Influence of Peristaltic Pump Tubes on Protein Particles Formation for Lipase Enzyme Therapeutic Protein

By

Salah Elden Omer

Degree Project in Pharmaceutical Technology, 2023
Department of Food Technology, Engineering, and Nutrition
Lund University Sweden
30 ECTS credits

Supervisor: Marie Wahlgren
Examiner: Lars Nilsson

Lund University
Department of Food Technology, Engineering, and Nutrition
Faculty of Engineering, LTH
P.O. Box 124
SE-221 00 Lund, Sweden

“The role of pump tubing types in making Protein cluster for Lipase drug.”

Popular Language Summary

Protein aggregation is a high molecular weight protein content cluster. Their presence in finished therapeutic protein products is considered risky for their significant effects on the quality of the medicine and the safety and health of the patient as it can induce adverse effects. Therefore, it is critical to identify the process that contributes to its formation and address the presence of particles in the development of therapeutic proteins.

From a formulation perspective, having profound knowledge of particle development mechanisms and identifying the causes and factors affecting particle formation is important. Knowing the factors that influence particle formation is advantageous to adopt control measures to mitigate risks associated with particles beginning from the initial production steps, through storage life duration.

This project was done to investigate the effect of pump tubing, Silicone, and PVC tubing, used for transporting protein during the production process, tubing of a peristaltic pump, on protein particle formation for lipase protein using Silicone and PVC tubing, and characterize the particle formed. Moreover, investigate the effect of using different surfactants on the formation of the particles. The surfactants, a commonly used compound in bio-pharmaceutical formulations to prevent aggregation, used were Polysorbate 20, Poloxamer 188, and Sodium Dodecyl Sulfate (SDS) surfactant. This was done by using a QICPIC instrument for particle analysis and Silicone and PVC tubing for pumped Lipase.

The lipase samples with or without surfactant were pumped in through the tubing for thirty minutes and measurements were taken every minute. Particles were characterized using ferret diameter and Sphericity. The results were compared to see the effect of tubing type and surfactants.

The results obtained from this investigation showed that peristaltic pumps can induce protein aggregation which depends on the tubing type and the presence of the surfactant. It was seen that Silicone tubing in most 7 cases induced the highest number of aggregates and that most surfactants tested except for polysorbate 20, gave protection against aggregation.

Abstract

Introduction: In Bio-pharmaceutical production, peristaltic pump tubing is considered one of the factors that influence protein aggregation.

Background: Protein particles present challenges for the control and analysis of various manufacturing processes in the biopharmaceutical industry for their significant effects on safety and quality.

Aim(s): This research aims to study the effect of different peristaltic pump tubing on particle formation as well as the Characterization of the particles and investigate the effect of surfactants on particle formation.

Methods: The study was conducted using a dynamic image analysis instrument (QICPIC) to characterize particles formed by Lipase protein samples while pumping through Silicone and PVC tubing using a peristaltic pump. Lipase samples concentration of 1mg/ml were investigated with and without polysorbate 20, Poloxamer 188, and SDS surfactants, while Lipase 10 mg/ml samples were investigated without using the surfactants.

Results: Pumped of all Lipase protein samples resulted in particle formation with varying degrees between Silicone and PVC tubing. The presence of Poloxamer 188 and Sodium Dodecyl Sulfate (SDS) surfactants reduced pumping-induced particles except for Polysorbate 20.

Conclusion: Silicone and PVC tubing generate particles even in the presence of surfactants and a great level of reduction the particle formation was achieved with Poloxamer 188 and SDS surfactants.

Keywords: Image analysis, Protein aggregation, Peristaltic pump tubing, Particle characterization, PVC tubing, Silicone tubing, Surfactant, QICPIC

Acknowledgments

I would like to offer my special thanks to my supervisor Professor Marie Wahlgren for her guidance and mentorship in this journey, her direction during the different stages of the project, her patience, and support in and out of the lab, and her valuable and prompt feedback during the experiments, and the report preparation and finalizing. I would like to thank her for guiding me toward an exciting area to research during the project. It has been a pleasure and an honor to be supervised by her.

I would also like to thank Anna Kjellström for all her help in facilitating my access to the lab, locating the chemicals, assisting me with instruments, and for her valuable advice. I am also thankful to LTH's Department of Food Technology, Engineering, and Nutrition and all its staff for all their considerate support.

Finally, a special feeling of gratitude to my loving parents, my beloved mother, and father, for all their unconditional love and faith during this intense academic journey. My dear siblings have never left my side and are very special.

Salah Elden Omer
June 2023

Dedication

To Sudan/Kush my home, my strength, my identity, and my pride.

Project Aim

The scope of this master thesis project aimed to investigate the effect of the peristaltic pump's tubing on lipase protein. More detailed aims are:

- Investigate the effect of the peristaltic pump's different tubing types on particle formation for lipase protein.
- Characterization of formed particles.
- Determine the effect of surfactants on particle formation.
- Comparison of the protection level gained by using different surfactants.
- Evaluate using DIA (QICPIC) for protein particle characterization.

Table of Contents

LIST OF ACRONYMS

SDS - Sodium Dodecyl Sulphate
DIA - Dynamic Image Analysis
PBS - Phosphate Buffer Saline
EQPC- Diameter of a Circle of Equal Projection Area
FERET_MAX- Ferret Diameter Maximum
FERET_MIN- Ferret Diameter Minimum
FERET_MEAN-Ferret Diameter Mean Value
PVC -Polyvinyl Chloride
MVQ- vinyl methyl silicone

1. Introduction

Protein aggregation is defined as a cluster of high molecular weight species, such as oligomers or multimers, formed by covalent or non-covalent bonds. It is considered one of the challenges for the biopharmaceutical industry in terms of control and analysis of various manufacturing processes (Mahler et al., 2009). The importance of the presence of particles from the bio therapeutics industry point of view relies on their impact on patient health and safety, as a source of adverse effects and induce of anti-drug antibodies and product quality which explains the need for robust and advanced analysis tools that enable characterization of particles in biopharmaceutical products in terms of number, size range, and distribution (Ripple and Dimitrova, 2012).

Protein particle formation is promoted by numerous mechanisms such as adsorption at interfaces, chemical environments, elevated temperature, the presence of nucleation sites, or mechanical stress (Ripple and Dimitrova, 2012). Stress such as stirring, pumping, and shaking on protein solutions induce particle formation due to encouraging complete or partial unfolding which is also induced by high temperatures through accelerated oxidation and deamination reactions or change protein conformation which promotes unfolding (Ripple and Dimitrova, 2012, Mahler et al., 2009).

Due to the dynamic nature of protein particles, as it is highly responsive to environmental change, different tools and various properties such as count, size, morphology, optical density, chemical composition, and other physicochemical properties were needed for characterization (Ripple and Dimitrova, 2012).

Particles are characterized into sub-visible particles ranging from 1µm to 100µm, and visible particles have a size range of more than 100 µm from a size perspective and were divided into homogeneous particles which have one predominant chemical entity, and heterogeneous in which protein coating a non-protein core according to their Chemical composition, and from a morphological view, particles have a highly variable shape ranging from nearly spherical aggregates to long, irregular fibers(Ripple and Dimitrova, 2012). Moreover, described as native, denatured, or partially denatured depending on the degree of loss in structure detected (Ripple and Dimitrova, 2012).

Therefore, the adoption of control measures and a consistent development approach aiming at the mitigation of risks associated with particles consider of great value for the successful development of therapeutic proteins, that achieved through the identification of the influence factors that contribute to the particle formation phenomenon starting from initial production steps, pathing through storage life duration, and ending at knowing the in vivo environment effects (Ripple and Dimitrova, 2012).

In the production process, the pump unit is considered one of the sources of protein aggregations, and their impact is different according to the type of pumping tube used and depends on their properties like hardness and surface chemistry, therefore, the pump traits are important (Deiringer and Friess, 2022b). Furthermore, the pump's operation unit could create large numbers of protein particles, out of which mobile pump parts generate small particles due to their acting as nucleation sites for protein particle formation, and could induce the development of homogeneous protein particles due to elevated shear stress or through an increase of liquid-solid or liquid-air interfaces of therapeutic base protein (Ripple and Dimitrova, 2012).

Different types of pumps are used in bioprocessing filling steps, such as rotary piston pumps, time-pressure pumps, rolling diaphragm pumps, and Peristaltic pumps (Her et al., 2020). The peristaltic pump is commonly used in biopharmaceutical production and other industries for transporting viscous fluids, works by positive displacement, depending on peristalsis, through mechanically enclosing a fixed volume repeatedly and consists of housing, flexible tube, rotor, and roller, as shown in Figure 1 (Mavrodontis, 2020). It is made from different polymers, platinum-cured silicone is commonly polymer used, and its optimization is achieved by adjusting the total pumped volume per cycle, speed, and pump acceleration, and it is considered the minimal source of particle formation (Her et al., 2020). The underlying cause of particle formation by peristaltic pump stems from the pump head heat leading to protein unfolds, cavitation produced by roller movement that induces oxidative stress, stretching of the tubing through mechanical stress, and interfacial adsorption to the tubing wall (Deiringer and Friess, 2022b).

Further, Peristaltic pump features include the capability of gentle pumping, which enables the transfer of shear-sensitive fluids, is more reliable and has less downtime due to their composition of simple and robust elements (Mavrodontis, 2020). Additionally, suitable for dosing by adjusting the flow rate by rotor speed, pumping of different kinds of fluids such as corrosive or high-temperature fluids due to the presence of flexible tubing, and prevention of cross-contamination during operation because of a tube rupture due to the use of a casing for enclose (Mavrodontis, 2020).

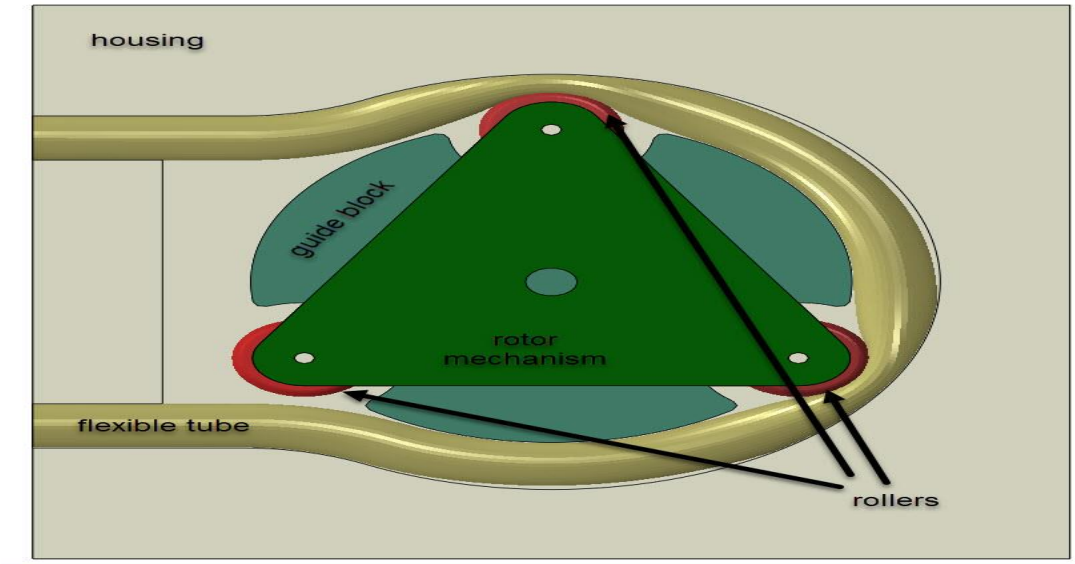

Figure. 1. Peristaltic pump geometry (Mavrodontis, 2020)

Surfactants are compounds of amphiphilic natures, characterized by having hydrophilic and hydrophobic heads and tails, respectively, commonly used in pharmaceutical formulations for their ability to decrease interfacial tension (Suhail et al., 2019). Generally, surfactants are used to reduce aggregation risks and instability of biopharmaceutics products (Khan, Mahler, and Kishore, 2015).

They are different types of surface-active compounds, a non-ionic surfactant group is commonly used in biopharmaceutics to prevent interfacial damage that has a positive impact on protein stability, and for their proven safety profile (Khan, Mahler, and Kishore, 2015). The extensively used non-ionic surfactants in biopharmaceutics are Polysorbate 20, and Poloxamer 188, a polyoxyethylene-based surfactant, shown in Figure 2 (Khan, Mahler, and Kishore, 2015). Both types of polysorbate prevent aggregation caused by an air-water interface, adsorption, and freeze-thaw stress. Moreover, Polysorbate 20 addition can prevent aggregation due to mechanical stress, while Polysorbate 80 prevents vortexing-induced aggregation (Khan, Mahler, and Kishore, 2015). Besides that, sugars such as Dodecyl Maltoside (DDM), and Sodium Dodecyl Sulfate (SDS) surfactant, a non-ionic surfactant, are also used for the prevention of particle formation. However, Sodium Dodecyl Sulfate should be removed before the final step for safety reasons (Haji Abdolvahab et al., 2014).

QICPIC is a dynamic image analysis (DIA) instrument that applies to particle characterization in terms of shape, and size, in the lab for size range starting from below 1 µm up to 34000 µm, as shown in Figure 3, by passing particles across a high-speed camera and get a digital image of individual particles, Demonstrating high-speed image analysis with high resolution by using a pulsed light source (Sympatec GmbH, 2023).

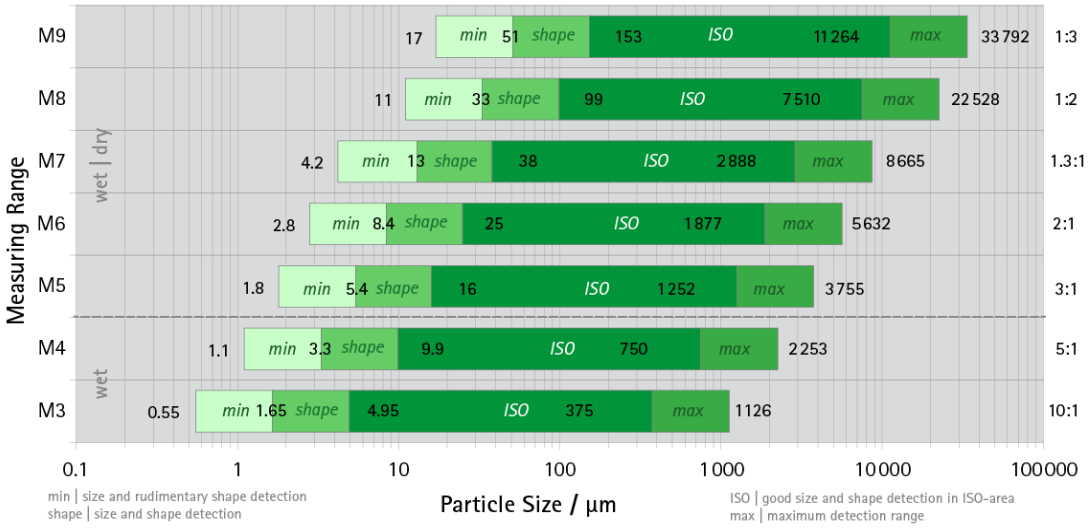

Figure. 2. QICPIC Measuring ranges with maximum resolution (Sympatec GmbH, 2023)

The scope of this master thesis project was aimed to use a QICPIC instrument for particle characterization to investigate the effect of peristaltic pumping on particle formation for different concentrations of Lipase protein using Silicone and PVC tubes. Moreover, underline the influence effects of using polysorbate 20, Poloxamer 188, Dodecyl Maltoside (DDM), and Sodium Dodecyl Sulfate surfactant (SDS), to prevent protein aggregation.

