Abstract

FISHER, SARAH MARIE. *A Method for the Encapsulation of MicroSpherical Particles. (Under the Direction of Dr. M. K. Ramasubramanian).*

Presently used single outlet air-driven droplet generators are incapable of producing sufficient numbers of microparticle-containing droplets in a sufficiently short time-period. A droplet generator was designed and fabricated, which increases the production rate by simultaneously producing multiple droplets. The encapsulator consists of several modular parts to produce the working system. A parametric study was done to study the effects of process parameters on the microcapsule geometry and uniformity. Microcapsules were produced with low-viscosity high-guluronic acid (LVG) at concentrations of 1.5 % and 1.8%, the gelled in a $BaCl_2$ cross-linking bath. The samples produced were done so with alginate alone and with polystyrene microspheres at a concentration of 500 beads per cubic milliliter. Microcapsules with diameters ranging from 0.4 to 0.9 mm were produced for both sets of samples. The polymorphism present varied between 20 and 75%. Although the testing included only a one-hole and six-hole configuration, the concept can be extended to multiple holes and hence an increase in production rate.

A Method for the Encapsulation for MicroSpherical Particles

by

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Biography

The author, Sarah M. Fisher was born on June 20, 1980 in Fayetteville, North Carolina. She was raised with her younger sister, Shannon Leah Fisher, in Moore County by her parents Barton and Cheryl Fisher. She graduated from Union Pines High School in May 1998. Upon graduation from high school, Sarah chose to attend North Carolina State University to pursue a degree in Mechanical Engineering. In May of 2003, she completed her Bachelor's degree with a minor in mathematics. During her undergraduate studies, she worked as a teacher and an engineering intern at various companies in Wake County.

Sarah chose to continue her education at North Carolina State University in pursuit of a Master of Science degree in Mechanical Engineering, immediately following the completion of her undergraduate degree. She had the opportunity to gain extensive teaching experience through teaching each of the undergraduate laboratories, including the senior design class. At the conclusion of the first year of her graduate program, Sarah received the Teaching Fellowship for the Future Award and Scholarship. She also had the pleasure of serving as the Graduate Student Representative for the Mechanical Engineering Department Lab Committee for two consecutive terms.

These experiences and her research in microencapsulation for the drive to cure diabetes, further emphasized Sarah's desire to work in the biomedical industry.

Acknowledgements

I would first like to thank my advisor, Dr. M. K. Ramasubramanian. He gave me a chance to work on this remarkable project, and supported me throughout the research. Though we had problems along the way, he has always showed me respect. I am truly thankful for his understanding and guidance throughout my thesis work.

Since I started my degree in mechanical engineering, there has been one professor, Dr. William Roberts, who has been a constant teacher, mentor, and friend. He exposed me to the breadth of engineering through his teachings in and outside the classroom. Dr. Roberts was an inspiration with his knowledge and guidance throughout my studies. He was a true friend, showing honesty and understanding. I will always be grateful for his constant encouragement and support.

I would like to acknowledge my family most of all. To my parents, Barton and Cheryl Fisher: Thank you for always believing in me, supporting all of my dreams, inspiring me to believe in myself, showing me unconditional love and understanding, teaching me to show respect and love for others, and always showing me that you are always there for me. Mom, you made me the person I am today. Daddy, you are my strength. To my sister, Shannon: You are my best friend. Thank you for always being there for me through the good times and bad, being truly optimistic for me, showing me unconditional love, spending time with me, giving me a smile, and making me truly proud of you each and every day of our lives. To my grandmother, Gloria Fisher: I truly could not ask for a better

person in my life. You are a true inspiration to me. You and I have always found those true moments together, and I will treasure them always. Thank you for giving me courage. To my love, Robert Hughes, thank you for giving me the courage to stand up for myself, believing in my abilities even when I do not, your endless love and commitment, our life together, and the future we will make together. Robert, I look forward to each and every day we spend together.

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1. Introduction

Due to the shortage of organs needed for transplantation, many patients die while waiting for an organ from a human donor. Those who receive an organ or tissue face serious issues related to the side effects of immunosuppression drugs administered to prevent organ rejection. Transplantation of cells and tissue rather than the whole organ has become an attractive option for increasing the chance of success and simplifying the surgical procedure to a minimally invasive one [1]. Further, in order to eliminate the organ shortage, use of tissues and organs from other species, known as xenotransplantation, has become very attractive for the treatment of life-threatening diseases. In order to avoid immunosuppression drugs, the tissue should be protected from attack by the host immune system while allowing the organ to function inside the host in a totally compatible manner. The process of entrapment of mammalian cells and tissue in biocompatible microcapsules, known as microencapsulation, has become the most popular technique for immunoprotection of cells [2]. This technique has shown promise in various systems of cell therapy for a wide spectrum of diseases.

The most promising application of this technology is the development of a reliable bioartificial pancreas in the form of microencapsulated islets, for the cure of diabetes mellitus [3]. Another important application is the development of a bioartificial liver in the form of encapsulated hepatocytes, for providing temporary but adequate metabolic support to allow spontaneous liver regeneration, or as a

bridge to orthotopic liver transplantation for patients with fulminant hepatic failure [4]. While the technique of encapsulation of cells and tissue has been shown to work through transplant studies with small animals and primates, and limited clinical trials, the barrier to offering the technique for human patients as a routine procedure is the lack of a mass production technique for the microencapsulation of cells to meet the demand (*Figure 1-1)*.

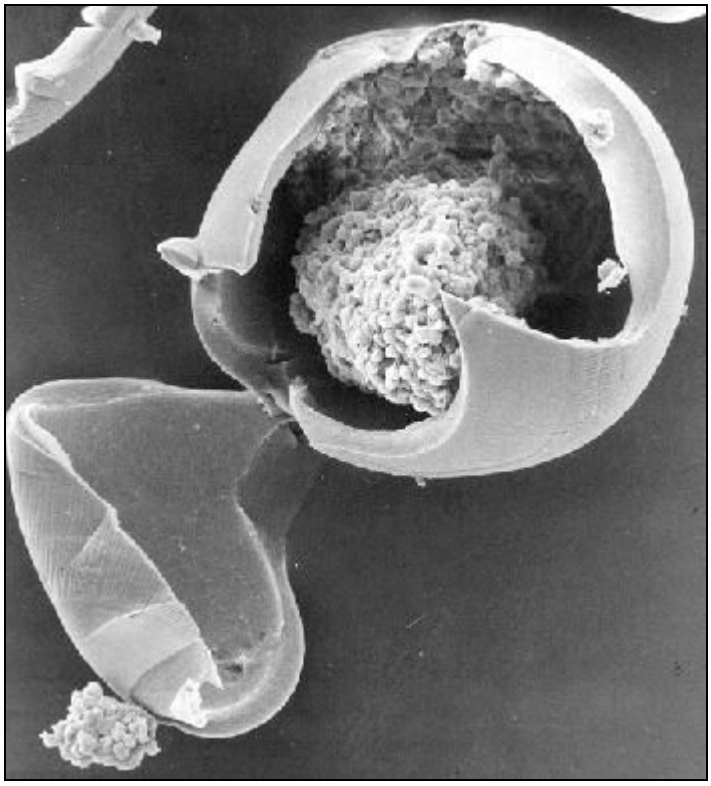

Figure 1-1: Islet Microencapsulated [5]

2. Background

2.1. Diabetes

Diabetes is a disease of carbohydrate metabolism defined by elevated blood sugar. There are two types of diabetes. In type 1 diabetes, the body has mistakenly destroyed its own islet cells of from the pancreas, which are the cells that produce insulin in response to glucose levels in the blood. This is known as the "Insulin-Dependent Diabetes Mellitus (IDDM)." In type 2 diabetes, the body has not destroyed its cells, but rather, cannot effectively use the insulin they are producing. This is known as the "Non-Insulin-Dependent Diabetes Mellitus (NIDDM)." Regardless, the end result is that the body is unable to regulate sugar levels in the blood due to lack of insulin production.

2.2. Islet Tissue

Islets are cells clusters located in the pancreas. It consists primarily of beta cells, alpha cells, delta cells, and pancreatic polypeptide cells. Roughly there are about ten thousand individual cells in an islet cluster. Beta cells are the most abundant, about 80%, in islet cells and they are the only cells in the body that make insulin. Alpha cells make glucagons, which is another hormone that affects the amount of glucose in the bloodstream. Delta cells make another hormone called "somatostatin." People with diabetes still have islets but the beta cells have been destroyed. An islet is anywhere from 50 μm to 150 μm in effective diameter (*Figure 2-1*). Replacing the beta cells (by replacing the islets)

would mean that people with type 1 diabetes would no longer need to take insulin shots [6]. About a million islet cells are needed for a human transplant. In general, four to six donor pancreas are needed for one human transplant as the islet comprise of only about 2% of all the tissue in a pancreas [3]. The process of isolating viable islet tissue from a donor pancreas is not efficient to capture all the islets since the process is a selective enzyme digestion of the rest of the organ. Porcine islets are available in abundance and are an attractive option to meet the demand.

Figure 2-1: Single Islet [6]

2.3. Cure for Diabetes

There are four general approaches that might accomplish a cure for diabetes [7]. The first is the complete pancreas and islet transplantation. In this case, a donor pancreas is implanted into a diabetic or islet cells from a donor are infused into a vein that drains in the pancreas. In this case, the primary problem is organ rejection. In order to prevent organ rejection, immunosuppression drugs have to be administered and their side effects and complications have to be dealt with. The second method involves a mechatronics solution where the

blood sugar level is measured using an embedded sensor and a microcomputer determines the amount of insulin needed and pumps the metered quantity into the blood stream from a reservoir. This method is still in its early stages of offering and faces a host of issues involving reliability and safe operation. The third approach is to genetically engineer the cells to evade the immune system of the host and avoid immunosuppressive drugs. This is not currently developed far enough to make an assessment of the effectiveness and side effects. The fourth approach is the use of encapsulated islets; bio-artificial pancreas has living, functional islets or cells in an artificial biocompatible material. This method has been shown to work when microencapsulated islets were transplanted into small animals and primates [8]. The transplantation of the organs and tissue across species boundaries is known as xenotransplantation. In the case of finding a cure for diabetes, the bioartificial pancreas seems to hold the most promise.

2.4. Microencapsulation

Microencapsulation of cells is a technique designed to entrap viable cells within the confines of semi-permeable membranes that are permeable to small molecules, such as nutrients and oxygen, which are essential for cell survival, but impermeable to large molecules, such as antibodies and immune cells [2, 4change, 8]. The bio-artificial pancreas is fabricated from living and nonliving components [2, 7, 8, 9]. The living component protects the islets from the diabetic's body yet permits the islets inside to thrive. A bio-artificial endocrine

pancreas replaces nonfunctional islets of Langerhans. It responds to changing blood composition with release of hormones including insulin. A bio-artificial pancreas is implanted into the peritoneal cavity of the diabetic and contains two to three million cells.

Microencapsulation has become a technique of significant interest in cell therapy for a variety of pathophysiologic conditions, including kidney failure, liver failure, CNS insufficiencies, and diabetes mellitus [2].

In the past, industry has used a number of techniques for the microencapsulation of cells. The two most widely used devices are the air-syringe pump droplet generator and the electrostatic bead generator. Each of these devices is fitted with a single syringe in which cells are suspended in alginate solution. The mixture is pushed through a single needle producing cross-linked spherical beads. Though each system has been proven to generate spherical beads without polymorphisms and relatively controllable diameter, there is one main drawback. They are incapable of producing sufficient numbers of microcapsules in a short time period to permit mass production of encapsulated and viable cells for transplantation in large animals and humans.

The focus of this research is to develop a device for the mass production of alginate microsphere encapsulations, and to determine the optimal conditions for the consistent production of alginate microsphere encapsulations. After building the bead generation device, a parametric study will be completed to

better understand the variables that affect the sphericity and polymorphism of the microspheres produced.

3. Literature Review

After deciding upon a research objective, a summary is developed of the tasks required to achieve that objective. First, the chemical composition of the mixing solutions required for optimal sphere viability is studied. Next, the factors influencing the properties and performance of microcapsules are studied. These factors are important to examine to better understand how the previous procedures can be applied. Finally, design parameters of each microencapsulation method are studied. The various parameters that control the size of the spheres from the previous methods are determined. Each of these areas are summarized and presented in the following sections.

3.1. Chemical Compositions

The swelling and shrinkage of microcapsules are important factors to investigate. The functional properties of alginates are based on the mannuronic acid and guluronic acid. The mechanical and swelling properties of alginate gel beads depend strongly on the mannuronic composition and block structure of the alginate molecules. Studies have shown that the greatest shrinkage is found in the beads made from low-G alginates [13]. Shrinkage, which is the defined as a decrease in the nominal diameter of a microcapsule, leads to a loss of water

and an increase in the polymer concentration relative to the alginate solution. Swelling, defined as the increase in nominal diameter of a microcapsule, leads to an increase in the shell concentration and increases the chances of the core shrinkage. Both shrinkage and swelling are expected with any alginates, but minimizing these problems is the ultimate goal.

A variety of sodium alginates, differing in mannuronic acid and viscosity have been studied for the purpose of encapsulating a variety of cell types [10]. Four different major groups that have been studied include:

- Low viscosity high-mannuronic acid (LVM)
- Medium viscosity high-mannuronic acid (MVM)
- Low viscosity high-guluronic acid (LVG)
- Medium viscosity high-guluronic acid (MVG)

These samples were gelled by cross-linking with 1.1% solution of either $BaCl_2$ or $CaCl_2$ [10]. From their results, major differences were found between the four different samples. The high M alginate samples (LVM and MVM) were more prone to swelling than the high G (LVG and MVG) samples. The swelling was determined by studying the samples over a period of sixteen days. The Ba^{++} and Ca^{++} both cause swelling, but the Ba^{++} is less potent for this parameter [10]. The study also revealed that the mannuronic acid-rich alginate beads swell more than the guluronic acid-rich alginate beads. While these properties have an important effect on the swelling, the viscosity did not have an effect on this parameter.

Alginates with high mannuronic acid (high M) or high guluronic acid (high G) content have been studied for additional mechanical properties [11]. The alginates with high-G materials, such as LVG and MVG, offer higher capsule stability [11]. From this study, they found that the capsules are more durable, shrink less, and provide permeability to the inner core [11].

Sodium alginates are important to study as a preparatory encapsulation medium. As shown from previous research, the chemical composition has a more significant effect than the physical properties on the bead swelling [10]. If swelling occurs, permeability decreases, and the chances that encapsulated islets will die increases [11]. From a similar study on sodium alginates, differing in molar mass and structural composition, were examined [12]. The chemical composition, specifically the guluronic acid content, was more significant than the molar mass for both mechanical and transport properties [12]. Capsules were produced using both high and low guluronic acid. Their results show that the high-G acids shrink less than the low-G acid materials.

3.2. Size, Shape, and Degree of Imperfections

The basic chemical composition has stronger effects than the physical properties, such as viscosity, on the mechanical strength and stability of beads for both long and short term periods [13]. After studying the chemical composition as a factor, previous research has shown that the physical properties are shown to have important effects on the bead diameter, ease of production, and other various application problems [10, 11, 12, 13]. The

viscosity levels affect the formation of beads. Finding the most favorable viscosity range can optimize the size and uniformity of the beads. Depending on the methods used to produce the beads, the alginate type and concentration will vary. Some examples of the methods used are gas shear, conventional "drip," and vibration. The gas shear method uses a concentric air stream to shear a drop from a needle tip. The drag force from the concentric flow of gas contributes to the gravitational force to shear the drop from the tip. The conventional, or drip, method allows the drop to fall from the needle tip randomly. With this method, the bead diameter is determined by the weight of a drop at the needle tip. Once the weight of the drop overcomes the surface tension forces, the drop detaches from the surface. The last method uses vibration as a mechanical disturbance to induce a controllable breakup of a liquid jet into droplets. The theory of capillary jet instability is used with this method. A wavelength of disturbance is applied to the jet, inducing the drop formation process [14]. The viscosity was studied as a property to measure for correlation to bead characteristics. Despite various chemical compositions, for the vibration method, the optimal viscosity range is 150-500 cP [14]. The goal of most studies is to produce microcapsules that are small in size. Initial studies show bead production of 300 - 500 μm in diameter. Production of inadequate microcapsules, partially enclosed capsules, is inversely related to capsule diameter [15]. Imperfections can have adverse effects on the viability of encapsulated islet transplants. These imperfections are difficult to quantify, but a number of factors can help to explain the phenomenon. The condition and

duration of storage of alginate samples may have negative effects on its ability to form spherical microcapsules [15]. Good spherical microcapsules are produced with a fresh batch of alginate, but after prolonged storage, polymorphism is observed, as its viscosity is reduced [15]. Therefore, viscosity of an alginate sample affects the morphology of microcapsules produced. An additional factor that can create imperfections is the amount of shear stress involved in the encapsulation process. When these stresses are too high, microartifacts are produced inside and outside the microcapsules [15].

An additional problem found in past research with the microcapsule production process is swelling, as covered in the previous section. The studies have shown that after treating the microcapsules with Na_2SO_4, the diameter will be held to less swelling [16]. Investigators have argued that solid gel microcapsules with perm-selectivity may be more stable than semi permeable microcapsules. This is argumentative at this point, but studies have shown that the Na_2SO_4 coating helps stabilize both types. An additional coating that has been shown to help the stability of microcapsules is isotonic sodium chloride solution (INaCIS) [17]. Washing the spheres once makes the microspheres more compact during incubation and keeps the final diameter of the microcapsules smaller. These steps are an important part of the microencapsulation process, but at this point, the most important factors are the alginate composition and viscosity.

3.3. Design Parameters and Size Control of Alginate Beads

The various devices used for microencapsulation are important to examine to better understand the various parameters that affect the size, shape, and morphology of the beads created. The first of these devices produced droplets through syringe pump extrusion, sometimes called an AirJet device [18]. This technique was used to encapsulate islets from adult Wistar rats. The steps taken include the following: (i) pancreatic islets are embedded in alginate droplets; (ii) droplets are transformed into rigid beads by cross-linking with calcium chloride; (iii) the beads are coated with polylysine and alginate, forming semipermeable capsules; and (iv) alginate core is liquefied with isotonic sodium citrate solution [18]. These steps are followed for each of the studies considered, with alterations for viability studies of the final microcapsules.

A droplet generator is composed of a droplet sizer and an alginate solution reservoir (*Figure 3-1*). The sizer consists of a cylindrical chamber holding the alginate nozzle. Air is applied to the alginate reservoir forcing the solution of a blunt hypodermic needle [19]. The alginate drops into a calcium chloride solution. After chelation takes place, the beads are measured.

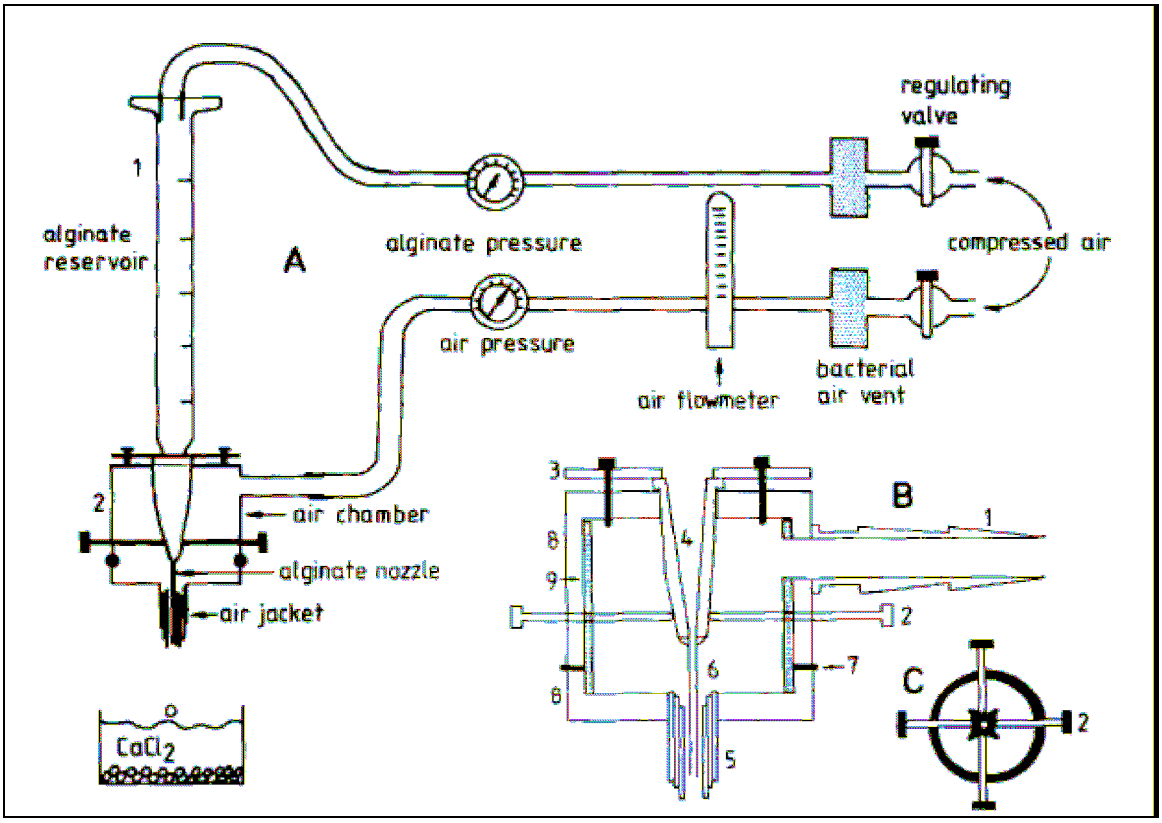

Figure 3-1: Alginate Droplet Generator [19]

This aperture can be altered to produce various bead sizes, but there is some standard deviation for any sample measured [19]. The diameter range is from 3.0 mm to 0.5 mm. In this study there were number a variables such as the air flow rate through the air jacket to the needle tip, the length, and wall thickness of the nozzle that were examined. The diameter of the nozzle and the pressure on the alginate solution in the syringe were found to be important factors that influenced the bead size. This technique used the gas shear methods discussed earlier to force the droplets to detach from the orifice. The experiments were run using both 25 and 22 gauge needles. These effects are shown below (*Figure 3-2*).

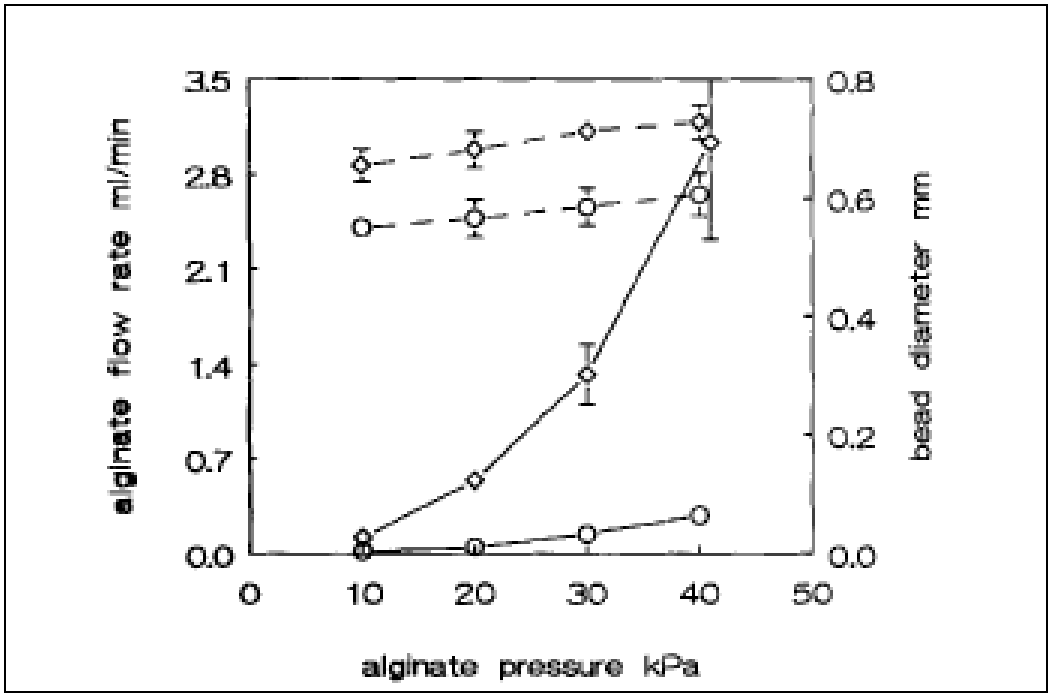

Figure 3-2: Influences affecting the flow rate (—) and beads size (---). The 25G needle (○—○) and 22G needle (◊—◊) are also shown. Data is given in mean + sem of five experiments [19].

Based on their results, they concluded the following [19]:

- As the air flow rate increases, bead diameter increases
- The length of the shaft had minimal influence on the diameter
- An increase in air flow rate through the air jacket at the nozzle tip compares with a decrease in the diameter of the beads
- Decreasing the diameter of the nozzle, decreases the size of the beads
- Standard deviations ranged from 2.2% up to 4.9% in these experiments.

These results are consistent for most of the experimentation done with this type of apparatus [15]. Additional studies showed smaller alginate microcapsules can be produced.

The JetCutting method of bead generation has been shown to be useful for microencapsulation of particles. A reservoir containing the material to be encapsulated is pressed through a nozzle and cut into identical cylinders [20]. The cylinders were cut using a rotating cutting tool. After the cutting procedure, the liquid cylinders become spherical and fall into a bath containing the crosslinking solution (*Figure 3-3)*.

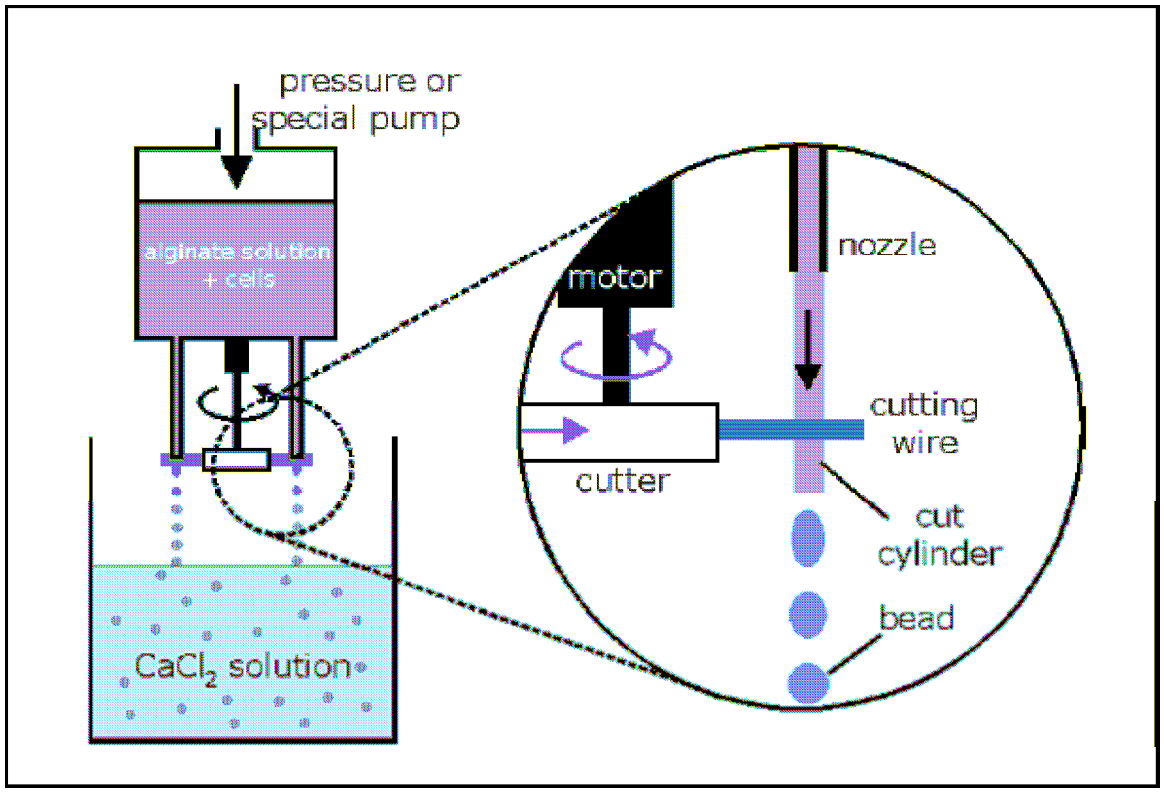

Figure 3-3: JetCutter [12]

There were many advantages to this method. For one, the alginate solutions used for microencapsulation were wide in variety. Another advantage was that the extrusion and bead formation steps were controlled by mechanical components. Therefore, the viscosity was not an important factor for this method because the steps are mechanically enforced. The two most influential variables were the nozzle and the fluid velocity speed. For small nozzle diameters, a low fluid velocity must be used to inflict minimum shear stress on the particles upon extrusion. The results of this study show that 60% of the beads were within the range of 295-325 μm. Other conclusions that were made from this study are as follows [20]:

- The beads can withstand the mechanical stress induced during the procedure.
- Bead size is dependent on the fluid flow velocity, nozzle diameter, number of cutting wires, and the number of nozzles used.
- Bead size reduction may be achieved by decreasing the size of the nozzle.
- The flow velocity and the speed of the cutting tool may be increased to increase the bead production, though the bead viability may decrease as well.

The basic production capacity of this technology was 5200 beads/s or approximately 330 ml bead suspension per hour [20]. The JetCutter technique

was developed in 2000 [14], and was also used in manufacturing practices for hydrogel production [14].

