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Calcium Alginate Microbead Production via an Air Assisted Shearing Process

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Honors Research Project

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Honors Research Project

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Abstract

The purpose of this project was to model the size of calcium alginate microbeads produced by extruding a solution of 1.5 wt.% alginate from a syringe and needle with air shear generated from a pneumatic line flowing in an annular tube around the extrusion needle. The air shear would create microdroplets that would fall into a solution of 2 wt.% calcium chloride in water, crosslinking the droplets into beads. These microbeads can be used in drug dispersion applications or in encapsulation of cells throughout the body due to their biocompatibility. The goal was to create uniform beads that ranged from 200 to 300 μm in diameter for injection based drug delivery and tissue engineering applications. Microbeads were successfully modeled and produced for diameters ranging from 100 to 1,000 μm. However, only microbeads in the 600 to 1,000 μm range were uniform. For a sample to be considered uniform, it must have had a coefficient of variance (CV) under 15%. Since the desired size range of the microbeads was not produced, no efforts were made to increase the production rates of the beads. The simplicity of the experimental setup may prove useful for applications where the desired microbead diameter ranges from 600 to 1,000 μm.

Executive Summary

One way to disperse drugs to patients or transport cells through the body is the use of microbeads. Many different types of beads exist, and calcium alginate beads are one example that is often chosen for reasons such as their biocompatibility and biodegradability. For many applications of these microbeads, the desired size ranges from 200 to 800 μm. One such application that falls in this range is for microbeads to be used to disperse drugs to surgical patients. The needle used to inject these drugs is about 500 μm in diameter, so the desired microbead diameter should be in the range of 200 to 300 μm. The goal of this project was to produce calcium alginate microbeads in this size range using a pneumatic line generating an annular airflow around a needle filled with an aqueous 1.5 wt.% alginate solution. The air shear generates microdroplets of the alginate solution, which fall into an aqueous 2 wt.% calcium chloride solution, crosslinking the microdroplet into a microbead. Not only do the beads need to fall within the size range, but their diameters must also be consistent. The size of the beads affects the drug dispersion mechanism, so uniform microbeads are critical. If uniform microbeads were produced in the preferred size range, measures would be investigated to increase the production rate of the beads.

A series of trials were conducted manipulating five parameters: alginate solution density, annular air shear generated, surface tension of the alginate solution, needle radius, and alginate solution viscosity. Of the five parameters tested, only the annular air shear generated and the needle radius had a significant effect on the microbeads size. Microbeads were successfully produced and ranged in size from about 100 to 1,000 μm in diameter. However, since uniform beads were determined to have a coefficient of variance (CV) less than 15%, only beads created in the 600 to 1,000 μm diameter range were classified as uniform. Therefore, scale-up of the

microbeads was not conducted using this technique. To make the smaller microbeads, high shear rates were required that would agitate the calcium chloride crosslinking solution causing the liquid to splash around, contributing to the higher size distribution of the microbeads.

As a result of this project, the author learned a technique for calibrating rotameters based on volumetric flowrates measured by timing how long balloons fill with air. The project also helped to develop organizational skills and good lab note taking practices. Working in the lab taught one to maximize the resources on hand and be resourceful. The writer's confidence and creativity improved from this endeavor. Not only benefiting the author, the work also may aid society. The experimental setup used to produce the alginate microbeads is easily constructed, and the cost to operate it is extremely low. If one desires to produce uniform alginate microbeads in the 600 to 1,000 μm range, this operation is easily repeated. Microbeads of this size could be used in other drug dispersion applications or in cell encapsulation/transportation scenarios.

For future work, different methods of calcium alginate microbead production should be investigated. Many other avenues exist such as the use of an electrostatic bead generator or using a stirred organic phase as a matrix while solutions of alginate and calcium chloride are added to the mixture (Zhou, 2009). Some of these processes should be used in an attempt to produce uniform calcium alginate microbeads in the desired size range of 200 to 300 μm.

Introduction

In today's medical field, researchers are continuously striving to improve how drugs are delivered to patients or how to design minimally invasive approaches for delivering cells for deep wound repair (Song et al, 2015). One way to accomplish this task is to use gel based beads or microbeads, which can carry and target medicine or cells to a particular location in the body after either being directly injected or applied to a surgical site or for drug dispersion applications.

One way to produce these beads is to use calcium alginate. In the past, calcium alginate microbeads have been formed by spray dying, spray cooling, extrusion, fluidized beds, coacervation, emulsification, photolithography, or micro-molding. However, these processes involve aspects that could have a negative impact on the final microbead product such as ultra-violet light exposure or use of organic solvents, which could cause contamination or affect the drug/cells to be released (Huang, 2010). Another approach described in this paper is to use shear flow to form microdroplets that are then contacted with a crosslinking solution, forming the microbeads. The low viscosity alginate solution (normally containing the medicine) is dripped from a needle while air shear flowing around the needle in an annulus connected to a pneumatic line helps to form the microdroplet, which falls into a calcium chloride solution crosslinking the microdroplet into a microbead.

Based on previous studies, optimal bead size was in the range of a couple hundred microns. "Bead size in the range of 200-800 microns is desired for most encapsulation applications" (Lee et al, 2013). More specifically, the needles (typically gauges 18 to 22) used to inject the beads into the surgical sites range in diameter from 410 to 840 microns, so beads with a size of 200 to 300 microns or less will easily flow through the needle (Sigma-Aldrich, 2016).

In terms of drug dispersion, the size of the bead alters the drug release behavior; so knowing how to consistently produce a certain bead size would benefit further studies in that area.

The goal of this project was to model the production of crosslinked alginate microbeads using a pneumatic line to generate air shear in annular flow around the needle tip, from which the low viscosity solution was expelled. Next, the fluid flow model could be used as a predictive tool to determine the processing parameters, such as air flow rate and needle tip diameter, for producing alginate microbeads of consistent dimension for various applications mentioned above. Finally, if beads of the right dimension could be consistently produced, increasing the production rate of the beads would be attempted to develop more materials to test.

