Polyelectrolyte Complex Nanoparticles for Protection and Delayed Release of Enzymes in Alkaline pH and at Elevated Temperature during Hydraulic Fracturing of Oil Wells

Reza Barati,[1] Stephen J. Johnson,[2] Stan McCool,[2] Don W. Green,[1] G. Paul Willhite,[1] Jenn-Tai Liang[1,2]

[1]*Department of Chemical and Petroleum Engineering, The University of Kansas, Lawrence, Kansas 66045*
[2]*Tertiary Oil Recovery Project, The University of Kansas, Lawrence, Kansas 66045*

Received 23 November 2010; accepted 18 January 2012
DOI 10.1002/app.36845
Published online in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: Polyethylenimine-dextran sulfate polyelectrolyte complexes (PECs) were used to entrap two enzymes used to degrade polymer gels following hydraulic fracturing of oil wells to obtain delayed release and to protect the enzyme from harsh conditions. Degradation, as revealed by reduction in viscoelastic moduli, of borate-crosslinked hydroxypropyl guar gel by commercial enzyme loaded in polyelectrolyte nanoparticles was delayed up to 11 h, compared with about 3 h for equivalent systems where the enzyme mixture was not entrapped. PEC nanoparticles also protected both enzymes from denaturation at elevated temperature and pH. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 000: 000–000, 2012

Key words: gels; nanoparticle; enzymes; polyelectrolytes; fracturing fluids; guar; hydraulic fracture cleanup

INTRODUCTION

Hydraulic fracturing of oil and gas production wells is often used to increase the available sand surface and, hence, rate of fluid production. Fracturing fluids are injected under pressure to propagate a fracture into the reservoir, performing two main functions: (i) opening the fracture and (ii) transporting propping agents to maintain the fracture aperture after the pressure is released.[1] Water-based fracturing fluids are preferred for economic, environmental, and occupational health and safety reasons. Guar gum and derivatives such as hydroxypropyl guar (HPG) are commonly used to viscosify water in fracturing fluids for conventional treatments, although hydraulic fracturing in unconventional reservoirs is shifting toward lower viscosity "slick water" treatments using polyacrylamides.[2]

The fracturing fluid must be highly viscous during the injection to minimize leak off of fluid and pressure into the surrounding matrix and to carry proppants effectively, and then "break" (degrade) to a water-like consistency to maintain high hydraulic conductivity in the fracture during subsequent production of hydrocarbons. Crosslinkers (e.g., borate) and breakers (either oxidizers or enzymes) are added to the fluid to fulfill each of these requirements. Enzymes have been used extensively to degrade both the fracturing fluid and the filter cake that forms on the faces of the fracture.[3–5] Enzymes used as breakers of guar gels have several benefits over chemical oxidizers: they are relatively inexpensive—especially considering that they are not consumed during their catalytic reaction with guar, they are environmentally benign, are easy to handle, and are chemically compatible with surface equipment and tubing.[1,3] However, enzymes become denatured and lose their catalytic activity at high temperature and extreme pH environments.[3] Such conditions are often encountered in the field and this limits the application of enzymes as breakers for fracturing fluids.

The use of high concentrations of enzymes or oxidizers mixed with fracturing fluid to ensure degradation of the filter cake increases costs and causes premature degradation of the fluid and hence loss of viscosity during injection. Encapsulation techniques have been used to inactivate breakers during injection. However, the relatively large size of the capsules, which are usually designed to break open when the fracture recloses at the end of the injection, may result in incomplete degradation of filter cake.[1,6] Polyethylenimine (PEI)—dextran sulfate (DS) polyelectrolyte complex (PEC) nanoparticles originally developed for drug delivery applications[7–9] and adapted for oilfield applications[10–12] were hypothesized to be capable of entrapping, releasing, and protecting

Correspondence to: S. J. Johnson (sjohn@ku.edu).

