

Device for Converting Elastin-Like Polypeptide into Soluble Form

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INTRODUCTION

The American Cancer Society estimates that cancer will affect approximately 1.4 million individuals in the United States in 2006. Genes, age, and behavioral factors (smoking and alcohol consumption) are believed to increase the risk of cancer. Currently, three treatments are available: chemotherapy, radiation therapy, and hormonal therapy. At least two of these therapies are required in conjunction for effective treatment. [1]

Chemotherapy uses chemicals that specifically target reproducing cells to prevent their growth ultimately to destroy the tumor. If complete eradication is not possible, chemotherapy attempts to contain the spread of a tumor; however, if containment is no longer possible, chemicals work to alleviate cancer symptoms including fever, fatigue, and pain. [1]

Radiation therapy utilizes ionizing radiation, either photons or particle radiation, to penetrate through deep tissues of the body where reproducing cancer cells often reside. This radiation is capable of either destroying cancerous cells or altering their genetic information, preventing their uncontrolled proliferation. In addition to targeting cancerous cells, chemotherapy and radiation therapy also harm normally dividing cells. [1]

Hormonal therapy is widely used to fight breast and prostate cancer. Estrogen and androgen are two hormones that manipulate the cell cycle in breast and prostate respectively. Hormonal therapy is carried out by either removing the hormone producing organs (ovaries and testis) or by inhibiting hormone function in cell proliferation by introducing materials that antagonize the hormone's

function. This technique is very effective in blocking hormone interaction in target tissues. For example, surgically removing ovaries from a woman with breast cancer will decrease estrogen production and thereby decrease the rate at which the tumor grows or even stunt the tumor growth. A hormone deficiency will have an adverse effect on certain tissues, in this case, bones and the liver. Hence, a treatment option that offers minimal loss of normal cells is desired. [1, 2]

Advances in DNA technology and genetic engineering have developed techniques for incorporating fragments of DNA into a cell's genome via viral and non-viral agents to restore a missing function or give the cell a new function. Although introduction of foreign materials into the cell, transfection, occurs at a higher rate with a viral agent, the non-viral agent is preferred. Elastin-Like Polypeptides (ELPs) may serve as the non-viral mode gene-carrier.

ELPs are synthetic polymers that consist of a repeated pentapeptide chain of amino acids: Valine – Proline – Glycine – X– Glycine (Figure 1) , where X is any amino acid except Proline. Every ELP has a Transition Temperature (T_t), above which it

aggregates due to hydrophobic collapse [5].

The transition temperature of ELP can be varied by altering the "X" amino acid, and thus allowing for different

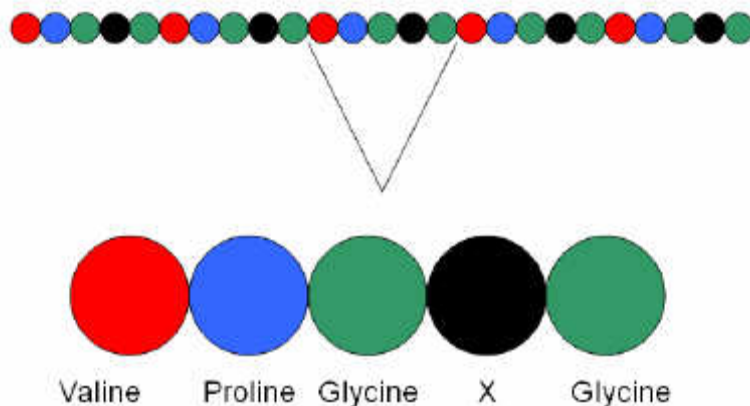


Figure 1: The basic structure of ELPs.

types of ELPs. Below T_t , ELP remains soluble when suspended in a solvent such as Phosphate Buffered Saline (PBS) [3]. This property can aid with tissue-specific gene delivery. An external heat source used to increase the temperature of a tumor causing ELP to migrate to the target cells. When ELP migrates to these cells, the elevated temperature will cause ELP to aggregate and allow the cells to accept the DNA through endocytosis [4]. This approach is minimally invasive, tissue-specific, and non-immunogenic. Currently, our client uses bacteria to synthesize ELPs.

Several steps must be carried out before ELP can be isolated from the bacteria. A genetically engineered vector genetically that contains the ELP gene is transformed into *E. coli* cells which are then grown in Luria Broth (LB) media. The cells increase in numbers while continuously transcribing and translating the ELP gene into ELP polypeptide. Then, the cells are isolated and lysed; ELP is separated from the endogenous DNA and bacterial proteins with centrifugation yielding an extremely viscous ELP pellet (Figure 2).

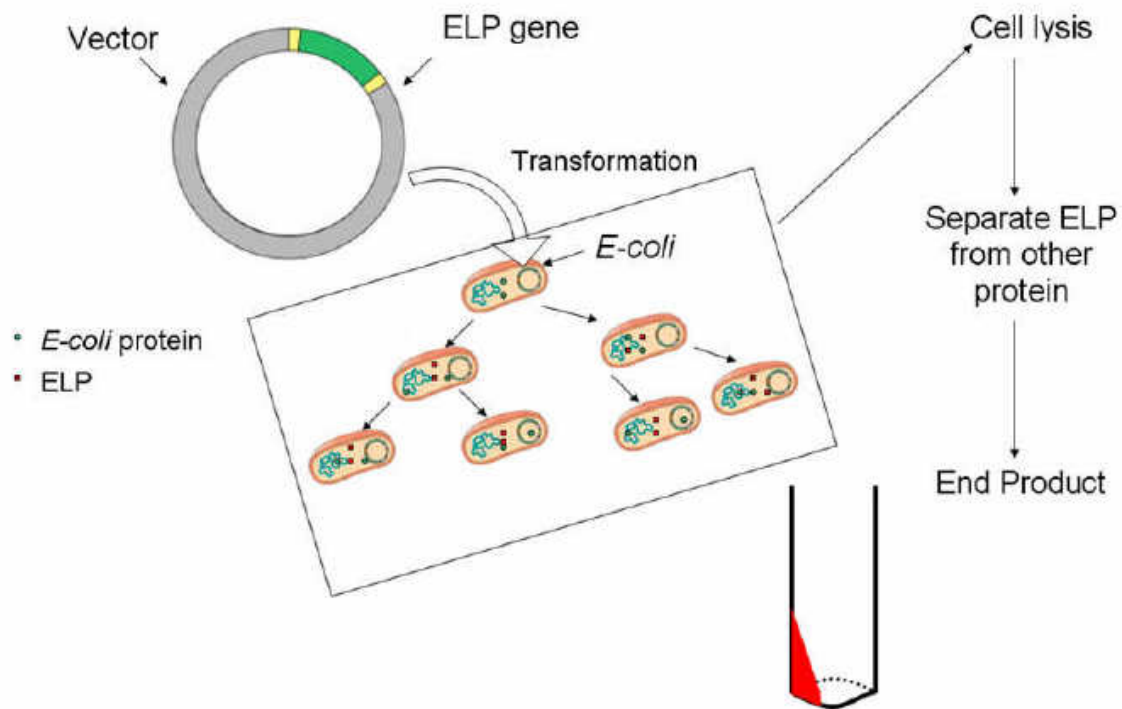


Figure 2: Current procedure used to isolate ELP. The red pellet is the viscous ELP pellet isolated at the end of the procedure.