A similar study shows that the JetCutting method had the highest flow rate capabilities in comparison to the free-fall dropping, electrostatic, vibration, and other methods used in the bead production research [21, 22]. By varying the cutting frequencies, viscosity, and nozzle diameter, the mean diameter of the beads could be generated. The mean diameters ranged from 0.15 up to 3.0 mm.

While the previous studies considered used syringe-pump or mechanical technologies, another technique to use is called an electrostatic potential bead generator [21, 22]. This technique is similar to the syringe-pump setup in that it also uses an alginate-particle reservoir and a droplet sizer. The reservoir is a syringe, and the sizer is a steel needle commercially produced for computer plotters. This instrument uses an electrostatic potential to pull droplets from a needle tip. Based on the free-fall techniques that allow the drops to fall due to its own weight, the diameters are too large [14]. By using a high voltage, the droplet size can be more controllable. The droplets are pulled from the needle tip by using an electrostatic potential. The voltage is applied between the needle and the solution below. The solution is electroconductive and contains gelling ions, usually Ca^{++}. For this technique the variables considered during the study included the flow rate from the reservoir, needle diameter, and electrostatic potential applied. The same variables from the previous studies considered were the alginate solution viscosity, alginate chemical composition, and the

molecular weight distribution. The experiments performed for this study consider each variable separately, in order to predict the effects on the bead diameter *(Figure 3-4).*

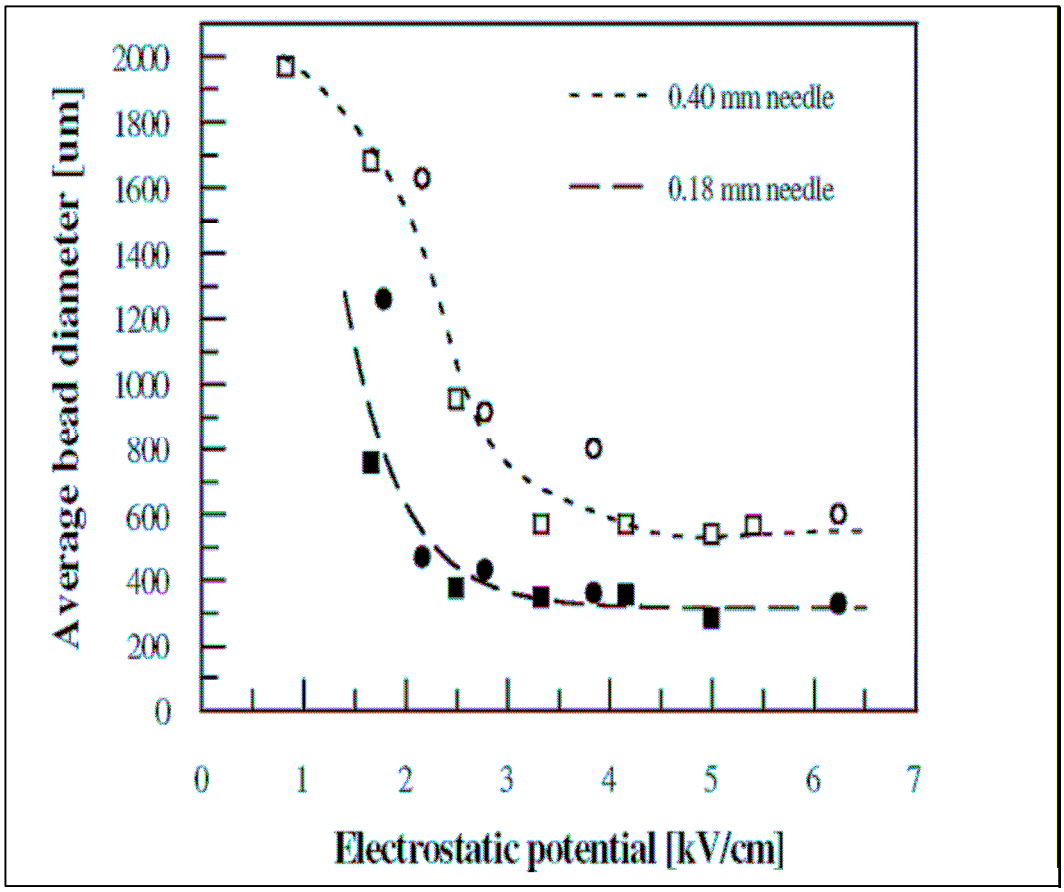

Figure 3-4: Effects of Electrostatic Potential on the Average Bead Diameter [22]

These results lead to the following conclusions:

- Bead diameter decreases with electrostatic potential until reaching a minimum threshold.
- For an alginate solution with low-G composition, the beads will be smaller than the solution with high-G content.

- With increases in alginate solution concentration, which includes an increase in viscosity, the bead diameter will increase as well.
- The relationship between bead diameter and flow rate is shown to be linear.

With this study, and similar studies in this area, the mean diameter produced at optimal settings was approximately 300 μm. The final results show that the molecular weight of the alginate samples had little influence on the size of the final beads measured. But as discussed earlier, there were other variables to consider with this type of bead generation.

Using this same technique, and similar alginate solutions, comparable results have been found in other studies. By varying the electrostatic voltage for 2 needle diameters, the bead diameters were wide-ranging. In the previous study utilizing electrostatic potential, bead diameters for a 0.40 mm needle ranged from 2000 down to 600 μm, and diameters for a 0.18 mm needle ranging from 1200 to 300 μm. The reported needle diameters are the inner diameter. The bead diameter decreased with the electrostatic potential/distance to bath ratio. In the second study, beads were produced using approximately the same size needles. For a 0.40 mm and a 0.2 mm needle, the bead diameter ranges were 775 to 525 μm, and 250 to 200 μm, respectively [24]. In both studies the electrostatic voltage was varied in approximately the same range. The second study also found a linear relationship between bead diameter and flow rate [23]. Though the variables range from electrical to chemical to mechanical this method has been shown to produce beads of various sizes consistently. Even

with various differences, this technique still requires the use of a syringe-needle model, similar to that of the free-fall and syringe-pump models.

Utilizing the laminar jet break-up technique, and incorporating an electrostatic potential can produce beads produced in a wide size distribution. For this design a range of 50-1000 μm nozzles can be applied, depending on the capsule diameter desired [25]. This technique has been used extensively in microencapsulation research, and also aids in understanding the variables that affect the bead diameters.

There are advantages and disadvantages for each of the three major techniques used for microencapsulation. Each of the three methods produce uniform spherical beads. While the vibrating nozzle method requires a relatively low viscosity (<0.2 Pa/s), the AirJet and JetCutter devices both work well with alginate of higher viscosity [26]. It is well known that beads with a small diameter, less than 300 μm, are more difficult to produce than beads with a large diameter, greater than 500 μm. There are advantages to producing smaller beads such as better oxygenation of encapsulated cells, smaller implant volume, and easier applications to organs [27]. Even with these advantages, cells must undergo high mechanical shear stress during the encapsulation process. Further stress is induced when using low viscosity alginate solutions, which lack mechanical stability in the alginate itself. In order to compensate for this problem, the beads must be covered with more alginate layers, protective polycationic shells, and crosslinked with Ba^{2+} instead of Ca^{2+}. With these

techniques, production of uniform spherical beads can be optimized, and with further research, the process can be used to produce beads on a large scale.

After studying the various techniques used for single droplet generation, it is important to consider upscaling the process. Transplantation of pancreatic islets into diabetic animals has been successful and it shows a promising technique for humans. In order for the human islet transplantation to work, one million islets would be required. At this rate, with the current designs, it would require over 100 hours of production time with approximately 1000 islets per milliliter. In order to increase this production rate multineedle generators have been studied. Using the AirJet technology, a four-needle device has been constructed and tested (27). The studies were completed with alginate-islet suspensions of 1000, 2000, 4000, 6000, and 10000 islets per milliliter alginate. Each solution was made using 3% alginate solution. As with any of these studies, there were inadequate capsules with each sample taken. Microscopic examination was performed to identify these inadequacies. The experiments were performed with beads of diameter of 500 and 800 μm. At the lowest number islets per milliliter, 1000, this portion amounts to 33 $\pm$ 3.4 and 6.7 $\pm$ 2.2 % viable encapsulations for 500 and 800 μm, respectively. As the number of islets per milliliter alginate is increased, the percentage of inadequate capsules increases as well (*Figure 1-3*). This is evident for both sizes of beads, though the percentage of inadequate capsules is lower for the larger beads. When using the 500 μm and 10,000 islets per milliliter alginate the portion of inadequate beads rises to 66 $\pm$ 9.7%, which is significantly larger than with the

range for 1000 to 6000 islets per milliliter alginate. With the 800 µm beads, the portion of inadequate beads is much lower with 500 µm capsules, but it starts to rise significantly higher with 4000 or more islets per milliliter alginate are used. As stated previously, a human patient will need over one million islets. With any clinical trial an accurate assessment of the amount injected into a patient is important. It is viable to understand how many viable microencapsulated islets can be produced per milliliter.

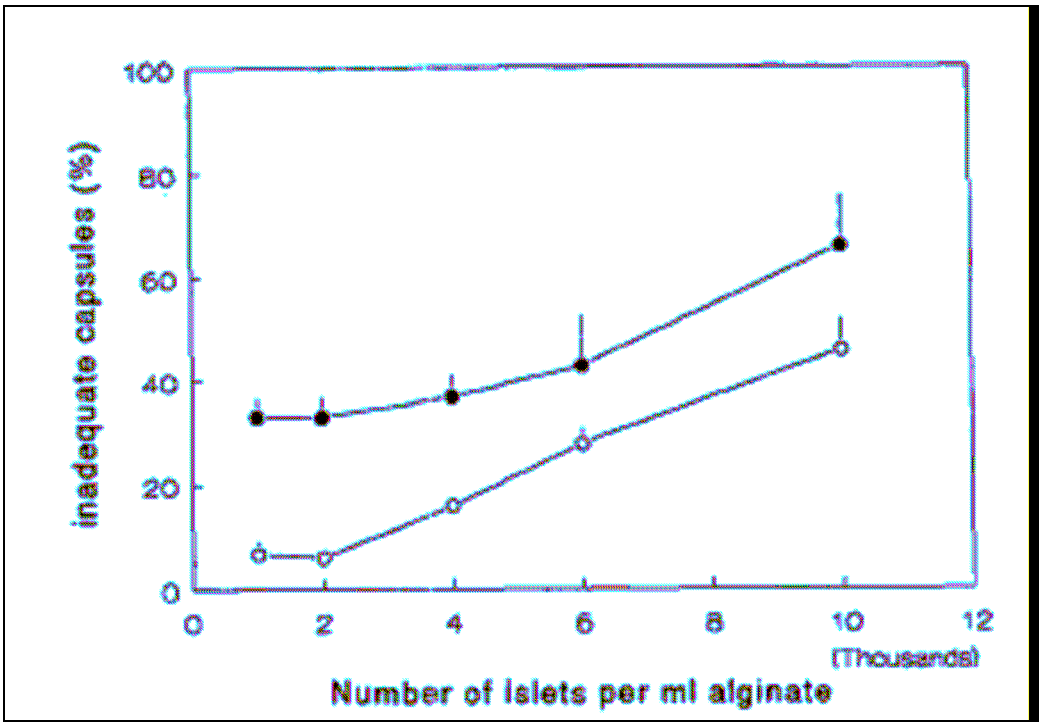

Figure 3-5: The numbers of islets per milliliter alginate have an effect on the adequacy of islet encapsulation in capsules of 500 µm (filled symbols) and 800 µm (open symbols). Data is presented as mean ± sem of five experiments [27].

This procedure is not fully developed since approximately 50% of the capsules with the diameter of 800 µm are empty [27]. By increasing the islet concentration, the number of inadequate capsules increases. As with any other

variable, there is an optimal threshold. And since the purpose of this research is to produce high quality microcapsules, the lower concentrations should be utilized. Even with the lower concentrations, the production time is reduced from earlier times required. The focus is also to produce smaller capsules, less than 500 μm. From this study using the 500 μm beads can produce the maximum number of islets per milliliter alginate, because from the same volume of alginate approximately three times more 500 μm than 800 μm capsules can be produced [19]. Now, with this in mind, this study shows that staying with the smaller beads, and lower islet concentrations, the process can be optimized.

A similar approach was taken when a multinozzle system was designed with the idea to produce large batches of microcapsules [20]. The device has 13 nozzles to which can be changed to produce beads within the 0.2-1 mm range [28]. The nozzles used in this experiment were 200 μm in diameter. The nozzles consist of a precision drilled sapphire stone on the tip of a stainless steel cone. With this device beads can be formed that are smaller than 340 μm, and the mean diameter of the viable beads is 0.368 mm. This is smaller than the other devices. The major problem with this device is coalescing of droplets in air before reaching the gelling solution. The problem with these results is particles with double volume, or ‘twin particles’ joined together. Though this is a new variable to consider, the range of error is low compared to the production distribution. From the samples taken, the error particles represented are between 3.1 and 6.4 %. Using micromachined components is a relatively new

technique with this research, and from these tests, it may be an option for mass production.

Not only is the bead diameter dependent on the variables considered during the encapsulation procedure, the incubation period is also considered. Each study may use a different incubation medium, but they all show dependence with this variable. After a certain period of time, the beads will start to decrease in size and deteriorate [13, 15, 24, 25, 28, 29]. For any given alginate suspension used in the bead formation, the hardening bath used is either $CaCl_2$ or BaCl. Both chemicals are used for the crosslinking phase. The divalent cations bind to G blocks. This selective binding of cations to alginate accounts for its capacity for ionotropic gels. The gel formation is based on the length of the G blocks of the alginate solution. Various studies have been done over a period of time to study the effects of gelling cations on bead swelling and viability. The mean diameter is a function of the incubation time; therefore, it should be limited.

4. Materials and Methods

4.1. Alginate Preparation

The encapsulation material used in this study was alginate, which consists of alginic acid and saline. The alginic acid used in these experiments, also known as sodium salt, is extracted from macrocystis pyrifera, which is a type of kelp. The alginate, or M. pyrifera, was obtained from Sigma, St. Louis, Mo. A secondary alginate was obtained from FMC BioPolymer, Philadelphia,

PA. The alginate types are low-viscosity high-guluronic acid (LVG), from Sigma; medium-viscosity high-mannuronic acid (MVM); and medium-viscosity high-guluronic acid (MVG); where the second two samples are from FMC BioPolymer. The molecular structure depends on the sequencing of the mannuronic acid (M) and the guluronic acid (G). The monomers are arranged in a pattern of blocks along a chain. The blocks may have different combinations such as MG, MM, or GG. The functional properties of the alginate as an immobilization matrix are in question, the composition and block structure are important to examine. The intrinsic inflexibility of the alginate molecules in solution increases in order MG<MM<GG [13]. The viscosity depends mainly on the molecular size, but the binding of cations and the gel-forming properties depend on composition and sequence. Macrocystis pyrifera is frequently used for immobilization, and yields gels with good strength and stability.

The samples were prepared using buffered saline, with 0.85% W/V isotonic Sodium Chloride solution from Fisher Scientific, Middletown, VA. Each sample was prepared by suspending the polymer of choice in the saline solution. A magnetic stirrer stirred each suspension for at least 6 hours. Once the samples were prepared, they were stored at room temperature.

After each sample was prepared, testing was done to find the dynamic viscosity and density. In order to do this testing, a Brookfield digital viscometer, LV model, was used (*Figure 4-1*). The readout on the viscometer was used to calculate the viscosity. To convert the reading to centiPoise (mPa*s), a slide

was used. Based on the viscometer model, spindle used (*Figure 4-2*), and RPMs, the scale factor is found.

$$Dial\,\mathrm{Re}ading \times Factor = Vis\cos ityincentiPoise$$

The spindle used in this experiment was number 2. The speeds correlating with the scale factor are listed below (*Table 4-1*).

Figure 4-1: Brookfield Viscometer

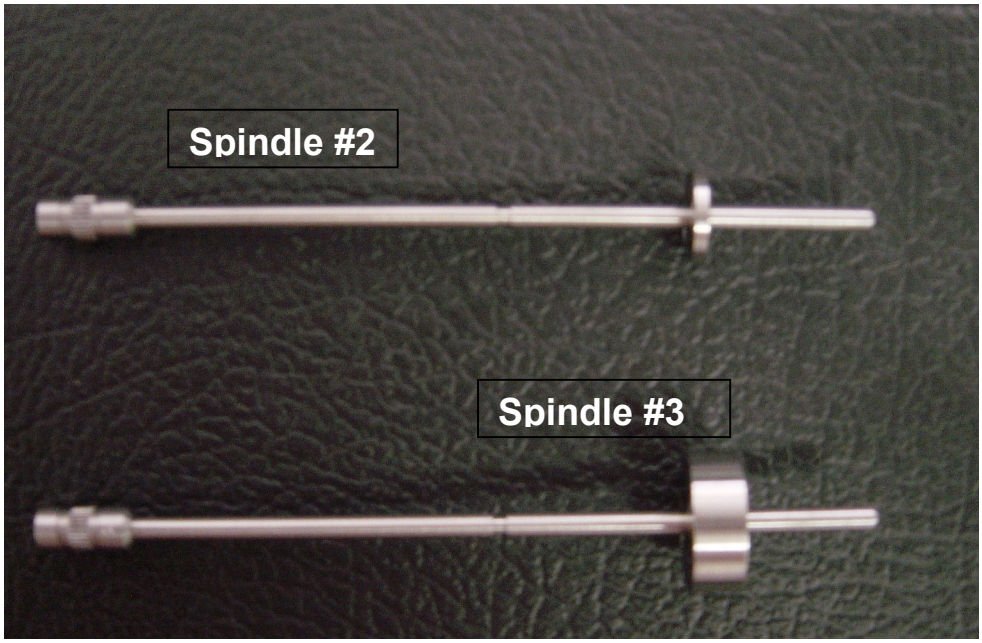

Figure 4-2: Spindles

Table 4-1: Viscometer model LV, Spindle #2

Speed (RPM)	Scale Factor
0.3	1M
0.6	500
1.5	200
3.0	100
6.0	50
12	25
30	10
60	5

Testing was completed on 600 mL samples, and the results are based on mean of five experiments (*Table 4-2*). The density was approximately the same for each sample. The important variable to examine is the viscosity. The viscosity increases with concentration for each sample, which includes LVM, MVM, and MVG. The viscosity also increases with each sample type, in the same corresponding order (*Figure 4-3*). The main objective is to assess which

viscosity will work in the encapsulator, therefore, only the minimum and maximum viscosities are tested for each sample.

Table 4-2: Properties of Alginates investigated

Sample Concentration [%]	TYPE	Density (g/mL)	Dynamic Viscosity (cP)	Kinematic Viscosity (P*cm3/g)
1.5	LVG	0.9956	40.00	0.4018
1.6	LVG	0.9948	45.10	0.4534
1.7	LVG	0.9940	48.00	0.4829
1.8	LVG	0.9946	58.90	0.5922
2.0	LVG	0.9959	77.50	0.7782
1.5	MVM	0.9940	222.50	2.2385
1.6	MVM	0.9950	275.50	2.7688
1.7	MVM	0.9969	352.90	3.5399
1.8	MVM	1.0002	433.10	4.3303
2.0	MVM	1.0005	492.00	4.9176
1.5	MVG	1.0016	346.00	3.4546
1.6	MVG	1.0005	426.50	4.2630
1.7	MVG	0.9959	528.00	5.3016
1.8	MVG	0.9905	636.40	6.4249
2.0	MVG	0.9885	720.00	7.2839

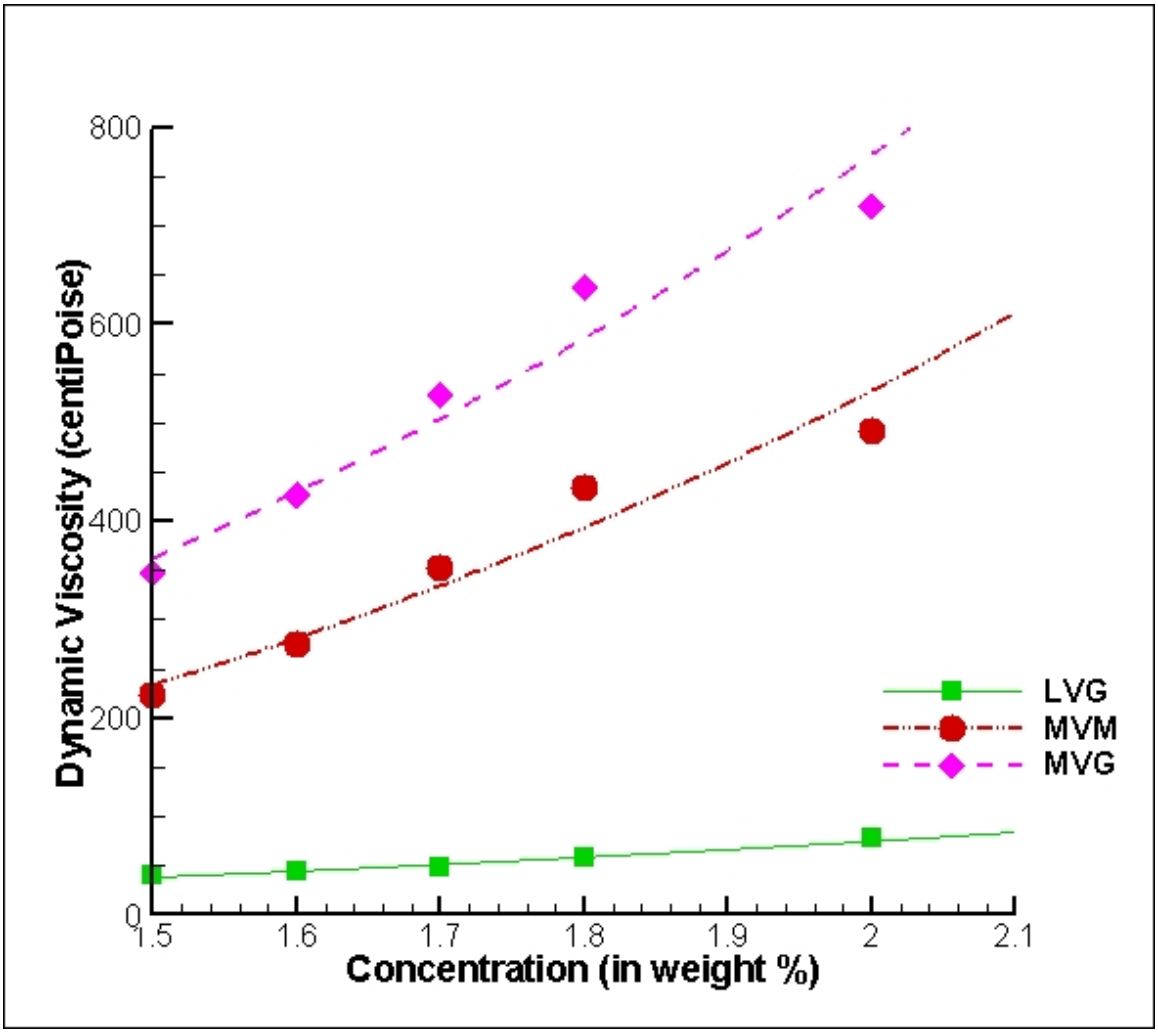

Figure 4-3: Dynamic Viscosity measurements based on samples

4.2. Cross-linking Solution

The cross-linking solution can either be made from calcium chloride or barium chloride. Either chemical works well with the alginate, but in this study the barium chloride is used in order to maintain bead durability and size for a longer period of time. With most studies that consider various coatings on the beads calcium chloride is used. With either of the chemicals the capsules will shrink or swell to either a smaller or larger size than the initial size upon exiting

the encapsulation model *(Figures 4-4 and 4-5)*. By using barium chloride, the samples are will have a constant diameter over a longer period of time [9]. The cross-linking solution is barium chloride dihydrate, 10% W/V from LabChem Inc, Pittsburg, PA. The alginate microspheres were made using the micro-encapsulation procedure and cross-linked in the barium chloride bath. In order to keep the polystyrene beads from agglomerating, a 0.1% solution of Triton-X100, (name of company), was prepared and the beads were dispersed in the solution before adding to the alginate solution prior to encapsulation.

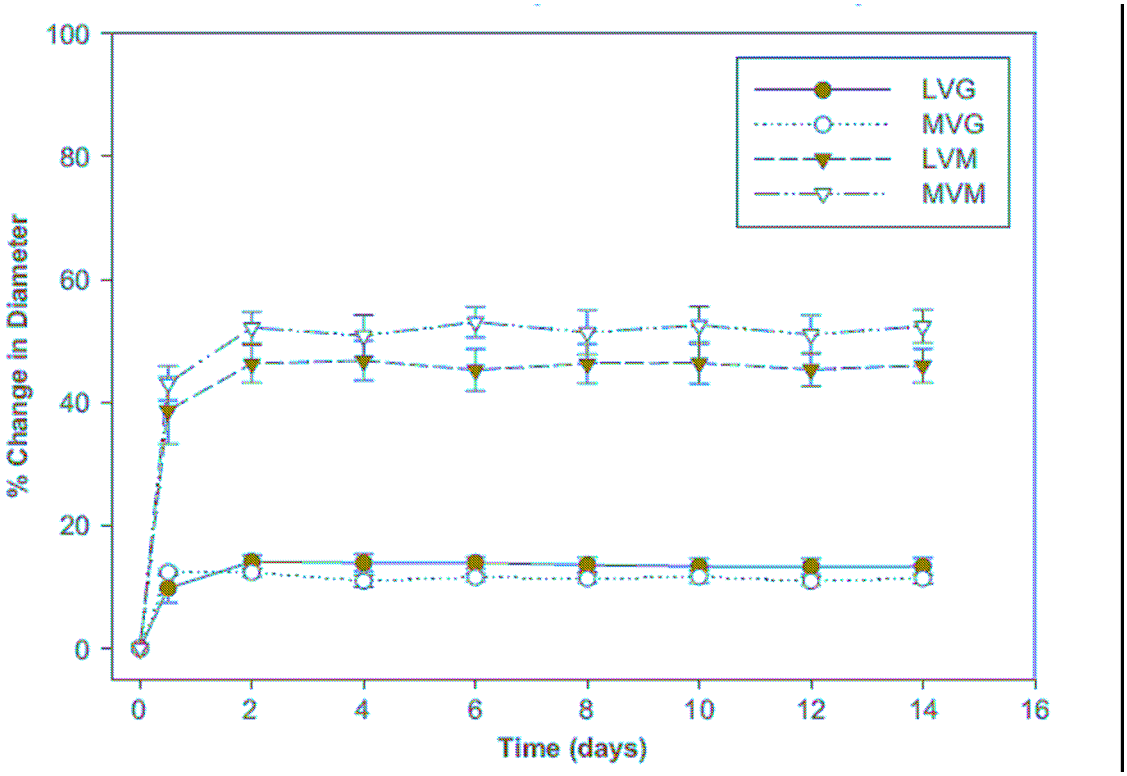

Figure 4-4: Changes in diameters of Ba^{++} -alginate microspheres during 14-day saline incubation at 37°C. Data represents mean±SEM of the percentage change in diameter with respect to initial measurements on day 0 (n=5) [10].

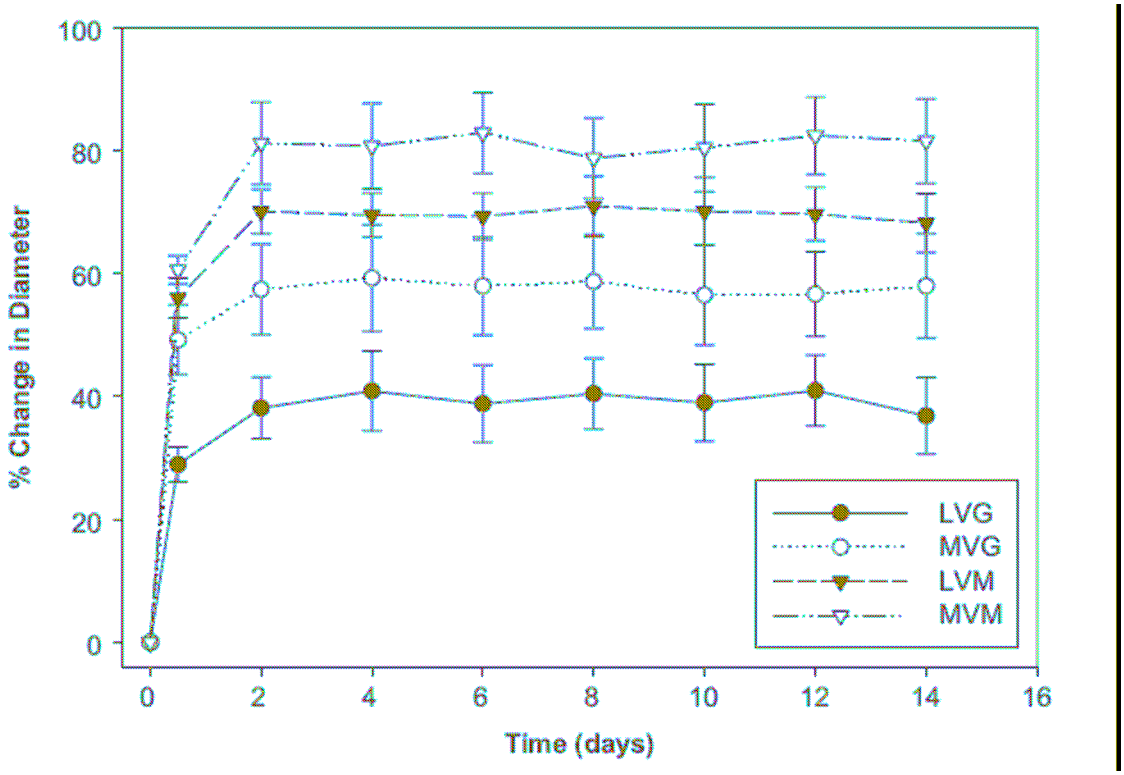

Figure 4-5: Changes in diameters of Ca^{++} -alginate microspheres during 14-day saline incubation at 37°C. Data represents mean±SEM of the percentage change in diameter with respect to initial measurements on day 0 (n=5) [10].