TABLE I
Polyelectrolyte Complex (PEC) Nanoparticle Formulations Differed in the Concentration of Enzymes and Order of Mixing

PEC	1% w/w PEI (aq.) (mL)	1% w/w DS (aq.) (mL)	25% w/w Pectinase (aq.) (mL)	EL2X (as supplied) (mL)	Make-up water (mL)	Order of addition
A	2	1	0.1	–	–	PEI, DS, pectinase
A′	2	1	0.1	–	–	PEI, pectinase, DS
H	2	1	–	0.1	–	PEI, DS, EL2X
H′	2	1	–	0.1	–	PEI, EL2X, DS
I	2	1	–	0.05	0.05	PEI, DS, EL2X
I′	2	1	–	0.05	0.05	PEI, EL2X, DS
J	2	1	–	0.025	0.075	PEI, DS, EL2X
J′	2	1	–	0.025	0.075	PEI, EL2X, DS

enzymes for fracturing fluids in a controlled manner. PECs are structures that form when aqueous solutions of polyanions and polycations are mixed together in nonstoichiometric ratios at low concentrations. Electrostatic attraction between oppositely charged polymers causes the polymers to self-assemble into particles and the excess charge on the outer surface contributes to their colloidal stability. If another charged entity is added during mixing of the polyelectrolytes, it may become incorporated into the particles where it is held by electrostatic and steric interactions. The size, charge, stability, and entrapment efficiency (EE) of the PECs may be controlled by varying the molecular weight, charge density, and relative concentrations of the polyelectrolytes, as well as the order of mixing of the various components, pH, and shear.[13] High EE of enzymes, homogeneous distribution, and flexible release time are desired for this system to be potentially applied in breaking of fracturing fluids.

This study builds on our previous work on the application of PEC nanoparticles to entrap and release pectinase for fracturing fluids.[10] Positively charged PEC nanoparticles were made by mixing the polyanion (DS) with excess (in terms of charge) polycation (PEI). Two enzymes with different temperature and pH optima used in the petroleum industry to break fracturing fluids were each entrapped in the PEC nanoparticles. A viscometric assay of the supernatant after centrifugation was used to calculate the EE for the nanoparticles loaded with enzymes. Order of mixing, concentration of enzymes, and pH of the nanoparticle systems were varied to maximize EE of the two enzymes. Retardation of enzyme activity was observed by monitoring the viscoelastic moduli of borate-crosslinked guar and HPG gels over time. Degradation times were compared with controls using equivalent concentrations of unentrapped enzymes.

MATERIALS AND METHODS

Materials

Chemicals were used as supplied: Sodium hydroxide, 1*N* hydrochloric acid, DS (M_w = 500 kDa), PEI (M_w = 25 kDa), sodium thiosulfate pentahydrate, potassium chloride, and sodium hydrate (Fisher Scientific, Pittsburgh, PA); sodium borate (J.T. Baker Chemical Co., Phillipsburg, NJ); HPG gum blend (Jaguar® 415, Rhodia, Paris, France); pectinase from *Aspergillus aculaceatus* (Sigma-Aldrich, St. Louis, MO) and Econo Gelbreak-EL2X (Economy Polymers and Chemicals, Houston, TX)—enzyme activities of 100% were assumed for concentration calculations.

Preparation of polyelectrolyte complex nanoparticles

Nanoparticles were made using the method presented previously.[10] In a typical formulation, 1 mL of a 1% w/w aqueous solution of DS (pH = 7.8) was pipetted rapidly into 2 mL of a 1% w/w aqueous solution of PEI (pH adjusted to 8.0 using 4*N* HCl) while stirring. The solution was then stirred for 20 min at 600 rpm. Enzymes were added rapidly from a 100 μL pipette as 0.1 mL of a 25% w/w pectinase solution or 0.1 mL of EL2X solution either before or after the DS (Table I). Enzyme-loaded nanoparticles were used as a breaker in polymer systems at a final concentration of 0.1% w/w pectinase or 0.4% EL2X.

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 in samples diluted $\sim 40\times$ by volume with deionized water. Zeta potential was also measured by phase analysis light scattering using the same instrument using samples diluted $\sim 20\times$ with 1.0 m*M* KCl solution. Zeta potential was estimated using the Smoluchowski approximation from the previously measured size and the electrophoretic mobility of the nanoparticles.[14]

Determination of enzyme activity and entrapment efficiency

EE of the enzyme in the PEC nanoparticles was determined by material balance [eq. (1)] using the

total enzyme concentration in a sample (C_T) and the enzyme concentration remaining in the supernatant of a centrifuged sample (C_S). Enzyme concentrations in the sample and in the supernatant were determined by a procedure to measure the activity of the enzyme to degrade an HPG solution using viscosity measurements. The time for the viscosity of the solution to fall to 50% of its initial value ($t_{1/2}$, h) was used to calculate the activity of the enzyme after the method reported by Bell and Etchells.[15] Activity was defined as the reciprocal of $t_{1/2}$. Enzyme concentration was previously determined to be a proportional to the activity[10] giving the relationship in eq. (1), in which A_T = activity of unentrapped enzyme at concentration C_T, and A_S = activity of supernatant.

