DNA-Encapsulated Silica Nanoparticle Tracers for Fractured Reservoir Characterization

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Abstract

This research investigated the use of DNA-encapsulated silica nanoparticles as a new type of tracer for fractured reservoir characterization. Silica nanoparticles with DNA embedded have been successfully synthesized by first adsorbing negatively charged DNA molecules onto positively charged silica seeds, and then coating the particles with silica. DNA was thereby “sandwiched” between the inner silica seed and outer silica layer, which protected the DNA from being affected by harsh environmental conditions (e.g. high temperature). The advantage of such DNA-embedded nanotracers is that the infinite number of possible sequences of DNA allows nanoparticles with different DNA “barcodes” to be uniquely identifiable, and hence can be applied in tracer testing to tag individual wellbores or flow paths.

The DNA-embedded silica nanoparticles were characterized through a series of DNA release, purification and quantification experiments, after which the encapsulated DNA was successfully extracted and quantified. The synthesis and characterization procedure as well as the mechanism of DNA handling techniques are explained in detail.

The behavior of DNA-encapsulated silica nanoparticles under simulated geological temperatures was investigated in this study. Heating experiments were conducted on plain silica nanoparticles to evaluate its capability of protecting the DNA while maintaining integrity of the particle at high temperature (198°C). Plain silica nanoparticles were also injected through packed sand under various temperatures to examine their flowability through porous media, their durability, and their influence on the permeability of porous media. DNA-encapsulated silica nanoparticles were finally injected through packed sand at high temperature (150°C) to investigate the feasibility to apply them in field tracer testing applications.

Problems associated with synthesizing DNA-encapsulated silica nanoparticles were also addressed and studied. The synthesis procedure was modified in order to enhance the particle stability in suspension.

The overall objective of this research was to synthesize and characterize DNA-silica nanotracer and to achieve a more complete understanding of the inherent mechanisms of their flow through porous rocks, as the study attempted to establish lab scale applications as a path toward the development of uniquely identifiable smart tracers at the field scale.
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Some of the material of this thesis has appeared in similar form in a joint paper coauthored by Yuran Zhang and Timothy Spencer Manley (Zhang et al. 2015).
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Chapter 1

1. Introduction

1.1. Motivation

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 fractures and their flow behavior. Techniques such as tracer tests 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 solute chemical tracers at the injection well and monitoring their breakthrough pattern at the production well. However, the number of solute tracers is limited, and hence presents a problem in situations where prior tracer residue within the tested region may cause ambiguity for subsequent tracer tests.

In recent years, there has been increasing amount of 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. For instance, by injecting polydispersed nanoparticles into fractured media, it is possible to infer fracture aperture by observing the size range of particles that went through (Alaskar 2013); By using dye-attached nanoparticles that release dye at a certain temperature, it is possible to infer temperature and flow information within the reservoir (Alaskar 2013). The high degree of control of their physical and chemical properties allows nanoparticle tracers to infer information that could not be achieved by conventional solute tracers.

In addition, nanoparticle tracers have less matrix diffusion compared with conventional solute tracers. The particles tend to stay in high velocity streamlines when being transported through porous media, hence travel more quickly than conventional solute tracers. The faster travel of nanoparticle tracers is even more prominent in fractured rocks because the particles are large compared to size of the pore throat, hence particles tend to be excluded from the low velocity regions of the rock matrix and remain in high velocity streamlines in the fractures (Smith et al. 1993). McCarthy et al. (2002) reported the breakthrough time of fluorescent carboxylate-modified latex microspheres through fractured shale saprolite to be five times shorter than solute bromide tracer.

Previous research in our group has looked at various nanoparticle tracer candidates such as dye-attached silica nanoparticles, irreversible thermochromic particles, tin-bismuth alloy particles, etc. Those nanoparticles have been transported successfully through porous or fractured rock samples. The specific goal was to develop thermally responsive particles to be transported through fractured reservoirs. By observing change in particles due to in-situ reservoir conditions, information about reservoir and fracture properties
could be inferred. The current research aimed to expand this work, searching for additional multifunctional nanoparticle tracer candidates that are able to carry abundant information about the reservoir formation it has traveled through.

1.2. Purpose of Study

The purpose of this study was to investigate 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 identifiability, 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 (Figure 1-1). Pioneering work by Paunescu et al. (2013) has proven that DNA protected by silica nanoparticles is able to withstand temperature as high as 200°C, and still can be amplified and quantified 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.

Figure 1-1. Schematic of DNA encapsulation into silica nanoparticles. (derived from Paunescu et al. 2013)

The advantage of a DNA-encapsulated nanotracer over other candidates is its uniquely identifiable nature. A DNA molecule is composed of four types of nucleotides, and the different arrangements of nucleotides that compose a long DNA double helix result in DNA molecules with almost infinite possible sequences. Hence by protecting various sequences of DNA into silica nanoparticles, we are able to obtain various uniquely identifiable DNA-silica nanoparticle tracers. The infinite number of DNA sequences leads to the unlimited number of uniquely identifiable nanoparticle tracers that could be applied to multiple wells or flow paths for tagging purposes without ambiguity.

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 own unique identifier, therefore enabling the identification of well connections, as illustrated in Figure 1-2.
In addition to the unique identifiability, the DNA-encapsulated silica nanotracer might also be used to infer temperature information about the formation. Although protecting DNA molecules with silica prevents the DNA from being denatured by high formation temperature, the DNA still undergoes some decay as temperature increases (Paunescu et al. 2013). Therefore, the level of decay that the protected DNA underwent can be analyzed and related to in-situ reservoir temperature, allowing additional information to be obtained.

![Figure 1-2. Schematic illustrating the well tagging application. If the same tracer were used in both wells, only the sum of the two return curves could be measured (red curve). Using unique tracers in each injection well allows us to distinguish return curves and identify the corresponding injection wells (blue and green curves). This would in turn provide more information about each interwell flowpath. Diagram by Morgan Ames.](image)

In this study, the synthesis procedure of DNA-encapsulated silica nanoparticles suggested by its original developers Paunescu et al. (2013) was carried out. Following preliminary silica seed preparation and surface functionalization, DNA molecules were adsorbed onto the silica seeds followed by a growth process that added a silica layer outside of the DNA, thereby “sandwiching” the DNA between inner silica seeds and outer silica layer. Particles at different stages of the synthesis were observed under SEM to confirm the effectiveness of the procedure.

The characterization of DNA-silica nanoparticles was also conducted. After the synthesis procedure that was used to produce the silica nanoparticles with DNA embedded, it was necessary to confirm that the DNA was actually in there. In addition, after flowing the DNA-silica nanoparticle tracer through reservoir rocks, an analysis technique is necessary to extract the information carried in the tracer. Hydrofluoric acid chemistry, DNA purification with spin column and qPCR quantification were conducted to release the DNA from the particles and identify its presence, and hence achieve particle characterization.
Besides the synthesis and characterization of DNA-silica nanoparticles, heating and injection experiments were conducted to test the particle durability under high temperature, flowability through porous or fractured media, as well as their influence on the permeability of the porous or fractured media it traveled through. Problems associated with the DNA-silica nanoparticles with respect to their transport through porous or fractured media were also encountered. The synthesis procedure was modified to enhance particle stability in suspension.

The overall goal of this research was to investigate the synthesis and characterization of DNA-silica nanotracer, to achieve full understanding of the inherent mechanism, as well as to establish lab scale applications as a path toward the development of DNA smart tracers at the field scale.
Chapter 2

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 2-1). Synthetic 113-base-pair single stranded DNA (ssDNA) with complementary sequences (Paunescu et al. 2013) was annealed according to standard annealing procedure to yield double stranded DNA (dsDNA) before being used for encapsulation.

After encapsulation, the DNA molecules are protected within the silica “fossil” and have been proven to withstand temperature as high as 200°C (Paunescu et al. 2013). The high temperature that is ubiquitous in geothermal reservoirs therefore would not be able to destroy the DNA once the DNA was embedded and protected within silica, and hence it is a feasible idea to apply such DNA-silica nanoparticles to field tracer testing applications.

In this chapter, the procedure and mechanism for the synthesis of DNA-silica nanoparticles are discussed in detail. The procedure is broken down into three stages, including silica seed synthesis, surface functionalization of silica seeds, and DNA encapsulation, in order to address the process with clarity.

2.1. Synthesis of Plain Silica Nanoparticles

In order to provide the supportive seed particles for DNA molecules to adsorb onto, plain silica nanoparticles in the 100~200 nm size range were first synthesized in our lab. The Stober method was used for the synthesis of plain silica nanoparticles, which involves the polycondensation of tetraethyl orthosilicate (TEOS) catalyzed by concentrated ammonium hydroxide (Stober, et al. 1968). Depending on the ratio of reagents involved...
and the reaction temperature, monodispersed silica nanoparticles with different diameters were yielded.

\[
\text{Si(OC}_2\text{H}_3\text{)}_4 + 2\text{H}_2\text{O} \xrightarrow{\text{NH}_4\text{OH}} \text{SiO}_2 + 4\text{C}_2\text{H}_5\text{OH}
\]  

(2-1)

Various modifications of the procedures were carried out to explore the effect of reagent ratio on the properties of silica nanoparticles (size, monodispersity, etc.). It is important to be able to control the size of nanoparticles so that we can eventually choose what size of nanoparticles to flow through porous or fractured media.

2.1.1. Procedure

Silica nanoparticles were prepared by first mixing ethanol, Milli-Q water and ammonium hydroxide in a 50 ml conical tube or a conical flask, then adding TEOS. The mixture was stirred overnight (>6 hours) in a shaker or on a magnetic stirrer plate at 900 rpm at room temperature (RT). When the reaction was complete, the resulting particles were washed by first centrifuging the particle solution at 9000 g for 20 min, then removing supernatant, and finally resuspending particles in ethanol or 2-propanol via vortexing and ultrasonication. The centrifugation and resuspension were repeated twice. Excess reagents were thereby removed and the silica nanoparticles could finally be preserved in ethanol or 2-propanol under room temperature for at least 6 months.

Various recipes with different reagent ratios, as listed in Table 2-1, were carried out. The ethanol served as solvent, and the ammonium hydroxide served as catalyst of the reaction. The reason why TEOS was added last is that we needed to make sure the ammonia catalyst was well mixed with other reagents before the reaction was triggered by TEOS, otherwise the nonuniform ammonia concentration in the solution would cause significant difference in silica nucleation rate, hence affect the monodispersity of resulting nanoparticles.