4.3. Polystyrene Microspheres

Polystyrene microspheres were obtained for the microencapsulation testing. The microspheres are 0.106 to 0.125 mm in diameter. The samples were obtained from PolySciences, Inc., Warrington, PA. Samples were made using the 1.5 % and 1.8 % LVG alginate, with a concentration of 500 beads/mL. The microspheres used in this application are spherical with a continuous wall surrounding the core. These samples were run with the microencapsulation procedure and cross-linked in the barium chloride bath.

4.4. Microscopy

The microspheres were studied using the Meiji EMZ-TR microscope from Meiji Techno CO., LTD., Santa Clara, CA. The microscope was fitted with a reticle in order to measure the bead diameters (*Figure 4-6)*.

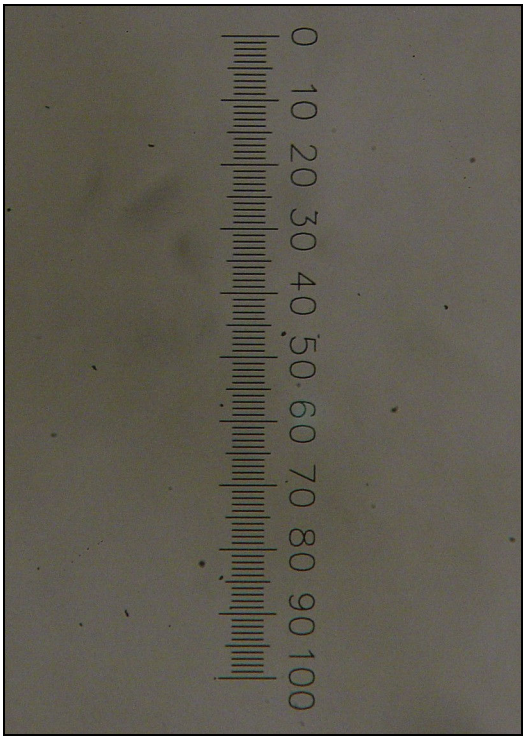

Figure 4-6: 5mm Reticle scale as seen through microscope eyepiece

A reticle is a flat piece of glass printed with a specific pattern. The glass is mounted in the microscope eyepiece, and the pattern is superimposed over the image of the specimen being studied. The reticle fitted for this particular microscope is 25 mm outer diameter, with a linear scale. The scale is a total 5 mm divided into 100 units. In order to accurately measure the bead diameters, the microscope was calibrated using a second reticle of the same size.

4.5. Statistical Analysis

From each sample, approximately 25 to 35 beads were measured manually. The diameter was measured, and observations were made on each bead to determine if a tail was present. The data was collected for each sample, and compiled for statistical analysis. The data, expressed as diameter (mm) and tail/no tail, was used for statistical analysis of variations between samples taken. Statistical evaluation of data was performed using an analysis of variance (ANOVA) computer program (SAS). The ANOVA was performed with Bonferroni correction for multiple comparisons to determine the significance of differences among groups of beads, and between sample variables. In all cases, a value of $p<0.05$ was accepted as significant. The p-value, probability value or observed significance level, is the probability of obtaining an F statistic greater than the computed F statistic when the null hypothesis is true. Therefore, with a smaller the p-value, the evidence will be stronger against the null hypothesis.

5. Microencapsulation System Design

The first system tested consisted of a syringe attached to a pneumatic cylinder. This system is similar to the syringe pump extrusion systems [11]. Needles of various gauges were attached to the syringe, and alginate was extruded through the needle. After studying the previous designs and this simple system, a large scale encapsulator was designed and fabricated. The various problems and variables of consideration are explained in the following sections.

5.1. Manual Piston Pump

The manual piston was used to understand how the plunging technique would produce beads, as opposed to a continuous pressure being applied to a syringe. By attaching a simple syringe to a pneumatic cylinder, the plunging motion was generated (*Figure 5-1)*. By plunging, the syringe is compressed, and retracted in a single cycle. The syringe was fitted with a hypodermic needle at the exit.

This method can produce droplets, with some complications. The force from the cylinder causes bending in the needle, and the system leaks around the fitting between the syringe and the needle. With this simple setup, a few alginate droplets are created.

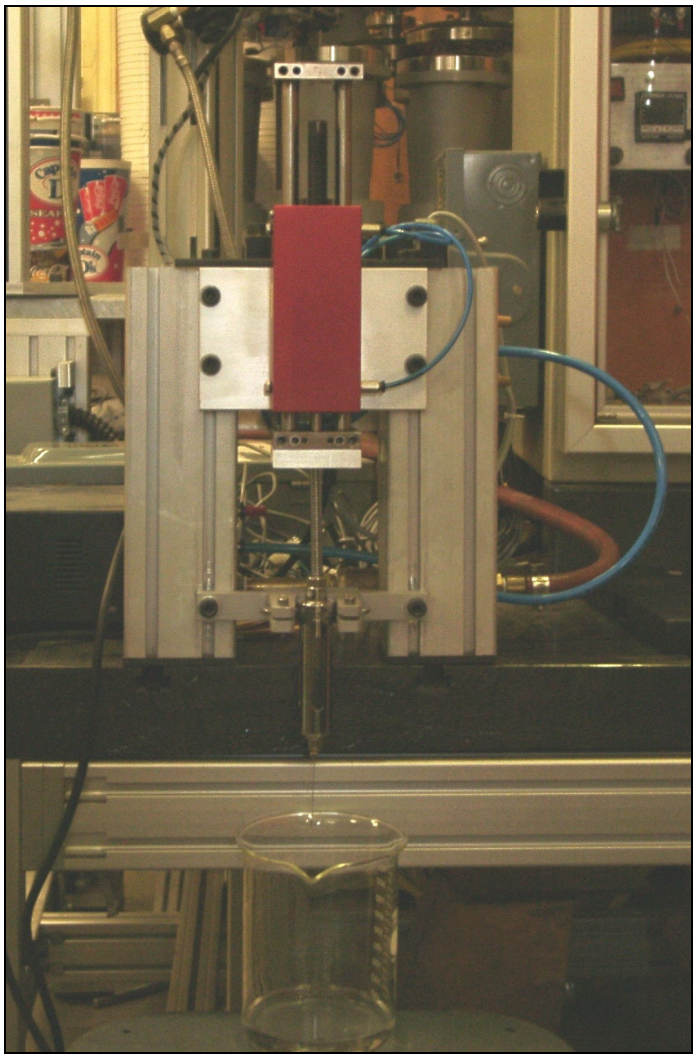

Figure 5-1: Syringe extrusion system with a pneumatic cylinder

From this investigation, the process is adapted into a large encapsulator. By using the basic syringe design, the large encapsulator is designed and fabricated.

5.2. Encapsulation Method

The encapsulator consists of several modular parts to produce the working system. These parts are as follows: Cell Chamber, drive rod, plunger, cell slide, bead ejector plate, and linear actuator (*Figure 5-2 and 5-3)*. To create a closed system the cell chamber is attached and sealed to the cell slide by a static o-ring (*Figure 5-2*). The piston uses a dynamic o-ring to seal the inside cell chamber bore. Lastly the cell ejector plate is bolted to the cell slide and sealed by another static o-ring.

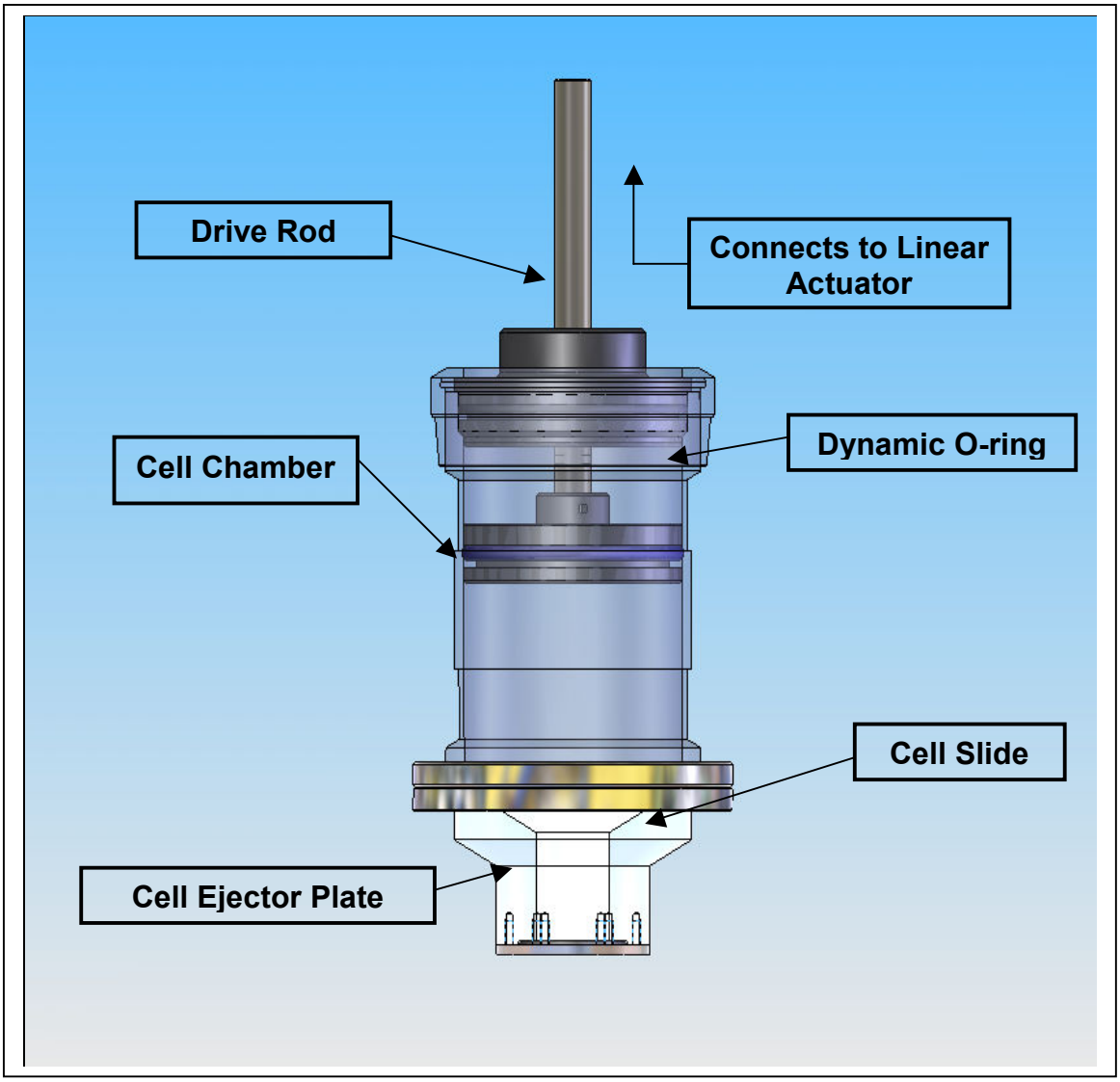

Figure 5-2: Transparent Microencapsulator Model

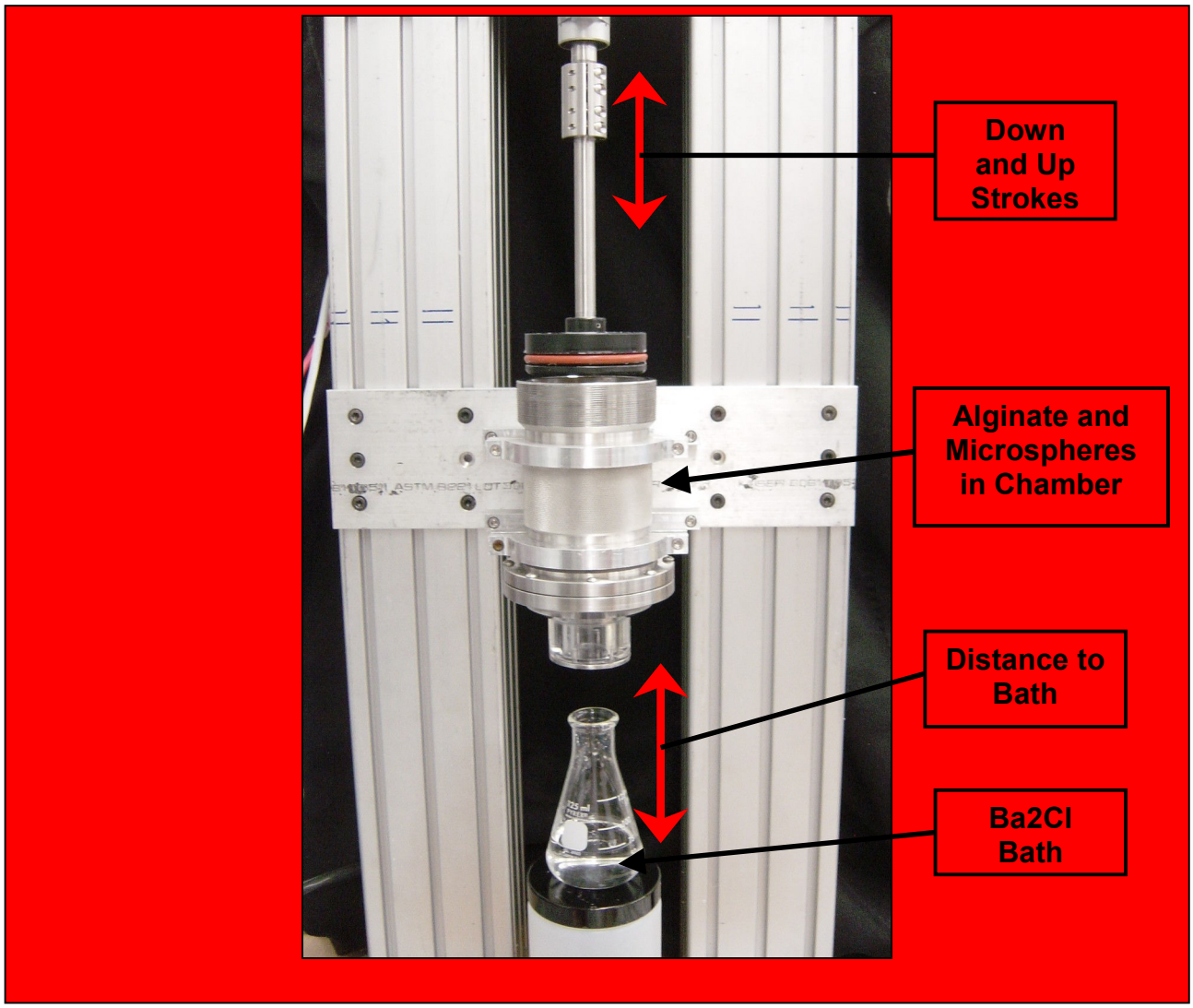

Figure 5-3: Encapsulator

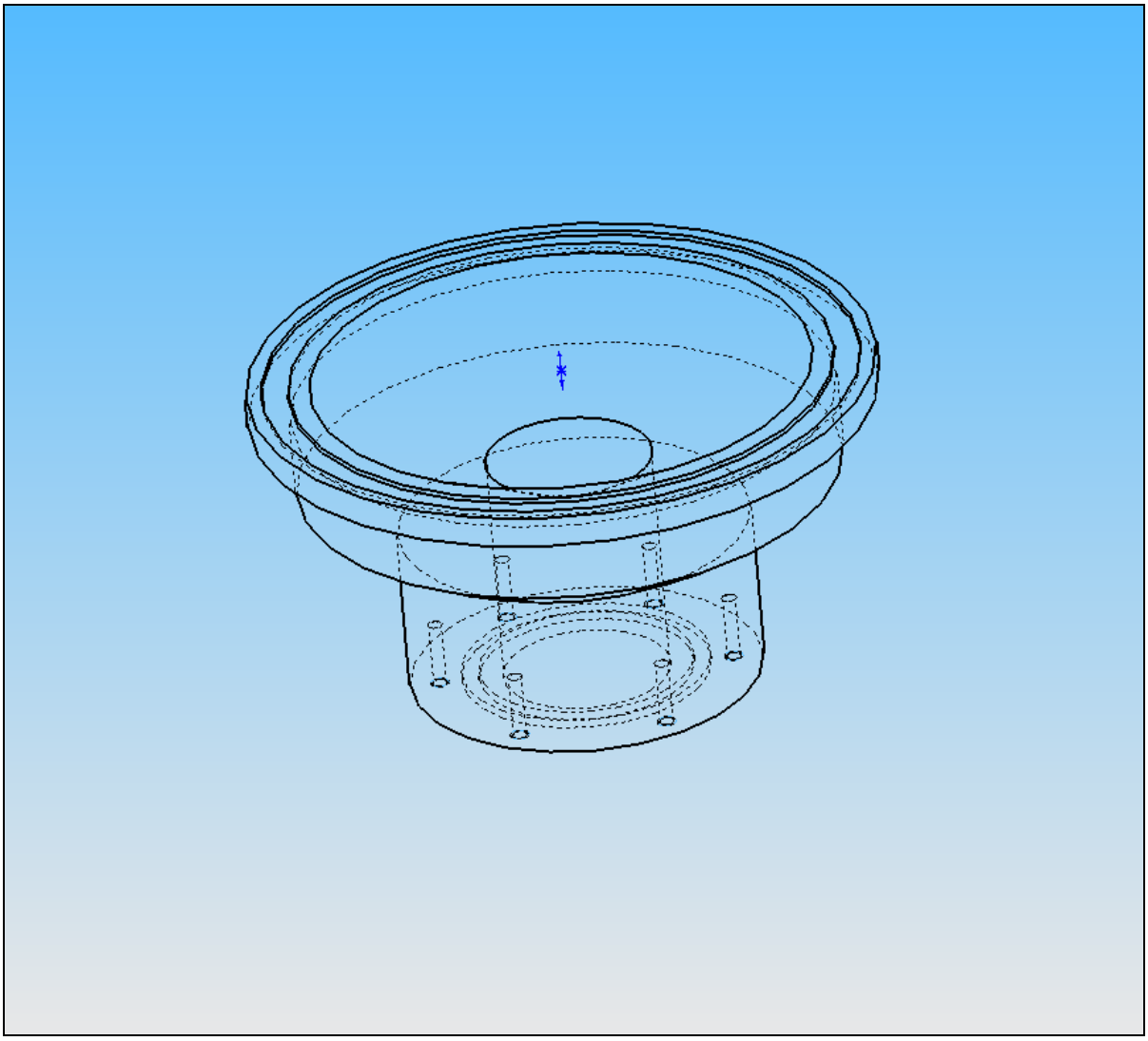

Figure 5-4: Cell Slide section

In order to purge air from the system, a hole was drilled through the plunger, and a purge plug is used to close the system after it is pressurized (*Figure 5-5*). A linear actuator is used to drive the plunger motion.

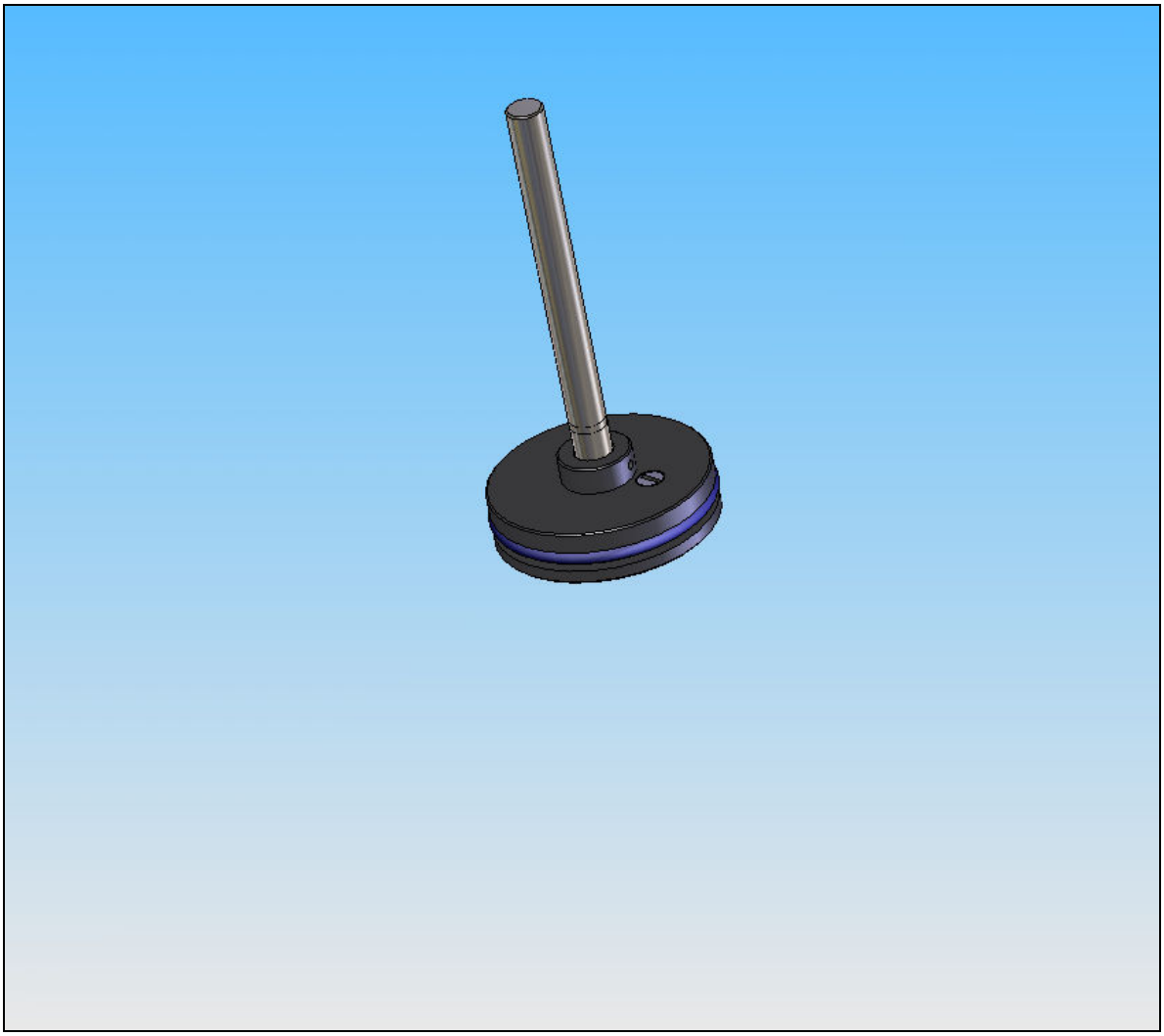

Figure 5-5: Plunger

Two cell ejector plate setups were used for this study. The working thickness of cell ejector plates was 0.05 inches, and the hole diameters used were 0.0156, 0.009, and 0.007 inches. Two different hole patterns were used. The first was a plate with one hole drilled through the center of the plate, and the

second was a plate with five holes on a 5/8 inch bolt circle with one hole in the center (*Figures 5-6 and 5-7)*. Images were taken of the individual holes drilled for each plate using a scanning electron microscope (*Figure 5-8*). Each hole was countersunk and drilled manually. For further accuracy these holes should be micromachined or drilled using laser technology.