$$\mathrm{EE} = \frac{C_T - C_S}{C_T} \times 100\% = \frac{A_T - A_S}{A_T} \times 100\% \qquad (1)$$

Aqueous suspensions of PEC nanoparticles were centrifuged at 14,000 × *g* for 1.5 h at 4°C to produce the supernatant. To perform the viscometric assay for the total enzyme concentration, 2.0 g of 5000 ppm HPG solution was mixed at room temperature with an enzyme solution to a total mass of 2.5 g. Additions were calculated to give concentrations in the final mixture of 0.02% pectinase or 0.08% EL2X and 4000 ppm HPG. The same procedure was used for the supernatants except the supernatants were diluted by the same factor as that used to control the concentration of enzyme of the nanoparticle suspensions before centrifugation. The enzyme-polymer mixtures (1.5 mL) were immediately placed in a Bohlin CS-10 rheometer (Malvern Instruments, Malvern, England) with a 40 mm/4° cone-and-plate geometry and the viscosity of the solution was measured over time as described below to determine $t_{1/2}$. Where needed, polymer solution pH was adjusted using 1*M* NaOH. Experimental temperature and pH are reported in the figure legend for each experiment.

Preparation of HPG solutions

Sufficient HPG to create a 5000 ppm solution was added slowly to the shoulder of a vortex of a vigorously (600 rpm) stirred solution of 2% KCl and 1.35 g/L of sodium thiosulfate in a 500 mL beaker. The solution was stirred at 600 rpm for an additional 5 min, then the stirring rate was reduced to 400 rpm for another hour, and the polymer was finally allowed to become hydrated for another 24 h at 200 rpm.[16] The solution was used as prepared (i.e., not filtered).

Preparation of borate-crosslinked HPG gel

Twenty-four milliliters of the 5000 ppm HPG was mixed with 7.5 mL of diluted EL2X-loaded PEC nanoparticles or an aqueous solution with equivalent concentration of free enzyme in a 40 mL scintillation vial. A total of 7.5 mL of a 2000 ppm borax solution was added to the mixture and the pH was adjusted to 9.2 with 0.1*M* NaOH. This yielded a gelant containing ~ 3076 ppm HPG, 385 ppm borax, and 0.08% w/w EL2X). Gel samples were sealed and incubated on a table shaker (LAB-LINE 3520 JR, Melrose, IL) at 150 rpm and 40°C.

Measurement of viscosity and viscoelastic moduli

HPG solution

A Bohlin CS-10 rheometer was used with a 40 mm/4° cone-and-plate geometry to measure the viscosity of HPG solutions. Viscometric assays were performed under a shear rate of 90 s^{-1} at 25°C for pectinase or 40°C for EL2X. To conduct the assay, 0.5 g of enzyme solution, nanoparticle suspension or supernatant was added to 2.0 g of 5000 ppm HPG solution. After mixing, 1.5 mL of the solution was removed for viscosity measurements.

Gelled HPG

A Bohlin CS-10 rheometer was used to measure the elastic (G') and viscous (G'') moduli for 30 mL gel samples over time at a frequency of 0.5 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

Characterization of enzymes

Activity was measured by viscometric assay for the two enzymes at different pH and temperature (Figs. 1 and 2) to identify optimal conditions for each. In contrast to pectinase, the commercial product, EL2X, showed significant activity at pH 9, consistent with its intended use at higher temperatures and pH, with activity claimed in the product data sheet of 50–160°F (10–71°C) or up to pH 10.[17]

Characterization and optimization of enzyme-loaded PEC nanoparticles

Triplicates of several formulations varying in enzyme concentration and in the order of reagent addition were prepared as shown in Table I. Size and zeta potential of the nanoparticles were measured over a range of pH (6.0–9.5) and are shown in Figures 3 and 4. Zeta potential of the pectinase-loaded nanoparticles (PEC A′) decreased with increasing pH in the range tested, whereas particle size showed a maximum near pH 7 then decreased

