Glass Siliconization Process Characterization for Insulin Delivery Device Performance

by

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B.S. Mechanical Engineering
University of Wisconsin-Madison, 2009

Submitted to the MIT Sloan School of Management and the Department of Mechanical Engineering in Partial Fulfillment of the Requirements for the Degrees of

Master of Business Administration and
Master of Science in Mechanical Engineering

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Abstract 
Baked-in siliconization of glass cylinders, primary insulin containers, is a manufacturing process that is critical to the performance of drug delivery devices. Delivery devices are evolving and placing additional burden on production processes. Variability in siliconization and baking processes affects the resulting friction characteristics that are related to device performance criteria such as injection time and velocity, which are critical to the patient experience. The principal objective is to better characterize the performance of siliconized and baked glass, enabling improvement of device injection performance. 

A controlled study was conducted in order to strip away extraneous variables and enhance experimental control. State-of-the-art nanoscale measurement techniques and tribological (friction) equipment were employed to test the physical characteristics of silicone-coated glass. Data collected was statistically analyzed to determine relative significance of primary factors as well as variable interactions, with respect to friction of the rubber versus coated glass system. 

Lack of silicone or “dry spots” were found to be a key concern for siliconized glass. Siliconization amount was empirically modeled and found to have an exponential relationship with the coefficient of friction. High velocities exacerbated issues arising from lack of silicone. Based on the test results, a clearer definition of proper baked-in glass siliconization has emerged. Recommendations included minimum siliconization amount and an awareness of significant variable effects and interactions on system friction. Groundwork has been laid for further work including process optimization in the pursuit of improving insulin delivery device injection performance. 

The opinions expressed herein are solely those of the author and do not necessarily reflect those of Sanofi. 

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1 Introduction

1.1 Background

1.1.1 Diabetes Epidemic and Insulin
Diabetes is an epidemic affecting approximately 9% of the world’s adult population according to the World Health Organization (WHO) resulting in 1.5 million deaths (2012 statistic) directly attributable to the disease\. The WHO further predicts that total deaths from diabetes will rise by more than 50% in the next 10 years, partly traceable to rapid increases in obesity and a lack of physical activity. The two major types of the disease are type 1 and type 2. Type 1-diabetes cannot be prevented and is associated with insufficient insulin production. Type 2 diabetes, which constitutes 90% of all cases, occurs when the body cannot effectively use insulin produced. Evidence suggests that type 2-diabetes can be prevented or delayed through maintenance of a healthy diet and exercise\. Although a healthy diet, exercise, and glucose monitoring remain important parts of the typical treatment plan for both types of diabetes, people suffering from the disease often require regular injection of insulin\.

Since the 1920’s, pharmaceutical companies have responded to the epidemic by developing and commercially producing insulin with different characteristics such as slower or faster acting profiles, or of different concentrations\. These drug developments have allowed insulin to become more suitable for individual patient needs. In parallel to improvement of the insulin itself, companies including Sanofi have engineered novel technologies and devices to improve insulin delivery.

1.1.2 Evolution of Insulin Delivery Devices
Over time insulin delivery methods and technologies have evolved to improve safety, efficacy, and convenience. Figure 1 shows an early version of insulin in a vial and its basic syringe for delivery from around 1925\. Even today, the basic vial and syringe combination remains the most widely used method of insulin delivery given its low unit cost and flexibility for patients in mixing formulations\. 

![Figure 1 – Insulin Novo and Syringe from 1925](image)
In 1949, through collaboration with the American Diabetes Association, Becton and Dickinson developed standard insulin syringes to reduce dosing errors that can result in hyperglycemia or hypoglycemia\(^4\). The standard insulin syringes are shown in Figure 2.

![Figure 2 - Standard Insulin Syringes from 1949](image)

In 1963, Dr. Arnold Kadish of Los Angeles, California developed a prototype of a portable insulin pump that could be carried in a backpack\(^7\). This concept, shown in Figure 3, was a further effort to improve insulin delivery.

![Figure 3 - Dr. Arnold Kadish's Portable Insulin Pump Prototype from 1963](image)

Then in 1976, smaller wearable infusion pumps were developed capable of administering insulin with improved convenience as an alternative to syringe injection. According to the American Diabetes Association, the Minimed 502 was the first commercially successful insulin pump and was introduced in 1983. Figure 4 is a picture of the Minimed 502 device\(^8\).
Durable insulin pens with replaceable cartridges were first introduced in 1985, followed by disposable prefilled syringes (pens) in 1989. These products are shown below in Figure 5 and Figure 6. Advantages of pens compared to the basic syringe and vial combination (per Figure 1) include “greater accuracy, convenience, patient preference, and adherence”. Furthermore, despite higher per unit cost of insulin, overall cost of treatment tends to be lower with pens versus basic syringes and vials, largely due to improved adherence. Adherence refers to the patient sticking to the caregiver-prescribed therapy.
Patients today can also consider newly engineered technologies including needle-free jet injectors, infusers, patches, and even an artificial pancreas. In the next couple of years, oral insulin may provide yet another alternative. In 2015, Sanofi and MannKind released a device for inhalation of insulin powder, which is shown below in Figure 7.

Although new technologies are becoming available to cater to different user needs and preferences, most patients with diabetes requiring insulin generally choose vials and syringes, pens, or infusion pumps. Different markets see different rates and trends of delivery system use. As of 2010, pumps were more widely used in the United States as compared to Europe. Further, although a majority of Europeans used pens as an alternative to basic syringes, only 15% of diabetes patients in America opted for pens. However, between 2005 and 2011, the incidence of patients with diabetes initiating vials and syringes within the United States fell from 90.5% to 31.3% while initiations of pens increased from 9.5% to 68.7%.

Insulin pens and syringe products today have a variety of styles and features, which have evolved since their introduction in the 1980s. There are single-use and reusable pens, which have a durable body and replaceable insulin cartridge, as well as single-dose and multi-dose pens. Some pens require manual injection, and others are mechanism-assisted (such as spring-driven). Certain products have a fixed needle and others are compatible with various needles for injection. An additional characteristic is interruptible versus uninterruptable dosing. Below in Figure 8 is a rendering of the SoloSTAR by Sanofi, of which one billion were produced in Frankfurt, Germany between 2007-2013.
Some newer products aim to integrate electronics and software to introduce a feedback loop with the healthcare provider to improve treatment efficacy by encouraging adherence, which is a big issue faced by the pharmaceutical industry and is strongly correlated with the total cost of care. Sanofi Executive Vice President Pascale Witz stated that half of diabetics stop taking insulin within their first year of treatment. Sanofi was the first large pharmaceutical company to integrate glucose monitoring with the Apple iPhone to enable information sharing with patients' families and doctors.

