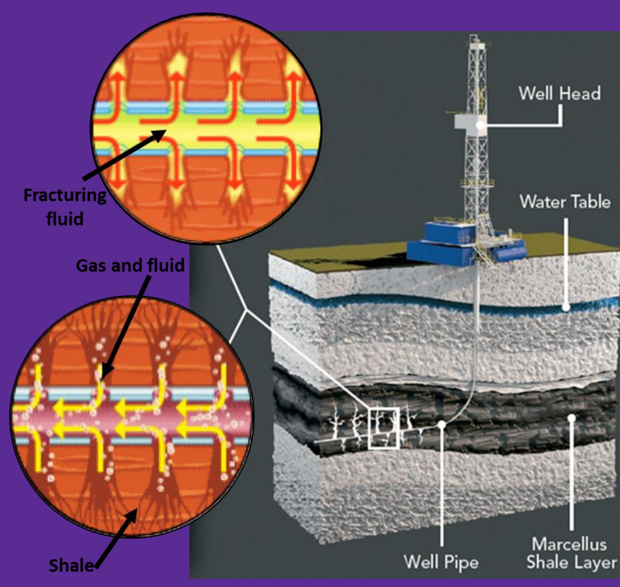

Synthesis and Characterization of β-mannanase and α-galactosidase Encapsulated Polymer Nanoparticles for Applications in Oil Industry

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Abstract

Hydraulic fracturing is often used in low-permeable oil reservoirs, which will account for about half of all oil resources by 2035. In hydraulic fracturing (also known as "fracking"), viscous fluids are pumped into wells under high pressure to fracture low permeability geologic formations. However, removal or degradation of fracturing fluid after the formation of fractures is critical to allow oil or gas flow to the well. Studies have shown that the use of high concentration of enzymes causes premature degradation of fracturing fluids while a low concentration of enzymes causes nonuniform degradation and results in filter cakes. Therefore, controlled release of enzymes during fracture formation is crucial to avoid premature degradation or formation of filter cakes in oil wells. β-mannanase and α-galactosidase have been widely used as enzyme breakers for guar gum-based polymers present in fracturing fluids. These enzymes are more eco-friendly and better breakers of guar-based fluids. The use of nanoparticles to encapsulate these enzymes can increase the effectiveness of these enzymes, as well as give more control over when they are released into the fracturing fluid.

This project focuses on the encapsulation and/or co-encapsulation of β-mannanase and α-galactosidase in various PLL-g-PEG (poly-L-lysine-graft-polyethylene glycol) co-polymers to produce nanoparticles to be used as an efficient enzymatic viscosity breaker for guar gum-based hydraulic fracturing fluids. PLL-g-PEG co-polymers are perfect candidates for this research because of its ability to co-encapsulate enzymes and its non-toxic nature. Four different PLL-g-PEG polymers were synthesized using different amounts of 5 kDa mPEG-NHS grafted onto 15–30 kDa PLL. Eight different sets of NPs were synthesized using β-mannanase and α-galactosidase enzymes. Current studies are focused on evaluating enzymatic activities and their ability to degrade guar gum polymers.

Introduction

Owing to depletion of conventional oil reservoirs and the increase in energy demand, oil resources recovered from low-permeability reservoirs play a vital role in satisfying world's growing energy needs. The U.S. Energy Information Administration (EIA) estimated that in 2021, about 7.22 million barrels per day of crude oil were produced directly from tight oil resources including low-permeable shale, sandstone, and carbonate rock formations in the United States. Hydraulic fracturing is one of the most prevalent and economically competitive technologies that has proved to improve productivity and maximize recovery in low-permeability oil reservoirs. This process utilizes high-viscosity fracturing fluids to create fractures in the rock formation surrounding the bore.

Over the last decade, a variety of methods have been investigated and proved to be useful as water-based fracturing fluids. These include guar-based polymers, synthetic polymers, and high-density brines. But guar-based polymers are widely used in the field because of their high shear stability and better clean up compared to other systems. A breaker (either an enzymatic or oxidative) is an important additive in fracturing fluids to extensively degrade the high-viscosity fluids into a low-viscosity fluid to increase fracture conductivity and to facilitate easy flow back to the reservoir surface.