The purpose of our project is to design an automated device that would efficiently resolubilize this pellet in Phosphate Buffered Saline (PBS) solution. Currently, the resolubilization process is labor intensive; the process involves pipetting the PBS solution onto the ELP pellet repeatedly until fully resolubilized. Over time, the ELP is suspended in the PBS solution, therefore, product loss increases with each iteration of pipetting.

DESIGN CONSTRAINTS

Our main goal in designing the device is to reduce the time required to resolubilize ELP in PBS solution and to reduce product loss. The device should minimize production of bubbles to decrease product loss. Since ELP aggregates above the transition temperature, T_t , overheating in the device could be problematic; hence, maintaining low temperature is critical in preventing ELP from aggregation. Therefore, the device should be able to operate within the temperature range 2° to 30° Celsius. The current process requires repeated pipetting for several hours, therefore, an automated device would benefit our client tremendously. ELP should be resolubilized in the centrifuge tube used by our client (opening diameter: 0.657 inches, body diameter: 1.097 inches, length: 4.054 inches). The device must be designed to accommodate the dimensions of the test tube. The final device should resolubilize ELP harvested from 2 liters of bacteria (1 liter of bacteria yields approximately 180 mg of ELP). The final ELP solution should be uniform in concentration at the end of each cycle. Finally, the prototype should function normally for at least 50 cycles (1 cycle is equivalent to resolubilizing ELP harvested from 2 liters of bacteria).

Designing a device that is easily cleaned would also reduce the manual labor required to operate the device. In the interest of safety, the device should have no exposed sharp edges that could harm the user. Since the device will be on a lab bench top, it should fit in should fit in a 1 meter x 1 meter area; the height should not exceed 0.5 meters. The device should weigh less than 20

pounds so it can be easily transported. See Product Design Specifications (PDS) in Appendix E.

AVAILABLE PRODUCTS

While there are many available products such as vortexers, rockers, and shakers, none are ideal for resolubilizing ELP. Another major drawback to these products is that they cost upwards of \$1500.

DESIGN ALTERNATIVES

Helix Model

This design is composed of intertwining spirals that form a helix. The helix, formed to fit inside the test tube, would be crossbraced by blades or bars that will facilitate the majority of the resolubilization of ELP. Since the opening of the tube is narrower than the body, the helix can collapse at the opening and re-expand once inside the test tube (Figure3).

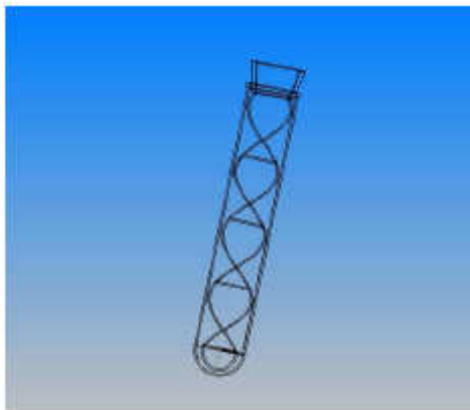


Figure 3: Wireframe model of Helix design inside test tube.

The helix will be fixed to a rubber stopper, yet allowed to rotate independently. An external motor will be used to rotate the helix component at high speeds (upwards of 500 revolutions per minute), effectively mixing the aggregate with the PBS.

Advantages and Disadvantages

The helix is easily cleaned and sanitized after each use. This design is also very cost effective (motor price: \$50), and resembles current industrial mixers. Since the helix is directly attached to the motor, a circuit can be built to allow for an adjustable rotation speed.

Disadvantages include the possibility of a difficult manufacturing process. Since the helix will be comprised of metal wire, welding is not an option, and we would need to epoxy or solder the blades in. The blades, if sharpened, could pose to be a safety hazard if the user is not careful.

Brush Model

This model consists of three distinct parts: the plastic centrifuge tube, a commercially available test tube brush, a lid for the centrifuge tube, and a motor to power the device. A hole through the lid allows the stem of the brush to fit into the centrifuge tube. The bristles of the brush are attached to a threaded rod; the brush will be 35 mm wide in diameter and 20 mm high. The stem that is attached to the brush will be 150 mm in length (Figure 4).