Devices for the treatment of diabetes will continue to evolve for the foreseeable future because the market for such products is so large and growing. The global market for injectables delivery for diabetes in 2014 was estimated to be nearly $28 Billion USD with the total diabetes drug delivery market growing at a compounded annual growth rate (CAGR) of 12.5%. The threat of patent expiry is also pushing companies to release more and more drug products. Pharmaceutical companies are expected to launch 400 new drug products between 2014 and 2017, a 146% increase since 2005. In the fight for market share and differentiation in a historically profitable sector, companies will continue to allocate resources to product development as well as the optimization of related manufacturing processes. To be able to produce the drug delivery devices of the future with the highest quality, thorough understanding of the manufacturing processes is needed. One such production process that continues to garner interest is baked-in glass siliconization.

1.1.3 Glass Siliconization: Old Process, New Challenges
Baked-in glass siliconization is a critical process in the production of glass cartridges, primary containers for insulin that are integrated into delivery devices. For decades, glassware has been coated with silicone oil or emulsion, approved material for medical devices and pharmaceutical containers. The inner barrel of cylindrical glass cartridges is sprayed with silicone emulsion, or “siliconized”, and baked for durability and depyrogenation (See 2.1.3) prior to filling of the drug products.

Baking of silicone emulsion results in an ultrathin coating, sometimes under 50 nanometers thick. After siliconization and baking, as part of the insulin filling operation, cartridges are assembled with a rubber stopper and cap, before being integrated into an injection device. Below in Figure 9 is a picture of glass cartridges, which are mass-filled with insulin by a number of pharmaceutical companies.

Figure 9 – 1.5mL Glass Cartridges, Primary Containers for Insulin
Siliconization of the glass cartridge serves several purposes. Friction is reduced between the inner glass surface and an elastomeric stopper, enabling the stopper to glide through the cartridge during insulin injection. Container evacuation and drainability characteristics are thus improved. Additionally, the siliconization yields a barrier film between the drug product and glass surface, which is important for certain formulations in preventing protein adsorption, critical for formulation efficacy. Improper siliconization can lead to problems when the cartridge is integrated into an injection device like an insulin pen. Common types of problems include “slip-stick” behavior of the rubber stopper during injection resulting in variability of critical injection device metrics like injection time and dose accuracy. Additionally, undersiliconization or process variability could affect the drug formulation efficacy if components are able to adsorb to unsiliconized glass surfaces. Oversiliconization may create new problems such as undesirable protein-silicone interactions. Silicone-free lubrication alternatives have been developed as a potential solution. However, higher unit costs are associated with silicone-free systems. Extensive studies are also needed to approve materials in pharmaceutical applications.

A well-processed cartridge has an inner barrel surface with a minimal coefficient of friction and performs consistently with respect to injection time and velocity when used in an injection device. Literature, which tends to focus on prefilled syringes that are siliconized with oil, commonly suggests that the silicone layer should be homogenous and minimal in thickness in order to optimize device performance. Existing testing reveals these trends but to date, there does not exist a complete definition of optimal baked-in siliconization, such as optimal silicone surface height, minimal coverage, or maximum unsiliconized area.

Next generation insulin delivery devices like pens will require greater understanding of the baked-in siliconization process for glass cartridges. Existing manual insulin pens and syringes can function properly without extremely tight control over the siliconization processes. This is because a manually driven pen inherently has closed-loop feedback and control. If a user injects insulin through an undersiliconized cartridge, they may have to apply more force due to higher friction and resistance, but this additional effort may not even be perceived. A schematic of manual injection is shown below in Figure 10.

![Figure 10 - Injection with Manual Insulin Pen](image-url)
However, for more convenient, next-generation mechanism-assisted pens without feedback and control, variability in the lubrication process can lead to undesirable injection time or improper dosing. Thus, efforts remain to understand and optimize glass siliconization in parallel to device development.

1.1.4 Sanofi Organizational Structure
This project was done in collaboration with various groups within Sanofi in Frankfurt, Germany. In order to develop a basic understanding of the key stakeholders, the following is a brief overview of Sanofi’s principal organizations concerned with insulin injection devices and primary containers, as well as glass siliconization.

Medical Devices (MED)
Sanofi Medical Devices (MED) is Sanofi’s group responsible for the design of, among other products, insulin delivery devices. Functions include project management, engineering, quality, usability, and laboratory product testing for research and development purposes. MED and its partners have the opportunity to translate patient needs into product requirements and innovative designs.

Site Frankfurt Insulin (SFI)
Site Frankfurt Insulin (SFI) is a manufacturing organization with responsibilities including production of primary containers for insulin. SFI has ownership of glass cartridge siliconization and baking, cartridge assembly (rubber stopper and cap), and insulin filling. Additionally, SFI conducts some bulk siliconization and sterilization of rubber stoppers. SFI utilizes high speed and high volume automation to compound, fill, and visually inspect primary containers of insulin. Quality is ensured through design, supplier engagement, in-process inspection, and routine testing.

Site Frankfurt Devices (SFD)
Site Frankfurt Devices receives finished primary containers of insulin (glass cartridges) from SFI and integrates them into pen devices. SFD utilizes high speed and high volume automation to handle the assembly, labeling, and packaging. The highest level of quality is ensured through in-process inspection.

1.2 Project Motivation and Business Case
In addition to producing safe and consistently performing products, investing to characterize and optimize glass siliconization processes makes good business sense. Profitability can be improved both by increasing revenue and decreasing costs. Additionally, the investment can also be viewed as a longer-term strategic opportunity to develop technological capability and build the company brand. Patients will opt for products that deliver consistent injection performance.

Increase Revenue
Optimizing siliconization processes can improve current and future revenue streams for insulin injection devices containing siliconized glass cartridges. More consistent insulin delivery device performance could be a differentiating characteristic and lead to higher market share. Even small increases in share of a growing $28 Billion market would be significant. Improved and consistent siliconization can also enable the development of future products with improved features or performance (such as lower injection force). With an estimated 400 million diabetics in the world, and potentially 176 million yet undiagnosed, the “profit potential for drug makers is enormous.”

**Decrease Cost**

Process improvement can also lead to cost reduction in a number of areas. First, manufacturing defects that can lead to scrap will be reduced. Reinvestment in capital equipment, which can cost millions of dollars, can be avoided or delayed by using existing equipment longer, even for cartridges used in newer insulin pen products. Finally, improved siliconization processes can mitigate the risk of adverse patient events and product recalls, which can also cost the company millions of dollars to resolve.

**Strategic Opportunity**

In addition to improving profitability, devoting resources to improving siliconization processes can lead to a longer-term competitive advantage. By better understanding the manufacturing processes, companies can advantageously leverage this knowhow to design and develop superior devices. In turn, becoming a leader in both device design and production can improve company image and brand in a move to defend and grow market share.