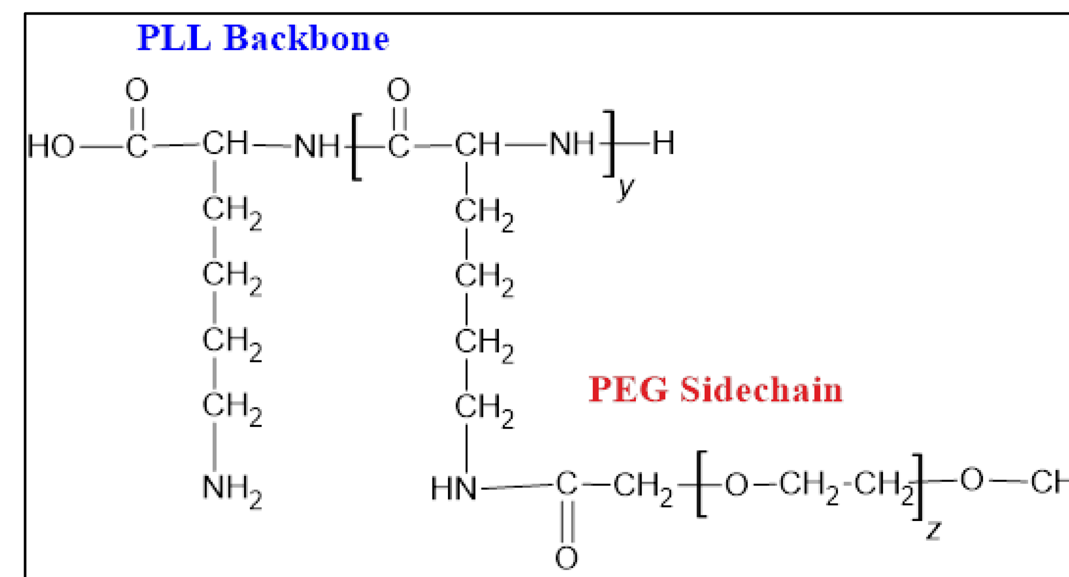

Figure 1: **Chemical structure of PLL-g-PEG.** A chemical reaction between N-Hydroxysuccinimide (NHS) group of mPEG-NHS and primary amine groups of PLL form the PEG grafted co-polymer, PLL-g-PEG.