Background

Alginate and alginate beads

Alginate is biopolymer extracted from seaweed often used in the medical industry due to its biocompatibility. Other benefits besides its biocompatibility include its low toxicity, relatively low cost, and mild gelation by addition of divalent cations (Lee & Mooney, 2013). The block copolymer consists of linear chains of (1-4)-linked monomers of β-D-mannuronic acid (M block) and α-L-guluronic acid (G block) (Amsden & Turner, 1999). Figure 1 shows the chemical structure of the two monomers. Different ratios of each block produce different properties of the alginate, especially gelling capability and gel strength. The ratio depends on the species of seaweed, part of the seaweed used, harvest location, and the harvest season (Kimica, 2009). For hydrogel formation in terms of crosslinking, only the G block of the polymer is believed to participate with the divalent cations. Therefore, the properties of the alginate gels depend on the ratio of M to G blocks and the length of the G blocks (Lee & Mooney, 2013).

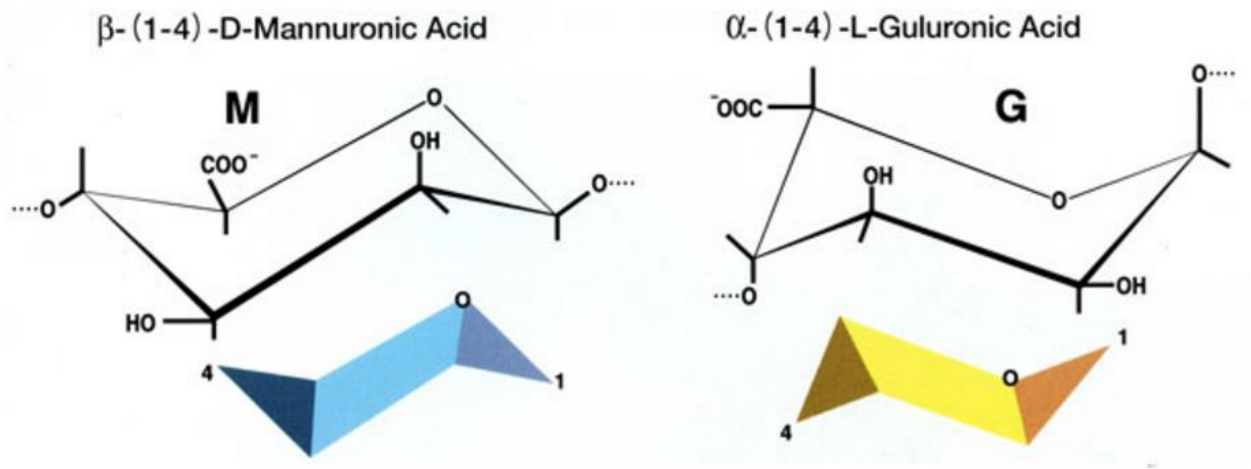


Figure 1. Alginate monomers' molecular structure (from Kimica, 2009).

For most biomedical applications, the hydrogel form of the alginate is used. "Hydrogels are three-dimensionally crosslinked networks composed of hydrophilic polymers with high water content" (Lee & Mooney, 2013). Crosslinking using divalent cations, as mentioned above, is the most common method for preparing alginate hydrogels. Calcium chloride is often used in the crosslinking of alginate, and the gel's uniformity and strength generally improves with slower gelation times as well as the molecular structure of the alginate as mentioned previously. Another feature of the hydrogels is their lack of long term stability. The gels can participate in exchange reactions swapping the divalent cations with monovalent cations in solution. This process breaks up the crosslinks in the hydrogel causing the gel to dissolve back into solution (Lee & Mooney, 2013). Depending on the application, this effect could be seen as positive or negative. In the case of using the hydrogel to slowly release drugs to a patient, this phenomenon becomes beneficial. One geometry that the microbeads can be crosslinked into is a spherical bead. If the beads are small enough, they can be injected into the body through a needle for example. Other applications for alginate beads besides drug delivery include uses in pollution control, delivery of angiogenic growth factors, crystallization of low and high molecular weight proteins, preservation of probiotics, and possibly biosensor applications (Rehm, 2009).

Modeling the air shear for generating alginate beads

To determine the shear generated from the pressurized air, appropriate equations for flow in an annulus were used. Equation 1 shows how to calculate the Reynolds number for annular flow where ρ is the fluid density, V is the velocity, D_2 is the outside tube's diameter, D_1 is the inside tube's diameter, and μ is the fluid viscosity (Bird, 2002).

$$Equation\ 1: Re = \frac{\rho V(D_2 - D_1)}{\mu}$$

Also, for laminar annular flow, the shear and velocity are determined by Equations 2 and 3, respectively, where R is the outside tube radius, L is the axial length of tubing, ΔP is the pressure drop across L, r is the distance from the center of the annulus, and κ is the ratio of the inside tube radius to the outside tube radius (Bird, 2002).

$$Equation\ 2: \tau_{rz} = \frac{R\Delta P}{2L}\left[\left(\frac{r}{R}\right) - \frac{(1-\kappa^2)}{\ln\left(\frac{1}{\kappa}\right)}\ln\left(\frac{R}{r}\right)\right]$$

$$Equation\ 3: v_z = \frac{R^2\Delta P}{4\mu L}\left[1 - \left(\frac{r}{R}\right)^2 - \frac{1-\kappa^2}{\ln\left(\frac{1}{\kappa}\right)}\ln\left(\frac{R}{r}\right)\right]$$

Combining Equations 2 and 3 gives the shear in terms of velocity while eliminating the pressure drop term, ΔP, and length term, L, yielding Equation 4.

$$Equation\ 4: \tau_{rz} = \frac{2\mu v_z\left[\left(\frac{r}{R}\right) - \frac{1-\kappa^2}{2\ln\left(\frac{1}{\kappa}\right)}\left(\frac{R}{r}\right)\right]}{R\left[1 - \left(\frac{r}{R}\right)^2 - \frac{1-\kappa^2}{\ln\left(\frac{1}{\kappa}\right)}\ln\left(\frac{R}{r}\right)\right]}$$