2. Method and Materials

The details of the methods and other specific materials, chemicals, amounts, and others can be found in Appendix A.

For pumping studies, Lipase was used in two concentrations, 1mg/ml and 10mg/ml, in 0.1 M Phosphate Buffer Saline (PBS) at pH 7. Lipase high-concentration samples were investigated without surfactants with Silicone and PVC tubing while the low-concentration samples were examined with and without surfactants. PBS was prepared by dissolving its ingredients in purified water obtained from the Milli-Q lab water system, after which the pH was adjusted with hydrochloric acid or sodium hydroxide using a pH meter.

The stock concentration of lipase was verified by a Nanodrop using UV absorption at 280 at the protein-specific extinction coefficient of 101300 m2 /mg and confirmed to be 25.25 mg/ml. The lipase samples were filtered with 0.2 µm polyether sulfone membrane syringe filters.

Tubing materials used were silicone and PVC purchased from Saveen and Werner AB (Sweden). Both tubings have an inner diameter of 2mm, outer diameter of 4mm, and wall thickness of 1mm, and were used with lengths for inlet and outlet of 35 cm and 30 cm, respectively, with a filling volume of 8 ml.

The surfactants used were polysorbate 20, Poloxamer 188, and Sodium Dodecyl Sulfate (SDS) surfactant obtained from Sigma Aldrich (Germany).

The surfactants concentration used were Polysorbate 20 0.1%, Poloxamer 188 0.1%, and Sodium Dodecyl Sulfate (SDS) surfactant 0.5% and 0/1%.

OLE DICH Instrumentmakers ApS Type 104 (Denmark) Peristaltic pump was used, and QICPIC using a wet dispersion unit called Lixell (Sympatec Inc., Germany) with Windox 5.0 software to analyze the size and shape of all particles of all images were used in the current study as analysis instrument.

All samples were freshly prepared and pumped in triplicate by the peristaltic pump with a speed of 10 and a rate of 14.8 ml/minute through Silicone and PVC tubes into QICPIC for thirty minutes, and measurement was taken every minute.

Sample Preparation:

All samples were prepared and filtered in the Laf-lab under a laminar aimed to avoid contamination with external particles and had a volume of 20 ml a minimum volume of wet dispersion that can be investigated by QICPIC to ensure a suitable volume for the particle-size measurement.

2.1 Blank

PBS was prepared by using Sodium chloride, Potassium Chloride, Sodium Phosphate Dibasic, and Potassium Phosphate from Merck (Sweden) and up to the volume with MQ water, and the pH was adjusted to 7. Six samples of 20 ml volume of PBS in a glass bottle of 25 ml were prepared and filtered in Laf-lab and investigated by the QICPIC instrument.

2.2 Lipase without Surfactants

Lipase was thawed at room temperature until no visible ice remains and gently mixed to prepare twelve samples six of which had a concentration of 1 mg/ml, and the rest had a concentration of 10 mg/ml were these filtered in a particle-free environment.

2.3 Sobi Lipase 1 mg/ml with Sorbitol 20

A stock solution of 1% w/w Polysorbate was prepared and pipetted into the lipase sample 1 mg/ml in a laminar flow hood to reach a final Polysorbate 20 concentration of 0.1 % (w/v). The lipase was divided into six samples of 20 ml for each. The samples were diluted with PBS buffer 0.1M, 0.15 M NaCl.

2.4 Lipase 1 mg/ml with Poloxamer 188

To prepare a sample of 0.1% concentration, the Poloxamer 188 was weighed and dissolved in PBS using magnetic stirring for 30 minutes. After that lipase was added and the total volume was used to prepare six samples of 20 ml for each.

2.5 Lipase 1 mg/ml with Sodium Dodecyl Sulfate (SDS)

SDS samples were prepared by dissolving in PBS while stirring for 30 minutes using a magnetic stirrer, then the lipase was added and placed in six glass bottles of 25 ml.

2.6 Protein Concentration

The Nanodrop instrument was used to verify the concentration of lipase concentration for all samples before measurements using the protein A280 method.

2.7 Experiment Setup

The measurements were carried out in a closed system by fixating the inlet tubing on the peristaltic pump rotor and connecting one of its sides to the QICPIC inlet and the other side with the glass bottle containing the sample. At the same time, the outlet tubing was connected to the sample bottle and QICPIC outlet. After installing the tubing, the washing protocol was implemented by running 200 ml of MQ water through the tubing. Fresh tubing was used after each measurement.

The Pump parameters were adjusted for all experimental measurements at a pump speed of 10 and a pump rate of 14.8 ml/minute. Before starting the measurement, the samples were inspected visually to evaluate the dispersion and presence of air bubbles and QICPIC connectivity with the computer, and the quality of measurement was evaluated.
QICPIC parameters were adjusted at 1.3500 g/cm3 as the Density value, Shape factor for EQPC value were set to 1. The parameters measured were Ferret max, Ferret min, and Ferret mean as Diameter values and Ferret max as standard diameter, Sphericity, Aspect ratio, Convexity, and Elongation as Shape value.

Continuous measurement to make a measurement every 60 seconds for 30 minutes and M4 measuring range (2um-668um) were selected. After measuring MQ water was run until no particle was seen on the QICPIC live Sensor Image window.

Figure.3. Experimental Setup at Lab, LTH, Lund University, (March 2023)

3. Results

3.1 Blanks

The results obtained when PBS had been pumped through Silicone and PVC tubing in a triplicate manner at 5, 10, 20, and 25 minutes are discussed below and shown in Table 1 and Figure 4-9:

Table 1: Average of total particle No, Median, Mean, Aspect ratio, and Sphericity (n=3) using PVC *and Silicone tubing (Average Standard deviation for* PVC *45, 2, 1, 0.004, 0.03 and 17, 7, 4, 0.04, 0.03 for Silicone, respectively)*

	Particle Number	Median	Mean	Aspect Ratio	Sphericity
PVC	157 ± 45	7 ±2 µm	9 ±1 µm	0.65 ± 0.004	0.85 ± 0.03
Silicone	33 ± 17	7 ±4 µm	10 ±3 µm	0.65 ±0.04	0.84 ± 0.3

Table 1 shows data of the average on the total number of particles, Sphericity, and aspect ratio for blanks. From the Sphericity and aspect ratio readings, both tubings generated particles with no difference in their degree of circularity and elongation. The particles show moderate circularity. However, PVC tubing showed 5-fold more particles, indicated by particle number, in comparison to Silicone tubing.

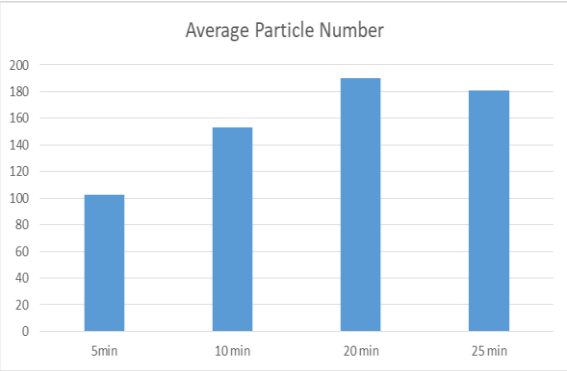

Figure 4: Average Particle Number (n=3) using PVC tubing – PBS Average standard deviation =45

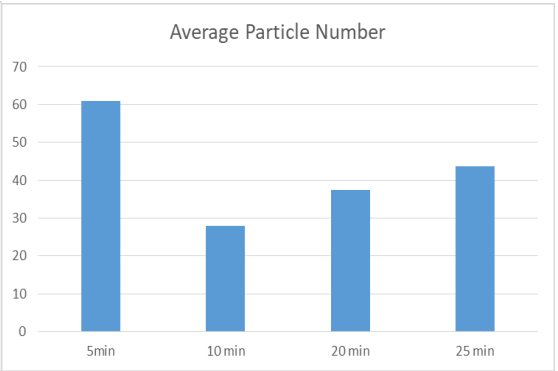

Figure 5: Average Particle Number (n=3) using Silicone tubing-PBS, average standard deviation=17

Figures 4 and 5 show the graphical representation of the average particle numbers obtained when PBS was pumped through PVC and Silicon tubing respectively. In Figure 4, for PVC tubing, there is a continuous increase in average particle number up to 20 minutes after which there is a slight decrease at 25 minutes in average particle number. However, in Figure 5,

with Silicone tubing the average particle number starts higher at 5 minutes but drops by more than half at 10 minutes, then increases gradually to 25 minutes. It is worth noting that with PVC tubing the average particle numbers are above 100 while for the Silicone tubing the average particle numbers are below 60.

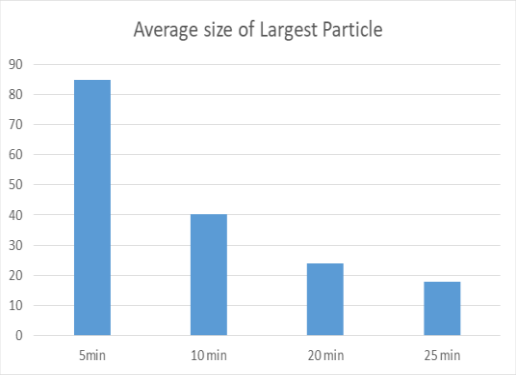

Figure 6: Average of Largest Particle (n=3) noted using PVC tubing – PBS, average standard deviation=13

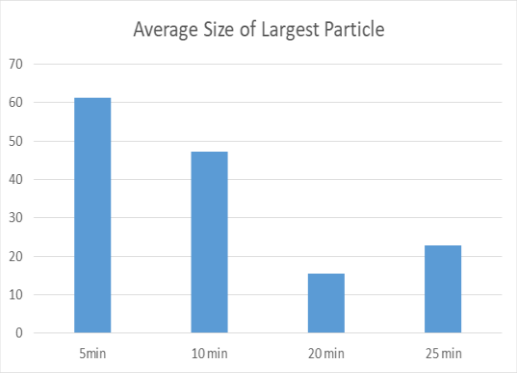

Figure 7: Average of Largest Particle (n=3) noted using Silicone tubing – PBS, average standard deviation=20

Figures 6 and 7 portray the graphs of the average of the largest particle size noted when PBS was pumped through PVC and Silicon tubing respectively. In Figure 6, using the PVC tubing, the highest particle size was the highest at 5 minutes, dropped by almost half at 10 minutes, and then continued to decrease up to 25 minutes. While in Figure 7, with the Silicone tubing, the average largest particle size noted started at above 60 micrometers at 5 minutes and slightly dropped at 10 minutes, then took a larger plunge at 10 minutes and continued to drop, only to increase slightly at 25 minutes. The trend in both graphs is a decreasing trend, and the average of the largest particle size at each interval is similar with both types of tubing.

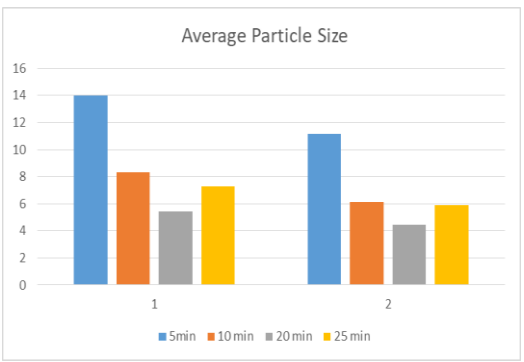

Figure 8: Average size of Particles (Mean (1), Median (2)) PVC tubing – PBS, average standard deviation= 1, 2 respectively.

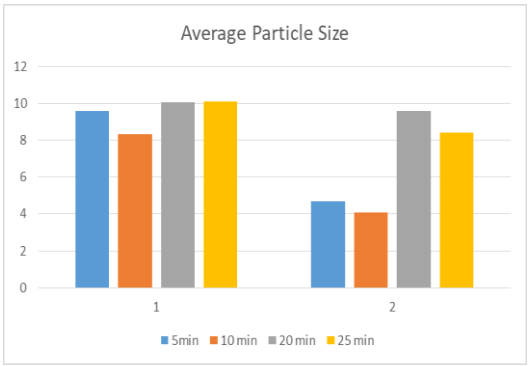

Figure 9: Average size of Particles (Mean(1), Median(2)) Silicone tubing – PBS, average standard deviation=3, 4 respectively.

Figures 8 and 9 are the graphs of the average size of particles as the median and the mean when PBS was pumped through PVC and Silicon tubing respectively. In Figure 8, when using the PVC tubing, the median particle size was highest at 5 minutes, and decreased up to

20 minutes, only to start increasing again at 25 minutes. The mean values were slightly lower than the median values, but the trend was represented in both average measures. Meanwhile, with the Silicone tubing, illustrated in Figure 9, the median particle size remains almost constant in the range between 8 -10 micrometers during the 25 minutes, while the mean shows considerable variation with readings at 5 and 10 minutes being similar and 20 and 25 minutes much higher and in the range of the median. The median size when using PVC tubing has a decreasing value unlike with silicone tubing where it is nearly constant, however, concerning the mean, PVC tubing starts with a large particle size at 5 minutes that declines as the experiment proceeds, unlike the silicone tubing the mean particle size starts at a less value and increases as the investigation proceeds.

3.2 Lipase 10mg

The results obtained when lipase 10 mg had been pumped through Silicone and PVC tubing in a triplicate manner at 5, 10, 20, and 25 minutes are discussed below and shown in Table 2 and Figure 10-15:

Table 2: Average of total particle No, Median, Mean, Aspect ratio, and Sphericity (n=3) using PVC and Silicone tubing (Average Standard deviation for PVC 254, 0.2, 00.2, 0.006, 0.002 and 63, 0.2, 0.3 0.01, 0.003 for Silicone, respectively).

	Particle Number	Median	Mean	Aspect Ratio	Sphericity
PVC	3332 ± 245	5 ± 0.2 µm	5 ± 0.2 µm	0.65 ± 0.006	0.89 ± 0.002
Silicone	1479 ± 63	4 ± 0.2 µm	5 ± 0.3 µm	0.60 ± 0.01	0.85 ± 0.003

Table 2 shows data on the average of the total number of particles, Sphericity, and aspect ratio for lipase 10mg. From the Sphericity and aspect ratio readings, both tubings generated particles with no major differences in their degree of circularity and elongation. The particles show moderate circularity. However, PVC tubing showed more than2 fold the number of particles, in comparison to Silicone tubing.