Table 2-1: Recipes carried out for synthesizing plain silica nanoparticles.

<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>5</td>
<td>0.5</td>
<td>0.5</td>
<td>RT</td>
<td>174~250</td>
<td>~210</td>
</tr>
<tr>
<td>2</td>
<td>97.4</td>
<td>8.05</td>
<td>1.55</td>
<td>5</td>
<td>RT</td>
<td>105~171</td>
<td>~140</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>0.5</td>
<td>0.8</td>
<td>0.8</td>
<td>RT</td>
<td>108~163</td>
<td>~140</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>1.5</td>
<td>0.5</td>
<td>1</td>
<td>RT</td>
<td>193~247</td>
<td>~220</td>
</tr>
<tr>
<td>5</td>
<td>18</td>
<td>0.8</td>
<td>0.5</td>
<td>0.8</td>
<td>RT</td>
<td>87~147</td>
<td>~120</td>
</tr>
<tr>
<td>6*</td>
<td>40</td>
<td>10</td>
<td>0.5</td>
<td>0.5</td>
<td>RT</td>
<td>224~329</td>
<td>~275</td>
</tr>
</tbody>
</table>

Apart from plain silica nanoparticle synthesis, a seeded particle growth process was also tested in order to provide a foundation for future silica coating outside of DNA-adsorbed silica nanoparticles. During the seeded particle growth process, condensation of TEOS
occurs directly onto silica nanoparticles that already exist in the solution. As the condensation goes on, particles thereby increase in size.

The resulting particle solution of recipe #1 was selected for the seeded growth test. The nanoparticle solution from recipe #1 was mixed with 40 ml ethanol, 10 ml H₂O, 0.5 ml NH₄OH and 0.5 ml TEOS and was stirred overnight at room temperature (recipe #6*). After the reaction was complete, the particles were washed by first centrifuging the particle solution at 9000 g for 20 min, then removing supernatant, and finally resuspending particles in ethanol via vortexing and ultrasonication. The centrifugation and resuspension were repeated twice to ensure that all excess reagents were removed.

The ammonium hydroxide had a concentration of 29.14% (wt/wt) and was purchased from Fisher Scientific. TEOS was 98% (wt/wt) purchased from Arcos Organics. Solvents used during reactions were 200 proof, anhydrous ethanol purchased from EMD Chemicals, and HPLC grade 99.9% 2-propanol purchased from Fisher Scientific. The shaker was a H5000-H model purchased from Benchmark Scientific. The centrifuge was a Sorvall Legend XFR model with Fiberlite F14-6*250 LE fixed angle rotor available in Lab 222, Green Earth Sciences Building.

2.1.2. Results and Discussion

The procedure yielded milky colloidal silica that was electrostatically stable for weeks at room temperature. The silica nanoparticles were hydrophilic because of the hydroxyl groups existing on particle surface (Kim et al. 2006), and carried negative charge because of the dissociation of surface silanol groups (Behrens et al. 2008). The resulting silica nanoparticles were further analyzed by observation under FEI XL30 Sirion Scanning Electron Microscope (SEM) available at the Stanford Nanocharacterization Laboratory (SNL).

The SEM samples were prepared according to the following procedure. Firstly, substrate was prepared by adhering a small piece of clean, dried silicon wafer which was previously preserved in ethanol onto an SEM pin mount using conductive carbon paint. Secondly, after being vortexed and ultrasonicated for dispersion, one drop of particle solution was dripped onto the silicon wafer substrate. After the drop of particle solution was dried, the silica nanoparticles were dispersed and fixed onto the substrate surface and the sample was ready for SEM observation.

During SEM observation, the 5 kV beam was used and live images were obtained through the Secondary Electron (SE) detector. As shown in Figure 2-2, the procedure yielded submicron-sized silica nanoparticles that were quite monodispersed. Depending on the specific recipe applied, resulting particles had different size ranges, as shown in Table 2-1. The particle sizes were calculated by picking SEM pictures that include at least 100 particles, and measuring particle diameter range according to the scale bar.
As indicated in Table 2-1 and Figure 2-2, altering the ratio of Ethanol, H₂O, NH₄OH and TEOS led to difference in the size range of resulting silica nanoparticles, but had almost no effect on the monodispersity of particles because particles from all recipes were in ±30 nm size range. The reason why particle size range could not be further reduced is that it is
hard to make every particle nucleate at the same time and grow at the same rate. Nevertheless, the ±30 nm size range was actually adequate for our study.

The sixth image of Figure 2-2 shows the result of the seeded particle growth conducted on particles from recipe #1. After seeded growth, average particle diameter increased from ~210 nm to ~275 nm (from 174~250 nm range to 224~329 nm range), indicating that silica outer shell around 33 nm thick had grown on the original particles. The sixth image of Figure 2-2 also contains tiny silica nanoparticles of ~50 nm, which is an indication of new condensation nuclei while the majority of particles were growing in size.

Silica nanoparticles yielded from recipe #3 (as highlighted in Table 2-1) with average diameter of ~140 nm were finally selected as the silica “seeds” for further DNA encapsulation, as suggested by Paunescu et al. (2013). The resulting particles of recipe #3 were washed three times with 2-propanol and were finally suspended in 4 ml of 2-propanol. Resulting particle concentration was ~50 mg/ml.

### 2.2. Surface Functionalization of Plain Silica Nanoparticles

As mentioned in Section 2.1.2, silica seeds synthesized through the Stober Method carry negative surface charge because of the dissociation of surface silanol groups. DNA molecules, however, also carry negative surface charge because of their phosphate backbone. The electrostatic repulsion between silica seeds and DNA molecules leads to an unfavorable condition for DNA to adsorb onto the surface of silica seed nanoparticles. We therefore modified the surface functionality of silica seeds to make them carry positive charge so that DNA molecules could adsorb onto their surface.

#### 2.2.1. Procedure

1 ml of silica seed solution from the previous step (recipe #3, ~140nm) was transferred into a 2-ml microcentrifuge tube and was ultrasonicated and vortexed for over 10 min until the particles became well dispersed and the solution became homogeneous. 10 μl of trimethyl[3-(trimethoxysilyl)propyl]ammonium chloride (abbreviated as TMAPS, 50% (wt/wt) in methanol purchased from TCI America) was then added to the silica seed solution, and the mixture was stirred in a shaker for over 12 h at 1400 rpm at room temperature. The mechanism of functionalization is illustrated in Figure 2-3. Note that it is necessary to use a 2-ml microcentrifuge tube rather than 1.5-ml tube to ensure perfect particle dispersion. After reaction was complete, the particles were washed with 2-propanol by centrifuging at 20817 g in an Eppendorf Microcentrifuge (Model 5430) for 4.5 min, carefully removing supernatant using a pipette, and resuspending in 1 ml of 2-propanol using vortexer and ultrasonic bath. The washing process was repeated twice and the particles were finally suspended in 1ml of 2-propanol. The concentration of surface functionalized silica seed solution, therefore, remained to be ~50 mg/ml.
In order to confirm that the surface charge of silica seeds became positive after surface functionalization, zeta potential of the silica nanoparticles before and after surface functionalization was measured using the Malvern Zetasizer Nano ZSP available at nSiL lab of Stanford Nanofabrication Facility (SNF).

Particle solution with original concentration (~50 mg/ml) was first vortexed and sonicated until particles became well dispersed, then was diluted to a factor of ten. 1 ml of the diluted sample was transferred into a cuvette, and the electrode was slowly dipped into the cuvette. Special care should be taken not to form any bubbles during this process, because bubbles would get in the path of the laser beam and render the measurement inaccurate.

2.2.2. Results and Discussion

As shown in the zeta potential measurement result (Figure 2-4), the silica seeds before surface functionalization carried -46.4 mV surface charge. After surface functionalization, however, the charge changed to +46.1 mV. The zeta potential measurement result indicates that: 1. Silica nanoparticle solutions before and after surface functionalization both had good electrostatic stability, because the absolute value of their zeta potentials exceeded 30 mV; 2. Surface charge of silica seeds was successfully modified, and DNA molecules carrying negative charge could therefore attach to the positively charged silica seeds to allow for subsequent encapsulation.
Figure 2-4. Zeta potential measurement of silica seeds before (top) and after (bottom) surface functionalization. Zeta potential value changed from -50mV to +40mV after the treatment.

2.3. DNA Encapsulation

This section describes how DNA molecules were encapsulated into the protective silica nanoparticles. Previously prepared silica nanoparticle seeds with positive charge were mixed with DNA solution and Milli-Q water to allow DNA molecules to adsorb onto silica seed surfaces, then the seeded particle growth process (previously tested on plain silica nanoparticles, as described in Section 2.1.1) was conducted to coat the DNA-adsorbed silica seeds with an outer silica layer, thereby “sandwiching” DNA molecules between inner silica seed and outer silica layer (Figure 2-1) so that DNA’s vulnerability to harsh environmental conditions (e.g. high temperature) would be mitigated.
2.3.1. Synthetic DNA and Preliminary Preparation

Three types of DNA, including plasmid DNA, genomic DNA and synthetic DNA, have been successfully encapsulated into silica nanoparticles by Paunescu et al. (2013). In our study, because we want to control the DNA sequence to investigate its potential for fracture or wellbore tagging purposes, we selected synthetic DNA, whose sequence can be decided by us, to be encapsulated into silica nanoparticles.

Synthetic ssDNA (synthetic single stranded DNA, also known as oligonucleotides) with 113 bases as well as its complementary oligonucleotides were purchased from Eurofins Genomics. The complementary oligonucleotides were dry, and had molecular weight of 34882.9 g/mole and 34814.9 g/mole, respectively. The sequence of the oligonucleotides (5’-3’, suggested by Paunescu et al. 2013) was:

ATT CAT GCG ACA GGG GTA AGA CCA TCA GTA GTA GGG GTA GTG CCA AAC CTC ACT CAC CAC TGC CAA TAA GGG GTC CTT ACC TGA AGA ATA AGT GTC AGC CAG TGT AAC CCG AT

Because we ultimately need to quantify the DNA encapsulated in the silica nanoparticles, the DNA that we use has to be amplifiable (i.e. double stranded), otherwise we would not be able to obtain the information desired out of the particles (details about DNA amplification is explained later in Section 3.2). However, synthetic DNA can only be purchased as single stranded, hence the complementary oligonucleotides that we purchased were first annealed in order to yield amplifiable dsDNA (double stranded DNA). The annealing is based on the fact that reverse-complementary DNA strands that are separated by heat (thermal denaturation) would re-pair by hydrogen bonds when temperature is lowered to a certain level.