Factors affecting bead size

Based on the above equations, the processing parameters that could affect the microbead size are listed as follows: the velocity of the air, the viscosity of the air, the needle radius, and the

tube radius. At which point the bead breaks away from the needle depends on a simple force balance shown in Equation 5, where ρ is the density of the sodium alginate solution, V_d is the volume of the droplet, g is gravitational acceleration, τ_{rz} is the shear force generated by the air, A_d is the area of the droplet that experiences shear, γ is the surface tension of the droplet, and r_n is the radius of the needle.

$$Equation\ 5: \rho V_d g + \tau_{rz} A_d = \gamma 2\pi r_n$$

If one assumes the droplet is a sphere, then Equation 5 can be written in terms of the droplet's radius, r_d. Equation 6 shows the spherical volume and surface area of the droplet exposed to the shearing force substituted into Equation 5 where C is a constant that falls somewhere between one and four based on how much the surface area of the drop experiences shear.

$$Equation\ 6: \frac{4}{3}\pi\rho g {r_d}^3 + C\pi\tau_{rz} {r_d}^2 = 2\pi\gamma r_n$$

In theory, Equation 6 shows which parameters will affect the size of the microbeads formed. From this equation, one can see that increasing the alginate solution density or the air shear generated should decrease the size of the beads while increasing the surface tension of the solution or radius of the needle should increase the bead radius. In addition, one might expect that the viscosity of the droplet might influence the detachment of the droplet from the needle tip. Therefore, these four parameters, along with the viscosity of the alginate solution, were examined in this project to assess their contribution to the produced bead size. Studies have shown that the viscosity of the solution affects the droplet's shape before detachment, giving reason for its analysis in this study (Lee et al, 2013).

Experimental Method

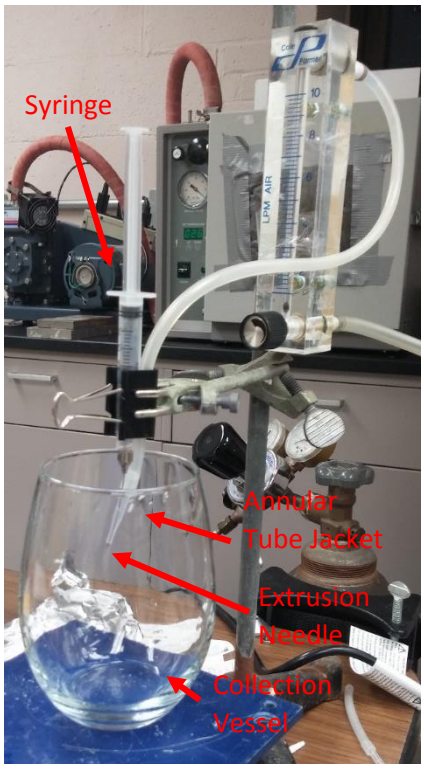
Materials and equipment

The materials used for these experiments are listed as follows: anhydrous calcium chloride (CAS# 10043-52-4), blue food dye, alginic acid sodium salt from brown algae (Sigma# 71238-250G), deionized water, sodium dodecyl sulfate (NaDDS), and poly(ethylene oxide-propylene oxide) MW 8,750 (Cat#16277 Polysciences) (P(EO-PO)). For these experiments, the following equipment was used: a rotameter; a pneumatic line; gauge-20, gauge-22, and gauge-25 flat needles; a syringe; an alligator clip; a wire mesh filter; a pair of tweezers; a 1.5 mL micro centrifuge tube with a snap cap; a ruler, chemwipes; a camera; balloons; a volumetric flask; a volumetric pipette; a ring stand; a ring clamp; a collection vessel; an XP2 pipette controller; and a Gilmont micrometer syringe GS-1100 0.2 mL.

Experimental procedure

Figure 2 shows the experimental set up for the microbead production. A rotameter was connected to a pneumatic line connected to the outside annular tube. A small incision was made in the side of this tube allowing the gauge-22 flat needle, which serves as the extruder, to be placed down the center creating an annulus. The needle was connected to a syringe, serving as a reservoir for the alginate solution, that was held in place with the tube using an alligator clip. This setup was suspended above a 2% solution of calcium chloride with a drop of blue food dye added to give the microbeads color, assisting with the characterization of the bead size. For each trial, the needle tip was a distance of 0.2 cm from the end of the annular tube. Figure 3 shows the needle distance from the annular tube. When the needle tip was flush with the annular tube, the alginate solution would adhere to the tubing distorting the shape of the bead. To actually produce beads, the syringe was filled with a 1.5 wt.% alginate solution. For each trial, the needle tip was

suspended 3.8 cm above the calcium chloride solution. The bowl containing the calcium chloride solution was placed on an adjustable platform to easily manipulate the microdrops' falling distance. For each trial, the rotameter was fixed to the desired setting by adjusting the air flow rate. Next, the syringe was filled with the alginate solution; and the bowl was filled with the calcium chloride solution. After the syringe was filled, a plunger was placed into it to create pressure to push out the solution to begin bead production. The plunger was left in until one milliliter of the alginate solution had been expelled. Next, the calcium alginate beads were filtered from the calcium chloride solution with a wire mesh filter. Using a pair of tweezers, the microbeads were collected into a micro centrifuge tube. To measure the bead size, the microbeads were placed on a ruler and pad-dried with a chemwipe to visualize the edges of the microbeads more easily with a magnification. Pictures of the beads were taken and analyzed using ImageJ software to determine the diameter of the microbeads.