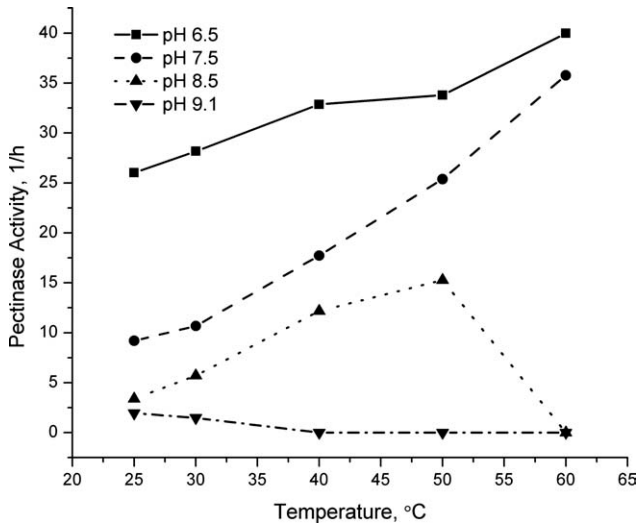

Figure 1 Activity of pectinase versus temperature at different pH values. Activity was measured using a viscometric assay for 5000 ppm HPG solutions degraded using 0.02% pectinase.

with further increase in pH. Both particle size and zeta potential of the EL2X-loaded PECs decreased with increasing pH.

This is not unexpected because the charge on the PEI is dependent on pH. PEI is a weak base with an isoelectric point (I_p) at pH 10.8.[18] At the I_p, PEI is uncharged and forms compact coils. As pH is decreased, more and more secondary amine groups are neutralized to —NH^+—; this increases the charge density on the PEI and electrostatic repulsion causes the polymer chains to adopt a more extended configuration, increasing the availability of the charged amine groups to interact with negative charges on the DS or enzyme. Conversely, as pH increases and

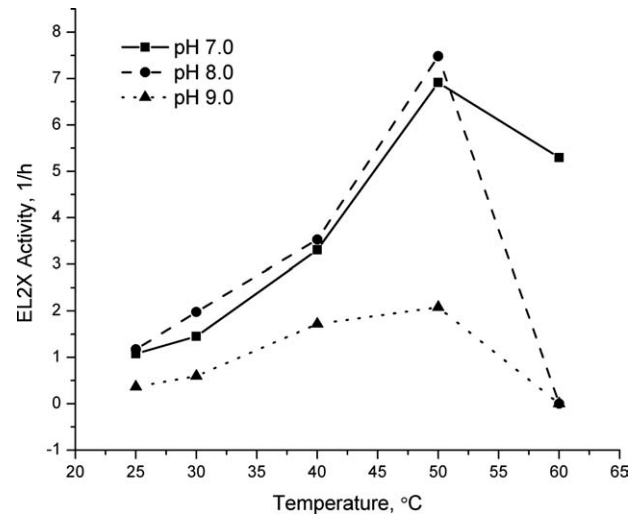

Figure 2 Activity of EL2X versus temperature at different pH values. Activity was measured using a viscometric assay for 5000 ppm HPG solutions degraded using 0.08% EL2X.

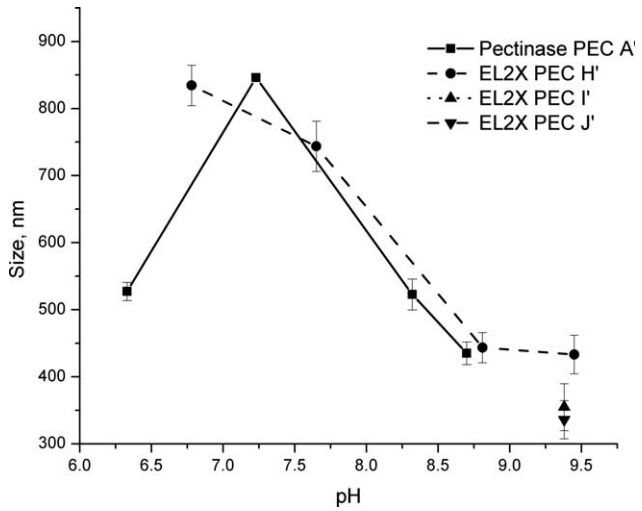

Figure 3 Mean size versus pH for pectinase (A′) and EL2X (H′, I′, J′) loaded PECs. Error bars = 1 SE, $n = 3$.

approaches the I_p, the PEI becomes less charged and more condensed, causing a reduction in magnitude of the net positive charge in the particles, resulting in less repulsion between PEI chains within each particle and hence decreased particle size.

