2023

Effect of filling volume and head space on the release of silicone oil from glass prefilled syringe with formulations containing polysorbate

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EFFECT OF FILLING VOLUME AND HEAD SPACE ON THE RELEASE OF SILICONE OIL FROM GLASS PREFILLED SYRINGE WITH FORMULATIONS CONTAINING POLYSORBATE

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy in Industrial Pharmacy
Department of Pharmaceutics and Industrial Pharmacy
Faculty Of Pharmaceutical Sciences
Chulalongkorn University
Academic Year 2023

ผลของปริมาตรบรรจุและปริมาตรอากาศเหนือสารละลายต่อการปลดปล่อยของน้ำมันซิลิโคนจากบรรจุภัณฑ์ประเภทแก้วรูปแบบกระบอกฉีดยาพร้อมฉีด ของสูตรตำรับที่มีโพลีซอร์เบทเป็นองค์ประกอบ

นางภัคศุภางค์ ตันติเสรีรัตน์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาเภสัชกรรมอุตสาหการ ภาควิชาวิทยาการเภสัชกรรมและเภสัชอุตสาหกรรม
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2566

Thesis Title	EFFECT OF FILLING VOLUME AND HEAD SPACE ON THE RELEASE OF SILICONE OIL FROM GLASS PREFILLED SYRINGE WITH FORMULATIONS CONTAINING POLYSORBATE
By	Mrs. Paksupang Tantisereerut
Field of Study	Industrial Pharmacy
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บรรจุภัณฑ์ประเภทแก้วรูปแบบกระบอกฉีดยาพร้อมฉีดมักจะถูกเคลือบพื้นผิวภายในของกระบอกยาฉีดและจุกยางด้วยน้ำมันซิลิโคนเพื่อเป็นสารหล่อลื่น ซึ่งหากน้ำมันซิลิโคนนั้นถูกปลดปล่อยออกมาที่สารละลายผลิตภัณฑ์จะส่งผลกระทบต่อความปลอดภัยต่อผู้ป่วยที่ได้รับผลิตภัณฑ์นั้น ๆ นอกจากนั้นการใส่สารลดแรงตึงผิวที่มีในตำรับเพื่อป้องกันการเกาะกลุ่มกันของโปรตีน ป้องกันการยึดติดของอนุภาคโปรตีนที่พื้นผิวภายในภาชนะบรรจุ และสามารถกระตุ้นทำให้เกิดการปลดปล่อยเพิ่มขึ้นของน้ำมันซิลิโคนออกจากพื้นผิวของบรรจุภัณฑ์ได้ การศึกษานี้มีวัตถุประสงค์ที่จะศึกษาผลของปริมาตรบรรจุและปริมาตรอากาศเหนือสารละลายต่อการปลดปล่อยของน้ำมันซิลิโคนจากบรรจุภัณฑ์ประเภทแก้วรูปแบบกระบอกฉีดยาพร้อมฉีด ของสูตรตำรับที่มีสารลดแรงตึงผิวโพลีซอร์เบทเป็นองค์ประกอบ โดยตัวอย่างที่จะใช้ในการศึกษาจะมีปริมาตรบรรจุและปริมาตรอากาศเหนือสารละลายที่แตกต่างกันไป จากนั้นตัวอย่างทดสอบผ่านสภาวะเร่งโดยถูกเขย่า ปริมาณน้ำมันซิลิโคนที่ถูกปลดปล่อยจากภาชนะบรรจุจะถูกตรวจวัดด้วยเครื่องไมโครโฟลวอิมเมจจิ้ง จากผลการทดสอบพบว่าภาชนะบรรจุที่มีอัตราส่วนของปริมาตรอากาศเหนือสารละลายต่อปริมาตรบรรจุสูงจะมีการปลดปล่อยน้ำมันซิลิโคนออกจากพื้นผิวของภาชนะบรรจุได้มากกว่า หรือสามารถสรุปในอีกทางได้ว่าในตัวอย่างที่มีปริมาตรอากาศเหนือสารละลายเท่ากันตัวอย่างที่มีปริมาตรบรรจุต่ำสุดจะพบการปลดปล่อยน้ำมันซิลิโคนออกได้มากกว่าเนื่องจากความสามารถในการเคลื่อนที่ของอากาศในกระบอกยาฉีดในตัวอย่างที่มีปริมาตรบรรจุต่ำนั้นเกิดขึ้นได้สูงกว่า ดังนั้นเพื่อที่จะลดโอกาสในการปลดปล่อยน้ำมันซิลิโคนออกจากพื้นผิวของภาชนะบรรจุจะต้องมีการควบคุมและติดตามตัวแปรของเครื่องบรรจุและปิดผนึกจุกยางเพื่อที่จะให้ผลิตภัณฑ์นั้นมีอัตราส่วนของปริมาตรบรรจุและปริมาตรอากาศเหนือสารละลายที่เหมาะสม

สาขาวิชา	เภสัชกรรมอุตสาหการ	ลายมือชื่อนิสิต .....
ปีการศึกษา	2566	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

6270022933 : MAJOR INDUSTRIAL PHARMACY

KEYWORD: Prefilled syringe, Polysorbate, Silicone oil, Sub-visible particle, Biopharmaceutical, Head space, Filling volume

Paksupang Tantisereerut : EFFECT OF FILLING VOLUME AND HEAD SPACE ON THE RELEASE OF SILICONE OIL FROM GLASS PREFILLED SYRINGE WITH FORMULATIONS CONTAINING POLYSORBATE. Advisor: Asst. Prof. NARUEPORN SUTANTHAVIBUL, Ph.D.

Prefilled syringe usually uses silicone oil as lubricant to coat and lubricate the inner surface of barrels and rubber stoppers. The release of coated silicone oil was found to affect the product quality and patient safety. Surfactants are added in many formulations to prevent protein aggregation and adhesion to surfaces of primary packaging. However, surfactants are also found to induce the release of silicone oil from glass prefilled syringe. This study aimed to examine the effect of filled volume and head space on the release of silicone oil from prefilled syringe containers containing polysorbate solution. Samples are prepared at different filling volume, level head space and agitated by orbital shaker at predefined speed and time. Amount of silicone oil released from containers was detected by Micro-flow Imaging (MFI™) technology. Results showed that the higher head space to filled volume ratio, more release of silicone oil was released from prefilled syringe was observed. In addition, when head space was held constant, the lower filling volume released more silicone oil than higher filling volume due to air bubble were allowed to move freely along the interior of the barrel of prefilled syringe. In conclusion, to minimize the risk of silicone oil leaching, setting appropriate parameters of the filling line and transferring machines must be monitored and controlled to the proper filling volume and head space of the product.

Field of Study: Industrial Pharmacy Student's Signature .....

Academic Year: 2023 Advisor's Signature .....

ACKNOWLEDGEMENTS

Firstly, I would like to express my sincere gratitude to my thesis advisor, Assistant Professor Narueporn Sutanthavibul, Ph.D., for the invaluable guidance, support, and motivation provided throughout the entire process, from research design and testing to the preparation of the final report.

Secondly, I would like to express my heartfelt appreciation to my thesis committee, Associate Professor Satit Puttipipatkhachorn, Ph.D., Assistant Professor Phanphen Wattanaarsakit, Ph.D. and Assistant Professor Wanchai Chongcharoen, Ph.D., for the useful guidance and insightful concepts to enhance the quality of my research.

Thirdly, I would like to express my sincere gratitude to Siam Bioscience Co., Ltd. for supplying the necessary materials and equipment required for the success of this research.

Lastly, I would like to express my love and thankfulness to my family for their unwavering trust and constant encouragement throughout my entire life.

Paksupang Tantisereerut

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LIST OF ABBREVIATIONS

Cat no.	Catalog number
cm^3	Cubic centimeter
COVID-19	Corona Virus Disease of 2019
cSt	Centistokes
HLB	Hydrophile-lipophile balance
Hr	Hour
MFI	Micro flow imaging
Mg	Milligram
mL	Millimeter
mRNA	Messenger RNA
pH	Potential hydrogen
rpm	Round per minute
SD	Standard deviation
µm	Micrometer
%	Percentage

Chapter I
INTRODUCTIONS

Component of glass prefilled syringe container that is in direct contact with the formulation is the barrel and the rubber stopper. Silicone oil is usually used as lubricant coating for both parts (1). Surface of rubber stoppers are coated with silicone oil to prevent adhesion of rubber to glass barrel during manufacturing process and to reduce the force applied when pushing the plunger. Also, inner surface of glass prefilled syringe is also coated with silicone oil in order to reduce the friction force between rubber stopper and barrel (2). The release of silicone oil is considered critical to the product quality as it is foreign matter and also can induce the immune system that can impact patient safety (3-6). In addition, presence of the silicone oil in biotherapeutic formulations can induce protein adhesion and protein aggregation (7, 8). This can cause lower response of the therapeutics and induce the immunologic reaction of patients.

Factors which can impact the release of silicone oil from prefilled syringe container are not only the quality of the packaging itself, but it can be results from formulation, stress condition and also filling volume and head space (8-12).

Since, the formulation of biotherapeutic proteins composed of buffer as pH controller to stabilized the proteins, salt to adjust the tonicity of the formulation, and also surfactants added to reduce the protein adhesion and protein aggregation. Surfactants in the formulation can generally induce the release of silicone oil on the surface of syringe barrel and rubber stopper (7, 10, 12, 13).

The presence of air bubble which is head space in the prefilled syringe container combined with the moving of the air bubble along the syringe barrel can also lead to the release of silicone oil (12-15).

Therefore, to evaluate the extent of silicone oil released from prefilled syringe container, micro-flow imaging (MFI) technique will be selected as the

analytical method. Since, the MFI technique can distinguish the silicone oil form aggregated proteins an also air bubbles in the sample (8, 11, 16, 17).

Objective

The aim of this study is to evaluate the effect of surfactant in formulation and the effect of different level of filling volume and head space on the extent of release of silicone oil from glass prefilled syringe.

Chapter II
LITERATURE REVIEW

Biopharmaceutical products

Biopharmaceutical products are the drugs that produced using biotechnology which is the use of living things, especially cells and bacteria, in industrial processes. These products may be produced through biotechnology in a living system, such as a microorganism, plant cell, or animal cell, and are often more difficult to characterize and complex than small molecule drugs (18). There are many types of biological products approved for use, including therapeutic proteins (such as filgrastim), monoclonal antibodies (such as rituximab), and vaccines (such as those for influenza and COVID-19 mRNA). Biopharmaceutical products are highly effective drugs due to the target specific properties and the pharmacokinetics properties (19). Using biopharmaceutical products has a risk on immunogenicity problem due to the product itself can be induced the immunogenic reaction as the foreign proteins from nature protein. And also, the subvisible particle, protein aggregated and misfolded protein can induce the immunogenic reaction to the product and can be caused the side effect and reduce the therapeutic efficacy and patient safety (3, 6, 20-22).

There are reports of the immunogenic reaction from the biopharmaceutical products. This can impact on the product quality and patient safety. The cause of the immunogenic reaction can be from the protein structure, host related factor and aggregated protein (3, 20).

Container closure for biopharmaceutical products

The biopharmaceutical products most are sterile solution or freeze dry product. The container closure available for this product group in the market are ampoule, vial, cartridge and prefilled syringe. To maintain the product quality through the product shelf lift, the container closure would be chosen during the development phase. The prefilled syringe has an advantage to patient to had an accurate dose and convenience for administration (10).

As Figure 1 (23), there are 3 components of prefilled syringe container consist of barrel, rubber stopper and plunger. The component that direct contact to the formulation is inner surface of barrel and the rubber stopper. The conventional prefilled syringe container usually coated the inner surface of barrel and rubber with the silicone oil. The inner surface of barrel usually coated with the silicone oil to prevent the protein adhesive on the inner surface and reduce the friction force between the rubber stopper and barrel during the stoppering process, and the administration. And also, the rubber stopper is coated with the silicone oil as the same purpose of the barrel. Moreover, coated silicone oil on the rubber stopper is to prevent the adhesion of rubber stopper during the manufacturing and reduce the friction force between the rubber stopper and machine part (1, 10, 12).

Figure 1 Prefilled syringe container

Subvisible particles of biopharmaceutical products

Subvisible particle is an insoluble particle with the particle size less than 100 μm and not able to detect by visible. The subvisible particle found in the product can be the primary packaging itself, the instability of product that form the insoluble matter, the contamination form manufacturing environment, and especially for biopharmaceutical product can found the aggregated protein, silicone oil droplet and formation of aggregated protein and silicone oil droplet (12, 24). The aggregated protein is high molecular weight of the native protein, it can be the dimer or multimers of native form or denatured monomers. The aggregated protein can be reversible or irreversible and can form the soluble or insoluble proteins. The aggregated protein can be induced by several factors such as ionic strength (e.g. pH of formulation), temperature changes (e.g. freeze-thaw condition) and interfacial exposure (9, 10, 21).

Formation of subvisible particles in biopharmaceutical products

There are several hypotheses that described the formation of subvisible particle in biopharmaceutical products. However, the subvisible particle related to biopharmaceutical products there are 2 mechanism which is the protein deformation and then the denatured protein form to dimer or multimers. And another mechanism is the released of silicone oil from inner surface of prefilled syringe container and form the particle with the protein.

The protein deformation

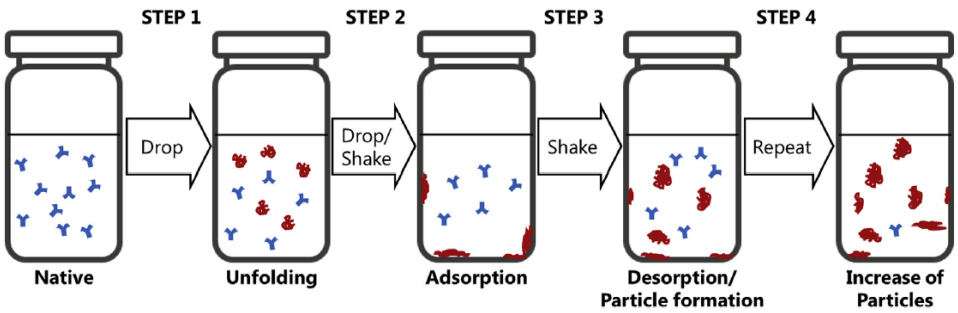


Figure 2 Particle formation of protein

When protein in the formulation is unfolded, the hydrophobic part of protein that used to folded in the designed formation faced to the solution as Figure 2, Step 2 Unfolding. When, the hydrophobic part of protein will attach with the hydrophobic part which is the inner surface of container as Figure 2, Step 3 Adsorption or attach to another protein molecule and become an aggregated protein Figure 2, Step 4 Particle formation. In the other hand when the present of shaking of container, it resulting in the desorption of unfolded protein from the surface of inner surface of container and the attach to another protein molecule as Figure 2, Step 4 Desorption (14).