The annealing procedure is as follows. Firstly, annealing buffer was prepared by mixing 20 ml 10x TE buffer (100 mM Tris + 10 mM EDTA, pH 8.0, purchased from G-Biosciences) and 0.5844 g NaCl (0.01 mole), then adding Milli-Q water until the total volume is 200 ml. Resulting annealing buffer therefore contained 10 mM Tris (pH 7.5~8.0), 50 mM NaCl, and 1 mM EDTA. Secondly, both complementary oligonucleotides were resuspended in the annealing buffer at 100 μM. Thirdly, 100 μl of the respective complementary oligonucleotides solution (total volume 200 μl) was mixed in a 1.5 ml microcentrifuge tube and was placed in a standard heat block at 95 °C for 5 min. The tube was then removed from the heat block and cooled to room temperature on the workbench, which took around 60 min. The oligonucleotides were thereby annealed to yield 200 μl dsDNA with a molar concentration of 50 μM. The dsDNA solution was then diluted to various concentrations and stored at -20 °C for future use.

2.3.2. DNA Encapsulation Procedure

In the DNA encapsulation process, DNA solution and functionalized particle solution were mixed to allow for negatively charged DNA molecules to adsorb onto the positively charged silica seed particles. Right after the adsorption, the seeded growth process, the
mechanism of which has been described in Section 2.1.1, was performed to add a silica outer layer to the DNA-attached silica seed.

Before encapsulation, the dsDNA solution prepared was diluted to a concentration of 717 nM, which corresponds to ~50 μg/ml. Surface functionalized silica seed solution suspended in 2-propanol at a concentration of ~50 mg/ml, as described in Section 2.2, was used for the encapsulation. The encapsulation procedure suggested by Paunescu et al. (2013) was applied with slight modifications.

1) 700 μl H₂O was mixed with 320 μl of the DNA solution in a 2-ml microcentrifuge tube, then 35 μl functionalized silica nanoparticle solution was added (particle size ~140 nm). The solution was well mixed by pipetting and vortexing and was left at room temperature for 3 min.

2) The mixture was centrifuged at 20817 g for 3.5 min at room temperature. Supernatant was carefully removed without losing particles.

3) The pellet was suspended in 1 ml of H₂O, vortexed and ultrasonicated in a bath until it turned into homogeneous solution. The solution was then centrifuged again at 20817 g for 3.5 min at room temperature. Supernatant was carefully removed without losing particles.

4) The pellet was suspended in 500 μl of H₂O, vortexed and ultrasonicated in a bath until it turned into homogeneous solution without visible particle aggregates. 0.5 μl TMAPS was added. The mixture was vortexed for a few seconds before 0.5 μl of TEOS was added to the solution.

5) The mixture was stirred in a shaker at 1400 rpm at room temperature for 4 h, then 4 μl of TEOS was added to the solution.

6) The mixture was stirred at 1400 rpm for a further 4 days at room temperature.

After completion of the 4-day reaction, the mixture was washed by centrifugation at 20817 g for 3.5 min at room temperature and the supernatant was removed. The pellet was then suspended in 500 μl of H₂O by vortexing and ultrasonication. The washing process was repeated twice and the particles were finally suspended in 100 μl of H₂O (resulting concentration ~17.5 mg/ml). Resulting particles were the final DNA-encapsulated silica nanoparticles, ready for further analysis and application.

Please note that the stirring velocity during the reaction must be high enough (1400 rpm), otherwise the particles would clump and fall to the bottom of the tube during the 4-day reaction, which would lead to suboptimal condition for the formation of silica layer and result in large, messy silica chunks that no longer maintain the spherical shape of particles (Figure 2-5).
Also note that no ammonia catalyst was used during the silica coating reaction, which was why the reaction took 4 days to complete. The reason for not adding ammonia catalyst was to prevent the DNA from hydrolyzing under basic environment (Paunescu et al. 2013) and maintain its integrity as much as possible.

Figure 2-5. SEM images of resulting “particles” when stirring velocity was not sufficient.

2.3.3. Results and Discussion

When the procedure described in Section 2.3.2 was strictly followed, the solution remained dispersed throughout the reaction. SEM samples were prepared according to the same procedure as described in Section 2.1.2 and SEM images of the resulting particles are shown in Figure 2-6.
Figure 2-6. SEM images of DNA-encapsulated silica nanoparticles at various degrees of magnification. Particle average size increased to ~160 nm.

As shown in Figure 2-6, the DNA encapsulation procedure yielded particles that increased in size to an average of 160 nm (145~178 nm range), and still maintained their spherical shape. The increase in particle diameter demonstrates the successful growth of
an outer silica layer outside of the DNA-attached silica seed. However, the DNA-silica nanoparticles were heavily agglomerated. Not only can this be reflected in the SEM images where the particles appear to have stuck with each other and formed micron-sized chunks, it could also be observed simply by setting the solution still right after the 4-day DNA encapsulation reaction. Particle sedimentation toward bottom of the reaction tube could be seen approximately 10 min after the stirring was stopped, and particles settled completely after the solution was set still for over 1 h. Photos of a sample tube were taken to show the sedimentation issue (Figure 2-7).

![Figure 2-7](image)

Figure 2-7. Photos for particle solution in sample tube. Top: DNA-silica nanoparticle solution right after synthesis (left) and 1 day after synthesis (right), particles settled completely to the bottom after 1 day. Bottom: Functionalized silica seed solution right after surface modification (left) and 1 day after surface modification (right), particles were stable and remained dispersed. Note that functionalized silica seed solution remain dispersed like shown in the photo even after setting still for 1 week.

Apparently, the agglomerated state of DNA-silica nanoparticles is unfavorable for transport through porous or fractured media because the agglomerated particle chunks are no longer submicron sized, no longer stable in solution (they tend to settle), and no longer spherically shaped. All three criteria are important for their transport through porous or fractured media (Alaskar, 2013). The agglomeration issue was also known to Paunescu et
al., the original developer of the DNA-silica nanoparticles, but did not affect their intended use for the particles. Therefore, apart from trying to successfully synthesize such DNA-encapsulated silica nanoparticles, attempts were also made to mitigate the agglomeration and enhancing their stability in solution. Details can be found later in Chapter 4.

2.4. Chapter Summary

This chapter described the processes by which DNA-encapsulated silica nanoparticles were synthesized step by step as instructed by their original developer but with slight modifications. The Stober method that synthesizes plain silica nanoparticles as the supporting seeds for DNA molecules was investigated. Various recipes were carried out, yielding monodispersed plain silica nanoparticles with varying average sizes. The silica seeds with ~140 nm diameter were used for subsequent DNA encapsulation.

The plain silica seeds were surface functionalized to yield positive charge, as confirmed by zeta potential measurement. Silica seed solution was then mixed with DNA solution and water to allow negatively charged DNA molecules to adsorb onto the seed surface. After washing away excess DNA, seeded growth process was conducted to coat the DNA-adsorbed silica seed with an outer silica layer. The coating was conducted without the presence of ammonia catalyst in order to maintain the integrity of DNA. After coating was complete, agglomerated DNA-encapsulated silica nanoparticles of around 160 nm were successfully synthesized as indicated by SEM analysis. The particles looked exactly the same as those produced by Paunescu et al. (2013), and were further characterized by DNA analysis, which will be discussed in Chapter 3.
Chapter 3

3. Characterization of DNA-encapsulated Silica Nanoparticles

In the DNA encapsulation procedure, DNA adsorption onto functionalized silica seeds and the growth of silica outer layer were performed in a single reaction with no pausing point. Therefore, it is tricky to demonstrate that the DNA adsorption was successful and that the resulting DNA-encapsulated silica nanoparticles actually contain DNA molecules within them. In order to prove the successful encapsulation of DNA molecules into the protective silica nanoparticles, a DNA release experiment according to the prescription of Paunescu et al. (2013) was performed and was followed by DNA quantification techniques that are commonly applied in the molecular biology field (e.g., qPCR). If DNA were successfully detected after the release experiment, not only would it prove that DNA was encapsulated successfully into the silica protective nanoparticles, it would also demonstrate that we had been able to synthesize and characterize the DNA-encapsulated silica nanoparticles in our own lab. That would mean that this specially manufactured nanoparticle with abundant information stored is ready for further investigations regarding its application in reservoir characterization.

The idea of the DNA release experiment is to dissolve the inner silica seed and outer silica layer of the DNA-silica nanoparticles using buffered hydrofluoric acid. DNA molecules would be freed from the particles into solution, and clean DNA could be captured by purifying the solution with a spin column. Purified DNA could then be quantified through qPCR. A schematic of the DNA release process is shown in Figure 3-1.

![Schematic of releasing DNA from synthesized DNA-silica nanoparticles](image)

Figure 3-1. Schematic of releasing DNA from synthesized DNA-silica nanoparticles

In this chapter, we will show that we were able to successfully prove the presence of DNA in our self-synthesized DNA-silica nanoparticles. The mechanism and procedure for DNA release, purification, and quantification along the process of testing the synthesized DNA-silica nanoparticles will be discussed in detail.
3.1. DNA Release Using Hydrofluoric Acid and DNA Purification

In order to recapture the DNA molecules that have been “sealed” within the protective silica nanoparticles, dissolving the protective silica nanoparticles is necessary. Although silica is not dissolvable in most chemical reactants, it is easily attacked by hydrofluoric acid (HF) to produce hexafluorosilicic acid (Equation 3-1). In addition, because aqueous hydrofluoric acid is a weak acid, it is expected to be relatively compatible with DNA (no influence even after 80 hours contact), as long as the pH of the hydrofluoric acid is controlled to a value that is not too low (Paunescu et al. 2013). Therefore, in principle it is feasible to release the DNA using hydrofluoric acid.

\[ \text{SiO}_2 + 6\text{HF} \rightarrow \text{H}_2\text{SiF}_6 + 2\text{H}_2\text{O} \] (3-1)

After dissolving the DNA-silica nanoparticles with hydrofluoric acid, the solution would be free of solids and the DNA molecules would be freed into the aqueous solution to allow for further purification and quantification. The hydrofluoric acid used was buffered oxide etch solution consisting of ammonium hydrogen difluoride and ammonium fluoride (\(\text{NH}_4\text{FHF}/\text{NH}_4\text{F}\)) with a pH of ~4.

