Characterization of Mechanical and Adsorption Properties of Silica Based Gels for Bacterial Encapsulation

Daniel P. Kieffer
Faculty Mentor: Alptekin Aksan
Graduate Student Supervisor: Jonathan K. Sakkos
UROP final report

Summary of experiments:

The overall objective of the research being conducted in this area is to create encapsulated bacteria that are used in a bioreactor to remediate contaminated fracking wastewater as an alternative to current methods that are expensive and inefficient[1]. Silica based encapsulation gels serve to protect bacteria from the potentially dangerous environment of the fracking wastewater. It has previously been shown that encapsulation may protect bacteria from predation, environmental stresses[2], and the toxicity of some fracking water pollutants[3]. Tunable properties based on the gel precursor chemicals is the focus of further exploration, but this report attempts to shed light on some of the mass transport and mechanical properties in gels with varying levels of chemical precursors. A variety of organic compounds have been identified in fracking wastewaters[4] in which it would be advantageous to have a hydrophobic gel used for encapsulation. Varying hydrophobicity within the gel was obtained by mixing different amounts of two different types of gel precursors, TMOS (tetramethylorthosilicate) and MTMS (methyltrimethoxysilane). Two different experiments are outlined in this report, the mechanical testing of the gels and an adsorption coefficient experiment.

Methods:

Regarding the procedure for the adsorption coefficient experiment, gels were formed at the bottom of 20mL scintillation vials by combining TMOS/MTMS mixtures, UPW (ultra pure water), and 1 M HCL in a ratio of 1:1:0.005 to form the gel precursor. Six gels were formed with MTMS/Total molar ratios of 0, 0.2, 0.4, 0.6, 0.8, and 1. The precursor was hydrolyzed by mixing for two hours on a stir plate. The precursor was then mixed with PBS (phosphate buffered saline) and TM-40 nanoparticles in a ratio of 4:1:1. Approximately 1mL of this mixture was pipetted into a 20mL scintillation vial and used as the container for this experiment. Gels were run in triplicate and were sealed with Teflon tape under the cap to prevent evaporation and any adsorption to the actual cap surface. Approximately 5mL of 10uM fluorene (a compound that mimic the aromatic compounds in fracking wastewater) or atrazine solution (a herbicidal chemical) in PBS was pipetted into the vial after the gels had completely solidified. Atrazine was used partially because another researcher had previously determined its partition coefficient in similar gels. The 10uM solutions of both fluorene and atrazine were made by first dissolving the solid compound in the organic solvent MTBE (methyl tert-butyl ether), and then a small amount was then transferred to the appropriate amount of PBS to make the 10uM solution. For fluorene, time points were taken at 0, 4, 24, 48, and 72 hours, as the time to reach the equilibrium concentration was not known (a separate container with gel and solution was made for each time point). Only one time point at 24 hours was used for atrazine.

For the extraction and measurement of fluorene, the 20mL scintillation vial was uncapped and the remaining 5mL of solution was poured into a 7ml scintillation vial, mixed with 1 mL of extraction solvent MTBE and vortexed for 10 seconds. Then approximately 300uL of MTBE was placed into a vial insert, and then into a 1.5mL vial, and it was measured on a Gas chromatograph (Agilent 7890A GC System) to determine the concentration of compound left in the bulk.