The released of silicone oil from inner surface of prefilled syringe container and form the particle with the protein

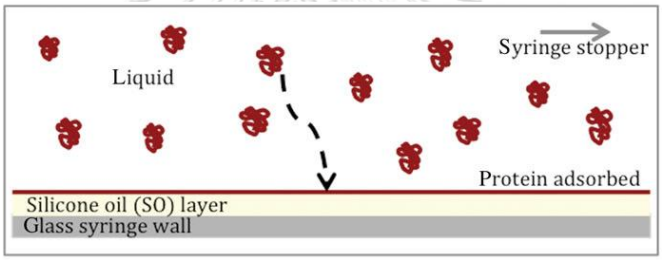


Figure 3 Step 1 Initial protein adsorption and gelation

In prefilled syringe container, the silicone oil is coated on the inner surface of barrel. When protein is denatured and unfold, it shown the hydrophobic surface to the environment. Then, it can be adsorbed on the surface of the barrel of prefilled syringe that coated with the silicone oil to form a silicone oil – protein gel layer as Figure 3 (12).

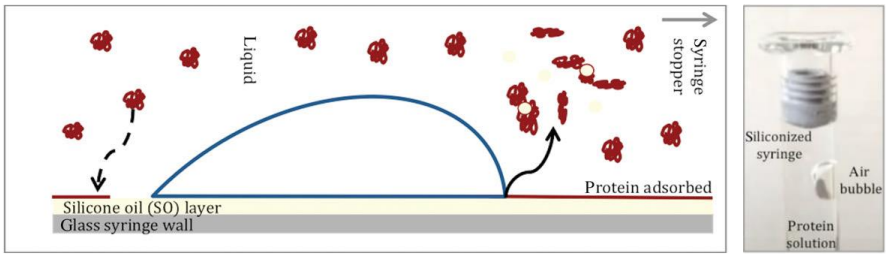


Figure 4 Step 2 Gelled protein and silicone oil removal from interface and further protein adsorption and gelation

When the air inside the prefilled syringe moving along the sinner surface of barrel, it can damage the silicone oil – protein gel layer and silicone oil layer can be removal from the surface to solution to form the subvisible particle as Figure 4 (12).

Factor influent the subvisible particle formation

Agitation and dropping

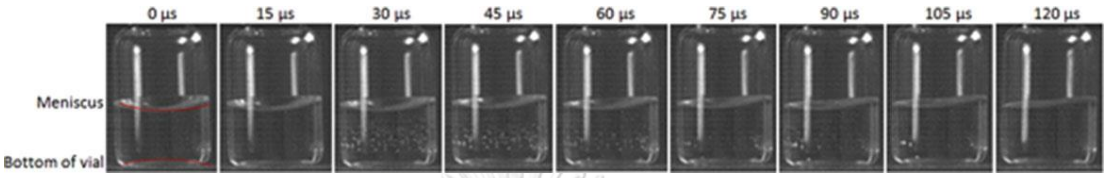


Figure 5 Air bubbles in the vial when it was suddenly dropped

Protein unfolding can occur when the product had an impact from agitation or dropping of container. The impaction has an effect to product the air bubble which is free radical as Figure 5. The free radical can cause the protein denature and change the structure or denatured. Moreover, when the headspace inside the container closure is presence and combination with the agitation it can produce more subvisible particles (15).

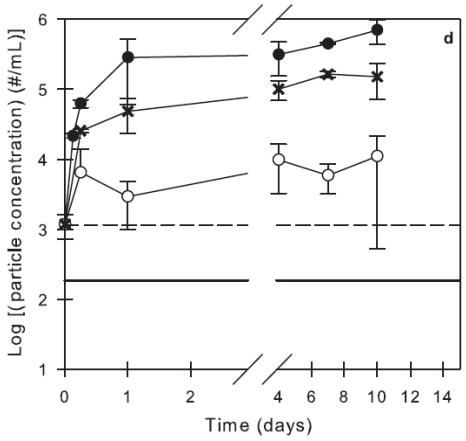


Figure 6 Particle concentration

Open symbols are syringes incubated with no air bubble, closed symbols are syringes incubated with an air bubble, and cross symbols are syringes incubated with no air bubble but with glass beads.

As shown in Figure 6, for prefilled syringe container when agitate the sample with headspace (air bubble) inside container (Closed symbols), it produces the subvisible particle higher than sample without headspace. While agitate sample without headspace (Cross symbols) or sample without headspace but with glass beads, there were lower subvisible particle formation. This can be concluded that the headspace inside prefilled syringe container can induce the subvisible particle formation (12).

As mentioned above, when the agitation combined with the headspace inside the prefilled syringe it can produce more subvisible particle and reduce the monomer in formulation more than container without headspace due to the moving of air bubble along the prefilled syringe barrel (12, 14).

The adsorption of protein on inner surface of container closure

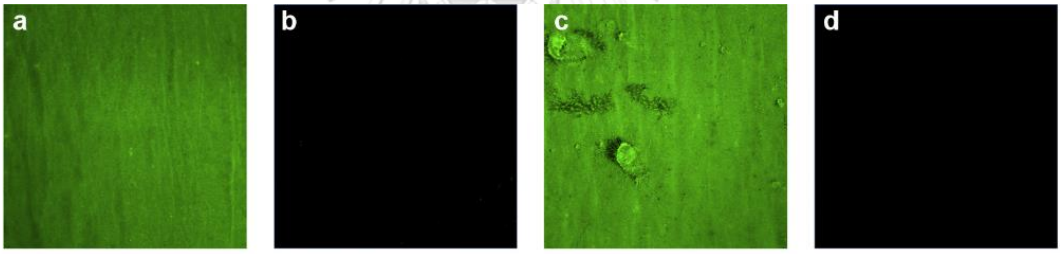


Figure 7 Picture show the protein adsorption on surface for each formulation
(a) Glycine buffer (b) Glycine buffer with 0.02% Polysorbate 20
(c) Phosphate buffer (d) Phosphate buffer with 0.02% Polysorbate 20 (8)

When protein is unfolded, the hydrophobic part of protein will expose to the environment and will be adsorbed on the inner surface of container closure and form the silicone oil – protein gel layer. To prevent the adsorption of protein on the inner surface of container closure, non-ionic surfactant such as polysorbate 20 or polysorbate 80 will be added to the formulation. The surfactant will reduce the surface tension between the formulation and inner surface of prefilled syringe barrel, this resulting in to prevention of protein adsorption to the hydrophobic surface (25).

As shown in Figure 7, when presence of surfactant which is Polysorbate 20 in the formulation contained it shown that no green fluorescence of adsorbed protein on the surface (8).

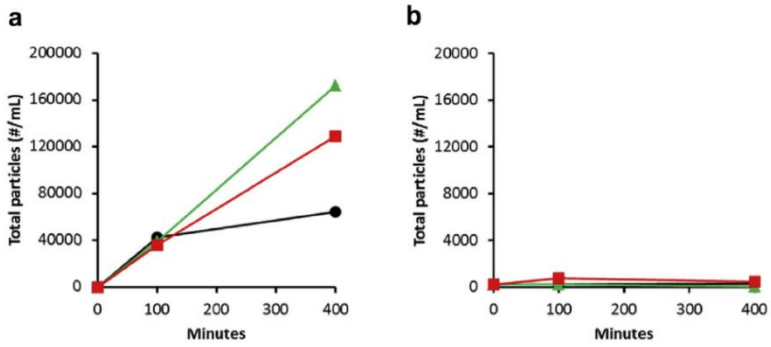


Figure 8 Subvisible particle found in sample at different filling volume and different formulation (with and without Polysorbate 80) (a) Formulation without Polysorbate 80 and (b) Formulation with Polysorbate 80, Circle is Filling volume 1 mL, Square is Filling volume 1.5 mL and Triangle is Filling volume 2.0 mL.

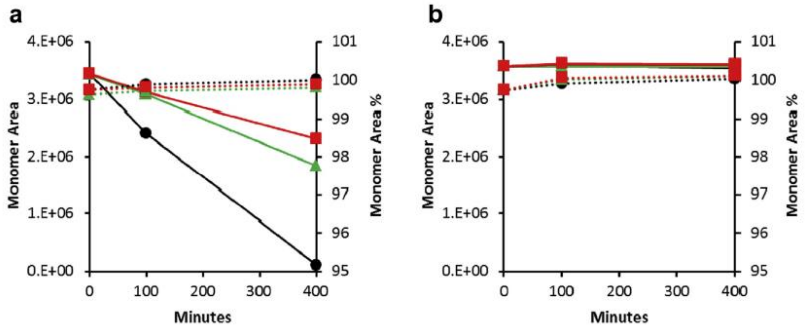


Figure 9 Monomer area in sample at different filling volume and different formulation (with and without Polysorbate 80) (a) Formulation without Polysorbate 80 and (b) Formulation with Polysorbate 80, Circle is Filling volume 1 mL, Square is Filling volume 1.5 mL and Triangle is Filling volume 2.0 mL

Formulation

Adding the surfactant to formulation can prevent protein aggregation and prevent the destabilization of proteins (26). There are several types of surfactant used in biopharmaceutical products such as non-ionic surfactant and ionic-surfactant.

Generally, non-ionic surfactants, such as polysorbate 20, polysorbate 80, poloxamer 188, etc. are used in formulations due to acceptable safety profiles and they were approved for used in biopharmaceutical product formulations and exhibited minimal cellular irritations (25-28). The concentration of surfactant in normal formulation is within range of 0.001 – 1% (w/v) and is sufficient to saturated all sites on the protein molecule (25).

As shown in Figure 8, the study of subvisible particle formation of formulation filled in the vial at different filling volume, when presence of Polysorbate 80 in formulation there was lower level of subvisible particle in the agitated sample. And also, when presence Polysorbate 80 in formulation, the monomer amount was reduced less than formulation without Polysorbate 80, as shown in Figure 9. Therefore, adding non-ionic surfactant to the protein formulation can reduce the subvisible formation and reduce the reduction of monomer in formulation (14).

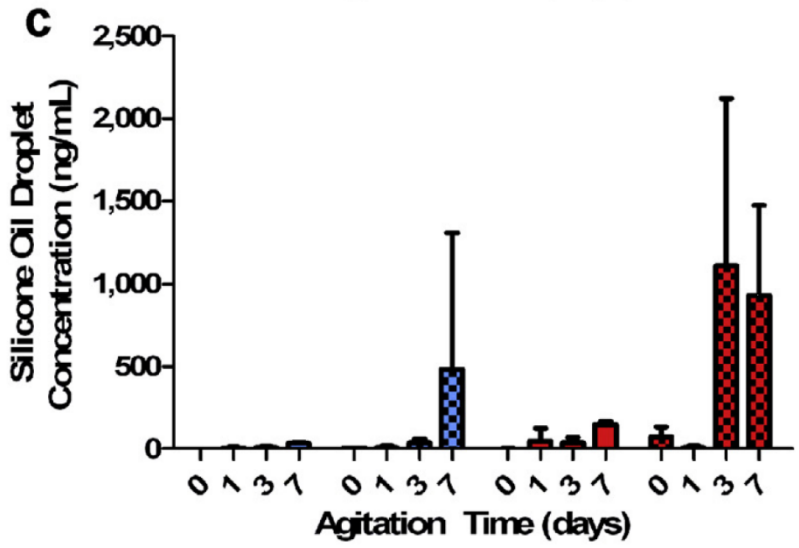


Figure 10 Silicone oil droplet found in difference formulation,
Blue is buffer without Polysorbate 20 filled in the Silicone oil free prefilled syringe, Blue with mark is buffer with 0.02% Polysorbate 20 filled in Silicone oil free prefilled syringe, Red is buffer without Polysorbate 20 filled with siliconized prefilled syringe and Red with mark is buffer with 0.02% Polysorbate 20 filled in siliconized prefilled syringe

However, presence of non-ionic surfactant in formulation can lead the leaching of silicone oil from inner surface of container to the formulation due to the emulsifier properties of surfactant. Therefore, when adding non-ionic surfactant to the formulation, it can find the silicone-oil droplet in the formulation as shown in Figure 10. Comparing the silicone oil droplet concentration, it found higher concentration in formulation contained polysorbate 20 (Red bar) than formulation without polysorbate 20 (Blue bar) (8).

Subvisible particle analysis

Subvisible particle is the particle that has size less than 100 μm. According to the pharmacopoeia, the criteria od subvisible particle for small volume injection is not more than 6000 particles per container for particle size ≥ 10 μm and not more than 600 particles per container for particle size ≥ 25 μm (29, 30). To analysis the subvisible particle there several techniques depend on the purpose of the analysis and the properties of the particle. The technique to detect the particle in biopharmaceutical product should be able to detect the transparent particle. Since, the subvisible particle from biopharmaceutical products can be the opaque such as the rubber stopper particle or particle from the environment and it can be the transparent particle such as aggregated protein or silicone oil droplet (11, 17, 31).

The subvisible particle counter will measure the size of particle and count the number of particles detected and report as the concentration of particle at each particle size. In pharmacopoeia there are several methods mentioned such as Light Obscuration Particle Count Test, Microscopic Particle Count Test and Flow imaging analysis. However, these methods are efficient to detect the opaque particles but not able to detect the translucent particles or protein like particles. Since another method that suggested in the pharmacopoeia for analysis subvisible particle for biopharmaceutical product which is flow imaging analysis (17, 30). The principle of Light Obscuration (LO) method is to detect the subvisible particle from the shadow of the particle when the particle flow through the detector and evaluate the particle size from the size of shadow as Figure 11 (Left) (16, 32). In the other hand, the

principle of microflow imaging (FI) method is to detect the subvisible particle from the digital image that capture when particle in sample flow through the detector. Therefore, the method which is microflow imaging that can detect the translucent particles would be advantage to use as the test method for detected protein particles and silicone oil droplet in the formulation.