Hydrofluoric acid, however, is a dangerous chemical because it is highly corrosive and penetrates skin tissue quickly to cause poisoning. The procedure that we adopted for the release experiment was suggested by Paunescu et al. (2013) and has been optimized to minimize the amount and concentration of hydrofluoric acid so that the user never needs to handle HF solutions with toxic potential upon oral uptake. Extreme care, however, is still necessary during the entire experiment. For instance, always wear double nitrile gloves, protective goggles and a lab coat during experiments, always operate in a fume hood, always keep HF antidote gel (calcium gluconate) in close proximity, etc.

Dissolving the silica protective particles with hydrofluoric acid would result in DNA in clear, solid-free solution. Although DNA is supposed to be compatible with the buffered etching solution that we used, it was still necessary to purify the DNA before further quantification to minimize the possibility for DNA to be affected by the impurities in the solution, especially when it could take several days for a DNA sample to be processed at a DNA quantification (qPCR) facility. The purification technique applied here involves the usage of a spin column, which is widely adopted in the molecular biology field for nucleic acid and protein purification from enzymes, primers and salts.

The mechanism of spin column purification relies on the fact that DNA molecule binds to the silica membrane in the center of spin column under certain pH and salt concentration. During the purification process, binding buffer (guanidine hydrochloride) is first added to the unpurified DNA solution to provide the optimal pH and ionic strength. The solution is then transferred into a spin column and is centrifuged to force the solution through the silica membrane. As solution passes through the silica microchannel, DNA molecules, under favorable ionic condition, would bind to the fibers of the membrane whereas the impurities (\(\text{SiF}_6^{2-}\), \(\text{NH}_4^+\), etc.) would flow through the membrane and reach the collection
tube. Wash buffer (various salts) is then added to the spin column and is forced through the silica membrane via centrifugation to wash away any remaining impurities from the membrane. Finally, prewarmed TE buffer is added to the center of the membrane to elute the clean DNA that has been bound to the membrane into TE buffer. DNA is thereby purified from impurities and is ready for further analysis.

The photo of a spin column and the schematic of the whole purification process are shown in Figure 3-2 and Figure 3-3. GET™ CLEAN DNA spin column kit purchased from G-Biosciences was used for the purification.

![Photo of a spin column and its collection tube](image1)

Figure 3-2. Photo of a spin column (green) and its collection tube (white).

![Schematic of DNA purification procedure](image2)

Figure 3-3. Schematic of DNA purification procedure using spin column.
The detailed procedure for DNA release (suggested by Paunescu et al. 2013 with slight modifications) and purification (suggested by the manufacturer of spin column kit) is as follows.

1) Etching solution (NH$_4$FHF/NH$_4$F) was first prepared by dissolving 0.46 g of NH$_4$FHF in 10 ml of H$_2$O and 0.38 g of NH$_4$F in 10 ml of H$_2$O and mixing the two solutions together. Resulting etching solution had a pH of ~4.

2) 100 μl of the DNA-silica nanoparticles synthesized as described in Chapter 2 (concentration ~17.5 mg/ml) was centrifuged at 20817 g for 3.5 min. Supernatant was discarded after centrifugation.

3) 300 μl of the NH$_4$FHF/NH$_4$F etching solution was added to the DNA-silica particle pellet. The etching solution and the particles were well mixed by pipetting, and were allowed to react until the cloudy particle solution became transparent, which was an indicator of complete removal of the silica.

4) 1.5 ml of binding buffer was added and mixed after all silica was removed, and the solution was transferred to a spin column. The spin column was centrifuged at 14000 g for 1 min and the flow-through was discarded.

5) The column was placed back into the same collection tube and 750 μl of DNA wash (precooled on ice) was added to the column. The column was centrifuged at 14000 g for 1 min and the flow-through was discarded.

6) The column was placed back into the same collection tube and was centrifuged at 14000 g for an additional 1 min, which ensured that all DNA wash was removed. The column was then placed into a clean collection tube (1.5 ml microcentrifuge tube).

7) 50 μl of TE buffer (preheated to 60 °C) was added to the center of the column membrane, and was incubated for 1 min. The column was then centrifuged at 14000 g for 1 min, and purified DNA was thereby eluted into the TE buffer and collected in the microcentrifuge collection tube, and was ready for further analysis.

The release experiment was conducted on three of the previously synthesized DNA-silica nanoparticle samples that underwent identical synthesis procedure, each of which contained 100 μl of DNA-silica nanoparticle solution (~17.5 mg/ml). Parallel experiments were conducted to investigate the uncertainty of the quantity of DNA molecules encapsulated in each batch of DNA-silica nanoparticles.
3.2. DNA Quantification Method

We are ultimately aiming to “tag” individual wellbores or fractures with our DNA-encapsulated nanoparticles that contain different DNA sequences. Therefore, when deciding which DNA quantification method to use for the analysis of the released DNA, we would expect the quantification method to not only tell us “how much” (measuring DNA concentration), but also tell us “which one” (specifying DNA sequence). For those purposes, qPCR (real-time quantitative polymerase chain reaction) is considered the best fit for our DNA analysis because of its low detection limit and its “selective” nature (detailed explanation follows).

3.2.1. Basic Mechanism of qPCR

The real-time quantitative polymerase chain reaction (qPCR) is a technique widely applied in molecular biology that allows fragments of DNA to be amplified and detected at the same time. The technique involves repeated heating and cooling cycles for the melting and enzymatic replication of DNA, with dsDNA template, forward and reverse primers, deoxyribonucleotides, thermostable DNA polymerase, and fluorescent dye as ingredients for the reaction.

In a qPCR cycle, a dsDNA (double stranded DNA) molecule is first heated to 95 °C so that the two strands separate and serve as the “template” for further amplification. Then the temperature is lowered to allow the primers to bind to their complementary sequence on the template DNA strand. Primer is defined as the short sequence of oligonucleotide (usually 15~20 bases) that is complementary to certain segment of a DNA template, serving as a starting point for the synthesis of new dsDNA molecules. The sequence of the forward and reverse primers we used is:

Forward primer (5’-3’): ATT CAT GCG ACA GGG GTA AG

Reverse primer (5’-3’): ATC GGG TTA CAC TGG CTG AC

Comparing the primer sequence with the dsDNA sequence (listed in Section 2.3.1), we can see that the forward and reverse primer we used are complementary to the 20 bases at the 5’ end of each DNA strand, respectively.

After the primers bind to the template, the DNA polymerase present would use the primers to synthesize the rest of the DNA strand complementary to the template. Therefore, two new dsDNA are synthesized out of every dsDNA template in each cycle, and the DNA template would be amplified exponentially as more and more heating cycles are undergone. A schematic of the DNA amplification process is shown in Figure 3-4.
In order for the amplified DNA to be detected in real time, or, in other words, in order to measure the amount of DNA after each heating cycle, fluorescent dye is used to enable the detection of DNA by measuring the intensity of fluorescence. The dye we chose was SYBR Green, which binds to the helix of double stranded DNA, and only fluoresces after it is bound to dsDNA. Therefore, as DNA amplification goes on, the increase in DNA product would lead to an increase in fluorescence intensity. The fluorescence intensity is measured at every heating cycle in qPCR, allowing a history of DNA amplification to be recorded.

Therefore, if we have, for instance, two DNA samples with different concentrations to be quantified, by comparing the number of heating cycles it takes for the fluorescence intensity to reach certain level (also defined as the cycle threshold), we would be able to infer the relative concentrations of the starting DNA samples: the smaller the starting DNA concentration, the more cycles it takes for the fluorescence level to reach the cycle threshold. And in order to know the absolute concentration of samples, DNA solution with known concentrations, also known as the standard, is prepared at 1:1, 1:10, 1:100, 1:1000, 1:10000 dilutions and is amplified simultaneously with the unknown samples.
(each sample is amplified in separate wells). Therefore, by comparing the cycle thresholds between the unknown and the standard DNA, the concentration of unknown samples can be inferred.

The amplification will eventually stop when the ingredient (deoxyribonucleotides) runs out, which is indicated by a plateau of fluorescence intensity on the amplification plot (Figure 3-7). All samples are analyzed in triplicate to ensure accuracy.

Apart from its capability of measuring DNA concentrations, qPCR is also highly “selective” because it requires the binding of primers in order for DNA templates to be amplified. In other words, if there are several DNA sequences in a DNA sample, only the DNA that contains sequence complementary to the primers would be amplified. This allows us to know not only how much DNA is present, but also which DNA it is.

3.2.2. Analyzing DNA Released from DNA-encapsulated Silica Nanoparticles

The DNA samples obtained after the DNA release experiment described in Section 3.1 were sent to the PAN Facility (Protein and Nucleic Acid Facility) at Stanford School of Medicine where qPCR analysis was performed. The instrument was a 96-well StepOnePlus™ Real-Time PCR System, and some of the qPCR ingredients, including deoxyribonucleotides, DNA polymerase, and fluorescence dye (SYBR Green), were supplied by the PAN facility according to their standard qPCR procedure.

As mentioned in the previous section, standard DNA with known concentrations is needed for the absolute quantification of unknown DNA samples. In addition, because primers are needed for every amplification cycle to start, it is important to select an appropriate primer concentration. If the primer concentration is too low, there will not be enough primer left as the amplification carries on, and the fluorescence intensity would not be able to reach the plateau. Primer used in qPCR is hence always in excess. However, if the primer concentration is too high, the primers would bump into each other when binding to the DNA template, causing suppression of the amplification. Therefore, before analyzing the DNA samples from the release experiment, a trial qPCR was conducted to test the standard curve, and meanwhile to determine the appropriate primer concentration.

According to the studies of Alaskar (2013) and Paunescu et al. (2013), a rough estimate for the concentration of released DNA is around 0.174 ng/μl (corresponding to 2.5 nM for the DNA sequence specified). We hence used the dsDNA with 113 base pairs that was annealed previously, and used 2.5 nM, 0.25 nM, 0.025 nM, 0.0025 nM, and 0.00025 nM as standard DNA concentrations, which should cover the range of possible unknown DNA concentrations. And in order to get an appropriate primer concentration, we tested primer concentrations of 50 nM, 100 nM and 300 nM. The resulting amplification plots are shown in Figure 3-5 ~ Figure 3-7.
Figure 3-5. Amplification plot of standard DNA using 50 nM primer concentration. 50 nM primer concentration is apparently not enough for the DNA template to be amplified.