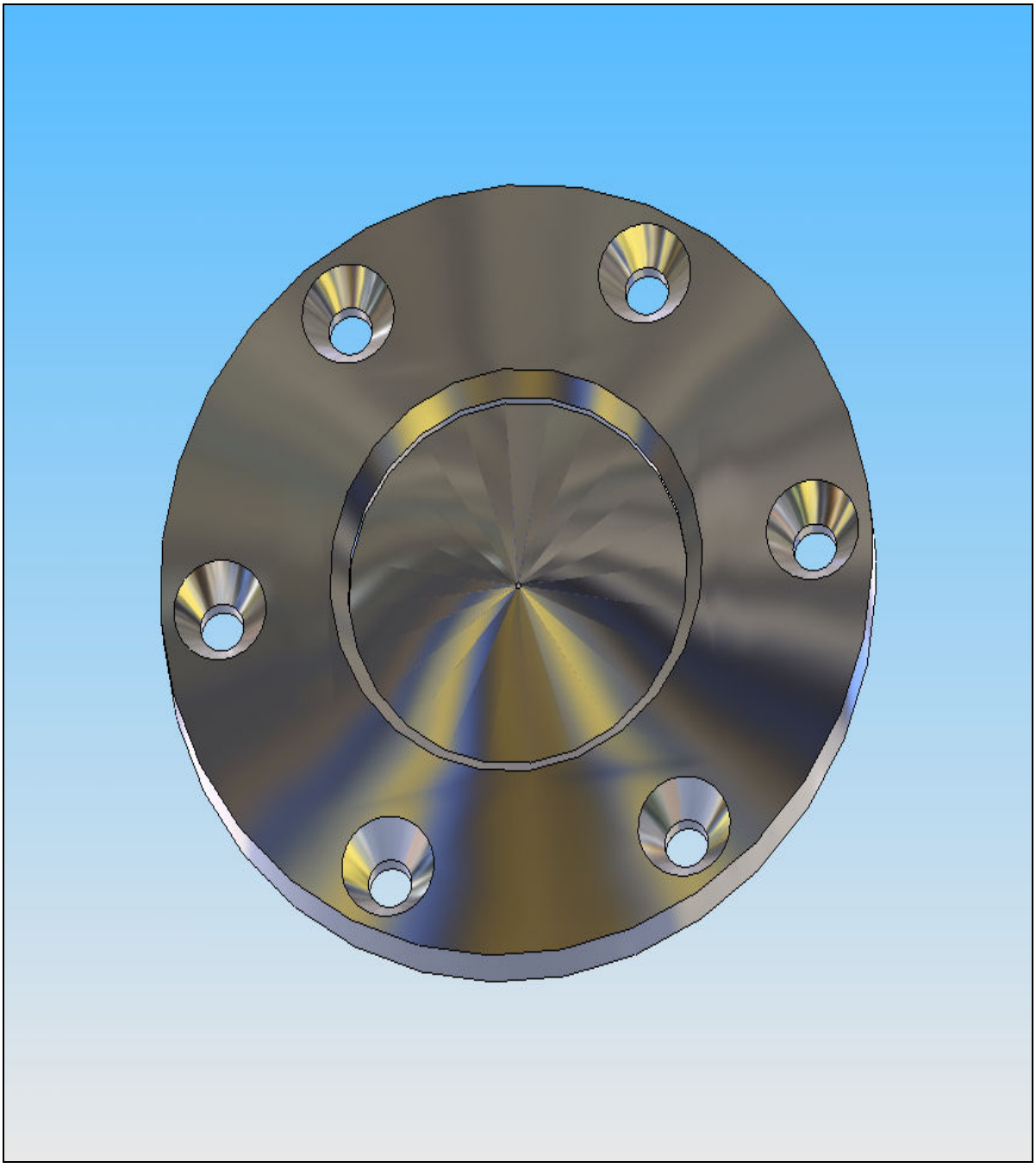

Figure 5-6: Bead Ejector Plate with 1 Hole

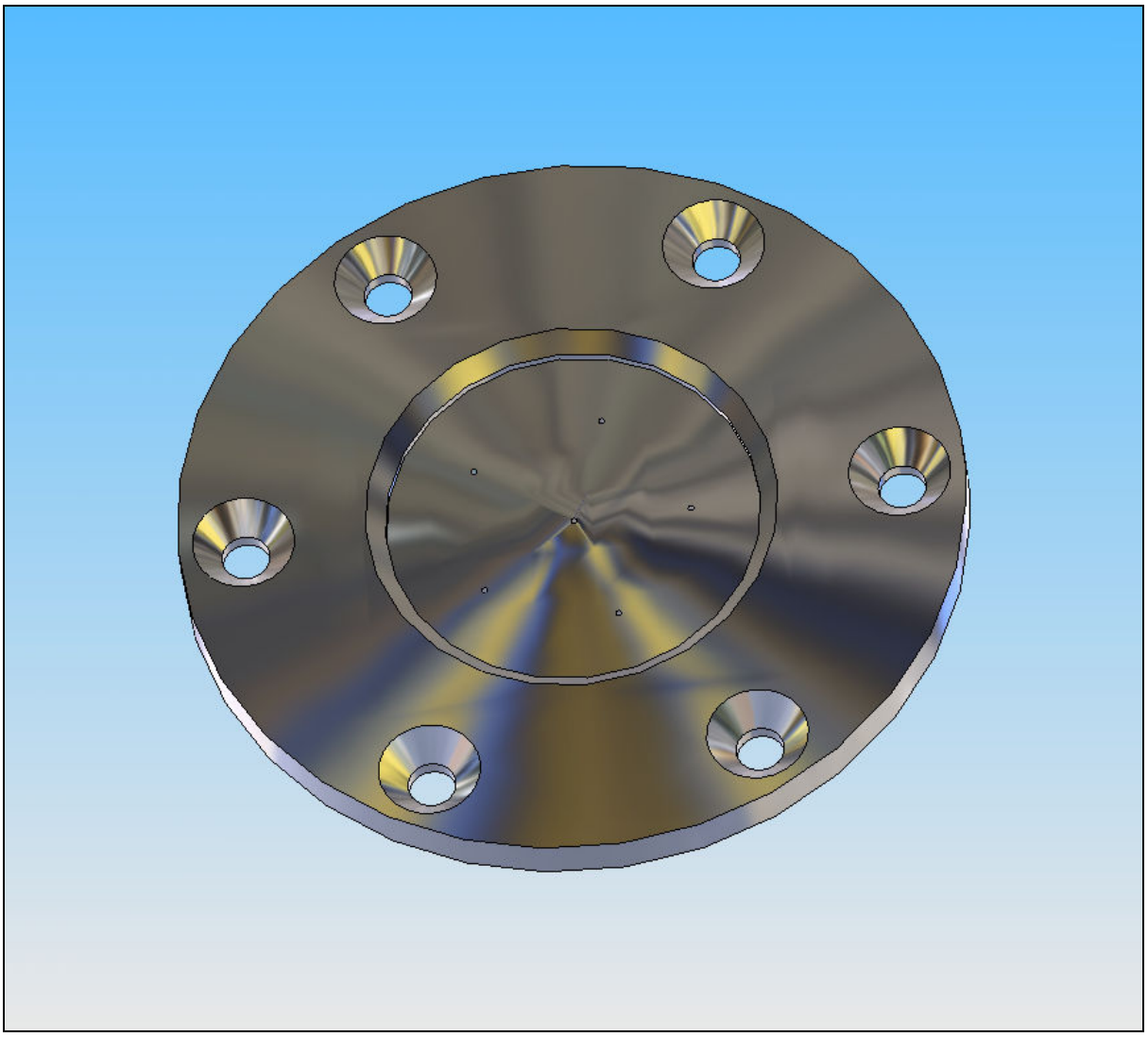

Figure 5-7: Bead Ejector Plate with 6 Holes

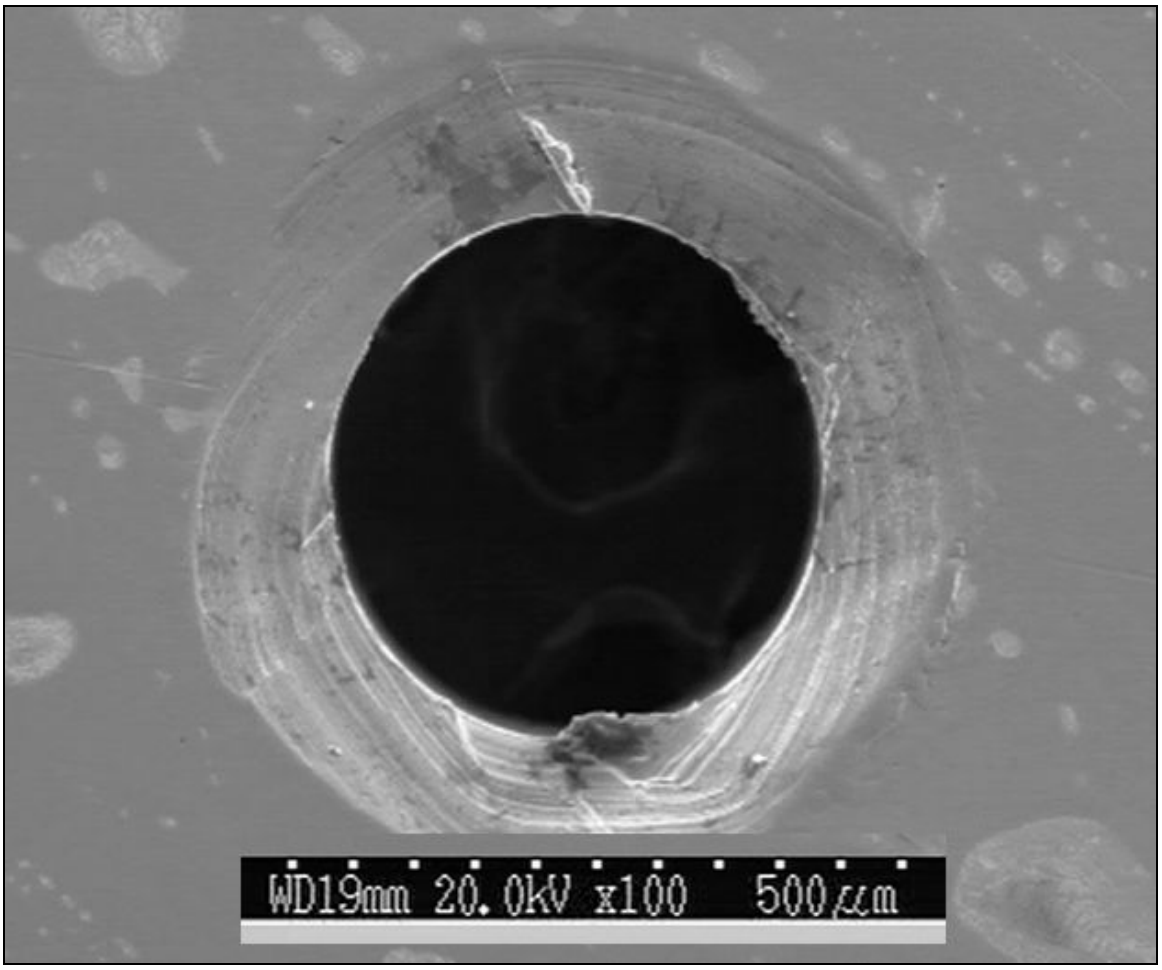

Figure 5-8: Image of individual hole

The system is controlled by using a software package called Motion Planner (Parker Hannifin). For this apparatus, a program was written to drive the plunger in the vertical direction. The parameters that are directly controllable or parameters that you can specify are: number of cycles, downstroke distance from datum, upstroke distance, velocity, and acceleration of the plunger in both directions.

Cycle: Number of complete down and upstroke per run.

Downstroke: The distance the plunger travels down, relative to the absolute position of the plunger from the previous cycle. This variable input is setup for a scaling factor of 1000, and the units are in inches. For example, for an input of 12, the distance down will be 0.012 inches.

Upstroke: The distance the plunger travels up after each downstroke, relative to the zero position. This variable input is also setup with a scaling factor of 1000, and units in inches.

Velocity: The speed at which the plunger moves on each down and up stroke. The units on this variable are rev/s.

Acceleration: The rate at which the plunger changes its velocity. The units for this variable are rev/s/s. For example, an acceleration of 200 will approach the set velocity slower than an acceleration of 800.

Each of these variables is considered during the parametric study with the encapsulator. As a result, the minimums and maximums for each variable are determined. Due to the viscosity and hole diameter, when the internal pressure exceeds the maximum motor output force, the stepper motor cannot stay in phase, and produces motor slippage. If the motor starts slipping, then the beads created are no longer valid, because then the variables are not met by the motor. Motor slippage can be determined during testing by the closed loop feedback from the motor, which is printed to the computer. When this occurs, the variables are modified to find the problems causing the motor slippage.

The parametric study considerers the variables listed above. The range for each variable is case sensitive. Therefore, each variable must be varied for each constant of the other variables. The case studies considered numbered over 500, but not all of the studies showed bead formation. Each case study that produced viable beads is reported with specific bead diameter and whether polymorphism, a tail, is present.

6. Results

Spherical bead diameter and polymorphism was studied with respect to each of the variables stated earlier. Polymorphism is described as a tail that forms on a bead upon exiting the encapsulator. These tails may cause problems for bead durability and stability; therefore, it is a variable to consider. Each parameter was studied to bette\r understand the effects on the bead production. Along with the parameters listed earlier, the distance from the encapsulator to the barium chloride bath was studied. After preliminary tests were completed, it was determined that some of the variables could be held constant for the study. The number of cycles only determines the number of beads that will be produced through one test. Since this is not part of the study, this variable is held constant. The second variable that is held constant is the distance to the bath. Previous studies have shown that a cross-linking bath must be placed under the encapsulation device so that the beads will not reach the bottom of the container [9, 10, 11, 14, 25]. From the preliminary tests, the

bath can be placed at four inches from the cell ejector plate. This is the distance from the exit to the surface of the bath. The volume of the barium chloride bath is 75 mL. Throughout the parametric study, this variable was held constant. The other variables, which include: downstroke, upstroke, acceleration, velocity, hole diameter, and hole frequency, are varied.

5.1. Variables

The variables each have an acceptable range to consider. In order to find this range, each alginate sample was run through the encapsulator, using the plate with a single 1/64 inch hole. The samples were taken and examined with the microscope. Based on the sample observations, the variable range was optimized. The first range considered was as follows:

Downstroke and Upstroke: 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, 0

Acceleration: 200, 400, 600, 800, 1000, 1200, 1400, 1800, 2000, 3000, 4000, 5000, 6000, 7000, 8000

Velocity: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.2, 1.4

Cycle: 10

Each of the possible combinations of these variables was considered. The observations varied with each sample, but the bad samples consisted of intense streams or large drops. The drops were usually larger than 3 mm. The main goal was to produce beads between 0.3 and 0.8 mm. The first set of samples taken included most of these combinations, and the 1.5 % LVG was used. This

sample has the lowest viscosity; therefore it should be the easiest to push through the outlet.

After these tests were completed, the variables were condensed. With these combinations, each sample concentration was tested with the encapsulator, through each cell ejector plate.

5.2. Sample Results

The viscosity was the variable that determined whether a sample could be used for the parametric study. For the higher viscosities, the sample could not be used to produce beads. The LVG alginate had viscosities ranging from 40 to 78 centiPoise. The other two types of alginate, MVM and MVG, were too viscous to use in the parametric study. None of the alginate samples could be used to produce beads for the cell plates with hole diameters of 0.007 and 0.009 inches. Therefore, the two plates used in this study both have hole diameters of 0.015 inches. The final combination of samples observed is given below (*Table 6-1*).

These problems are attributed to two variables that need to be considered at a later time. The linear motor used in this study pushes 75 lbf. If the linear motor is changed to allow for a higher force, then this problem may not arise. The other variable to consider is the number of holes. If the number of holes is increased, then the sample viscosity range can be increased as well. Despite these problems, the parametric study showed promising results with the LVG alginate.

Table 6-1: Samples Taken for Examination

Class	Levels	Values
Cycles (#)	2	5, 10
DownStroke (x 1/1000 inch)	1	12
Upstroke (x 1/1000 inch)	4	2, 4, 6, 8
Velocity (rev/s)	3	0.2, 0.6, 1.0
Acceleration (rev/s/s)	3	800, 4000, 8000
Sample Type	1	LVG
Concentration (% weight)	2	1.5, 1.8
Hole Diameter (inches)	1	0.015
Hole Pattern (#)	2	1, 6
Samples Encapsulated	2	Empty Alginate Microcapsules, Alginate Microcapsules containing microspheres

5.3. Microcapsule Examples

In order to better understand visually how the microcapsules are examined, pictures were taken of various samples throughout the experimentation. Examples of the alginate spheres with and without tails are given below (*Figures 6-1 through 6-3).* Each image is taken at a magnification level. For a magnification level of 2, the defined magnification factor is 4. For a sample reading of 80.0 lines, the actual reading is

$$\left(\frac{80.0}{4}\right)\times 0.05 = 1.00mm$$

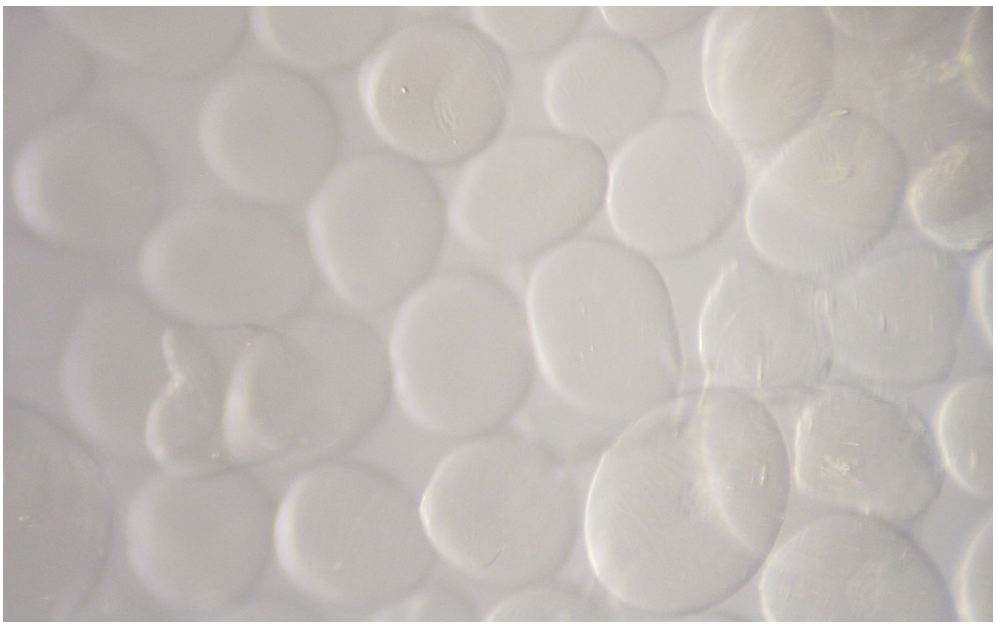

Figure 6-1: Alginate spheres with and without a tail (M = 4).

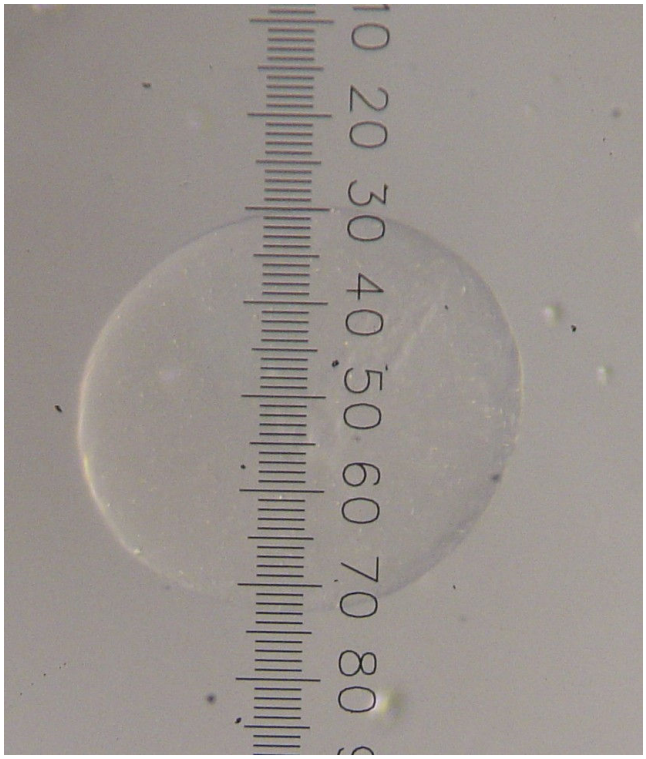

Figure 6-2: Alginate sphere without a tail (M = 4, d ~ 0.54 mm).

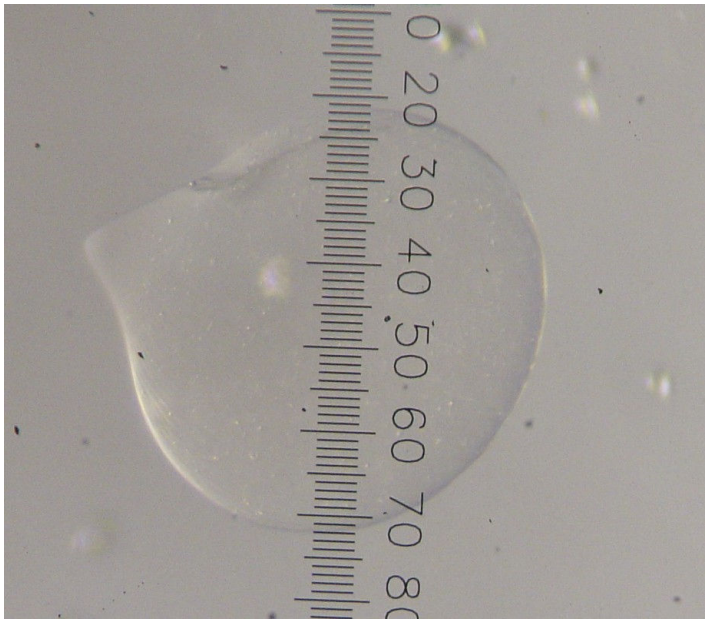

Figure 6-3: Alginate sphere with a tail (M = 4, d ~ 0.64 mm).

The images below are taken using a Scanning Electron Microscope (*Figures 6-4 through 6-8*). The images show good spherical shapes as well as some fragmentation and tailing effects.

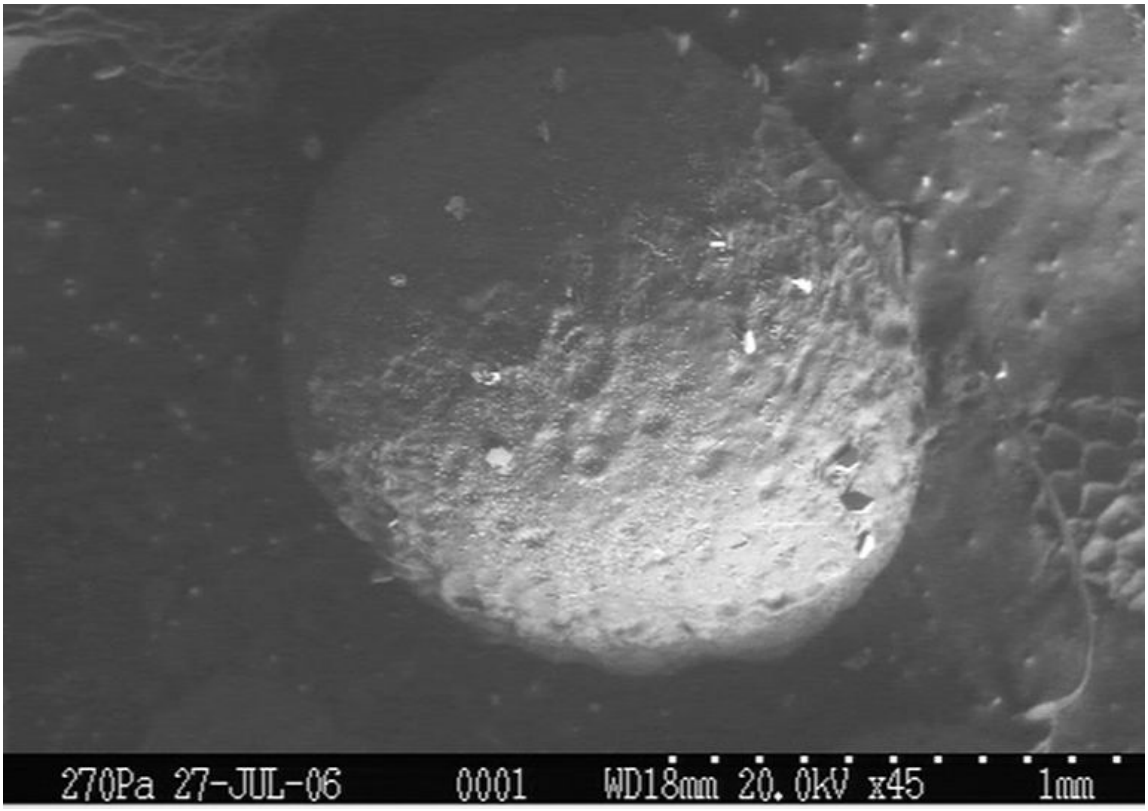

Figure 6-4: Microencapsulation example #1.

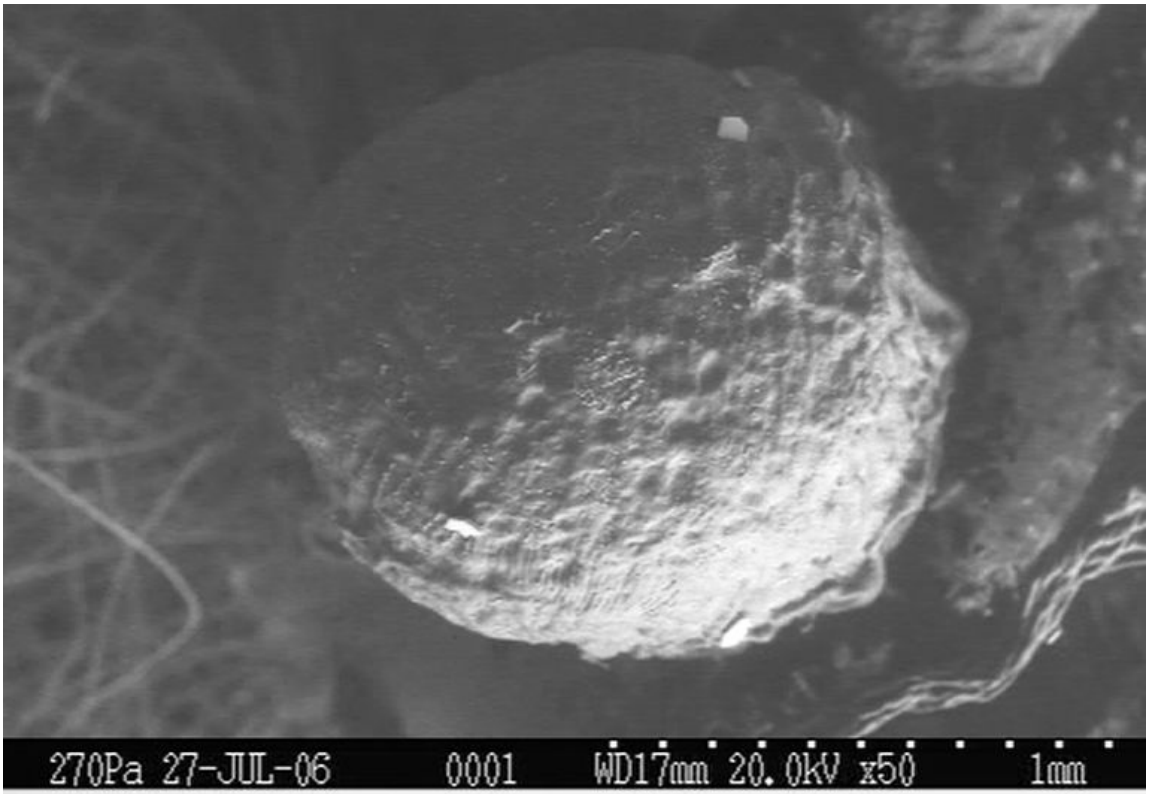

Figure 6-5: Microencapsulation example #2.

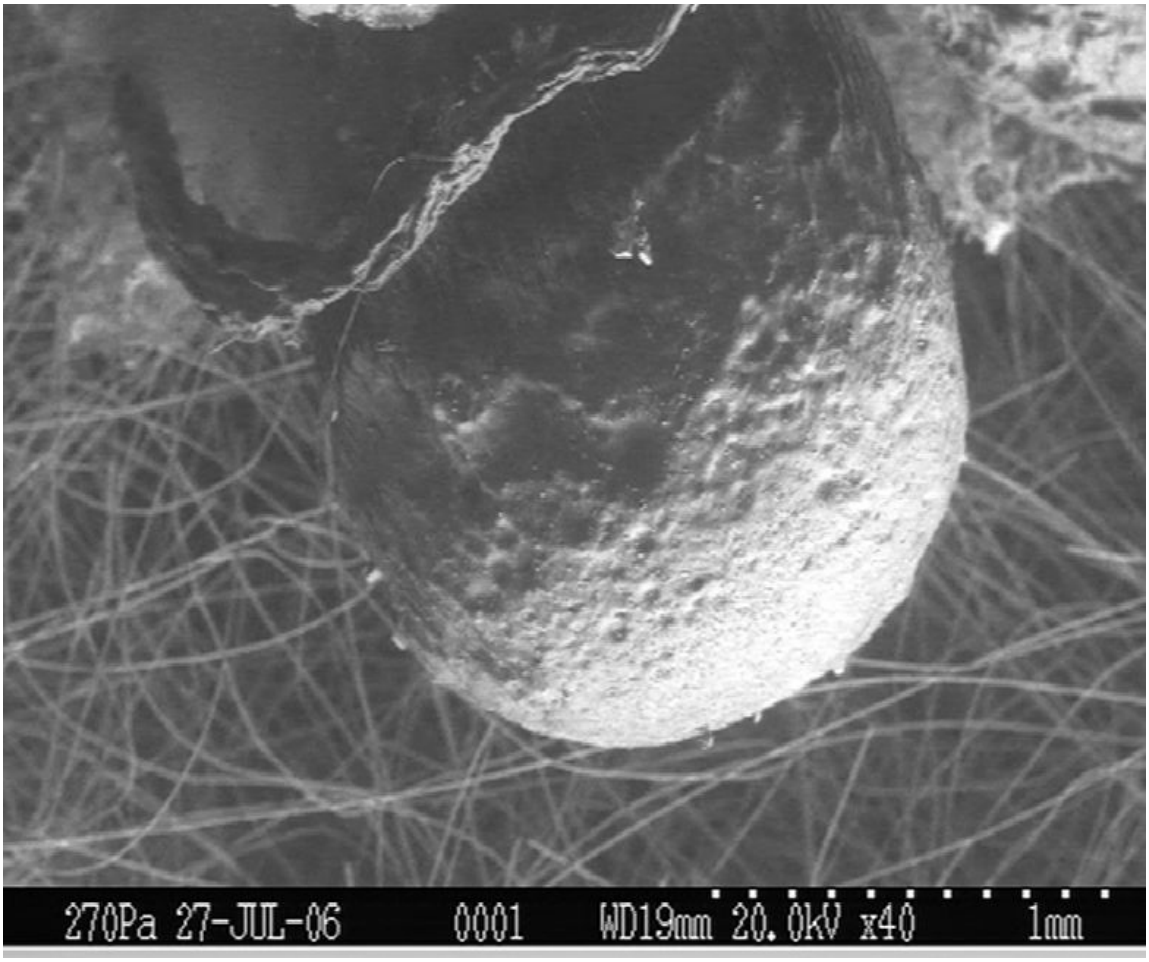

Figure 6-6: Microencapsulation example #3 with tailing.

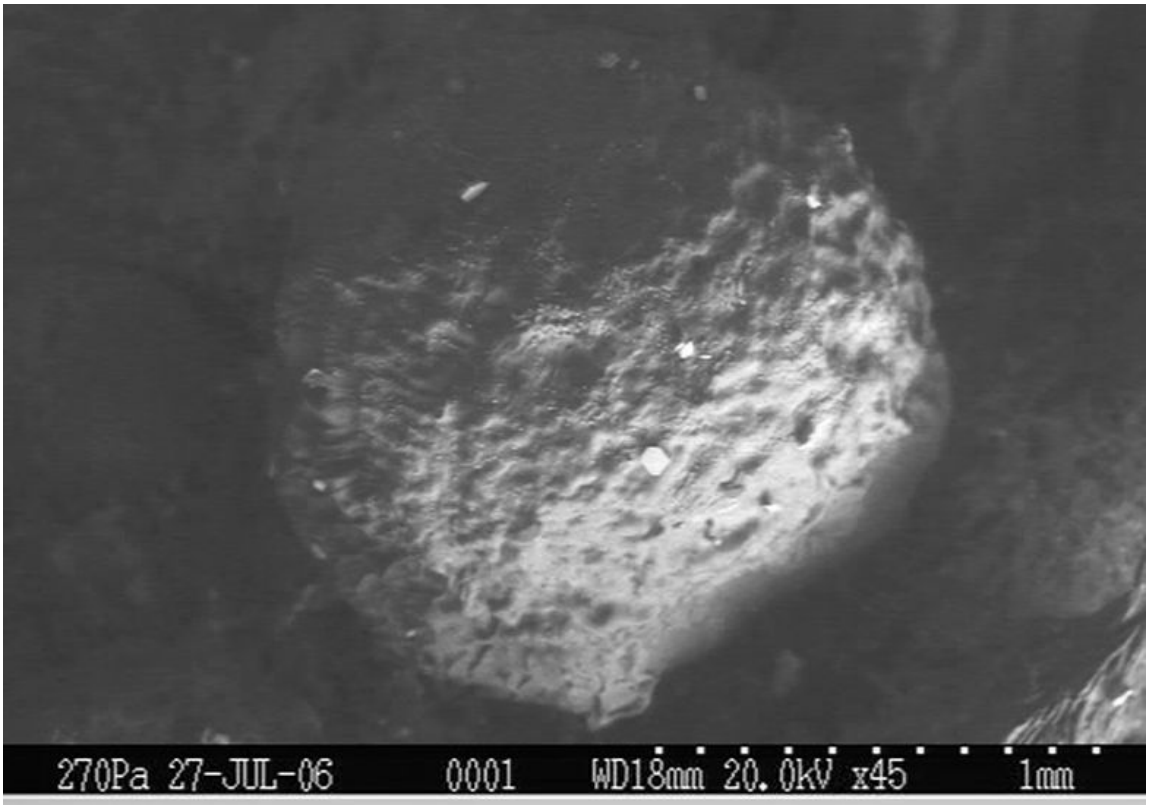

Figure 6-7: Microencapsulation example #4 with fragmentation.