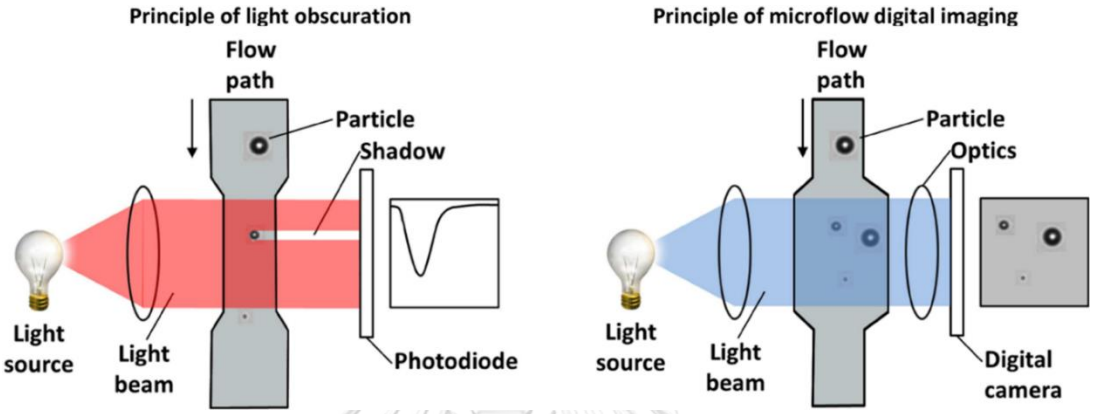


Figure 11 Picture show principle of Light Obscuration (Left) and Microflow Digital Imaging (Left)

Chapter III
MATERIALS AND METHODS

Materials

a. Sorbitol Cat. No. 1.03140.9028 from Merck KGaA
b. Sodium acetate trihydrate Cat. No. 141632 from AppliChem GmbH
c. Polysorbate 20 Cat. No. 817072 from Merck KGaA
d. Polysorbate 80 Cat. No. 817061 from Merck KGaA
e. Acetic acid Cat. No. 100056 from Merck KGaA
f. Milli-Q water from Merck KGaA
g. 0.2 µm cellulose acetate filter Part no. SFC025022NA from Membrane solution
h. Pipette tip with filter Cat no. TFLR1121000-Q from Thermo Scientific.
i. Prefilled syringe 1 mL with 25G ½ needle Part no. 47365319 from BD Medical Barrels made from clear borosilicate glass type I and the inner surface coated with lubricant DC 360, Silicone oil 1000 cSt viscosity. Each lot of prefilled syringe was controlled for silicone oil coating level by the manufacturer.
j. Rubber stopper from West 4023/50 Grey Rubber (Bromobutyl) Part no. 472844110 from BD Medical

Equipments

a. Micropipette 1000 µL
b. Orbital Shaker IKA™ Model KS260 Control
c. Micro-flow imaging (MFI™) ProteinSimple Model MFI 520 with software MVSS (Version 5.0.0), USA.
d. Biosafety cabinet Class II (BSC Class II)

Methods

I. Subvisible particle analysis by Microflow Digital Imaging Technique

1.1 Environment control

All samples were prepared and tested under BSC class II to prevent the contamination from the environment. The BSC was turned on at least 30 minutes to replace the contaminated air with cleaned air. The air velocity of cleaned air was set as 0.45 m/s. During sample preparation and testing operators wore mask and non-powdered gloves to prevent the contamination from personnel.

The storage temperature of samples was set within the range of 2 – 8 °C similar to the formulation. The room temperature was controlled at 23 – 25 °C for sample preparation and testing as the cleanroom condition for drug product manufacture.

1.2 Silicone oil detection

Sample analysis was performed under BSC class II to prevent particle contamination from environment. The Micro-flow imaging (MFI™) ProteinSimple Model MFI 520 with software MVSS (Version 5.0.0), USA, was used to analyse silicone oil particle in the solution. Prior to test on each day, the environment check was performed using particle-free water injected using glass syringe as shown in Figure 12, to indicate the suitability of environment, glassware and water for the test. The criteria of environment check were number of particle size ≥ 10 µm is not more than 1 particle/mL.

For all test samples, the plunger rod was inserted into the prefilled syringe. Then, the plunger rod was pushed to release the test solution from syringe to 1 mL tube. Then, the sample solution was transferred using pipette tip with air filter and the sample solution was injected to Micro-flow imaging (MFI™) as shown in Figure 13.

Figure 12 Connection of the syringe at the inlet port of MFI™ instrument

Figure 13 Connection of the pipette tip at the inlet port of MFI™ instrument

The parameters to measure the particle counts were set as recommended by the manufacturers to collect all particles from the sample,

1. Purge volume 0.10 mL
2. Sample Analyzed Volume 0.20 mL
3. Particle detection parameter
    a. ECD, Equivalent circular diameter (μm): 1 – 100
    b. Circularity: 0 – 1

c. Intensity mean: 0 – 1023

d. Aspect ratio: 0 – 1

Then, the particle count data is interpreted using parameter filter to distinguish silicone oil droplet from other particulate matters. Since the samples were prepared without proteins, so, particles found in the test samples were deficient of proteins and not interfere with the interpretation. Then, the filter criteria were set as the decision tree according to the following parameters specific to the silicone oil droplet (11, 17).

a. Aspect ratio ≥ 0.70,

b. Intensity mean ≤ 600.00,

c. Circularity ≥ 0.86 and

d. Equivalent circular diameter (ECD).

The particle size will be sorted as 2 μm range, from 0 to 2 μm, > 2 μm to 4 μm and the last interval is > 24 to 26 μm shown in Table 1. Then, each particle of silicone oil droplet was calculated for their volume (Equation 1) and convert to the total amount of silicone oil released from surface of prefilled syringe and rubber stopper per milliliter of solution as Equation 2.

$$Silicone\ oil\ volume\ (\mu m^3) = No.\ of\ silicone\ oil\ particle \times \frac{4}{3} \times \pi \times \left(\frac{r}{2}\right)^3$$

r = Radius of particle (μm)

••• Equation 1

Table 1 Estimated silicone oil volume for each particle size range

Interval (μm)	r (μm)	Silicone oil volume (μm^3)
0-2	1	0.52
>2-4	3	14.14
>4-6	5	65.48
>6-8	7	179.67
>8-10	9	381.86
>10-12	11	697.19
>12-14	13	1150.81

Interval (μm)	r (μm)	Silicone oil volume (μm^3)
>14-16	15	1767.86
>16-18	17	2573.48
>18-20	19	3592.81
>20-22	21	4851.00
>22-24	23	6373.19
>24-26	25	8184.52

$$Silicone\ oil\ amount\ of\ each\ sample\ per\ mL = (No.of\ particle_{0-2\mu m} \times silicone\ oil\ volume_{0-2\mu m}) + (No.of\ particle_{>2-4\mu m} \times silicone\ oil\ volume_{>2-4\mu m}) + \cdots + (No.of\ particle_{>24-26\mu m} \times silicone\ oil\ volume_{>24-26\mu m}) \quad \bullet\bullet\bullet \text{ Equation 2}$$

1.3 Preparation of solution

Prior to the solution preparation, glassware was rinsed with Milli-Q water before use. Then, prepare the solution by dispensing materials to 250 mL volumetric flask and dissolve the dispensed materials with 100 mL of water. Then, add 0.125 g of acetic acid to the flask and swirl it until homogeneous. The total volume was adjusted to 250 mL with water. Then, filter the solution through 0.2 μm cellulose acetate membrane filter. The filtration process is performed under BSC class II to prevent foreign particle contamination. The solutions compositions are shown in Table 2.

Table 2 Compositions of the test solutions

Material	Formulation 1	Formulation 2	Formulation 3	Formulation 4
Sorbitol	-	12.5 g	12.5 g	12.5 g
Sodium acetate	-	0.0308 g	0.0308 g	0.0308 g
Polysorbate 20	-	-	0.010 g	-
Polysorbate 80	-	-	-	0.010 g
Acetic acid	-	0.125 g	0.125 g	0.125 g
Water qs to	250 mL	250 mL	250 mL	250 mL

1.4 Sample preparation for MFI™ measurement

Use the micropipette to measure the desired volume of solution and transferred the solutions into prefilled syringe. The rubber stopper was inserted into the syringe barrel using the inserting applicator as shown in Figure 14 to the desired head space level. The filling operation was executed under BSC class II to prevent foreign particle contamination. Then, the sample was placed on the plastic tray to hold test prefilled syringe sample and then the tray was placed on the orbital shaker, IKA™ Model KS260 Control. The orbital shaker was operated at desired speed and time. After the agitation cycle is finished, samples were kept at 2 – 8 °C. Each test is conducted in triplicates.

When samples from 2 – 8 °C storage condition were taken for measurements, they were kept at ambient condition for at least 30 minutes before starting any tests.

Figure 14 The manual rubber stopper inserting applicator

II. The effect of solution components on silicone oil release

To study the impact of formulations on the release of silicone oil from prefilled syringe containers. The samples were prepared by filling 0.6 mL of each formulation in Table 2 into prefilled syringe. The rubber stopper was inserted to obtained 0.16 cm^3 head space. Then, shaking the samples at 180 rpm for 120 minutes with orbital shaker. After 120 minutes, samples were kept at 2 - 8°C

before measurements. For controlled samples, 0.6 mL of the solution of each formulation in Table 2 was filled into prefilled syringe and, the rubber stopper was inserted to 0.16 cm^3 head space, then, samples were kept at 2 – 8 ℃ but without shaking.

III. The effect of shaking parameters on silicone oil release

To study the impact of shaking parameters, which are speed and time, on the release of silicone oil from prefilled syringe containers. The samples were prepared by filling 0.6 mL of solution obtained according to Table 2 into prefilled syringe. Then, the rubber stopper was inserted to 0.16 cm^3 head space. Agitate samples with speed and time as shown in Table 3. Samples were kept at 2 - 8℃ prior to measurement. For controlled samples, were obtained the same way but without shaking.

Table 3 Shaking speed and time

Test no.	Time (minutes)	Speed (rpm)
1	90	160
2	90	180
3	90	200
4	120	160
5	120	180
6	120	200
7	150	160
8	150	180
9	150	200
10 (Control)	0 (No shaking)	0 (No shaking)

Figure 15 Samples placed on orbital shaker

IV. The effects of filling volume and head space on silicone oil release

The solutions obtained as in Table 2 were filled into prefilled syringe with filling volume and head space shown in Table 4. Then, the rubber stopper was inserted to desired head space. Shake samples using resulting speed and time from part III. Samples were kept at 2 - 8°C. For controlled samples, 0.6 mL solution obtained as in Table 2, then, the rubber stopper was inserted to obtained 0.16 cm^3 head space. Controlled samples were kept at 2 – 8 °C but without shaking.

Table 4 Filling volume and head space

Test no.	Ratio of Head space : Filling volume	Filling volume (mL)	Head space (cm^3)	Shaking
1	0:10	0.4	0.00	Shake
2	0:15	0.6	0.00	Shake
3	0:25	1.0	0.00	Shake
4	4:10	0.4	0.16	Shake
5	4:15	0.6	0.16	Shake
6	4:25	1.0	0.16	Shake

Test no.	Ratio of Head space : Filling volume	Filling volume (mL)	Head space (cm^3)	Shaking
7	8:10	0.4	0.32	Shake
8	8:15	0.6	0.32	Shake
9	8:25	1.0	0.32	Shake
10 (Controlled)	4:15	0.6	0.16	No

V. Data analysis

Data were presented as means ± SD. The 1-Way ANOVA followed by Tukey's test for multiple comparison was used to evaluate differences at the significant level 0.05.

Chapter IV
RESULTS AND DISCUSSIONS

Subvisible particle counted from measurement were filtered by the software and method as described in Chapter III. The representative image of silicone oil droplets found from the sample are shown in Figure 16. The number of silicone oil particle is translated to silicone oil amount from each sample by calculating by Equation 1 and 2.

Figure 16 Representative silicon oil droplets

I. Effect of solution composition on silicone oil release

The quantity of silicone oil released from glass prefilled syringe containers are shown in Figure 17.

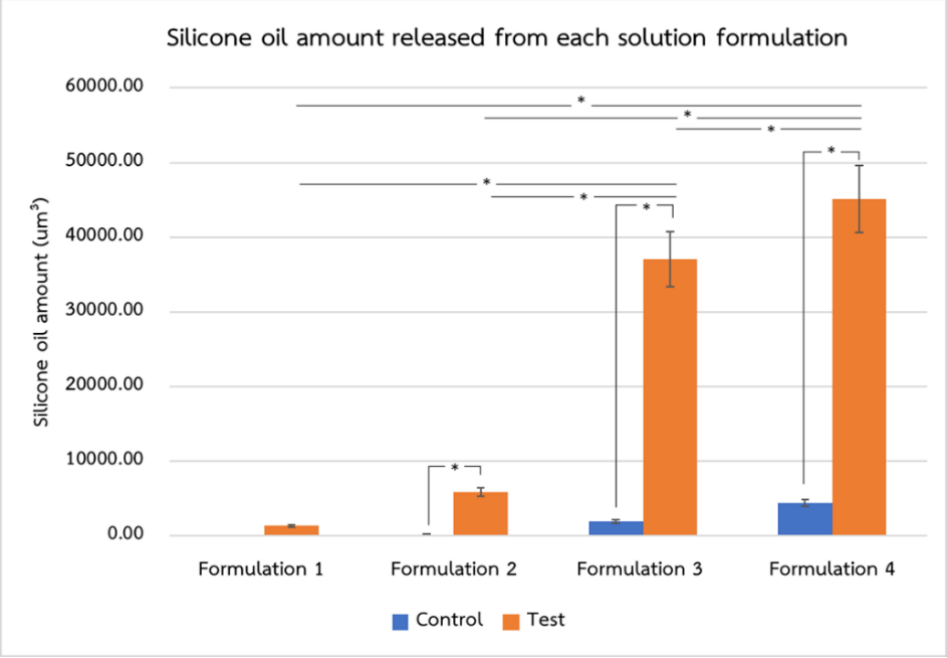


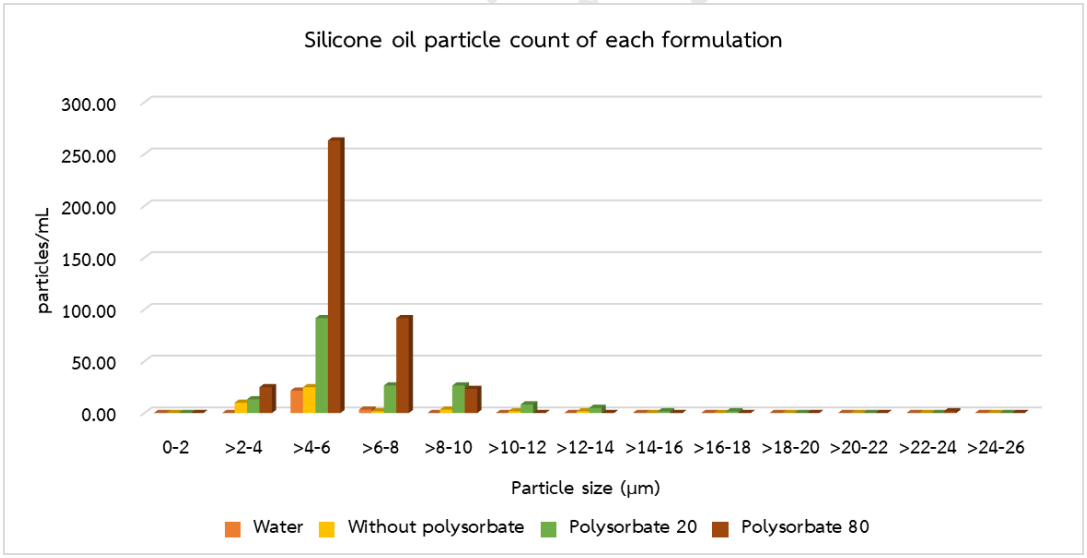
Figure 17 Silicone oil amount released from each formulation (n=3, p≤0.05)

Table 5 Amount of silicone oil amount released obtained from each formulation

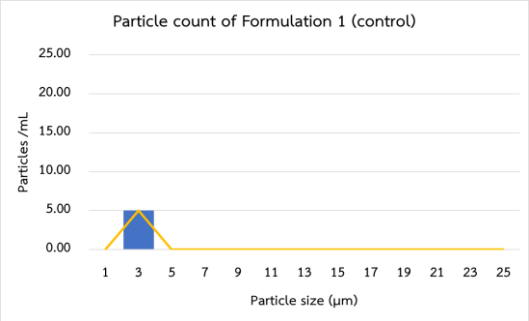
Formulations	Silicone oil released (μm^3 ± SD)	
	Control	Shaken samples
1 (Water)	70.71 ± 0.00	1,324.68 ± 84.85
2 (Buffer without polysorbate)	182.41 ± 25.62	5,631.17 ± 875.96
3 (Buffer with polysorbate 20)	1,961.11 ± 195.18	37,012.88 ± 1,774.42
4 (Buffer with polysorbate 80)	4,397.04 ± 344.92	45,157.12 ± 3,647.83

To evaluate the impact of formulation on the released of silicone oil from glass prefilled syringe, the sample from each formulation were prepared and were shake at speed 180 rpm for 120 minutes. Results are shown in Figure 17 and Table 5, the controlled samples were kept at 2 – 8 °C without shaking. The silicone oil amount from test samples (orange bar) were higher than controlled samples (blue bar) for every formulation. In addition, the silicone oil amount from test sample of formulation 2, formulation 3 and formulation 4 were significantly higher than the controlled sample with confidential level of 95% ($p \leq 0.05$). Moreover, the silicone oil released in formulations contained polysorbate (formulations 3 and 4) were significantly higher than formulation without polysorbate (formulations 1 and 2).