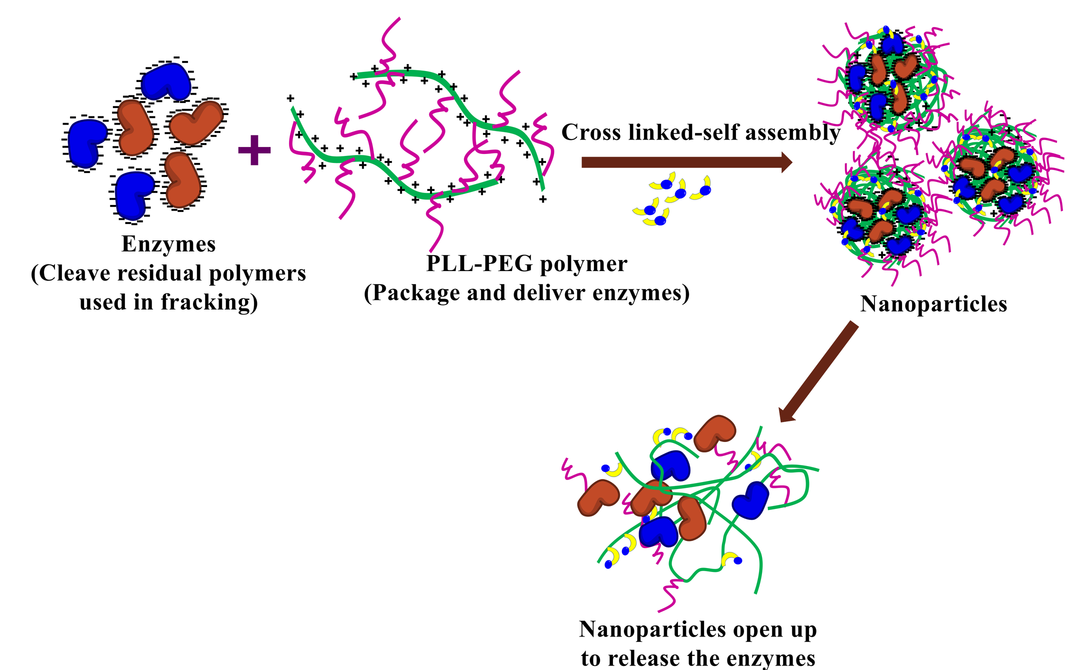

***Figure 3:*Assembly of polymer-enzyme nanoparticles followed by crosslinking with DTSSP, and release of enzymes under reducing conditions.**

Methods

- Four different PLL-g-PEG co-polymers were prepared with different grafting ratios of 2%, 5%, 10%, and 15% of mPEG-NHS by weight. The molecular weights of mPEG-NHS was 5 kDa and PLL was 15-30 kDa.
- NPs were synthesized by adding 6 mg/mL PLL-g-PEG solution to a 0.266 mg/ml solution of either α-galactosidase or β-mannanase enzymes, followed by 0.025% glutaraldehyde after 1 hour.
- Dynamic light scattering was used to determine the size and dispersity of the nanoparticles immediately after NP synthesis and followed by centrifugation for 3 minutes at 5,000 rpm.
- SDS-PAGE gel electrophoresis was used to determine the encapsulation efficiency of α-galactosidase or β-mannanase enzymes in nanoparticles.