### 1.3 Project Goals

The principal objective of this work supported by Sanofi was to improve the understanding of baked-in glass cartridge siliconization with respect to insulin injection device performance. Heightened understanding enables development of improved process specifications, process optimization, and ultimately more consistent device performance. The following questions guided the project:

1. What is the state of the art in terms of baked-in glass cartridge siliconization and relevant inspection technology?
2. What should be the definition of acceptable baked-in siliconized glass with respect to insulin injection device performance? (Height, evenness, adhesion, friction coefficient...?)
3. Both in the lab and production how should siliconized and baked glass cartridges be inspected?
4. How can baked-in glass cartridge siliconization processes be characterized and improved to achieve the desired output?

### 1.4 Project Scope
Siliconization of glass is a large topic area and with limited time and resources, not all aspects were within the scope or focus of the project. The siliconized containers of interest were 1.5ml and 3.0ml glass cartridges used for the primary packaging of insulin. Prefilled syringes siliconized with silicone oil were not directly investigated. There are also many different types of rubber stoppers and stopper siliconization processes that play a role in system performance, and not all were tested. The materials section of the paper (Section 4) describes the specific components used for testing. As described in the previous section, the principal objective was to improve the understanding of the performance of baked-in and siliconized glass. Since Sanofi currently employs several siliconization techniques, it was not feasible to characterize all inputs and variants.

1.5 Project Approach

It is difficult to improve what is not understood and measurable. Thus, the first step in the project was to define the current state and conduct a literature review. As part of current state definition, meetings were held with key stakeholders from Sanofi’s manufacturing and engineering organizations. Documents obtained enabled process mapping and understanding of the flow of information and materials relevant to baked-in glass siliconization. Testing and inspection methods currently utilized were compared against possibilities suggested in literature and by suppliers of promising new technology. An industry conference was attended to learn about newest developments in the field.

In parallel to the current state and literature review, system physics and theoretical models along with their assumptions and limitations were considered. Theory was compared against a bulk of existing test data generated internally by Sanofi and externally by its partners. Physical theory and existing test data provided the basis for identification of design factors for further experimentation. Once hypotheses were developed, experiments were planned, followed by execution, analysis, and iteration. Results supported data-driven recommendations for process improvement and potentially further work.

1.6 Acronyms

The following table covers acronyms used in the paper.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
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<tbody>
<tr>
<td>3D-LSM</td>
<td>3D Laser Scanning Microscope</td>
</tr>
<tr>
<td>AFM</td>
<td>Atomic Force Microscope</td>
</tr>
<tr>
<td>CAD</td>
<td>Computer-Aided Design</td>
</tr>
<tr>
<td>DOE</td>
<td>Design of Experiments</td>
</tr>
<tr>
<td>MED</td>
<td>Sanofi “Medical Devices” Engineering Group</td>
</tr>
<tr>
<td>PDMS</td>
<td>poly-dimethylsiloxane</td>
</tr>
<tr>
<td>PFS</td>
<td>Prefilled Syringes</td>
</tr>
<tr>
<td>RTS</td>
<td>Ready to Sterilize; refers to rubber stoppers</td>
</tr>
<tr>
<td>SFD</td>
<td>Sanofi “Site Frankfurt Devices” Manufacturing Group</td>
</tr>
<tr>
<td>SFI</td>
<td>Sanofi “Site Frankfurt Insulin” Manufacturing Group</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WFI</td>
<td>Water for Injection</td>
</tr>
<tr>
<td>XPS</td>
<td>X-Ray Photoelectron Spectroscopy, also known as</td>
</tr>
</tbody>
</table>
2 Review of Current State, Physics, and Metrology

2.1 Glass Cartridge Siliconization Processes

2.1.1 Upstream Processes
Empty glass cartridges are received from third party suppliers in various volumes such as 1.5mL and 3.0mL versions. Cartridges are washed and dried several times prior to siliconization. Rubber stoppers of appropriate geometry and material are procured in two different conditions. Ready-to-Sterilize (RTS) stoppers are formed and siliconized by a third party supplier. Sanofi then steam-sterilizes the RTS stoppers prior to assembly in the glass cartridges. Other non-RTS stoppers are received, washed, siliconized, and sterilized. Dow Corning silicone emulsion is received in a condensed state and must be appropriately diluted with Water for Injection (WFI) prior to glass cartridge siliconization.

2.1.2 Glass Siliconization
The inner barrels of glass cartridges are coated with silicone emulsion using one of several siliconization techniques provided by companies like Bosch and Bausch + Ströbel. Below in Figure 11 is a picture of an automated siliconization and washing machine for glass cartridges.

![Figure 11 - Example of Siliconization Machine from Bausch + Ströbel](image)

The oldest technique utilized since around 1990, referred to as "Flushing", applies silicone emulsion inside the cartridge barrel. Compressed air blown through the cartridge removes extra emulsion and is critical in controlling the amount of silicone emulsion remaining on the glass surface. Despite having fewer controls than newer systems, the Flushing process is considered established and stable.

The so-called "Time-Pressure" siliconization process variant, as the name suggests, uses principal controls of time and pressure to atomize silicone emulsion, spraying it into the glass cartridges. The Time-Pressure method is newer than Flushing and has been used since the early 1990s.
The newest siliconization technology, available since around 2007, uses individual micro-dosing pumps to accurately and precisely spray silicone emulsion into glass cartridges. Systems utilizing “diving nozzles” dynamically move through the cartridge while delivering atomized emulsion. Studies have demonstrated the benefits of more consistent silicone coverage using diving versus fixed nozzles. This system is the most complicated and thus requires the greatest effort for process development and improvement given the higher number of factors.

2.1.3 Down-Stream Processes
Following siliconization, regardless of technique, glass cartridges are transferred through automation into a baking tunnel. Cartridges move steadily through the tunnel on a conveyor belt for a minimum of 10 to 20 minutes to ensure sufficient time for depyrogenation. Depyrogenation refers to the removal of fever-causing substances such as bacterial endotoxins and exotoxins. The manufacturer of the silicone emulsion, Dow Corning, recommends baking temperatures under 250 degrees Celsius for less than two hours, though higher temperatures are often used for shorter baking times. In practice, downstream issues can cause delays, stopping the conveyor belt. In this situation, cartridges are allowed to sit in the baking tunnel for up to a maximum time that has been qualified through testing on a per-line basis, after which time they must be scrapped. At Sanofi, temperatures around 300 degrees Celsius are utilized.

Siliconized and baked glass cartridges move into a clean environment where they are assembled with a rubber stopper, filled with insulin, and capped. The metal cap is crimped around the neck of the cartridge to seal the system. Glass cartridges are bulk packaged and marked as a lot. Automated inspection equipment checks for defects such as cracks in the glass or foreign material present in the cartridge. After inspection, bulk-packaged cartridges are transferred from SFI to another facility at SFD where they are integrated into delivery devices.