Figure 3-6. Amplification plot of standard DNA using 100 nM primer concentration. 100 nM primer concentration is apparently not enough for the DNA template to be amplified.
At this point, we had everything needed for the released DNA quantification. qPCR analysis was therefore conducted at Stanford PAN Facility using the 113 base pair dsDNA at 2.5 nM, 0.25 nM, 0.025 nM, 0.0025 nM, and 0.00025 nM as standard DNA, and the 20 base pair forward and reverse primers at 300 nM concentration.

### 3.3. Results and Discussion

The release experiment, as described in Section 3.1, was conducted on three DNA-silica nanoparticle samples, each of which contained 100 μl of DNA-silica nanoparticle solution (~17.5 mg/ml) that underwent identical synthesis procedure. Hence we had three parallel samples to study the amount of DNA released from the DNA-silica nanoparticles. The three released DNA samples were amplified via qPCR along with the standard DNA.

However, the result did not make sense at first, as shown in Figure 3-8 and Figure 3-9. Figure 3-8 is the overall amplification plot of both standard DNA and unknown DNA, and Figure 3-9 splits the unknown DNA amplification curves from standard DNA curves, in order to distinguish them better. As shown in the figures, the standard DNA was amplified with correct-looking amplification curves, whereas the unknown DNA just showed some incorrect-looking lines, making it impossible to compare the relative cycle threshold with the standard curves.
Closely observing the amplification plot, however, it does not seem as if the unknown DNA sample underwent any amplification at all. Conversely, it looks like the fluorescence intensity for the unknown DNA sample was high even at the very beginning. Hence it was suspected that there was DNA successfully released from the DNA-silica nanoparticles, however the concentration was too high compared with the standard DNA concentration, and that the deoxyribonucleotide ingredient present during the reactions was not sufficient for the DNA to further amplify and hence a correct-looking amplification curve similar to the standard curve was not obtained.

The released DNA sample was hence diluted to a factor of 1000 in order to deal with the issue. The 1/1000 diluted unknown DNA sample was amplified with standard DNA again via qPCR, and correct-looking amplification curves for the unknown DNA were obtained, as shown in Figure 3-10 and Figure 3-11.

Color legends for all standard curves are shown in Table 3-1.

Table 3-1: Color legends of standard amplification curves in Figure 3-8 ~ Figure 3-11.

<table>
<thead>
<tr>
<th>Color Legend Name</th>
<th>Standard DNA Concentration</th>
<th>Cycle Threshold (Ct)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.5 nM (~1.74×10^4 ng/μl)</td>
<td>9</td>
</tr>
<tr>
<td>B</td>
<td>0.25 nM (~1.74×10^2 ng/μl)</td>
<td>15</td>
</tr>
<tr>
<td>C</td>
<td>0.025 nM (~1.74×10^3 ng/μl)</td>
<td>21</td>
</tr>
<tr>
<td>D</td>
<td>0.0025 nM (~1.74×10^4 ng/μl)</td>
<td>27</td>
</tr>
<tr>
<td>E</td>
<td>0.00025 nM (~1.74×10^5 ng/μl)</td>
<td>33</td>
</tr>
</tbody>
</table>
Figure 3-8. Amplification curves of released (unknown) DNA samples together with amplification curves of standard DNA. Sample concentration was not within the appropriate range for amplification (too high).
Figure 3-9. Amplification curves of standard DNA (top) and amplification curves of unknown DNA (bottom). This figure separates the plot in Figure 3-8 into two components to make it easier for readers to tell from standard amplification curves and unknown DNA amplification curves. Sample concentration was not within the appropriate range for amplification (too high).
Figure 3-10. Amplification curves of released (unknown) DNA samples diluted to a factor of 1000 together with amplification curves of standard DNA.
Figure 3-11. Amplification curves of standard DNA (top) and amplification curves of unknown DNA diluted to a factor of 1000 (bottom). Curves with three different colors. This figure separates the plot in Figure 3-10 into two components to make it easier for readers to tell from standard amplification curves and unknown DNA amplification curves. Concentration of released DNA could be determined by comparing cycle threshold number with that of the standard curves.
It is shown in Figure 3-10 and Figure 3-11 that the concentration of released DNA sample lies around $1.74 \times 10^{-1}$ ng/μl~$1.74 \times 10^{-2}$ 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 via qPCR (original concentration of released DNA around $1.74 \times 10^2$ ng/μl~$1.74 \times 10^1$ ng/μl), indicating that even if the original nanoparticle solution were diluted to a factor of $1 \times 10^8$, there is still a good chance that the DNA can be detected and amplified after the release experiment. Given the mass concentration of silica nanoparticles in the solution and the amount of DNA encapsulated, the concentration could be calculated that the particles were loaded with 0.2% (wt/wt) DNA.

The abundance in encapsulated DNA leaves sufficient room for applying the DNA-silica nanoparticles in flow experiments, and ultimately field applications, because the particles would go through a large degree of dilution while traveling through porous or fractured media.

It is also noticed from the qPCR result, however, that the three unknown DNA samples had different cycle thresholds, or in other words, the DNA concentrations released from the particles were not the same. Unknown DNA sample #2 took three less cycles than sample #1 and #3 for the amplification curve to reach the cycle threshold, meaning that sample #2 carried more DNA than sample #1 and #3. This was not expected because all three samples came from DNA-silica nanoparticles that underwent identical synthesis procedure.

Possible reasons for the difference in released DNA concentration could be:

1) There is uncertainty in the amount of DNA encapsulated during DNA-silica nanoparticle synthesis process. For instance, it was impossible to perfectly control the amount of DNA adsorbed onto surface functionalized silica seeds after the supernatant was washed away.

2) In the release experiment, DNA underwent purification via spin column before being sent to qPCR analysis. The recovery rate of spin column leads to another uncertainty in the released DNA concentration. In addition, during DNA purification, the 1.5 ml binding buffer added to the reaction mixture led to a total solution volume of ~1.8 ml, which exceeded the capacity of the spin column (spin column could only contain ~750 μl fluid).
In order to deal with the issue, the ~1.8 ml solution was split into three aliquots. The binding process was repeated three times on the same column, each time binding one aliquot of solution. The repeated binding process could have caused uncertainty in spin column recovery rate.

The fluctuation in released DNA amount could be dealt with by mixing together the DNA-silica nanoparticles from different reaction vials, in order to form a larger batch. A small aliquot of the batch could be analyzed to determine the amount of DNA encapsulated in certain volume of solution, which could be considered representative of the DNA content of the whole batch.

In our application aim, however, the small fluctuation of encapsulated DNA amount between different samples does not matter a lot. If the DNA-silica nanoparticle is ultimately flowed between wells to determine the interwell connectivity or to characterize the fracture network, we would be more likely to face difference in DNA concentrations that are by orders of magnitude. The small difference in encapsulated DNA amount in the starting material, therefore, would not have significant impact on the results.

### 3.4. Chapter Summary

In this chapter, the characterization method for DNA-encapsulated silica nanoparticles was discussed. The structure of DNA-encapsulated silica nanoparticles is relatively complex and involves knowledge from other disciplines. It is necessary that we have the ability to extract the information carried by the DNA-silica nanoparticles, so that we can apply the DNA-silica nanoparticles in reservoir characterization applications.

The DNA encapsulated within silica nanoparticles was first extracted via buffered hydrofluoric acid that dissolved the protective silica particles and thereby released free DNA into solution. The solution was then purified via spin column to remove all impurities (SiF$_6^{2-}$, NH$_4^+$, etc.) and elute clean DNA into the TE buffer. The clean DNA was then amplified and quantified via qPCR, which indicated that abundant DNA had been encapsulated and that the particles were loaded with approximately 0.2% (wt/wt) DNA. The mechanism for spin column purification and qPCR quantification was explained in detail. The selective nature of qPCR allows unique identification of specific DNA sequence within the particles. This can be applied in geothermal fields for wellbore or flow path tagging purposes.

With the successful synthesis of DNA-encapsulated silica nanoparticles as well as the capability to extract and quantify the DNA embedded, we are ready to establish the path toward applying such particles as tracer in porous or fractured media for reservoir characterization.
Chapter 4

4. Modification of DNA-encapsulated Silica Nanoparticles

Having successfully synthesized the DNA-encapsulated silica nanoparticles and shown that we are able to characterize the DNA-silica nanoparticles via DNA release, spin column purification followed by qPCR quantification, we were then ready to investigate the use of such particles in reservoir flow experiments. However, as shown in the figures in Chapter 2, although the silica seeds before DNA encapsulation had great stability in water or alcohol solution and looked well dispersed under SEM, our synthesized DNA-silica nanoparticles were heavily agglomerated into micron-sized chunks, and could not remain stable in solution for more than 10 min. The agglomeration issue was also known to Paunescu et al., the original developer of the DNA-silica nanoparticles, but did not affect their intended use for the particles.

Previous studies in our group by Alaskar (2013) have indicated that spherical shape is more favorable in transporting nanoparticles through porous or fractured media, and that particles with nonspherical shapes tend to have more retention. Therefore, in order for the DNA-silica nanoparticles to be transported through porous or fractured media, the agglomeration problem would need to be addressed.

In this chapter, several experiments aiming to investigate the possible cause for the agglomeration of DNA-silica nanoparticles will be described and discussed. In addition, the attempts that have been made to mitigate the agglomeration of DNA-silica nanoparticles will be discussed.

4.1. Cause for Agglomeration

During the process of synthesizing DNA-silica nanoparticles, the particles were well dispersed in solution (stable for days) until the protective silica layer was added to the DNA-adsorbed silica seed. Therefore, it must have been a process somewhere in the DNA encapsulation step that caused the particles to aggregate. As described in Section 2.3, during the DNA encapsulation step, functionalized silica seed solution was first mixed with DNA solution to allow DNA molecules to adsorb onto silica seed surfaces; the particles were then washed to remove excess DNA solution and the seeded growth reaction was performed to coat the DNA-adsorbed silica seed with an outer silica layer of ~10 nm thick.

Looking at the DNA encapsulation procedure, two possible causes for the agglomeration were proposed:
1) The absence of ammonia catalyst during the silica coating process led to insufficient electrostatic repulsion among particles (Wang et al. 2011).