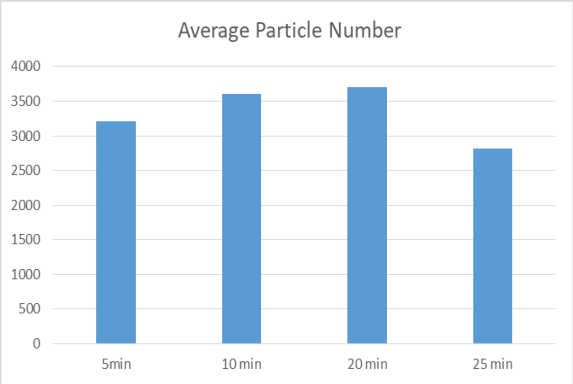

Figure 10: Average Particle Number using PVC tubing: Lipase 10mg, Average stander deviation = 254

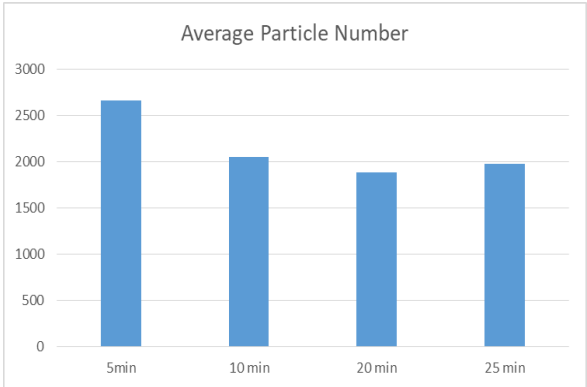

Figure 11: Average Particle Number using Silicone tubing: Lipase 10mg, Average stander deviation = 63

Figures 10 and 11 display the graphs of the average particle numbers obtained when lipase 10mg was pumped through PVC and Silicon tubing respectively. In the PVC tubing, in Figure 10, the average particle numbers remained above 2500 throughout the experiment, where at 5 minutes it was a little above 3000 particles and continued to increase up to 20 minutes; however, in the following 5 minutes, it declined by around 1000 particles. Meanwhile, the average particle numbers of lipase 10mg pumped through the silicone tubing, were lower starting at around 2600 particles at 5 minutes and decreasing up to the 20-minute-point only to increase again slightly at 25 minutes. When using the PVC tubing the average particle numbers are above 2500 particles while with the Silicone tubing, the average particle numbers are at around 2500 and lower.

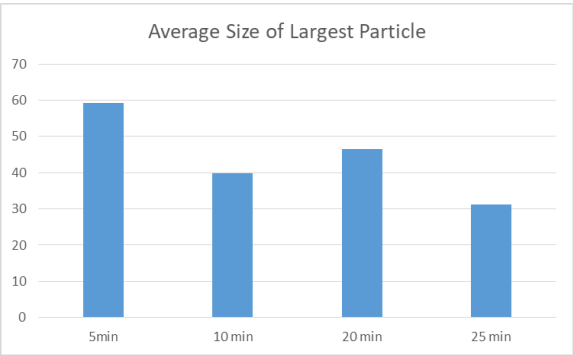

Figure 12: Average of Largest Particle noted using PVC tubing: Lipase 10mg, average standard deviation= 12.

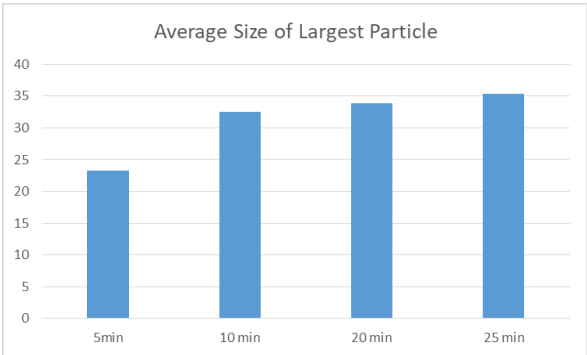

Figure 13: Average of Largest Particle noted using Silicone tubing: Lipase 10mg, average standard deviation=3

Figures 12 and 13 show the graphs of the average of the largest particle size noted when lipase 10mg was pumped through PVC and Silicon tubing respectively. In Figure 12, using the PVC tubing, the highest particle size was the highest at 5 minutes, declined at 10 minutes, increased at 20 minutes, then again decreased at 25 minutes. With the Silicone tubing, as shown in Figure 13, the average largest particle size noted increased gradually with each measurement interval leading to the 30 micrometers noted at 25 minutes. The trend in both

graphs is quite different as it fluctuates in Figure 12, while it increases gradually in Figure 13, moreover, the average of the largest particle size for the PVC tubing remains above 30 micrometers while with the silicon tubing, it remains below 30 micrometers.

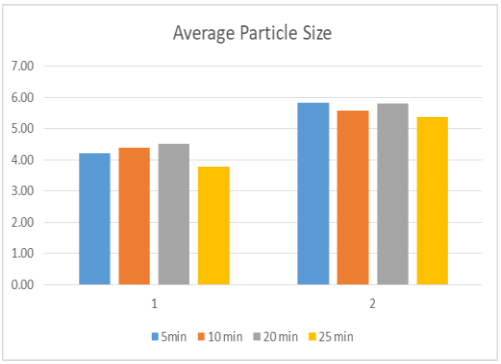

Figure 14: Average size of Particles (Median (1), Mean (2)) PVC tubing – PBS, average standard deviation= 0.2, 0.2 respectively.

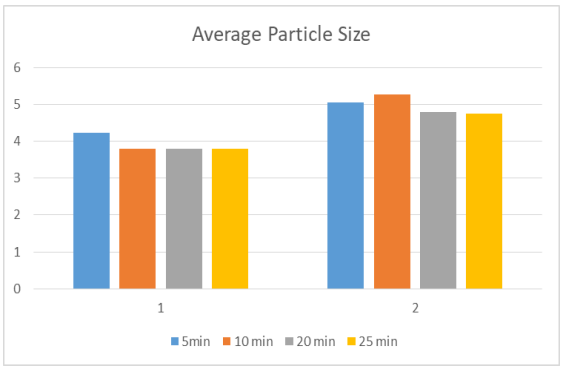

Figure 15: Average size of Particles (Median(1), Mean (2)) Silicone tubing – PBS, average standard deviation=0.2, 0.3 respectively

Figures 14 and 15 show the graphs of the average size of particles as the median and the mean when lipase 10mg was pumped through PVC and Silicon tubing respectively. In Figure 14, when using the PVC tubing, the median particle size was about 4 micrometers at 5 minutes, and increased up to 20 minutes, only to start decreasing again at 25 minutes. The mean values using the PVC tubing started high at 5 minutes, decreased at 10 minutes, showed a slight increase at 20 minutes, and decreased again at 25 minutes. Meanwhile, with the Silicone tubing, illustrated in Figure 15, the median particle size is also about 4 micrometers at 5 minutes but decreases at 10 minutes slightly and stays almost constant in the range during the remaining 20 minutes, while the mean is higher at 5 and 10 minutes at about 5 micrometers and again remains constant at 20 and 25 minutes. The median size when using PVC tubing has an increasing value unlike with silicone tubing where it is decreasing, however, concerning the mean, PVC tubing starts with a large particle size at 5 minutes that declines as the experiment proceeds, while with the silicone tubing, the mean particle size starts at a higher value and decreases as the investigation proceeds.

3.3 Lipase 1mg

The results obtained when lipase 1 mg had been pumped through Silicone and PVC tubing in a triplicate manner at 5, 10, 20, and 25 minutes are discussed below and shown in Table 3 and Figure 16-21:

Table 3: Average of total particle No, Median, Mean, Aspect ratio, and Sphericity (n=3) using PVC and Silicone tubing (Average Standard deviation for PVC 1376, 0.1, 0.4, 0.003, 0.004 and 1889, 0.5, 0.6 0.05, 0.001 for Silicone, respectively)

	Particle Number	Median	Mean	Aspect Ratio	Sphericity
PVC	1813 ± 1376	4 ± 0.1 µm	5± 0.4 µm	0.63 ± 0.003	0.87 ± 0.004
Silicone	4908 ± 1889	3 ± 0.5 µm	5 ± 0.6 µm	0.65 ± 0.05	0.87 ± 0.001

Table 3 shows data on the average of the total number of particles, Sphericity, and aspect ratio for lipase 1mg. From the Sphericity and aspect ratio readings, both tubings generated particles that are similar in their degree of circularity and elongation. The particles show moderate circularity. However, PVC tubing showed less than 3-fold the number of particles, in comparison to Silicone tubing.

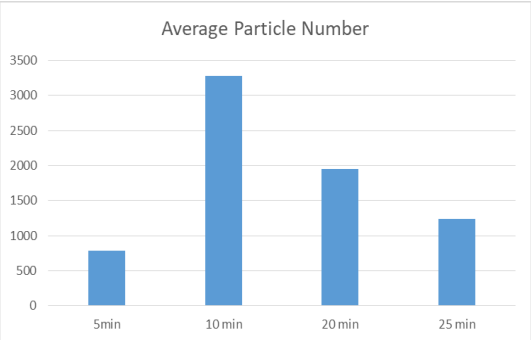

Figure 16: Average Particle Number using PVC tubing: Lipase 1mg, Average stander deviation = 1376

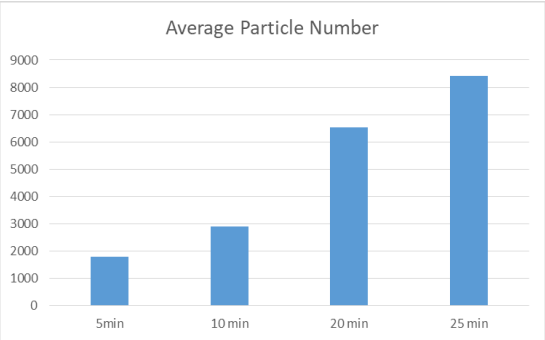

Figure 17: Average Particle Number using Silicone tubing: Lipase 1mg, Average stander deviation =18899

Figures 16 and 17 demonstrate the graphs of the average particle numbers obtained when lipase 1mg was pumped through PVC and Silicon tubing respectively. In the PVC tubing, in Figure 16, the average particle numbers were around 700 particles, which increased drastically from 10 minutes to over 3000 particles, after which it started declining at 20 and 25 minutes respectively. Meanwhile, the average particle numbers of lipase 1mg pumped through the silicone tubing, Figure 17 were low beginning at 5 minutes and rose gradually to reach over 8000 particles in 25 minutes. When using the PVC tubing, the average particle numbers showed an increasing then decreasing trend while with the Silicone tubing, the average particle numbers only increased.

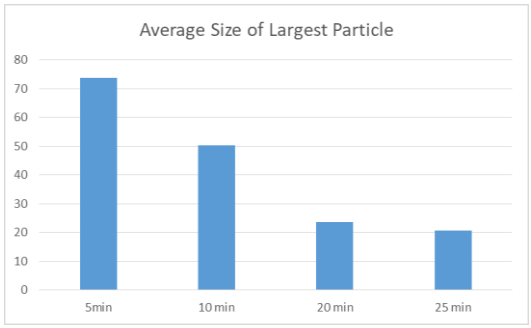

Figure 18: Average of Largest Particle noted using PVC tubing: Lipase 1mg, average standard deviation= 25

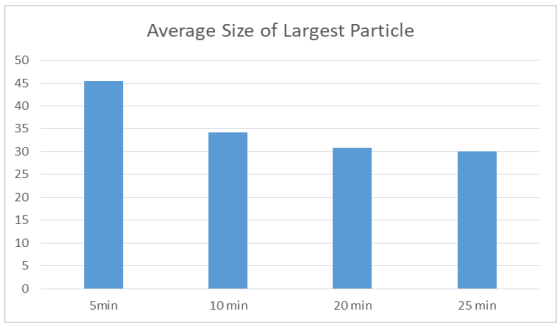

Figure 19: Average of Largest Particle noted using Silicone tubing: Lipase 1mg, average standard deviation=6

Figures 18 and 19 show the graphs of the average of the largest particle size noted when lipase 1mg was pumped through PVC and Silicon tubing respectively. In Figure 18, using the PVC tubing a declining trend is seen, with the highest particle size being the highest at 5 minutes and decreases up to the 25-minute interval. With the Silicone tubing, as shown in Figure 19, the average largest particle size noted shows a declining trend as well, starting at 45 micrometers and reducing to 30 micrometers at 25 minutes. The trend in both graphs is a decreasing one, with a wider particle size range noted when using PVC tubing compared to Silicone tubing.

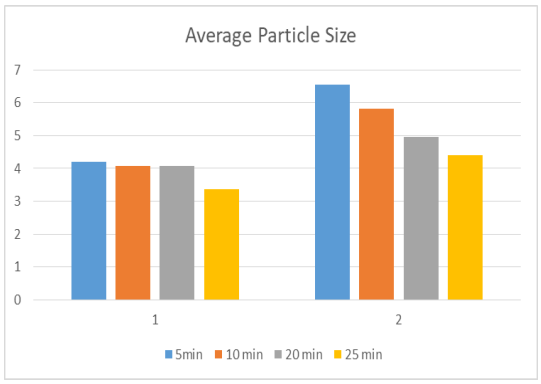

Figure 20: Average size of Particles (Median (1), Mean (2)) PVC tubing – PBS, average standard deviation= 0.1, 0.4 respectively.

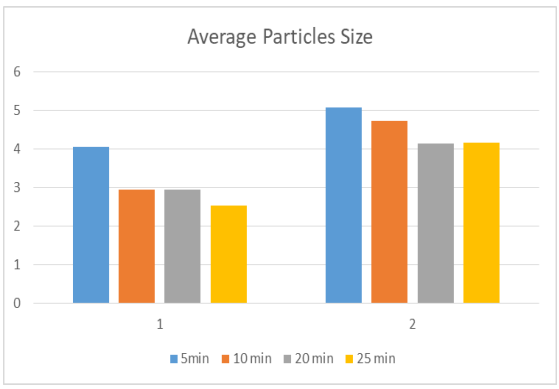

Figure 21: Average size of Particles (Median (1), Mean (2)) Silicone tubing – PBS, average standard deviation=0.5, 0.6 respectively

Figures 20 and 21 display the graphs of the average size of particles as the median and the mean when lipase 1mg was pumped through PVC and Silicon tubing respectively. In Figure 20, when using the PVC tubing, the median particle size was about 4 micrometers at 5-20 minutes and decreased slightly at 25 minutes. The mean values using the PVC tubing started high at 5 minutes and decreased gradually up to 25 minutes. Meanwhile, with the Silicone tubing, depicted in Figure 21, the median particle size is also about 4 micrometers at 5 minutes but decreases at 10 minutes slightly to stay almost constant at 10 and 20 minutes, and again declines at 25 minutes. While the mean is higher at 5 minutes at about 5 micrometers, it decreases gradually up to 20 minutes and remains constant between 20 and 25 minutes. The

median particle size when using PVC tubing has an almost constant value that decreases at 25 minutes unlike with silicone tubing where it is decreasing. Nevertheless, for the mean, using the PVC tubing starts with a large particle size at 5 minutes which declines gradually as the experiment proceeds, while with the silicone tubing, the mean particle size starts at a higher value and decreases then remains constant as the investigation proceeds.