Order of mixing had a profound effect on particle formation and enzyme EE, with best results seen when enzyme was mixed with the PEI before the addition of DS [formulations designated by a prime (′) symbol]. This contrasts with previous experiences with Cr(III) as the entrapped material, where order of mixing did not have a significant effect. The difference is almost certainly due to the inability of the high M_w enzymes to be intercalated into preformed PEI-DS particles, unlike the much smaller chromium atoms. Only enzyme formulations assembled in this order were considered further.

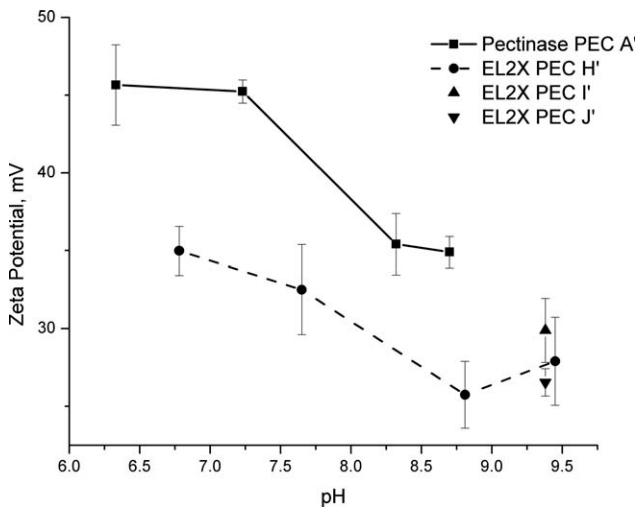

Figure 4 Mean zeta potential versus pH for pectinase (A′) and EL2X (H′, I′, J′) loaded PECs. Error bars = 1 SE, $n = 3$.

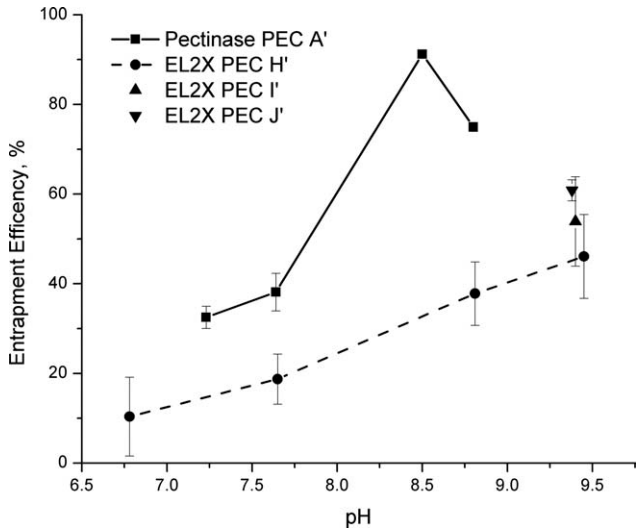

Figure 5 Entrapment efficiency versus pH for pectinase (A′) and EL2X (H′, I′, J′) loaded PECs at 25°C and 40°C, respectively. Error bars = 1 SE, $n = 3$.

To measure enzyme activity and EE, viscometric assay was performed on nanoparticle suspensions, supernatants, and enzymes as described above. Entrapment efficiency (EE_A) of the pectinase-loaded nanoparticles (PEC A′) was 91% at pH 8.5. EL2X systems showed EE_A of 46% when 0.1 mL of 100% EL2X was added (PEC H′), suggesting that the loading capacity of the PEC system had been exceeded. Because PEC formation only occurs at low polyelectrolyte concentrations, the amount of EL2X was reduced to 0.5 mL (I′) and 0.025 mL (J′), EE_A was slightly improved to about 54% and 61%, respectively (Fig. 5). Decreasing the loading of EL2X also decreased the particle size for the EL2X-loaded systems (Fig. 4).

Degradation of borate crosslinked HPG gels by EL2X-loaded nanoparticles at 40°C

Viscosity is commonly measured during field application because it is a relatively simple and fast assay. To further characterize the degradation process, a rheometric study was also performed. Delayed release of pectinase using system A with pH of 8.7 at 25°C had been demonstrated previously.[10] In this work, we investigate delayed release of EL2X from the H′ PEC system at pH 9.4 (EE = 54%) and 40°C.

