

# Diffusion of Calcium Phosphate Crystals Into Sodium Alginate Gel

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**Abstract:**

This report encapsulates my role as a research assistant at Carleton University, Ottawa, Canada, contributing to a project focused on studying precipitations within sodium alginate. Guided by Professor Andrew Speirs and in collaboration with master's student Aaryn Lavallee, our main goal was to induce calcium phosphate crystal precipitation in hydrogel compounds, achieved through a double diffusion system. Initial experiments centered on simple diffusion, employing sodium alginate samples submerged in calcium phosphate solutions. Despite multiple trials, no substantial weight gain or precipitation was observed, prompting the development of a new double diffusion chamber. Designed using CAD software and fabricated by the Carleton Machine Shop, this chamber accommodated dual solutions while enabling continuous fluid circulation. Subsequent experimentation utilized the newly devised double diffusion chamber to stimulate precipitation. Placing the sample between two solutions, this approach encountered issues including leakage and sample decomposition. Adjustments to the assembly were enacted to address these challenges. In summation, this report traces the progression from simple diffusion exploration to the establishment of a double diffusion system for promoting calcium phosphate crystal precipitation in hydrogel compounds. Despite hurdles faced, insights from courses like MAAE2001, CHEM1001, and CHEM1002 facilitated adaptation. This experience enriched my experimental skills, deepening comprehension of research methodologies and effective problem-solving techniques.

**Introduction:**

During the summer, my role as a research assistant at Carleton University in Ottawa, Ontario, Canada, granted me the opportunity to contribute to a scientific/engineering research project under the mentorship of Professor Andrew Speirs and alongside the master's student, Aaryn

Lavallee. Our collective focus was on investigating the mechanical properties of sodium alginate, and my involvement was stemmed around introducing a new dimension to this research, namely, the facilitation of calcium phosphate crystal precipitation within the hydrogel compounds developed by Aaryn. Building upon prior work that had explored single diffusion, I tried using double diffusion to facilitate crystal formation within the samples. To achieve this, several problems needed to be addressed, including the creation of a custom diffusion chamber to conduct the experiments and the establishment of chemical solutions to enable the diffusion process to proceed. Using skills learned in class and through some extracurricular work I was able to come up with solution for various problems tackled during the term. Research was conducted into diverse solutions and approaches and employing computer-aided design software (CAD), I designed a diffusion chamber model, which was subsequently sent to the Carleton Machine Shop for realization. During the span of the report, I will cover the various phases of my work over the summer which includes the research phase of my work, the first and second experimentation phases as well as the design phase.

**Research Phase:**

The preliminary phase of this study spanned a duration of two weeks, during which insights emerged from research that I conducted. This notably included a significant departure from the initial hypothesis put forth by the preceding researcher. Contrary to the original approach, which sought to induce calcium phosphate precipitation in the hydrogel by dissolving it in water and observing the resultant precipitation i.e., using a single diffusion system, my findings indicated that a double diffusion methodology yielded more promising results. [1][2][3] In the double diffusion system, two distinct solutions, one containing calcium and the other containing phosphate, were introduced to the hydrogel, creating the precipitation of crystals within the sodium

alginate gel. [6] This new approach exhibited noteworthy distinctions in the precipitation chemistry and required a new diffusion system to be created and tested.

### **First Experimentation Phase:**

During the initial phase of experimentation, I immersed samples of sodium alginate in beakers containing calcium phosphate, aiming to investigate the possibility of inducing precipitation within the hydrogel using simple diffusion. This investigation involved four distinct samples, each with varying compositions: one sample comprising sodium alginate and 10.3% gelatin, two samples comprising sodium alginate and 14.2% gelatin, and a final sample composed solely of sodium alginate. In the case of one of the two samples containing 14.2% gelatin, the calcium phosphate solution was replenished daily for one sample whereas for the other sample the solution was left for the entire duration of the experiment. Using the experimental skills, I learned during my chemistry classes I formulated a very basic experiment to test the above-mentioned hypothesis. The sodium phosphate solution utilized in the experiment was prepared by combining distilled water and sodium phosphate, resulting in a 0.1M solution. [1][2] To monitor potential precipitation, the initial weight of each sample was recorded before the commencement of the experiments. Subsequently, daily measurements were taken to observe any weight fluctuations, with the hypothesis being that the occurrence of precipitation would lead to a discernible increase in weight. Upon the conclusion of the initial five days of experimentation, a meticulous analysis of the data was conducted. Regrettably, the results did not yield any conclusive evidence of significant weight gain within any of the samples. Instead, fluctuations in weight were observed across all samples, making it challenging to discern any clear pattern or definitive indication of

precipitation formation. The inconclusive outcomes from this preliminary investigation underscore the need for a double diffusion system versus the attempted single diffusion.