3.4 Lipase 1mg with SDS 0.1%

The results obtained when lipase 1mg with SDS 0.1% had been pumped through Silicone and PVC tubing in a triplicate manner at 5, 10, 20, and 25 minutes are discussed below and shown in Table 4 and Figure 22-27:

Table 4: Average of total particle No, Median, Mean, Aspect ratio, and Sphericity (n=3) using PVC and Silicone tubing (Average Standard deviation for PVC 26, 0.4, 0.4, 0.003, 0.02 and 200, 0.2, 0.3, 0.002, 0.004 for Silicone, respectively)

	Particle Number	Median	Mean	Aspect Ratio	Sphericity
PVC	416 ± 26	7 ± 0.4 µm	9 ± 0.4 µm	0.60 ± 0.003	0.81 ± 0.02
Silicone	722 ± 200	4 ± 0.2 µm	6 ± 0.3 µm	0.60 ± 0.002	0.84 ± 0.004

Table 4 shows data on the average of the total number of particles, Sphericity, and aspect ratio for lipase 1mg- SDS 0.1%. From the Sphericity and aspect ratio readings, both tubings generated particles that are similar in their degree of circularity and elongation. The particles show moderate circularity. However, PVC tubing generates almost half the number of particles generated by Silicone tubing.

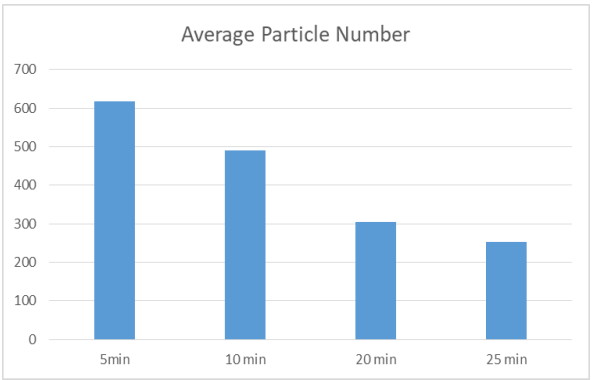

Figure 22: Average Particle Number using PVC tubing: Lipase-SDS 0.1%, Average stander deviation = 26

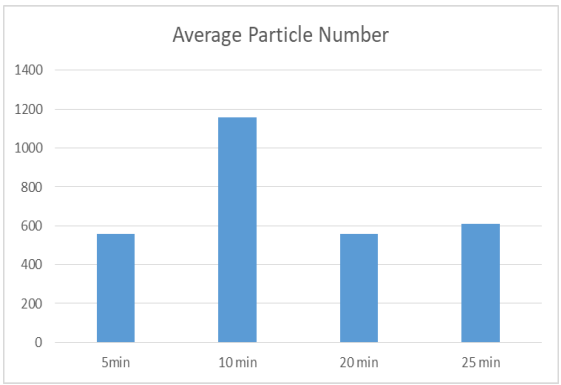

Figure 23: Average Particle Number using Silicone tubing: Lipase-SDS 0.1%, Average stander deviation =200

Figures 22 and 23 present the graphs of the average particle numbers obtained when lipase1mg -SDS 0.1% was pumped through PVC and Silicon tubing respectively. In the PVC

tubing, in Figure 22, the average particle numbers start at above 600 particles at 5 minutes, which reduces gradually up to the 25-minute interval to just above 200 particles. While the average particle numbers of lipase 1mg -SDS 0.1% pumped through the silicone tubing, Figure 23 at 5 minutes was at its lowest at about 500 particles and doubled at 10 minutes to reach its highest level, then again reduced by around half, then increased again at 25 minutes. When using the PVC tubing, the average particle numbers were between 250-600 particles. Meanwhile, with the Silicone tubing, the average particle numbers fluctuated between 500-1100 particles during the time intervals of the experiment.

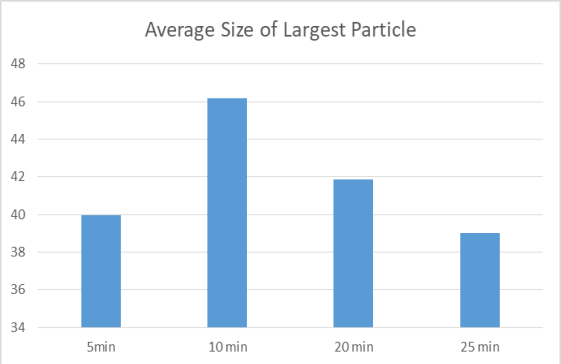

Figure 24: Average of Largest Particle noted using PVC tubing: Lipase-SDS 0.1%, average standard deviation= 5

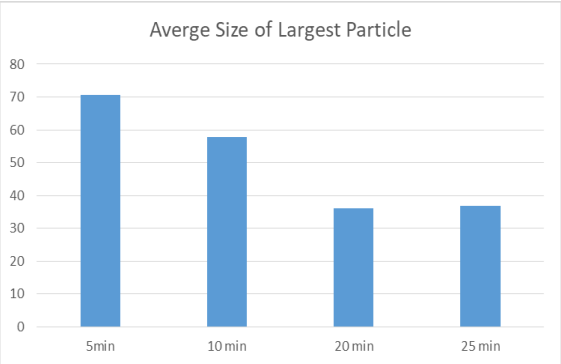

Figure 25: Average of Largest Particle noted using Silicone tubing: Lipase-SDS 0.1%, average standard deviation= 12

Figures 24 and 25 show the graphs of the average of the largest particle size noted when lipase 1mg- SDS 0.1% was pumped through PVC and Silicon tubing respectively. In Figure 24 showing the PVC tubing, the highest particle size noted was 46 micrometers at 10 minutes, which declines to reach 39 micrometers at 25 minutes. Meanwhile, in the Silicone tubing, shown in Figure 25, the average largest particle size noted shows a declining trend starting at 70 micrometers in the 5-minute interval to decrease by almost half by the last interval of the experiment. A wider particle size range was noted when using Silicone tubing compared to PVC tubing.

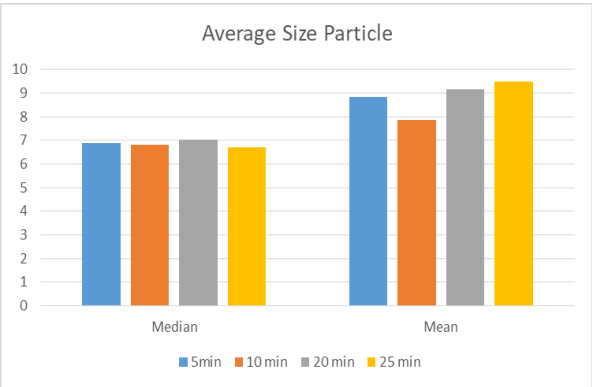

Figure 26: Average size of Particles (Median (1), Mean (2)) PVC tubing: Lipase-SDS 0.1%, average standard deviation= 0.4, 0.4 respectively.

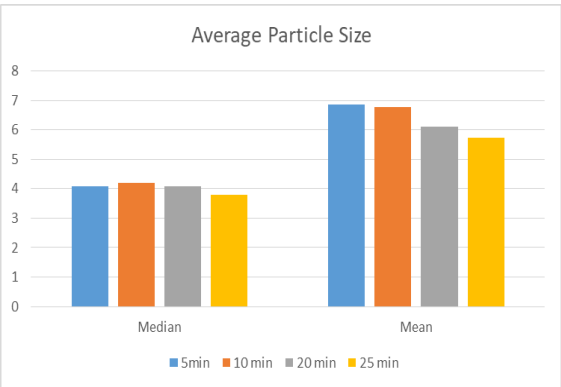

Figure 27: Average size of Particles (Median (1), Mean (2)) Silicone tubing: Lipase-SDS 0.1%, average standard deviation= 0.2, 0.3 respectively

Figures 26 and 27 demonstrate the graphs of the average size of particles as the median (1) and the mean (2) when lipase 1mg- SDS 0.1% was pumped through PVC and Silicon tubing respectively. In Figure 26, when using PVC tubing, the median values remained at 6.8-7 micrometers up to the 25-minute interval. The mean particle size was about 8.9 micrometers at 5 minutes, decreased at 10 minutes, only to continue increasing up to the 25-minute interval. As for the Silicone tubing, represented in Figure 27, the median remains almost constant between 5 to 20 minutes, then peaks at 25 minutes. While the mean particle size slightly decreases from 5 to 10 minutes, then starts a gradual decrease as it approaches the 25-minute mark. The mean particle size when using PVC tubing fluctuates between 7.9- 9.4 micrometers, with silicone tubing where it is decreasing starting from 7.9 micrometers. However, for the median, using the PVC tubing remains somewhat constant during the experiment. While with the silicone tubing, the mean particle size starts almost constant for 20 minutes but increases in the final interval of the investigation.

3.5 Lipase 1mg with SDS 0.5%

The results obtained when lipase 1mg with SDS 0.5% had been pumped through Silicone and PVC tubing in a triplicate manner at 5, 10, 20, and 25 minutes are discussed below and shown in Table 5 Figure 28-33:

Table 5: Average of total particle No, Median, Mean, Aspect ratio, and Sphericity (n=3) using PVC and Silicone tubing (Average Standard deviation for PVC 22, 0.7, 0.6, 0.008, 0.009 and 145, 0.6, 0.3, 0.0008, 0.002 for Silicone, respectively)

	Particle Number	Median	Mean	Aspect Ratio	Sphericity
PVC	115 ± 22	6 ± 0.7 μm	8 ± 0.6 μm	0.60 ± 0.008	0.80 ± 0.009
Silicone	445 ± 145	6 ± 0.6 μm	6 ± 0.3 μm	0.60 ± 0.0008	0.84 ± 0.002

Table 5 shows data on the average of the total number of particles, Sphericity, and aspect ratio for lipase 1mg- SDS 0.5%. It appears that both tubings generated a few particles with no difference in their degree of circularity and elongation. The particles show low circularity in PVC tubing and moderate circularity in Silicone tubing. However, the Silicone tubing shows more than 4-fold the number of particles generated by the PVC tubing.

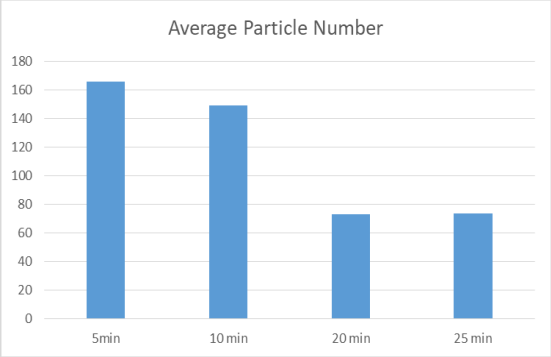

Figure 28: Average Particle Number using PVC tubing: Lipase-SDS 0.5%, Average stander deviation = 22

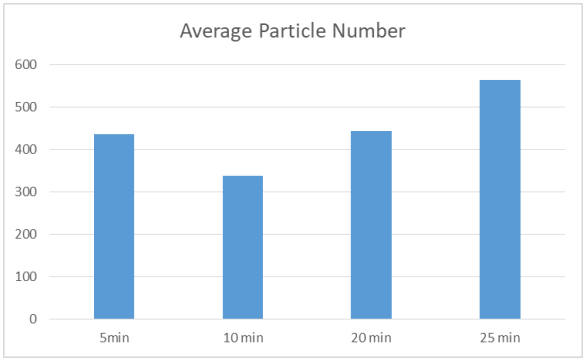

Figure 29: Average Particle Number using Silicone tubing: Lipase-SDS 0.5%, Average stander deviation = 145

Figures 28 and 29 show the graphs of the average particle numbers obtained when lipase1mg -SDS 0.5% was pumped through PVC and Silicon tubing respectively. In the PVC tubing, in Figure 28, the average particle numbers at 5 minutes, begin at just above 160 particles and decrease by less than half as the experiment proceeds to the 25-minute interval. On the other hand, the average particle numbers of lipase 1mg -SDS 0.5% pumped through the silicone tubing, shown in Figure 29, the average particles start at around 450 particles at 5 minutes, then decreased at 10 minutes at about 350 particles, to increase gradually up to the 25-minute interval. When using the PVC tubing, the average particle numbers were between 70-165 particles, meanwhile with the Silicone tubing, the average particle numbers fluctuated between 350-650 particles during the time intervals of the experiment.

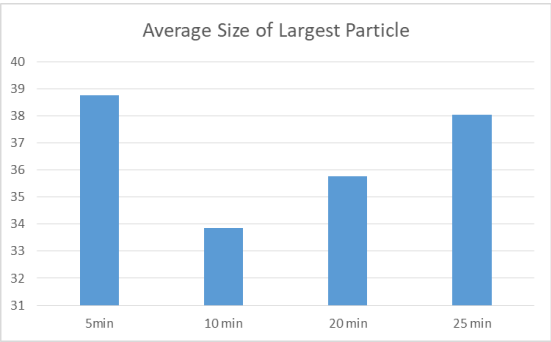

Figure 30: Average of Largest Particle noted using PVC tubing: Lipase-SDS 0.5%, average standard deviation= 1.6

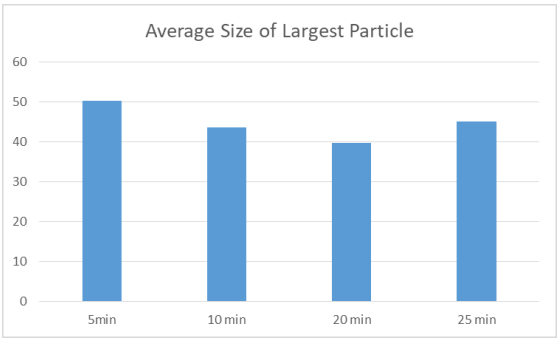

Figure 31: Average of Largest Particle noted using Silicone tubing: Lipase-SDS 0.5%, average standard deviation= 2.3

Figures 30 and 31 display the graphs of the average of the largest particle size noted when lipase 1mg- SDS 0.5% was pumped through PVC and Silicon tubing respectively. In Figure 30, showing the PVC tubing, the highest particle size noted was around 38.8 micrometers at 5 minutes, declines to reach 33.9 micrometers at 10 minutes, and then increases gradually up to the 25-minute interval. While, in the Silicone tubing, shown in Figure 31, the average largest particle size noted shows a declining trend between 50 -40 micrometers in the first 3 intervals up to 20 minutes, then starts to increase at the 25-minute interval. A similar particle size

range was noted when using Silicone and PVC tubings.