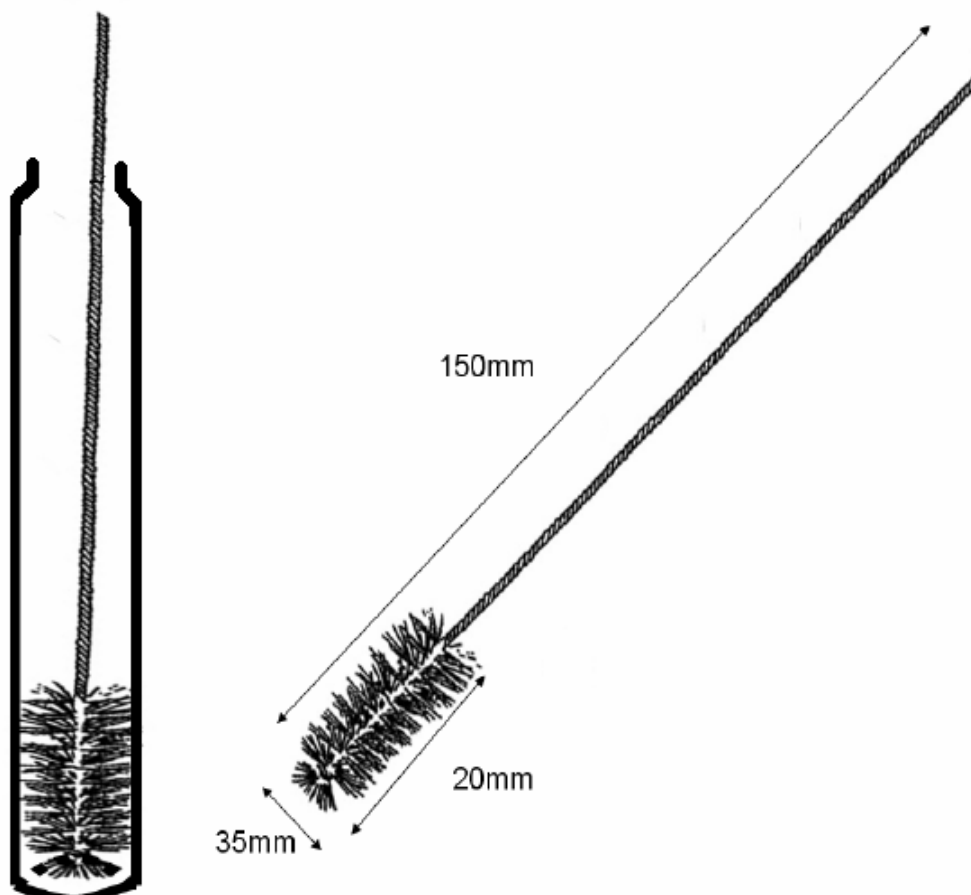


Figure 4: Brush model in test tube and with approximate dimensions.

The dimensions of the brush were determined based on the dimensions of the test tube so that the brush is custom fit to effectively resolubilize ELP. On top of the lid, a small motor is connected to the rod of the brush and allows the brush to rotate to solubilize the ELP sample. The motor could either be powered by a battery or through AC current by plugging into an outlet. When the motor is powered, the brush rapidly rotates around the vertical axis and scrapes off ELP attached to the sides of the tube. One of the strengths to this design is the high surface area exposure of the bristles to ELP and PBS, which allow for faster and

more efficient mixing. Since the bristles fit tightly against the centrifuge tube, ELP aggregate will be scraped off of the sides and will get incorporated into the PBS solution. This feature is especially important since the ELP will be centrifuged before resolubilization, and will therefore be attached to one side of the test tube.

Advantages and Disadvantages

Fabricating the device could be relatively simple since most of the parts are readily available in the market; however, we would still need to make adjustments to the brush to customize fit in the centrifuge tube provided by our client. While this model has some advantages such as simple fabrication, the test tube brush model also has several weaknesses. First, the bristles of the brush may be difficult to clean after use, this may also lead to reduced product yield. Since one of our client's requirements was to minimize product loss, this could potentially be a problem. Moreover, it is uncertain whether forces applied by the bristles would be enough to convert the ELP aggregate into a soluble form. In addition, the bristles of the brush are made of plastic and may wear out easily after use. Overall, the design has several positive aspects as well as drawbacks; however, more experimental work is required to get a better idea of force required to resolubilize ELP.

Dual Scraper Model

The Dual Scraper model (Figure 5) consists of two scrapers that are designed to scrape the ELP aggregate from the surface of the test tube and mix

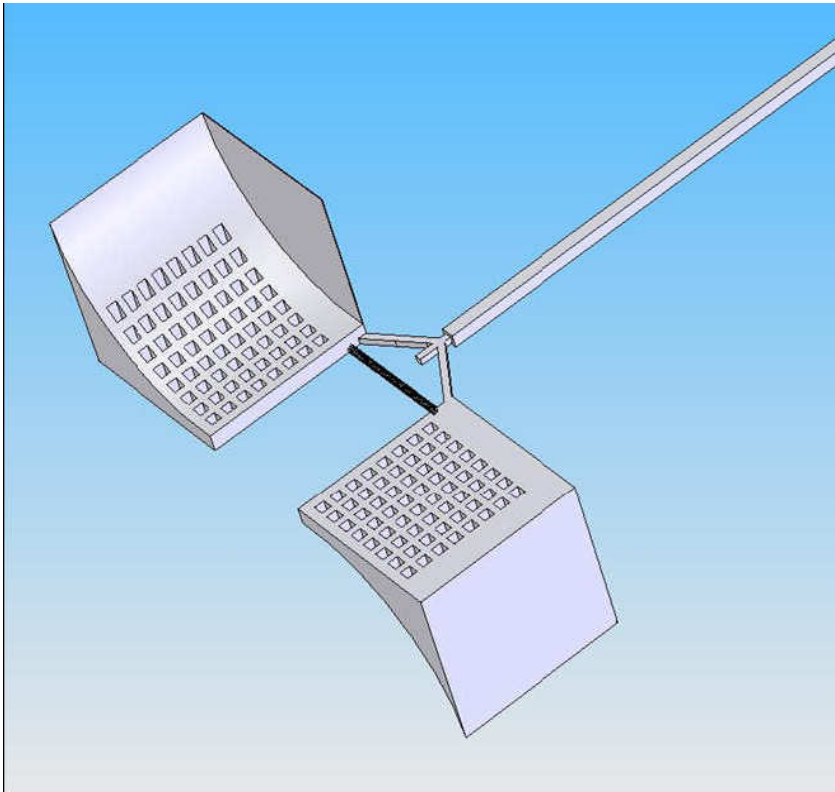


Figure 5: The dual scraper model consists of two scrapers to scrape the ELP from the inside surface of the centrifuge tube.

this aggregate with PBS solution. Since the opening of the test tube and the diameter of the bottom of the test tube have different dimensions, 18mm and 29 mm respectively, the scrapers must be able to collapse to

enter the test tube and extend to the edges once inside. Therefore, the overall width of the collapsed scrapers must be less than 18mm and must extend to approximately 28.5mm in order to reach the ELP on the side of the tube. This device also must have a mechanism external to the test tube to collapse the scrapers so that they can be removed from the test tube. These scrapers will rotate about a central axis powered by an external variable speed AC motor. Variable speed is ideal for this device because it allows us determine a speed that will effectively mix the solution but will not produce many bubbles as this

often results in product loss. The scrapers will be coated in Teflon or an alternative non-stick material to prevent the ELP from adhering to their surface for easy clean up. They will also have holes to allow ELP to pass through them as it mixes into the solution.

Advantages and Disadvantages

The slits in the scrapers will allow for greater surface area at the ELP-PBS interface, which will rapidly increase the rate of resolubilization. The holes will also decrease the resistance on the scrapers thereby putting less strain on the motor. One drawback to this design is its difficulty to manufacture.

DESIGN MATRIX

We constructed a design matrix to determine which of our three alternative designs to choose for the final design. The matrix scored five categories on a scale of 1-10 for each, with 1 being poor and 10 being the most desirable. The matrix itself is located in Appendix B. In summary, the dual scraper model scored the highest overall, with top scores in product yield and prototype life. We have modified the design matrix to include our final design. This design received the top overall score (Appendix D).