In the Stober process, the polycondensation of TEOS takes place in the presence of ammonium hydroxide that serves as catalyst for the reaction. However, when coating DNA-adsorbed silica seed with the protective silica layer, the ammonia catalyst was not adopted, which was why the encapsulation reaction took 4 days to be completed. The reason for the absence of ammonia catalyst is that DNA molecules go through hydrolysis under basic environment, so the absence of ammonia provides a neutral environment for the reaction to take place rather than basic. The neutral environment would maintain an optimal integrity of DNA encapsulated into the DNA-silica nanoparticles (Paunescu et al. 2013).

However, in a typical Stober process, the ammonium hydroxide not only serves as the catalyst, but also helps the resulting particles maintain sufficient electrostatic repulsion, leading to colloidal stability of silica nanoparticle solution (Wang et al., 2011). The absence of ammonia catalyst during DNA encapsulation step, therefore, may have also led to the particle aggregation.

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. In other words, the DNA-adsorbed silica seeds may already have the tendency to aggregate even before the protective silica layer was coated.

Controlled experiments were conducted to study the cause for the agglomeration of DNA-silica nanoparticles. Experimental conditions of the controlled experiments are listed in Table 4-1 and illustrated in Figure 4-1.

Compared with the original synthesis procedure (EXP#1, Figure 4-2), EXP#2 excluded DNA from the reaction: the silica layer was directly coated onto the surface functionalized silica seed with no DNA adsorbed, with no ammonia catalyst present either. The total volume of reaction system was kept unchanged by replacing DNA solution with water. Hence if the absence of ammonia catalyst was causing particles to agglomerate, we should also be able to observe particle agglomeration in EXP#2. Resulting particles of EXP#2 were observed under SEM (Figure 4-3).

EXP#3 had exactly the same procedure as the original (EXP#1), except that the reaction was stopped after adsorbing DNA onto surface functionalized silica seeds (step 4, Section 2.3.2). Resulting particles were observed under SEM looking for possible impact that the DNA molecules could have on the state of silica seeds (Figure 4-4).
**Figure 4-1.** Schematic illustrating the conditions for the controlled experiments.

**Table 4-1:** Controlled experiments design investigating the cause for particle agglomeration: EXP#1) DNA-silica nanoparticle synthesis as described in Section 2; EXP#2) Same procedure as EXP#1 except that no DNA was encapsulated, shell grown as usual; EXP#3) Same procedure as EXP#1 but stopped the process after DNA adsorption onto silica seeds.

<table>
<thead>
<tr>
<th>Experiment#</th>
<th>DNA</th>
<th>NH$_3$•H$_2$O</th>
<th>Agglomeration?</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXP#1</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>EXP#2</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>EXP#3</td>
<td>Y</td>
<td>N</td>
<td>Y*</td>
</tr>
</tbody>
</table>
Figure 4-2. SEM image of DNA-silica nanoparticles from EXP#1 (original procedure). Particles are heavily agglomerated.

Figure 4-3. SEM image of particles from EXP#2 (no DNA). Slight aggregation could be observed at some locations of the SEM sample, but particles are in general still quite well dispersed.
Figure 4-4. SEM image of particles from EXP#3 (DNA adsorbed silica seeds). Particles are stuck together after DNA adsorption, and DNA molecules attaching to two particles on each end could be observed (shown in red circles).

As shown in the SEM photos, the resulting particles of EXP#2 were in general still well dispersed, although slight aggregation could be observed at some locations of the SEM sample. The cause for this slight aggregation could be the absence of ammonia catalyst during the silica layer coating step, which led to insufficient electrostatic repulsion among particles (Wang et al. 2011). However, compared with the resulting particles of the original procedure, the level of agglomeration is minor and negligible. And the particle solution right after the reaction remained stable and well dispersed. Therefore the absence of ammonia catalyst is not likely to be the cause for the agglomeration of DNA-silica nanoparticles, or at least not the major cause.

Looking at the SEM photo of EXP#3, however, it can be seen that although the particles have not aggregated to the same extent as the original DNA-silica nanoparticles, they have been attached to each other even before the silica layer was coated. Looking closer, we are even able to spot some tiny DNA strands that have attached to two different particles with their two ends (red circles in Figure 4-4). This observation corroborates the second possible reason we proposed, that DNA molecules dragged silica seeds together during the process.

Given that the particles were attached to each other by DNA molecules even before the protective silica layer was coated, it can be inferred that the subsequent seeded growth process was actually adding silica layer to clusters of particles, rather than individual particles, which further severed the aggregation problem, leading to resulting DNA-silica nanoparticles that are heavily agglomerated and unable to remain dispersed in solution.
4.2. Modification Attempts

4.2.1. Modification with Surfactant

In previous research, Alaskar (2013) used polyvinyl pyrrolidone (PVP) as surfactant to modify the surface properties of hematite nanorice in order to investigate its ability to transport through porous media. PVP is also known to have the ability to stabilize formed nanoparticles against agglomeration (Ahlberg et al. 2014). We therefore tried using PVP solution to see if it was able to separate the aggregated particles from each other, or at least disperse the aggregated chunks to some extent to make them more stable in suspension.

PVP solution was prepared by dissolving 1.1 g PVP powder (55000 g/mole, obtained from Stanford Department of Materials Science) into 20 ml Milli-Q water, resulting in PVP solution of 0.001 M. 100 μl of previously synthesized DNA-silica nanoparticles were mixed with 1 ml PVP solution and the mixture was stirred overnight at 1350 rpm at room temperature. Another sample was prepared by adding 1ml PVP solution to the DNA-silica synthesis reaction tube right after the 4-day encapsulation reaction, and further stirring overnight. If the particles started aggregating right after the 4-day reaction, the latter sample would still allow the PVP to act on individual particles before they aggregate. Resulting particle solution was later observed under SEM (Figure 4-5).

After PVP coating, it did take longer for the DNA-silica nanoparticles to start settling. However, particles would still start settling to the bottom of the tube eventually after 20 min. SEM images also indicated that the DNA-silica nanoparticles were still in clusters. This shows that the prolonged stirring was not able to separate individual particles and that PVP merely acted on clusters of particles. In addition, the effect of PVP was not able to prevent the clusters from settling.
Figure 4-5. SEM images of DNA-encapsulated silica nanoparticles after PVP coating. Particles are still agglomerated. PVP seems to have acted merely on clusters of particles and did not enhance particle stability to a noticeable extent.

Given the result of PVP coating, it seems that we would need to adjust the DNA encapsulation process, rather than merely the product, to try to eliminate agglomeration before it took place.

4.2.2. Modification by Adjusting Reagent Ratio During Synthesis

It has been discussed in Section 4.1 that the most likely reason for the agglomeration of DNA-silica nanoparticles is that, when adsorbing DNA onto functionalized silica seeds, after mixing functionalized silica seeds with DNA solution and water, the DNA molecules dragged particles together before silica layer was coated. Hence the layer was grown outside of clusters of particles, which further exacerbated the problem. Therefore, looking at the DNA-encapsulation step of the synthesis procedure, if there were fewer silica seeds present in a certain volume of solution, it could be harder for DNA molecules to drag together silica seeds that were further apart, and hence the resulting particles may not be as heavily agglomerated.
Therefore, additional DNA-encapsulation experiments were conducted with adjusted ratio of water, DNA solution and functionalized silica seeds. The idea was to reduce the concentration of silica seeds. Reagent ratios have been listed in Table 4-2. EXP#1 was the original reagent ratio as described previously in Section 2.3, EXP#4 and EXP#5 reduced the percentage of functionalized silica seeds solution, while maintaining the total solution volume to be the same. All experiments were carried out according to the same procedure as described in Section 2.3, only difference being the ratio of reagents in the starting mixture.

Resulting particles of all experiments were sampled and observed under SEM (Figure 4-6 ~ Figure 4-9).

**Table 4-2: Ratios of water, DNA solution and functionalized silica seeds solution.** EXP#1 is the original ratio; EXP#4 and EXP#5 reduced the percentage of functionalized silica seeds solution, while maintaining the total solution volume to be the same.

<table>
<thead>
<tr>
<th></th>
<th>EXP#1</th>
<th>EXP#4</th>
<th>EXP#5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Water</strong></td>
<td>700</td>
<td>700</td>
<td>700</td>
</tr>
<tr>
<td><strong>DNA Solution</strong></td>
<td>320</td>
<td>335</td>
<td>345</td>
</tr>
<tr>
<td><strong>Functionalized Particle Solution</strong></td>
<td>35</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>

Figure 4-6. SEM images of resulting DNA-silica nanoparticles from EXP#1. (original procedure)
Figure 4-7. SEM images of resulting DNA-silica nanoparticles from EXP#4.
Figure 4-8. SEM images of resulting DNA-silica nanoparticles from EXP#5. Note the tiny nucleates next to the DNA-silica nanoparticle chunks.

Figure 4-9. Zoomed-in SEM images of tiny nucleates from EXP#5.
SEM images show that resulting particles of EXP#4 are almost the same as the particles resulting from EXP#1, which indicates that reducing functionalized silica seeds solution volume from 35 μl to 20 μl during the DNA encapsulation step was not able to separate the silica seeds far enough to prevent agglomeration. EXP#5, which reduced functionalized silica seeds solution to 10 μl during DNA encapsulation step, also resulted in DNA-silica nanoparticles that are agglomerated to a similar extent. However we were able to find smaller particle chunks that were less than 500 nm in size, which indicates that it is a viable approach to at least reduce the size of agglomerated DNA-silica chunks by reducing the concentration of silica seeds during the DNA-encapsulation step.

However, apart from the DNA-silica nanoparticles that were yielded from the seeded growth process of DNA-encapsulation step, a number of tiny particles with average diameter of around 20 nm were observed in the resulting particles of EXP#5 (as shown in Figure 4-8 and Figure 4-9). As mentioned in Chapter 2.1, the Stober process involves the formation of silica nuclei (seeds) and subsequent growth of those nuclei, resulting in particles growing in size. However, when the silica nuclei present in a certain volume of solution are too few, or in other words, when the average distance between seeds exceeds some critical value, secondary nucleation would occur (Chou, et al. 2007), resulting in newly formed silica nuclei that are much smaller than the particles present from the very beginning. Therefore, those tiny particles of around 20 nm are very likely to be newly formed silica nanoparticles due to secondary nucleation.

