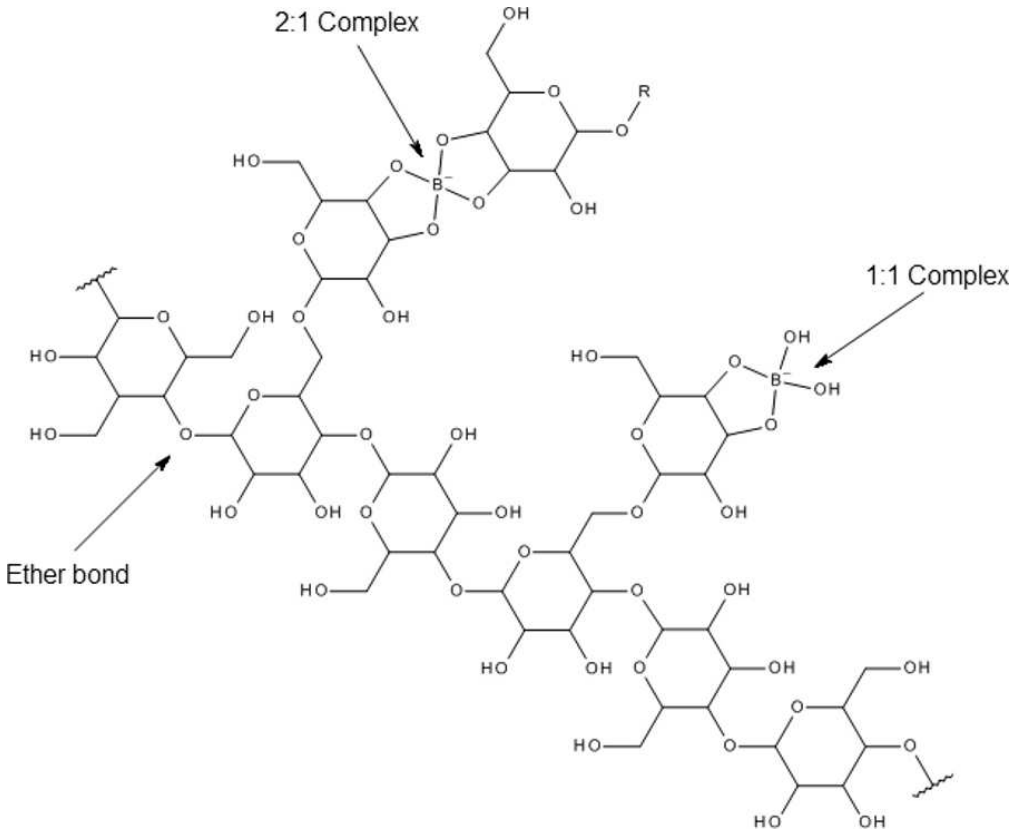
Figure 1 Chemical structure of guar showing mechanism of crosslinking by borate, and ether bond vulnerable to cleavage by pectinase (-R denotes another guar molecule).

Figure 2 SDS-PAGE gel. Contents of each lane are described in Table 2. Numbers on left are molecular weight of marker proteins in Lane 1.

Figure 3 Comparison of effect of shear on the activity of 0.1% pectinase solution and pectinase-loaded nanoparticles (PEC nanoparticle System *A*) at 25 °C and pH 8.8. Activity = 1/time taken to degrade 5000 ppm HPG solution to 50% of its initial viscosity.

Figure 4 Viscosity of borate cross-linked guar gel mixed with 0.1% pectinase or pectinase-loaded PEC nanoparticles vs. time at 25 °C and pH 9. N.B. the viscometer is only able to measure viscosity up to 1028 cP so graph indicates the latest measurement for which viscosity exceeded this value.

Figure 5 G′and G″ vs. time for HPG solution, and gel degraded using either 0.1% pectinase or nanoparticle-entrapped pectinase (PEC nanoparticle System *A*) at 25 °C and pH 9.