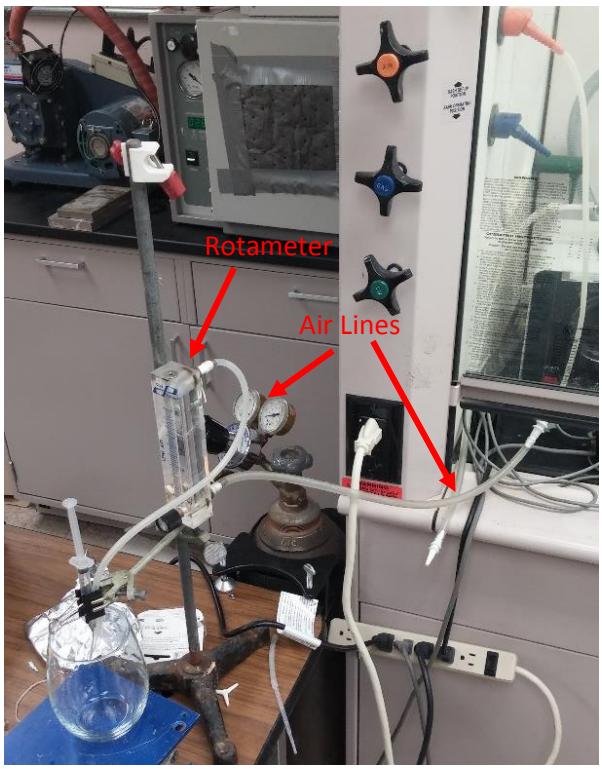


Figure 2. Experimental setup for calcium alginate bead production.

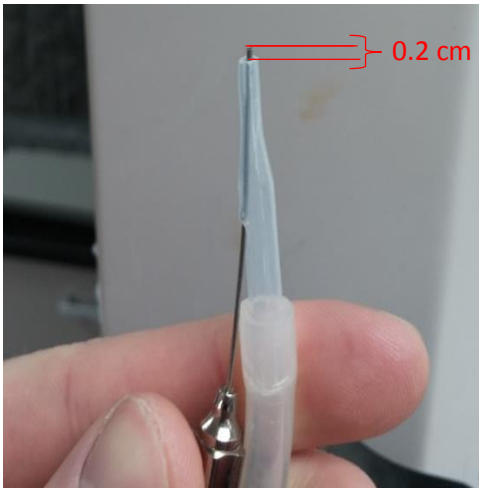


Figure 3. Needle distance from the annular tubing, approximately 0.2 cm.

To determine the shear generated by the annular air flow, the rotameter was calibrated to determine the velocity of airflow in the annulus. A balloon was attached to the outside tube to complete this calibration, and the air flow was adjusted to the desired rotameter setting and allowed to fill the balloon while being timed. This process was videotaped; and the tape was analyzed using ImageJ software to determine the volume of the balloon for a certain time interval, yielding the volumetric flowrate of air.

Density measurements for the samples were made to study if the dissolved alginate caused a change. To accomplish these measurements, the mass of an empty volumetric flask shown in Figure 4 was recorded. Next, the flask was filled with DI water and massed again. The mass of DI water in the flask was used to determine the total volume of the flask. Then, solutions of alginate were poured into the flask; and the mass was measured. The densities were determined using the mass of the solution along with the volume of the vial. For each measurement, the same flask was used; and it was dried using a pneumatic line between samples.

Figure 4. The volumetric flask used for density determination of various liquids used in this study.

Viscosity of the alginate solutions was also tested. Figure 5 shows the volumetric pipette used for the testing. First, the pipette was suspended in the air using a ring stand and clamp. Next, the sample solutions were drawn up into the pipette with the pipette controller. The time for each sample to fall between two marked lines on the pipette was recorded. As a reference, DI water was first tested. The sample viscosities were then determined based on their times relative to the water's time.

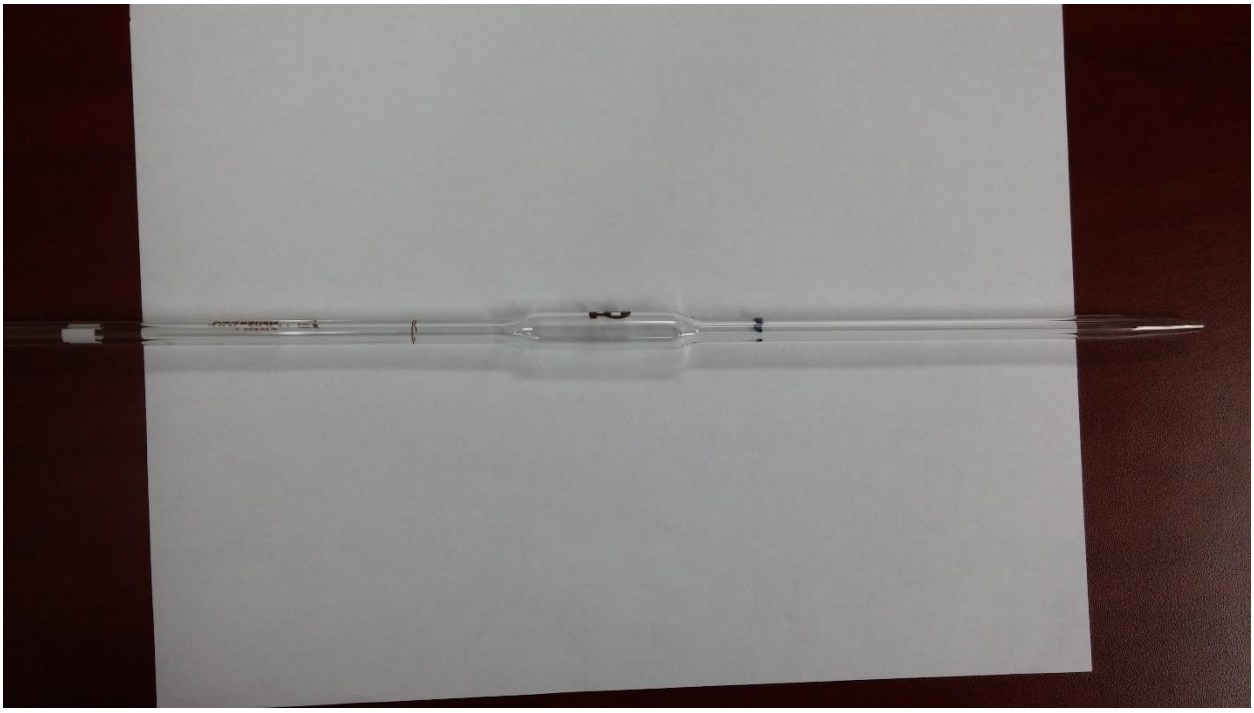


Figure 5. Volumetric pipette for viscosity measurements (time for solution to flow between the two marked lines was recorded).