FINAL DESIGN

While all three possible design ideas were viable, certain criteria such as ease of construction and lifetime were reconsidered. Besides the difficulty of manufacturing such designs as the helix and dual scraper models, we decided that since these devices would be assembled from very small components we may encounter issues such as durability. A lack of durability would drastically decrease the lifetime of our product. Therefore, through further brainstorming we developed the rock tumbler design which was inspired by an existing rock tumbler device.

The rock tumbler device utilizes an existing rock tumbler design that has been modified to suit our specific needs. It consists of a rubber drum which houses an apparatus that holds the test tube in place and at an angle. This apparatus consists of a sheet of high density Polyethylene with an angled hole to accommodate the test tube. It is supported by three peripheral bolts that serve as legs, which allow for its removal from the drum for easy cleaning. A rubber lined primary cover fits into the opening of the drum which seals the drum closed. The cover has a bolt in the center of its outer surface which serves as a handle for opening the drum and as an axle for the drum to spin on. An aluminum secondary cover is placed over this cover and allows the bolt to pass through its center. A customized nut is placed on the bolt which holds the two covers in place. Also, this nut has a groove on its outer surface that fits into a notched piece of metal on the rock tumbler base. This holds the drum in position and gives it an axis to spin from. The rock tumbler base consists of two parallel rods,

one of which is spun by an attached AC motor. The sides of the drum rest on these rods which cause the drum to spin in a clockwise direction about its central axis. See Appendix A.

Advantages and Disadvantages

The current design incorporates several of our clients requirements including: operating below the transition temperature of ELP, resolubilizing ELP within the centrifuge tube, minimizing the production of bubbles, as well as resolubilizing ELP efficiently. Testing the device with ELP demonstrated that the motor does not overheat; rather it maintains ideal temperature that effectively resolubilizes the aggregate. In addition, since the device is designed to accommodate the centrifuge tube, there was no need to transfer the aggregate to a different tube for resolubilization. Hence, incorporating the centrifuge into the device aids in minimizing product loss. Moreover, the current method that includes repeated pipetting produces many bubbles and leads to product loss; however, when the device was tested with ELP, there were only a small amount of bubbles produced. A key component to the design is the use of stainless steel beads that aid in resolubilization; these beads can be autoclaved after use to ensure that the ELP is sterile. Overall, the device does resolubilize the aggregate at a faster time than the prior method used, considerably reducing the time and manual labor required.

While the prototype operates within our client's needs, the device does have some disadvantages associated with it. Our design includes the use of

stainless steel beads for effective mixing. While they are effective in resolubilization, it is challenging to remove the beads from the centrifuge tube without incurring some product loss. Moreover, the device only accommodates our client's current needs to resolubilize ELP obtained from 2 liters of bacteria. In the future, when resolubilization of larger quantities is required, the device would need to be modified accordingly.

Expenses

The rock tumbler required for this device is available for \$145.00 from various carriers. The carriage bolts and nuts are available from farm and fleet or any hardware store for about \$3.75. The sheet of high density polyethylene is about \$5.00 also from Farm and Fleet.

TESTING

ELP Testing

One of our major concerns with this project was whether ELP would be available for us to test our prototype. Fortunately, after our prototype was



Figure 6: Prototype testing in Dr. Furgeson's lab. Shown is drum when filled ice for temperature testing.

built, two batches of ELP were purified in the following three weeks and we were granted permission to test our device with this ELP. The first time we carried out testing (April 10, 2006), we packed the drum with ice, added approximately 12 zinc-plated BB gun beads to the tube, and added 1 mL of cold PBS to the tube. By filling the drum with ice, we could maintain the temperatures considerably



Figure 7: ELP pellet in test tube.

below the transition temperature of the ELP to prevent any ELP aggregation.

After about 10 minutes of mixing, we found that the ELP was not resolubilizing. Instead of

mechanically breaking the viscous ELP pellet, the beads just adhered to the ELP. We hypothesized that the low temperatures of the tube increased the viscosity of the ELP. We decided to remove the ice and run our device at room temperature. This step was taken only after verifying that the transition temperature of this particular ELP was above room temperature. After mixing the ELP with PBS in our device for about 7 minutes, the entire ELP pellet was resolubilized

Testing with Creamed Honey

To find the best suitable condition to resolubilize ELP, we carried out extensive testing by varying several parameters such as bead type, number of beads and mass of creamed honey.

Experimental Setup

Since ELP was unavailable, we used creamed honey for testing purposes. Although the viscosity of creamed honey at room temperature may be slightly less than that of ELP, it was the best available alternative. Moreover, our client approved of this alternative. We used approximately 400 mg of creamed honey with 2mL of water and because these ratios are consistent with those specified by our client. See Appendix B.

We decided to carry out our experiments at room temperature because all of the ELP synthesized by our client has a transition temperature above room temperature. The first variable we tested was bead type. First we tested 400 mg of creamed honey with 2mL of water and varied the number of beads (10 and 20). We took absorption data using a spectrophotometer at 2 minute intervals followed by two one minute intervals to confirm that the absorption values stabilized. We repeated this process with 10 and 20 glass beads. These beads are very light and may take longer to resolubilize ELP. Lastly, we tested with 2.3 mm diameter – stainless steel beads that we ordered from Biospec Products, Inc. These beads are made from Type 316 Stainless Steel, the same steel used to

manufacture artificial hips and other artificial joint replacements, therefore they are sterile, and autoclavable.

Our main goals with these tests were to show that the heavier the beads, the faster the creamed honey would solubilize. For these experiments, we decided to use double the usual amounts of honey and water because this made it easier to weigh the honey into the tube and also provide more solution to expedite the process. For the last two experiments, we chose the amount of honey and water that corresponds to the amount of ELP and PBS used in our client's lab. Table 1 in the appendix shows the experimental set up. Following this table are the data acquired from these tests. By checking absorbance spectra from 400nm to 700nm at certain time intervals, we can estimate the time required for our device to resolubilize the sample.

Results

These graphs show the absorbance values at 400 nm over the course of several minutes. Graphs that stabilize show that the solution has reached a uniform concentration. These graphs are shown in Appendix C. We discovered that as time progressed, the absorption levels rose unexpectedly for Test 2. We realized that zinc had scraped off of the BB gun beads and thus increasing absorbance values over time. This was later confirmed by members of our client's lab, who realized this after our test with ELP. This did not occur in Test 1 because we used fewer beads in this test thereby reducing the amount of zinc in the solution. Surprisingly, the amount of time that the glass beads took to solubilize the honey was equivalent to that of the BB gun beads or the stainless

steel beads (Tests 3,4). This can be attributed to the fact that the creamed honey used in our testing was at room temperature and considerably less viscous than anticipated. With the ELP pellet, however, these beads would not be effective; this could be verified by further testing. Lastly, the absorbance graphs for stainless steel beads (Tests 5, 6) make it clear that for practical purposes, these beads should be used because they are inert and aid in quick resolubilization of ELP.