### **Design Phase:**

The initial experimentation's outcomes underscored the unsuitability of single diffusion for crystal formation within hydrogel samples, necessitating the conception of a double diffusion system. This system was required to address specific criteria: the inclusion of two distinct chambers for separate solutions and provision for securely holding the hydrogel sample, ensuring contact with both fluids to facilitate diffusion. [6] Guided by these prerequisites, a Delrin-based design with specified dimensions was formulated (refer to Figure 1 and Figure 2). The resultant design not only allocated space for both fluids and the sample but also showcased potential adaptability for future experiments by integrating a pump system. This feature would enable a continuous circulation of fluids through the chambers, maintaining a consistent concentration throughout the experiment's duration. Using insights from MAAE2001 and ECOR1047, I utilized my design skills to develop a CAD model using both SolidWorks and Fusion360. Following the completion and evaluation of the CAD model by Professor Spiers, it was dispatched to the Carleton Machine Shop for manufacturing, with requisite hardware components procured from sources such as Home Depot and Home Hardware. Upon receiving the fabricated part, the project transitioned into the subsequent phase of experimentation.

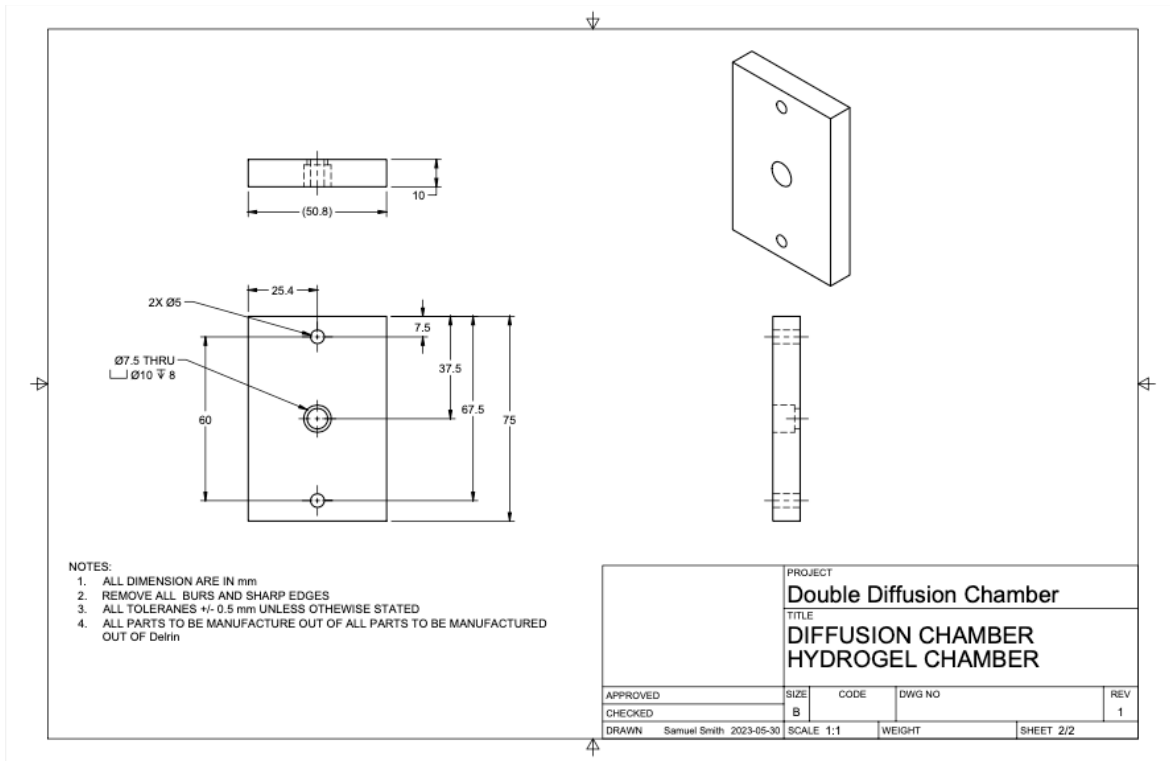


Figure 1: Hydrogel Chamber

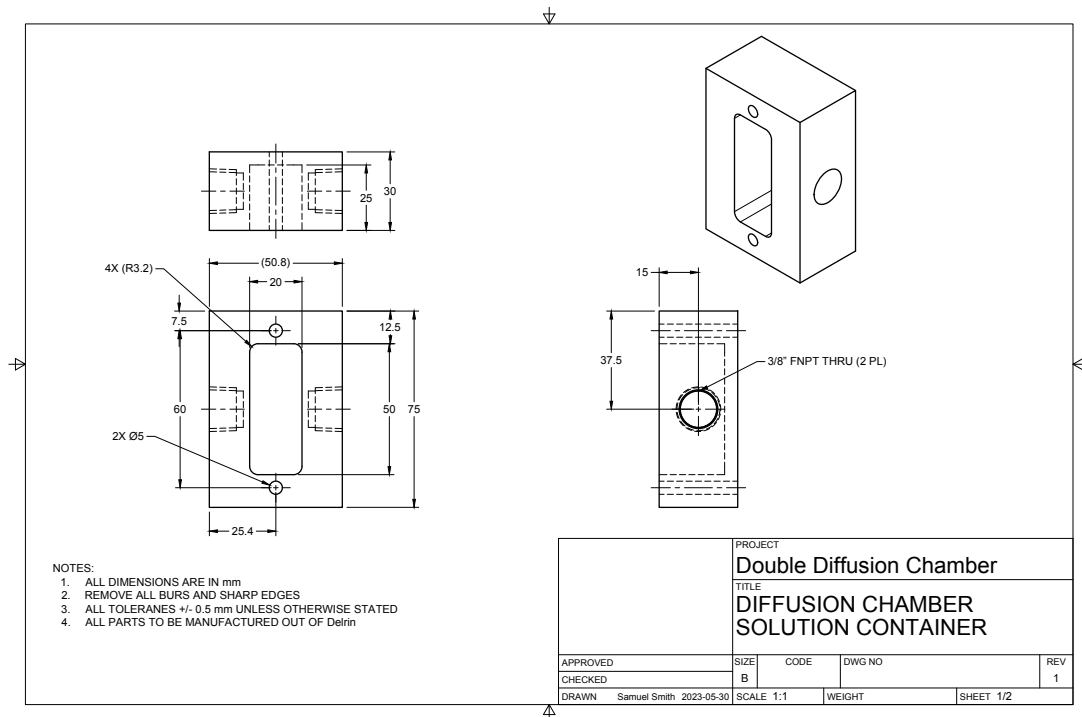


Figure 2: Solution Chamber