The next property tested was the surface tension of the samples, which was modified using surfactants, sodium dodecyl sulfate and poly(ethylene oxide-propylene oxide), each at a concentration of 0.015 wt.%. To measure the surface tension, the pendant drop method was applied by taking pictures of the pendant drop from a needle before it detaches from a needle tip using a Gilmont micrometer syringe. Figure 6 shows an example of one of the pictures. From the dimensions of the droplet, one can determine the surface tension of the liquid based on Equation 7, where γ is the surface tension, ρ_D is the density of the droplet, ρ_M is the density of the matrix (air for these tests), g is gravitational acceleration, D_e is the equatorial diameter of the drop, and H is a correction factor that depends on the ratio of the drop diameter measured horizontally at a distance of the equatorial diameter from the apex of the drop to the equatorial diameter (D_s/D_e).

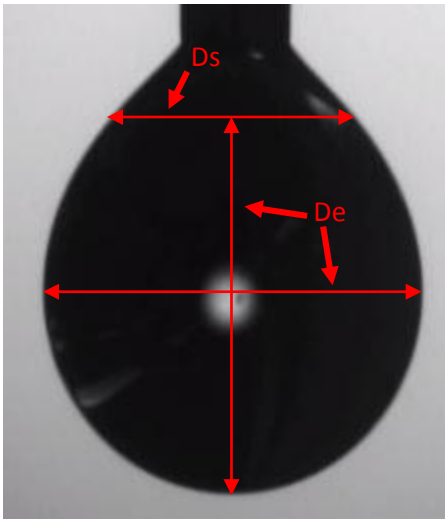


Figure 6. An image of the pendant drop of an alginate solution, where the equilateral diameter (De), and the diameter at a distance of De from the apex, Ds, are marked.

$$Equation\ 7: \gamma = \frac{(\rho_D - \rho_M) g D_e^2}{H}$$

Data and Results

Figure 7 shows different pictures of the calcium alginate microbeads formed placed on a 12-inch ruler.

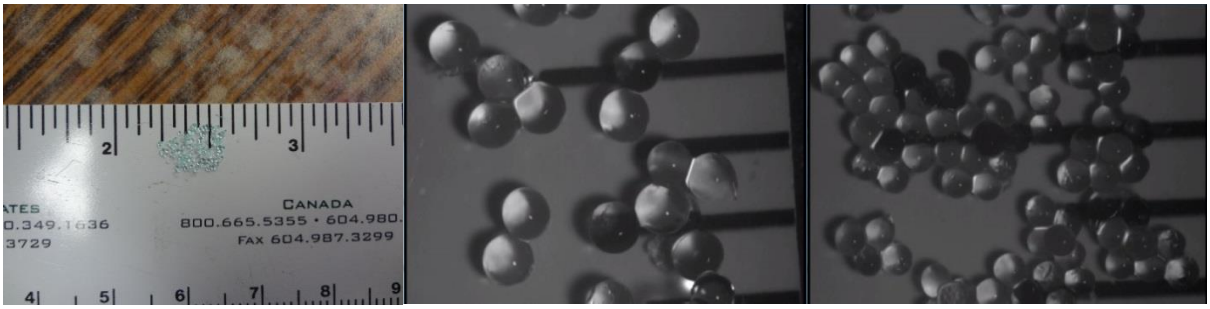


Figure 7. Images of calcium alginate microbeads formed by extrusion with air shear placed on a ruler.

Before any measurements of the microbeads were conducted, the rotameter was calibrated to determine the appropriate shear rate at the inner annulus wall (the needle) for each

setting. Figure 8 shows the results. After the volumetric flow rate was determined, the average air velocity was found by dividing the volumetric flow rate by the cross-sectional area of the annulus. Equation 4 was used to determine the shear generated based on the velocity of the air. The results show a fairly linear relationship between the rotameter setting and the shear.

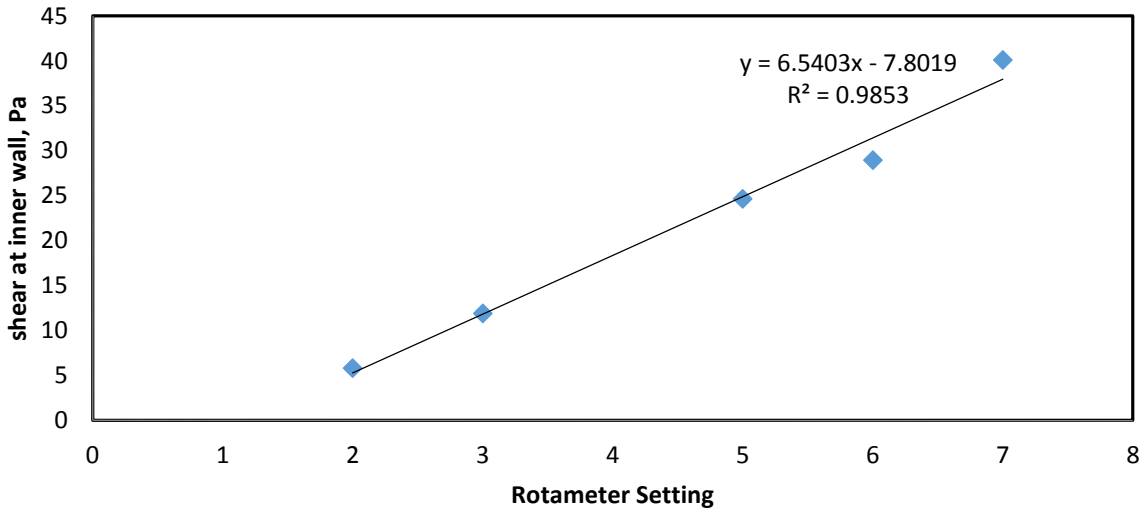


Figure 8. Shear stress at the inner annulus wall (the needle) versus rotameter setting. The shear stress increases almost linearly with the increase of the rotameter setting from 2 to 7.

The actual bead size for different shear rates using a gauge-22 needle is shown in Figure 9. The results also show that the bead diameter is inversely related to the air shear with a linear trend. A verification run is also shown to demonstrate the repeatability of the process. Table 1 and Table 2 show the statistical results for the original run along with the verification trial. Along with the average bead size, the tables give the standard deviation (Stdev), standard error (Error), and coefficient of variance (CV).