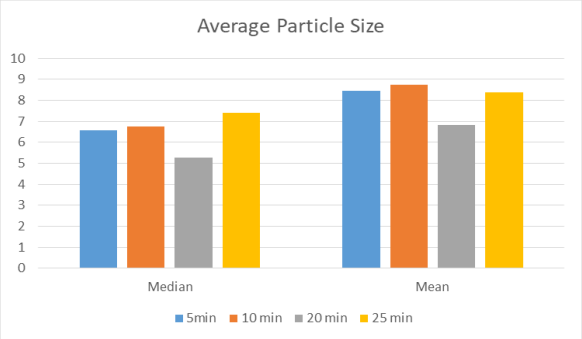

Figure 32: Average size of Particles (Median (1), Mean (2)) PVC tubing: Lipase-SDS 0.5%, average standard deviation= 0.7, 0.6 respectively.

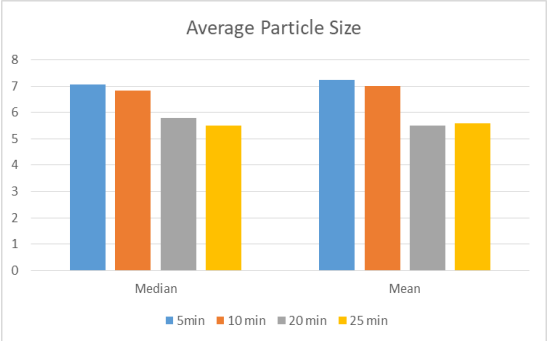

Figure 33: Average size of Particles (Median (1), Mean (2)) Silicone tubing: Lipase-SDS 0.5%, average standard deviation= 0.6, 0.3 respectively.

Figures 32 and 33 show the graphs of the average size of particles as the median (1) and the mean (2) when lipase 1mg- SDS 0.5% was pumped through PVC and Silicon tubing respectively. In Figure 32, when using the PVC tubing, the median particle size is at around 6.5 micrometers at 5-10 minutes, plunges to 5.2 micrometers at 20 minutes, then increases to 7.2 micrometers at 25 minutes. As for the Silicone tubing, represented in Figure 33, the median particle sizes the median decreases gradually from 7 micrometers at 5 minutes to 5.3 micrometers at 25 minutes. While the mean remains almost constant at 5-10 minutes, then drops to remain almost constant at 5.5 micrometers at 20-25 minutes. The median particle size when using PVC tubing fluctuates between 5.2- 7.2 micrometers like the range of 5.4-7 micrometers with silicone tubing. Using the PVC tubing the mean fluctuates between 6.9 micrometers (20 minutes) and 8.9 micrometers (10 minutes) while with the silicone tubing, the mean particle size remains almost constant at around 7 micrometers (5-10 minutes), then at around 5.5 micrometers (20- 25 minutes).

3.6 Lipase 1mg with Poloxamer 188

The results obtained when lipase 1mg with Poloxamer 188 0.1% had been pumped through Silicone and PVC tubing in a triplicate manner at 5, 10, 20, and 25 minutes are discussed below and shown in Table 6 and Figure 34-39:

Table 6: Average of total particle No, Aspect ratio, and Sphericity (n=3) using PVC and Silicone tubing (Average Standard deviation for PVC 19, 1.2, 1.2, 0.02, 0.007 and 173, 0.4, 0.6, 0.0004, 0.01 for Silicone, respectively)

	Particle Number	Median	Mean	Aspect Ratio	Sphericity
PVC	134 ± 19	9 ± 1.2 µm	11 ± 1.2 µm	0.62 ± 0.02	0.83 ± 0.007

Silicone	423 ± 173	5 ± 0.4 µm	8 ± 0.6 µm	0.60 ± 0.0004	0.82 ± 0.01

Table 6 shows data on the average of the total number of particles, Sphericity, and aspect ratio for lipase 1mg- Poloxamer 188. It appears that both tubings generated a few particles with almost no difference in their degree of circularity and elongation. The particles show moderate circularity in PVC tubing and low circularity in Silicone tubing. However, the PVC tubing shows less than 3-fold the number of particles generated by Silicone tubing.

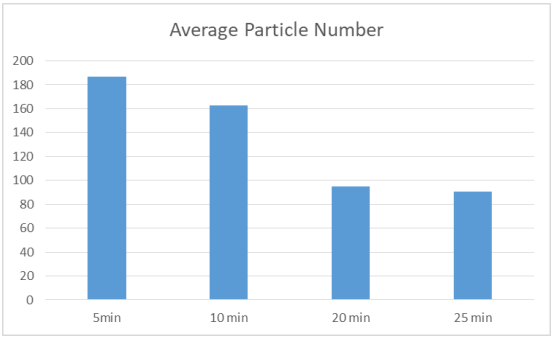

Figure 34: Average Particle Number using PVC tubing: Lipase Poloxamer 188, Average stander deviation =19

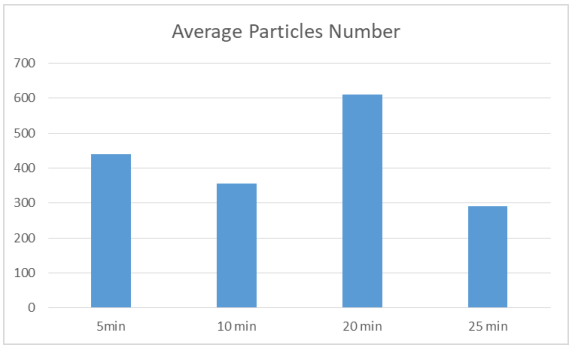

Figure 35: Average Particle Number using Silicone tubing: Lipase- Poloxamer 188, Average stander deviation = 173

Figures 34 and 35 show the graphs of the average particle numbers obtained when lipase1mg - Poloxamer188 was pumped through PVC and Silicon tubing respectively. In the PVC tubing, depicted in Figure 34, the average number of particles starts high at 5 minutes, and decreases in the following intervals of the experiment to be reduced by more than half by the end of the experiment. Meanwhile, the average particle numbers of lipase 1mg -Poloxamer 188 pumped through the silicone tubing shown in Figure 35, starts higher at 5 minutes, decrease at 10 minutes, yet rise to their highest at 600 particles at 20 minutes, to decrease again at 25 minutes by around half. When using the PVC tubing, the average particle numbers were between 90-180 particles, meanwhile with the Silicone tubing, the average particle numbers fluctuated between 290-600 particles during the time intervals of the experiment.

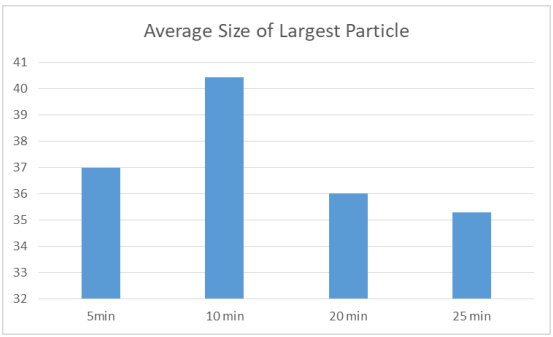

Figure 36: Average of Largest Particle noted using PVC *tubing: Lipase-Poloxamer 188, average standard deviation= 3.6*

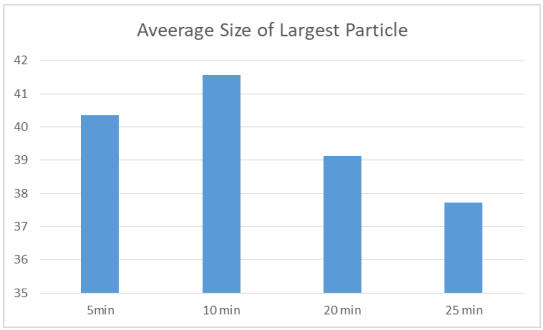

Figure 37: Average of Largest Particle noted using Silicone tubing: Lipase-Poloxamer, average standard deviation=2.3

Figures 36 and 37 illustrate the graphs of the average of the largest particle size noted when lipase 1mg- Poloxamer 188 was pumped through PVC and Silicon tubing respectively. In Figure 36, showing the PVC tubing, the particle size noted at 5 minutes was around 37 micrometers increases to reach 40.2 micrometers at 10 minutes, and then decreases gradually leading up to the 25-minute interval. While, in the Silicone tubing, shown in Figure 37, the average largest particle size noted at 5 minutes was around 40.3 micrometers, increases to reach 41.5 micrometers at 10 minutes, and then decreases gradually leading up to the 25-minute interval. A similar particle size range was noted when using Silicone and PVC tubings.

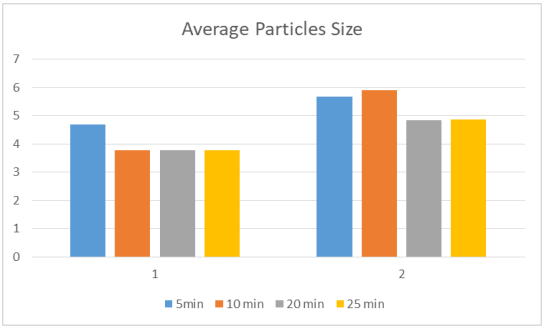

Figure 38: Average size of Particles (Median (1), Mean (2)) PVC tubing: Lipase-Poloxamer 188, average standard deviation= 1.2, 1.2 respectively.

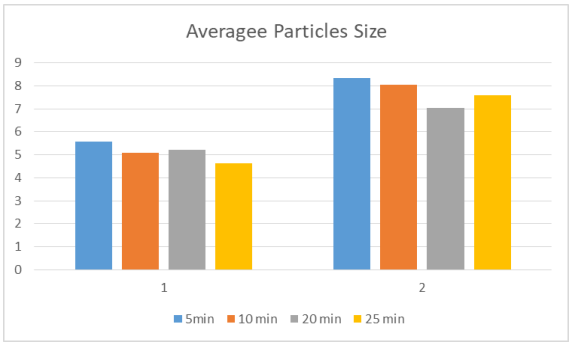

Figure 39: Average size of Particles (Median (1), Mean (2)) Silicone tubing: Lipase-Poloxamer 188, average standard deviation= 0.4, 0.6 respectively.

Figures 38 and 39 depict the graphs of the average size of particles as the median (1) and the mean (2) when lipase 1mg- Poloxamer 188 was pumped through PVC and Silicon tubing respectively. In Figure 38, using the PVC tubing, the median particle size is at around 4.7 micrometers at 5 minutes, plunges to 3.9 micrometers at 10 minutes, then remains constant for up to 25 minutes. The mean slightly increases between 5-10 minutes to about 5.9 micrometers, plunges at 20 minutes to 4.9 micrometers then remains constant up to the 25-minute interval. As for the Silicone tubing, represented in Figure 39, the median particle size fluctuates between 4.5 micrometers (25 minutes) and 5.5 micrometers (5 minutes). On the other hand, the mean is in a higher range where it is 8.2 micrometers at 5 minutes and decreases to 7 micrometers at 20 minutes but increases again at 25 minutes to around 7.5 micrometers. The median particle size when using PVC tubing fluctuates between 3.9- 4.7 micrometers less than the range of 4.5- 5.5 micrometers with silicone tubing. Using the PVC tubing the mean fluctuates between 4.9 micrometers (20-25 minutes) and 5.9 micrometers (10 minutes) while with the silicone tubing, the mean particle size fluctuates between 8.2 micrometers (5 minutes) and 7 micrometers (20 minutes).

3.7 Lipase 1mg with Polysorbate 20

The results obtained when lipase 1mg with Polysorbate 0.1% had been pumped through Silicone and PVC tubing in a triplicate manner at 5, 10, 20, and 25 minutes are discussed below and shown in Table 7 and Figure 40-45:

Table 7: Average of total particle No, Median, Mean, Aspect ratio, and Sphericity (n=3) using PVC and Silicone tubing (Average Standard deviation for PVC 14036, 0.00, 0.3, 0.003, 0.003 and 4261, 0.3, 0.04, 0.002, 0.002 for Silicone, respectively)

	Particle Number	Median	Mean	Aspect Ratio	Sphericity
PVC	24673 ± 14036	4 ± 0.00 µm	5± 0.3 µm	0.61 ± 0.003	0.87 ± 0.003
Silicone	5457 ± 4261	3 ± 0.3 µm	4 ± 0.04 µm	0.63 ± 0.002	0.88 ± 0.002

Table 7 shows data on the average of the total number of particles, Sphericity, and aspect ratio for lipase 1mg- Polysorbate 20. It appears that both tubings had generated particles with almost no difference in their degree of circularity and elongation. The particles show moderate circularity in both tubings. However, the PVC tubing has more than 4-fold the number of particles in comparison to the Silicone tubing.

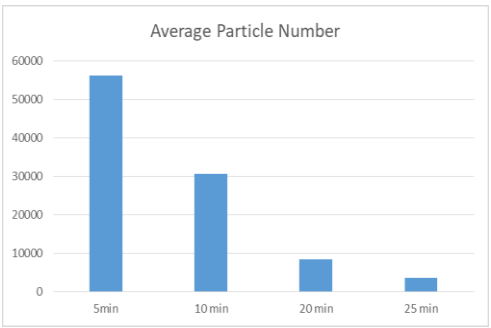

Figure 40: Average Particle Number using PVC tubing: Lipase-Sorbate20, Average stander deviation = 1436

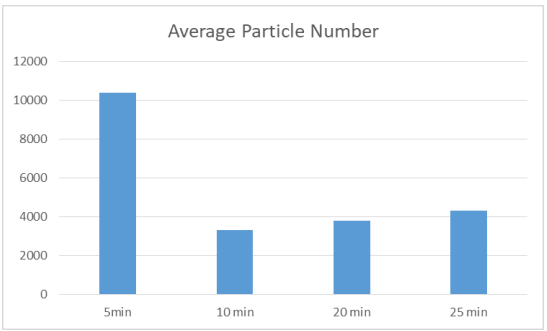

Figure 41: Average Particle Number using Silicone tubing: Lipase-Sorbate20, Average stander deviation =4261

Figures 40 and 41 illustrate the average particle numbers obtained when lipase1mg – Polysorbate 20 was pumped through PVC and Silicon tubing respectively. In the PVC tubing, Figure 40, the average number of particles starts extremely high at 5 minutes above 10000 particles, which declined to 3000 particles at 10 minutes, to increase slightly leading up to just above 4000 particles at the 25-minute interval. While the average particle numbers of lipase 1mg -polysorbate pumped through the silicone tubing, shown in Figure 41, showed a declining trend. It starts with 55000 particles at 5 minutes, and reduces to 30000 particles at 10 minutes, to continue declining to below 10000 particles at 20 and 25-minute intervals. When using the PVC tubing, the average particle numbers were between 10000-3000

particles, meanwhile with the Silicone tubing, the average particle numbers fluctuated widely between 550000-3000 particles during the time intervals of the experiment.

