DNA-Encapsulated Silica Nanoparticle Tracers for Fracture Characterization

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ABSTRACT

The objective of our experiments has been to develop and evaluate a uniquely identifiable particle tracer for use in geothermal applications. Following the work of Paunescu et al. (2013), DNA-tagged nanotracers have been made by adsorbing synthetic DNA onto silica nanoparticles which were then coated with silica to protect the DNA. The silica nanoparticles were then evaluated for durability with a controlled heat experiment at 198°C and flowed through packed sand at 25°C, 120°C, and 150°C while monitoring permeability changes. All were subsequently analyzed with SEM imaging.

1. Introduction

In geothermal fields, energy extraction is dependent on the network of fractures in the reservoir. Therefore, it is essential to understand the characteristics of the reservoir and its flow behavior. Data collection techniques have been developed to understand fracture connectivity, thereby enabling engineers to better enhance the development and production of the field. Tracer tests have been applied widely in the industry to map fracture distribution, mostly by injecting chemicals at the injection well and monitoring its breakthrough pattern at the production well. In recent years, there has been research on solid particle tracers (Alaskar 2013; Frane et al. 2014; Li et al. 2014). Specially manufactured micro or nanoscale particles are promising tracer candidates because of the high degree of control of their physical and chemical properties compared with conventional solute tracers. Particle tracers also have less matrix diffusion, and hence travel more quickly through the reservoir as they tend to remain in high-velocity fluid streamlines. In this work, we investigated a DNA-based nanoparticle tracer that has good thermal stability and great capacity for information storage due to its uniquely identifiable character.

DNA is well-known for its unique identifyability, a feature that could be applied to geothermal fracture network characterization. A DNA-based nanotracer can be produced by attaching synthetic DNA molecules to the surface of a silica nanoparticle seed and adding a protective outer silica layer, which is used to alleviate the DNA’s vulnerability to high reservoir temperatures. Pioneering work by Paunescu et al. (2012) has proven that DNA protected by silica nanoparticles is able to withstand temperature as high as 200°C, and still can be amplified through qPCR (real-time quantitative polymerase chain reaction) after being released into the suspension by dissolving the outer silica layer. Therefore, it should be possible to apply such particles to geothermal tracer testing to investigate the connectivity of fracture networks.

The advantage of a DNA-encapsulated nanotracer over other candidates is its uniquely identifiable nature, which could be used to tag individual flow paths. The qPCR process to amplify and quantify DNA is also selective. In other words, the process can selectively amplify the DNA that contains a specific segment of sequence. Therefore, by applying such nanotracers with varying DNA sequences to different wellbores and/or fractures, we would be able to understand the flow path of the tracer by identifying the DNA sequence within the fluid. The essentially infinite number of DNA sequences...
allows every flow path to have its unique identifier, therefore enabling the identification of well connections, as illustrated in Figure 1.

In this study, DNA-encapsulated silica nanoparticles were synthesized successfully. These tracers and plain silica nanoparticles were subjected to simulated geological stresses in order to evaluate their durability in a geothermal setting. The particles were heated at temperatures of 198°C for up to 30 minutes, and injected through packed sand at temperatures as high as 150°C. Controlled experiments were also conducted to enhance particle suspension (details in Section 6). The intent of this research has been to establish a path toward the development of DNA smart tracers at the field scale.

2. Synthesis of DNA-Encapsulated Silica Nanoparticles

The synthesis of DNA-encapsulated silica nanoparticles was performed according to the procedure suggested by Paunescu et al. (2013) with slight modifications. DNA was first adsorbed onto positively charged silica seed particles, after which seed particle growth method was applied to coat the DNA-adsorbed seeds with silica layer, thereby “sandwiching” DNA molecules between inner seed and outer shell (Figure 2). Synthetic 113-base-pair single stranded DNA (ssDNA) with complementary sequences (Paunescu et al. 2012) purchased from Eurofins Genomics was annealed according to standard annealing procedure to yield double stranded DNA (dsDNA) before being used for encapsulation.