4.3. Chapter Summary

Although we were able to successfully synthesize DNA-encapsulated silica nanoparticles, the particles were heavily agglomerated into micron-sized chunks and were unstable in suspension (i.e. the particles tend to settle). The agglomeration issue was obviously unfavorable for application as reservoir tracer because we need tracer particles to be small enough (nanometer-sized) to be able to travel through micron-sized pore throats, and meanwhile stable enough to be able to remain in high-velocity streamlines. This chapter described the research conducted in order to address the issue.

By designing controlled experiments looking at the effect of different synthesis stages on the properties of resulting DNA-silica nanoparticles, we came to the conclusion that the agglomeration was mainly caused by DNA molecules that attached to two silica seeds with their two ends during DNA encapsulation step. DNA molecules may have dragged silica seeds together even before the protective silica layer was coated, hence the protective silica layer was actually coated onto clusters of DNA-adsorbed silica seeds, rather than individual ones, which led to resulting DNA-encapsulated silica nanoparticles that were heavily agglomerated. The absence of ammonia catalyst during silica coating step, may also have contributed to the agglomeration. However, the contribution was found to be negligible, and hence not our main focus for investigation.
Attempts were made in order to mitigate the agglomeration issue. The ratio of water, DNA solution and functionalized silica seeds during DNA adsorption step was adjusted. The idea was to reduce the concentration of functionalized silica seeds in the mixture. With fewer silica seeds present in certain volume of solution, it could be harder for DNA molecules to drag different silica seeds together.

The results indicated that it is possible to reduce the size of the agglomerated DNA-silica nanoparticle chunks by significantly reducing the concentration of functionalized silica seeds in the reaction mixture. However, secondary nucleation occurred when silica seed concentration was sufficiently low, and newly formed silica nucleates with average diameter of ~20 nm was observed in the resulting particles. Currently, research is still looking at optimizing the reagent ratio during DNA adsorption step aiming to resolve the agglomeration issue, or at least reduce the size of agglomerated DNA-silica nanoparticle chunks to facilitate their transport through micron-sized pore throats.
Chapter 5

5. Heating and Injection Experiments

Apart from the synthesis and characterization of DNA-encapsulated silica nanotracer, additional research has been done looking into the behavior of such nanotracers under simulated reservoir flow conditions. As shown in previous chapters, the structure of DNA-silica nanotracer is rather complex. Various stages in the synthesis process may have caused significant change in particle surface properties, flowability, etc., as compared with the original plain silica seeds. Therefore, it is necessary to fully understand the flow behavior of DNA-silica nanoparticles through step-by-step research.

In this chapter, we describe how plain silica nanoparticles were subjected to simulated geothermal temperature and flow conditions. Our self-synthesized plain silica nanoparticles were heated to 198 °C to evaluate their behavior under high temperature and pressure conditions that are ubiquitous in geothermal reservoirs. This also provides information on how well the outer silica layer of DNA-silica nanoparticles can protect the DNA molecules underneath. Plain silica nanoparticle solution was also injected through a sand pack under various temperatures while monitoring permeability change of the sand pack. This allows us to understand not only the flowability and breakthrough pattern of silica nanoparticles under simulated geothermal reservoir conditions, but also the influence of nanoparticles on the permeability of the sand pack. Finally, our self-synthesized DNA-encapsulated silica nanoparticles were injected through sand pack under high temperature to investigate their feasibility to be applied as smart tracers in geothermal reservoirs.

The heating and injection experiments described in this chapter were conducted by Timothy Spencer Manley, who is a graduate student also at the Stanford Geothermal Program. Further details about the experiments can be found in Manley (2015).

5.1. Plain Silica Nanoparticles Heating Experiments

Plain silica nanoparticles (~200 nm) synthesized as described in Section 2.1 were tested at a reasonable geothermal temperature (198°C) to investigate their survival rate under simulated reservoir conditions. As the particles were used in water suspension, water loss due to evaporation under high pressure and temperature may cause the experiment to fail (Brinton et al. 2011). In order to avoid water leakage, five identical stainless steel tubes fitted with end caps were used as experiment vessels. The tubes were filled completely with the silica nanoparticle solution and the end caps were tightly fitted onto the tubes to avoid leakage. With the fixed volume of the vessel, the fluid inside would pressurize itself to prevent boiling at high temperature. All tubes were placed into an oil bath heated to 198°C, and were heated at 198°C for 5 min, 10 min, 15 min and 25 min, respectively.
Each sample tube was cooled in water after heating and was then sampled and observed under SEM (Figure 5-1, photos taken by Manley (2015)).

Figure 5-1. SEM images of silica nanoparticles (~200 nm) heated at 198°C for varying lengths of time (5 min, 10 min, 15 min, 25 min). There was an abundance of amorphous silica observed along with original spheres. Photos were taken by Manley (2015).

As indicated in the SEM images, the silica nanoparticles underwent varying degrees of dissolution and precipitation with varying heat exposure. All samples were further analyzed with Auger Electron Spectroscopy (AES), which confirmed that the amorphous precipitate surrounding the nanoparticles was also silica. It was therefore concluded that, during the heating experiment, silica spheres may have partially dissolved under high temperature, and yet precipitated again utilizing the silica spheres present in the solution as nucleation sites (Manley 2015). Despite the dissolution and precipitation observed, the particle integrity was quite well maintained.
5.2. Plain Silica Nanoparticles Injection Experiments

Plain silica nanoparticles around 200 nm in diameter synthesized as described in Section 2.1 were flowed through a sand-packed stainless steel tube in order to evaluate their flowability through porous media, breakthrough pattern and influence on the permeability of the sand pack. The experimental apparatus is illustrated in Figure 5-2. The particles were flowed under 25°C, 120°C and 150°C to examine the durability of the particles and the influence of temperature on the flow characteristics. Detailed experimental design is shown in Table 5-1.

![Figure 5-2. Apparatus for silica nanoparticle injection experiment. Both system pressure and differential pressure across the sand pack were monitored. Figure was drawn by Manley (2015).](image)
Table 5-1: Summary of plain silica nanoparticle injection experiments. Experiments designed and conducted by Manley (2015). Note that the flow rate of experiment 1 was 1.2 ml/min, and experiment 2~7 had a flow rate of 2.5 ml/min

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Experiment</th>
<th>Permeability [darcy]</th>
<th>Percent Change</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 25°C</td>
<td>Baseline</td>
<td>54</td>
<td>-8.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post-Silica Injection</td>
<td>49.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 120°C</td>
<td>Baseline</td>
<td>93</td>
<td>-7.7</td>
<td>pH = 6.9 Effluent</td>
</tr>
<tr>
<td></td>
<td>Post-Silica Injection</td>
<td>85.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 150°C</td>
<td>Baseline</td>
<td>39.7</td>
<td>11.1</td>
<td>pH = 7.4 Effluent</td>
</tr>
<tr>
<td></td>
<td>Post-Silica Injection</td>
<td>44.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 150°C</td>
<td>Baseline</td>
<td>48.5</td>
<td>20</td>
<td>Silica Slug Injected</td>
</tr>
<tr>
<td></td>
<td>Post-1 Hour Bake</td>
<td>58.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 150°C</td>
<td>Baseline</td>
<td>45.9</td>
<td>46.2</td>
<td>Water Only</td>
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<tr>
<td></td>
<td>Post - 1 Hour Bake</td>
<td>67.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 150°C</td>
<td>Baseline</td>
<td>37</td>
<td>12.4</td>
<td>Water Only</td>
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<tr>
<td></td>
<td>Post - 6 Hour Bake</td>
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<tr>
<td>25°C</td>
<td>Water Flush</td>
<td>66.9</td>
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</tr>
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<td>7 150°C</td>
<td>Baseline</td>
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<tr>
<td></td>
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<tr>
<td>25°C</td>
<td>Water Flush</td>
<td>70.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each experiment was conducted using fresh sand packed into the stainless steel tube that was cleaned beforehand. During each experiment, distilled water was first injected at constant flow rate until the differential pressure stabilized. The stabilized differential pressure was recorded as the “baseline” value. This baseline value provides a reference so that we are able to observe the influence of subsequent nanoparticle injection.

Nanoparticle solution was then injected into the system by turning the three-way valve at the particle injection loop. The same amount of nanoparticle solution was used in each experiment. Each experiment was run for 30 min, with differential pressure values recorded every minute, and effluent samples taken every two minutes. With the differential pressure value, flow rate, dimensions of the sand pack and the viscosity of water at specified temperature, permeability values for the sand pack were calculated, as listed in Table 5-1. Effluent samples were observed under SEM individually to study the breakthrough pattern of injected silica nanoparticles as well as the change in particle appearance after being flowed through the porous medium under high temperature (Figure 5-3).

Note that in experiments 1~3, fluid was flowed continuously throughout the experiments. However in experiments 4~5 the flow was stopped for one hour after nanoparticle solution reached the sand pack and in experiments 6~7 the flow was stopped for six hours. The purpose of stopping the flow was to test particle durability during prolonged periods in simulated reservoir conditions. After the prolonged stop, flow was restarted, effluent was sampled and permeability measurements were taken. Each experiment also lasted for 30 min apart from the holding time.
The results listed in Table 5-1 indicate that the injection of silica nanoparticles reduced permeability of the sand pack. Although experiment 3 showed increased permeability, the result of following experiments that were also conducted at 150°C indicated that permeability was also increased in pure water (due perhaps to rock dissolution at elevated temperature) when no nanoparticle was injected. The permeability increase for pure water injection was less than that of the nanoparticle injection. It can therefore be concluded that the injection of silica nanoparticles had a reducing effect on the permeability of sand pack. The particles, however, did survive the journey through the sand pack as indicated in Figure 5-3, confirming their ability to be transported through sand pack as well as their durability.

5.3. DNA-encapsulated Silica Nanoparticle Injection Experiments

It was mentioned in Chapter 2 that the synthesized DNA-encapsulated silica nanoparticles were heavily agglomerated. The reason may be DNA strands dragging together functionalized silica seeds during the DNA adsorption step, leading to subsequent silica coating onto clusters of DNA-adsorbed nanoparticles (discussed in Chapter 4). While research is still looking at how to resolve or at least mitigate the agglomeration issue, it is still possible that the DNA-silica nanoparticles are able to be transported through fractured rock because the agglomerated chunks are still micron-sized, and hence smaller than expected fracture apertures. The packed sand with high permeability could be used to test this.