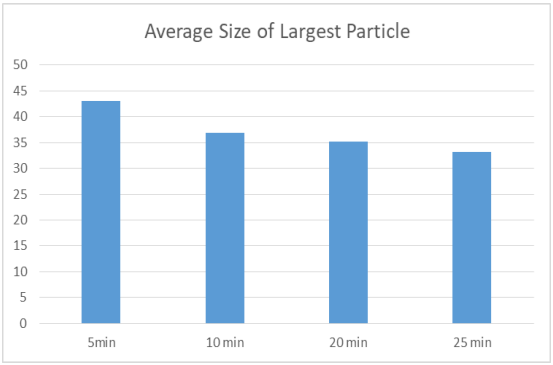

Figure 42: Average of Largest Particle noted using PVC tubing: Lipase-polysorbate, average standard deviation= 7

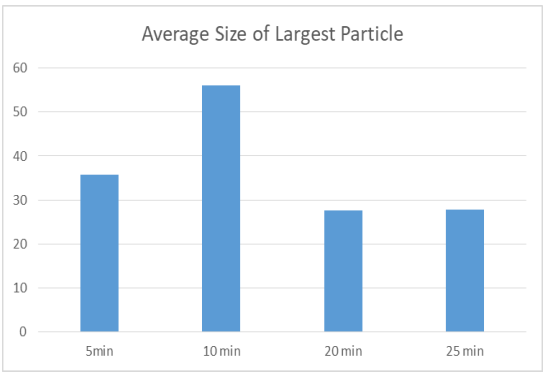

Figure 43 Average of Largest Particle noted using Silicone tubing: Lipase-Polysorbate 20, average standard deviation= 11

Figures 42 and 43 illustrate the graphs of the average of the largest particle size noted when lipase 1mg- Twin was pumped through PVC and Silicon tubing respectively. Figure 42 displays a declining trend when using the PVC tubing, the particle size noted at 5 minutes was around 44 micrometers, which decreases gradually leading up to 35 micrometers at the 25-minute interval. Meanwhile, in the Silicone tubing, shown in Figure 43, the average largest particle size noted at 5 minutes was around 35 micrometers, increases to reach around 56 micrometers at 10 minutes, and then decreases gradually leading up to the 25-minute interval. A wider particle size range was noted when using Silicone tubing compared to PVC tubing.

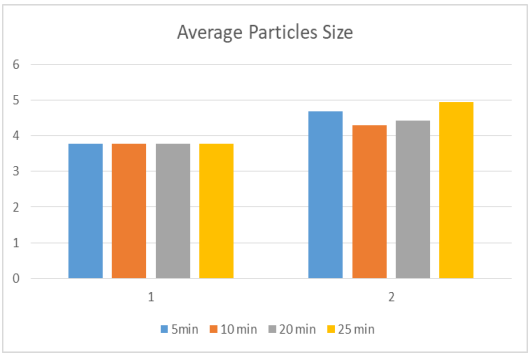

Figure 44: Average size of Particles (Median (1), Mean (2)) PVC tubing: Lipase-Polysorbate 20, average standard deviation= 0.0, 0.3 respectively. M

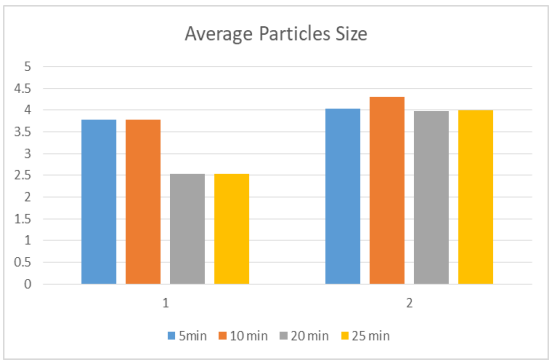

Figure 45: Average size of Particles (Median (1), Mean (2)) Silicone tubing: Lipase-Polysorbate 20, average standard deviation= 0.3, 0.04 respectively.

Figures 44 and 45 show the graphs of the average size of particles as the median (1) and the mean (2) when lipase 1mg- Polysorbate 20 was pumped through PVC and Silicon tubing respectively. In Figure 44, using the PVC tubing, the median particle size is constant at all the

reading intervals at around 3.8 micrometers. The mean, on the other hand, is 4.7 micrometers at 5 minutes, drops to 4.2 micrometers at 10 minutes then increases gradually to reach 5 micrometers at 25 minutes. Represented in Figure 45, for the Silicone tubing, the median particle size is almost constant at 3.6 micrometers at 5-10 minutes, then plunges to 2.5 micrometers at 20-25 minutes. On the other hand, the mean is almost constant throughout the experiment at around 4 micrometers, except for a slight peak at 10 minutes. The median particle size when using PVC tubing is constant at 3.8 micrometers and is divided between 2.5 - 3.6 micrometers for Silicone tubing. Using the PVC tubing the mean fluctuates between 4.2 micrometers (10 minutes) and 5 micrometers (25 minutes) while with the silicone tubing, the mean particle size fluctuates between 4.2 micrometers (10 minutes) and 4 micrometers (5, 20, and 25 minutes).

4. Discussion

Pumping has a great impact on the quality and safety of therapeutic protein products as a cause of the formation of particles. The associated risk is greater as there is no filtration performed after filling which leads the formed particle to end up in the finished product. Therefore, it is important to understand and control the process of particle formation by the pump unit. Effective control depends on the assessment of particles formed during pump operation using a suitable analytical method (Her and Carpenter, 2020).

In this experiment, in addition, to calculating the shape descriptor and assessing the presence of sub visible particles and particle size distribution, quantifying the formed particles provides an important clue about the difference between the results of tubing material obtained from the same conditions. For instance, both tubings had fewer particles when PBS was pumped than when lipase with or without surfactants was pumped and silicone tubing had fewer particles in comparison to PVC tubing, but the pattern was changed when lipase was circulated. The Silicone tubing had lower particles only when lipase 10mg, and lipase 1 mg with polysorbate20 were pumped, while PVC tubing had lower particles when pumped with lipase 1mg free of surfactants, and lipase 1mg with Poloxamer 188, SDS 0.1%, and SDS 0.5%. Thus, without investigating different concentrations and surfactants one can mistakenly recommend one of the tubing as the least particle formation. Moreover, the detected particle in the background during the PBS pump indicates that the observable level of the particle was not a result of the tubing shed as also discussed by Her and Carpenter (2020).

The tubing used has different traits in terms of chemical composition, temperature range, and degree of hardness. Silicone tubing was made from Vinyl Methyl Silicone, has a wide temperature range [-60°C +200°C], and has a hardness value of 60 shore A which indicates its softness and flexibility, while the other is made of Polyvinyl Chloride (PVC) material, has Temperature resistant up to +60°C, stiffer, and has greater abrasion and extrusion resistance as it has a hardness value of shore A80.

High hardness and abrasion characteristics explain low particle performance for the PVC tubing as literature linked a decrease in formed particles with an increase in tubing hardness and high abrasion with low shedding of tubing particles (Deiringer and Friess, 2022b).

The study demonstrated a fluctuation in the average of the particle count and the size of the largest particle with time for both tubing as shown in figures 5,6,11,12,17,18,23,24,29,30,35, 36,41, and 42 for the average largest particle and in figures 3,4,9,10,15,16,21,22,27,28,33,34, 39, and 40 for the particle number. In general, this fluctuation could be a result of the dynamic nature of the protein aggregates as it is responsive to a minor change in their environment in terms of size and number and the reduction of size with the increase in time could be due to their weak association during protein particle growing that led to broken into smaller particle during measurement (Ripple and Dimitrova, 2012). In most cases we could either see a decrease in particle size with time or that it fluctuated around a rather similar value. Thus, for this protein, we do not see an increase in size with pumping time but in some cases a decrease. This could either be due to compaction or attrition of particles formed initially during the pumping.

From the size perspective, both tubings generated particles were characterized as sub-visible particle as it has a size range below 100 μm as demonstrated in all figures of the average size of the largest particle (Ripple and Dimitrova, 2012). The average number of particles has a large standard deviation which is consistent with many similar protein particles studies that reveal large differences in particle count for the same pumped product such as those performed by Deiringer and Friess (2022a) and Her and Carpenter (2020).

From a morphology perspective, data in tables1-7 showed that all Silicone tubing results demonstrated an average Sphericity value ranging between 0.84-0.88 which indicates moderate circularity, and small particles size except for lipase with Poloxamer 188 had a Sphericity value of less than 0.83 which indicates low circularity and slightly bigger particles. For PVC tubing measurements, moderate circularity was obtained only with SDS results. While aspect ratio values demonstrate slight elongation particles for both tubing measurements as it ranges from 0.6 to 0.65 (Durand et al., 2023). Shape variability is one of the protein particle traits as it ranges from nearly spherical aggregates to long, irregular fibers (Ripple and Dimitrova, 2012). Both tubing exhibit distribution skewed to the right as the mean values were greater than the median values as the average particle size data reveal which indicates the dominance of the small particle in the population and more abundant particle size (mode) will have a value of less than the median value obtained for any of the data set i.e. will be less than 9 μm for each experiment result.

The experiments demonstrated that both tubings generate protein particles that differ in number according to the presence of surfactants and protein concentration used. Silicone tubing showed interesting results regarding lipase concentration as it expresses an inverse relation with lipase concentration (1mg and 10mg), while PVC tubing showed a positive

relation with concentration. Furthermore, the particle formed decreased remarkably for both tubing when surfactants were used except for polysorbate 20 which attained the highest particle, 24673 particles for PVC and 5457 particles for Silicone.

Compression of the performance of the surfactants reveals that SDS surfactant concentrations exhibit sensational protection levels in both tubings. The protection was increased with the increase in its concentration as SDS 0.5% achieved the lowest particle when compared to 0.1% concentration in the measurements. The high SDS concentration achieved more than 3-fold protection in PVC tubbing and more than 1-fold in silicone tubing in comparison to the low concentration SDS.

Poloxamer 188 had the second-highest prevention capacity for particle formation in both tubings as the particle count was 423 and 134 in silicone and PVC tubing, respectively, when used. In contrast, the highest particle number on both tubings was achieved in the presence of Polysorbate 20. This indicates that Polysorbate 20 is not a good protectant when it comes to pumping materials used in this study. In difference from SDS polysorbate is not likely to form a complex with the protein. Thus it has a less stabilizing effect in solution. However, it is well known to adsorb to surfaces and could have had a protective effect on surface-induced aggregation. This was, however, not the case in this study. Lipase could have to some degree created an enzymatic degradation of the Polysorbate, but this cannot fully explain the results. It is interesting to note that Poloxamer 188 which probably has a similar surface protection mechanism as polysorbate, gives a very good protection. Polymeric surface components are known to be less dynamic in their adsorption/desorption pattern and this could be one explanation for the observed results.

The degree of protection of the Poloxamer 188 was nearly the same as the SDS 0.5% in both tubings. In the Silicone tubing particle count was 423 particles for Poloxamer 188 and 445 particles for SDS0.5%, while in the PVC tubing particle count was 134 particles for Poloxamer 188 and 115 particles for SDS0.5%.

The remarkable control of particle formation by Poloxamer 188 and SDS 0.05% suggests these two surfactants as a potential alternative for lipase formulation. The SDS 0.1% in PVC tubing had more than 3-fold particle number and nearly 2-fold in Silicone tubing compared to Poloxamer 188.

The importance of the selection of tubing for peristaltic pumps stems from the quality and safety issues associated with it as a source for particle formation. The major advantage of the tubings is single-use, avoid cross-contamination and extensive cleaning is unnecessary.

General recommendation on the tubing is difficult as it depends on different factors tubing base material, process requirements, availability, and cost. Therefore, an understanding of the relationship between protein aggregation and tubing materials would be beneficial for tubing

selection in biologics formulation. Moreover, During formulation development, testing with the actual pump, brand, and tubings provides assurance that particle levels are controlled throughout the development, scaling up, and manufacturing processes to give the highest quality products (Her and Carpenter, 2020).

The QICPIC instrument used in this project is a benefit of advancement in technology that helps in developing automated methods for particle characterization in terms of size and shape using dynamic image analysis techniques. It combined image capture, and viewing approaches, and uses software for image interpretation that enables easy use, the reduction of the time required for analysis, and size determination. The result obtained in this research demonstrates that QICPIC can be relied on for investigation of the presence of particles and characterization.
In this study, QICPIC errors were minimized by choosing suitable parameters for insertion after many pilot studies, improper dispersion air bubbles were avoided, the quality of the measurement and the communication between the computer and the instrument were always checked, and proper cleaning protocol was followed.
Increase frequency of using QICPIC instrument for the detection and characterization of protein particles would facilitate the execution of research in this area and contribute in developing methods and advancements in equipment for rapid and simultaneous measurement.

5. Conclusion

From a biopharmaceutical perspective, the tubing tubes of peristaltic pumps are of great importance for their role in particle formation during the pumping process.
In this degree project Silicone and PVC peristaltic pump were investigated using lipase 1mg and 10 mg free surfactant and lipase 1 mg with Polysorbate20, Poloxamer 188, and two concentrations of SDS 0.1% and 0.5%.

The experiments showed that peristaltic tubing type has a significant effect on particle formation as the presence of particles was differ in number, size, and the effect of the surfactants according to the tubing type used. Both tubings revealed substantially lower particles when PBS was pumped in comparison to lipase.
In general, PVC tubing induces fewer particles in most experiments, while silicone tubing generates fewer particles only with lipase 10 mg and lipase with polysorbate 20.

The particles produced by both tubings were characterized by size to sub visible particles (below 100 µm) and by shape into Low to moderate circularity for Sphericity and slight elongation for their aspect ratio.
The study revealed that the presence of surfactant was associated with the reduction of particle formation except for polysorbate 20. Poloxamer 188 and SDS surfactants showed a great level of inhibiting particle formation which suggests using as a potential alternative in the formulation and development after more investigation.
The project results demonstrate the potentiality of using a QICPIC instrument for particle characterization and reveal its advantages in terms of the viewing approaches, reduction of the time required for analysis, and size determination.

6. Future Research

For the future of this project, investigate other tubing and protein type with different peristaltic pump speeds, and buffers, and Compare the QICPIC result obtained, with other techniques such as the Laser diffraction technique or scanning electron micrograph (SEM) images for validation and combined it with measuring the activity of the lipase enzyme would be of great value for biopharmaceutical industry in term of development and production and protein transportation.

7. Reference List

Deiringer, N. and Friess, W. (2022a). Proteins on the Rack: Mechanistic Studies on Protein Particle Formation during Peristaltic Pumping. *Journal of Pharmaceutical Sciences*, 111(5), pp.1370–1378. Doi https://doi.org/10.1016/j.xphs.2022.01.035.

Deiringer, N. and Friess, W. (2022b). Reaching the Breaking Point: Effect of Tubing Characteristics on Protein Particle Formation during Peristaltic Pumping. *International Journal of Pharmaceutics*, 627(2022), p.122216. Doi https://doi.org/10.1016/j.ijpharm.2022.122216.