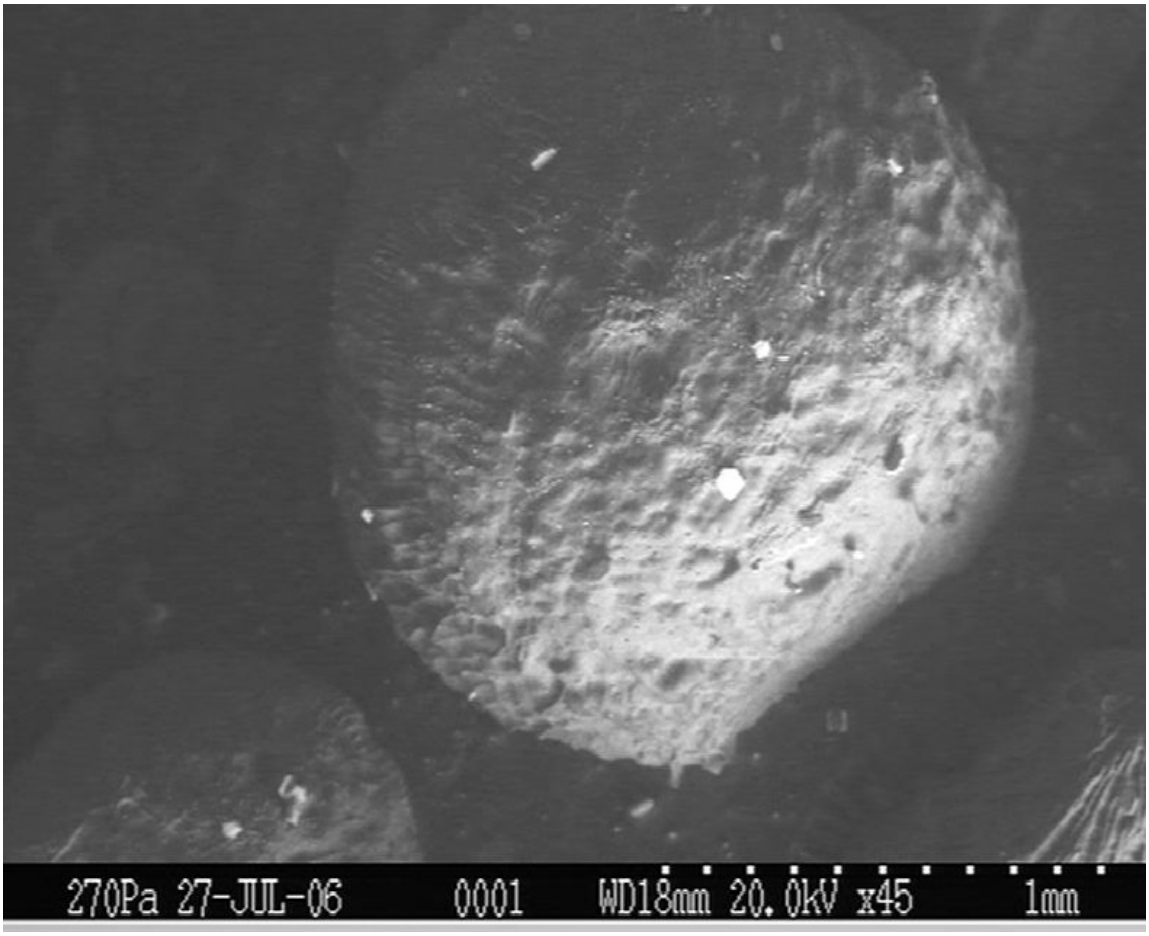

Figure 6-8: Microencapsulation example #5 with fragmentation.

For the polystyrene microspheres encapsulated in alginate, the beads range from 1 to 3 beads in a good spherical bead. Some of the samples show that the non-spherical beads contain more beads than normal, but these beads are not measured for the sample. A single polystyrene microsphere is shown below before encapsulation (*Figure 6-9)*. Examples of the polystyrene microspheres after encapsulation are shown below (*Figures 6-10 through 6-13).* Beads that seem to be “out of focus” are actually in another plane within the same encapsulation.

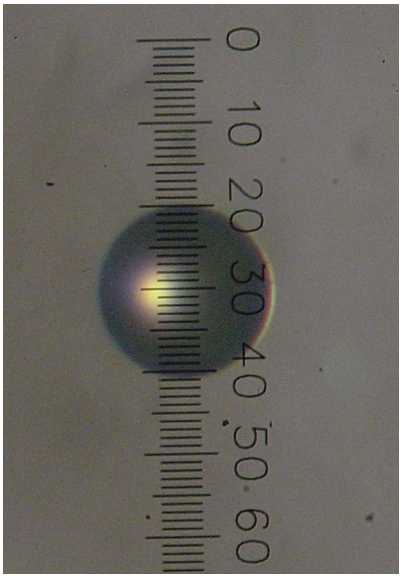

Figure 6-9: Polystyrene microsphere before encapsulation (M = 8.75, d ~ 0.120 mm).

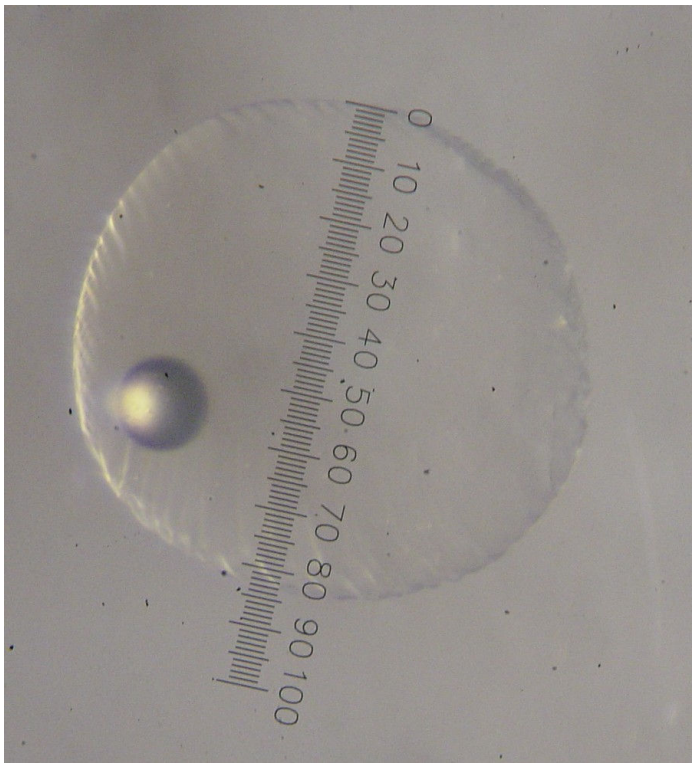

Figure 6-10: Polystyrene microsphere after encapsulation without a tail (M = 8.75, d ~ 0.47 mm).

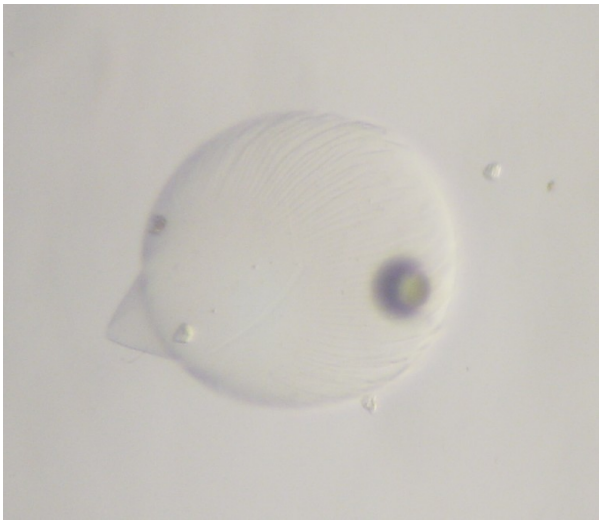

Figure 6-11: Polystyrene microspheres after encapsulation with a tail (M = 8.75).

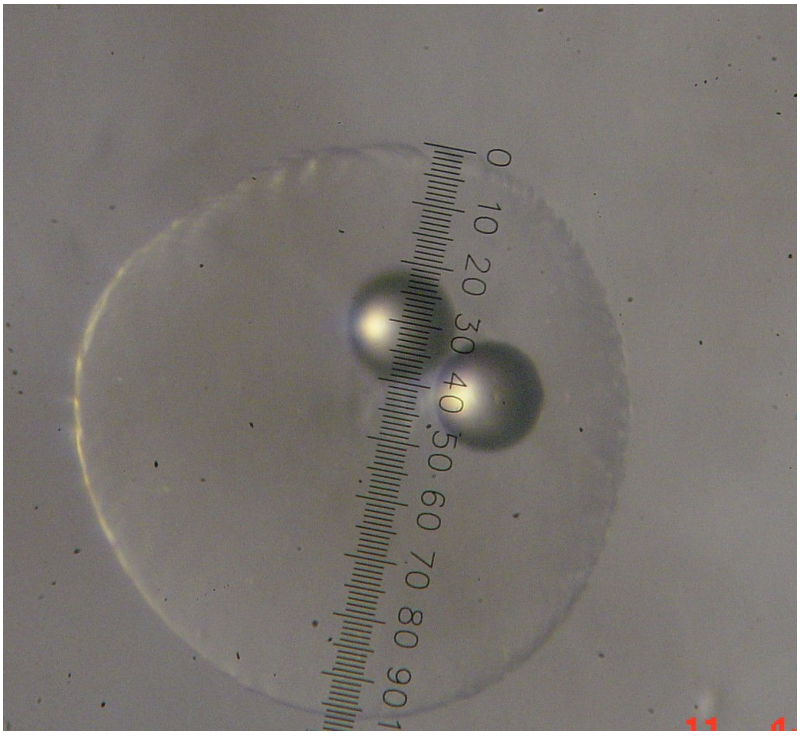

Figure 6-12: Polystyrene microspheres after encapsulation without a tail, and multiple beads (M = 8.75, d ~ 0.53 mm).

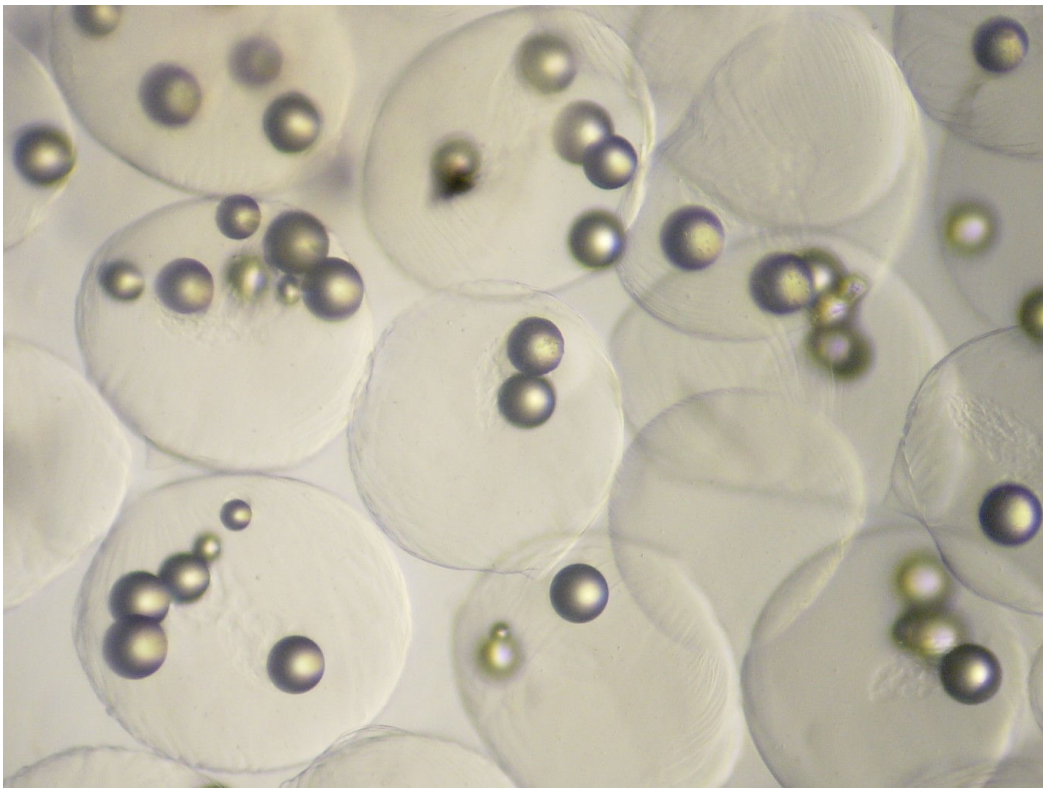

Figure 6-13: Polystyrene microspheres after encapsulation from a sample (M = 4).

6.4. Result Analysis

The results are quantified below according to the variables considered. Each section includes the ANOVA analysis and the variable interactions, which govern the bead diameters measured. The variables that are analyzed include:

- DF is the degrees of freedom for each variable or combination of variables.
- P-value measures the consistency by calculating the probability of observing the results from the sample of data.
- F-value indicates the strength of the variable effect on the sample.

- Least Square Means, or adjusted means, are the means that have been corrected for imbalances in other variables.

6.5. Alginate Microspheres

Empty alginate microspheres were produced with LVG alginate concentrations of 1.5 and 1.8 %. The samples were run with the test parameters described above, using both cell ejector plates (*Table 6*-1). The bead diameters were measured, and the polymorphism was observed. With each sample, the beads were selected randomly for measurement.

6.5.1. 1.5% LVG through Cell Ejector Plate with 1 Hole

The LVG alginate is used to run through the encapsulation process using the cell ejector plate with a single 0.015 inch hole. The variables considered are velocity, upstroke, and acceleration, while the cycle is constant. From the ANOVA analysis, the levels of variation are listed by variable (*Table 6-2).*

Table 6-2: Class level information for 1.5% LVG through 1 hole cell ejector plate

Class	Levels	Values
Velocity	3	0.2, 0.6, 1
Upstroke	4	2, 4, 6, 8
Acceleration	3	800, 4000, 8000

The results of the analysis show the interactions between variables (*Tables 6-3).* By using the type III sum of squares, the differences between the predicted bead diameters over a balanced velocityXaccelerationXupstroke sample or population. Therefore with this test the differences between the LS-means are being tested. The three variables including velocity, upstroke, and acceleration, are tested to find the effect of each on the bead diameter and tail presence. Again, this test is done by examining the p-value for each variable effect. Given a p-value less than 0.05 is accepted as significant. If the p-value is significant, the f-value is examined.

The test is done to see the effect of each variable alone as well as with the other variables. When two of the variables have a significant effect together, this is a 2-way interaction. When all three variables have a significant effect together, this is a 3-way interaction. If a 3-way interaction is present, then this is the effect that will be examined because then the rest of the effects of void. If a 3-way is not present, then the 2-way interactions are examined. If neither type of interaction is present, then each of the three variable effects are examined. Velocity and upstroke have a strong effect on the bead diameter based on the p-value. But there are 2-way and 3-way interactions between all three parameters. Since a 3-way interaction is present, this will be the effect of most interest. Therefore, all three variables have an effect on the mean diameter. There are also 2-way and 3-way interactions on the tail presence data. The upstroke looks to have the strongest effect based on the f-value, but again the 3-way interaction effect on the tail presence is examined.

Table 6-3: ANOVA Analysis for 1.5 % LVG through 1 hole cell ejector plate

Dependent		Diameter		Tail	
Source	**DF**	**F Value**	**P Value**	**F Value**	**P Value**
Velocity	2	61.67	<0.0001	0.75	0.4729
Upstroke	3	142.75	<0.0001	9.82	<0.0001
Velocity*Upstroke	6	7.93	<0.0001	6.49	<0.0001
Acceleration	3	0.99	0.3959	1.83	0.1401
Velocity*Acceleration	4	10.71	<0.0001	2.03	0.0876
Upstroke*Acceleration	6	18.81	<0.0001	2.5	0.021
Velocity*Upstroke*Acceleration	11	9.1	<0.0001	2.29	0.0092

The following graphs show: the mean diameter as a function of the acceleration with the upstroke = 2, 6 (*Figure 6-14)* and upstroke = 4, 8 (*Figure 6-15)*. The second set shows: The % of beads without tails as a function of the acceleration with the upstroke = 2, 4 (*Figure 6-16)* and upstroke = 6, 8 (*Figure 6-17).*

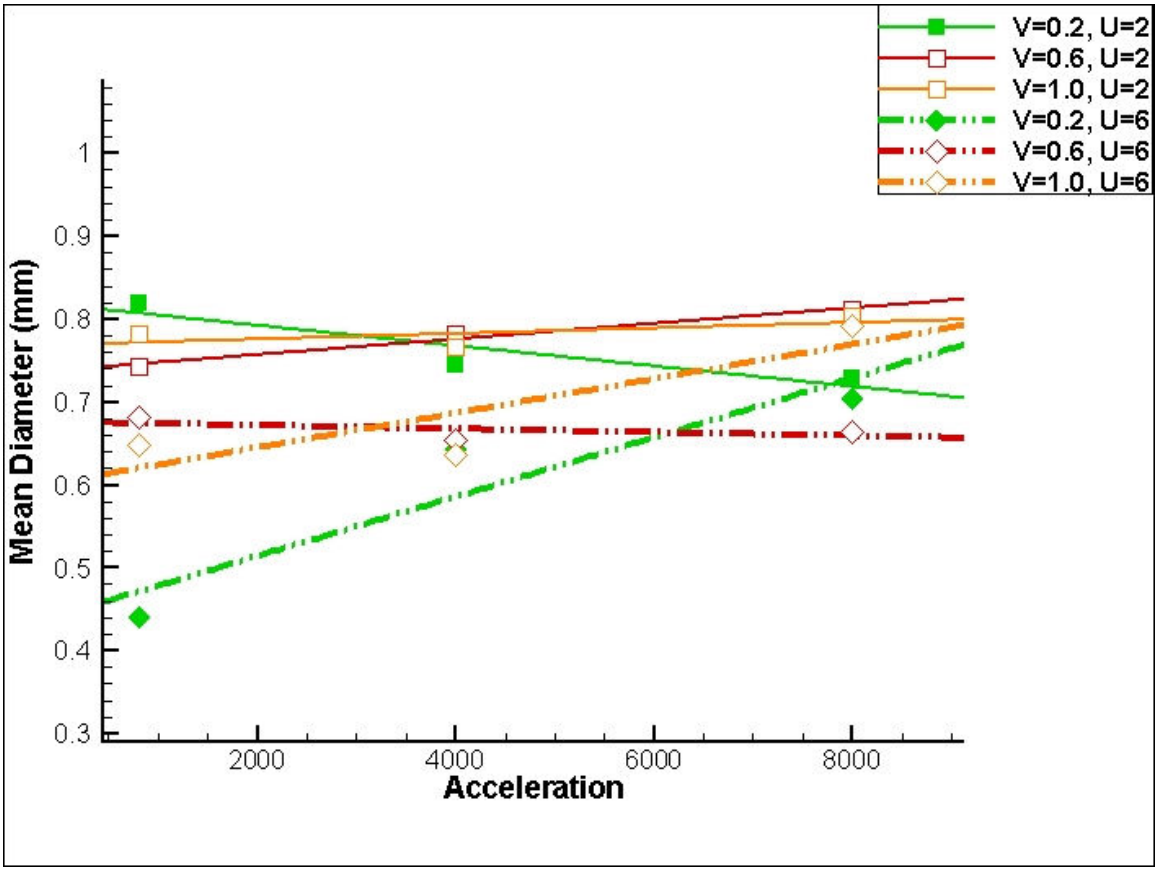

Figure 6-14: Mean Diameter as a function of Acceleration for Upstroke = 2 and 6. (1.5% LVG)

From this graph, there is an obvious linear relationship based on the acceleration. The slope increases as the velocity increases for an upstroke of 2. The inverse is the case for an upstroke of 6. As the acceleration increases, the variability of the bead diameter decreases. Therefore, at higher accelerations, the velocity has a lower effect on the change in bead diameter. By just examining the bead diameters, the values are within an acceptable range of 0.4 to 0.9 millimeters.

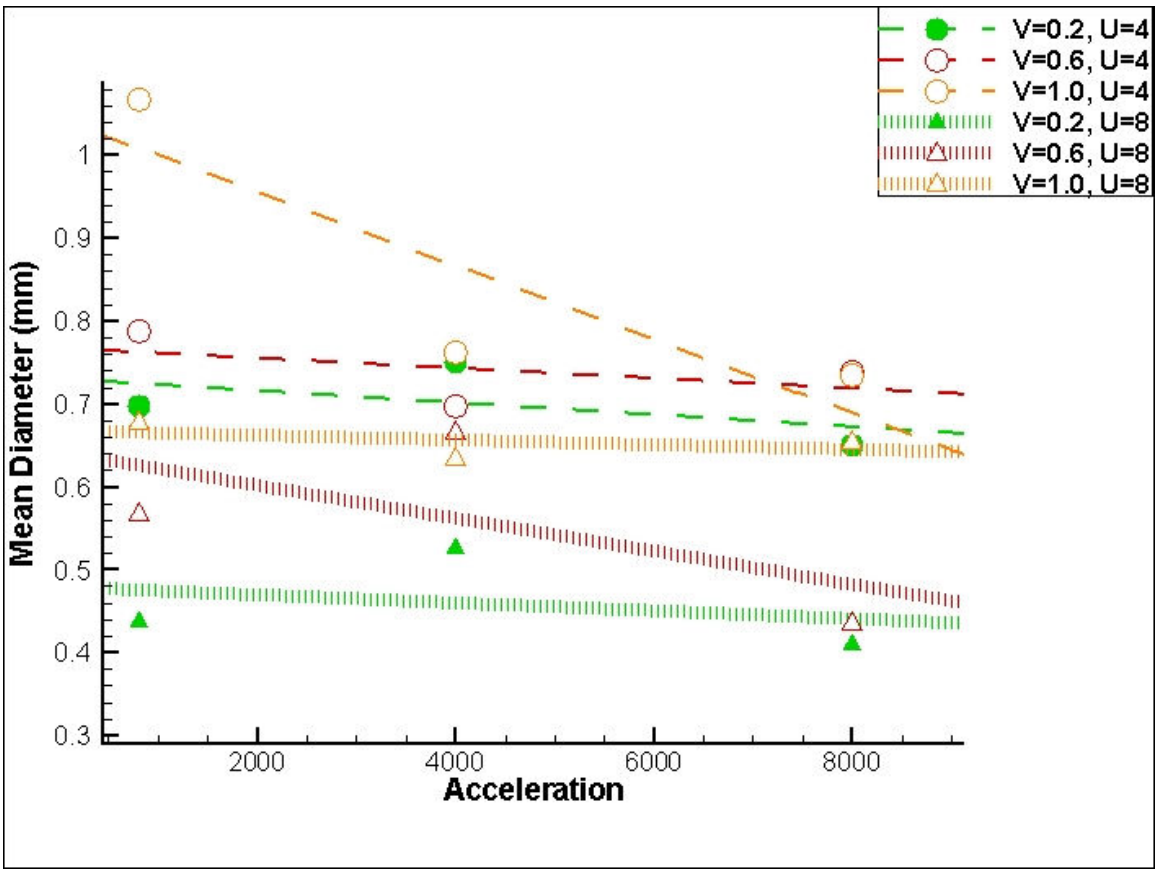

Figure 6-15: Mean Diameter as a function of Acceleration for Upstroke = 4 and 8. (1.5% LVG)

From this second graph, there is also a linear relationship between the mean diameter and acceleration. The variability also decreases with acceleration across the velocity range for both upstroke values. The bead diameter increases as velocity increases for both upstroke values. The bead diameters cover a larger range, from 0.4 to 1.1 mm, but the range does decrease with acceleration down to the accepted range of 0.4 to 0.9 mm.

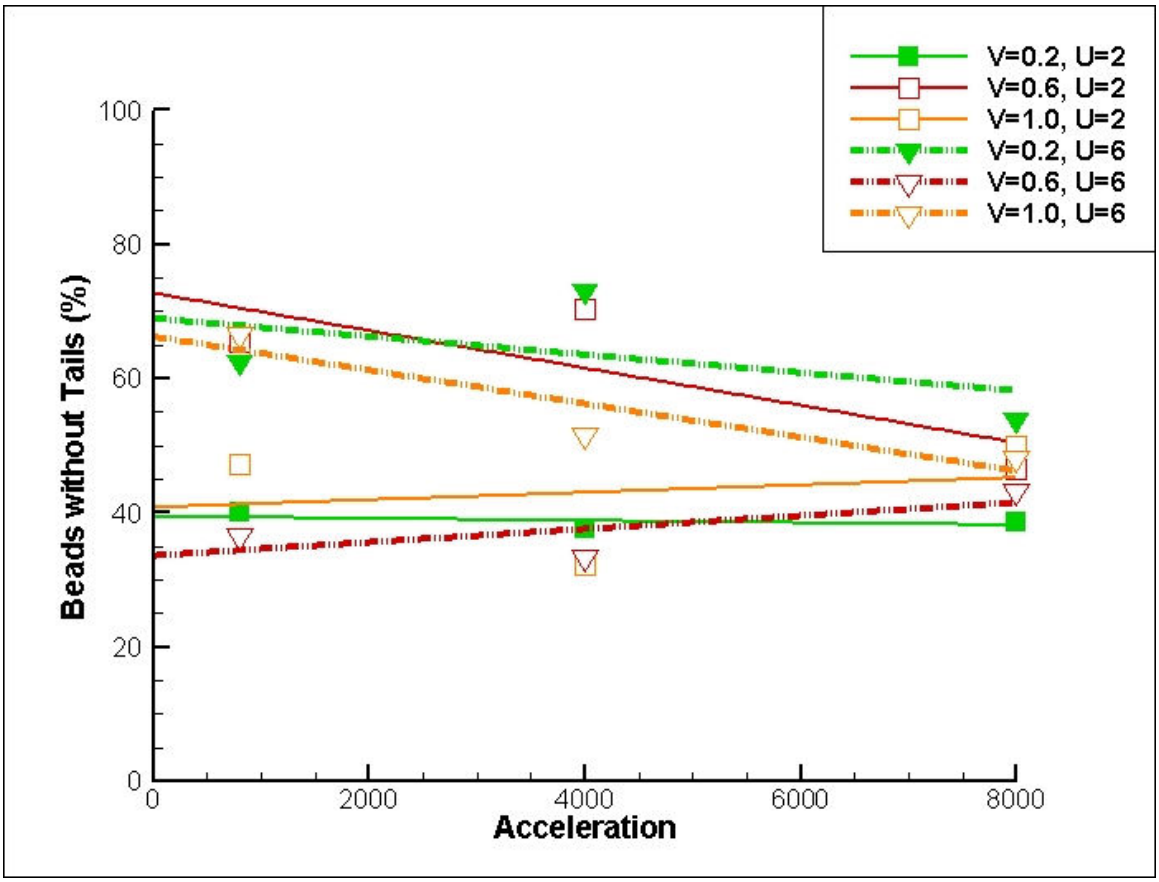

Figure 6-16: Beads without Tails as a function of Acceleration for Upstroke =2 and 6. (1.5% LVG)

This graph shows a linear relationship between acceleration and beads present without tails. The variability decreases with acceleration. There is an increasing trend for:

- U = 2, V = 0.2 and 1.0
- U = 6, V = 0.6

A decreasing trend is present for the rest of the cases:

- U = 6, V = 0.2 and 1.0
- U = 2, V = 0.6

The range of beads produced without tails is 30 to 80 %.

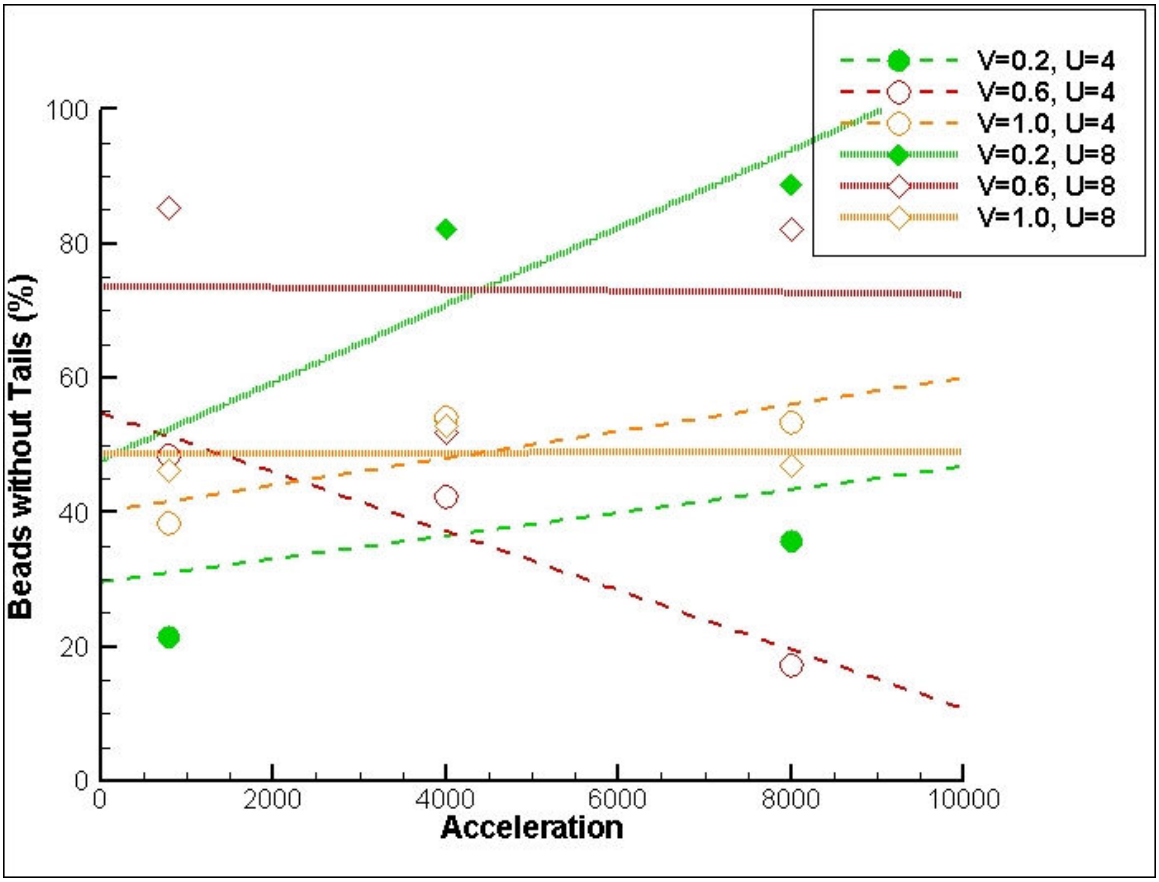

Figure 6-17: Beads without Tails as a function of Acceleration for Upstroke =4 and 8. (1.5% LVG)

From this graph, a high level of variability is present across the acceleration values. The linear relationships are present for the most part, except for an upstroke of 8. From this graph and the previous, there are linear trends for an upstroke of 4 and 2. Unfortunately, these results do not give a predictable pattern for the presence of beads without tails.