EL2X-loaded nanoparticles (PEC J′) with final EL2X concentration of 0.4% were mixed with 5000 ppm HPG solution in 2% KCl and 2000 ppm borax crosslinker was added. The mixture gelled immediately upon addition of the borax at room temperature. Gels with enzymes and control gels without enzyme added were shaken at 150 rpm and 40°C; samples were removed at intervals and viscoelastic moduli were measured using a Bohlin rheometer. Elastic (G') and viscous (G'') moduli were plotted versus time for a frequency of 0.5 Hz (Fig. 6). Delay in degradation of both moduli was observed; G' and G'' reached values equal to or lower than those of an equivalent HPG gel degraded with unentrapped enzyme in 7 h and 11 h, respectively, indicating a significant delay in activity of enzyme (by 4 and 5 h). Plots of both G' and G'' were shifted toward longer delays for the systems degraded with nanoparticles compared to the systems degraded with unentrapped enzyme, indicating a delay in transition from gel to a low viscosity solution. Faster decline of G' compared with G'' is typical for enzymatic degradation of guar solutions because the gel structure is attacked first, followed by the degradation of the guar back bone.[19]

Protective effect of nanoparticles on enzymes

Viscometric assays were performed for pectinase and EL2X enzymes at pH values of 9.1 and 9.75 and temperatures of 40°C and 50°C, respectively. Both enzymes are rapidly denatured under these conditions. The assay was repeated three times and a representative plot of viscosity versus time is shown in Figure 7. The enzymes retained their activities for a significantly longer time under similar conditions after being entrapped by the PEC nanoparticles. It would appear that entrapment of enzymes within the nanoparticles confers protection from the pH of the bulk solution, presumably by a local buffering effect by the PEI, which dominates the pH of the nanoparticle preparations (8.3 for PEC A′ and 9.3 for PEC I′).

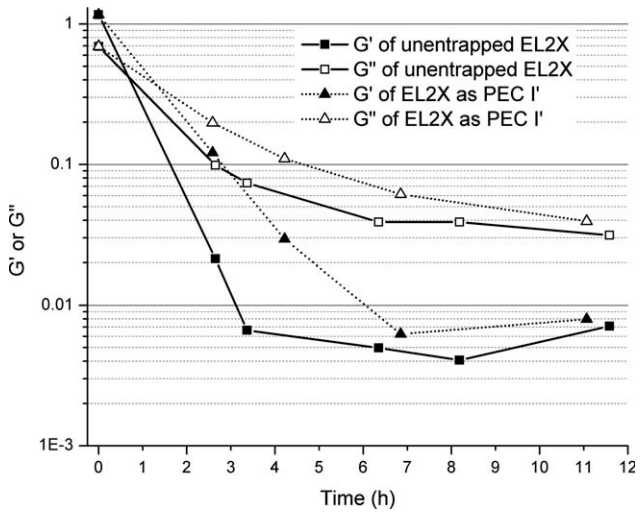

Figure 6 G' and G'' versus time for borate-crosslinked HPG gel degraded using either 0.08% unentrapped EL2X or an equivalent amount of EL2X PEC I′ (pH 9.2, 40°C).

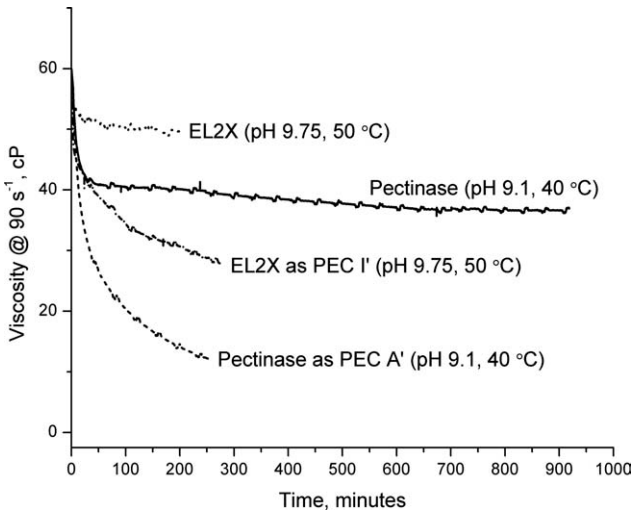

Figure 7 Entrapment of enzymes in PEC nanoparticles protects activity of pectinase (pH 9.1, 40°C) and EL2X (pH 9.75, 50°C).