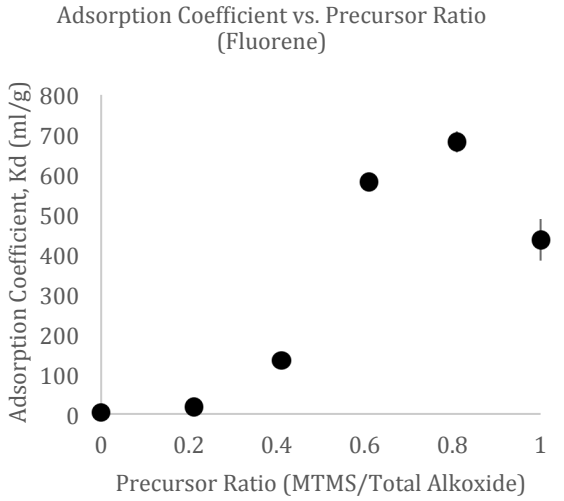
Calibrations were run to determine the conversion of GC peak area to concentration. Atrazine concentration was measured by taking 1mL of solution and passing it through a 0.2 μm Teflon syringe filter, which was then placed in a GC vial and measured on HPLC (Hewlett-Packard HP 1090 Liquid Chromatograph system equipped with a photodiode array detector with a C18 column).

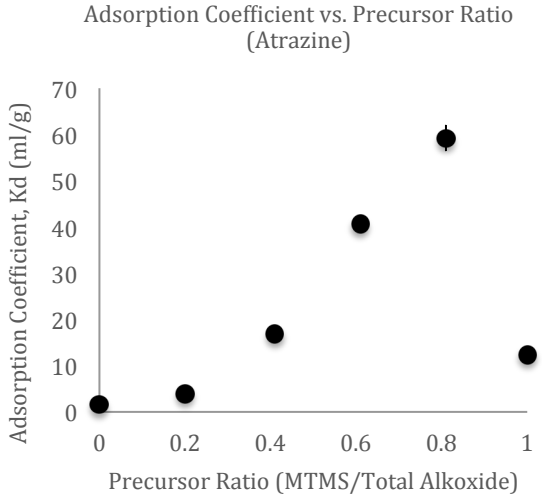
It was assumed that there was minimal evaporated compound (fluorene or atrazine) in the headspace of the container and adsorbed to the glass or cap of the scintillation vial. The difference between the measured concentration put into the vial at t=0 and the measured concentration at a certain time point was assumed to have adsorbed into the gel. Because the gels are porous, their specific surface area is taken into account, and is used for the calculation for the adsorption coefficient.

For mechanical compression testing, gels were formed using the same chemical formulas used for the adsorption experiment, but were formed in flexible gels molds to produce a cylindrical sample ideal for mechanical testing. Compressing testing was performed with an MTS system, and the raw data was analyzed by Matlab.

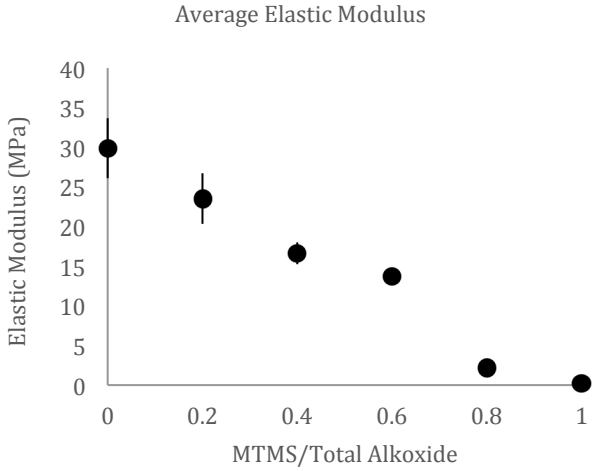
Results:

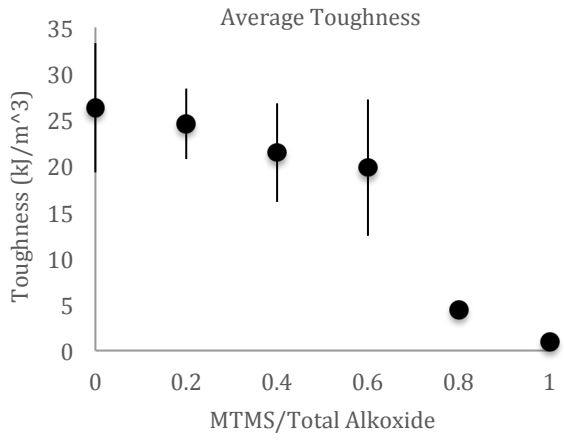
Mass Transport Properties

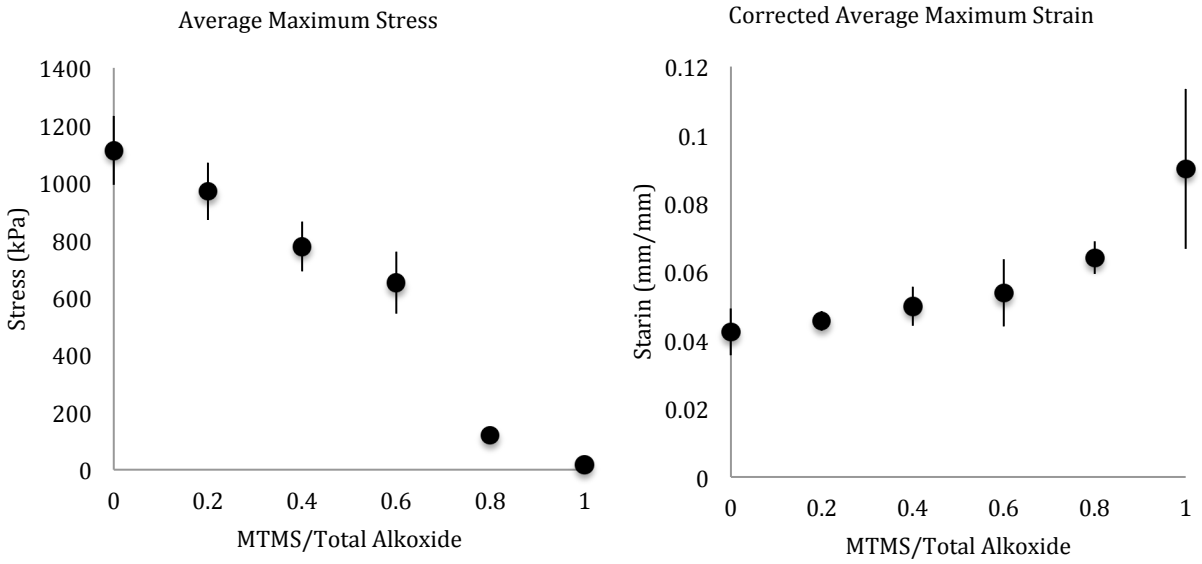




Mechanical Testing Results







The adsorption coefficients for both fluorene and atrazine are depicted; the densities of each gel were previously determined and used in the calculation for the adsorption coefficient. The increasing trends for both graphs confirm the increasing hydrophobicity of the gels (as both compounds are hydrophobic and therefore attracted to more hydrophobic gels). One anomaly however, is the decreased adsorption coefficient for the R=1 gel, where it is suspected that the low specific surface area due to poor pore structure actually decreases partitioning and adsorption, even though the gel is the most hydrophobic. Even between the two different solutes, there was a discrepancy between where the R=1 gel falls in relation to the other gels, which is something important to note regarding the mass transport properties. Because fluorene is a much more non-polar hydrophobic compound than atrazine, it could be expected that more would partition and adsorb into gel R=1 ($\log_{OW}$ 4.18 for fluorene versus $\log_{OW}$ 2.3 for atrazine). As can be seen in the following results, the mechanical integrity of the gels sharply falls off after gel R=.6, and therefore gels R=.8 and R=1 will not be considered in any subsequent experiments as these gels are too weak to be a serious candidate to be used for bacterial encapsulation. Ideal gels must have the intended hydrophobic nature, but keep mechanical integrity to a reasonable standing.