Conclusion

Ideally, these tests should be conducted on ELP. Conditions that solubilize honey may not resolubilize ELP. Even though the 2.3 mm stainless steel beads solubilized honey, heavier beads might be needed to speed up the resolubilization of ELP.

ETHICS

Since our client is interested in using ELPs as a mode of drug delivery, there is a potential ethical concern about testing on animals. Another possible ethical issue concerns the design itself. While we modified the device to customize its use for resolubilization of ELP, the basic drum and the motor are originally components of a commercially available rock tumbler. Consequently, if our client would like the device to be mass produced and available for purchase, it is important to ensure that the prototype is not violating any patent rights for the rock tumbler. If there were some patent issues associated, then it is imperative to make enough alterations to avoid patent violations, and at the same time to preserve key design aspects of the current prototype.

FUTURE WORK

Throughout the construction of this prototype, we came up with some ideas for the future of this project. Our current model is designed to hold only one ELP-containing test tube. The main focus of this was to test the ability of the machine to resolublize the aggregated ELP automatically in the lab setting. A short-term goal would be to modify the design to accommodate two test tubes containing the ELP pellet, as each liter of culture the transformed plasmid produces two test tubes of final product. Since the future of ELP in medicine is promising, but uncertain, we believe our design could provide a basis for an industrial resolublization machine, capable of transforming mass quantities (i.e. many tubes and/or much greater amounts of ELP per tube) of ELP into the usable form.

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Appendix A - Drawings of Prototype

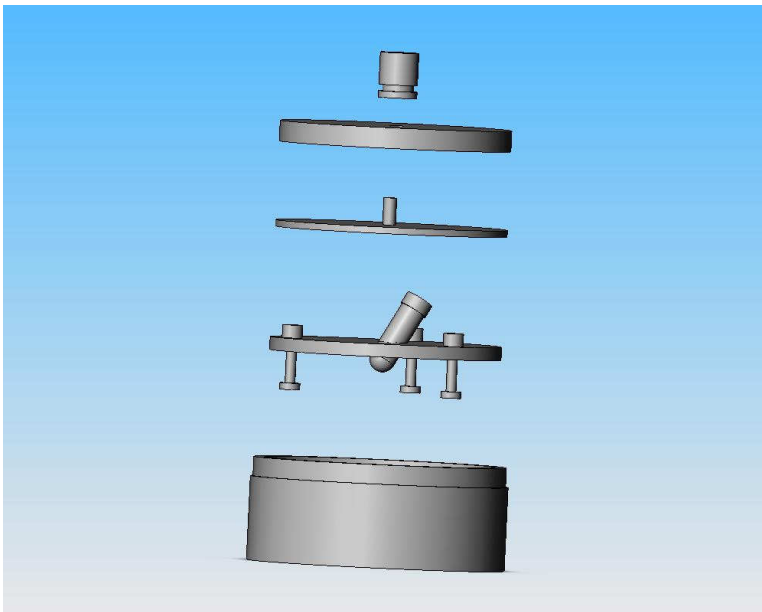


Figure 8: Side View: This side view displays each of the components that compose the drum of our device. From the top down: Customized grooved nut; Secondary Plate; Primary plate; Test tube holding apparatus including angled test tube; drum.

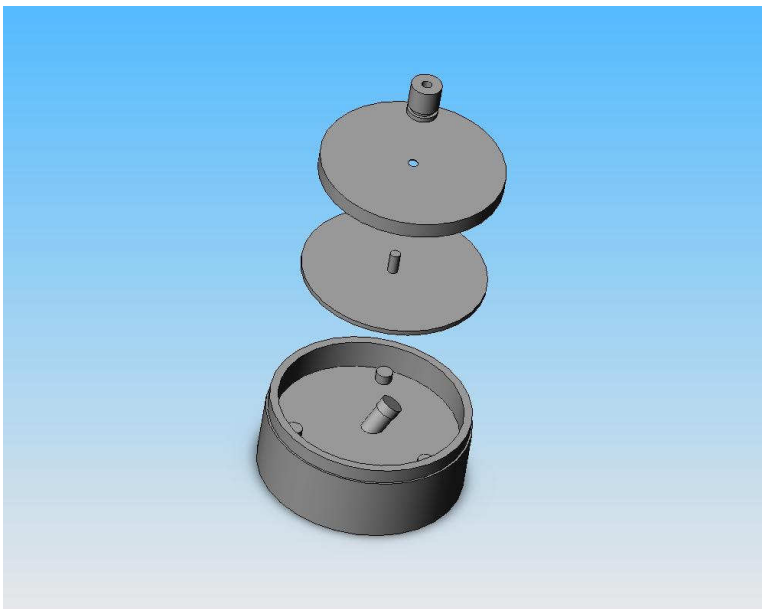


Figure 9: Top View: Same order of components as listed in Figure 1. Shows Test tube holding apparatus in place in the drum.

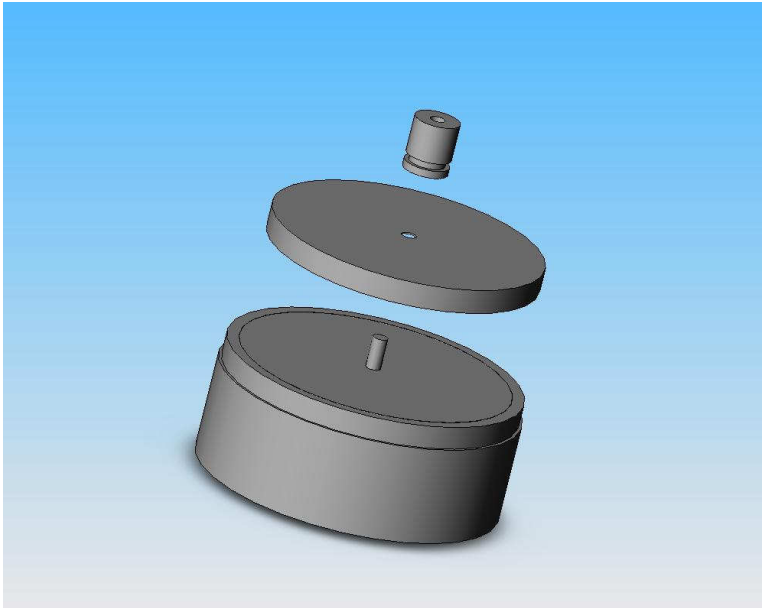


Figure 10: Shows primary cover in place.