2.1.4 Glass Siliconization Process Map
Figure 12 portrays a process map of baked-in glass siliconization, including upstream and downstream processes.
2.2 System Physics

2.2.1 Siliconized Syringe Model

In order to develop a plan to characterize a coated surface, it is critical to understand its role in the physical system. In his journal article, Rathore covered high-level theory and the depiction of a siliconized syringe in Figure 13 highlights the key components and factors with respect to friction\(^2\). The syringe model is also applicable to a siliconized glass cartridge assembled with a rubber stopper and installed in an insulin pen.

\[ \text{Force} \]

\[ \text{Plunger Rod} \]

\[ 2r_b \]

\[ \text{Needle} \]

\[ L_n \]

\[ 2r_n \]

\[ d \]

\[ l_{stopper} \]

\[ \text{Stopper} \]

\[ \text{Silicone Oil} \]

\[ \text{Glass} \]

\[ \text{Barrel} \]

Figure 13 - Physical Model of Siliconized Syringe
For a cartridge installed in a pen, the force depicted above would be generated by a mechanism such as a spring. Additionally, syringes are typically siliconized with oil as shown, while glass cartridges are sprayed with silicone emulsion and baked. The reference Table 2 provides a description of the variables used in Figure 13 by Rathore.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>$\bar{v}$</td>
<td>Injection speed</td>
</tr>
<tr>
<td>$r_b$</td>
<td>Radius of inner barrel of cartridge or syringe</td>
</tr>
<tr>
<td>$l_{stopper}$</td>
<td>Length of rubber stopper</td>
</tr>
<tr>
<td>$d$</td>
<td>Thickness of silicone lubrication</td>
</tr>
<tr>
<td>$r_n$</td>
<td>Inner radius of needle</td>
</tr>
</tbody>
</table>

**Force** Transverse force applied to rubber stopper

The force that is required to move the rubber stopper is opposed by friction and hydrodynamic force. The hydrodynamic force is the force to overcome the pressure drop across the needle and can be modeled using the Hagen-Poiseuille law given the appropriate assumptions. This is not repeated here, while the focus is on the friction element.

### 2.2.2 Basic Friction Considerations

Without silicone coating the inner barrel of the glass cartridge, the rubber stopper would be unable to glide through the glass cylinder under normal insulin injection conditions. Just as automotive windshield wipers function better when it is raining, the silicone provides lubrication and acts to reduce the coefficient of friction between the rubber and glass materials. As the silicone film reduces the coefficient of friction, it follows that lower forces are required to displace the rubber stopper inside the glass cylinder. The basic friction model in Equation 1 assumes that friction force $F_F$ is simply proportional to the coefficient of friction $\mu$ and the normal force $F_N$ pressing the materials together. Table 3 describes the model variables.

$$F_F = \mu * F_N$$  

### Table 3 - Basic Friction Model Parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_F$</td>
<td>Friction force</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Coefficient of friction</td>
</tr>
<tr>
<td>$F_N$</td>
<td>Normal force</td>
</tr>
</tbody>
</table>
The compression fit of the intentionally oversized stopper in the glass cylinder generates a normal force acting outwards radially. Minimum radial stopper compression is needed to avoid leakage of fluid past the stopper. As lateral force is applied to the stopper by a plunger or spring, insulin on the other side (assumed to be incompressible fluid) cannot rapidly flow through the thin-gauge needle, resulting in lateral stopper compression. Lateral compression of the rubber stopper results in transverse force related to the Poisson ratio of the rubber material, per the illustration below by Sebastian Hasenclaver\textsuperscript{25} in Figure 14. This effectively increases the radially acting normal force between the rubber stopper and glass cylinder.

![Figure 14 - Lateral Force $F_k$ Increases Normal Force $F_n$ Between Rubber Stopper and Glass Cylinder](image)

While the basic friction model is useful as a starting point, it has been demonstrated through classical experimentation that friction is complex and depends on additional variables. In fact, entire fields of study exist today to investigate friction such as contact mechanics and tribology. In 1902, Richard Stribeck presented his results on the dependence of friction on multiple factors for lubricated journal bearings. He developed what is now considered a fundamental friction curve, relating the friction coefficient to lubricant viscosity, relative sliding velocity, and normal load (as shown below: $\eta$, $V$, and $P$, respectively). Different friction "regimes" are described in the diagram\textsuperscript{26} in Figure 15.

After the dry friction condition, Stribeck described a boundary lubrication condition in which friction decreases and then remains stable with an increasing lubrication parameter. Then, the friction coefficient decreases through the mixed lubrication regime and reaches a minimum. After the minimum, there is a transition to the hydrodynamic lubrication regime, where the friction coefficient increases with an increasing lubrication parameter. The key takeaway from Stribeck is that the friction force is more complex than simply having a linear relationship with normal force.
Later, Arvanitaki demonstrated a significant reduction (roughly two orders of magnitude) in the friction coefficient $\mu$ in silicone-lubricated contacts compared to dry contacts between rubber and glass under various sliding velocity conditions, which is shown below in Figure 16\textsuperscript{27}. The contact was lubricated with silicone oil (110 mPa-sec viscosity). Figure 16 also shows the friction coefficient to have a dependence on velocity for a given lubrication condition. It is worth emphasizing that similar to Stribeck, Arvanitaki also showed the significance to friction of factors such as velocity, lubricant viscosity, and normal load.
Although Arvanitaki found contact area to be a function of normal load and velocity, basic theory assumes the area of contact plays no role in friction. However, cases of extremely small contact area or high surface roughness provide examples of how the area of contact can matter. In these cases, unless they break down, “hills and valleys” of the opposing surfaces can mesh, increasing the friction force. Excluding very smooth surfaces, only a small percentage of the opposing surfaces may be in contact. This is referred to as the “real” area of contact, as opposed to the “apparent” area of contact, and is illustrated in Figure 17. In the cartridge application, increasing the normal load and compressing the rubber stopper onto glass should increase the real contact area, as the rubber deforms into the interstitial valleys.

![Image: Illustration of Real Contact Area Between Materials](image)

Taking into account additional variables, Rathore suggested use of the model in Equation 2 for the friction force. The model variables are described in Table 4.

\[ F_F = \left( \frac{2\pi \mu_{oil} r_b l_{stopper}}{d_{oil}} \right) \bar{v} \]  

**Equation 2 - Updated Friction Model**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( F_F )</td>
<td>Friction force</td>
</tr>
<tr>
<td>( \mu_{oil} )</td>
<td>Viscosity of silicone lubrication</td>
</tr>
<tr>
<td>( r_b )</td>
<td>Radius of inner barrel of cartridge or syringe</td>
</tr>
<tr>
<td>( l_{stopper} )</td>
<td>Length of rubber stopper in contact</td>
</tr>
<tr>
<td>( d_{oil} )</td>
<td>Thickness of silicone lubrication</td>
</tr>
<tr>
<td>( \bar{v} )</td>
<td>Injection speed</td>
</tr>
</tbody>
</table>
While this model includes additional factors compared to the basic friction model in Equation 1 there are a few remarks. First, the model cannot be valid in large ranges of velocity, based on the Stribeck curve. Normal force is absent, which is expected to increase friction. The inversely proportional relationship between friction force and lubrication thickness is intuitive: very low silicone amounts would significantly increase friction. This is to be further investigated. There are additional coating characteristics not present in the model, such as variation in thickness or percent coated area.