6.5.2. 1.8% LVG through Cell Ejector Plate with 1 Hole

The results from the ANOVA analysis are listed below (*Table 6-4 and 6-5).* The bead diameters in this study are also affected by all three variables independently. But they are also affected by 2-way and 3-way interactions, and the 3-way interactions take precedence. The 3-way interaction is examined below (*Figures 6-18 and 6-19)*. The tail presence is affected by the upstroke, but also by two other 2-way interactions. These 2-way interactions are examined below (*Figures 6-20 and 6-21)*.

Table 6-4: Class level information for 1.8% LVG through 1 hole cell ejector plate

Class	Levels	Values
Velocity	3	0.2, 0.6, 1.0
Upstroke	4	2, 4, 6, 8
Acceleration	3	800, 4000, 8000

Table 6-5: ANOVA Analysis for 1.8 % LVG through 1 hole cell ejector plate

Dependent		Diameter		Tail	
Source	**DF**	**F Value**	**P Value**	**F Value**	**P Value**
V	2	11.81	<0.0001	1.51	0.2206
U	3	22.69	<0.0001	5.13	0.0016
V*U	6	6.84	<0.0001	3.11	0.005
Accel	2	9.04	0.0001	0.72	0.4851
V*A	4	9	<0.0001	2.41	0.0475
U*A	6	7.63	<0.0001	1.47	0.1869
V*U*A	12	10.71	<0.0001	1.3	0.2121

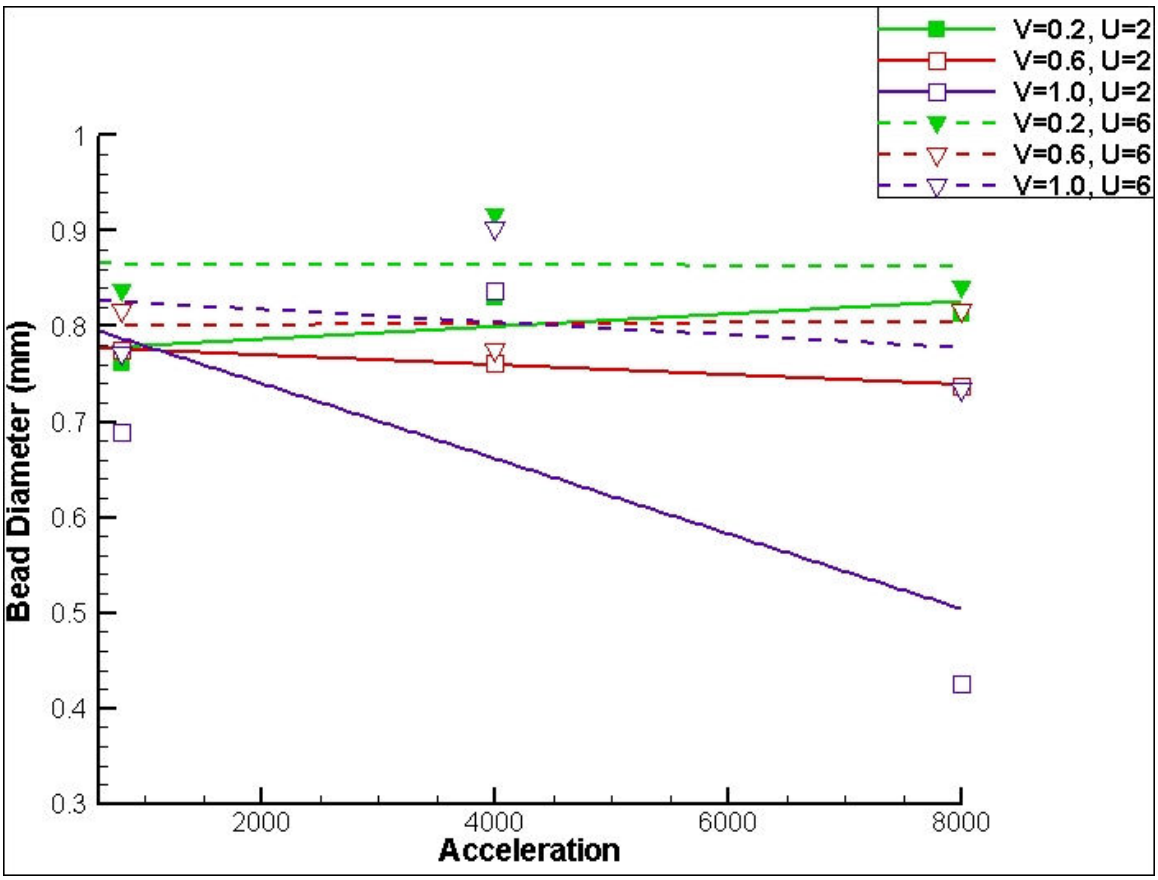

Figure 6-18: Mean Diameter as a function of Acceleration for Upstroke = 2 and 6. (1.8% LVG)

This graph shows similar results as those obtained from the 1.5 % sample.

- Slope increases with acceleration as velocity increases for U = 2
- As acceleration increases, variability increases as well.

The bead diameter increases as the upstroke increases. By examining the bead diameters produced, the range smaller than the range produced from the 1.5 % sample, but they are also slightly higher.

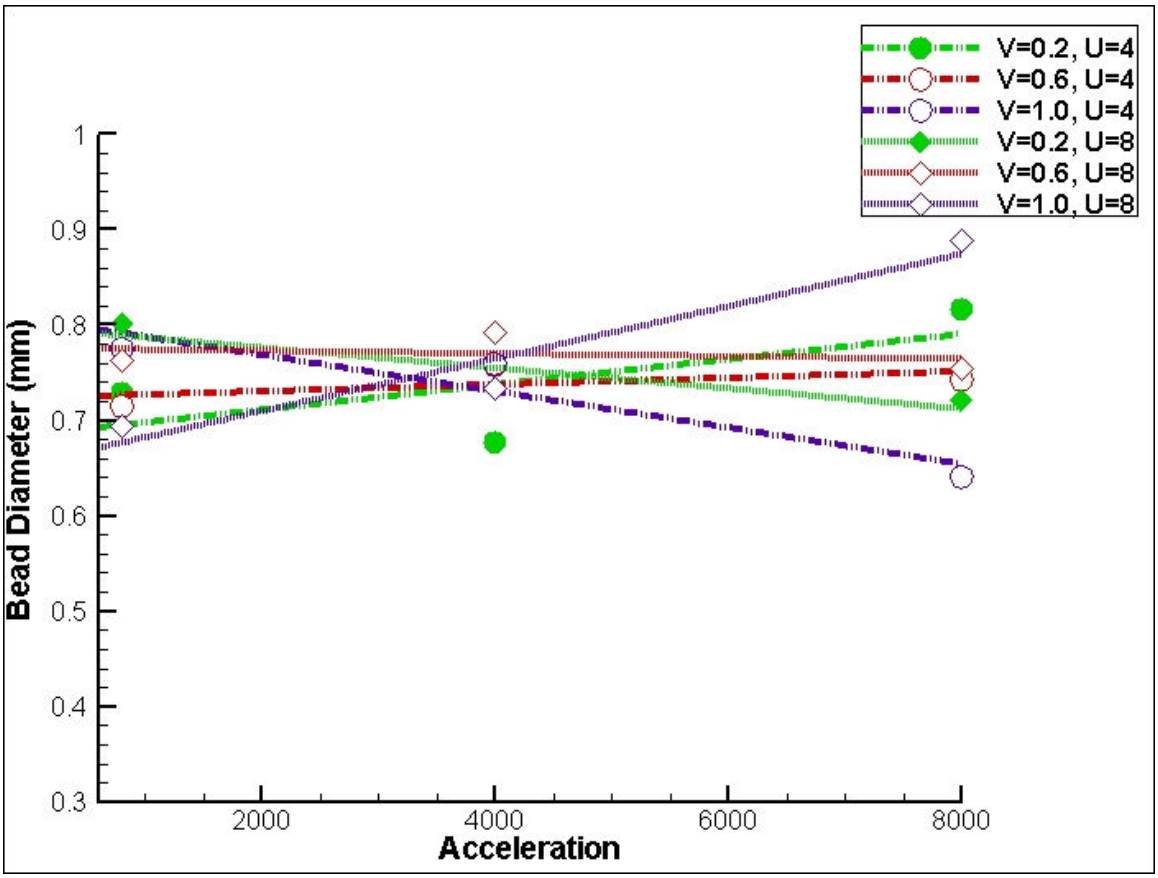

Figure 6-19: Mean Diameter as a function of Acceleration for Upstroke = 4 and 8. (1.8% LVG

The variability in this data increases at extremum of acceleration. The slope increases for an upstroke value of 4, as velocity increases. The range of these values is also much smaller than the range from the 1.5 % sample.

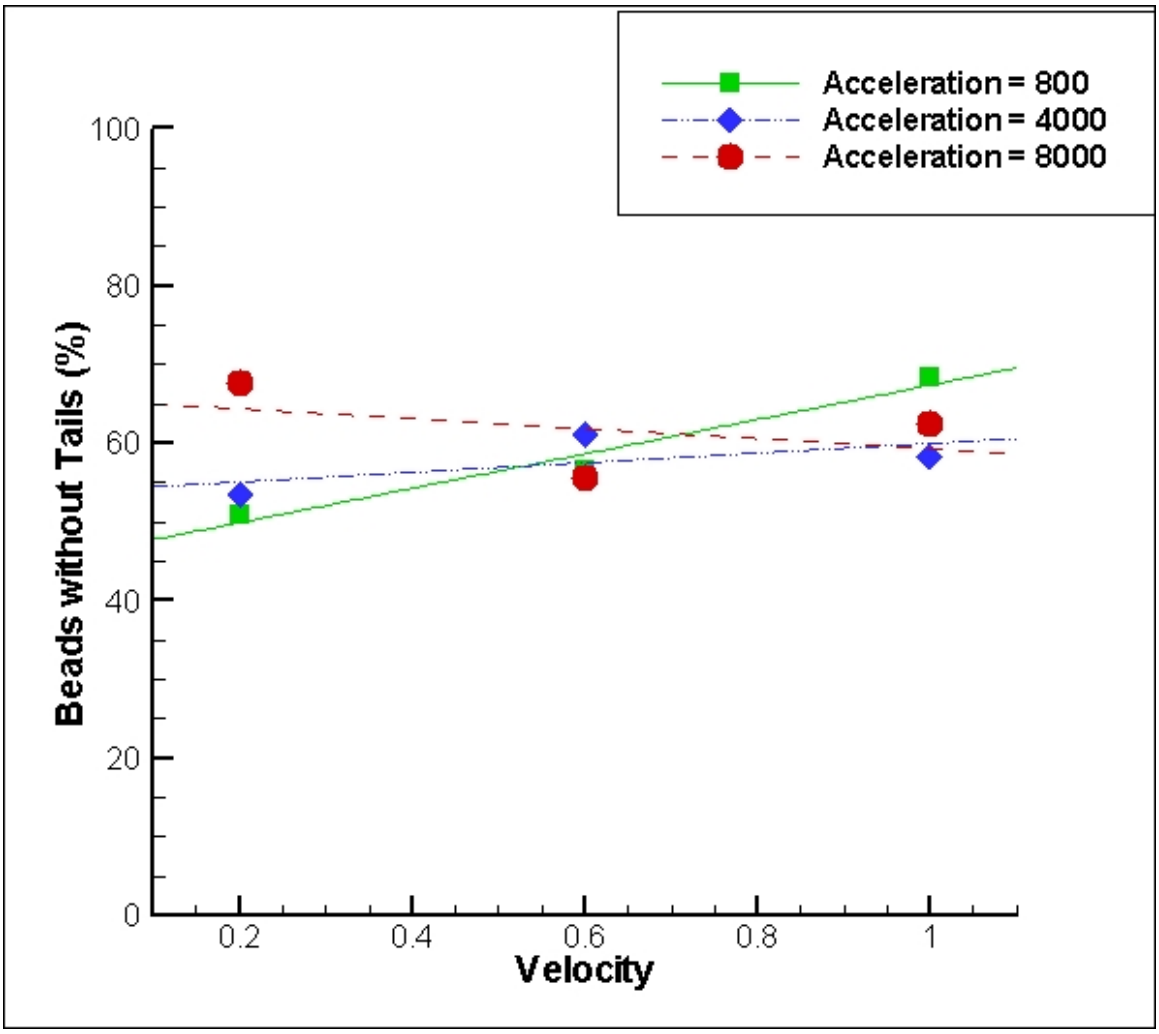

Figure 6-20: Beads without Tails as a function of Velocity for Acceleration. (1.8% LVG)

This analysis is based on a 2-way interaction; therefore the only variables of interest are acceleration and velocity. From this graph, the trend shows a decrease as acceleration increases. The range is higher than that of the 1.5 % sample, which shows improvement on the samples.

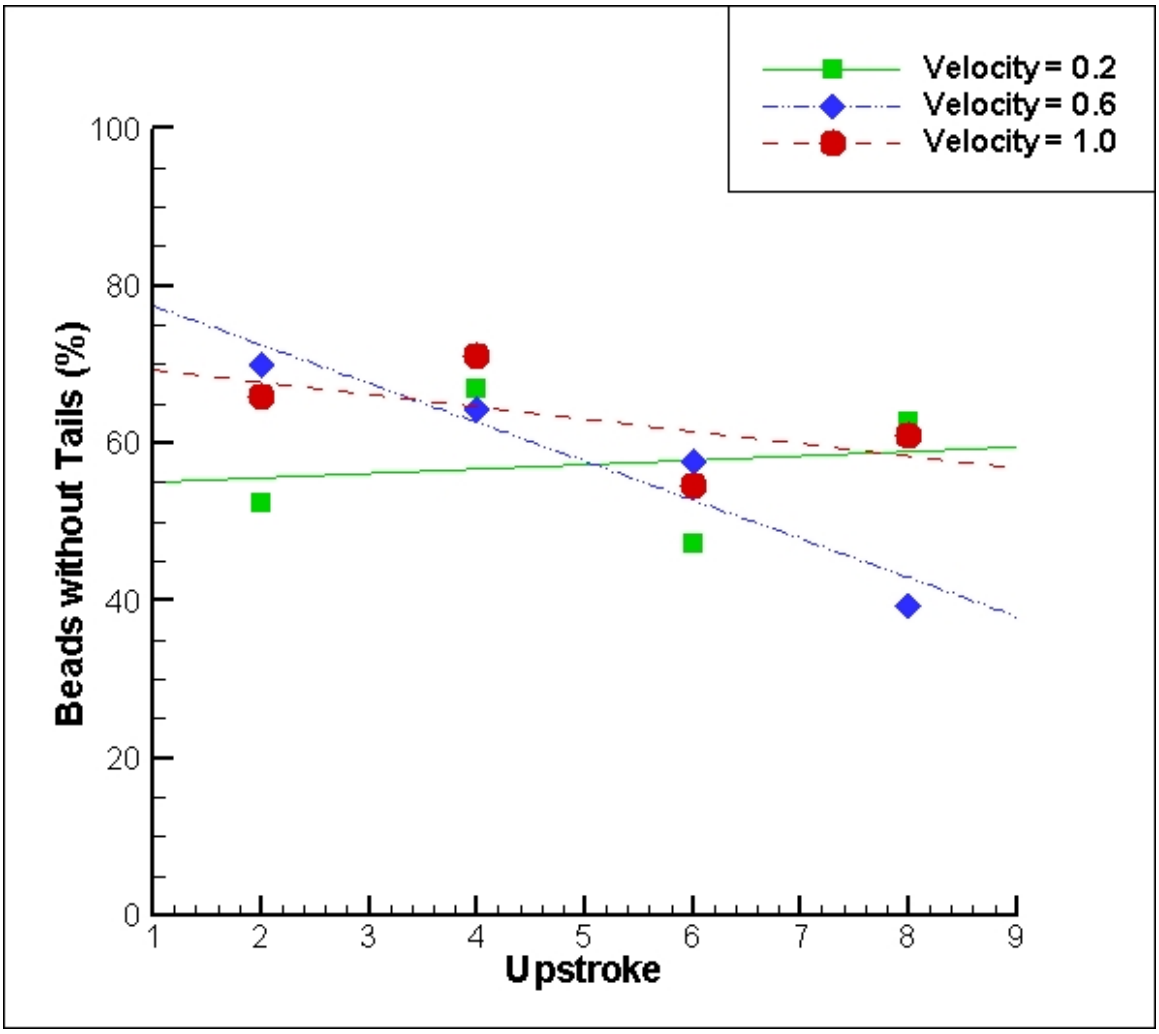

Figure 6-21: Beads without Tails as a function of Upstroke for Velocity =0.2, 0.6, and 1.0. (1.8% LVG)

From these results, a linear relationship is present for each velocity value. Though the slopes are different for each, the linearity is evident. The variability stays relatively constant as the upstroke increases. The percentage of the sample without tails is much higher than that produced with the 1.5 % sample.

6.5.3. 1.5% LVG through Cell Ejector Plate with 6 Holes

As stated earlier, the cell ejector plate used in these studies has 6 holes drilled in a given pattern. The study was done using the same variables as the previous studies, but alginate beads were not produced with all of the combinations. This data is shown to have a lower number of degrees of freedom (*Table 6-6).* The tests did not work for upstroke = 8 or for a velocity = 0.2. During the tests that did not work, a solid stream was produced instead of droplets.

Table 6-6: Class level information for 1.5% LVG through 6 hole cell ejector plate

Class	Levels	Values
Velocity	2	0.6, 1.0
Upstroke	3	2, 4, 6
Acceleration	3	800, 4000, 8000

This decreases the levels of differences for the data. As is shown in the table below, there is a two-way interaction between velocity and upstroke, and a three-way interaction, in the bead diameter data (*Table 6-7*). Unfortunately, nothing can be predicted on the tail data, because the p-values are too high. Therefore, the data is not comparable across variables. The graph for the bead diameter is shown below (*Figures 6-22)*.

Table 6-7: ANOVA Analysis for 1.5 % LVG through 6 hole cell ejector plate

Dependent		Diameter		Tail	
Source	**DF**	**F Value**	**P Value**	**F Value**	**P Value**
V	1	0.1	0.7499	3.18	0.0752
U	2	12.73	<0.0001	1.54	0.2152
V*U	2	22.9	<0.0001	0.03	0.971
Accel	2	0.76	0.4662	2.19	0.1132
V*A	2	1.39	0.2512	1.8	0.167
U*A	4	1.25	0.2871	1.66	0.1584
V*U*A	4	2.12	0.0769	0.68	0.6078

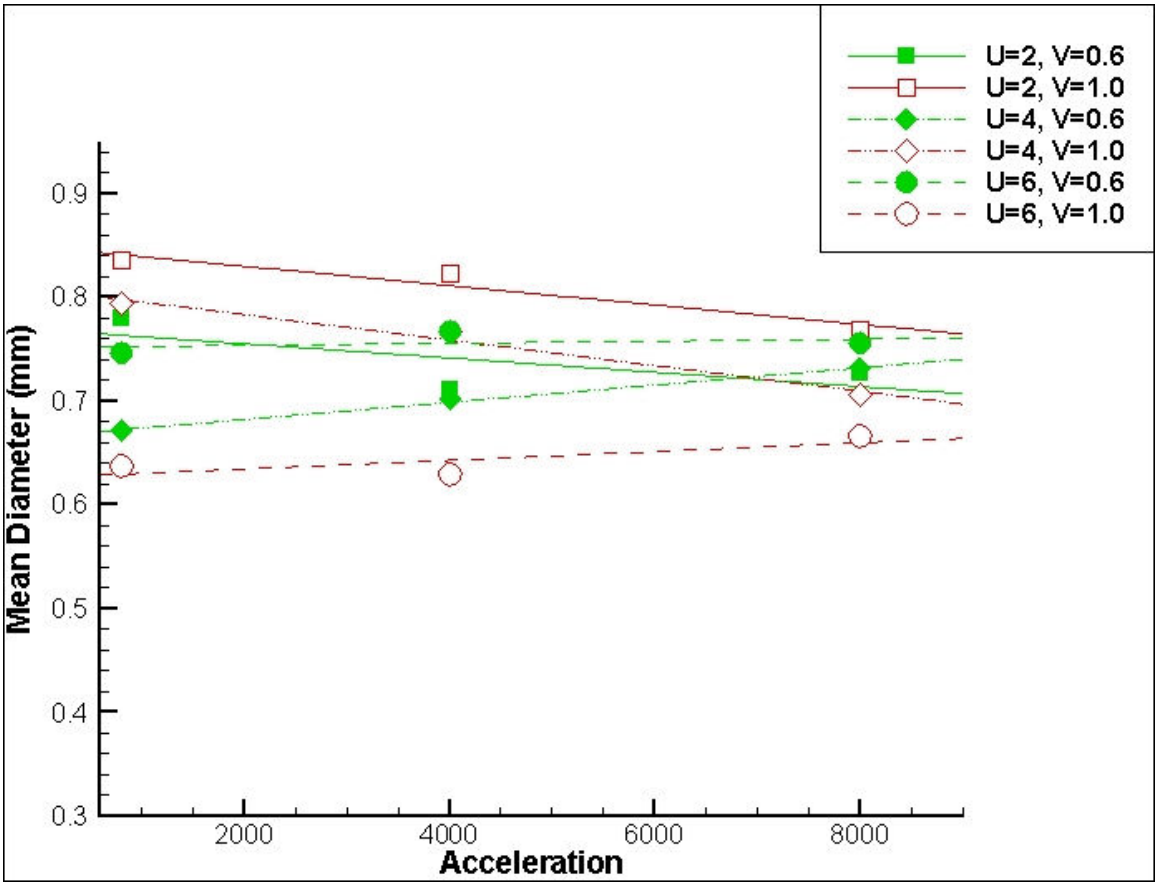

Figure 6-22: Mean Diameter as a function of Acceleration all values of Upstroke and Velocity. (1.5% LVG, through 6 holes)

From this graph, a strong linearity is observed for all values of upstroke and velocity. The variability decreases as acceleration increases. Therefore the range of mean bead diameter decreases as the acceleration increases. As the velocity increases the bead diameter decreases, at the extremum of upstroke values. The mean diameters observed from this data is well within the range specified, 0.4 to 0.9 mm.

6.5.4. 1.8% LVG through Cell Ejector Plate with 6 Holes

The same cell ejector plate is used in this study, and the velocities are still limited to 0.6, and 1.0. The beads did not exit the plate independently for a velocity of 0.2 or an upstroke of 8. By using six holes, the beads are produced at a higher rate, but at the lower velocity or the higher upstroke, the streams come together to form one large stream. This does not produce beads. The ANOVA analysis is given in the following tables (*Tables 6-8 and 6-9).*

Table 6-8: Class level information for 1.8% LVG through 6 hole cell ejector

Class	Levels	Values
Velocity	2	0.6, 1.0
Upstroke	3	2, 4, 6
Acceleration	3	800, 4000, 8000

The interactions of interest for the bead diameters are all three possible 2-way interactions. The strongest influence will be from the velocity-acceleration interaction because the f-value is the highest among the 2-way interactions. Unfortunately the p-value for the bead tails is still too high to examine any possible interactions. The bead diameter data is given below (*Figures 6-23 and 6-24).*

Table 6-9: ANOVA Analysis for 1.8 % LVG through 6 hole cell ejector plate

Dependent		Diameter		Tail	
Source	**DF**	**F Value**	**P Value**	**F Value**	**P Value**
V	1	5.31	0.0217	0.85	0.3582
U	2	12.48	<0.0001	1.83	0.1617
V*U	2	3.77	0.0238	0.96	0.3842
Accel	2	15.06	<0.0001	0.48	0.6217
V*A	2	20.1	<0.0001	0.46	0.6346
U*A	4	4.12	0.0027	0.95	0.4327
V*U*A	4	1.55	0.186	0.5	0.733

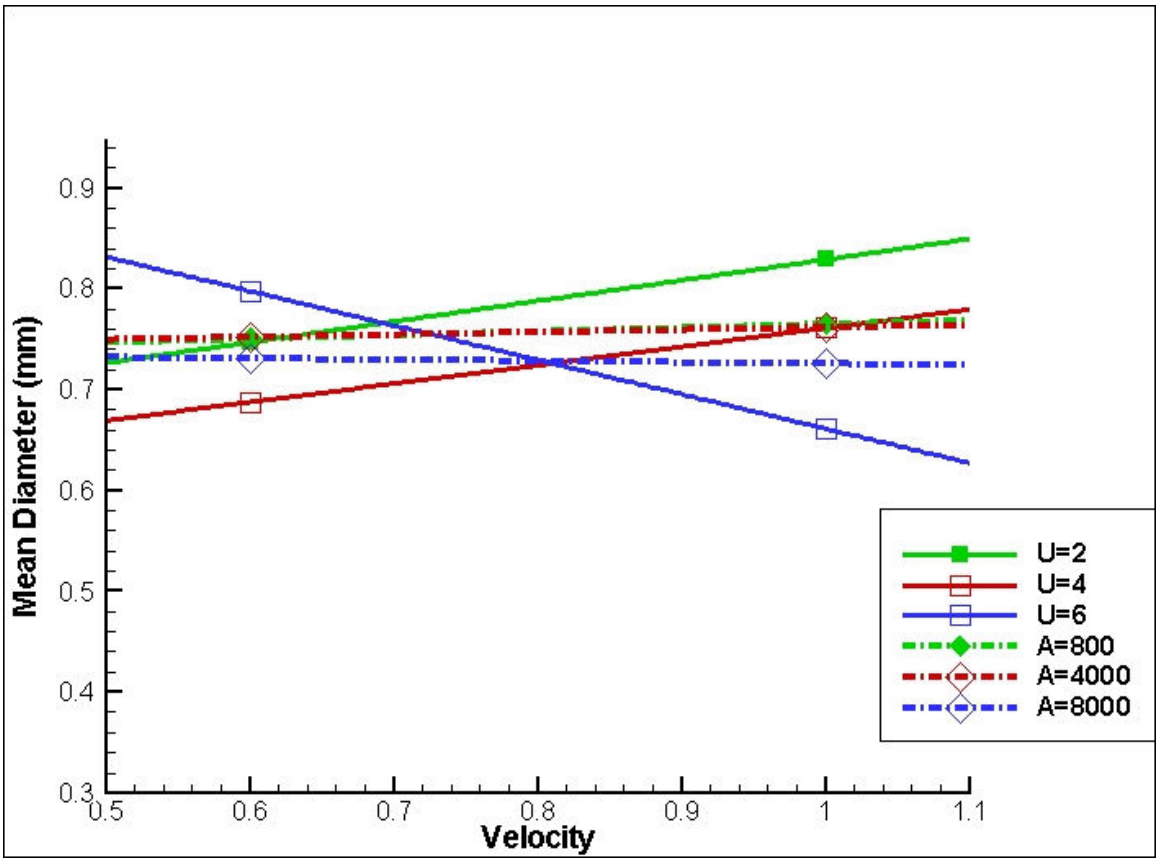

Figure 6-23: Mean Diameter as a function of Velocity, for Upstroke and Acceleration. (1.8% LVG, through 6 holes)

For the acceleration range, the variability is low. Therefore, as the velocity increases, the mean diameter of the beads stays approximately constant for each acceleration level. The mean diameter is also approximately constant over the velocity range. This graph also shows that the bead diameter increases as upstroke and velocity increase. The mean diameter is well within the given range for this data. Therefore, the 6 hole plate can be used to produce the same size beads as the 1 hole plate.