Ultimately this data helps determine which gel has the best mass transport properties, and yet holds sufficient mechanical integrity to be used for bacterial encapsulation. It is the undesirable change in the decreased mechanical integrity of gels with increasing hydrophobicity that calls for a TMOS/MTMS mixture instead of a pure MTMS gel. Higher adsorption of hydrophobic solutes should aid in their removal by degradation, and therefore hydrophobicity is an equally important trait as mechanical integrity. An area of further examination and experimentation is the mass transport properties of the same gels, but with more compounds of interest. Gels R=.8 and R=1, although the most hydrophobic, have poor mechanical properties, and are most likely to be left out of any subsequent encapsulation degradation assays and diffusion studies. In separate experiments, it was observed that these gels crumble and fall apart with the slightest of agitation, and are therefore a poor choice to use for encapsulation of bacteria.

Accomplishing objectives:

The initial objective of this project was to determine the diffusivity of the silica gels, but experimental roadblocks prevented this to be conducted in a timely manor. Only one diffusion cell was available to use, and the difficulties of mounting the very fragile gel membranes proved to have many complications that included cracking and leaking. Approximately one time point could be taken per day, so it quickly became clear that the characterization of 6 different gel membranes would most likely have to come in a different form because of the complications and bad data that had been acquired during the first part of summer. Different diffusion methods were explored, such as "test tube" diffusion method in which the two chambers separated by the membrane is abandoned completely for a test tube in which the a gel and compound in solution are the only two components. Current experiments are being run with a new diffusion apparatus, and the samples and results seem promising.

Ultimately the adsorption coefficient data was the best data obtained that showed a clear trend that gave insight into the differences in the mass transport properties of the different gels. Guidance from a graduate student, Jonathan Sakkos, greatly expedited the process of the experiments, and the process of coming up with new experiments when the original plan did not work out. Had the original experimental plan been successful, the length of the summer term would have been more than enough time to accomplish the original objective. I would encourage other UROP students to do some preliminary testing to make sure that there procedure outlined in their proposal has a high chance of producing meaningful results before they procede into the actual testing and experiments.

The mechanical evaluation of the gels was a straightforward process with most of the work going into analyzing the data. Much time was spent developing a code that automatically analyzed the raw data results from the mechanical testing experiment. Although tedious to make, the code will help for future mechanical testing experiments, and hopefully save a lot of time. The mechanical testing data was helpful in confirming which gels were worth evaluating in further experiments as well as suitable for bacterial encapsulation.

Reflection and Evaluation:

The UROP program was a great opportunity to conduct research under a faculty mentor in a setting where I was guided through many basic laboratory protocols and procedures. I learned many things about a variety of both engineering and biochemistry practices from how to handle toxic chemical waste to putting data together in a presentable manor. Throughout the summer, I kept a detailed laboratory notebook and had the opportunity to put together and present data on a few occasions. For anyone who is interested in research and graduate school, a UROP summer experience is a great way to get exposure to the ins and outs of research.

The information for UROP students is very clearly displayed on the UROP website, and it is a very easy process for students to apply to the program, given that they already have a faculty mentor set up ahead of time. In my case, I had already been working under a faculty mentor, and the proposal was just a matter of pulling together an experiment under the guidance of a graduate student. Overall I enjoyed my summer as a UROP student, and I plan to continue conducting research under the same faculty mentor.

References

1. Dickson DJ, Ely RL. Silica sol-gel encapsulation of cyanobacteria: lessons for academic and applied research. Appl Microbiol Biotechnol 97 (2013):1809–1819

2. Adlercreutz, P. Oxygen supply to immobilized cells. (1985).

3. Cassidy, M. B., Shaw, K. W., Lee, H. & Trevors, J. T. Enhanced mineralization of pentachlorophenol by κ-carrageenan-encapsulated Pseudomonas sp. UG30. *Appl. Microbiol. Biotechnol.* **47,** 108–113 (1997).

4.Strong LC, Gould T, Kasinkas L, Sadowsky MJ, Aksan A, Wackett LP. Biodegradation in Waters from Hydraulic Fracturing: Chemistry, Microbiology, and Engineering. *American Society of Civil Engineers* (2013)