2.1 Silica Seed

Plain silica nanoparticles of ~150 nm size were first synthesized at room temperature by the polycondensation of tetraethyl orthosilicate (TEOS) (Stober et al. 1968). 0.8 ml TEOS was added to a mixture of 18 ml ethanol, 0.8 ml of 29.14% concentrated ammonia solution and 0.5 ml of Milli-Q water. The mixture was then stirred using a Benchmark Multi-Therm shaker at 1000 rpm at room temperature for 6 h. The resulting particles were then washed three times with 2-propanol by centrifugation at 9000g for 20 min and resuspension using ultrasonic bath, and were finally suspended in 4 ml of 2-propanol to yield a particle concentration of ~50 mg/ml. The silica seeds were characterized using Scanning Electron Microscopy (SEM), which indicated average particle diameter of ~140 nm, as shown in Figure 3. The particles served as the silica “seeds” for subsequent synthesis.

2.2 Surface Functionalization

Because both the synthesized silica seeds and DNA molecules carry negative surface charge under experimental conditions, each 1 ml of silica seeds was stirred with 10 µl trimethyl[3-(trimethoxysilyl)propyl]ammonium chloride (TMAPS) at 1400 rpm in a 2-ml micro tube for over 12 h to yield positive surface charge (Paunescu et al. 2012). The particles were
then washed three times with 2-propanol by centrifugation at 20817 g for 4.5 min and resuspension using ultrasonic bath, and were finally suspended in 1ml of 2-propanol.

In order to demonstrate the change in surface charge of silica seeds after surface functionalization, zeta potential measurement was conducted, as shown in Figure 4.

The zeta potential measurement indicates that the silica seeds before surface functionalization carried -50 mV charge. After surface functionalization, however, the charge changed to +40 mV. DNA molecules carrying negative charge could therefore attach to the surface functionalized silica seeds to allow for subsequent encapsulation.

2.3 DNA Encapsulation

700 μl Milli-Q water, 35 μl functionalized silica seed solution and 320 μl of 717 nM dsDNA solution were mixed in a 2-ml micro tube and left at room temperature for 3 min to allow for the adsorption of DNA onto the functionalized silica seed. 0.5 μl TMAPS as co-interacting species and 4.5 μl TEOS as silicon source were added, followed by a 4-day stirring at 1400 rpm, to allow the growth of ~10 nm silica coating onto the DNA-adsorbed silica seeds (Figure 5). The DNA molecules were thereby sandwiched between the silica seed and silica coating, obtaining greater thermal stability because of the silica protection (Paunescu et al. 2012). Resulting particles were then washed three times with water by centrifugation at 20817g for 3.5 min and resuspended using an ultrasonic bath, and were finally suspended in 100 μl of water. As indicated in Figure 5, the seeded growth process yielded agglomerated particles with average diameter of ~160 nm, and the monodispersity of particles was slightly enhanced compared with Figure 3.

Although resulting DNA-silica nanoparticles look almost identical to those produced by the original developer (Paunescu et al. 2013), they are not optimal for reservoir applications because the particles were agglomerated into micron-sized chunks that are not stable in solution (i.e. they tend to settle to the bottom of container). It would be more desirable if the particles were separated from each other and well dispersed in solution, because this would allow the particles to transport easily through porous rocks (Alaskar 2013). Therefore, further experimentations were performed aiming to resolve this issue (Section 6).

3. Testing on DNA-Silica Nanoparticles

In order to quantify the DNA encapsulated inside the particles, DNA release experiments were conducted according to the procedure suggested by Paunescu et al. (2013). After centrifuging 100 μl of synthesized DNA-silica nanofluid and discarding the supernatant, the particles were reacted with 300 μl NH$_4$HF/NH$_4$F etching solution (0.23 g NH$_4$HF + 0.19 g NH$_4$F + 10ml H$_2$O, pH~4) until the solution became transparent (i.e. silica completely dissolved, DNA released into solution). Released DNA was then purified using GET CLEAN DNA spin column supplied by G-Biosciences according to manufacturer’s instructions in order to remove excessive acid and salts. The DNA released from each 100 μl of DNA-silica nanofluid was finally eluted in 50 μl of TE buffer, and was diluted to a factor of 1000 before running qPCR quantification on StepOnePlus™ Real-Time PCR System available at Stanford Protein and Nucleic Acid Facility.