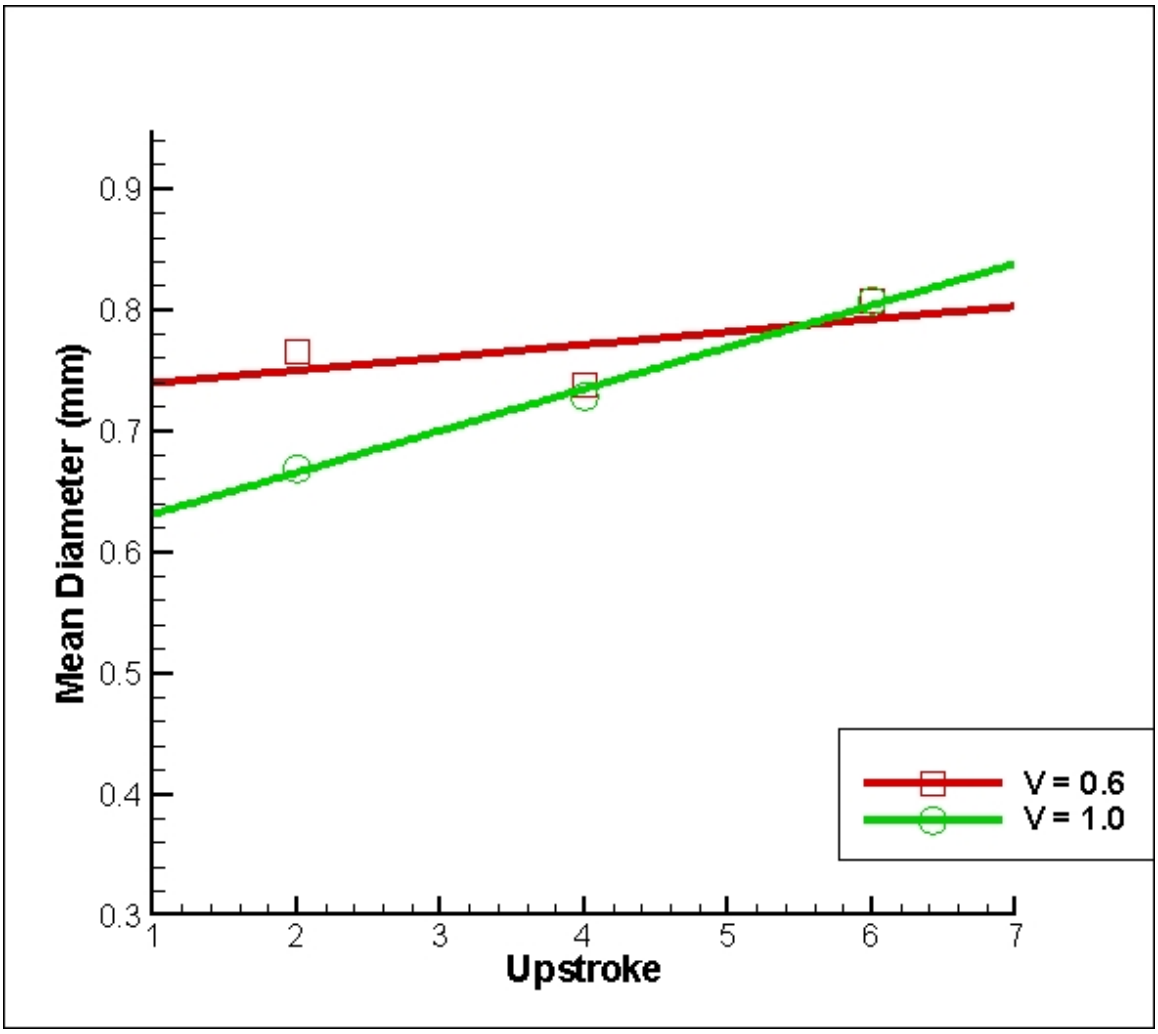

Figure 6-24: Mean Diameter as a function of Upstroke, for Velocity = 0.6 and 1.0. (1.8% LVG, through 6 holes)

This data set shows that the variability decreases as upstroke increases. The mean diameter is approximately the same for both velocities at the upstroke maximum. Again, the mean diameter is within the same range.

6.6. Microencapsulation of Polystyrene Microspheres

The second object for this project was to encapsulate microspheres. This demonstrates that the encapsulator will be useful for the encapsulation of

biological cells at a later time. The samples were run with the same test parameters as the previous tests, using the cell ejector plate with 1 hole. The bead diameters were measured, and the polymorphism was observed. With each sample, an average of 2 microspheres was encapsulated in each alginate capsule. This was expected based on the diameter of the microspheres used in this study.

6.6.1. 1.5 % LVG with Polystyrene Microspheres

As stated earlier, the variables are limited on this study, in the same manner as the two previous studies. The samples could not be tested for a velocity = 0.2 or an upstroke = 8. The results of this section are given in the ANOVA tables (*Tables 6-10 and 6-11).*

Table 6-10: Class level information for 1.5% LVG, polystyrene microspheres

Class	Levels	Values
Velocity	2	0.6, 1.0
Upstroke	3	2, 4, 6
Acceleration	3	800, 4000, 8000

The only effects from this analysis on the beads diameter are from upstroke and a 2-way interaction between velocity and upstroke on the bead diameter (*Table 6-11*). The p-values are too high, showing that the effects of the

three variables are not predictable on the presence of tails. The bead diameter data is shown in the figure below (*Figure 6-25)*. The mean bead diameter decreases with upstroke for a velocity of 1.0. Inversely, the mean bead diameter increases with upstroke for a velocity of 0.6. The mean bead diameter in these graphs does not show a trend that matches the trends from the previous data (*Figure 6-25*). With this data, the tests need to be rerun to find a trend.

Table 6-11: ANOVA Analysis for 1.5 % LVG, polystyrene microspheres

Dependent		Diameter		Tail	
Source	**DF**	**F Value**	**P Value**	**F Value**	**P Value**
V	1	0.2	0.6536	0.74	0.39
U	2	8.33	0.0003	1.18	0.3086
V*U	2	25.47	<0.0001	0.85	0.4281
Accel	2	1.87	0.1558	0.85	0.4281
V*A	2	0.18	0.8384	0.52	0.5943
U*A	4	0.32	0.8642	1.17	0.3255
V*U*A	4	2.16	0.0724	0.59	0.6704

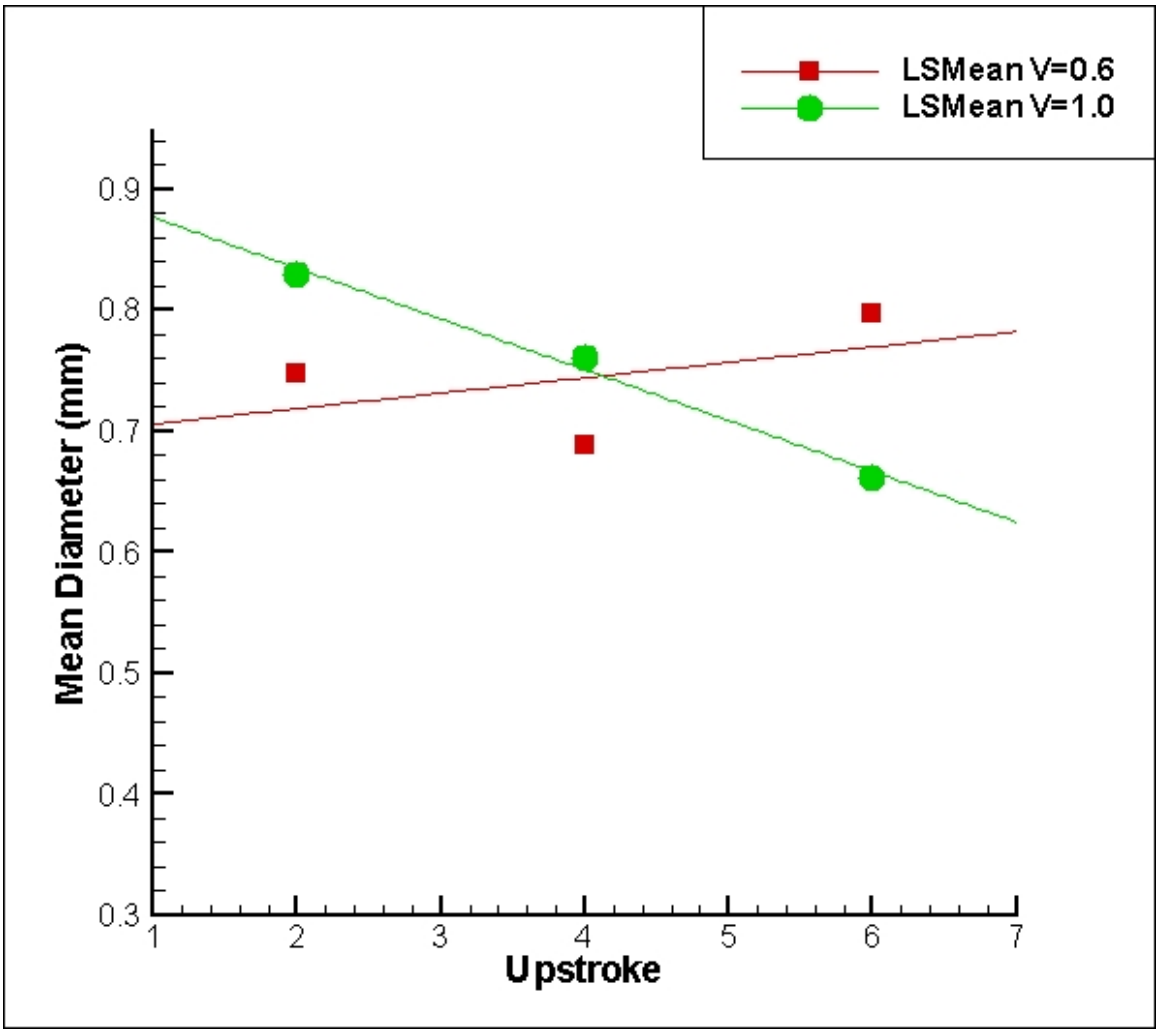

Figure 6-25: Mean Diameter as a function of Upstroke, for Velocity. (1.8% LVG, through 6 holes)

6.6.2. 1.8% LVG with Polystyrene Microspheres

These tests were run with the same setup as the previous test run, but with 1.8 % LVG alginate. The ANOVA results are shown below (*Tables 6-12 and 6-13*). There are large effects on the mean bead diameter from the upstroke and the acceleration. The 2-way interactions are present from all three variables, and the 3-way interaction is present. The p-values from the tail data is

too high for testing with this data. The 3-way interaction is shown below (*Figure 6-22*). From this data, the variability of the mean bead diameter is higher at the maximum acceleration. The slope trend decreases as velocity increases for upstroke values of 2 and 4, but it is constant as velocity increases for an upstroke value of 6. The mean bead diameters are within the given range for all values of acceleration.

For both concentrations of alginate, beads are consistently being produced with a mean diameter of approximately 0.8 mm (*Figures 6-13, 6-14, 6-17, and 6-18*). For all cases using 1.8 % LVG, the trends are the same (*Figures 6-17, 6-18, and 6-25*). Therefore, the addition of polystyrene beads to the mixture does not have significant effects on the bead diameter produced.

Table 6-12: Class level information for 1.8% LVG, polystyrene microspheres

Class	Levels	Values
Velocity	2	0.6, 1.0
Upstroke	3	2, 4, 6
Acceleration	3	800, 4000, 8000

Table 6-13: ANOVA Analysis for 1.8 % LVG, polystyrene microspheres

Dependent		Diameter		Tail	
Source	**DF**	**F Value**	**P Value**	**F Value**	**P Value**
V	1	2.63	0.1054	0.74	0.39
U	2	13.54	<0.0001	1.18	0.3086
V*U	2	11.23	<0.0001	0.85	0.4281
Accel	2	18.31	<0.0001	0.85	0.4281
V*A	2	19.49	<0.0001	0.52	0.5943
U*A	4	2.67	0.0317	1.17	0.3255
V*U*A	4	4.7	0.001	0.59	0.6704

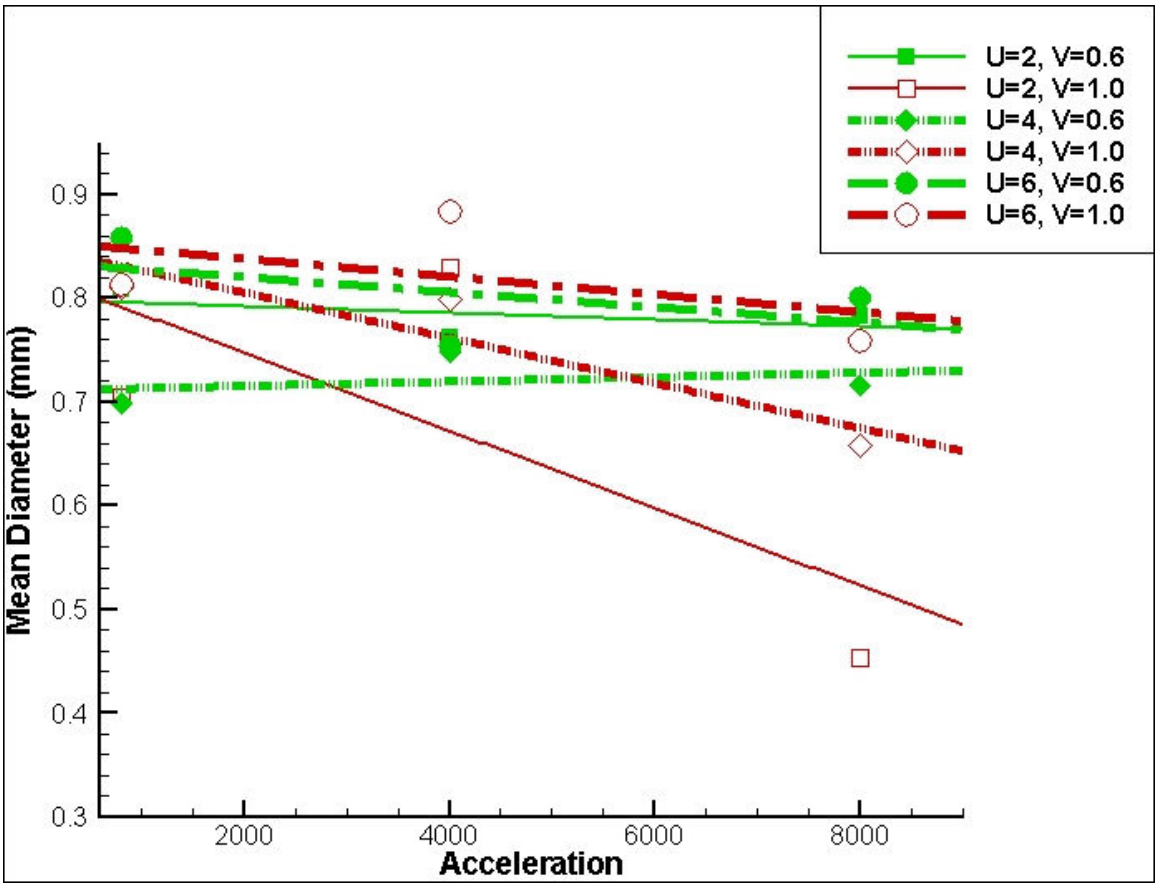

Figure 6-26: Mean Diameter as a function of Acceleration, for Velocity and Upstroke. (1.8% LVG, polystyrene microspheres)

6.7. Bead Diameter Comparisons across Experimental Sets

From the above data, comparisons should be made based on the two-way interaction variables. Since each of the data sets has the same two-way variable interaction, velocity to upstroke, the LS Means can be compared. The comparisons are made based on the bead diameter for each of the data sets with respect to viscosity. The first two sets are separated based on the viscosity, while the second two sets are based on the cell ejector plate used in the experiment. These sets are shown in the figures below (*Figures 6-27 and 6-28).*

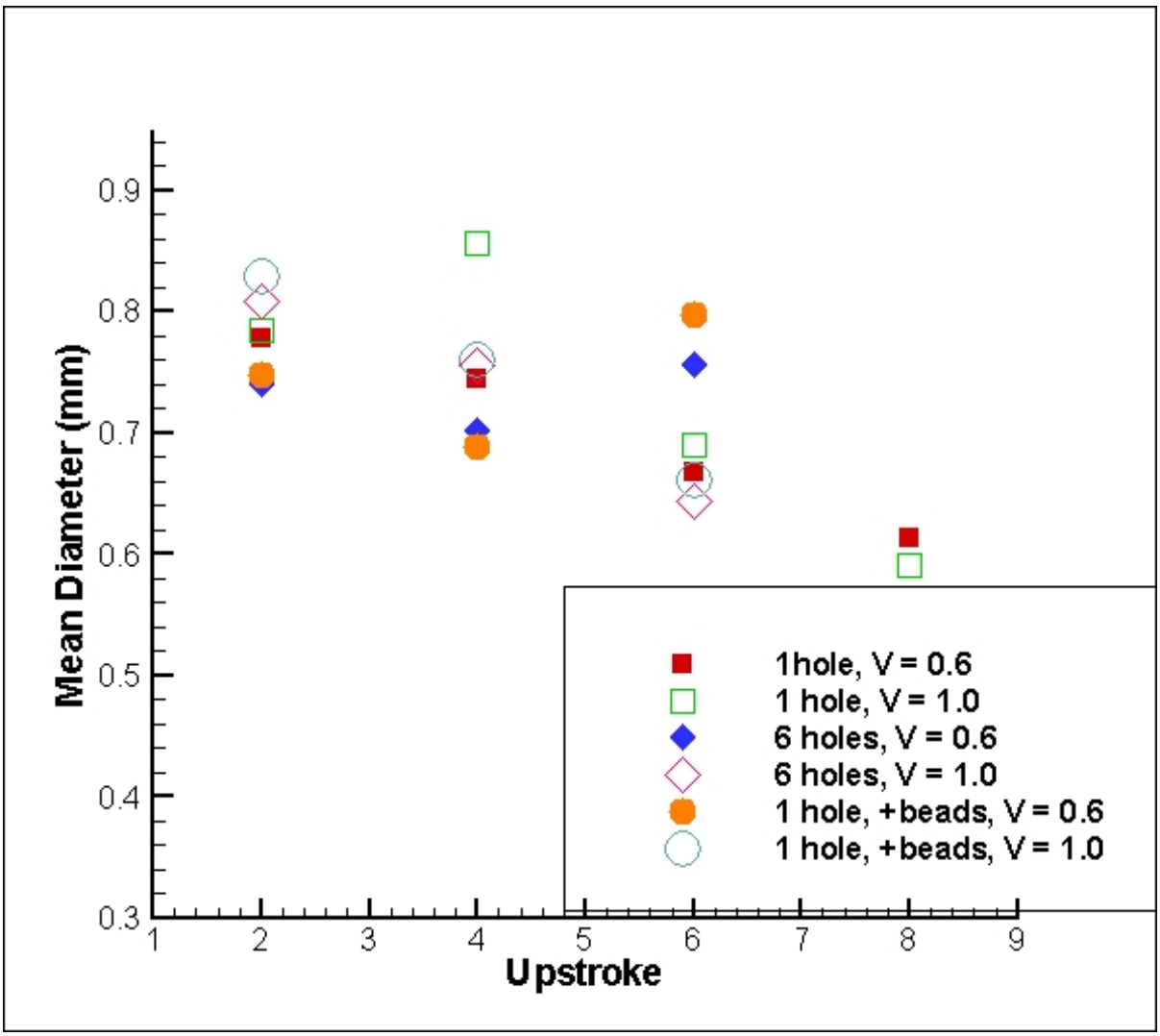

Figure 6-27: Mean Diameter as a function of Upstroke. (1.5 % LVG)

The mean diameter decreases as the upstroke increases for most cases (*Figure 6-27)*. This is inversely true for the following cases:

- 1 hole, V = 0.2, 0.6
- 6 holes, V = 0.6.

The variability is lowest at the minimum upstroke value and increases as the upstroke increases. The mean diameter values are within the given range, 0.4 to 0.9 mm, for all cases of 1.5 % LVG in this study.

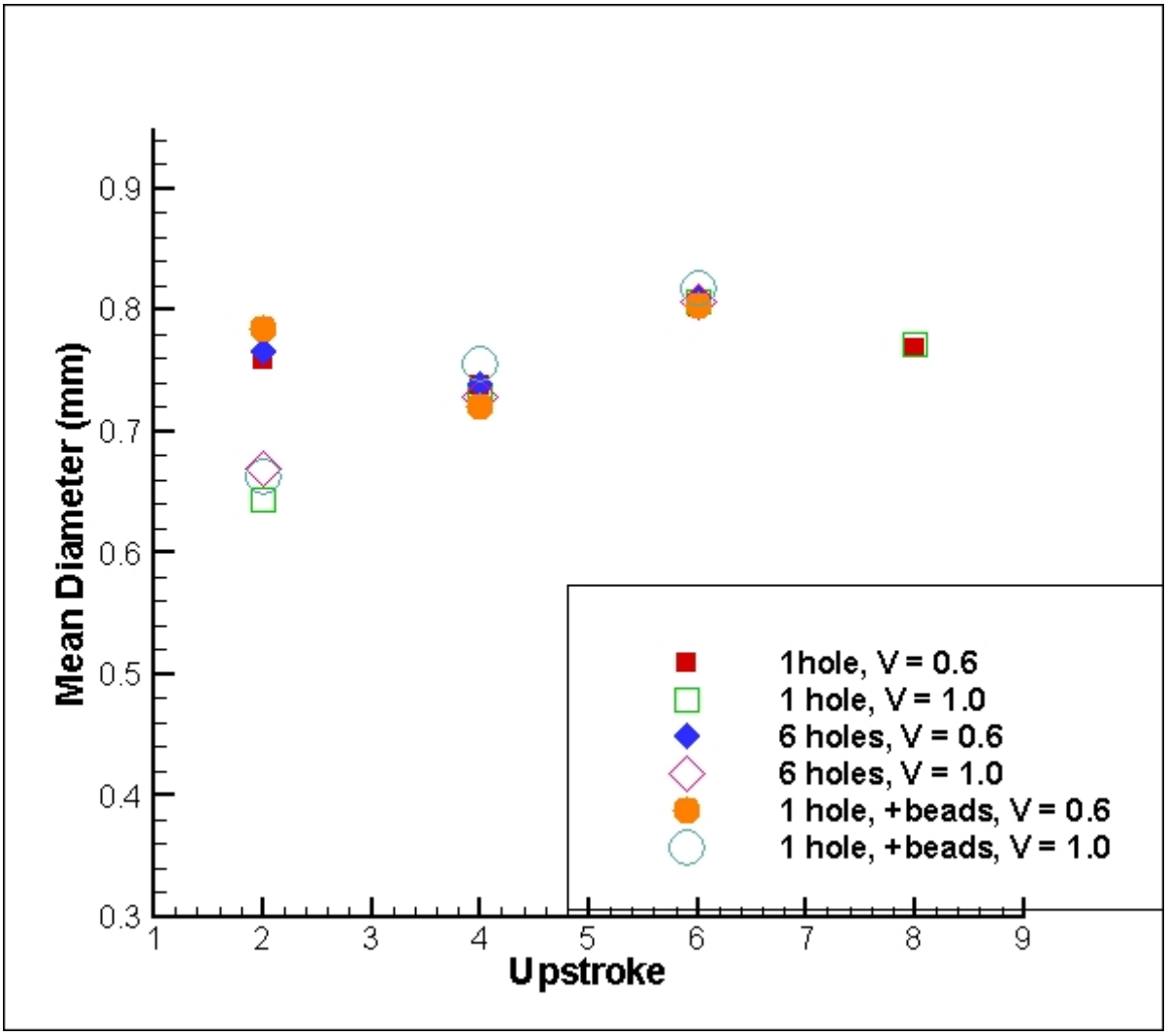

Figure 6-28: Mean Diameter as a function of Upstroke. (1.8 % LVG)

The variability decreases as the upstroke increases with these cases (*Figure 6-28*). A positive slope describes all cases where the velocity is 1, and as the upstroke increases, the mean diameter increases. The slope is approximately constant for all cases where the velocity is 0.6, and further the mean diameter is independent of the upstroke. The mean diameter is within the given range for all cases with 1.8 % LVG in this study.

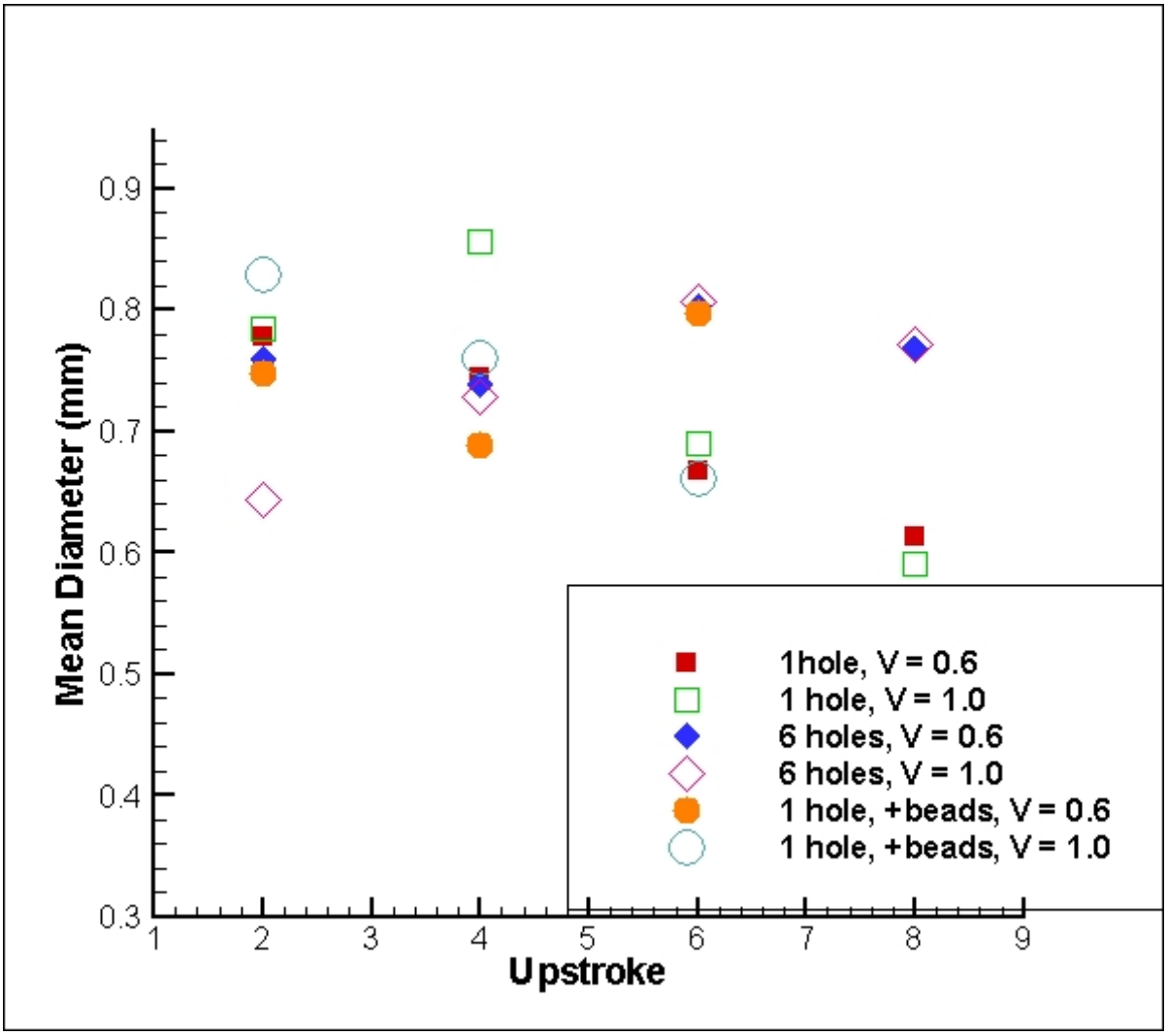

Figure 6-29: Mean Diameter as a function of Upstroke. (1 hole plate)

The mean diameter decreases as the upstroke increases for the following cases (*Figure 6-29*):

- 1.5 % LVG, V = 0.6, 1.0
- 1.5 % LVG + beads, V = 0.6

The inverse is true, meaning the mean bead diameter increases as the upstroke increases for the following:

- ALL samples made with 1.8 % LVG
- 1.5 % LVG + beads, V = 1.0

There are some outliers with this data, which could further be studied by repeating these tests.

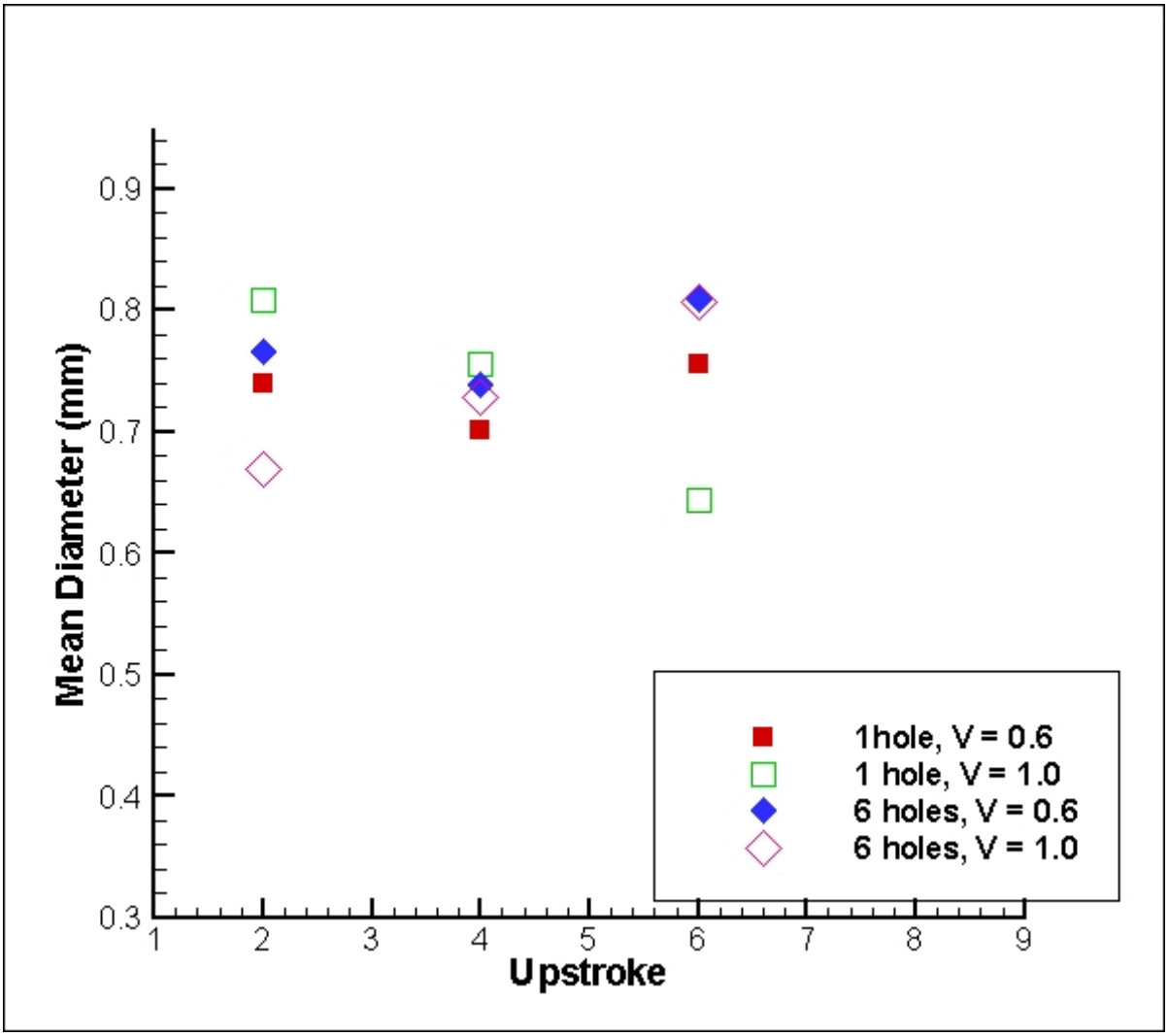

Figure 6-30: Mean Diameter as a function of Upstroke. (6 hole plate)

This final graph shows that the mean diameter increases as the upstroke increases for the 1.8 % LVG samples (*Figure 6-30*). The results from the 1.5 % LVG samples show that the mean diameter either decrease or stay relatively constant as the upstroke increases. The variability is highest at the extremes of the upstroke. These trends could be further studied by repeating the tests as well.