Chemical structure of guar showing mechanism of crosslinking by borate, and ether bond vulnerable to cleavage by pectinase (-R denotes another guar molecule).
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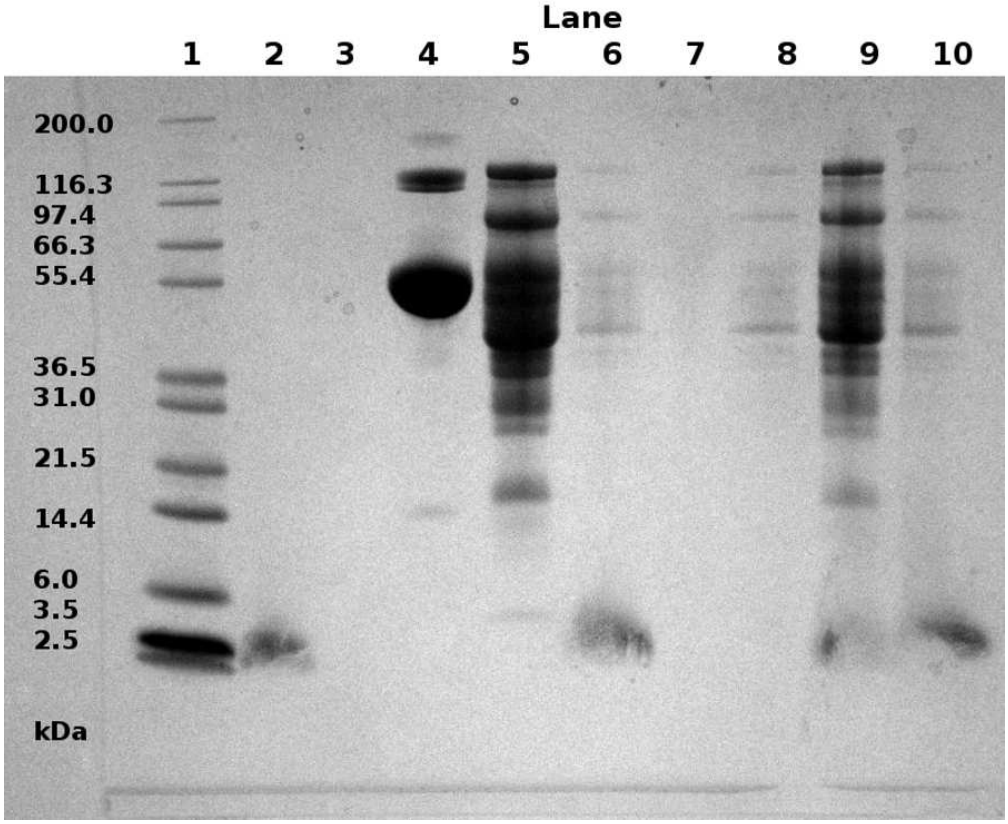
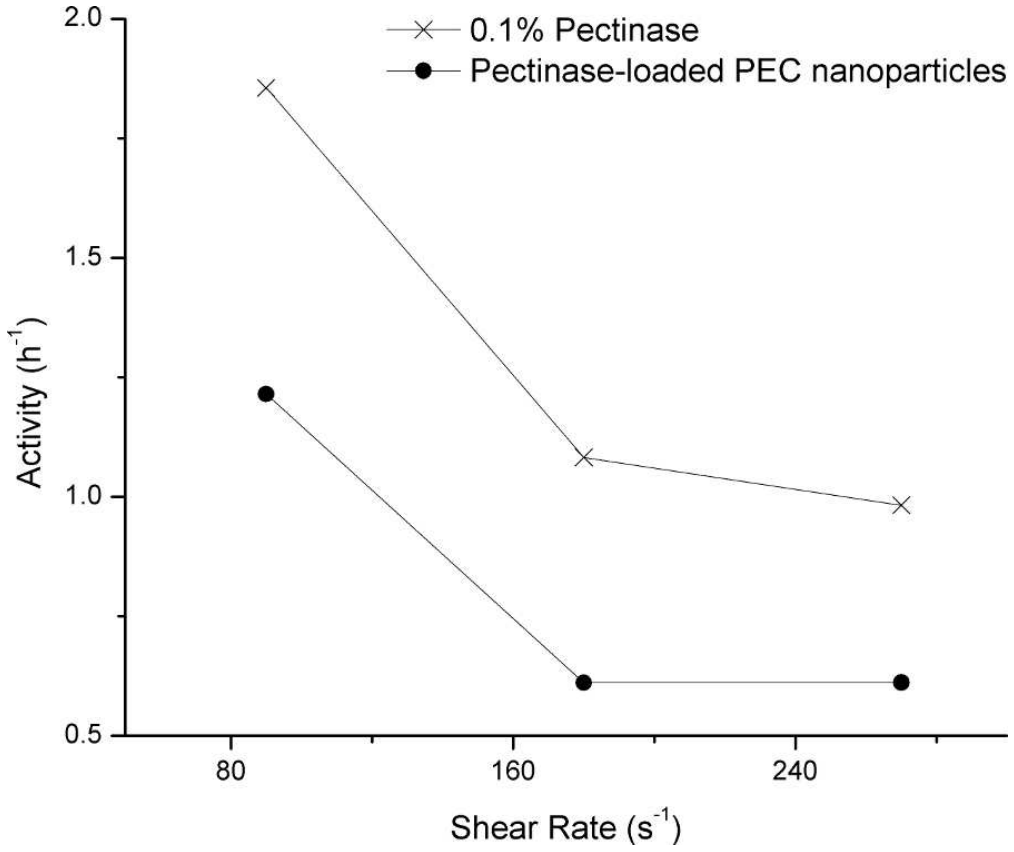
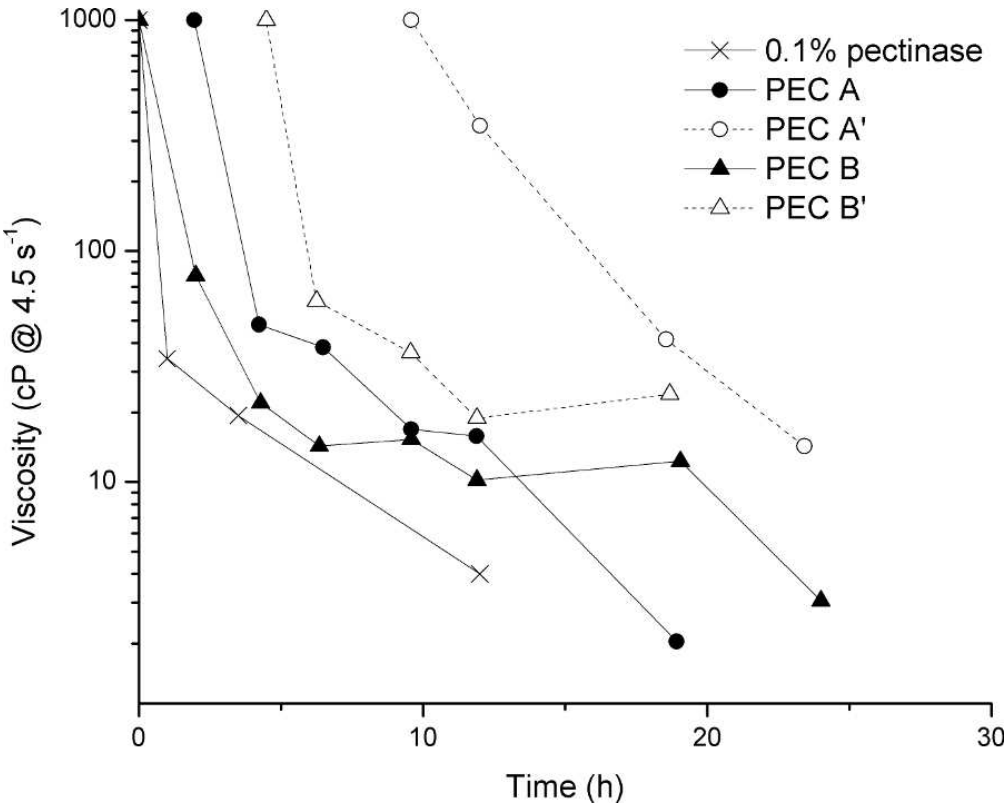