With well-controlled pH of etching solution and appropriate DNA purification, the DNA integrity was not affected much, as proven by qPCR quantification results as shown in Figure 6. Figure 6a is the overall amplification plot. Those curves with equal intervals are standard curves that came from the amplification of the same 113bp DNA with known
concentrations, and the rest of the curves represent the amplification of DNA with unknown concentration (DNA released from the particles).

Figure 6. a) Overall amplification plot of qPCR run that quantifies DNA released from DNA-silica nanoparticles. b) Standard curves of qPCR run that serve as calibration curve for DNA with unknown concentration. c) Amplification curve of DNA released from DNA-silica nanoparticles.

As shown in Figure 6a~c, the overall amplification plot was separated into standard-curve-only (Figure 6b) and unknown-DNA-only (Figure 6c) plots for clarity. The legends for standard amplification curve in Figure 6b are listed in Table 1.

It is shown in Figure 6 that the concentration of released DNA sample lies between 0.1 ~ 0.01 ng/μl, which is well within the range of amplifiable DNA concentration for qPCR (detection limit ~10^{-6} ng/μl). Also note that the released DNA samples were diluted to a factor of 1000 before being amplified, indicating that there were actually plenty of DNA molecules encapsulated inside the silica nanoparticles. The abundance in encapsulated DNA leaves sufficient room for applying the DNA-silica nanoparticles in flow experiments, because the particles would go through large extent of dilution as traveling through porous media.

### 4. Heating Experiments

The first objective was to evaluate silica particles at a reasonable geothermal reservoir temperature to evaluate their survival rate under reservoir conditions. Brinton et al. (2011) conducted experiments on quartz sand proppants,
but water loss from the pressure vessel evaporated the water suspension. A sample of SiO₂ spheres with 2 µm diameter, suspended in water, was purchased for the initial heating experiment. Five identical stainless steel tubes, fitted with end caps to withstand the pressure, were constructed and completely filled with the silica nanoparticle suspension. An oil bath was heated to 198°C and all five tubes were placed in the bath simultaneously. At five minute increments, a single tube was removed until none remained in the bath. Upon removal they were placed in a container of room temperature water and labeled according to the order of removal. The tubes were then opened, and a sample was removed from each and analyzed under the SEM (Scanning Electron Microscope). Each tube was found to be still fully filled with nanoparticle suspension upon opening (so none had leaked). The images revealed varying degrees of dissolution and precipitation in accordance with the time of exposure.

It was suspected that the chemical content of the precipitate included components other than silica. Therefore samples were analyzed under Auger Electron Spectroscopy (AES) to determine composition. It was concluded that the composition of the spherical silica particles was consistent with that of the amorphous precipitate surrounding it. Therefore they were both considered to be silica. It was concluded that the silica spheres dissolved partially into the suspension and then later precipitated, utilizing the suspended silica spheres as nucleation sites.

A Matlab code was written to process the SEM images and estimate the true size of the silica spheres in the image. Figure 8 shows the 2µm diameter silica spheres after 15 minutes of heat exposure.

Fifteen circles were found by the code, and their diameter was calculated by converting pixels to microns using the scale bar in the image. The smallest diameter was 1.769 µm and the largest was 2.141 µm; the average was 1.995 µm. This indicates a certain degree of dissolution and precipitation. The original suspension likely also contained dissolved silica which would have aided precipitation.