## **Second Experimentation Phase:**

In this experimental phase, the primary objective was to use the newly designed double diffusion chamber for the purpose of inducing precipitation. This was achieved by establishing two distinct solutions to place in their respective chambers. Both sides of the system were filled with a singular solution, while the hydrogel sample was positioned between these solutions. The experimental framework drew inspiration from prior investigations documented across diverse sources. Collectively, the research indicated that a solution composition comprising 150 mM of NaCl, 10 mM of Tris HCl, and either 8 mM of CaCl<sub>2</sub> or NaPO<sub>4</sub> yielded the most favorable conditions for precipitate formation within the sample. [3][4][5][6] As illustrated in Figure 3, the experimental setup was set up using fasteners to hold the system together. The chambers were filled via the connected tubing, which was subsequently sealed to counteract any potential solution evaporation. Following the initial trial, certain issues necessitated rectification before proceeding to subsequent trials. Notably, the assembly exhibited leakage, a challenge that was effectively resolved through the implementation of gasket material, tailored to match the shapes of the chambers within the system. Simultaneously, ensuring the system's components were adequately tightened assisted in mitigating leakage. An additional concern was identified, where fluid from one side of the chamber permeated to the other side. This issue was addressed through the application of silicone gel, creating a secure seal that effectively anchored the sample and prevented leakage.