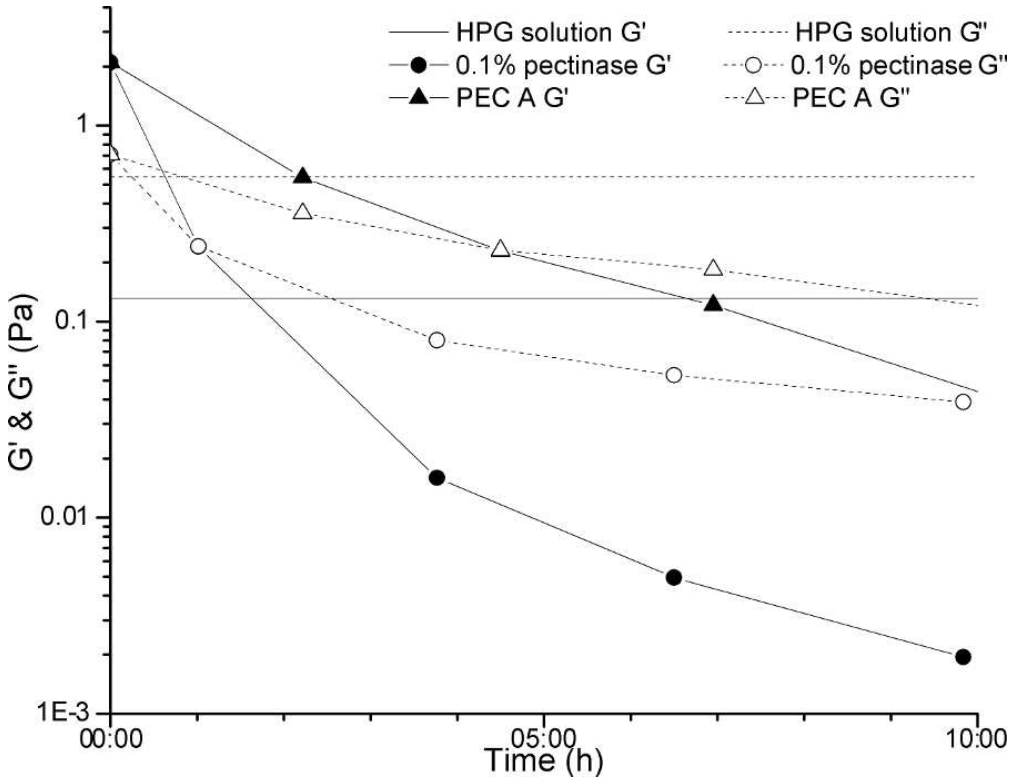
SDS-PAGE gel. Contents of each lane are described in Table 2. Numbers on left are molecular weight of marker proteins in Lane 1.
76x62mm (300 x 300 DPI)



Comparison of effect of shear on the activity of 0.1% pectinase solution and pectinase-loaded nanoparticles (PEC nanoparticle System A) at 25 °C and pH 8.8. Activity = 1/time taken to degrade 5000 ppm HPG solution to 50% of its initial viscosity.
76x63mm (300 x 300 DPI)



Viscosity of borate cross-linked guar gel mixed with 0.1% pectinase or pectinase-loaded PEC nanoparticles vs. time at 25 °C and pH 9. N.B. the viscometer is only able to measure viscosity up to 1028 cP so graph indicates the latest measurement for which viscosity exceeded this value.
76x60mm (300 x 300 DPI)



G´ and G´´ vs. time for HPG solution, and gel degraded using either 0.1% pectinase or nanoparticle-entrapped pectinase (PEC nanoparticle System A) at 25 °C and pH 9.
76x58mm (300 x 300 DPI)