Therefore, an injection experiment using DNA-silica nanoparticles as synthesized in Chapter 2 was conducted at 150°C to investigate whether DNA-silica nanoparticles can be transported through packed sand and whether simulated geological temperature together with flow through porous media would adversely affect the DNA-silica nanoparticles.
The injection experiment was conducted by Spencer Manley (Manley, 2015) using the same apparatus as illustrated in Figure 5-2. Freshly packed sand was used and the flow rate was 2.5 ml/min. Because of the unexpected failure of the differential pressure gauge, permeability changes could not be monitored. However the effluent was still sampled regularly and subjected to SEM analysis. In addition to those samples, all the rest of the effluent was collected for further analysis. The SEM photos of effluent samples are presented in Figure 5-4.

Figure 5-4. SEM images of DNA-encapsulated silica nanoparticle injection effluent. a) and b): Particles found in the effluent that are likely to be the injected DNA-silica nanoparticles. c) Particles much smaller than the injected DNA-silica nanoparticles were also present in the effluent. Particles this small may not be part of the influent, but could be dissolved silica that has precipitated out of solution following the temperature reduction in the water jacket. Images taken by Manley (2015).

The DNA-silica nanoparticles were observed to have transported through the sand pack successfully. The number of particles that went through, however, was significantly lower than was injected, and the particle chunks appeared to have been broken down into
individual particles. The small recovery rate was anticipated because the injected DNA-silica nanoparticles were agglomerated into micron-sized chunks with nonspherical shape, which was unfavorable for their transport through porous media as suggested by Alaskar (2013). In addition, the instability of DNA-silica nanoparticles in suspension may have caused the particles to settle even before they were injected into the sand pack, and hence trapped somewhere in the system. The disappearance of particle clusters may be due to the high temperature and the travel through the porous sand pack that disaggregated the particle chunks. This may adversely affect the DNA embedded because the DNA that used to be sandwiched in between different particles that were clustered by the outer silica layer may be exposed.

With confirmation that at least some DNA-silica nanoparticles did go through, the next crucial step was determining whether the DNA survived and whether it was still detectable. Apart from the sampled effluent for SEM, all the rest of the effluent was collected in a conical flask. If we were able to detect DNA within the effluent, it would prove that not only did the DNA-silica nanoparticles make it through the sand pack, but the DNA itself also survived the journey.

If DNA indeed survived the journey, it would still need to be released from the silica capsule in order to be detected. Hence a release experiment as described in Chapter 3 was conducted on the effluent.

The effluent, however, looked transparent. In other words, no particles could be observed by naked eye due to the significant dilution as well as the reduced number of particles that went through the sand pack. The effluent was transferred to a 50 ml centrifuge tube, and even after 20-min centrifugation at 3000 g, no particle pellet could be observed at the bottom of the centrifuge tube.

The release experiment was hence conducted on the transparent sample. To try to concentrate the particles as much as possible, the effluent was centrifuged for 20 min at 3000 g. Centrifuged fluid was removed of its supernatant with care, leaving only a small amount of fluid at the bottom of the tube. The same release and purification procedure as described in Section 3.1 was carried out. The only difference was that in the original experiment, we knew the reaction with hydrofluoric acid was complete when the particle solution turned from whitish to transparent, whereas in this case, because no particles could be seen by naked eye, we could only estimate the reaction time based on previous experience with the original release experiment. Therefore, after adding buffered hydrofluoric acid to the concentrated effluent sample, the solution was well mixed by pipetting, and allowed to react for ~6 min (chosen based on past experience) before being purified with the spin column.

The purified sample was later analyzed via qPCR. However, no DNA was detected. There are several possible reasons:

1) The number of DNA-silica nanoparticles that went through the sand pack was too few to allow sufficient amount of DNA to be extracted. This could be caused by particle
retention in the sand pack either because of the particle size or unfavorable surface charge. To address this issue, the agglomeration of the DNA-silica nanoparticles would need to be mitigated. In addition, the surface properties of DNA-silica nanoparticles need to be investigated and modified to prevent the particle from sticking to the sand grains.

2) DNA did not survive the high temperature of the flow experiment, or had decayed to the extent that it could no longer be detected by qPCR. To address this issue, the thermal stability of the DNA-silica nanoparticles would need to be reevaluated and enhanced. The dismantlement of agglomerated particle chunks after transport through packed sand needs to be taken into account.

Future research will be conducted to determine what caused our inability to detect DNA in effluent sample. The packed sand after the DNA-silica injection experiment will be sampled both at the inlet and the outlet, and will be subjected to SEM analysis looking for trapped DNA-silica nanoparticles. If we are able to find trapped DNA-silica nanoparticles in the packed sand, release experiment will be conducted on the sampled sand to see if DNA could be extracted and amplified.

5.4. Chapter Summary

This chapter focuses on the feasibility of flowing the proposed DNA-encapsulated silica nanotracer through porous or fractured media under high temperature. Plain silica nanoparticles have great durability under high temperature and great flowability through packed sand, as proven by the heating and injection experiments conducted by Manley (2015). DNA-encapsulated silica nanoparticles were also injected through packed sand under high temperature and a small number of them went through successfully, as proven by SEM images of the effluent. However the DNA that was supposed to remain intact in the effluent particles was not detected, which could be due either to insufficient number of DNA-silica nanoparticles in the effluent or destruction of the DNA due to temperature or exposure as the aggregated particle chunks were broken apart.

Future research will look into the apparent absence of DNA in the effluent. The packed sand from the DNA-silica nanoparticle injection experiment will be sampled and observed under SEM to look for trapped DNA-silica nanoparticles. But overall, given the fact that a number of DNA-silica nanoparticles were still able to travel through packed sand even before the agglomeration issue was resolved, the proposed DNA-encapsulated silica nanoparticles shows promise in being applied as tracer for geothermal fracture characterization.
Chapter 6

6. Conclusions and Future Work

6.1. Conclusions

The overall objective of this research was to investigate the development of a uniquely identifiable DNA-based nanoparticle tracer to map fracture distributions in geothermal reservoirs. Following the procedure suggested by their original developer (Paunescu et al. 2013) with slight modifications, DNA-encapsulated silica nanoparticles of ~160 nm diameter were successfully synthesized by first adsorbing negatively charged synthetic DNA molecules onto positively charged silica seeds, and then coating the particles with silica. Sandwiched between the inner silica seed and outer silica layer, the DNA was protected by the “fossil” surrounding it and was able to withstand temperature as high as 200°C in the original experiments by Paunescu et al. (2013). However, the DNA-embedded particles were heavily agglomerated into micron-sized chunks because of the DNA strands that dragged silica seeds together during DNA adsorption step. The agglomeration issue was also known to Paunescu et al., but did not affect their intended use of the particles. Attempts have been made to mitigate the agglomeration issue, and it was found that reducing the concentration of silica seeds during the DNA encapsulation step appeared to have reduced size of the chunks to some extent, yet caused secondary nucleation that produced newly formed silica nanoparticles much smaller than the ones already present.

Despite the agglomeration issue, the DNA-silica nanoparticles were characterized by extracting the DNA embedded using buffered hydrofluoric acid and amplifying the DNA using qPCR (real-time quantitative polymerase chain reaction), which is a process that selectively quantifies target DNA with specified sequence. Particle characterization indicated abundant DNA to have been encapsulated. Even if the synthesized DNA-silica nanoparticles solution were diluted to a factor of $1 \times 10^8$, the DNA would still be able to be detected and amplified through qPCR. This is essential to reservoir applications because tracer material is generally expected to be highly diluted.

Heating and injection experiments by Spencer Manley (Manley, 2015) indicated excellent durability and flow characteristics of plain silica nanoparticles. Particle dissolution under high temperature appeared to have minimum impact on overall particle integrity. Following those preliminary investigations on plain silica nanoparticles, DNA-encapsulated silica nanoparticles, despite their agglomeration, have been transported through packed sand successfully, but the amount of particle recovered was limited, possibly due to trapping or destruction. DNA was not detected after dissolving silica nanoparticles in the effluent, which may be due to the limited number of effluent particles that did not provide a sufficient amount of DNA to be detectable. Research is still looking at how to transport more DNA-silica nanoparticles through the sand pack and how to
recover the embedded DNA from the effluent. But given the fact that abundant DNA was successfully encapsulated into the silica nanoparticles, and that the particles were successfully transported through the sand pack despite the heavy agglomeration, the DNA-encapsulated silica nanoparticles still shows realistic viability to application in geothermal reservoir characterization as uniquely identifiable tracer.

6.2. Future Work

Future work will first aim at resolving the issues encountered so far in applying DNA-encapsulated silica nanoparticles as reservoir tracer. Agglomeration of the DNA-silica nanoparticles will need to be resolved or at least mitigated, possibly by optimizing reagent ratio during the DNA encapsulation stage of the particle synthesis. Smaller silica seeds could also be synthesized (below 100 nm) to look at the effect of reduced seed size on the resulting DNA-silica nanoparticles. Surface property of DNA-silica nanoparticles will be adjusted to enhance their stability in suspension.

The inability to see effluent nanoparticles by naked eye after large dilution is another problem. Although particles can be easily concentrated via centrifugation in the lab, a better approach is needed to allow particle tracers to be gathered at the field scale. The DNA release and extraction procedure also needs to be improved to account for the fact that observing solution transparency to determine whether extraction is completed may not always be feasible.

In addition, despite the tremendous value of information inherent in the DNA-silica nanotracer, it would still be beneficial if the synthesis procedure were simplified, and if the DNA-silica nanoparticles could be synthesized in larger batches. Other nanoparticle tracer candidates, for instance, magnetically recoverable DNA/silica encapsulates (Puddu et al. 2014), polymer-based fluorescent particles (Frane et al. 2014), etc., could also be looked into.

Additional thoughts could be given to the possibility of applying such DNA-based nanotracer in fractured shale oil reservoirs, in which case the behavior of DNA-silica nanoparticles in the presence of oil phase would need to be investigated.
Nomenclature

Abbreviations:

TEOS: Tetraethyl orthosilicate
RT: Room Temperature
SEM: Scanning Electron Microscope
TMAPS: trimethyl[3-(trimethoxysilyl)propyl]ammonium chloride
bp: base pair
ssDNA: single stranded DNA
dsDNA: double stranded DNA
qPCR: real-time Quantitative Polymerase Chain Reaction
AES: Auger Electron Spectroscopy
References