7. Conclusions

This study has identified important factors that affect the diameter and polymorphism of alginate microcapsules and alginate coated polystyrene microspheres. An encapsulator has been designed and fabricated in order to produce microencapsulated particles. An effective procedure has been developed for the production of large quantities of microcapsules if viable microcapsules. By examining the variables that control the diameter and sphericity of the microcapsules, the procedure can be optimized to consistently produce stable, viable samples.

The question that was sought to answer in this study was what effect the mechanical variables of the encapsulator would have on the size and sphericity of the microbead that is generated. The data shows that the spherical beads can be produced with mean diameters between 0.4 and 0.9 mm. The percentage of the samples without tails ranges between 20 and 90 %. The number of beads produced is easily increased with an increased number of

holes in the cell ejector plate, while keeping the mean diameters between the same range, and limiting the polymorphism.

The microcapsules were produced again with the addition of polystyrene beads. With the same sample experimental variables, the mean diameters in the range of 0.4 to 0.85 mm. In addition, the microparticles encapsulated were approximately 1 to 3 per encapsulated beads. At this rate, the bead production is increased six-fold, and the beads are kept to the same size. Despite the low number of samples without tails, the percentages are still the same or better than previous studies have shown. Therefore, based on the previous report of production standards, the production time is decreased from over 200 hours to approximately 35 hours. This time can be reduction additional by increasing the number of holes in the cell ejector plate, increasing the number of beads per sample without tails, and increasing the cross-linking bath volume.

A new encapsulation device allows for consistent microcapsule production, which can be modified for further optimization. In order to use higher viscosity alginate samples, the motor should be resized. With this change, and keeping the cell ejector plates the same, microcapsules of a similar diameter range can be produced.

8. References

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Appendices

9.1. Compumotor 6K8 Onboard Programmable I/O's

Table 0-1 - Onboard Programmable I/O's for Axis 5-8

6K8 Control Function	Bit #	Pin #	Function for Brim Curling Machine
Positive End of Travel Limit, Axis 5	1	23	Positive Limit
Negative End of Travel Limit, Axis 5	2	21	Negative Limit
Home Limit, Axis 5	3	19	Home for Ultramotion Linear Actuator
Positive End of Travel Limit, Axis 6	4	17	n/c
Negative End of Travel Limit, Axis 6	5	15	n/c
Home Limit, Axis 6	6	13	n/c
Positive End of Travel Limit, Axis 7	7	11	n/c
Negative End of Travel Limit, Axis 7	8	9	n/c
Home Limit, Axis 7	9	7	n/c
Positive End of Travel Limit, Axis 8	10	5	n/c
Negative End of Travel Limit, Axis 8	11	3	n/c
Home Limit, Axis 8	12	1	n/c

Note: All even pin #'s correspond to earth ground (GND)

9.2. S Drive Connections to 6K8 Controller

Table 0-2 – Parker S Drive Stepping Motor Connections

S-Drive Signal Name	Pin #	Compumotor 6K8 Signal Name	Pin #
Step +	8	Step +	1
Step -	9	Step -	9
Direction +	10	Direction +	2
Direction -	11	Direction -	10
Enable	1	Shut uo	7
Ground	17	Shut Common	14
Stall Out	46	Stall	4
Fault Out	43	Fault	5
Ground	44	Iso Command	13

9.3. Microencapsulator Motion Program

Motion Program file for Microencapsulator
Written by: Sarah Fisher

```
; WIZID = 00010040
; WIZID = 00010100
;Scaling Setup
;Distance Units - counts,counts,counts,counts,counts,counts,counts,counts
SCLD 300000,1,1,1,300,1,1,1
;Velocity Units - rev/s,rev/s,rev/s,rev/s,rev/s,rev/s,rev/s,rev/s
SCLV 25000,8000,1,8000,25000,8000,8000,8000
;Acceleration Units - rev/s/s,rev/s/s,rev/s/s,rev/s/s,rev/s/s,rev/s/s,rev/s/s,rev/s/s
SCLA 25000,8000,1,8000,25000,8000,8000,8000
SCALE1


;----------
;Setup Program
DEL SETUP
DEF SETUP
FOLMAS 0,0,0,0,0,0,0,0
FOLEN00000000
```

```
  ; WIZID = 00010060
  ;Enable Mode Code
  DRIVE00000000
  ; WIZID = 00010080
  ;Drive Setup
  ;Axis 1, Servo Control, No Drive
  ;Axis 2, Servo Control, No Drive
  ;Axis 3, Servo Control, No Drive
  ;Axis 4, Servo Control, No Drive
  ;Axis 5, Stepper Control, Gemini GT Drive, ULTRAMOTION
  ;Axis 6, Servo Control, No Drive
  ;Axis 7, Servo Control, No Drive
  ;Axis 8, Servo Control, No Drive
  AXSDEF 11110111
  DRFLVL 11111111
  DRFEN 00001000
  KDRIVE 0000X000
  DRES ,,,,25000,,,
  PULSE ,,,, 0.3,,,
  DSTALL XXXX0XXX

  ; WIZID = 00010100
;Scaling Setup
;Because scaling commands are not allowed in a program,
;the scaling commands will be placed at the beginning
;of the program file.  This insures that motion programs
;in subsequent programs will be scaled correctly.

  ; WIZID = 00010120
  ;Feedback Setup
  SFB 1,1,1,1,,1,1,1
  ERES 4000,4000,4000,4000,,4000,4000,4000
  SMPER 0,4000,0,4000,,4000,4000,4000
  EFAIL 0000X000
  ENCPOL 0000X000
  ENCSND 0000X000
  ESTALL XXXXXXXX
  ESK XXXXXXXX
  ENCCNT XXXXXXXX
  ; WIZID = 00010140
  ;Hardware Limit Setup
  LH 3,3,0,3,3,3,3,3
  LHAD 100,100,100,100,100,100,100,100
  LHADA 100,100,100,100,100,100,100,100

  ;Software Limit Setup
```

```
LS 0,0,0,0,0,0,0,0
LSAD 100,100,100,100,100,100,100,100
LSADA 100,100,100,100,100,100,100,100
LSNEG 0,0,0,0,0,0,0,0
LSPOS 0,0,0,0,0,0,0,0

;Home Limit Setup
HOMA 10,10,10,10,10,10,10,10
HOMAA 10,10,10,10,10,10,10,10
HOMV 1,1,1,1,1,1,1,1
HOMAD 10,10,10,10,10,10,10,10
HOMADA 10,10,10,10,10,10,10,10
HOMBAC 10000000
HOMZ 01111111
HOMDF 10000000
HOMVF 1,0,0,0,0,0,0,0
HOMEDG 00000000

LIMLVL 111000000000110000000000
; WIZID = 00010160
;Variable Setup
VAR2 = 0.00000000 ;DOWNSTROKE
VAR3 = 0.00000000 ;UPSTROKE
VAR4 = 0.00000000 ;FSVARR04
VAR6 = 0.00000000 ;FSVARR06
VARI1 = 0 ;FSVARI01
VARI2 = 0 ;FSVARI02
VARI3 = 0 ;FSVARI03
VARI4 = 0 ;FSVARI04
VARI5 = 0 ;ACCELERATIONLIN
VARI6 = 0 ;VELOCITYLIN
VARS1 = "0" ;PISTONCYCLES
VARS2 = "0" ;PISTONDOWNSTROKE
VARS3 = "0" ;PISTONUPSTROKE
VARS4 = "0" ;REPEAT
VARS5 = "0" ;ACCELERATION
VARS6 = "0" ;VELOCITY

; WIZID = 00010180
;Command Processing Code

; WIZID = 00010200
;Enable Mode Code
DRIVE00001000
;Error Setup
;To modify the error bits,
```

```
  ;Please double-click on your Error Program

  ; Error program setup
  ERRORP CRASH ; Error program setup
  ERROR XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX ; Bits tested
END

; WIZID = 00010220
;-------------
;Main Program
DEL MAIN
DEF MAIN

  ; WIZID = 00010240
  RUN SETUP
  ; WIZID = 00010260
  RUN HOMLIN
  ; WIZID = 00010280
  RUN PMSUCK
  ; WIZID = 00010300
  RUN AGAIN
  ; WIZID = 00010320
  ;Loop Action
  REPEAT
    ; WIZID = 00010340
    ;If Code
    IF(VARI3=1)
      ; WIZID = 00010360
      ;Assignment Code
      VARI2 = VARI1 +1 ;

      ; WIZID = 00010380
      ;Loop Action
      REPEAT
        ; WIZID = 00010400
        ;Motion Code
        MA XXXX0XXX
        A ,,,,(VARI5),,,
        AA ,,,,0,,,
        AD ,,,,(VARI5),,,
        V ,,,,(VAR6),,,
        D ,,,,(VAR2),,,
        MC XXXX0XXX

        ; WIZID = 00010420
        ;Go Code
```

```
      GO 00001000
      ; WIZID = 00010440
      ;Native Code module
      write"Down"

TPE


      ; WIZID = 00010460
      ;Motion Code
      MA XXXX0XXX
      A ,,,,(VARI5),,,
      AA ,,,,0,,,
      AD ,,,,(VARI5),,,
      V ,,,,(VAR6),,,
      D ,,,,(VAR3),,,
      MC XXXX0XXX

      ; WIZID = 00010480
      ;Go Code
      GO 00001000
      ; WIZID = 00010500
      ;Assignment Code
      VARI2 = VARI2 -1 ;

    ; WIZID = 00010520
    UNTIL(VARI2=1)
    ; WIZID = 00010540
    RUN AGAIN
  ; WIZID = 00010560
  ELSE
  ; WIZID = 00010580
  NIF
  ; WIZID = 00010600
  ;If Code
  IF(VARI3=2)
    ; WIZID = 00010620
    RUN PUMPCY
    ; WIZID = 00010640
    RUN PISTDN
    ; WIZID = 00010660
    RUN PISTUP
    ; WIZID = 00010680
    RUN SETACC
    ; WIZID = 00010700
    RUN SETVEL
```

```
    ; WIZID = 00010720
    ;Assignment Code
    VARI2 = VARI1 +1 ;

    ; WIZID = 00010740
    ;Native Code module
    write"I am fixin to git r done"


    ; WIZID = 00010760
    ;Wait Code
    T 5.000


    ; WIZID = 00010780
    ;Loop Action
    REPEAT
      ; WIZID = 00010800
      ;Motion Code
      MA XXXX0XXX
      A ,,,,(VARI5),,,
      AA ,,,,0,,,
      AD ,,,,(VARI5),,,
      V ,,,,(VAR6),,,
      D ,,,,(VAR2),,,
      MC XXXX0XXX

      ; WIZID = 00010820
      ;Go Code
      GO 00001000
      ; WIZID = 00010840
      ;Native Code module
      write"Down"

TPE


      ; WIZID = 00010860
      ;Motion Code
      MA XXXX0XXX
      A ,,,,(VARI5),,,
      AA ,,,,0,,,
      AD ,,,,(VARI5),,,
      V ,,,,(VAR6),,,
      D ,,,,(VAR3),,,
      MC XXXX0XXX
```

```
        ; WIZID = 00010880
        ;Go Code
        GO 00001000
        ; WIZID = 00010900
        ;Assignment Code
        VARI2 = VARI2 -1 ;

      ; WIZID = 00010920
      UNTIL(VARI2=1)
      ; WIZID = 00010940
      RUN AGAIN
    ; WIZID = 00010960
    ELSE
    ; WIZID = 00010980
    NIF
    ; WIZID = 00011000
    ;If Code
    IF(VARI3=3)
      ; WIZID = 00011020
      RUN HOMLIN
      ; WIZID = 00011040
      RUN PMSUCK
      ; WIZID = 00011060
      RUN AGAIN
    ; WIZID = 00011080
    ELSE
    ; WIZID = 00011100
    NIF
  ; WIZID = 00011120
  UNTIL(VARI3=4)
END
STARTP CLR

; WIZID = 00011140
;-------------
;User Program
;Subroutine to Home ULTRA Motion Actuator
DEL HOMLIN
DEF HOMLIN


  ; WIZID = 00011160
  ;Native Code module
  Write"Homing ULTRA Motion Actuator"
```

```
  ; WIZID = 00011180
  ;Home Code
  HOM xxxx0xxx

  ; WIZID = 00011200
  ;Motion Code
  MA XXXX0XXX
  A ,,,,10,,,
  AA ,,,,0,,,
  AD ,,,,10,,,
  V ,,,,1,,,
  D ,,,,-150,,,
  MC XXXX0XXX

  ; WIZID = 00011220
  ;Go Code
  GO 00001000
  ; WIZID = 00011240
  ;Motion Parameter Code
  ;Distance
  D ,,,,50,,,


  ; WIZID = 00011260
  ;Go Code
  GO 00001000
  ; WIZID = 00011280
  ;Set Position Code
  PSET ,,,,0,,,
END

; WIZID = 00011300
;-------------
;User Program
;Subroutine to Test ULTRA Motion Movement in negative Z direction.
DEL GOIN
DEF GOIN


  ; WIZID = 00011320
  ;Motion Code
  MA XXXX0XXX
  A ,,,,10,,,
```

```
  AA ,,,,0,,,
  AD ,,,,10,,,
  V ,,,,1,,,
  D ,,,,250,,,
  MC XXXX0XXX

  ; WIZID = 00011340
  ;Go Code
  GO 00001000
END

; WIZID = 00011360
;-------------
;User Program
;Subroutine to test ULTRA Motion Actuator movement in negative Z direction
DEL GOOUT
DEF GOOUT


  ; WIZID = 00011380
  ;Motion Code
  MA XXXX0XXX
  A ,,,,10,,,
  AA ,,,,0,,,
  AD ,,,,10,,,
  V ,,,,1,,,
  D ,,,,-250,,,
  MC XXXX0XXX

  ; WIZID = 00011400
  ;Go Code
  GO 00001000
END

; WIZID = 00011420
;-------------
;User Program
;Subroutine to read in how many times to cycle piston
DEL PUMPCY
DEF PUMPCY


  ; WIZID = 00011440
  ;Loop Action
  REPEAT
    ; WIZID = 00011460
```

```
    ;Native Code module
    Write"Input number of Piston Cycles -- <!'number>"
VARS1=">"
VARI1 = READ1
WRVARI1

; WIZID = 00011580
;-------------
;User Program
;Subroutine to read in piston down stroke variable
DEL PISTDN
DEF PISTDN


  ; WIZID = 00011600
  ;Loop Action
  REPEAT
    ; WIZID = 00011620
    ;Native Code module
    Write"Input Piston Stroke (negative Z direction) -- <!'number>"
VARS2=">"
VAR2 = READ2
WRVAR2



    ; WIZID = 00011640
    ;If Code
    IF(VAR2>=0)
      ; WIZID = 00011660
      ;Native Code module
      write"Hello I need a negative Z input to start making your droplets"
    ; WIZID = 00011680
    ELSE
    ; WIZID = 00011700
    NIF
  ; WIZID = 00011720
  UNTIL(VAR2<0)
END

; WIZID = 00011740
;-------------
;User Program
;Subroutine to ask if run again with same parameters
DEL AGAIN
DEF AGAIN
```

```
  ; WIZID = 00011760
  ;Loop Action
  REPEAT
    ; WIZID = 00011780
    ;Native Code module
    Write"Option 1 - Run Again With Same Parameters From Current Z location --
<!'number>"
Write"Option 2 - Run Again With New Parameters From Current Z location --
<!'number> "
Write"Option 3 - Run Again With New Parameter REHomed - <!'number>"
Write"Option 4 - Exit -- <!'number>"
VARS4=">"
VARI3 = READ4
WRVARI3



    ; WIZID = 00011800
    ;If Code
    IF(VARI3<>1 AND VARI3<>2 AND VARI3<>3 AND VARI3<>4)
      ; WIZID = 00011820
      ;Native Code module
      Write"Make a decision it's not difficult"


    ; WIZID = 00011840
    ELSE
    ; WIZID = 00011860
    NIF
  ; WIZID = 00011880
  UNTIL(VARI3=1 OR VARI3=2 OR VARI3=3 OR VARI3=4)
END

; WIZID = 00011900
;-------------
;User Program
;Subroutine to read in piston up stroke variable
DEL PISTUP
DEF PISTUP


  ; WIZID = 00011920
  ;Loop Action
  REPEAT
```

```
    ; WIZID = 00011940
    ;Native Code module
    Write"Input Piston  Up Stroke (positive Z direction) -- <!'number>"
VARS3=">"
VAR3 = READ3
WRVAR3



    ; WIZID = 00011960
    ;If Code
    IF(VAR3<=-0.0001)
      ; WIZID = 00011980
      ;Native Code module
      write"Hello I need a positive Z input for the up stroke"

    ; WIZID = 00012000
    ELSE
    ; WIZID = 00012020
    NIF
  ; WIZID = 00012040
  UNTIL(VAR3>=0)
END

; WIZID = 00012060
;-------------
;User Program
;Subroutine to Oscillate ULTRA Motion Actuator to control piston stroke
DEL PUMP
DEF PUMP


  ; WIZID = 00012080
  ;If Code
  IF(LIM=bxxxxxxxxxxxxxx1)
    ; WIZID = 00012100
    ;Native Code module
    Write"ULTRA Motion is in the house"
    ; WIZID = 00012120
    RUN PUMPCY
    ; WIZID = 00012140
    ;Motion Code
    MA XXXX1XXX
    A ,,,,10,,,
    AA ,,,,0,,,
    AD ,,,,10,,,
```

```
V ,,,,1,,,
D ,,,,-0.5,,,
MC XXXX0XXX

; WIZID = 00012160
;Go Code
GO 00001000
; WIZID = 00012180
;Assignment Code
VARI2 = VARI1 +1 ;

; WIZID = 00012200
;Wait Code
T 5.000


; WIZID = 00012220
;Loop Action
REPEAT
  ; WIZID = 00012240
  ;Motion Code
  MA XXXX1XXX
  A ,,,,10,,,
  AA ,,,,0,,,
  AD ,,,,10,,,
  V ,,,,1,,,
  D ,,,,-1,,,
  MC XXXX0XXX

  ; WIZID = 00012260
  ;Go Code
  GO 00001000
  ; WIZID = 00012280
  ;Wait Code
  T 2.000


  ; WIZID = 00012300
  ;Motion Code
  MA XXXX1XXX
  A ,,,,10,,,
  AA ,,,,0,,,
  AD ,,,,10,,,
  V ,,,,1,,,
  D ,,,,-0.5,,,
  MC XXXX0XXX
```

```
      ; WIZID = 00012320
      ;Go Code
      GO 00001000
      ; WIZID = 00012340
      ;Wait Code
      T 2.000


      ; WIZID = 00012360
      ;Assignment Code
      VARI2 = VARI2 -1 ;

    ; WIZID = 00012380
    UNTIL(VARI2=1)
  ; WIZID = 00012400
  ELSE
    ; WIZID = 00012420
    GOSUB HOMLIN
  ; WIZID = 00012440
  NIF
END

; WIZID = 00012460
;-------------
;User Program
;Subroutine to set Linear motor accelerations
DEL SETACC
DEF SETACC


  ; WIZID = 00012480
  ;Loop Action
  REPEAT
    ; WIZID = 00012500
    ;Native Code module
    Write"Input Linear Motor Acceleration -- <!'number>"
VARS5=">"
VARI5 = READ5
WRVARI5


    ; WIZID = 00012520
    ;If Code
    IF(VARI5<=0)
      ; WIZID = 00012540
```

```
      ;Native Code module
      write"Hello I need a positive acceleration input to move"


    ; WIZID = 00012560
    ELSE
    ; WIZID = 00012580
    NIF
  ; WIZID = 00012600
  UNTIL(VARI5>0)
END

; WIZID = 00012620
;-------------
;User Program
;Subroutine to set linear motor velocity
DEL SETVEL
DEF SETVEL


  ; WIZID = 00012640
  ;Loop Action
  REPEAT
    ; WIZID = 00012660
    ;Native Code module
    Write"Input Linear Motor Velocity -- <!'number>"
VARS6=">"
VAR6 = READ6
WRVAR6

    ; WIZID = 00012680
    ;If Code
    IF(VAR6<=0)
      ; WIZID = 00012700
      ;Native Code module
      write"Hello I need a positive velocity to move"


    ; WIZID = 00012720
    ELSE
    ; WIZID = 00012740
    NIF
  ; WIZID = 00012760
  UNTIL(VAR6>0)
END
```

```
; WIZID = 00012780
;-------------
;User Program
;Subroutine to pump and suck the fluid
DEL PMSUCK
DEF PMSUCK


  ; WIZID = 00012800
  ;If Code
  IF(5PC=0)
    ; WIZID = 00012820
    RUN PUMPCY
    ; WIZID = 00012840
    RUN PISTDN
    ; WIZID = 00012860
    RUN PISTUP
    ; WIZID = 00012880
    RUN SETACC
    ; WIZID = 00012900
    RUN SETVEL
    ; WIZID = 00012920
    ;Assignment Code
    VARI2 = VARI1 +1 ;

    ; WIZID = 00012940
    ;Native Code module
    write"I am fixin to git r done"

    ; WIZID = 00012960
    ;Wait Code
    T 5.000


    ; WIZID = 00012980
    ;Loop Action
    REPEAT
      ; WIZID = 00013000
      ;Motion Code
      MA XXXX0XXX
      A ,,,,(VARI5),,,
      AA ,,,,0,,,
      AD ,,,,(VARI5),,,
      V ,,,,(VAR6),,,
      D ,,,,(VAR2),,,
      MC XXXX0XXX
```

```
      ; WIZID = 00013020
      ;Go Code
      GO 00001000
      ; WIZID = 00013040
      ;Native Code module
      write"Down"

TPE


      ; WIZID = 00013060
      ;Motion Code
      MA XXXX0XXX
      A ,,,,(VARI5),,,
      AA ,,,,0,,,
      AD ,,,,(VARI5),,,
      V ,,,,(VAR6),,,
      D ,,,,(VAR3),,,
      MC XXXX0XXX

      ; WIZID = 00013080
      ;Go Code
      GO 00001000
      ; WIZID = 00013100
      ;Assignment Code
      VARI2 = VARI2 -1 ;

    ; WIZID = 00013120
    UNTIL(VARI2=1)
  ; WIZID = 00013140
  ELSE
  ; WIZID = 00013160
  NIF
END


; WIZID = 00013180
;-------------
;Error Program
DEL CRASH
DEF CRASH

END
```

9.4. SAS Programs

Program 1

```
***pgm1.sas;
options formdlim='_' pageno=1;
data a;
infile 'results_reorg.csv' dlm=',' dsd missover firstobs=2;
input name $ diam PM $ Visc upstroke acc velocity;
if pm='T' then tail=1;
else tail=0;
run;

proc freq data=a nlevels; by visc;
tables pm--velocity pm*tail;
*tables Visc*acc*velocity*upstroke/list out=temp;
run;



/*
NAME,DIAMETER,T/NT,VISCOSITY,UPSTROKE,ACCEL,VELOCITY
1BH1,0.60,NT,1.5,8,400,0.2
1BH2,0.50,NT,1.5,8,400,0.2
*/


***Visc=1.8;
title 'Visc=1.8';
proc glm data=a;
class velocity upstroke acc;
where visc=1.8 ;
model diam = velocity|upstroke|acc/ss3;
lsmeans velocity*upstroke*acc / adjust=bon pdiff;
ods output lsmeans=Visc1_8_Diam_Means;
run; quit;

proc glm data=a;
class velocity upstroke acc;
where visc=1.8 ;
model  tail= velocity|upstroke|acc/ss3;
*means velocity*upstroke velocity*acc;
lsmeans velocity*upstroke velocity*acc / adjust=bon pdiff;
ods output lsmeans=Visc1_8_Tail_Means;
run; quit;



***Visc=1.5;
title 'Visc=1.5 without 200,700, 2400';
proc glm data=a;
class velocity upstroke acc;
where visc=1.5 and acc not in(200,700,2400);
model diam tail = velocity|upstroke|acc/ss3;
lsmeans velocity*upstroke*acc / adjust=bon pdiff;
```

```
ods output lsmeans=Visc1_5_Means;
run;quit;

***visc=1.5, upstroke=8 and all acc levels;
title 'Visc=1.5 and upstroke=8';
proc glm data=a;
class velocity upstroke acc;
where visc=1.5 and upstroke=8;
model  diam tail= velocity|acc/ss3;
lsmeans velocity*acc / adjust=bon pdiff;
ods output lsmeans=Upstroke8_Means;
run; quit;

proc export data=Upstroke8_means file='Upstroke8.xls' replace;
run;

proc export data=Visc1_5_Means2 file='Visc1_5_Means.xls' replace;
run;
proc export data=Visc1_8_Tail_Means file='Visc1_8_Tail_Means.xls'
replace;
run;

proc export data=Visc1_8_Diam_Means file='Visc1_8_Diam_Means.xls'
replace;
run;
```

Program 2

```
***pgm1.sas;
options formdlim='_' pageno=1;
data a;
infile 'results_reorg_a.csv' dlm=',' dsd missover firstobs=2;
input name $ diam PM $ Visc upstroke acc velocity;
if pm='T' then tail=1;
else tail=0;
run;

ods listing close;
ods rtf file="pgm1a.rtf" style=journal;


proc freq data=a nlevels; by visc;
tables pm--velocity pm*tail;
*tables Visc*acc*velocity*upstroke/list out=temp;
run;



/*
NAME,DIAMETER,T/NT,VISCOSITY,UPSTROKE,ACCEL,VELOCITY
1BH1,0.60,NT,1.5,8,400,0.2
1BH2,0.50,NT,1.5,8,400,0.2
*/


***Visc=1.8;
```

```
title 'Visc=1.8';
proc glm data=a;
class velocity upstroke acc;
where visc=1.8 ;
model diam = velocity|upstroke|acc/ss3;
lsmeans velocity*upstroke*acc / adjust=bon pdiff;
ods output lsmeans=Visc1_8_Diam_Means2;
run; quit;

proc glm data=a;
class velocity upstroke acc;
where visc=1.8 ;
model  tail= velocity|upstroke|acc/ss3;
*means velocity*upstroke velocity*acc;
lsmeans velocity*upstroke  velocity*acc / adjust=bon pdiff;
ods output lsmeans=Visc1_8_Tail_Means2;
run; quit;



***Visc=1.5;
title 'Visc=1.5';
proc glm data=a;
class velocity upstroke acc;
where visc=1.5 ;
model diam tail = velocity|upstroke|acc/ss3;
lsmeans velocity*upstroke*acc / adjust=bon pdiff;
ods output lsmeans=Visc1_5_Means2;
run;quit;

***visc=1.5, upstroke=8 and all acc levels;
title 'Visc=1.5 and upstroke=8';
*proc glm data=a;
*class velocity upstroke acc;
*where visc=1.5 and upstroke=8;
*model  diam tail= velocity|acc/ss3;
*lsmeans velocity*acc / adjust=bon pdiff;
*ods output lsmeans=Upstroke8_Means2;
*run; quit;

ods rtf close;
ods listing;

*proc export data=Upstroke8_means file='Upstroke8.xls' replace;
*run;

proc export data=Visc1_5_Means2 file='Visc1_5_Means2.xls' replace;
run;
proc export data=Visc1_8_Tail_Means2 file='Visc1_8_Tail_Means2.xls'
replace;
run;

proc export data=Visc1_8_Diam_Means2 file='Visc1_8_Diam_Means2.xls'
replace;
run;
```

Program 3

```
***pgm2.sas;
options formdlim='_' pageno=1;
data a;
infile 'results_reorg1.csv' dlm=',' dsd missover firstobs=2;
input name $ diam PM $ Visc holes upstroke acc velocity beads;
if pm='T' then tail=1;
else tail=0;
Exp=substr(name,1,1);
if velocity=.2 then delete;
run;

ods listing close;
ods rtf file="pgm2.rtf" style=journal;

proc freq data=a;
tables exp;
run;

proc sort data=a; by exp;
proc freq data=a nlevels; by exp;
tables visc holes pm--velocity;
run;

**without upstoke=8 and without velocity=.2;
proc glm data=a; by exp; where upstroke ne 8;
class velocity upstroke acc;
model diam tail= velocity|upstroke|acc/ss3;
lsmeans velocity|upstroke|acc / adjust=bon pdiff;
ods output lsmeans=LSMEANS3456;
run; quit;

ods rtf close;
ods listing;

proc export data=lsmeans3456 file='pgm2.xls' replace;
run;
```