Theoretical equations represent the silicone layer with a single thickness variable, whereas in practice the layer is a complex surface. Presumably a complete lack of silicone would lead to a dysfunctional system, and excessive silicone is also undesirable due to potential interactions with the drug product\textsuperscript{17}. In an extreme sense, excessive silicone could potentially increase the interference between opposing bodies, which would also be expected to eventually increase friction. In between these extreme cases, there lies an opportunity to better define the role of the silicone layer in the overall system.

2.2.3 Rubber Stopper Contact Pressure

When the oversized rubber stopper is assembled into the cylindrical cartridge following glass siliconization and baking processes, it is radially compressed. This compression results in contact pressure between the stopper and glass cylinder. This pressure can be calculated using first-order models for a shaft inserted into a hub. An equation adopted from Schmid is shown below in Equation 3\textsuperscript{29}. It takes into account the geometry and material properties of the components. The formula simplifies given the high modulus of glass, eliminating the \((1-\nu_{\text{Shaft}})/E_{\text{Shaft}}\) term. Intuitively, the pressure is proportional the interference between the components, represented by \(\delta_{\text{Radial}}\). The equation is also sensitive to assumptions of rubber stopper material properties of modulus and Poisson ratio.

\[
P = \frac{\delta_{\text{Radial}}}{r_{\text{Hub-inner}}} \left( \frac{r_H^2_{\text{Hub-Outer}} + r_H^2_{\text{Hub-inner}}}{E_{\text{Hub}}(r_H^2_{\text{Hub-Outer}} - r_H^2_{\text{Hub-inner}})} + \frac{1 - \nu_{\text{Shaft}}}{E_{\text{Shaft}}} \right).
\]

Equation 3 – Shaft in Hub Pressure

Table 5 summarizes sample values used to calculate the rubber stopper compression in the glass cartridge. Material properties were taken from material datasheets or assumed based on discussion of a similar topic in Marc Schader’s thesis\textsuperscript{30}. One addition to the formula was estimates of the surface roughness of the glass and rubber, which effectively adjusted the nominal interference. Surface roughness values were estimated based on data from Sanofi.

Table 5 – Rubber Stopper Compression Parameters

<table>
<thead>
<tr>
<th>“Hub” Parameters – 1.5 mL Glass Cartridge</th>
</tr>
</thead>
<tbody>
<tr>
<td>(E_{\text{Hub}})</td>
</tr>
<tr>
<td>(\nu_{\text{Hub}})</td>
</tr>
<tr>
<td>(R_{Z-Hub})</td>
</tr>
<tr>
<td>(D_{\text{Hub-inner}}(2\times r_{\text{Hub-inner}}))</td>
</tr>
</tbody>
</table>
Calculated pressure values were used as a starting point to translate normal forces expected in the glass cartridge to normal forces later specified in tribological testing. To complete the necessary translation, a simple ANSYS simulation was conducted with help from a colleague from Sanofi MED. The stopper, laid on its side, was compressed between two rigid parallel plates with a range of forces. This simulated what the stopper would experience when used as a test tip for the linear tribometer, a topic to be discussed in the coming sections. The resulting contact area and average pressure was calculated for each force. Thus, normal forces specified for testing with a linear tribometer would be roughly representative of the normal forces expected for rubber stopper assembled to glass cartridges, forces that are based on the designed interference fit and dimensional tolerances. Figure 18 illustrates the basic simulation.
2.3 Inspection Methods and Technology

2.3.1 Background
Metrology was a critical aspect of the project given the challenge of measuring a 100-nanometer, baked-on silicone coating residing on the inner surface of a transparent glass cylinder. An early effort of the project was to identify methods and technologies suitable to inspect the silicone coating and assessing its characteristics and performance. Each technique identified was found to have advantages and disadvantages. Some methods assessed a characteristic of the coating directly, while others measured an aspect of its performance indirectly within a greater system. Some otherwise capable technologies were found to be currently more suitable for the laboratory as opposed to a high-speed production environment.
Only in the past decade have standards for nanometrology started to emerge. In 2005, the International Organization for Standardization (ISO) Technical Committee 229 on Nanotechnology published a number of standards and guidance relevant for some of the techniques described in this section. The National Institute of Standards and Technology (NIST) also has resources directed at development of standards for nanoscale characterization and measurement.

Section 5 covers methods actually used for this project in greater detail. Additionally, Funke recently provided a comprehensive review of techniques suitable for baked-in siliconization\(^3\). Therefore, only a brief summary of methods is provided below. Test methods are arranged categorically though technically speaking, some methods apply to multiple categories.

### 2.3.2 Coverage Visualization

One approach is to enhance the ability to visualize the silicone coverage. Areas without silicone would be expected to have higher friction characteristics. Though subjective, coverage of the silicone on glass can be visualized through the use of glass powder or gold nanoparticles. Glass powder is shaken in a syringe or cartridge and selectively sticks to silicone oil to provide a visual indication of coverage. Figure 20 below from Chan shows syringes that were not properly siliconized in the lower (right) third and would likely have higher resulting friction in this area\(^{23}\).

![Figure 20 - Visualization of Silicone Coverage with Glass Powder](image)

Alternatively, similar visualization was demonstrated through the use of gold nanoparticles. These particles selectively stick to proteins that have adsorbed to unsiliconized glass. Figure 21 below from Eu provides an example of this more-involved approach\(^{32}\).
A third coverage visualization technique utilizes Schlieren optics, commercialized by ZebraSci\textsuperscript{33}. This technology was evaluated and discussed separately by Wen and Funke\textsuperscript{34,31}. It may be more suitable for prefilled syringes siliconized with silicone oil because droplets are required for detection. According to Funke, "thin baked-on silicone layers, however, do not form large silicone droplets upon contact with aqueous media, therefore they are hardly accessible by Schlieren visualization." That being said, ZebraSci does offer in-line inspection capabilities suitable for production processing speeds above 500 units per minute. Further developments may make this approach worth considering in the future.

### 2.3.3 Mass Determination
Funke also discussed a number of methods to determine the mass of silicone coating applied to the substrate. Achieving a certain mass of silicone will not alone guarantee proper performance, as illustrated by insufficient coverage in the previous section. However, it is another metric to consider that, along with other requirements, could be a valuable aspect of a specification. Apart from use of a basic mass balance, more advanced techniques include Atomic Absorption Spectrometry (AAS), Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), Reverse Phase Liquid Chromatography - Evaporative Light Scattering Detection (RPLC-ELSD), Calorimetry, and Solvent Extraction + Fourier Transform Infrared Spectroscopy\textsuperscript{31}.