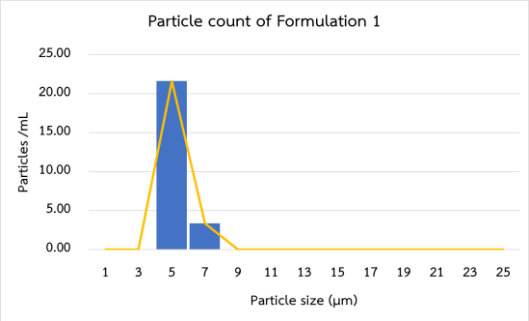
(a) Silicone oil particle size distributions obtained for each formulation



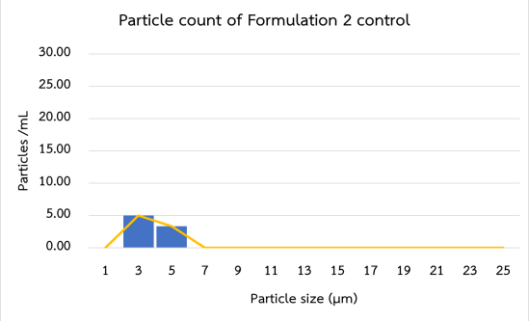
(b) Silicone oil particle count from formulation 1 (control)



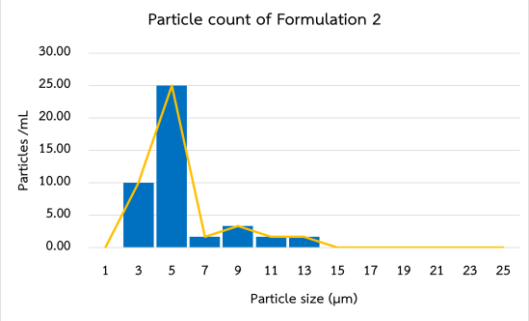
(c) Silicone oil particle count from formulation 1



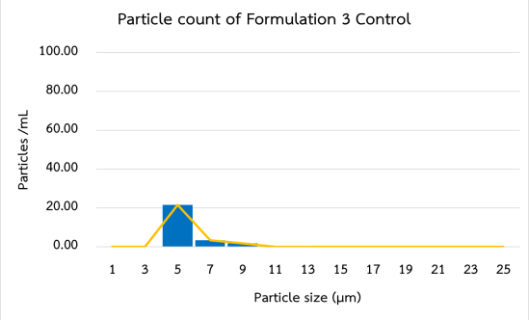
(d) Silicone oil particle count from formulation 2 (control)



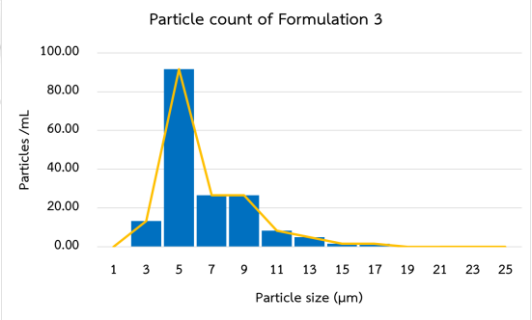
(e) Silicone oil particle count from formulation 2



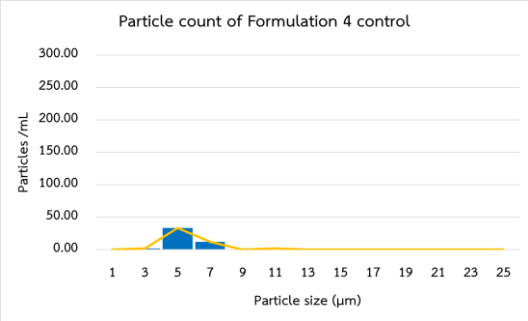
(f) Silicone oil particle count from formulation 3 (control)



(g) Silicone oil particle count from formulation 3



(h) Silicone oil particle count from formulation 4 (control)



(i) Silicone oil particle count from formulation 4

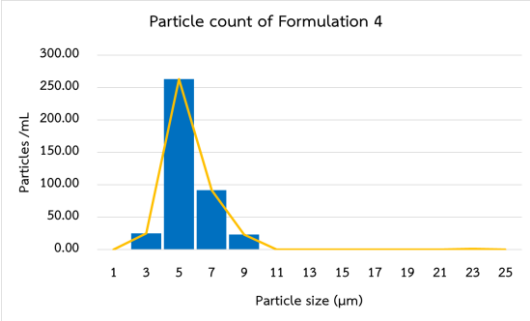


Figure 18 Silicone oil particle released of each formulation

(a) Silicone oil particle size distributions obtained for each formulation (b) Silicone oil particle count from formulation 1 (control) (c) Silicone oil particle count from formulation 1 (d) Silicone oil particle count from formulation 2 (control) (e) Silicone oil particle count from formulation 2 (f) Silicone oil particle count from formulation 3 (control) (g) Silicone oil particle count from formulation 3 (h) Silicone oil particle count from formulation 4 (control) (i) Silicone oil particle count from formulation 4

Table 6 Particle size distribution of silicone oil droplets obtained from each formulation

Parameter	Formulation							
	1 (control)	1	2 (control)	2	3 (control)	3	4 (control)	4
D10	1.20	3.23	1.33	1.87	3.25	3.09	3.19	3.12
D50	2.00	4.15	2.67	3.93	4.23	4.62	4.35	4.35
D90	2.80	5.50	4.50	8.40	6.40	8.94	6.46	6.66
Span	0.80	0.55	1.19	1.66	0.75	1.27	0.75	0.82

In addition, from Figure 18 and Table 6 comparing the silicone oil particle count shown that formulations with polysorbate 80 have the highest number of particles, more than formulations with polysorbate 20, buffer and water, in reducing order. It can be concluded that formulations contained polysorbate (both polysorbate 20 (formulation 3) and polysorbate 80 (formulation 4)) induced the

release of silicone oil from glass prefilled syringe. Due to the properties of the surfactant in the formulation can play roles as the emulsifier of the silicone oil layer coated on the inner surface of prefilled syringe container and released the silicone oil particles to the formulation (10, 11).

Moreover, results showed that polysorbate 80 effect the release of silicone oil more than polysorbate 20 significantly ($p \leq 0.05$). Due to the hydrophilic/lipophilic balance (HLB) of both surfactants are different. HLB of polysorbate 20 is 16.7 which is higher than the HLB of polysorbate 80 which is 15.0 exhibiting more lipophilic (33, 34). And as the chemical structure of polysorbate 20 and polysorbate 80 are shown in Figure 19, when hydrocarbon chain of polysorbate 80 (monooleate) is longer than polysorbate 20 (monolaurate). According to the properties of polysorbate it can be concluded that polysorbate 80 is more lipophilic and able to dissolve the silicone oil from surface of prefilled syringe more than polysorbate 20. In addition, the concentrations of polysorbate 20 and polysorbate 80 in all formulations are 0.004 %w/v which the concentration of polysorbate 80 is higher than the critical micelle concentration (CMC) value of 0001 %w/v and free polysorbate 80 will dissolve the silicone oil (33). However, the concentration of polysorbate 20 is lower than CMC value which is 0.006 %w/v (35). Then, the silicone oil released from the prefilled syringe container can form emulsion droplets in the solution contained polysorbate 80 in formulation 4 more than polysorbate 20 in formulation 3. Therefore, the polysorbate 80 can affect the release of silicone oil to formulation higher than polysorbate 20. Then, formulation containing polysorbate 80 was selected to be further investigated in future studies.

However, this study used formulations without proteins which results may be different from above. Then, the free polysorbate in formulations containing proteins should be evaluated for the impact on the release of silicone oil from prefilled syringe container.

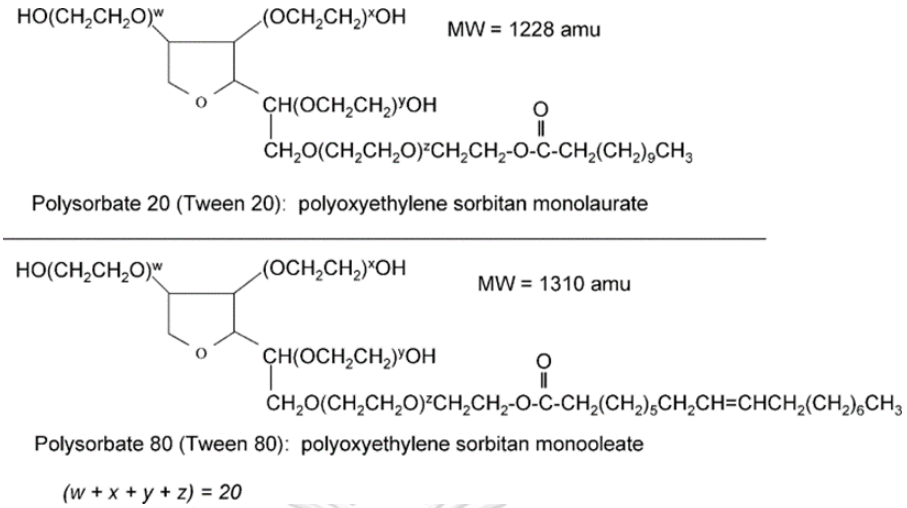


Figure 19 Chemical structures of polysorbate 20 and polysorbate 80 (36)

II. Effect of shaking parameters on silicone oil release

To evaluate the impact of shaking speed and time on the release of silicone oil from glass prefilled syringe, samples were prepared with various shaking speed and time. The result of varied shaking speed and time can be concluded that both parameters induced the release of silicone oil from prefilled syringe as shown in Figure 20 and Table 7. At 90 minutes, the silicone oil released from glass prefilled syringe were not significantly different (at significant level 0.05) for all shaking speeds. At 120 minutes and shaking speed at 180 rpm and 200 rpm showed that the silicone oil released from glass prefilled syringe were higher than shaking speed at 160 rpm at significantly ($p \leq 0.05$). Also, the silicone oil released was higher than shaking time of 90 minutes for all shaking speeds.

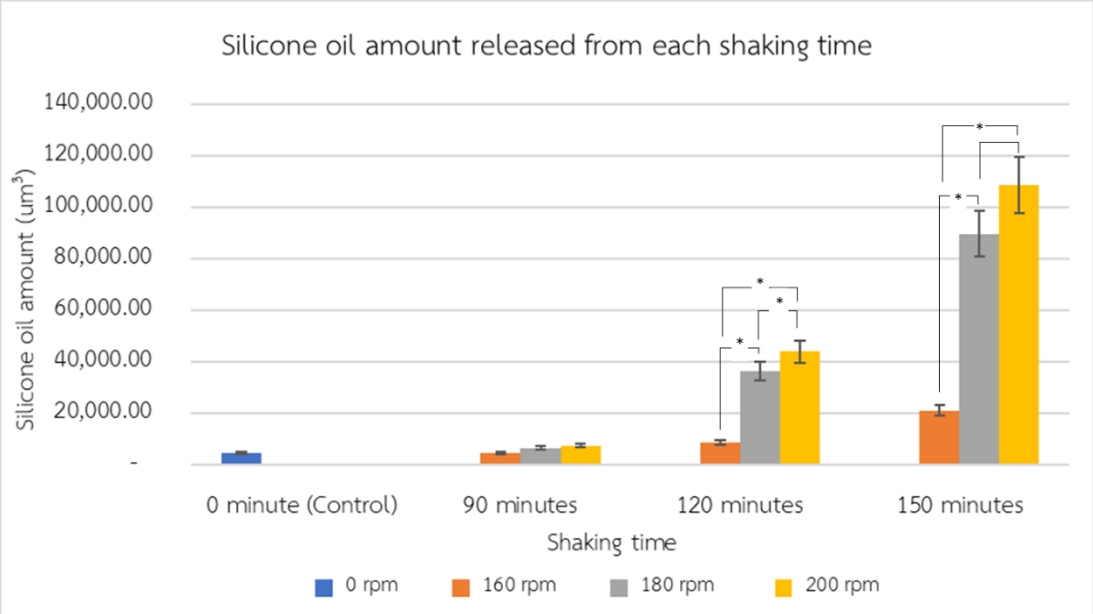
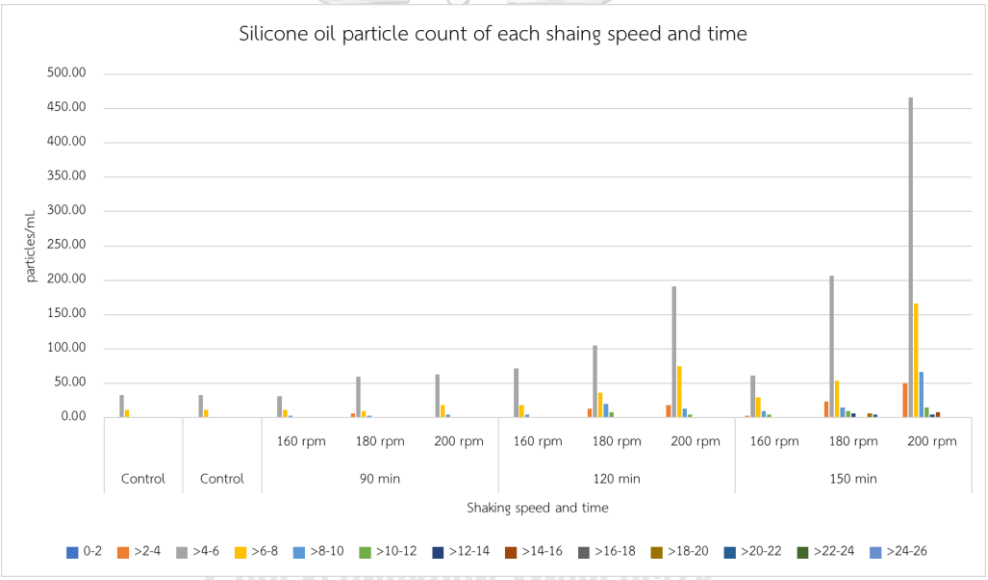