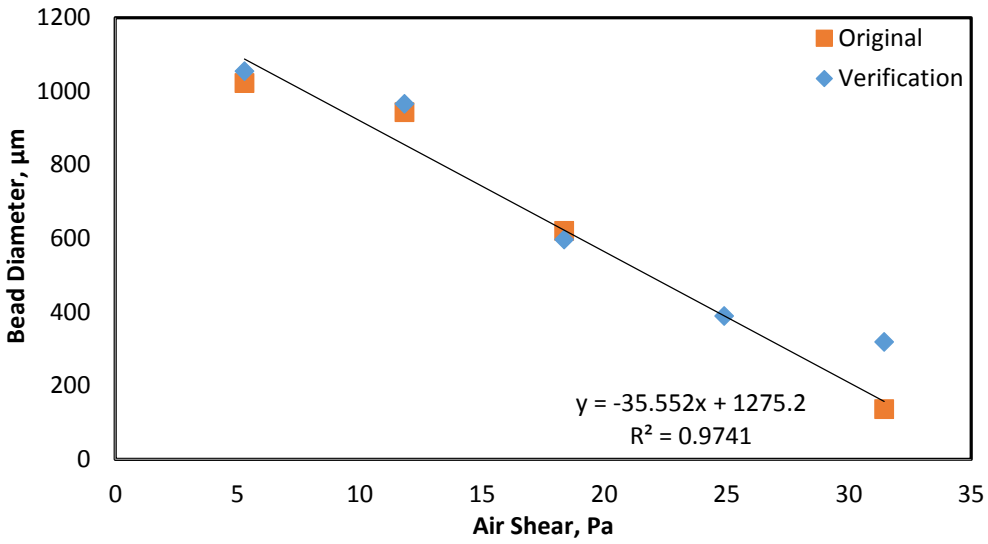


Figure 9. Testing and verification of average bead diameters at various air shear rates showing a linear trend between the two parameters.

Table 1. Statistical Data for the Original Trial

Shear, Pa	Bead D, µm average	Stdev, µm	Error, µm	CV
5.28	1022	191.6	27.95	18.7%
11.82	944	133.5	22.89	14.1%
18.36	621	84.7	7.70	13.6%
31.44	136	98.4	4.93	72.3%

Table 2. Statistical Data for the Verification Trial

Shear, Pa	Bead D, µm average	Stdev, µm	Error, µm	CV
5.28	1054	55.4	12.10	5.3%
11.82	966	67.8	10.72	7.0%
18.36	597	48.7	3.30	8.1%
24.90	389	98.8	14.11	25.4%
31.44	319	98.6	4.91	30.9%

When modeling a system in engineering, often dimensionless numbers are used to gain perspective on which parameters affect the modeled system. Figure 10 shows the bead diameter versus Reynolds number for the air flowing in the annulus. From the linear fit of the data, Equation 8 was developed to relate the bead diameter in µm to the Reynold's number.

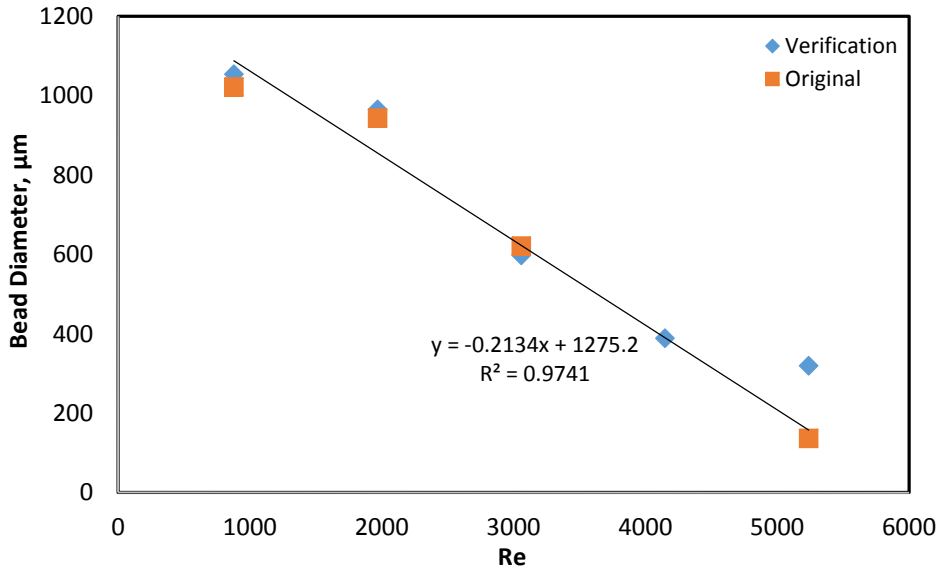


Figure 10. The bead diameter decreases with the increase of Reynold's number, and a rough relationship of D (µm) = – 0.2 Re + 1275.

$$Equation\ 8: D = -0.2134\mu m * Re + 1275.2\mu m$$

Figure 11 shows the results for the density testing for DI water, 1.5 wt.% alginate, 1.5 wt.% alginate with 0.015 wt.% P(EO-PO), and 1.5 wt.% alginate with 0.015 wt.% NaDDS. Neither the alginate nor the surfactants affected the solution density much.