### 2.3.4 Chemical Composition
A few chemical analysis techniques were evaluated and one was briefly tested during the project. These techniques allow for the identification of elements present in a material or on a surface. Solvent Extraction + FTIR mentioned in the previous section can provide this type of information. Additionally, both X-Ray Photoelectron Spectroscopy (XPS), also known as Electron Spectroscopy for Chemical Analysis (ESCA), and Time of Flight Secondary Ion Spectrometry (TOF-SIMS) were considered. Both techniques are highly sensitive and can be used for nanoscale measurements. According to Evans Analytical Group (EAG), both methods can be used in tandem with sputtering to perform depth profiling\textsuperscript{35}. For additional information on XPS, see Section 5.6.

### 2.3.5 Coating Topography – Non-Contact Methods
Coating topography is an additional category of methods that directly measures the coating to gain insights into surface height, variability, and roughness. Within this area, there are contact and non-contact types. For visualization of the ultrathin silicone coating, it is important to note the Abbe Diffraction limit for conventional optical systems that limits feature resolution to about 200 nanometers, about half the wavelength of visible light used for imaging. However, several techniques exist to image surfaces beyond the diffraction limit. A group of inherently non-contact optical methods for scanning surface topography includes reflectometry, interferometry, 3D Laser Scanning Microscopy (3D-LSM), Optical Coherence Tomography (OCT), Ellipsometry, and Raman Spectroscopy.

RapID of Berlin, Germany offers a purpose built machine called the Layer Explorer to measure film thickness in siliconized cartridges and syringes. RapID suggests that the reflectometry function reliably measures silicone layer height from 80 nanometers to 4 micrometers. For thinner baked-in silicone, the interferometry function is preferred for layer height measurement down to 15 nanometers with repeatability of approximately 25%. RapID’s machine is purpose built for testing cylindrical samples and is thus not suitable for flat, coated substrates, though reflectometry and interferometry are theoretically suitable for both geometries. See Section 5.4 for additional details on the method used for this project.

Keyence’s 3D-LSM is a versatile optical technology suitable to inspect baked-in silicone on glass, with display resolution for height measurement as good as 0.5 nanometer. While more suitable for flat substrates, Funke demonstrated its relevance for cylindrical geometries by breaking glass cartridges prior to measurement. For more on 3D-LSM, see Section 5.5.

Electron microscopes are another class of inspection equipment worthy of consideration given the needed scale of measurement, though currently these options fall in the laboratory end of the spectrum and are not suitable for production. Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and Scanning Tunneling Microscopy (STM) are a few examples. Given that they were not employed on the project, further discussion is not provided, though future work to characterize thin-film baked-on siliconization might consider such techniques.

2.3.6 Coating Topography – Contact Methods

Atomic Force Microscopy (AFM) is one of the workhorses of nanoscale metrology, providing “images with atomic or near-atomic-resolution surface topography.” Because a contact probe is typically used, this method may be destructive, though no special sample treatment is required to conduct testing. To test the absolute coating thickness, it would necessary to have a substrate reference point, achieved for example, by scratching away the coating to the surface.

Nanoindentation is method in which a force is applied through a test probe that presses into a film or coating in a controlled manner. The force and displacement data could be used to characterize a portion of the surface and thus an aspect of the topography.

2.3.7 Coating Performance
Siliconized glass cartridges are commonly tested at the system level, meaning once assembled with a rubber stopper. Cartridges may be routinely pulled from production and evaluated using a universal testing machine like an Instron or Zwick. A universal testing machine is used to apply a constant force, constant velocity, or even a simulated force to the rubber stopper resulting in its displacement through the siliconized glass cylinder. Below in Figure 22 is a picture of a Zwick universal testing machine and fixture for testing cartridges.

![Zwick testing machine](image)

Figure 22 - Zwick Universal Testing Machine

Depending on the specific method, a profile of injection velocity or force versus position is obtained. Figure 23 provides an example of force profiles from Rathore in which a constant velocity was applied to the stopper while the resulting force was measured and plotted against displacement. For a given profile, a "break-away" force spike is followed by a gliding force regime. It should be noted that this particular syringe was first emptied to eliminate the hydrodynamic force and measure only the friction between the stopper and siliconized glass syringe. Here, $F_F$ is represented by $f_{friction}$. Significant variation can be observed in the figure both within a given profile, and from profile to profile.

![Vendor 2](image)

Figure 23 - Typical Force Profiles for Syringe Tested at Constant Velocity
Although testing the glass cartridge + rubber stopper system with a universal testing machine appears simple, there are many factors at play influencing the outputs of friction and associated injection characteristics (time, velocity, etc.). This method is typically associated with high variability, and it is not just due to variability in the glass siliconization process. In addition to the siliconized glass surface, the rubber stopper is itself siliconized. Furthermore, the components have production dimensional tolerances resulting in variability in the interference fit (normal force) between the stopper and glass. Tolerances arise from production processes such as rubber stopper molding and glass cartridge forming. Variability in raw or processed material properties such as elastic modulus of the rubber stopper, and surface characteristics like roughness and energy, also complicate the situation. The impact is that it is challenging to precisely define the role of the siliconized glass surface when variability is observed in the output obtained from this approach, despite the fact that existing testing from a number of sources highlights that siliconization is a key factor. Even so, the question remains: how much of the noise seen in results such as those in Figure 23 resulted from variability in the glass siliconization process, as opposed to other sources? Did the cartridge tested with the highest gliding force have the lowest silicone layer thickness, highest variability in silicone coverage, or perhaps was it a result of the test equipment alignment? The point is that this chart illustrates variation, but not cause.

A tribometer, which can be used to test coatings and lubricants, measures friction and wear between materials in sliding contact. This type of machine was evaluated as an alternative to a universal testing machine to characterize baked-in siliconized glass surfaces. There are rotary and linear acting tribometers designed for different applications. Rotary applications include, for example, bearings in the automotive or aerospace industries. Typically test variables include normal load, velocity, test tip material, and number of cycles. Friction force is measured, and the friction coefficient can be calculated because the normal force is specified. Some tests for automotive applications run for thousands of cycle, though the applicability to siliconized glass would constitute far fewer cycles. By using a tribometer, it could be possible to take a more fundamental approach to focus in on the glass siliconization process, while stripping away or controlling the other potentially confounding variables. However, there are some tradeoffs that will be discussed in the next sections. Because this equipment was used in the project, Section 5.7 contains specific details about the method. A basic configuration of a linear tribometer from Nanovea is shown below in Figure 24.39

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Figure 24 – Basic Schematic of a Linear Tribometer
3 Design of Experiments

3.1 General Information
Design of experiments (DOE) is a systematic method to explore process or design parameters in order to screen for significant factors or optimize with respect to a defined output. According to Montgomery, “the effective use of sound statistical experimental design methodology can lead to products that are easier to manufacture, have higher reliability, and have enhanced field performance.”\textsuperscript{40} DOE, combined with empirical modeling, is a robust and systematic approach for process characterization that was leveraged to better understand the key factors for glass cartridge siliconization and baking processes. Successful implementation of the DOE and subsequent statistical analysis led to confirmation of key design factors and estimates of main and interaction effects of variables. Several testing iterations were conducted to more deeply explore certain parameters.