Figure 20 Amount of silicone oil released when the glass prefilled syringe is shaken with different rates and durations

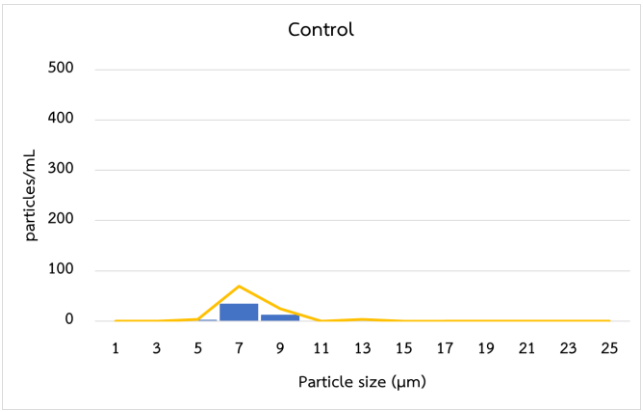
Table 7 Silicone oil amount obtained from each shaking parameter (μm^3)

Speed (rpm)	Silicone oil released (μm^3 ± SD)		
	Time (minutes)		
	90	120	150
160	4,451.55 ± 392.06	8,524.59 ± 104.73	21,005.55 ± 839.53
180	6,334.41 ± 359.76	36,268.49 ± 1,945.02	89,668.98 ± 4,378.78
200	7,321.12 ± 180.86	43,857.93 ± 3,998.00	108,611.05 8,766.36
Control	4,397.04 ± 334.92		

(a) Silicone oil particle size distributions obtained for each shaking speed and time



(b) Control sample



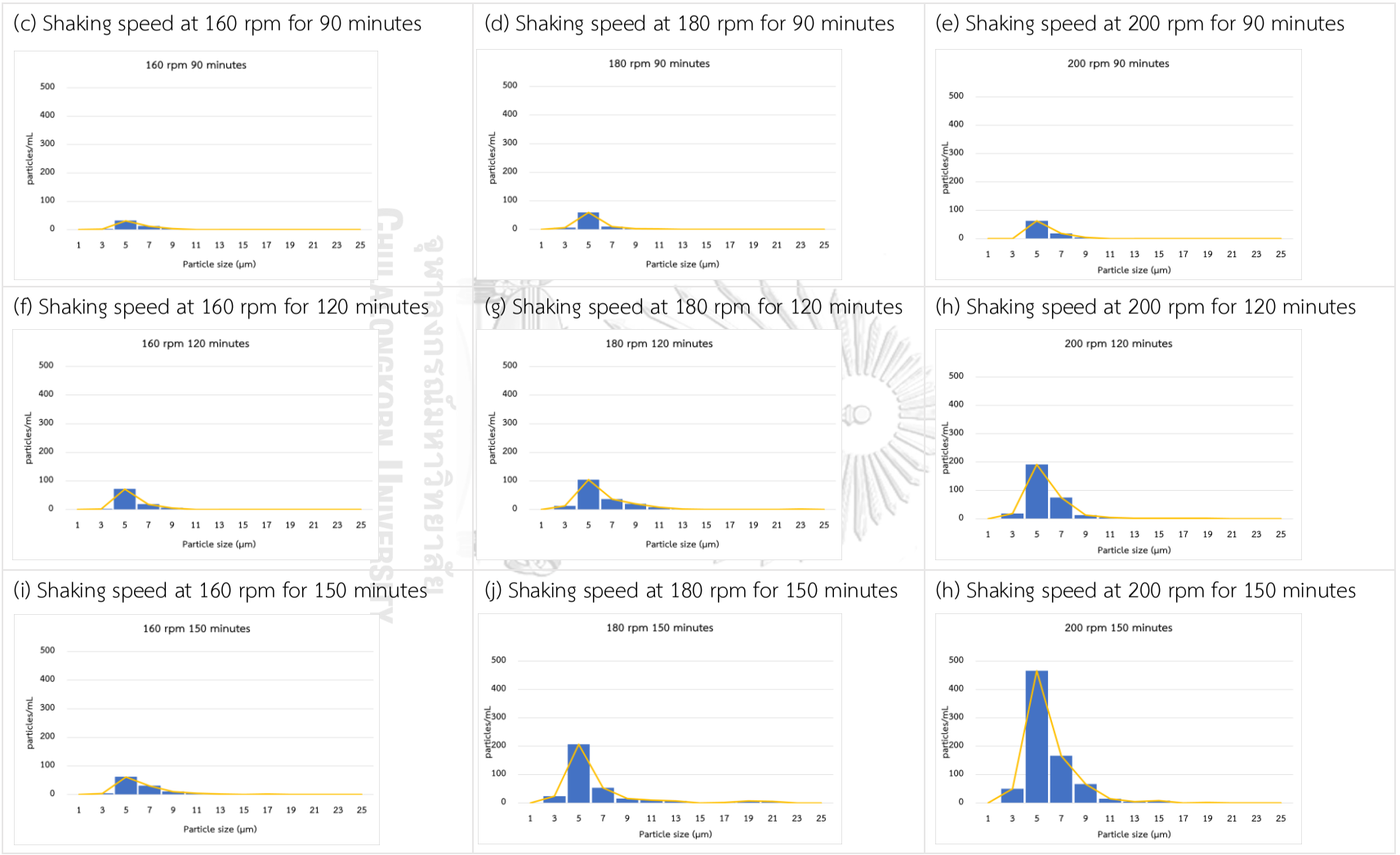


Figure 21 Silicone oil particle count released when the glass prefilled syringe was shaken with different rates and durations

(a) Silicone oil particle size distributions obtained for each shaking speed and time (b) Control sample (c) Shaking speed at 160 rpm for 90 minutes (d) Shaking speed at 180 rpm for 90 minutes (e) Shaking speed at 200 rpm for 90 minutes (f) Shaking speed at 160 rpm for 120 minutes (g) Shaking speed at 180 rpm for 120 minutes (h) Shaking speed at 200 rpm for 120 minutes (i) Shaking speed at 160 rpm for 150 minutes (j) Shaking speed at 180 rpm for 150 minutes (k) Shaking speed at 200 rpm for 150 minutes

Table 8 Particle size distribution of each shaking speed and time

Parameter	Shaking speed and time									
	Control	160 rpm 90 min	160 rpm 120 min	160 rpm 150 min	180 rpm 90 min	180 rpm 120 min	180 rpm 150 min	200 rpm 90 min	200 rpm 120 min	200 rpm 150 min
D10	3.19	3.20	3.05	3.27	3.22	3.10	3.13	3.26	3.09	3.12
D50	4.35	4.42	4.14	4.37	4.30	4.52	4.43	4.73	4.36	4.46
D90	6.46	6.74	6.37	6.60	6.49	8.30	6.84	8.40	8.62	7.56
Span	0.75	0.80	0.80	0.76	0.76	1.15	0.84	1.09	1.27	1.00

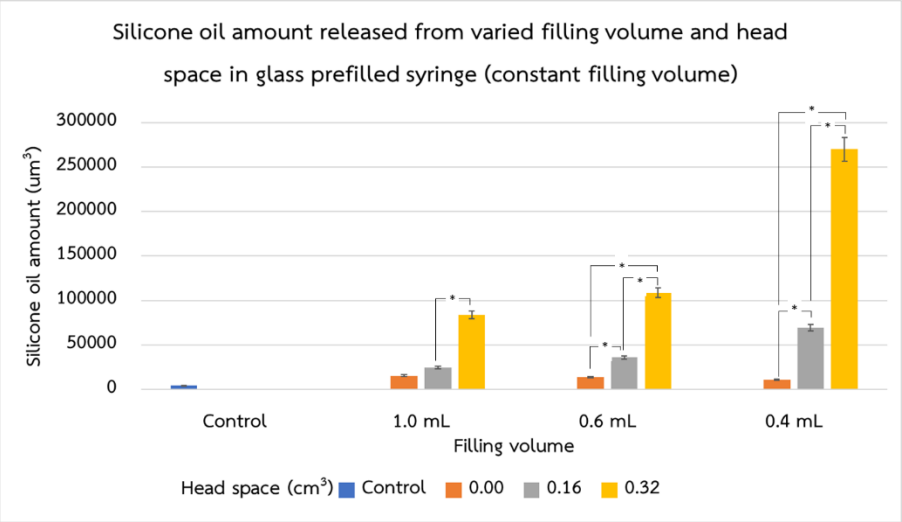
Comparing the particle amount as shown in Figure 21 and Table 8, when increasing the time and the speed resulting in the increase in the amount of silicone oil particles at every particle size. In addition, at higher shaking time it was found that silicone oil particle at larger size was increased due to the agglomeration of the particles. Due to the agitation of prefilled syringe container leading to the movement of the air bubble which is the head space inside the prefilled syringe container. The movement of air bubble along the prefilled syringe barrel can cause the cavitation inside the prefilled syringe and disrupt the silicone oil coating layer and released as droplets to the solution (12).

In summary, comparing the shaking time, the higher speed can increase the release of silicone oil from glass prefilled syringe. Likewise, at same shaking speed when increase the shaking time it can induce higher release of silicone oil from glass prefilled syringe. In conclusion, at high shaking speeds of 180 rpm and 200 rpm for 120 minutes silicone oil was released more than when 160 rpm was used at every shaking time at significant level 0.05. Therefore, shaking speed at 180 rpm for 120 minutes was selected as process parameter for future study.

III. Effects of filling volume and head space on silicone oil release

To study the impact of filling volume and head space on the release of silicone oil from glass prefilled syringe. The sample were prepared using polysorbate 80 formulation (formulation 4) then the sample were shaken at 180 rpm for 120 minutes.

(a) Constant filling volume



(b) Constant head space

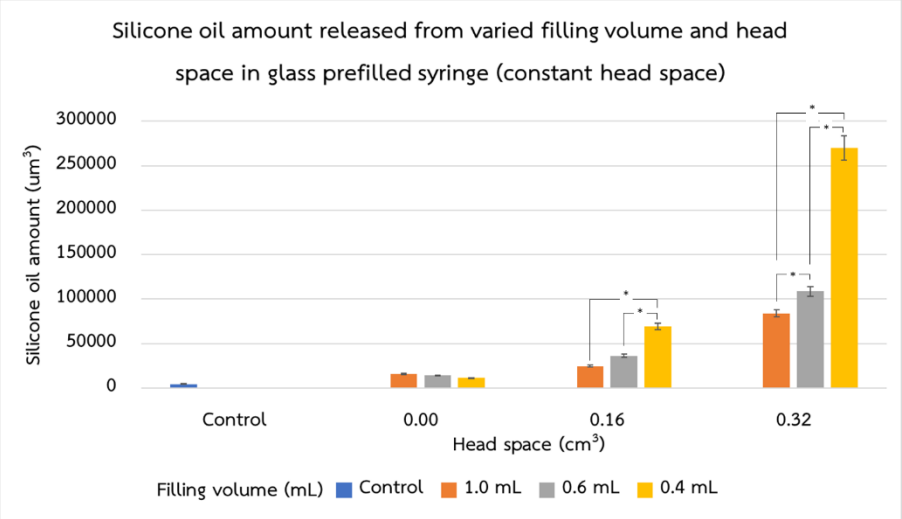


Figure 22 Amount of silicone oil released from varied filling volume and head space (n = 3, p≤0.05) (a) Constant filling volume (b) Constant head space

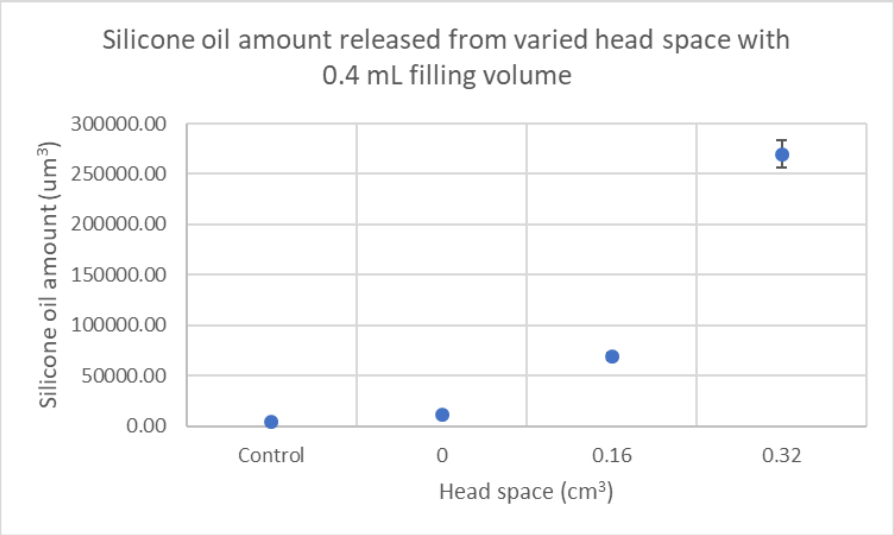


Figure 23 Amount of silicone oil released from study of prefilled syringe with various head space at 0.4 mL filling volume