Results

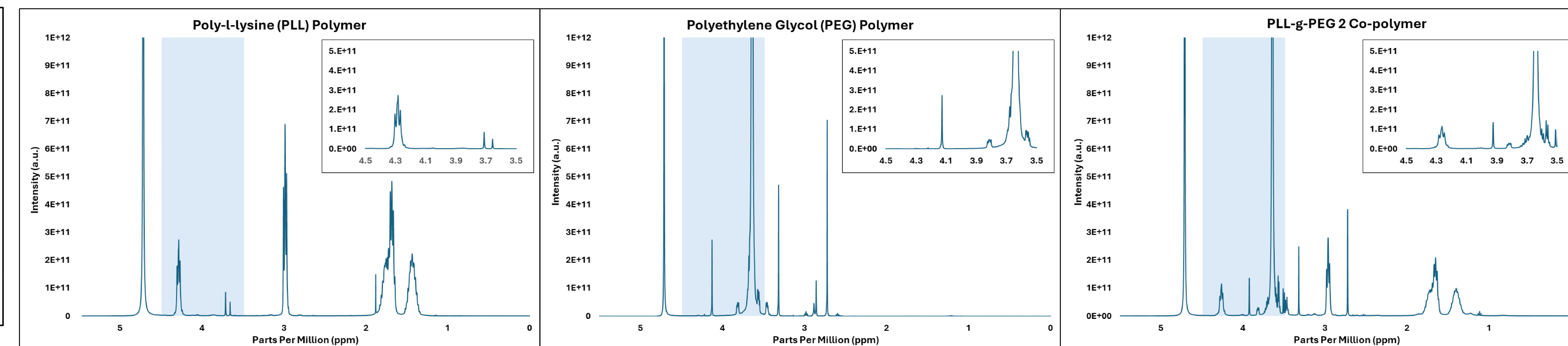

Figure 2: **^{1}H NMR Spectra of PLL, PEG, and PLL-g-PEG**

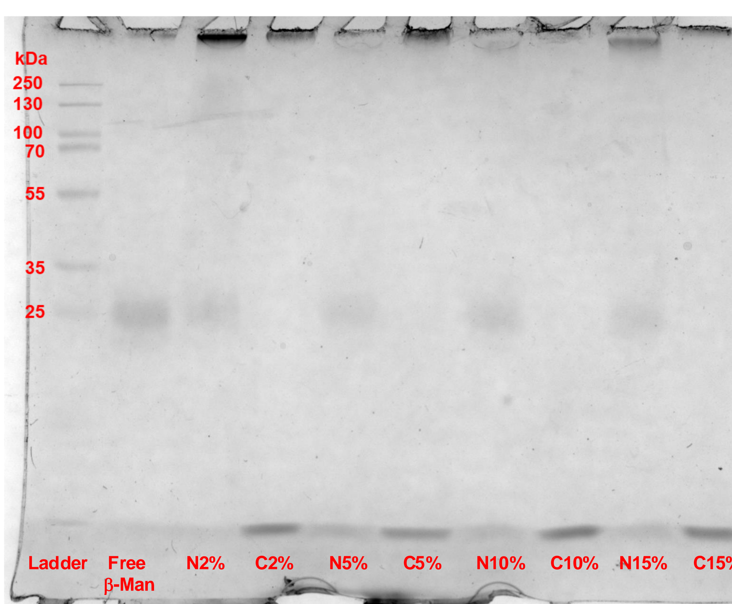

Figure 4: **SDS-PAGE gel for β-Mannanase nanoparticles and co-polymers of the same grafting ratios.** This image shows the gel used to evaluate β-Mannanase encapsulation in the nanoparticles. From left, the protein ladder, free β-Mannanas, and the NPs and co-polymers; N2%, C2%, N5%, C5%, N10%, C10%, and N15% (C: copolymers; N: nanoparticles) are shown in lanes 1-10 respectively.

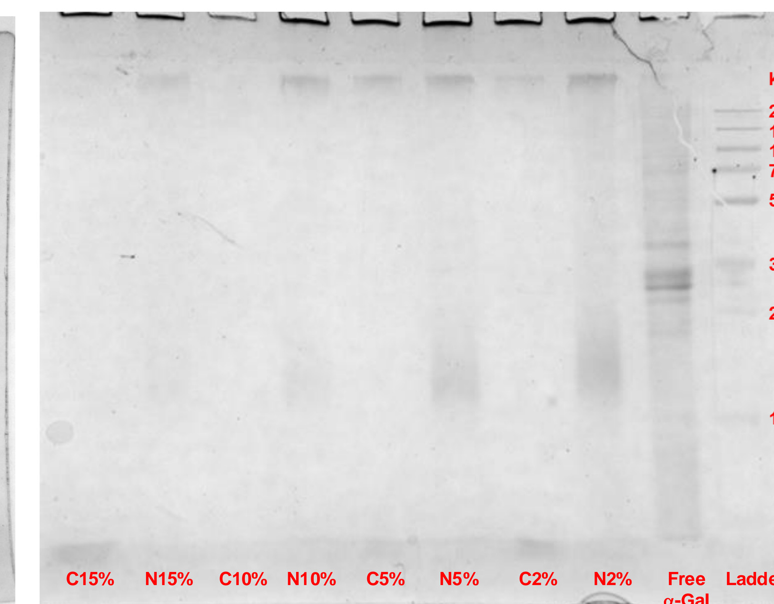

Figure 5: **SDS-PAGE gel for α-galactosidase nanoparticles and co-polymers of the same grafting ratios.** This image shows the gel used to evaluate α-galactosidase encapsulation in the nanoparticles. From right, the protein ladder, free α-galactosidase, and the NPs and co-polymers; N2%, C2%, N5%, C5%, N10%, C10%, and N15% (C: copolymers; N: nanoparticles) are shown in lanes 1-10 respectively.