3.2 Sources of Variability
An early step in experiment design is to identify potential sources for variability or perceived variability in the performance of the siliconized glass surface. Performance in this context refers back to the primary goal of silicone as a lubricant: reduction of friction to achieve optimal and consistent injection characteristics (e.g. time and force) when the cartridge is used in a delivery device by a patient. Perceived variability suggests that, for example, some measured variability in performance could actually be attributable to other factors such as measurement equipment error.

A Cause-and-Effect or Fish Bone Diagram in Figure 25 summarizes some of the potential causes for variability. Although the process map in Figure 12 was used as an input to the Fish Bone Diagram, a wider range of concepts is also introduced.
Factors Contributing to Variability in Silicone Layer Performance and Consequent Injection Device Metrics (Time, Force, etc.)

Figure 25 - Fish Bone Diagram of Factors vs. Variability in Silicone Layer Performance

The Fish Bone Diagram highlights some of the difficulty of defining what good siliconization looks like. Without care taken to control for the noise, it can be challenging to draw conclusions specific to the glass siliconization process. For example, if a particular cartridge tested has an unusually high force profile when subjected to constant velocity testing, was it because the glass cartridge siliconization was poor, or perhaps because the stopper siliconization was insufficient? Or, perhaps the component geometry resulted in a tolerance stack-up leading to a tighter interference fit between the rubber stopper and glass cartridge, resulting a higher normal force pressing the components together?

3.3 High-Level Experiment Planning

3.3.1 Glass Plate Testing
In order to heighten experimental control and reduce potentially confounding variables in the system, a simplified physical setup was proposed in order to focus on the siliconized glass surface. In addition to directly testing some siliconized cartridges, flat glass plates were precisely siliconized and baked, before they were tested for layer characteristics and frictional properties (silicone layer performance). Testing on flat glass had its tradeoffs that will be discussed in the next section. However, this approach enabled some new methods to investigate the performance of siliconized glass. Figure 26 presents a high-level vision of the major aspects of the study along with possible inputs and outputs to provide a framework for the proposed experimentation.

**High-Level Glass Plate Test Planning**

**Inputs and Outputs: Possible Design Factors**

<table>
<thead>
<tr>
<th>Glass Plates from Schott</th>
<th>Siliconeization Rubróder/SonoTek</th>
<th>Baking Sanofi</th>
<th>Silicone Layer Geometry Sanofi or Fraunhofer AFM, XPS, 3DLSM</th>
<th>Silicone Layer Performance Fraunhofer Linear Tribometer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Input Parameters</td>
<td>Input Parameters</td>
<td>Input Parameters</td>
<td>Input Parameters</td>
<td>Input Parameters</td>
</tr>
<tr>
<td>- Mask Pattern</td>
<td>- Silicone Emulsion %</td>
<td>- Bake Temperature [°C]</td>
<td>- Normal Force [N]</td>
<td>- Linear Velocity [mm/sec]</td>
</tr>
<tr>
<td>Mask plates with different defect patterns, e.g. lines of variable width</td>
<td>- Set Spray Amount [g]</td>
<td>- Bake Time [min]</td>
<td>- Test Tip Geometry (Stopper, Modified Stopper, Spherical Probe)</td>
<td>- Test Tip Geometry (Stopper, Modified Stopper, Spherical Probe)</td>
</tr>
<tr>
<td>Output Parameters</td>
<td>Output Parameters</td>
<td>Output Parameters</td>
<td>Output Parameters</td>
<td>Output Parameters</td>
</tr>
<tr>
<td>- Plate Weight [g]</td>
<td>- Vary emulsion % to effectively change silicone amount / height. Hold other factors constant (e.g. sprayed amount, spray time, spray pressure, linear velocity of sprayer).</td>
<td>- If production baking tunnel used it may not be possible to vary bake temperature, but bake time could be varied.</td>
<td>- Baked Silicone Amount [g]</td>
<td>- Coverage (% Area with at least X)</td>
</tr>
<tr>
<td>Plate weight to be used as reference to determine amount silicone</td>
<td>Alternatively vary spray time while holding other factors constant.</td>
<td>Measure new plate weight and subtract original plate weight to determine baked silicone amount</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Output Parameters</td>
<td>Output Parameters</td>
<td>Output Parameters</td>
<td>Output Parameters</td>
</tr>
<tr>
<td></td>
<td>- Actual Spray Amount [g]</td>
<td>- Normal Force [N]</td>
<td>- Test Tip Geometry (Stopper, Modified Stopper, Spherical Probe)</td>
<td>- Friction Coefficient (average, min, max, standard deviation)</td>
</tr>
<tr>
<td></td>
<td>Measure new plate weight and subtract original plate weight to determine actual spray amount</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 26 - High-Level Glass Plate Test Planning and Possible Design Factors

**3.3.2 Tradeoffs with Testing Flat Glass**

There are some tradeoffs inherent in testing with flat glass compared to glass cartridges, which are discussed below in Table 6. Testing a different physical system will inherently produce results that are not completely transferrable. For the challenges with the approach that were identified, risk mitigation was proposed.

**Table 6 - Tradeoffs with Approach**

<table>
<thead>
<tr>
<th>Advantages of Approach</th>
<th>Challenges with Approach</th>
</tr>
</thead>
</table>

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<table>
<thead>
<tr>
<th>Precisely control siliconization layer sprayed onto glass using advanced equipment</th>
<th>Results from flat glass system testing may not be one-to-one comparable with those from cylindrical system. For example, in the cylindrical system, the silicone is constrained to be either plowed forward by the stopper or move backward in-between the glass and rubber, increasing the contact pressure — both of these scenarios likely increase the friction. On a flat plate without added constraints, the silicone is free to spread outwards, perpendicular to the direction of travel.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precisely control normal force between rubber test tip and glass surface (difficult to control in cylinder given interference fit between stopper and glass affected by variability in dimensions and material)</td>
<td>New materials and conditions are introduced with a different physical system. Mitigation — plan additional testing to help translate or validate results. Even if results are not one-to-one, they are expected to be “directionally relevant”. For example, if a surface coverage of X% area is found to have acceptable frictional characteristics, the absolute friction values measured may be different between systems, but the X% coverage may be valuable insight regardless of system.</td>
</tr>
<tr>
<td>Reduce extraneous variables effect on output, e.g. effect of assembly</td>
<td>No infrastructure existing to siliconize flat glass substrates. Existing infrastructure established to siliconize cylindrical components like syringes and cartridges. Mitigation — Develop new relationships with suppliers for precision siliconization of flat substrates.</td>
</tr>
<tr>
<td>Increases number of measurement techniques available to measure surface characteristics such as AFM and 3D-LSM. Introduces new testing possibilities such as controlled defect creation (e.g. test effect of dry-spot size)</td>
<td>Limited in-house knowledge of friction testing equipment (tribology). Mitigation — Develop new relationship with experts in tribology to access expertise and equipment.</td>
</tr>
</tbody>
</table>

### 3.3.3 Curved Glass Sections from Cartridges
In addition to testing flat glass, an additional concept was explored to test curved glass sections cut out of production siliconized glass cartridges utilizing a customized linear tribometer setup. The silicone layer height of glass cartridge was first measured using interferometry. Then, cartridges were laser cut to gain access to the inner surface. Finally, the surface was tested for friction using a linear tribometer test setup, specifying normal force and velocity. This concept is illustrated in Figure 27.