Durand, S., Jackson, B.E., Fonteno, W.C. and Michel, J.C., 2023. Quantitative Description and Classification of Growing Media Particle Morphology through Dynamic Image Analysis. *Agriculture*, *13*(2), p.396.

Haji Abdolvahab, M., Fazeli, A., Fazeli, M.R., Brinks, V. and Schellekens, H. (2014). The Effects of Dodecyl Maltoside and Sodium Dodecyl Sulfate Surfactants on the Stability and Aggregation of Recombinant Interferon Beta-1b. *Journal of Interferon & Cytokine Research: the Official Journal of the International Society for Interferon and Cytokine Research*, [online] 34(11), pp.894–901. Doi https://doi.org/10.1089/jir.2013.0131.

Her, C. and Carpenter, J.F. (2020). Effects of Tubing Type, Formulation, and Postpumping Agitation on Nanoparticle and Microparticle Formation in Intravenous Immunoglobulin Solutions Processed with a Peristaltic Filling Pump. *Journal of Pharmaceutical Sciences*, 109(1), pp.739–749. Doi https://doi.org/10.1016/j.xphs.2019.05.013.

Her, C., Tanenbaum, L.M., Bandi, S., Randolph, T.W., Thirumangalathu, R., Mallela, K.M.G., Carpenter, J.F. and Elias, Y. (2020). Effects of Tubing Type, Operating Parameters, and Surfactants on Particle Formation during Peristaltic Filling Pump Processing of a mAb Formulation. *Journal of Pharmaceutical Sciences*, 109(4), pp.1439–1448. Doi https://doi.org/10.1016/j.xphs.2020.01.009.

Khan, T.A., Mahler, H.-C. And Kishore, R.S.K. (2015). Key Interactions of Surfactants in Therapeutic Protein Formulations: a Review. *European Journal of Pharmaceutics and*

Biopharmaceutics, [online] 97(2015), pp.60–67. Doi https://doi.org/10.1016/j.ejpb.2015.09.016.

Mahler, H.-C., Friess, W., Grauschopf, U. and Kiese, S. (2009). Protein aggregation: Pathways, Induction Factors, and Analysis. *Journal of Pharmaceutical Sciences*, 98(9), pp.2909–2934. Doi https://doi.org/10.1002/jps.21566.

Mavrodontis, N. (2020). Performance Assessment of a Peristaltic Pump. *SIMULEON Blog*. Available at: https://simulation-blog.technia.com/simulation/performance-assessment-of-a-peristaltic-pump?utm_campaign=Simuleon%20Blogartikelen&utm_medium=email&_hsmi=88345559&_hsenc=p2ANqtz-8qaT8TcOm_B0Tu7n5TMxXNuFzWjqEUbQ6Am6J-3abAz5KjPI6G-85ZPgijjjAMzidhkNXMX7TxeWsMaqeQa1_JuyHYyA&utm_content=88345559&utm_source=hs_email [Accessed Feb. 2023].

Ripple, D.C. and Dimitrova, M.N. (2012). Protein particles: What We Know and What We Do Not Know. *Journal of Pharmaceutical Sciences*, 101(10), pp.3568–3579. Doi https://doi.org/10.1002/jps.23242.

Suhail , M., Janakiraman, A.K., Khan, A., Naeem, A., Badshah, S.L. and Ommega Internationals (2019). Surfactants and Their Role in Pharmaceutical Product Development: an Overview. *J Pharm. Pharm*, 6(2), pp.72–82. Doi https://doi.org/10.15436/2377-1313.19.2601.

Sympatec GmbH (2023). *QICPIC*. [Online] Sympatec.com. Available at: https://www.sympatec.com/en/particle-measurement/sensors/dynamic-image-analysis/qicpic/ [Accessed 11 Feb. 2023].

8. Appendix

8.1 Appendix A: Lab Protocol

General Aspects

- Concentration of 10 mg/ml and 1 mg/ml of Sobi Lipase will be used
- Samples of 20 ml will be used to ensure a suitable volume for the particle-size measurement (Minimum volume required (20 ml) for wet dispersion to be investigated by QICPIC)
- Samples will be dispersed in Phosphate Buffer Saline PBS (0.1 M pH 7)
- Triplicate manner will be used for measurements.
- Each tubing type will be tested with buffer (Blank) and lipase with and without surfactant.
- The length of the inlet and outlet tubing will be 35 cm and 30 cm respectively, with a filling volume of 8 ml.
- The pump speed will be 10 and the rate will be 14.8 m/minute.
- Glass bottle of 25 ml to Place and collect the sample will be used.
- The bottle cap had a hole drilled into it, of sufficient diameter to provide a tight fit for the peristaltic pump tubing.

Buffer Preparation

- Prepare 800 mL of distilled water in a suitable container.
- Add 0.8 g of Sodium chloride to the solution.
- Add 0.02 g of Potassium Chloride to the solution.
- Add 0.144 g of Sodium Phosphate Dibasic to the solution.
- Add 0.0245 g of Potassium Phosphate Monobasic to the solution.
- Adjust solution to desired pH (7).
- Add distilled water until the volume is 1 L.
- Stored in a cool and dry place.

Samples without surfactants

A-Lipase 1 mg/ml:

- Thaw lipase 25.25 mg/ml at 2°C - 8°C until no visible ice remains.
- Gently mix before use. (Invert the bottle up and down several times)
- Use to prepare 120 ml of lipase 1 mg/ml in PBS without surfactants as flow:

  1- Put 5 ml of thawed lipase in a suitable container

  2- Add 121 ml of PBS

  3- Gently mix

- Use Nanodrop to check the concentration.
- Filter the sample in a laminar flow hood using a 0.2 µm syringe filter.
- Divided into six samples of 20ml for each.
- Store in the refrigerator.

B-Lipase 10 mg/ml:

- Thaw lipase 25.25 mg/ml at 2°C - 8°C until no visible ice remains.
- Gently mix before use. (Invert the bottle up and down several times)
- Use to prepare 120 ml of lipase 10 mg/ml in PBS without surfactants as flow:

  1- Put 50 ml of thawed lipase in a Suitable container

  2- Add 76 ml of PBS

  3- Gently mix
- Use Nanodrop to check the concentration.
- Filter the sample in a laminar flow hood using a 0.2 µm syringe filter.
- Divided into six samples of 20ml for each.
- Store in the refrigerator.

Sample with surfactants

A-Polysorbate 20

- Prepare a stock solution of 1% w/w of surfactants.

- Pipette 12 ml of the Polysorbate 20 into 108 ml of lipase sample in a laminar flow hood to reach a final surfactant concentration of 0.1 % (w/v).
- Filter surfactant solutions and the sample in a laminar flow hood using a 0.2 µm syringe filter.
- Use Nanodrop to check the concentration.
- Store and freeze the sample containing surfactants at -70°C until use.

B-Poloxamer 188

- Weigh 0.121g of Poloxamer 188.
- Dissolve in 121 ml of PBS using magnetic stirring for 30 minutes.
- Add 5 ml of lipase 25.25mg/ml.
- Use Nanodrop to check the concentration.
- Store and freeze the sample containing surfactants at -70°C until use.

C-Sodium Dodecyl Sulfate (SDS) Surfactant

- Weigh 0.1126g of SDS.
- Dissolve in 121 ml of PBS using magnetic stirring for 30 minutes.
- Add 5 ml of lipase 25.25mg/ml.
- Use Nanodrop to check the concentration.
- Store and freeze the sample containing surfactants at -70°C until use.

Measurement of Protein Concentration

A-Nanodrop

- Start the ND-1000 software.
- Choose method type protein a 280.
- Clean the pedestal using water and tissue.
- Left the sampling arm and add 2µl of dH2o into the lower pedestal.
- Lower the sampling arm again gently.
- Press Ok, and wait for the instrument to start up.
- Lift the sampling arm and wipe off the liquid with a tissue.
- Place 2µl of sample buffer into the pedestal.

- Lower the arm and press Ok.
- Wipe off and add 2µl of the protein sample.
- Press measurement.

Measurements Matrix

Tubing	PBS	Lipase 1 mg	Lipase 10 mg	Lipase with Surfactants	
Silicone	**3**	**3**	**3**	**12**	**No. of Measurement**
	60 ml	**60 ml**	**60 ml**	**240 ml**	**Volume**
PVC	**3**	**3**	**3**	**3**	**No. of Measurement**
	60 ml	**60 ml**	**60 ml**	**240 ml**	**Volume**
Total Volume	**120 ml**	**120 ml**	**120 ml**	**480 ml**	**840 ml**

Analysis

Precautions:

- Inspect the sample visually to evaluate the dispersion.
- Check the instrument set-up (e.g. warm-up).
- Avoid air bubbles, evaporation of liquid, and inhomogeneities in the dispersion.
- The blank measurement must be performed using the same method as that used for the measurement of the samples.
- Before installing tubing in the pump, wash the tube using the following protocol:
    - Run 200 ml of MQ water through the tubing.
    - Remove tubing after each pumping run.

Measurements:

1. Start the computer and create a new folder in Windox Database.
2. Register a new measurement in Database Administration.
3. Open the QICPIC sensor control and locate your database to the measurement database.
4. Click on EXTRAS and click Redetect Hardware to test the communication between the computer and the instrument.
5. Click on EXTRAS and click Self Sensor Test to know the quality of the measurement.
6. Select product parameters as follows:
    - Use 1.3500 g/cm3 as the Density value and 1 as the Shape factor for product properties.
    - Use EQPC, FERET_MAX, FERET_MIN, and FERET_MEAN as Diameter values and use Sphericity, Aspect ratio, Convexity, and Elongation as Shape values for calculation properties.
    - Use FEERET_MAX as the Standard diameter.
    - Click on ISO conformance.
    - Create a standard QICPIC class by selecting M4 ISO measuring range.
7. Select M4 as the measuring range.
8. Select trigger parameters as flow:

- Frame rate10hz.
- Measurement start by the start button, finish after 60 s, and repeat measurement is 29 (Continuous measurement).

9. Select disperser as flow:

- Disperser LIXELL
- CUVETTE 0.2
- LIQUID Default

10. Select the Evaluation button to set up the desired format of outputs.
11. Start pumping Silicon particles disperse into MQ water to fill the volume.
12. Stop Pumping and select Autofocus in a disperser.
13. Run at least 200 ml of MQ water for cleaning or until no particle is seen on the live Sensor Image window.
14. Run the sample.
15. Start the measurement.
16. Evaluate and save the data.
17. Run at least 200 ml of MQ water for cleaning until no particle is seen on the live Sensor Image window.

18. Measurement Schedule

Day	PBS	Lipase 1mg	Lipase 10 mg	Lipase with Poloxamer	Lipase with SDS 0.1%	Lipase with SDS 0.5%	Lipase with Polysorbate 20	Tubing
1	✓	X	X	X	X	X	X	Silicone
2	X	X	X	X	X	X	X	PVC
3	X	X	X	X	X	X	X	Silicone
4	X	X	X	X	X	X	X	PVC
5	X	X	✓	X	X	X	X	Silicone
6	X	X	✓	X	X	X	X	PVC
7	X	X	X	✓	X	X	X	Silicone
8	X	X	X	✓	X	X	X	PVC
9	X	X	X	X	✓	X	X	Silicone
10	X	X	X	X	✓	X	X	PVC
11	X	X	X	X	X	✓	X	Silicone
12	X	X	X	X	X	✓	X	PVC
13	X	X	X	X	X	X	✓	Silicone
14	X	X	X	X	X	X	✓	PVC

8.2 Appendix B: Results

8.2.1 Nanodrop Measurements

Sample	Absorbance 1	Absorbance 2	Absorbance 3	Average Absorbance	Concentration
Lipase Stock	**30.00**	**30.66**	**30.59**	**30.42**	**25.25 mg/ml**
Lipase 10 mg/ ml	**12.013**	**12.150**	**12.045**	**12.07**	**10 mg/ml**
Lipase 1mg/ml	**1.227**	**1.266**	**1.06**	**1.184**	**0.98 mg/ml**
Lipase 1mg/ml with Polysorbate 20 0.1%	**1.230**	**1.222**	**1.233**	**1.23**	**1 mg/ml**
Lipase 1mg/ml with Poloxamer 0.1%	**1.344**	**1.262**	**1.337**	**1.314**	**1 mg/ml**
Lipase 1mg/ml with SDS 0.5%	**1.243**	**1.283**	**1.265**	**1.264**	**1 mg/ml**
Lipase 1mg/ml with SDS 0.1%	**1.323**	**1.293**	**1.281**	**1.299**	**1 mg/ml**

8.2.2 QICPIC Measurements

8.2.2.1 Blank PVC:

Time	Particle No.	Mean	Median	Size of Largest Particle	Particle No.	Mean	Median	Size of Largest Particle	Particle No.	Mean	Median	Size of Largest Particle	Average Particle No.	A.Mean	A.Median	A.Size of Largest Particle	Stdv Particle No	Stdv Mean	Stdv Median	Stdv Largest Particle
5min	193.00	10.06	6.72	90.42	7.00	18.88	19.45	33.65	107.00	13.10	7.36	131.05	102.33	14.01	11.18	85.04	93.09	4.48	7.17	48.92
10 min	130.00	6.19	3.79	62.43	38.00	13.14	10.85	28.77	291.00	5.70	3.79	29.23	153.00	8.34	6.14	40.14	128.06	4.16	4.08	19.30
20 min	96.00	4.13	3.79	12.87	231.00	4.69	3.79	33.67	244.00	7.46	5.83	24.82	190.33	5.43	4.47	23.79	81.95	1.79	1.18	10.44
25 min	81.00	4.33	3.79	9.55	28.00	11.79	9.14	30.76	434.00	5.66	4.68	12.93	181.00	7.26	5.87	17.75	220.70	3.98	2.87	11.40

8.2.2.2 Blank Silicone:

Time	Particle No	Mean	Median	Size of Largest Particle	Particle No.	Mean	Median	Size of Largest Particle	Particle No.	Mean	Median	Size of Largest Particle	Average Particle No	A.Mean	A.Median	A.Size of Largest Particle	Stdv Particle No	Stdv Mean	Stdv Median	Stdv Largest Particle
5min	11.00	8.99	5.57	21.72	140.00	7.46	4.68	33.79	32.00	12.26	3.79	128.09	61.00	9.57	4.68	61.20	69.22	2.45	0.89	58.25
10 min	20.00	12.83	4.68	42.65	31.00	4.68	3.79	11.73	33.00	7.48	3.79	87.40	28.00	8.33	4.08	47.26	7.00	4.15	0.52	38.05
20 min	3.00	21.20	21.21	22.73	83.00	4.28	3.79	11.38	26.00	4.72	3.79	12.42	37.33	10.07	9.60	15.51	41.19	9.65	10.06	6.27
25 min	4.00	19.14	17.92	29.93	77.00	4.72	2.52	11.70	50.00	6.47	4.86	26.88	43.67	10.11	8.44	22.84	36.91	7.87	8.30	9.76