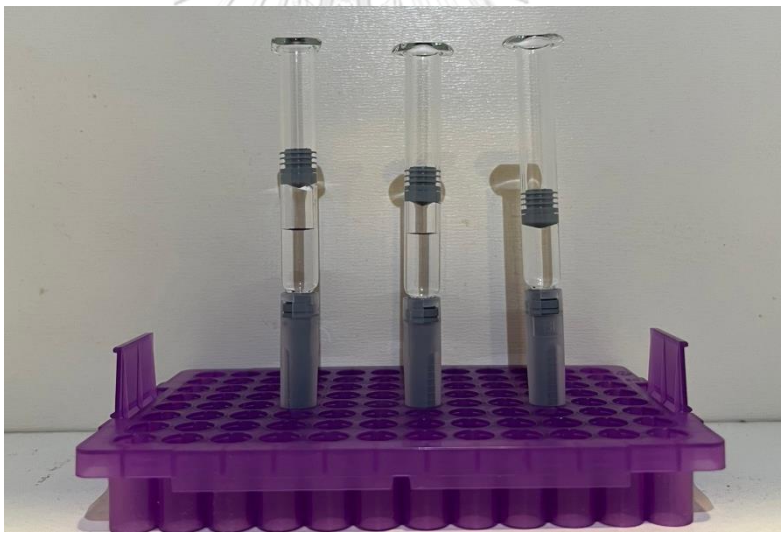


Figure 24 Sample filled with 0.4 mL of buffer at various head space.
Left: Head space 0.32 cm^3 Middle: Head space 0.16 cm^3 Right: Head space 0 cm^3

At filling volume 0.4 mL, the sample with head space 0.32 cm^3 was released silicone oil droplets higher than sample with head space 0.16 cm^3 and 0 cm^3 accordingly as shown in Figure 23

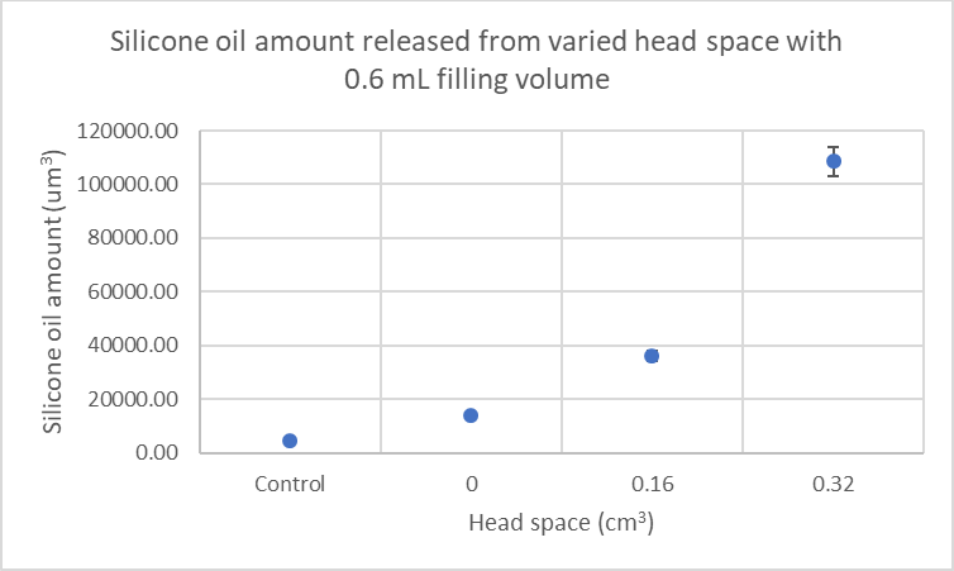


Figure 25 Silicone oil amount released from study of prefilled syringe with various head space at 0.6 mL filling volume

Figure 26 Sample filled with 0.6 mL of buffer at various head space.
Left: Head space 0.32 cm^3 Middle: Head space 0.16 cm^3 Right: Head space 0 cm^3

At filling volume 0.6 mL, the sample with head space 0.32 cm^3 was released silicone oil droplets higher than sample with head space 0.16 cm^3 and 0 cm^3 accordingly as shown in Figure 25,

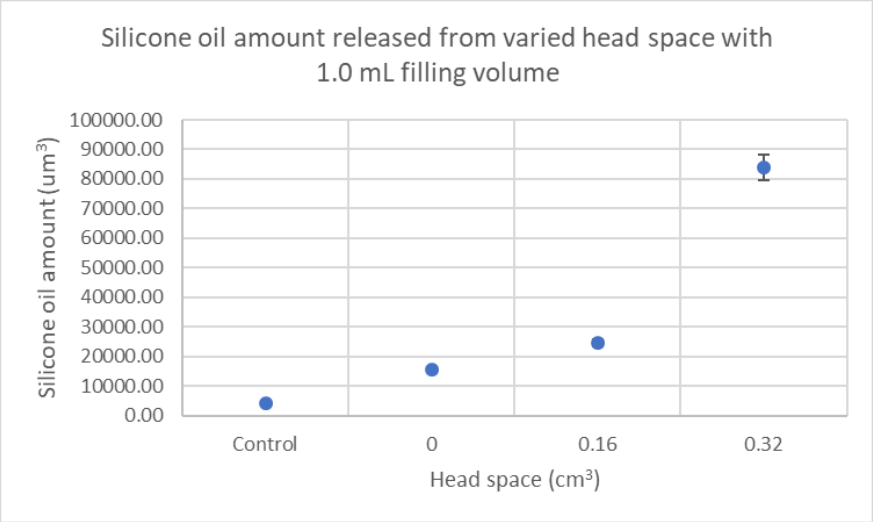


Figure 27 Silicone oil amount released from study of prefilled syringe with various head space at 1.0 mL filling volume

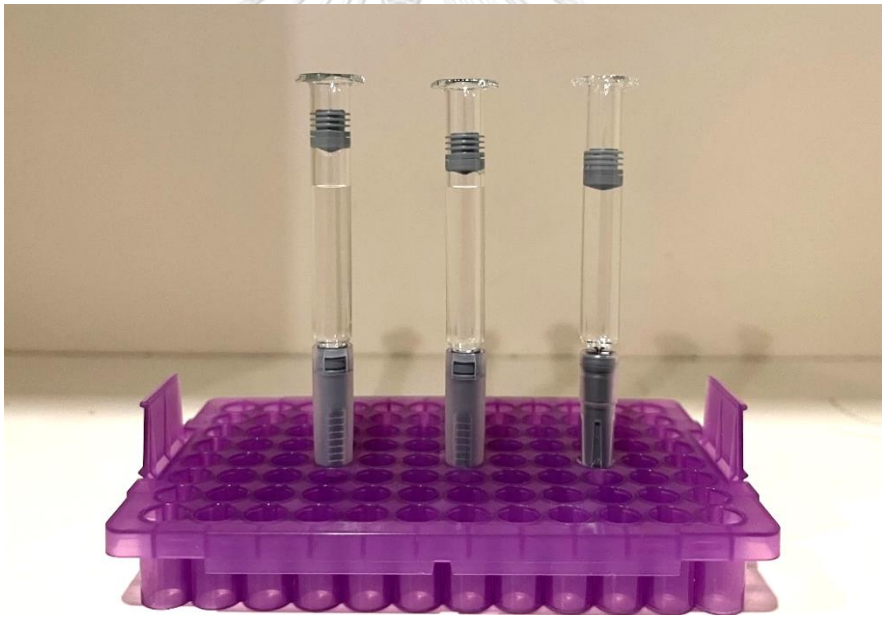


Figure 28 Sample filled with 1.0 mL of buffer at various head space.
Left: Head space 0.32 cm^3 Middle: Head space 0.16 cm^3 Right: Head space 0 cm^3

Likewise, filling volume 0.4 mL and 0.6 mL, at filling volume 1.0 mL the sample with head space 0.32 cm^3 was released silicone oil droplets higher than sample with head space 0.16 cm^3 and 0 cm^3 accordingly as shown in Figure 27.

Table 9 Silicone oil amount released from varies filling volume and head space

Filling volume	Silicone oil released (μm^3 ± SD)		
	Head space		
	0.00 cm^3	0.16 cm^3	0.32 cm^3
0.4 mL	11,158.27 ± 765.73	69,427.88 ± 1,043.90	269,815.81 ± 19,931.29
0.6 mL	14,006.39 ± 891.13	36,268.496 ± 1,945.02	108,646.44 ± 6,973.38
1.0 mL	15,671.19 ± 557.28	24,700.83 ± 2,171.45	83,904.85 ± 2614.46
Control	4,397.04 ± 344.92		

In conclusion, as Figure 22 (a), Figure 23, Figure 25 and Figure 27 showed, comparing at constant filling volume, the silicone oil released from the prefilled syringe is increased when the head space is increased.

At filling volume 0.4 mL, the silicone oil released from controlled sample and sample with 0 cm^3 head space were not significantly different. However, when comparing the controlled sample and sample with 0 cm^3 head space with the samples with 0.16 cm^3 and 0.32 cm^3 head space, it was shown that samples with head space released the silicone oil from prefilled syringe higher than the sample with 0 cm^3 head space. And when compared the sample with 0.32 cm^3 head space with the sample with 0.16 cm^3 head space, it was shown that the sample with 0.32 cm^3 head space released the silicone oil more than the sample with 0.16 cm^3 head space.

Likewise, at filling volume 0.6 mL and 1.0 mL showed the same results. Except, the sample filled 1.0 mL with 0.16 cm^3 that the silicone oil released was not significantly higher that the sample with 0 cm^3 head space (at significant level 0.05). Since the sample at filling volume 1 mL with 0.16 cm^3 head space had the lowest ratio of head space to filling volume and the air bubble had limited space to move along the syringe barrel. Therefore, the head space at 0.16 cm^3 inside the prefilled syringe container with filling volume 1 mL had the low impact on the released of silicone oil to the formulation.

Meanwhile, when comparing at constant head space, from Figure 22 (b), the silicone oil released from sample with 0 cm^3 head space of every filling volume were

not significantly different from the control sample (no shaking) at significant level 0.05. As mentioned in the previous section, when presence of the head space which is air bubble inside the prefilled syringe container and when the air bubble move along the syringe barrel it can induced the release of silicone oil from the prefilled syringe container (1, 11). When the head space is present, at 0.4 mL filling volume it was show that the highest silicone oil released were 0.6 mL and 1.0 mL, respectively.

Table 10 Ratio of head space to filling volume

Test no.	Ratio of volume of Head space to Filling volume	Ratio of contact surface of Head space to Filling volume	Contact surface of Head space (cm^2)	Contact surface of Filling volume (cm^2)	Filling volume (mL)	Head space (cm^3)
1	0:10	N/A	0	2.25	0.4	0.00
2	0:15	N/A	0	3.78	0.6	0.00
3	0:25	N/A	0	6.30	1.0	0.00
4	4:10 (0.40)	0.40	1	2.25	0.4	0.16
5	4:15 (0.27)	0.26	1	3.78	0.6	0.16
6	4:25 (0.16)	0.16	1	6.30	1.0	0.16
7	8:10 (0.80)	0.79	2	2.25	0.4	0.32
8	8:15 (0.53)	0.53	2	3.78	0.6	0.32
9	8:25 (0.32)	0.32	2	6.30	1.0	0.32
10 (Controlled)	4:15	0.26	1	3.78	0.6	0.16

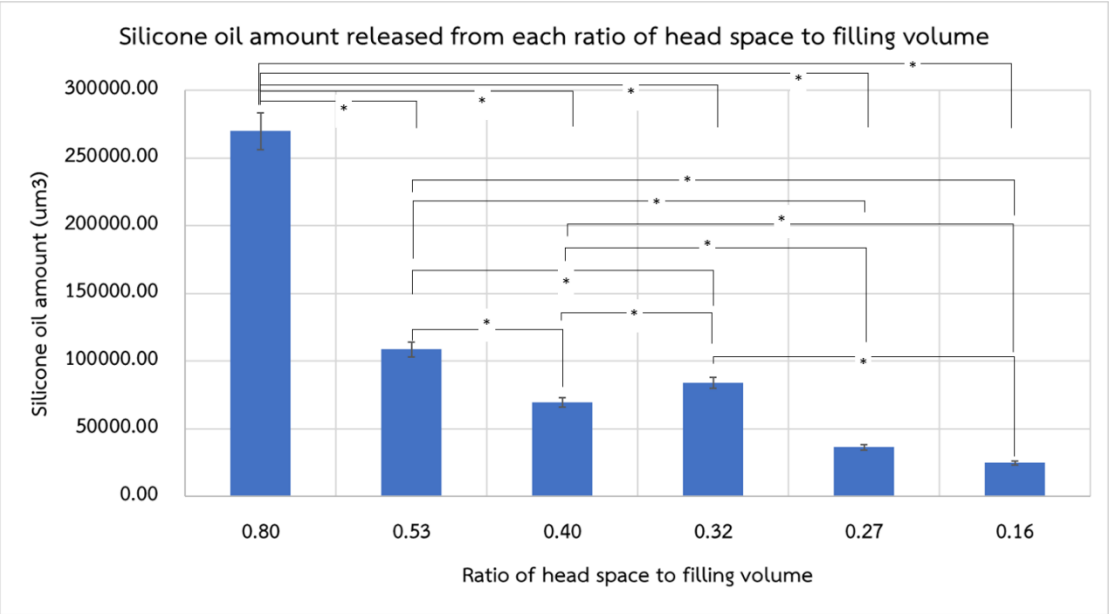


Figure 29 Silicone oil amount released from each ratio of head space to filling volume (n = 3, p≤0.05)

Figure 30 Sample with 0.32 cm^3 head space
(a) Filling volume 1 mL (b) Filling volume 0.4 mL

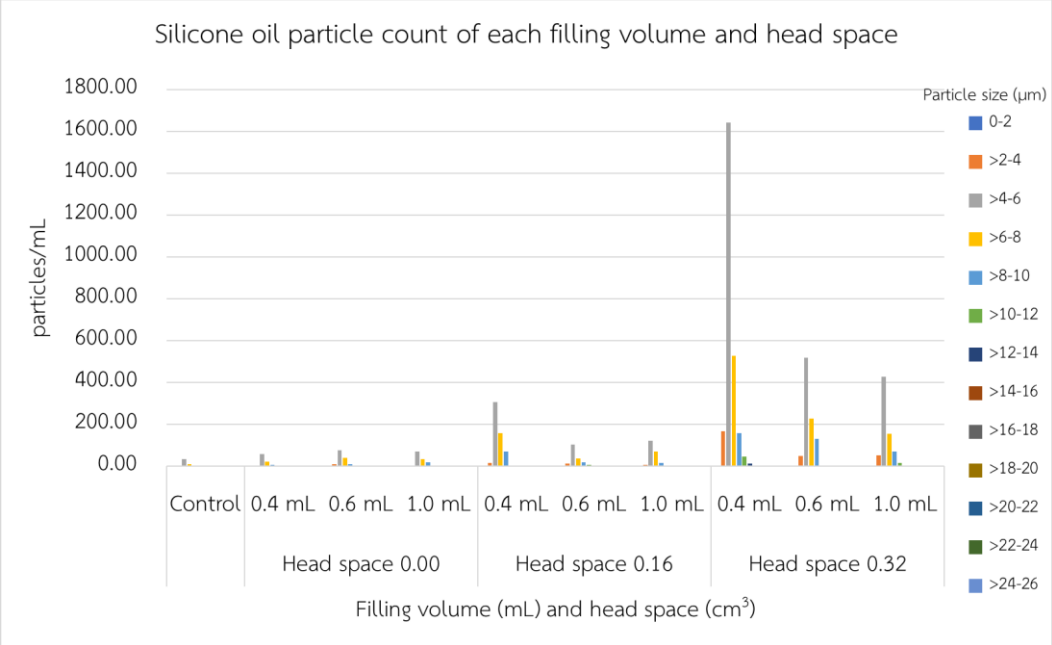
Comparing the silicone oil released from each ratio of head space to filling volume, sample of ratio 0.80 had the highest amount of silicone oil released. Sample of ratio 0.16 had the lowest amount of silicone oil released. Due to the air bubble inside the prefilled syringe is able to move along the prefilled syringe barrel at higher ratio of head space as shown in Figure 30. When the air bubbles moving at higher speed it can induced the cavitation of air to inner surface of the prefilled syringe.