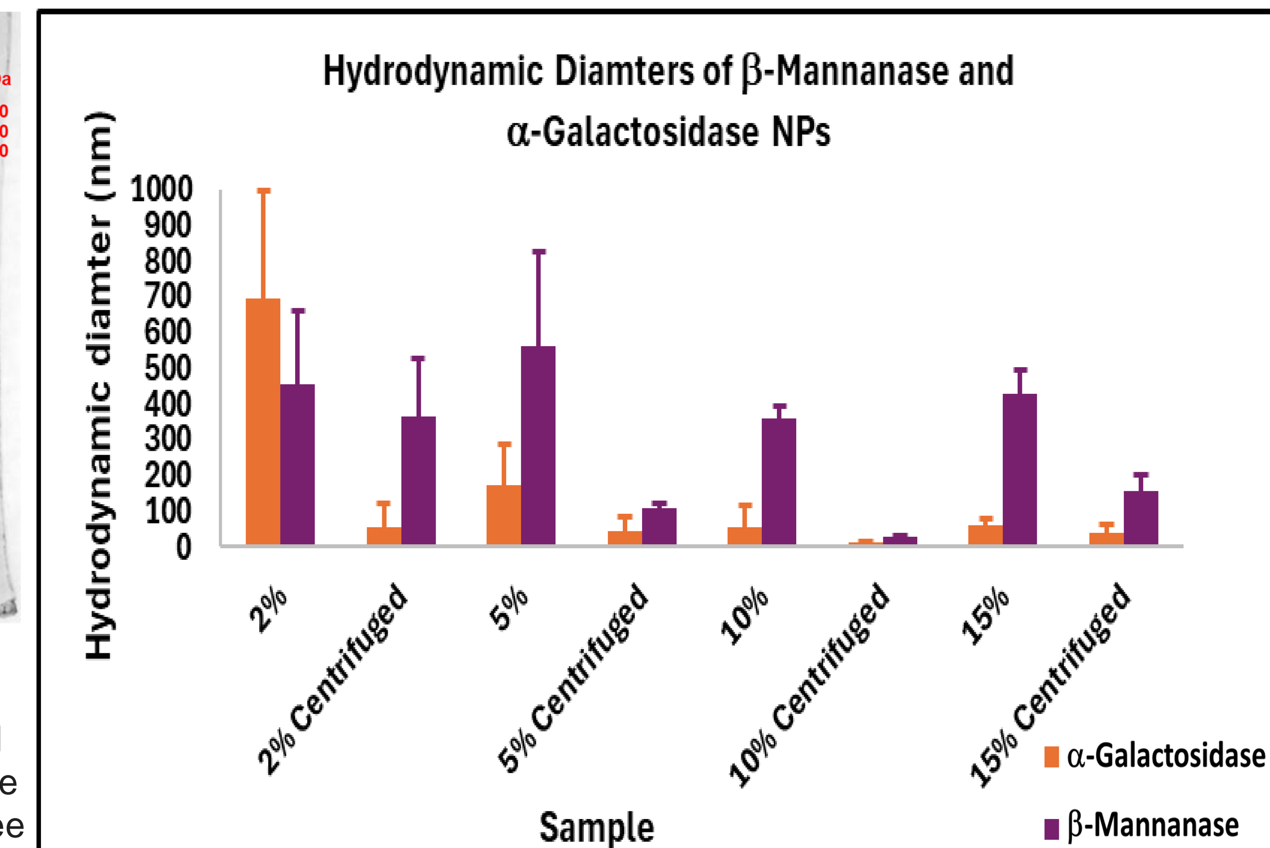

Figure 6: **Hydrodynamic diameters of β- mannanase and α-galactosidase nanoparticles pre- and post-centrifugation.**

Conclusions & Future Work

- Hydrodynamic diameter of NPs significantly decreased across all samples after centrifugation. NPs were polydisperse across all the samples.
- Dynamic light scattering data shows that the average sizes of β-mannanase NPs are significantly larger than that of α-galactosidase NPs.
- The SDS-PAGE gels suggest a partial encapsulation of both of the enzymes in their respective NP samples.
- Current research studies are focused on evaluating the two enzyme breakers' ability to successfully degrade guar gum polymers.
- Future research intends to evaluate the controlled release of enzymes in a fracturing environment.

References

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