8.2.2.3 Lipase 10 mg PVC:

Time	Particle No.	Median	Mean	Size of Largest Particle	Particle No.	Median	Mean	Size of Largest Particle	Particle No.	Median	Mean	Size of Largest Particle	Average Particle No	Median	Mean	Average Size of Largest Particle	Stdv Particle No	Stdv Median	Stdv Mean	Stdv Largest Particle
5min	1362.00	3.79	5.59	34.27	3165.00	3.79	5.66	102.62	5113.00	5.05	6.19	40.63	3213.33	4.21	5.81	59.17	1875.97	0.73	0.33	37.76
10 min	1399.00	3.79	5.34	39.05	3974.00	4.68	5.67	38.34	5436.00	4.68	5.67	42.24	3603.00	4.38	5.56	39.88	2043.91	0.19	0.52	2.07
20 min	1312.00	3.79	5.53	59.66	3346.00	4.68	5.80	42.79	6439.00	5.05	6.03	37.09	3699.00	4.51	5.79	46.51	2581.66	0.25	0.65	11.74
25 min	1262.00	3.79	5.23	30.46	2398.00	3.79	5.44	33.49	4779.00	3.79	5.40	29.88	2813.00	3.79	5.36	31.28	1794.85	0.11	0.00	1.94

8.2.2.4 Lipase 10 mg Silicone:

Time	Particle No	Median	Mean	Size of Largest Particle	Particle No.	Median	Mean	Size of Largest Particle	Particle No.	Median	Mean	Size of Largest Particle	Average Particle No	A.Median	A.Mean	A.Size of Largest Particle	Stdv Particle No.	Stdv Median	Stdv Mean	Stdv Largest Particle
5min	146.00	6.83	9.21	28.38	3275.00	4.68	5.68	21.10	2045.00	3.79	4.43	25.40	2660.00	4.23	5.05	23.25	869.74	0.63	0.88	3.66
10 min	171.00	7.25	9.58	25.79	2781.00	3.79	5.66	38.26	1328.00	3.79	4.89	26.87	2054.50	3.79	5.27	32.56	1027.43	0.00	0.55	6.91
20 min	151.00	8.47	12.42	50.76	2486.00	3.79	4.96	40.15	1289.00	3.79	4.61	27.61	1887.50	3.79	4.79	33.88	846.41	0.00	0.25	11.59
25 min	129.00	5.57	11.41	48.18	2583.00	3.79	4.82	42.60	1365.00	3.79	4.67	28.06	1974.00	3.79	4.74	35.33	861.26	0.00	0.11	10.39

8.2.2.5 Lipase 1 mg PVC:

Time	Particle No	Median	Mean	Size of Largest Particle	Particle No.	Median	Mean	Size of Largest Particle	Particle No.	Median	Mean	Size of Largest Particle	Average Particle No.	Median	Mean	A.Largest Particle	Stdv Particle No	Stdv Median	Stdv Mean	Stdv Largest Particle
5min	387.00	5.05	6.62	30.56	417.00	3.79	8.23	147.01	1548.00	3.79	4.82	43.85	784.00	4.21	6.55	73.80	661.81	0.73	1.71	63.74
10 min	318.00	3.79	5.41	22.62	382.00	4.68	5.82	21.86	9150.00	3.79	6.25	106.14	3283.33	4.08	5.83	50.21	5080.78	0.52	0.42	48.44
20 min	316.00	4.68	5.57	26.45	346.00	3.79	5.13	28.47	5192.00	3.79	4.20	15.82	1951.33	4.08	4.97	23.58	2806.54	0.52	0.70	6.80
25 min	276.00	3.79	4.83	25.97	207.00	3.79	5.04	22.24	3222.00	2.52	3.33	14.12	1235.00	3.37	4.40	20.78	1721.14	0.73	0.93	6.06

8.2.2.6 Lipase 1 mg Silicone:

Time	Particle No	Median	Mean	Size of Largest Particle	Particle No.	Median	Mean	Size of Largest Particle	Particle No.	Median	Mean	Size of Largest Particle	Average No.Particles	A.Median	A.Mean	A.Size of Largest Particle	Stdv No	Stdv Median	Stdv Mean	Stdv Largest Particle
5min	2055.00	3.79	4.28	49.56	2980.00	2.52	4.11	24.34	357.00	5.83	6.82	62.45	1797.33	4.05	5.07	45.45	1330.35	1.67	1.52	19.38
10 min	2694.00	2.52	4.10	35.21	5667.00	2.52	4.12	28.15	309.00	3.79	5.97	39.49	2890.00	2.95	4.73	34.28	2684.37	0.73	1.07	5.73
20 min	4467.00	2.52	3.98	30.60	12561.00	2.52	4.20	30.18	2586.00	3.79	4.25	31.81	6538.00	2.95	4.14	30.87	5300.18	0.73	0.15	0.85
25 min	6060.00	2.52	4.08	26.84	15515.00	2.52	4.22	31.65	3650.00	2.52	4.17	31.52	8408.33	2.52	4.16	30.00	6271.41	0.00	0.07	2.74

8.2.2.7 Lipase 1 mg - SDS 0.1% PVC:

Time	Particle No	Median	Mean	Size of Largest Particle	Particle No	Median	Mean	Size of Largest Particle	Particle No.	Median	Mean	Size of Largest Particle	Average Particle No.	A.Median	A.Mean	A.Size of Largest Particle	Stdv Particle No	Stdv Median	Stdv Mean	Stdv Largest Particle
5min	432.00	7.10	6.14	35.02	701.00	4.68	8.13	41.89	717.00	8.88	12.28	42.96	616.67	6.88	8.85	39.96	160.13	2.11	3.13	4.31
10 min	234.00	6.83	4.11	25.31	643.00	5.05	7.60	55.54	591.00	8.51	11.93	57.62	489.33	6.80	7.88	46.16	222.65	1.73	3.92	18.08
20 min	199.00	5.05	5.26	30.32	235.00	8.10	11.15	54.68	479.00	7.99	11.10	40.60	304.33	7.04	9.17	41.87	152.33	1.73	3.39	12.23
25 min	116.00	6.31	6.39	38.08	227.00	6.31	10.51	34.27	414.00	7.50	11.60	44.70	252.33	6.71	9.50	39.01	150.61	0.69	2.75	5.28

8.2.2.8 Lipase 1 mg - SDS 0.1% Silicone:

Time	Particle No	Median	Mean	Size of Largest Particle	Particle No.	Median	Mean	Size of Largest Particle	Particle No.	Median	Mean	Size of Largest Particle	Average Particle No.	A.Median	A.Mean	A.Size of Largest Particle	Stdv Particle No.	Stdv Median	Stdv Mean	Stdv Largest Particle
5min	515.00	3.79	6.07	61.78	644.00	3.79	7.83	111.73	510.00	4.68	6.67	38.48	556.33	4.08	6.86	70.67	75.96	0.52	0.89	37.43
10 min	2049.00	3.79	6.36	86.48	580.00	3.79	7.09	49.18	849.00	5.05	6.84	37.92	1159.33	4.21	6.76	57.86	782.13	0.73	0.37	25.42
20 min	307.00	3.79	5.58	34.44	453.00	4.68	7.52	43.78	917.00	3.79	5.24	30.16	559.00	4.08	6.11	36.13	318.52	0.52	1.23	6.97
25 min	370.00	3.79	5.25	41.75	450.00	3.79	6.23	42.17	1015.00	3.79	5.69	26.48	611.67	3.79	5.72	36.80	351.58	0.00	0.49	8.94

8.2.2.9 Lipase 1 mg - SDS 0.5% PVC:

Time	Particle No	Median	Mean	Size of Largest Particle	Particle No.	Mean	Median	Size of Largest Particle	Particle No.	Mean	Median	Size of Largest Particle	Average Particle No.	A.Median	A.Mean	A.Size of Largest Particle	Stdv Particle No	Stdv Median	Stdv Mean	Stdv Largest Particle
5min	258.00	3.79	6.14	35.02	141.00	10.26	6.72	35.86	98.00	11.30	6.83	45.41	165.67	6.57	8.45	38.77	82.80	0.37	4.07	5.77
10 min	196.00	2.52	4.11	25.31	133.00	11.57	8.81	35.86	118.00	12.08	7.28	40.40	149.00	6.73	8.72	33.86	41.39	2.40	5.38	7.74
20 min	70.00	3.16	5.26	30.32	50.00	8.64	3.79	36.89	99.00	8.64	6.72	40.13	73.00	5.26	6.81	35.78	24.64	1.47	3.17	5.00
25 min	86.00	3.79	6.39	38.08	61.00	10.98	7.99	35.96	74.00	10.33	7.80	40.06	73.67	7.39	8.37	38.03	12.50	0.87	3.98	2.05

8.2.2.10 Lipase 1 mg - SDS 0.5% Silicone:

Time	Particle No	Median	Mean	Size of Largest Particle	Particle No.	Mean	Median	Size of Largest Particle	Particle No.	Mean	Median	Size of Largest Particle	Average Particle No	A.Median	A.Mean	A.Size of Largest Particle	Stdv Particle No	Stdv Median	Stdv Mean	Stdv Largest Particle
5min	1069.00	6.46	10.91	60.66	141.00	7.39	4.68	42.58	98.00	7.81	5.57	47.34	436.00	7.05	7.22	50.19	548.62	3.37	0.69	9.37
10 min	763.00	6.83	10.85	42.77	133.00	6.44	3.79	38.66	118.00	7.73	5.83	49.40	338.00	6.82	7.00	43.61	368.14	3.64	0.66	5.42
20 min	1182.00	5.57	8.52	40.94	50.00	5.46	3.79	35.40	99.00	5.46	5.05	42.75	443.67	5.78	5.50	39.70	639.88	2.45	0.06	3.83
25 min	1555.00	5.05	7.70	51.85	61.00	4.88	3.79	34.77	74.00	6.78	5.05	48.56	563.33	5.51	5.57	45.06	858.83	2.00	1.05	9.06

8.2.2.11 Lipase with Poloxamer 188 PVC:

Time	Particle No	Median	Mean	Size of Largest Particle	Particle No	Median	Mean	Size of Largest Particle	Particle No.	Median	Mean	Size of Largest Particle	Average Particle No.	A.Median	A.Mean	A.Size of Largest Particle	Stdv Particle No	Stdv Median	Stdv Mean	Stdv Largest Particle
5min	110.00	13.18	14.01	39.58	223.00	5.94	10.71	43.56	227.00	4.68	5.68	27.82	186.67	7.93	10.13	36.99	66.43	4.58	4.19	8.19
10 min	68.00	15.17	18.42	43.87	175.00	7.94	10.61	46.70	246.00	3.79	5.90	30.64	163.00	8.97	11.64	40.41	89.60	5.76	6.32	8.57
20 min	40.00	14.26	16.12	43.68	109.00	10.46	13.00	46.20	135.00	3.79	4.84	18.13	94.67	9.51	11.32	36.00	49.10	5.30	5.82	15.53
25 min	67.00	19.89	21.23	49.86	124.00	7.10	9.99	37.14	81.00	3.79	4.86	18.86	90.67	10.26	12.03	35.29	29.70	8.51	8.38	15.58

8.2.2.12 Lipase with Poloxamer 188 Silicone:

Time	Particle No	Median	Mean	Size of Largest Particle	Particle No.	Median	Mean	Size of Largest Particle	Particle No.	Median	Mean	Size of Largest Particle	Average Particle No.	A.Median	A.Mean	Size of Largest Particle	Stdv Particle No	Stdv Median	Stdv Mean	Stdv Largest Particle
5min	266.00	5.83	9.43	39.26	469.00	5.83	8.52	39.52	582.00	5.05	7.06	42.31	439.00	5.57	8.34	40.36	160.12	0.45	1.19	1.69
10 min	152.00	5.89	9.43	38.15	310.00	4.68	7.51	40.20	603.00	4.68	7.18	46.33	355.00	5.08	8.04	41.56	228.84	0.70	1.22	4.26
20 min	96.00	7.20	9.43	40.57	1329.00	3.79	4.38	29.31	404.00	4.68	7.28	47.50	609.67	5.22	7.03	39.13	641.71	1.77	2.53	9.18
25 min	122.00	5.05	8.18	41.23	331.00	5.05	7.77	34.86	417.00	3.79	6.86	37.05	290.00	4.63	7.60	37.71	151.71	0.73	0.68	3.24

8.2.2.13 Lipase with Polysorbate 20 PVC:

Time	Particle No.	Median	Mean	Size of Largest Particle	Particle No.	Median	Mean	Size of Largest Particle	Particle No.	Median	Mean	Size of Largest Particle	Average Particle No.	A.Median	A.Mean	A.Size of Largest Particle	Stdv Particle No	Stdv Median	Stdv Mean	Stdv Largest Particle
5min	96742.00	3.79	4.98	35.16	48649.00	3.79	4.46	23.62	22910.00	3.79	4.61	70.48	56100.33	3.79	4.68	43.09	37475.76	0.00	0.27	24.41
10 min	71489.00	3.79	4.07	27.81	16454.00	3.79	4.31	30.30	3771.00	3.79	4.48	52.34	30571.33	3.79	4.29	36.82	35998.70	0.00	0.20	13.50
20 min	22673.00	3.79	4.13	33.90	2107.00	3.79	4.06	32.23	609.00	3.79	5.09	39.57	8463.00	3.79	4.43	35.23	12328.99	0.00	0.57	3.84
25 min	9325.00	3.79	4.30	38.94	877.00	3.79	4.62	28.46	468.00	3.79	5.92	32.04	3556.67	3.79	4.95	33.15	4999.71	0.00	0.86	5.33

8.2.2.14 Lipase with Polysorbate 20 Silicone:

Time	Particle No.	Median	Mean	Size of Largest Particle	Particle No.	Median	Mean	Size of Largest Particle	Particle No	Median	Mean	Size of Largest Particle	Average Particle No.	A.Median	A.Mean	A.Size of Largest Particle	Stdv Particle No.	Stdv Median	Stdv Mean	Stdv Largest Particle
5min	4909.00	2.52	4.09	32.06	1996.00	3.79	4.54	40.80	24299.00	3.79	4.04	34.60	10401.33	3.37	4.22	35.82	12123.54	0.73	0.27	4.50
10 min	4291.00	2.52	4.07	32.82	2151.00	3.79	4.24	42.43	3463.00	3.79	4.31	92.89	3301.67	3.37	4.21	56.05	1079.08	0.73	0.12	32.27
20 min	3984.00	2.52	4.13	25.83	3421.00	3.79	4.33	29.99	4009.00	2.52	3.97	26.95	3804.67	2.95	4.14	27.59	332.50	0.73	0.18	2.15
25 min	4115.00	2.52	3.93	23.66	3564.00	2.52	4.22	26.59	5276.00	2.52	4.00	33.36	4318.33	2.52	4.05	27.87	873.92	0.00	0.15	4.98