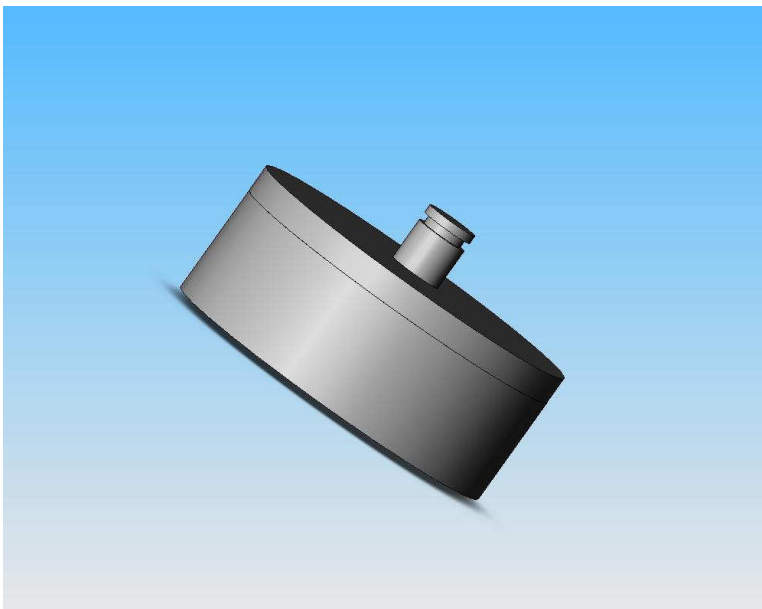


Figure 11: Shows completely closed drum.

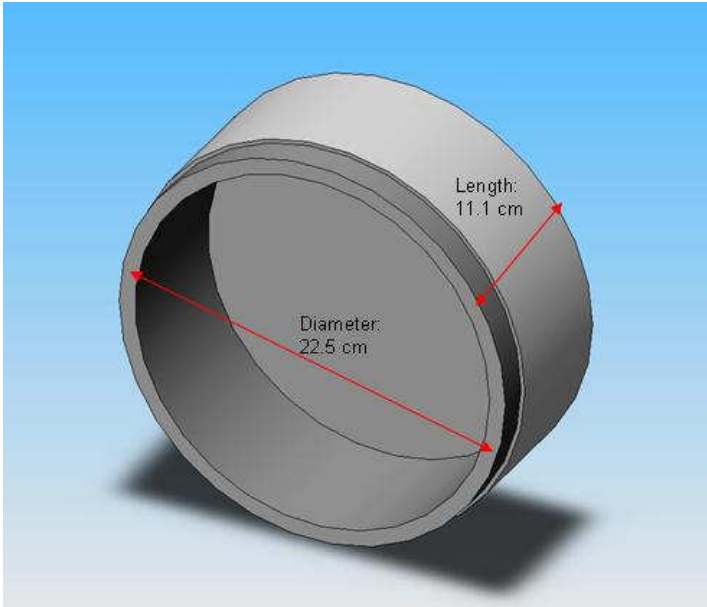


Figure 12: Drum dimensions.

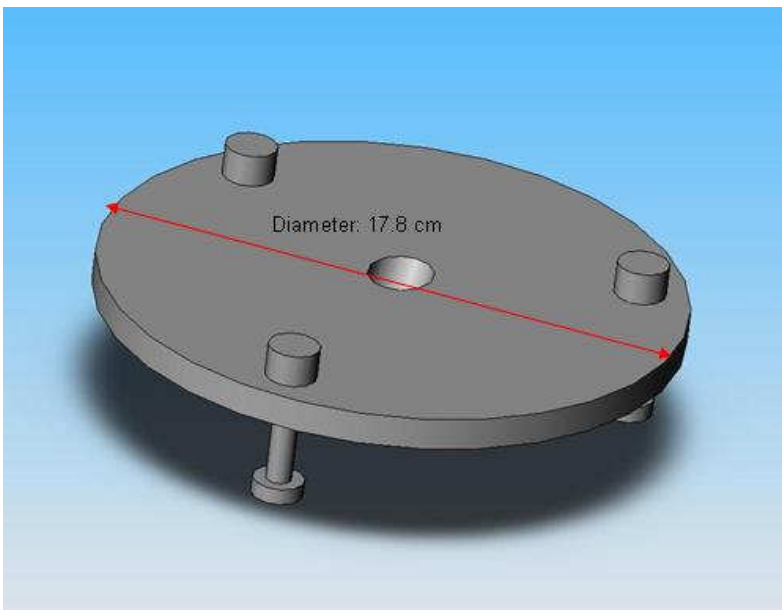


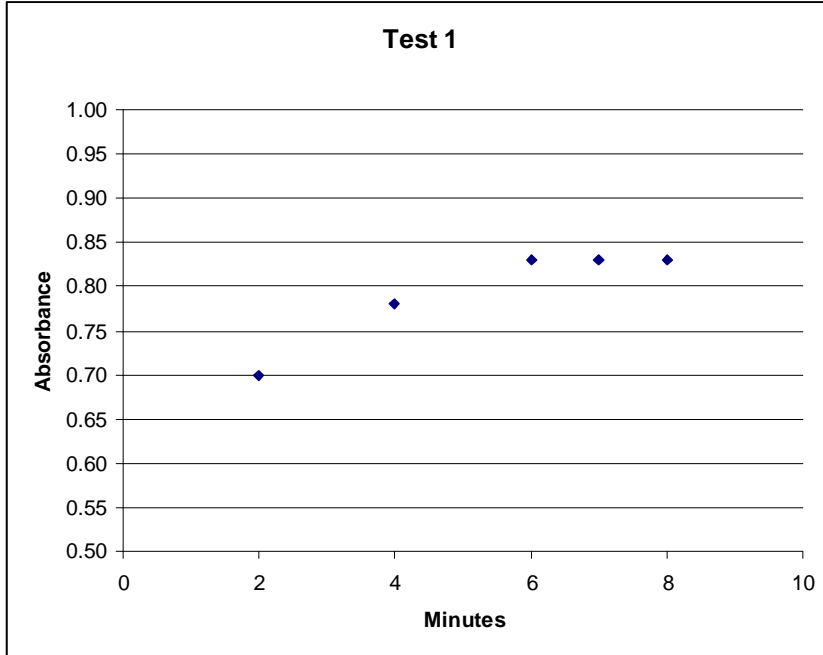
Figure 13: Dimensions of test tube holding apparatus.