Then, the higher ratio released the silicone oil to formulation more than sample at lower ratio.

Nonetheless, sample at ratio of head space to filling volume 0.32 (0.32 cm^3 to 1.0 mL) can released the silicone oil to formulation higher than sample at ratio of head space to filling volume 0.40 (0.16 cm^3 to 0.4 mL). Due to the contact surface of sample with 1.0 mL is higher than sample with 0.4mL. Then, at higher contact surface of solution can impact on the released of silicone oil higher than lower contact surface.

As shown in Table 10, the ratio of volume of head space to filling volume and the ratio of contact surface of head space to filling volume were not different.

(a) Silicone oil particle size distributions obtained for each filling volume and head space



(b) Control sample

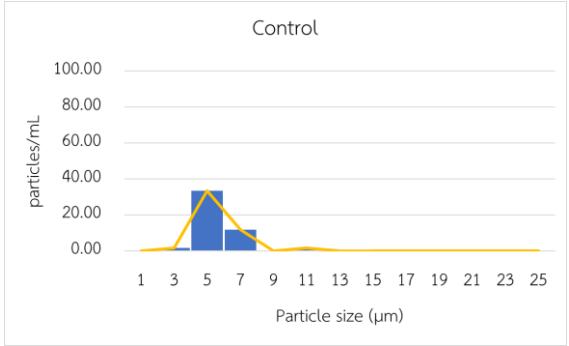


Figure 31 Silicone oil particle size distribution obtained for various filling volume and head space

(a) Silicone oil particle size distributions obtained for each filling volume and head space (b) Control sample (c) Filling volume 0.4 mL with 0 cm^3 head space (d)) Filling volume 0.4 mL with 0.16 cm^3 head space (e)) Filling volume 0.4 mL with 0.32 cm^3 head space (f)) Filling volume 0.6 mL with 0 cm^3 head space (g)) Filling volume 0.6 mL with 0.16 cm^3 head space (h)) Filling volume 0.6 mL with 0.32 cm^3 head space (i)) Filling volume 1.0 mL with 0 cm^3 head space (j)) Filling volume 1.0 mL with 0.16 cm^3 head space (k)) Filling volume 1.0 mL with 0.32 cm^3 head space

Table 11 Particle size distribution of each filling volume and head space

Parameter	Control	Filling volume and head space								
		0.4 mL, 0 cm^3	0.6 mL, 0 cm^3	1.0 mL, 0 cm^3	0.4 mL, 0.16 cm^3	0.6 mL, 0.16 cm^3	1.0 mL, 0.16 cm^3	0.4 mL, 0.32 cm^3	0.6 mL, 0.32 cm^3	1.0 mL, 0.32 cm^3
D 10	3.19	3.21	3.10	3.27	3.25	3.10	3.22	3.11	3.17	3.10
D 50	4.35	4.44	4.54	4.71	4.71	4.52	4.66	4.35	4.60	4.45
D 90	6.46	6.91	6.82	7.62	7.55	8.30	6.86	6.88	7.58	7.46
Span	0.75	0.83	0.82	0.92	0.91	1.15	0.78	0.87	0.96	0.98

In addition, as shown in Figure 31, at constant filling volume the number of silicone oil particle count found in sample with higher head space more than sample with lower head space. Moreover, comparing at constant head space the lower filling volume found the silicone oil particle higher than the higher filling volume. These were results of the ability of movement of air bubble inside the prefilled syringe container. It can be inferred that at higher ratio of head space combined with lower ratio of filling volume can impact on the released of silicone oil form prefilled syringe container.

As shown in Table 11, the size of silicone oil particle mostly found were at 4 μm. For sample with filling volume 0.4 mL and 0.6 mL, with the head space, the size of silicone oil droplet was increased. This can be influence from the movement of solution inside the prefilled syringe when the air bubble is moving. When the solution is moving, it enhances the agglomeration of silicone oil droplets and form the bigger size of particle.

In conclusion, the amount of silicone oil released from each filling volume without head space was found not be significantly different at significant level 0.05. Comparing samples with head space, silicone oil released were increased. This is due to results from the movement of air bubbles can be cause of the cavitation inside the prefilled syringe as shown in Figure 30. From the study, filling volume 0.4 mL with 0.32 cm^3 head space, which is the highest ratio of head space to filling volume shown in Figure 31 and Table 11, had the highest amount of silicone oil released. It can be concluded that condition with no headspace, high filling volume could induce higher release of silicone oil than when low filling volume was used.

Chapter V
CONCLUSION

Formulation containing surfactant either polysorbate 20 or polysorbate 80 significantly impact the release of silicone oil from prefilled syringe when filled with head space. When each ratio of filling volume and head space were compared, higher ratio was shown to induce higher release of silicone oil due to cavitation of air bubble from greater motion of air bubbles inside the prefilled syringe. However, at the same headspace 1 mL filling volume initiated smaller sized air bubble. Therefore, lower filling volume affect the release of silicone oil more than higher volume when the head space was held constant.

Moreover, when the agitation presence, there will be a movement of air bubbles and solution inside the prefilled syringe. This movement has affected to the cavillation of air bubble inside the prefilled syringe and resulting in the release of silicone oil from surface of prefilled syringe container.

The recommendation to determine the filling condition at each filling volume the headspace should be minimized to the optimum level of each machine in order to prevent the movement of air bubble along the syringe barrel. And also, should minimized the agitation that would be occurred during the manufacturing process.

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APPENDIX

Table 12 Silicone oil amount obtained from each formulation (μm^3)

Formulation	Control	Shaken samples
1 (Water)	70.71 ± 0.00	1,324.68 ± 84.85
2 (Buffer without polysorbate)	182.41 ± 25.62	5,631.17 ± 875.96
3 (Buffer with polysorbate 20)	1,961.11 ± 195.18	37,012.88 ± 1,774.42
4 (Buffer with polysorbate 80)	4,397.04 ± 344.92	45,157.12 ± 3,647.83

Table 13 Data of silicone oil amount obtained from each formulation (μm^3)

Sample	Sample 1	Sample 2	Sample 3	Average	SD
Formulation 1 control	70.71	70.71	70.71	70.71	0
Formulation 1	1,339.95	1,400.86	1,233.24	1,324.68	84.85
Formulation 2 control	212.00	167.62	167.62	182.41	25.62
Formulation 2	4,791.37	5,562.86	6,539.29	5,631.17	875.96
Formulation 3 control	2,070.66	1,735.76	2,076.90	1,961.11	195.18
Formulation 3	38,964.41	35,496.56	36,577.66	37,012.88	1,774.42
Formulation 4 control	4,033.15	4,438.77	4,719.19	4,397.04	344.92
Formulation 4	41,265.42	48,498.55	45,707.39	45,157.12	3,647.83

Table 14 Test of homogeneity of variances of silicone oil amount obtained from each formulation

	df	Sum of Squares	Mean Square	F	Sig
Between Groups	7	6960306100	994329443	457.49	0.000
Within Groups	16	34774840	2173427		
Total	23	6995080940			

Table 15 Multiple comparison of variances of silicone oil amount obtained from each formulation

Difference of Levels		Difference of Means	SE of Difference	95% CI	T-Value	P-Value
Water	Water CT	1254	1204	(-1298, 3806)	1.04	0.313
Pre CT	Water CT	112	1204	(-2440, 2663)	0.09	0.927
Pre	Water CT	5560	1204	(3009, 8112)	4.62	0.000
PS 20 CT	Water CT	1890	1204	(-661, 4442)	1.57	0.136
PS 20	Water CT	36942	1204	(34390, 39494)	30.69	0.000
PS 80 CT	Water CT	4326	1204	(1775, 6878)	3.59	0.002
PS 80	Water CT	45086	1204	(42535, 47638)	37.46	0.000
Pre CT	Water	-1142	1204	(-3694, 1410)	-0.95	0.357
Pre	Water	4306	1204	(1755, 6858)	3.58	0.003
PS 20 CT	Water	636	1204	(-1915, 3188)	0.53	0.604
PS 20	Water	35688	1204	(33136, 38240)	29.65	0.000
PS 80 CT	Water	3072	1204	(521, 5624)	2.55	0.021
PS 80	Water	43832	1204	(41281, 46384)	36.41	0.000
Pre	Pre CT	5449	1204	(2897, 8001)	4.53	0.000
PS 20 CT	Pre CT	1779	1204	(-773, 4330)	1.48	0.159
PS 20	Pre CT	36830	1204	(34279, 39382)	30.60	0.000
PS 80 CT	Pre CT	4215	1204	(1663, 6766)	3.50	0.003
PS 80	Pre CT	44975	1204	(42423, 47526)	37.36	0.000
PS 20 CT	Pre	-3670	1204	(-6222, -1118)	-3.05	0.008
PS 20	Pre	31382	1204	(28830, 33933)	26.07	0.000
PS 80 CT	Pre	-1234	1204	(-3786, 1318)	-1.03	0.320
PS 80	Pre	39526	1204	(36974, 42078)	32.84	0.000
PS 20	PS 20 CT	35052	1204	(32500, 37604)	29.12	0.000
PS 80 CT	PS 20 CT	2436	1204	(-116, 4988)	2.02	0.060
PS 80	PS 20 CT	43196	1204	(40644, 45748)	35.89	0.000
PS 80 CT	PS 20	-32616	1204	(-35168, -30064)	-27.10	0.000
PS 80	PS 20	8144	1204	(5592, 10696)	6.77	0.000
PS 80	PS 80 CT	40760	1204	(38208, 43312)	33.86	0.000

Table 16 Particle count from each formulation (Particles/mL)

Particle size (μm)	Formulation							
	1 Control	1	2 Control	2	3 Control	3	4 Control	4
0-2	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
>2-4	5.00±0.00	0.00±0.00	5.00±8.65	9.99±10.00	0.00±0.00	13.32±12.57	1.67±2.89	24.98±14.99
>4-6	0.00±0.00	21.65±16.06	3.33±2.89	24.98±26.44	21.65±15.26	91.60±46.42	33.31±20.80	263.14±240.04
>6-8	0.00±0.00	3.33±2.89	0.00±0.00	1.67±2.89	3.33±2.89	26.65±7.63	11.66±5.77	91.60±46.42
>8-10	0.00±0.00	0.00±0.00	0.00±0.00	3.33±2.89	1.67±2.89	26.65±2.88	0.00±0.00	23.32±23.61
>10-12	0.00±0.00	0.00±0.00	0.00±0.00	1.67±2.89	0.00±0.00	8.33±2.88	1.67±2.89	0.00±0.00
>12-14	0.00±0.00	0.00±0.00	0.00±0.00	1.67±2.89	0.00±0.00	5.00±5.00	0.00±0.00	0.00±0.00
>14-16	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.67±2.89	0.00±0.00	0.00±0.00
>16-18	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.67±2.89	0.00±0.00	0.00±0.00
>18-20	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
>20-22	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
>22-24	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.67±2.89
>24-26	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Table 17 Data of silicone oil amount from each shaking parameter (μm^3)

Agitation speed and time	Sample 1	Sample 2	Sample 3	Average	SD
160 rpm 90 min	4,876.48	4,103.86	4,374.30	4451.55	392.06
160 rpm 120 min	8,645.44	8,460.31	8,468.02	8524.59	104.73
160 rpm 150 min	21,793.10	21,101.30	20,122.25	21005.55	839.53
180 rpm 90 min	6,190.43	6,068.94	6,743.86	6334.41	359.76
180 rpm 120 min	36,794.85	34,114.46	37,896.17	36268.49	1945.02
180 rpm 150 min	92,197.07	92,197.07	84,612.80	89668.98	4378.78
200 rpm 90 min	7,396.49	7,452.09	7,114.76	7321.12	180.86
200 rpm 120 min	41,907.34	41,209.65	48,456.81	43857.93	3998.00
200 rpm 150 min	105,684.72	118,466.27	101,682.16	108611.05	8766.36
Control	4,033.15	4,438.77	4,719.19	4397.04	344.92

Table 18 Test of homogeneity of variances of silicone oil amount obtained from each shaking parameter

	df	Sum of Squares	Mean Square	F	Sig
Between Groups	9	38410429820	4267825536	364.96	0.000
Within Groups	20	233880938	11694047		
Total	29	38644310758			
Remark: Data specified in Table 7.					