To confirm the content of the precipitate, a repeat experiment was conducted using silica nanoparticles (~200 nm diameter) created as described in Section 2. These were delivered in an ethanol suspension, a byproduct of the process utilized to create the silica spheres. The suspension was centrifuged and washed, by pouring out the supernatant, adding distilled H₂O, and centrifuging again. This process was repeated several times, and finally the suspension was sonicated to ensure adequate dispersion. Five new stainless steel tubes were constructed and the suspension was pipetted into them, filling each completely. The experiment was conducted exactly as before, and the results were very similar.

This sample was further analyzed using Dynamic Light Scattering (DLS), which attempts to determine the size distribution of particles in suspension using light refraction.

Figure 8. SEM image processed and analyzed by Matlab code, showing silica spheres after 15 minutes heated at 198°C. Circles were detected, outlined in blue and measured by converting pixels to micrometers.

Figure 9. Silica 200nm diameter particles heated at 198°C for varying lengths of time. Clockwise from top left: 5min, 10 min, 15 min, 25 min. An abundance of amorphous silica was observed along with original spheres.
Results indicated a decrease in average particle size in tubes 1-3 (5 – 15 min exposure) and an increase in size for the last two tubes (20-25 min exposure).

5. Injection Experiments

Silica nanoparticles were then evaluated by injection through a tube of packed sand. Experimentation temperatures were 25°C, 120°C, and 150°C. For experiments above the boiling point of water, a backpressure regulator was utilized to ensure the water did not boil. For 120°C the system pressure was 32 psi (2.2 atm) and for 150°C it was 90 psi (6.1 atm).

For each experiment, fresh sand was packed into a stainless steel tube. Distilled water was injected at a constant flow rate through the apparatus shown in Figure 10. After temperature and differential pressure stabilized, this “baseline” value was recorded. A three-way valve was then turned to push the nanoparticle “slug” through the system. The same slug volume was used in each experiment. Differential pressure values were recorded at one-minute intervals, and effluent samples were taken roughly every two minutes. The experiments ran for 30 minutes.

Effluent samples were pipetted from their individual containers and placed on an SEM stand for imaging. Utilizing the flow rate, measured length of the tube, differential pressure values and the viscosity of water at each temperature, rough permeability values were calculated for the packed sand. A comparison was made between the “baseline” permeability and the stabilized permeability following nanoparticle injection.

Experiments 1-3 were flowed continuously over the course of 30 minutes. In order to simulate a longer period in the reservoir, it was decided to stop the flow when the nanoparticle slug was inside the packed sand, and retain the fluid inside the sand for a gestation period before resuming the injection and flowing the fluids out. In

### Table 2. Summary of injection experiments for silica nanoparticles.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Experiment</th>
<th>Permeability [darcy]</th>
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<th>Comments</th>
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<tr>
<td>1</td>
<td>25°C Baseline</td>
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<td></td>
</tr>
<tr>
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<td>11.1</td>
<td>pH = 7.4 Effluent</td>
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<td>Silica Slug Injected</td>
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<td>25°C Water Flush</td>
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</table>

*Note that experiments 1-5 each used a different set of packed sand, while experiments 6-7 utilized the same sand.

** All flow rates were a constant 2.50mL/min except for experiment 1 (1.20mL/min).
experiments 4-5 the flow was stopped for one hour, and in experiments 6-7 it was stopped for six hours. In both cases the air bath remained at the specified temperature. After the baking period, flow was restarted and samples were taken along with permeability measurements. Each experiment lasted 30 minutes plus the baking time. Once the sand had cooled to room temperature, distilled water was flushed through the sand and permeability was calculated.

Note that permeability comparisons for the red and green shaded experiments in Table 2 were made between packed sand with baseline water and nanoparticle suspension. Results indicate that in every case the nanoparticle suspension has a reducing effect on permeability. Experiment 3 shows an increase in permeability, but the following experiments confirmed that permeability was increased in a purely water environment. However, permeability increased less when nanoparticles were injected.