This is consistent with the protective effects of PEC nanoparticles reported for several drugs and proteins.[9,20]

CONCLUSIONS

Enzymes entrapped in PEC nanoparticles optimized for maximum enzyme EE are able to break borate-crosslinked HPG gel, with the action being delayed significantly compared with unentrapped enzymes at the same concentration. The delayed release of the enzyme allows the loaded particles to be mixed with the gelant with a reduced risk of failure to gel or premature breaking. PEC entrapment of gel-breaking enzymes shows potential for improving the performance of hydraulic fracturing treatments in conventional oil and gas reservoirs.

Pectinase is inactivated rapidly at pH 9.1 and 40°C and the commercial enzyme mixture (EL2X) at pH 9.75 and 50°C. Enzyme activity observed in PEC preparations under these conditions suggest that PEC entrapment provides a protective environment for both enzymes and this may allow application in conditions which were previously untenable.

References

1. Economides, M. J.; Nolte, K. G. Reservoir Stimulation, 3rd ed.; Wiley, New York & Chichester, 2000.
2. Palisch, T. T.; Vincent, M. C.; Handren, P. J. SPE Prod Oper 2010, 25, 327.
3. Armstrong, C. D.; Stevens, R. F.; Lee, H.; Stephenson, C.; Qu, Q. In Society of Petroleum Engineers International Symposium and Exhibition on Formation Damage Control, Lafayette, LA, 2010.
4. Fodge, D. W.; Anderson, D. M.; Pettey, T. M. U.S. Pat. 5,551,515, September 3 (1996).
5. Tjon-Joe-Pin, R. M. U.S. Pat. 5,201,370, April 13 (1993).
6. Ayoub, J. A.; Hutchins, R. E.; Van der Bas, F.; Cobianco, S.; Emiliani, C. N.; Glover, M.; Kohler, M.; Marino, S.; Nitters, G.; Norman, D.; Turk, G. Presented at the Society of Petroleum Engineers International Symposium and Exhibition on Formation Damage Control, Lafayette, LA, 2006.
7. Tiyaboonchai, W. Development of a New Nanoparticle Delivery Vehicle Based on an Aqueous Polymer System: Polyethylenimine and Dextran Sulfate. The University of Kansas: Lawrence, KS, 2003.
8. Tiyaboonchai, W.; Woiszwillo, J.; Middaugh, C. R. Eur J Pharm Sci 2003, 19, 191.
9. Tiyaboonchai, W.; Woiszwillo, J.; Sims, R. C.; Middaugh, C. R. Int J Pharm 2003, 225, 139.
10. Barati, R.; Johnson, S. J.; McCool, S.; Green, D. W.; Willhite, G. P.; Liang, J.-T. J Appl Polym Sci 2011, 121, 1292.
11. Cordova, M.; Cheng, M.; Trejo, J.; Johnson, S. J.; Willhite, G. P.; Liang, J.-T.; Berkland, C. Macromolecules 2008, 41, 4398.
12. Berkland, C.; Cordova, M.; Liang, J.-T.; Willhite, G. P. U.S. Pat. WO 030,758 A1, March 13 (2008).
13. Koetz, J.; Kosmella, S. Polyelectrolytes and Nanoparticles; Springer-Verlag: Berlin, 2007; p 105.
14. Hunter, R. J. Zeta Potential in Colloid Science: Principles and Applications; Academic Press: Waltham, MA, 1981; p 386.
15. Bell, T. A.; Etchells, J. L. A Method for Testing Cucumber Salt-Stock Brine for Softening Activity; Agricultural Research Service, US Department of Agriculture, Washington DC, 1955.
16. Tayal, A.; Kelly, R. M.; Khan, S. A. Macromolecules 1999, 32, 294.
17. Anonymous, Product Data Sheet for ECONO GelBreak-EL2X. Economy Polymers and Chemicals: Houston, TX, 2000.
18. Hostetler, R. E.; Swanson, J. W. Polym Chem 1974, 12, 29.
19. Cheng, Y.; Prud'homme, R. K. Biomacromolecules 2000, 1, 782.
20. Hartig, S. H.; Greene, R. R.; Dikov, M. M.; Prokop, A.; Davidson, M. Pharm Res 2007, 24, 2353.