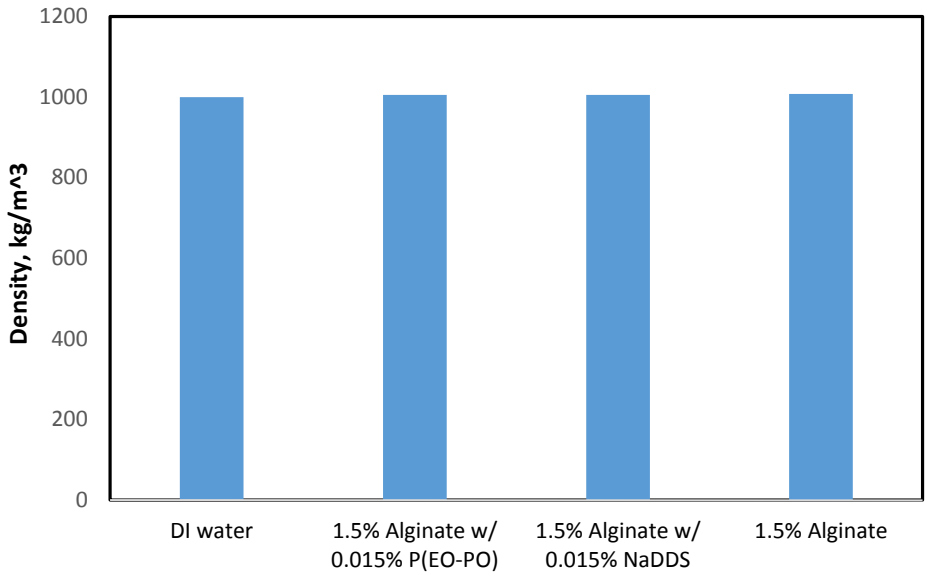


Figure 11. Densities of deionized water, an alginate solution, and an alginate solution with added surfactants. The density was not affected much by the alginate or surfactants.

Viscosity of the alginate solutions was also measured to determine if the bead size was affected by viscosity. Figure 12 shows the resulting microbead diameter as the alginate solution viscosity was modified by using different concentrations of sodium alginate, while under a constant air flow rate (setting "4" on the rotameter) for shear. The results show that the alginate solution viscosity has little to no effect on the bead size produced. As mentioned above, previous studies have shown that the viscosity plays more of a role in the shape of the alginate beads than in the size of the beads (Lee et al, 2013).

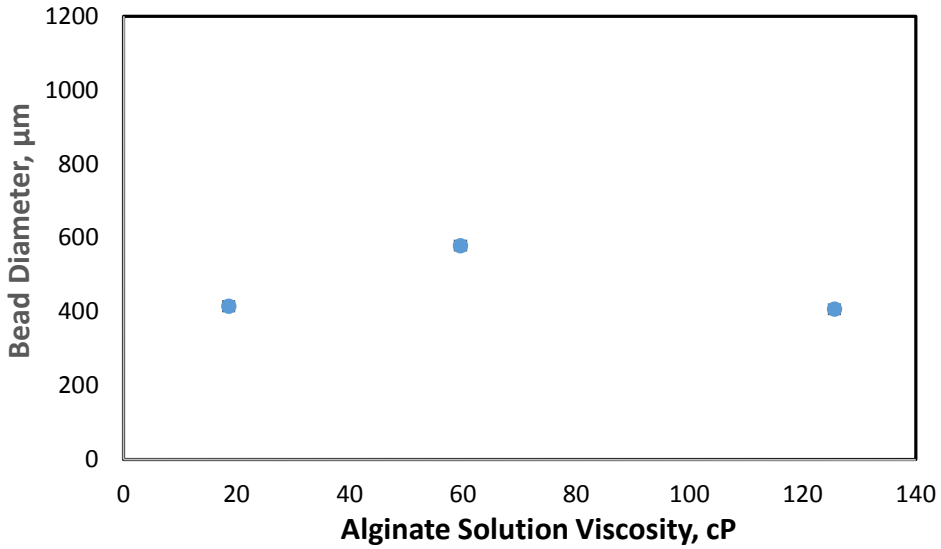


Figure 12. Microbead diameter versus alginate solution viscosity at "4" rotameter setting, bead size is almost independent of the alginate solution viscosity.

Figure 13 shows different bead diameter versus air shear rate for samples whose surface tension was modified using the two different surfactants in the alginate solution. Table 3 shows the surface tension values for the for the different alginate solutions.

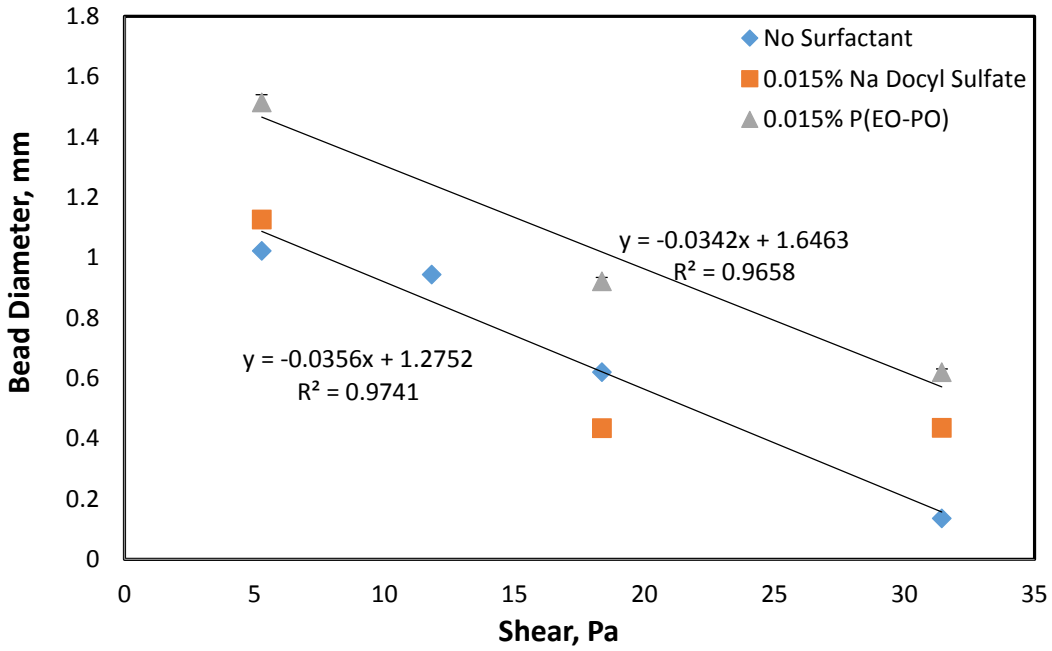


Figure 13. Microbead diameter versus air shear using different surfactants. Trends are counter intuitive as the lower surface tension of the surfactants with the beads is expected to decrease bead size, but the bead size is actually larger in most cases

Table 3. Surface Tension Measurements

species	Average γ, mN/m
1.5 wt.% Alginate	72.0
1.5 wt.% Alginate w/ 0.015% P(EO-PO)	54.0
1.5 wt.% Alginate w/0.015% SDDS	50.9

The last parameter tested in these trials was the needle radius. No air shear was used when running these trials. According to Equation 6, when no air shear is present, the needle radius will be proportional to the drop radius cubed. Figure 14 shows this trend.