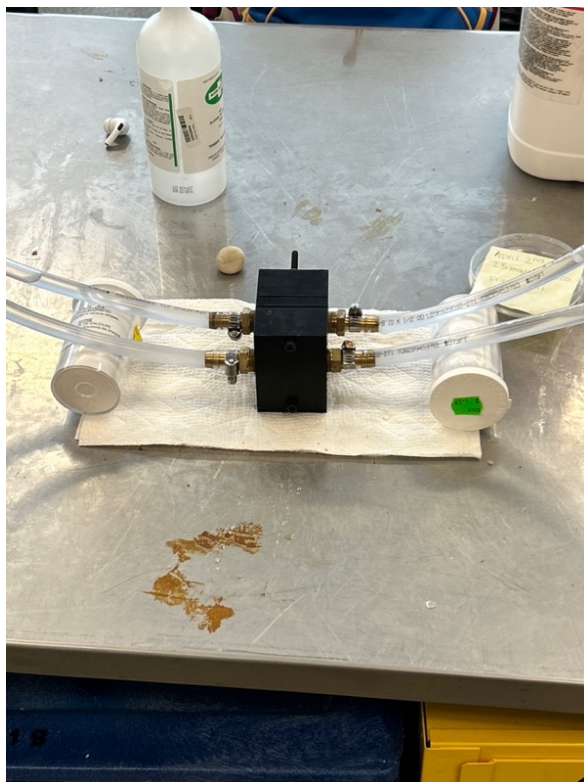


Figure 3: Experimental Setup 2<sup>nd</sup> trial

However, the second trial yielded an unexpected outcome: the sample underwent pronounced decomposition after its immersion in the solution. Consequently, a reiteration of the test was conducted, this time excluding the NaCl and Tris HCl buffers, while exclusively utilizing solutions containing the precipitates. Regrettably, the sample's disintegration persisted, prompting further considerations for future steps. To this end, a new approach will be taken, involving the isolation of samples in distinct solutions. One sample will be exclusively immersed in a sodium chloride solution, while the other will be subjected solely to a calcium phosphate solution. This should establish what the underlying cause of the dissolution is and lead to a new way to tackle the issue.

**Conclusion:**

In conclusion, my work as a research assistant during this co-op term at Carleton University has provided valuable insights and experiences in the realm of scientific exploration and engineering experimentation. Guided by Professor Andrew Speirs and collaboratively working with master's student Aaryn Lavallee, I contributed to a research project aimed at inducing calcium phosphate crystal precipitation within hydrogel compounds through the implementation of a double diffusion system. The initial experimentation phase underscored the limitations of single diffusion for crystal formation within hydrogel samples. This prompted the design and construction of a double diffusion chamber, developed based on specific criteria. The Delrin-based design accommodated two separate solutions and the hydrogel sample, allowing for efficient diffusion while holding the potential for future enhancements, such as integration with a pump system for fluid circulation. The experimentation phase, conducted using the double diffusion chamber, encountered challenges, including leakage and sample decomposition. These issues were addressed through gasket material, silicone gel, and modified solutions. Despite the setbacks, these experiences underscored the iterative nature of engineering and scientific experimentation and the adaptability required to address unforeseen challenges. There are many future steps that I will be exploring in the remaining weeks of my work term: I will be looking into different concentrations of solutions to achieve the formation of crystals, I will also explore the possibility to have the calcium chloride already mixed in the sample allowing for single diffusion using sodium phosphate and finally I will look into sourcing a peristaltic pump that will allow for constant fluid flow through the system. This co-op term has provided an opportunity to apply theoretical and practical knowledge gained from courses like MAAE2001, CHEM1001, and CHEM1002 to real-world research scenarios. The experience has deepened my understanding of experimental

methodologies, problem-solving strategies, and the practical implications of design and fabrication processes. Through this endeavor, I have not only expanded my skill set but also gained a deeper appreciation for the complexities and rewards of scientific inquiry.

**Acknowledgements:**

I would like to thank Professor Andrew Speirs for this fantastic opportunity and for his guidance throughout my work term. I would also like to thank Arryn Lavalley for her support and guidance as well. This experience has been a great first taste of what research and design has to offer and has given me a great appreciation of the work and more future aspirations to one day work in this field.

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