A purpose for these experiments was to confirm the durability of the silica nanoparticles. SEM imaging confirmed that the particles did indeed survive the journey through the packed sand, as shown in Figure 11.

6. Modification of DNA-Silica Nanoparticles

As shown in Section 2.3, the synthesis procedure suggested by Paunescu et al. (2013) yielded agglomerated DNA-Silica nanoparticles that would start settling to the bottom of solution within 10 min. However, it would be optimal if the particles were dispersed in order for them to transport through porous media, which is possible because plain silica nanoparticles without DNA are well dispersed (stable in solution for over 1 week) and have been transported successfully through Berea sandstone (Alaskar, 2013) as well as packed sand (as described in Section 5).

Two possible causes for the agglomeration could be: (1) the absence of ammonia catalyst during silica coating process led to insufficient electrostatic repulsion among particles (Wang et al., 2011); (2) the DNA molecules dragged particles together during adsorption process because the two ends of the DNA helix could have adsorbed onto two different particles. Controlled experiments were conducted to study the agglomeration causes (Table 3).

As shown in Table 3, EXP#2, which applied exactly the same procedure as the original synthesis process described in Section 2 except replacing the 320 µl DNA solution with equal volume of water, yielded particles with no agglomeration. This indicates that the absence of ammonia catalyst was not the cause of agglomeration. Hence it is more likely that the presence of DNA molecules in the original process led to particle aggregation, which can also be corroborated by EXP#3, where the original synthesis was performed, but ceased at the point when DNA was adsorbed onto silica seeds (Figure 12). The SEM images indicate that silica seeds were brought together by DNA molecules even before silica coating, and that the silica shell was coated onto clusters of silica seeds, which even worsened the aggregation.

To address the aggregation issue, attempts are being made to adjust the ratio of water, DNA solution and functionalized silica seeds in the procedure described in Section 2.3. It is possible that if fewer particles were present in a unit volume of mixed solution, it would be harder for them to get together and form clusters.

7. DNA-Encapsulated Silica Nanoparticle Injection Experiments

Despite the agglomeration of synthesized DNA-encapsulated silica nanoparticles, it is still possible that the particles may be able to be transported through fractured rock because the agglomerated particle chunks are still micron-sized and therefore smaller than expected fracture apertures. To test this a sand pack with high permeability could be used. Therefore, a final injection experiment using DNA-silica nanoparticles was conducted to investigate whether the DNA-silica nanoparticles could be transported through packed sand and whether simulated geological temperatures (150°C) combined with flow through porous media would adversely affect DNA-encapsulated silica nanoparticles.
A new sand pack was prepared and the injection experiment was conducted with a flow rate of 2.50 ml/min. Unfortunately, the differential pressure gauge failed, eliminating the ability to monitor permeability changes. The effluent, however, was sampled regularly and subjected to SEM image analysis (Figure 13).

The particles were observed to have traveled through the packed sand successfully, although the number of particles observed was significantly lower than in previous injection experiments, which was anticipated because of the nonspherical shape of the DNA-silica nanoparticles, as well as its instability in suspension (i.e. the particles tend to settle). Whether the DNA encapsulated within the particles survived the journey has not been determined yet, and research is still looking at whether the DNA can be detected via qPCR despite the significantly lower number of particles that transported through the packed sand.

8. Conclusion

Initial investigations into silica nanoparticles indicate excellent flow characteristics through porous media at high temperatures. Particle dissolution, while present, appears to have minimal impact on overall particle integrity. DNA has been successfully encapsulated within silica particles at ~160 nm diameter, and the qPCR quantification after the release experiments indicates abundant DNA within the particles. Although DNA-encapsulated silica nanoparticles are agglomerated into clusters, they were transported successfully through packed sand as proven by SEM analysis of the effluent. Given the rather low detection limit of qPCR, the particles show good promise in being detectable after traveling through the porous or fractured media of a geothermal reservoir. Therefore, current results indicate realistic viability of DNA-encapsulated silica nanoparticles as tracers for geothermal flow path analysis.

References