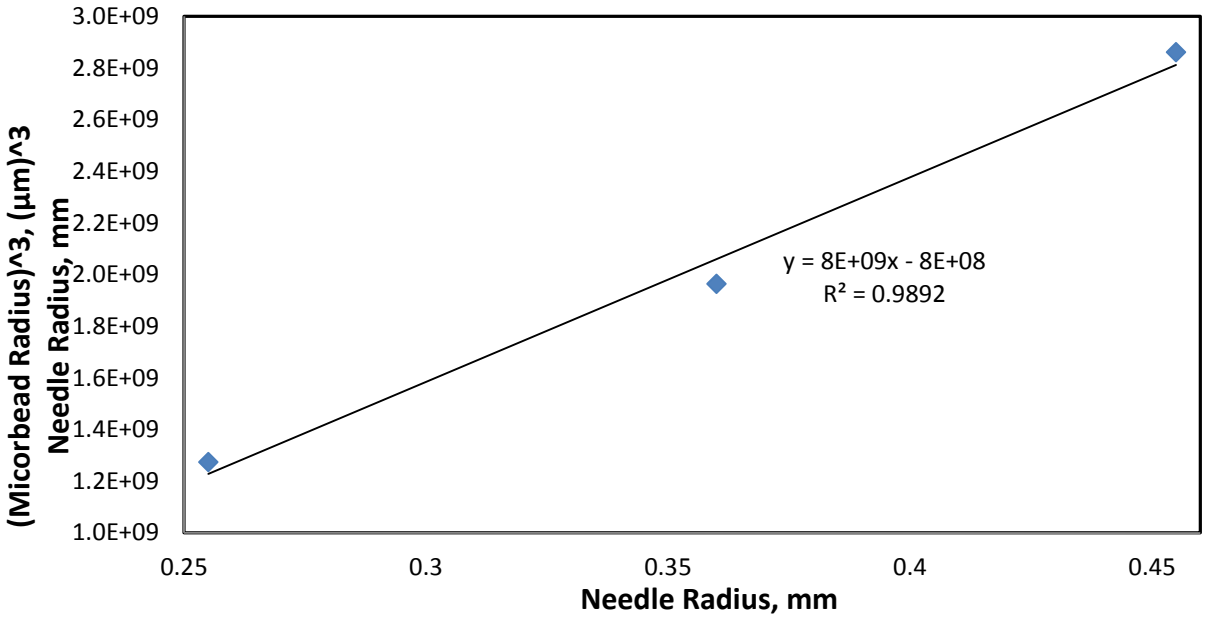


Figure 14. Needle radius versus microbead radius cubed. This trend matches the relationship described in equation 6 when no shear is generated showing roughly that $[r_d\,(\mu m)]^3\, r_n\,(mm) = 8E+09 * r_n\,(mm) - 8E+08$

Discussion/Analysis

Calcium alginate microbeads were successfully made using the experimental setup described above. Since the system is very simplistic, a major benefit is that it can be easily reproduced in other labs. Based on Equation 6, four parameters were analyzed to determine the effect on alginate bead size: air shear, density, surface tension, and extruder needle radius. Viscosity was also studied. The initial testing results make sense theoretically as the beads' diameter decreased with the increasing shear rate. An empirical relationship was also developed, shown in Equation 8, relating the microbead diameter to the Reynolds numbers. This equation shows that increasing the density of the shearing fluid, velocity of the shearing fluid (which increases the shear rate), or outside diameter of the tube should lead to smaller diameter

microbead production. If the needle diameter or shearing fluid's viscosity is increased, the microbead diameter is expected to increase. Further testing should be conducted to determine if two trend lines are actually developing in Figure 10, one for laminar flow and one for turbulent flow. In the figure, one can make an argument that two different linear lines are forming above and below the Reynolds number of 2100; but more data points are needed to reach a definite conclusion.

For reasons such as consistency in the drug release mechanism of other applications, one wants the size distribution of the microbeads to be as uniform as possible. According to literature, many applications desire a CV of less than 15% for the microbeads (Lee et al, 2013). As shown in the table and figures above, consistent beads were formed in the size range of about 600 to 1,000 μm based on the CV value. However, as this technique was desired to be used for production of beads in the 200 to 300 μm range for injection into surgical sites, scale up of the production rate of the beads was not attempted. Microbeads were created in the desired range; but the diameters fluctuated greatly, as seen in Figure 9 and from the high CV values. For other applications of microbeads in the 600 to 1,000 μm range, this technique would prove quite useful. To make the smaller microbeads, high shear rates were required that would agitate the calcium chloride crosslinking solution causing the liquid to splash around, possibly contributing to the higher size distribution of the microbeads. Measurements made on the alginate solutions with and without surfactants showed that the density of the solutions remained fairly consistent, so density effects on the microbead size were not studied. Also, no trend developed when the alginate solution viscosity was varied as expected because the viscosity plays more of a role in forming the shape of the alginate beads. One would expect that as the surface tension of the alginate solution was decreased, the size of the microdroplet would also decrease forming a

smaller microbead. However, Figure 12 shows results to the contrary with larger beads forming when the surfactants were added in most cases. Further testing should be conducted on the surface tension to try to explain this trend. Figure 14 shows the trend between needle tip radius and bead size, which corresponds to Equation 6. Based on these results, the air shear rate generated and needle tip diameter should be manipulated in future studies to produce consistent beads in the desired size range of 200 to 300 μm. Lastly, other methods for producing beads in the desired range may be an electrostatic bead generator or using a stirred organic phase as a matrix while solutions of alginate and calcium chloride are added to the mixture (Zhou, 2009). These methods and others should be investigated.

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