Table 19 Multiple comparison of variances of silicone oil amount obtained from each shaking parameter

Difference of Levels		Difference of Means	SE of Difference	95% CI	T-Value	P-Value
160 rpm 90 min	Control	55	2792	(-5770, 5879)	0.02	0.985
160 rpm 120 min	Control	4128	2792	(-1697, 9952)	1.48	0.155
160 rpm 150 min	Control	16609	2792	(10784, 22433)	5.95	0.000
180 rpm 90 min	Control	1937	2792	(-3887, 7762)	0.69	0.496
180 rpm 120 min	Control	31871	2792	(26047, 37696)	11.41	0.000
180 rpm 150 min	Control	85272	2792	(79448, 91096)	30.54	0.000
200 rpm 90 min	Control	2924	2792	(-2900, 8748)	1.05	0.307
200 rpm 120 min	Control	39461	2792	(33637, 45285)	14.13	0.000
200 rpm 150 min	Control	104214	2792	(98390, 110038)	37.32	0.000

Difference of Levels		Difference of Means	SE of Difference	95% CI	T-Value	P-Value
160 rpm 120 min	160 rpm 90 min	4073	2792	(-1751, 9897)	1.46	0.160
160 rpm 150 min	160 rpm 90 min	16554	2792	(10730, 22378)	5.93	0.000
180 rpm 90 min	160 rpm 90 min	1883	2792	(-3941, 7707)	0.67	0.508
180 rpm 120 min	160 rpm 90 min	31817	2792	(25993, 37641)	11.40	0.000
180 rpm 150 min	160 rpm 90 min	85217	2792	(79393, 91042)	30.52	0.000
200 rpm 90 min	160 rpm 90 min	2870	2792	(-2955, 8694)	1.03	0.316
200 rpm 120 min	160 rpm 90 min	39406	2792	(33582, 45231)	14.11	0.000
200 rpm 150 min	160 rpm 90 min	104160	2792	(98335, 109984)	37.30	0.000
160 rpm 150 min	160 rpm 120 min	12481	2792	(6657, 18305)	4.47	0.000
180 rpm 90 min	160 rpm 120 min	-2190	2792	(-8014, 3634)	-0.78	0.442
180 rpm 120 min	160 rpm 120 min	27744	2792	(21920, 33568)	9.94	0.000
180 rpm 150 min	160 rpm 120 min	81144	2792	(75320, 86969)	29.06	0.000
200 rpm 90 min	160 rpm 120 min	-1203	2792	(-7028, 4621)	-0.43	0.671
200 rpm 120 min	160 rpm 120 min	35333	2792	(29509, 41158)	12.65	0.000
200 rpm 150 min	160 rpm 120 min	100086	2792	(94262, 105911)	35.85	0.000
180 rpm 90 min	160 rpm 150 min	-14671	2792	(-20495, -8847)	-5.25	0.000
180 rpm 120 min	160 rpm 150 min	15263	2792	(9439, 21087)	5.47	0.000
180 rpm 150 min	160 rpm 150 min	68663	2792	(62839, 74488)	24.59	0.000
200 rpm 90 min	160 rpm 150 min	-13684	2792	(-19509, -7860)	-4.90	0.000
200 rpm 120 min	160 rpm 150 min	22852	2792	(17028, 28677)	8.18	0.000
200 rpm 150 min	160 rpm 150 min	87605	2792	(81781, 93430)	31.38	0.000
180 rpm 120 min	180 rpm 90 min	29934	2792	(24110, 35758)	10.72	0.000
180 rpm 150 min	180 rpm 90 min	83335	2792	(77510, 89159)	29.85	0.000
200 rpm 90 min	180 rpm 90 min	987	2792	(-4838, 6811)	0.35	0.727
200 rpm 120 min	180 rpm 90 min	37524	2792	(31699, 43348)	13.44	0.000
200 rpm 150 min	180 rpm 90 min	102277	2792	(96452, 108101)	36.63	0.000
180 rpm 150 min	180 rpm 120 min	53400	2792	(47576, 59225)	19.13	0.000
200 rpm 90 min	180 rpm 120 min	-28947	2792	(-34772, -23123)	-10.37	0.000
200 rpm 120 min	180 rpm 120 min	7589	2792	(1765, 13414)	2.72	0.013
200 rpm 150 min	180 rpm 120 min	72343	2792	(66518, 78167)	25.91	0.000
200 rpm 90 min	180 rpm 150 min	-82348	2792	(-88172, -76524)	-29.49	0.000
200 rpm 120 min	180 rpm 150 min	-45811	2792	(-51635, -39987)	-16.41	0.000
200 rpm 150 min	180 rpm 150 min	18942	2792	(13118, 24766)	6.78	0.000
200 rpm 120 min	200 rpm 90 min	36537	2792	(30713, 42361)	13.09	0.000
200 rpm 150 min	200 rpm 90 min	101290	2792	(95466, 107114)	36.28	0.000
200 rpm 150 min	200 rpm 120 min	64753	2792	(58929, 70577)	23.19	0.000

Table 20 Particle count from each shaking speed and time

Particle size (µm)	Control	Shaking time								
		90 minutes			120 minutes			150 minutes		
		Shaking speed			Shaking speed			Shaking speed		
		160 rpm	180 rpm	200 rpm	160 rpm	180 rpm	200 rpm	160 rpm	180 rpm	200 rpm
0-2	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
>2-4	1.67±2.89	1.67±2.89	6.66±2.88	0.00±0.00	1.67±2.89	13.32±2.89	18.32±23.61	3.33±5.77	23.32±2.88	49.96±13.22
>4-6	33.31±20.80	31.64±10.40	59.95±21.78	63.29±10.40	71.61±20.80	104.92±78.52	191.52±128.39	61.62±36.14	206.51±75.00	466.32±180.31
>6-8	11.66±5.77	11.66±2.89	9.99±10.00	18.32±10.40	18.32±14.42	36.64±25.64	74.95±30.39	29.98±5.00	53.29±20.19	166.55±113.30
>8-10	0.00±0.00	3.33±2.89	3.33±2.89	5.00±5.00	5.00±5.00	19.99±19.99	13.32±2.89	9.99±8.65	14.99±8.65	66.62±37.83
>10-12	1.67±2.89	0.00±0.00	1.67±2.89	0.00±0.00	0.00±0.00	8.33±10.40	5.00±5.00	5.00±5.00	9.99±8.65	14.99±21.78
>12-14	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.67±2.89	1.67±2.89	1.67±2.89	6.66±5.77	5.00±5.00
>14-16	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.67±2.89	0.00±0.00	0.00±0.00	8.33±7.63
>16-18	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.67±2.89	1.67±2.89	1.67±2.89	0.00±0.00
>18-20	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.67±2.89	0.00±0.00	6.66±2.88	1.67±2.89
>20-22	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	5.00±0.00	0.00±0.00
>22-24	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.67±2.89	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
>24-26	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Table 21 Data of silicone oil amount released from varies filling volume and head space (μm^3)

Sample description	Sample no.			Average	SD	%RSD
	Sample 1	Sample 2	Sample 3			
Control	4,033.15	4,438.77	4,719.19	4,397.04	344.92	7.84
0.4 mL, 0 cm^3	10,330.42	11,841.17	11,303.23	11,158.27	765.73	6.86
0.6 mL, 0 cm^3	13,788.93	13,244.12	14,986.12	14,006.39	891.13	6.36
1.0 mL, 0 cm^3	15,716.83	15,092.49	16,204.24	15,671.19	557.28	3.56
0.4 mL, 0.16 cm^3	69,229.07	70,556.89	68,497.69	69,427.88	1,043.90	1.50
0.6 mL, 0.16 cm^3	36,794.85	34,114.46	37,896.17	36,268.49	1,945.02	5.36
1.0 mL, 0.16 cm^3	27,036.16	24,323.66	22,742.68	24,700.83	2,171.45	8.79
0.4 mL, 0.32 cm^3	287,081.76	248,004.39	274,361.29	269,815.81	19,931.29	7.39
0.6 mL, 0.32 cm^3	104,306.02	116,690.20	104,943.11	108,646.44	6,973.38	6.42
1.0 mL, 0.32 cm^3	84,020.12	86,459.77	81,234.67	83,904.85	2,614.46	3.12

Table 22 Test of homogeneity of variances of silicone oil amount obtained from varies filling volume and head space

	df	Sum of Squares	Mean Square	F	Sig
Between Groups	9	174816000000	19423991392	418.51	0.000
Within Groups	20	928235194	46411760		
Total	29	175744000000			

Table 23 Multiple comparison of variances of silicone oil amount obtained from varies filling volume and head space

Difference of Levels		Difference of Means	SE of Difference	95% CI	T-Value	P-Value
0.4 mL, 0 cm^3	Control	6761	5562	(-4842, 18364)	1.22	0.238
0.6 mL, 0 cm^3	Control	9609	5562	(-1994, 21212)	1.73	0.099
1.0 mL, 0 cm^3	Control	11274	5562	(-329, 22877)	2.03	0.056
0.4 mL, 0.16 cm^3	Control	65031	5562	(53428, 76634)	11.69	0.000
0.6 mL, 0.16 cm^3	Control	31871	5562	(20268, 43475)	5.73	0.000
1.0 mL, 0.16 cm^3	Control	20304	5562	(8701, 31907)	3.65	0.002
0.4 mL, 0.32 cm^3	Control	265419	5562	(253816, 277022)	47.72	0.000
0.6 mL, 0.32 cm^3	Control	104249	5562	(92646, 115853)	18.74	0.000
1.0 mL, 0.32 cm^3	Control	79508	5562	(67905, 91111)	14.29	0.000

Difference of Levels		Difference of Means	SE of Difference	95% CI	T-Value	P-Value
0.6 mL, 0 cm^3	0.4 mL, 0 cm^3	2848	5562	(-8755, 14451)	0.51	0.614
1.0 mL, 0 cm^3	0.4 mL, 0 cm^3	4513	5562	(-7090, 16116)	0.81	0.427
0.4 mL, 0.16 cm^3	0.4 mL, 0 cm^3	58270	5562	(46666, 69873)	10.48	0.000
0.6 mL, 0.16 cm^3	0.4 mL, 0 cm^3	25110	5562	(13507, 36713)	4.51	0.000
1.0 mL, 0.16 cm^3	0.4 mL, 0 cm^3	13543	5562	(1939, 25146)	2.43	0.024
0.4 mL, 0.32 cm^3	0.4 mL, 0 cm^3	258658	5562	(247054, 270261)	46.50	0.000
0.6 mL, 0.32 cm^3	0.4 mL, 0 cm^3	97488	5562	(85885, 109091)	17.53	0.000
1.0 mL, 0.32 cm^3	0.4 mL, 0 cm^3	72747	5562	(61143, 84350)	13.08	0.000
1.0 mL, 0 cm^3	0.6 mL, 0 cm^3	1665	5562	(-9938, 13268)	0.30	0.768
0.4 mL, 0.16 cm^3	0.6 mL, 0 cm^3	55421	5562	(43818, 67025)	9.96	0.000
0.6 mL, 0.16 cm^3	0.6 mL, 0 cm^3	22262	5562	(10659, 33865)	4.00	0.001
1.0 mL, 0.16 cm^3	0.6 mL, 0 cm^3	10694	5562	(-909, 22298)	1.92	0.069
0.4 mL, 0.32 cm^3	0.6 mL, 0 cm^3	255809	5562	(244206, 267413)	45.99	0.000
0.6 mL, 0.32 cm^3	0.6 mL, 0 cm^3	94640	5562	(83037, 106243)	17.01	0.000
1.0 mL, 0.32 cm^3	0.6 mL, 0 cm^3	69898	5562	(58295, 81502)	12.57	0.000
0.4 mL, 0.16 cm^3	1.0 mL, 0 cm^3	53757	5562	(42154, 65360)	9.66	0.000
0.6 mL, 0.16 cm^3	1.0 mL, 0 cm^3	20597	5562	(8994, 32200)	3.70	0.001
1.0 mL, 0.16 cm^3	1.0 mL, 0 cm^3	9030	5562	(-2573, 20633)	1.62	0.120
0.4 mL, 0.32 cm^3	1.0 mL, 0 cm^3	254145	5562	(242541, 265748)	45.69	0.000
0.6 mL, 0.32 cm^3	1.0 mL, 0 cm^3	92975	5562	(81372, 104578)	16.71	0.000
1.0 mL, 0.32 cm^3	1.0 mL, 0 cm^3	68234	5562	(56631, 79837)	12.27	0.000
0.6 mL, 0.16 cm^3	0.4 mL, 0.16 cm^3	-33159	5562	(-44763, -21556)	-5.96	0.000
1.0 mL, 0.16 cm^3	0.4 mL, 0.16 cm^3	-44727	5562	(-56330, -33124)	-8.04	0.000
0.4 mL, 0.32 cm^3	0.4 mL, 0.16 cm^3	200388	5562	(188785, 211991)	36.02	0.000
0.6 mL, 0.32 cm^3	0.4 mL, 0.16 cm^3	39219	5562	(27615, 50822)	7.05	0.000
1.0 mL, 0.32 cm^3	0.4 mL, 0.16 cm^3	14477	5562	(2874, 26080)	2.60	0.017
1.0 mL, 0.16 cm^3	0.6 mL, 0.16 cm^3	-11568	5562	(-23171, 35)	-2.08	0.051
0.4 mL, 0.32 cm^3	0.6 mL, 0.16 cm^3	233547	5562	(221944, 245150)	41.99	0.000
0.6 mL, 0.32 cm^3	0.6 mL, 0.16 cm^3	72378	5562	(60775, 83981)	13.01	0.000
1.0 mL, 0.32 cm^3	0.6 mL, 0.16 cm^3	47636	5562	(36033, 59239)	8.56	0.000
0.4 mL, 0.32 cm^3	1.0 mL, 0.16 cm^3	245115	5562	(233512, 256718)	44.07	0.000
0.6 mL, 0.32 cm^3	1.0 mL, 0.16 cm^3	83946	5562	(72342, 95549)	15.09	0.000
1.0 mL, 0.32 cm^3	1.0 mL, 0.16 cm^3	59204	5562	(47601, 70807)	10.64	0.000
0.6 mL, 0.32 cm^3	0.4 mL, 0.32 cm^3	-161169	5562	(-172772, -149566)	-28.97	0.000
1.0 mL, 0.32 cm^3	0.4 mL, 0.32 cm^3	-185911	5562	(-197514, -174308)	-33.42	0.000
1.0 mL, 0.32 cm^3	0.6 mL, 0.32 cm^3	-24742	5562	(-36345, -13138)	-4.45	0.000

Table 24 Silicone oil particle count released from various filling volume and head space

Particle size (μm)	Control	Head space								
		0.00 cm^3			0.16 cm^3			0.32 cm^3		
		Filling volume			Filling volume			Filling volume		
		0.4 mL	0.6 mL	1.0 mL	0.4 mL	0.6 mL	1.0 mL	0.4 mL	0.6 mL	1.0 mL
0-2	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.67±1.15	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
>2-4	1.67±2.98	3.33±2.89	10.00±8.65	3.33±2.89	16.65±16.06	13.32±2.89	8.33±10.40	168.21±109.95	48.30±36.83	51.63±41.90
>4-6	33.31±20.80	59.96±18.01	76.61±24.64	69.95±18.01	306.44±98.20	104.92±78.52	121.58±101.21	1642.11±840.96	517.95±333.38	428.02±230.82
>6-8	11.66±5.77	21.65±16.06	41.64±2.89	34.97±10.00	159.88±18.01	36.64±25.64	71.61±16.06	526.28±135.02	228. 17±53.89	154.89±34.98
>8-10	0.00±0.00	8.33±5.77	9.99±5.00	18.32±5.77	69.95±17.31	19.99±19.99	14.99±8.65	159.88±26.44	129.91±66.10	69.95±45.79
>10-12	1.67±2.89	0.00±0.00	0.00±0.00	0.00±0.00	5.00±8.65	8.33±10.40	0.00±0.00	46.63±28.41	0.00±0.00	14.99±14.99
>12-14	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.67±2.89	1.67±2.89	13.32±10.40	0.00±0.00	3.33±2.89
>14-16	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.67±2.89	0.00±0.00	0.00±0.00
>16-18	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
>18-20	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.67±2.89	0.00±0.00	0.00±0.00
>20-22	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
>22-24	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.67±2.89	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
>24-26	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

VITA

NAME	Paksupang Tantisereerut
DATE OF BIRTH	15 Jun 1989
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