Appendix B - Experimental Setup

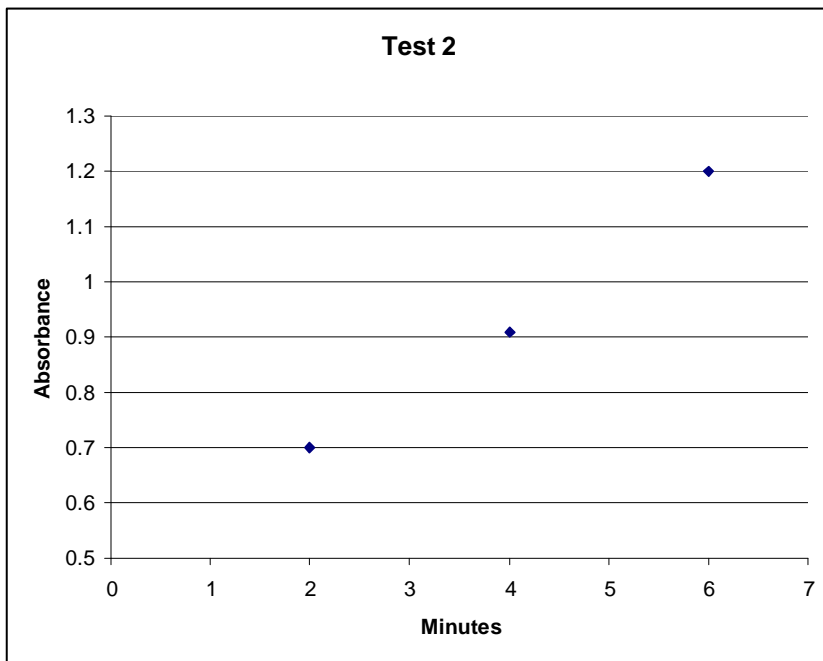
Test #	Amount of Honey (mg)	Amount of water (mL)	Type of Beads	Number of beads
1	417	2	Zinc-platted BBs	10
2	404	2	Zinc-platted BBs	20
3	407	2	Light glass beads	10
4	397	2	Light Glass Beads	20
5	193	1	Stainless Steal Beads	20
6	204	1	Stainless Steel Beads	40

Appendix C - Testing Data

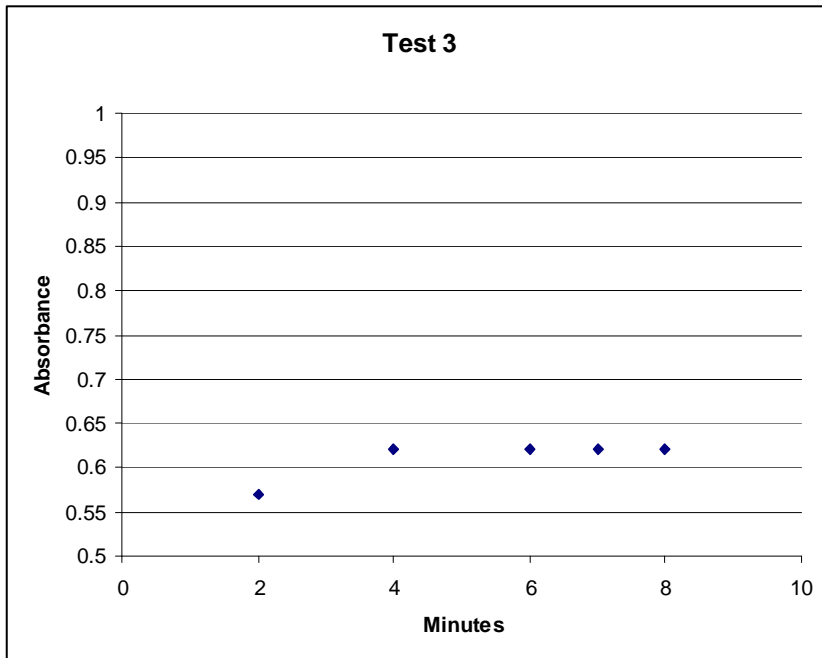
Test 1



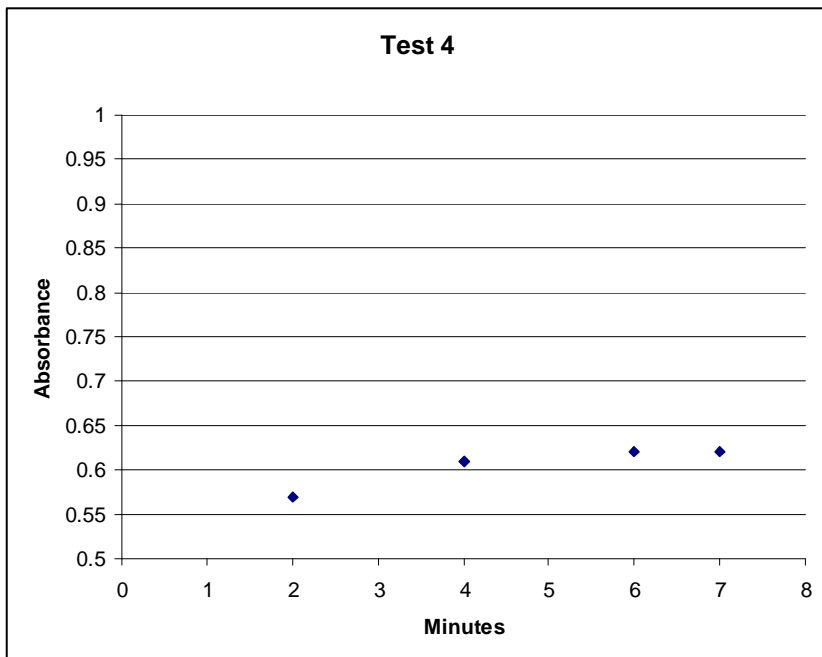
Test 2



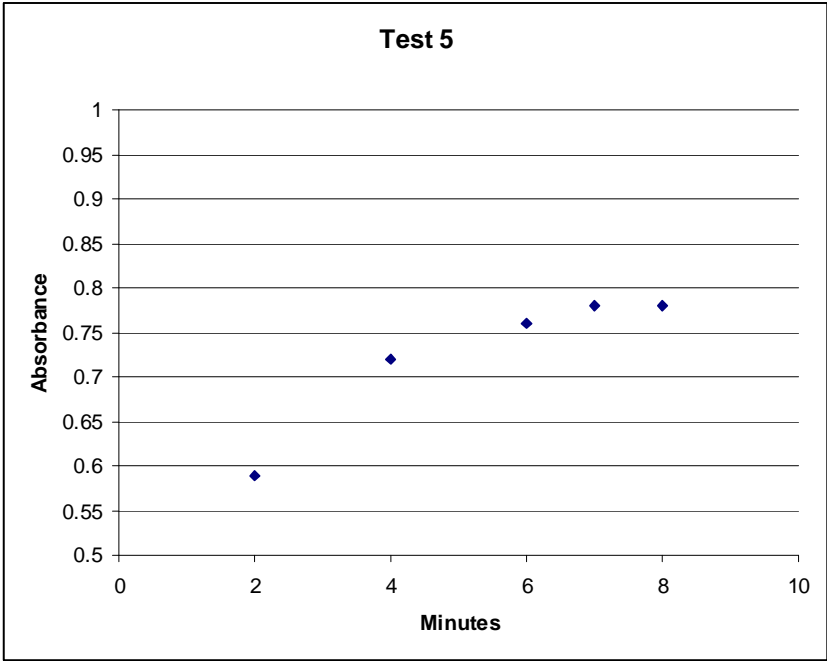
Test 3



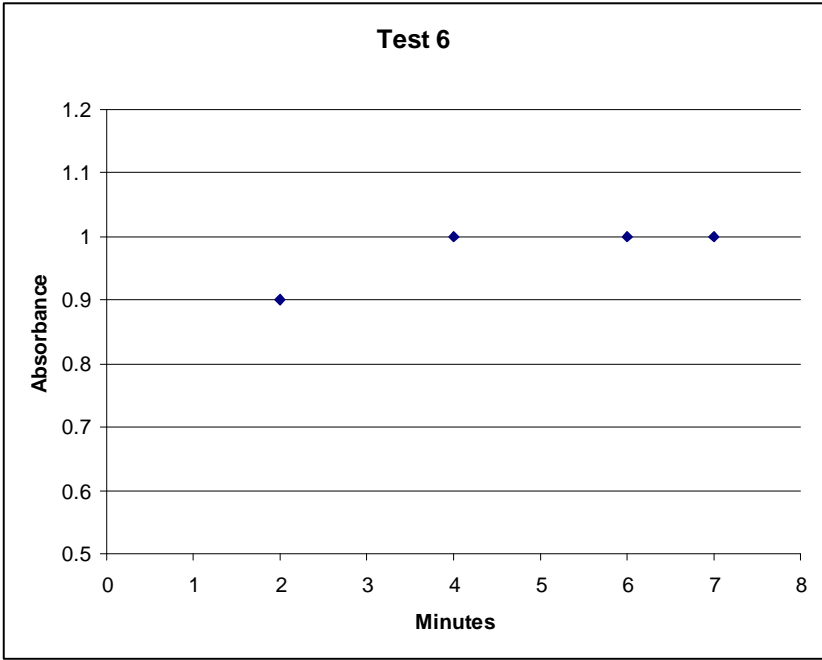
Test 4



Test 5



Test 6



Appendix D – Revised Design Matrix

Criteria	Brush Model	Helix Model	Dual Scraper Model	Rock Tumbler Model
Ease of Cleaning	2	7	6	8
Product Yield	2	5	7	6
Cost	8	6	5	5
Ease of Manufacturing	8	4	7	10
Prototype Life	2	6	8	9
Total	22	28	33	38

Appendix E - PDS

Product Design Specification

Title: Converting Elastin-Like Polypeptide (ELP) aggregate into soluble form

Team:

Dhaval Desai – Team Leader
Lee Linstroth – Communicator
Malini Soundarrajan – BSAC
Nathan Kleinhans – BWIG

Date: April 27th, 2006

Function: Elastin-Like Polypeptides (ELPs) may serve as promising drug-delivery agents to treat various types of cancers. After harvesting ELPs from bacteria, ELPs become aggregated. In order for our client, Dr. Furgeson, to conduct research on ELPs, soluble form of ELP is required. ELP aggregation depends on temperature and salt concentration. Our device should efficiently convert ELP aggregate to soluble form.

Client Requirements:

- Device should be automated and require minimal manual labor.
- At least 80% of ELP should be recovered after it is converted into a soluble form.
- PBS should be used as the solvent in minimal amounts.
- Accommodate test tube (10.3 cm x 2.8 cm)

Design Requirements:

1. Physical and Operational Characteristics

a) *Performance Requirements*

The design should be able to resolubilize ELP harvested from 4 liters of bacteria (1 liter of bacteria yields approximately 180 mg of ELP).

b) *Safety*

The device should have no exposed sharp edges that harm the user. Any moving parts should be shielded to prevent the user from harm and all electrical elements should be contained to prevent unwanted shock or contact.

c) *Accuracy and reliability*

The final ELP solution should be uniform in concentration with complete resolublization. Concentration uniformity will be measured by a spectrophotometer three times to verify concentration. Complete resolublization will be measured visually with total rehydration of aggregate pellet.

d) *Life in service*

The device should function normally for a minimum of 5 years if kept in optimal conditions.

e) *Operating Environment*

Device will be used in a normal laboratory setting.

- Normal room temperature operation ($\sim 22^{\circ}\text{C}$) for the majority of product life, but should continue to function normally within temperature range of 2°C to 30°C .
- Normal pressure ("the standard atmosphere" (1 atm) = 101.325 kPa)
- Low humidity
- Dirt and dust levels are low and negligible
- Fluid corrosion will may be a factor, as the laboratory setting may produce volatile fluid that could affect the product
- Vibrations from device or other equipment may cause loosening and detachment of parts and should be inspected regularly

f) *Ergonomics*

The device should be user friendly and easy to clean. Sterile equipment is a must, so design should allow for easy and quick cleanup to be ready for multiple batches. The device should be easy to hold (if handheld) and not require extraneous movement to operate.

g) *Size*

The device should fit in a 1 meter x 1 meter laboratory bench top. The height of the device should not exceed 0.5 meters.

h) *Weight*

The weight of the device should not exceed 20 pounds.

i) *Materials*

The materials should withstand the temperature range mentioned earlier in this PDS. The materials should withstand forces applied to it from motion, test tubes, or ELP. For example, if a motor is incorporated, it should be powerful enough to withstand any forces applied to the attached mechanism.

j) *Aesthetics, Appearance, and Finish*

The chamber, in which the ELP solution will be made, should be transparent. Product is not required to be aesthetically pleasing as long as functional conditions are met.

2. Production Characteristics

- a) *Quantity*
Only one unit is desired.
- b) *Target Product Cost*
The total product cost should be less than \$100.

3. Miscellaneous

- a) *Customer*
Our client prefers that the resolubilization process takes less than 3 hours. Also, the client would like the final product to be as concentrated as possible.
- b) *Competition*
Many kinds of laboratory mixers, shakers, emulsifiers, and vortexers are available through equipment supply companies including Bionexus, Diager, and IncubatorShaker. This equipment is not currently ELP specific, and costs anywhere from \$200 to \$2500 each.