![Image](image)

**Figure 27 - Schematic of Intended Use for Curved Glass Cartridge Sections**

The advantages with curved glass sections were threefold. First, glass cartridges from production were evaluated, though this was not intended to be a production testing method. Testing curved glass in addition to flat glass provided additional application-specific insights. Second, the siliconized surfaces of the cartridges were tested for layer height leveraging the purpose built RapID Layer Explorer interferometry (see 5.4). The current implementation of interferometry by RapID is not suitable for measuring coating thickness on flat glass. Third, after cutting, the siliconized and sectioned cartridges were tested with a special setup of the tribometer enabling a more granular comparison silicone layer height data to friction along the length of the sample. Conversely, current test methods attempt to compare 365 degrees worth of silicone layer height data to a single profile of force versus distance (see Figure 23). Section 5.7 describes specific test methodology.

### 3.4 Design Factors

The duration and cost of studies are often proportional to the number of factors investigated. However this relationship may not linear, but instead exponential. Unless techniques such as fractional factorial experimental design are implemented, the number of tests is (number of replicates) x (number of levels per factor)^n(number of factors). For example, an experiment with three replicates, two levels, and three factors would have 3x2^3 = 24 tests. Adding a factor would double the number of tests to 48. Replication is critical to assess test variability and factor significance. It is thus critical to select a manageable number of parameters to test. Considering the Cause-and-Effect diagram in Figure 25, it would not be feasible to test all factors. Thus prior knowledge, initial testing, and reasonable engineering judgment are needed to pare down the study. Furthermore, the Pareto principal suggests that just a few factors account for the majority of the effect on an output.

Table 7 summarizes the factors considered for the study. Some of these were variable, and given resource constraints, some ended up be fixed.

**Table 7 - Experiment Design Factors**
<table>
<thead>
<tr>
<th>Category</th>
<th>Design Factor</th>
<th>Units</th>
<th>Example Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass Type</td>
<td>A Glass Substrate</td>
<td>[-]</td>
<td>Flat Plate or Curved Glass Cartridge Section</td>
</tr>
<tr>
<td>Siliconization Parameters</td>
<td>B Dry Spot Size / Mask</td>
<td>[mm]</td>
<td>Variable</td>
</tr>
<tr>
<td></td>
<td>C Silicone Emulsion %</td>
<td>[%]</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>D Spray Deposition Density</td>
<td>[mg/cm²]</td>
<td>Variable</td>
</tr>
<tr>
<td>Baking Parameters</td>
<td>E Bake Temperature</td>
<td>[°C]</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>F Bake Time</td>
<td>[hours]</td>
<td>0.5</td>
</tr>
<tr>
<td>Silicone Layer</td>
<td>G Tribometer Test Tip Material</td>
<td>[-]</td>
<td>Siliconized Rubber Stopper</td>
</tr>
<tr>
<td>Performance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>H Glass-Rubber Diametrical</td>
<td>[mm]</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Interference Fit in Cartridge</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Only</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Corresponding Cartridge Contact</td>
<td>[N/mm²]</td>
<td>Variable</td>
</tr>
<tr>
<td></td>
<td>Pressure (Ref. Only)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tribometer Normal Force -</td>
<td>[N]</td>
<td>Variable</td>
</tr>
<tr>
<td></td>
<td>Stopper on Flat Plate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>I Tribometer Linear Velocity</td>
<td>[mm/sec]</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>J Tribometer Test Distance</td>
<td>[mm]</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>K Tribometer Number of Cycles</td>
<td>[cycles]</td>
<td>1</td>
</tr>
</tbody>
</table>

Variable “H” refers to the normal force acting to press the rubber stopper to the glass substrate. This was theoretically calculated based on the interference fit and is discussed in Section 2.2.3. Variable “I”, Tribometer Linear Velocity, is related to the rate of travel of the rubber stopper through the cartridge. Sanofi tests various speeds as part of typical development and production testing.

3.5 Replicates and Randomization

Per good experimental design protocol, experimental runs were replicated and randomized when feasible. Experiment replicates are needed when performing analysis of variance (ANOVA) in order to test if measured differences between samples are due to actual factors and not chance. Randomization is needed to mitigate the risk of unknown or uncontrolled time effects. For example, if the humidity is uncontrolled and significant with respect to experimental output, it can create artificial differences in non-randomized testing between first and last test runs.

4 Project Materials

4.1 Borosilicate Glass Plates
Borosilicate glass plates were procured from Schott AG (Mainz, Germany). Schott also manufactures cylindrical cartridges for insulin primary packing using clear Type I borosilicate glass called Fiolax. The glass forming processes for plates and cartridges are different but a material with similar chemical composition and physical properties was selected for the plates per recommendation from Schott. Table 8 and Table 9 summarize typical material composition and property data found in Schott datasheets for the respective materials.

| Table 8 - Chemical Composition of Fiolax Glass vs. Borosilicate Glass Plate Material |
|-----------------------------------|-----------------------------------|
|                                    | Fiolax Clear | Borosilicate Plate |
| SiO₂                               | 75%          | 80.4%              |
| B₂O₃                               | 10.5%        | 13.0%              |
| Al₂O₃                              | 5%           | 2.4%               |
| Na₂O                               | 7%           |                    |
| Na₂O + K₂O                         |              | 4.2%               |

| Table 9 - Further Physical Data for Fiolax Glass vs. Borosilicate Glass Plate Material |
|---------------------------------|---------------------------------|
|                                  | Fiolax Clear | Borosilicate Plate |
| Coefficient Thermal Expansion (ISO 7991) | 4.9 x 10⁻⁶ K⁻¹ | 3.3 x 10⁻⁶ K⁻¹ |
| Transformation Temp.             | 565 °C       | 516 °C             |
| Density at 25 °C                 | 2.34 g-cm⁻³  | 2.23 g-cm⁻³       |
| Refractive Index                 | 1.492        | 1.472              |

A single large plate with approximate dimensions of 1000 x 1500 x 2 mm was first manufactured. Then smaller plates with dimensions of 24 x 75 x 2 mm were cut out from the larger plate. The dimensions were selected for transportability and suitability with the tribometer used for silicone layer performance testing. The surface characteristics of the large original plate depend on raw materials and the manufacturing process. Although these characteristics are expected to be relatively homogenous, they can vary slightly between the top and bottom sides of the plate. To mitigate this issue and improve consistency in testing, Schott engraved an “x” in the corner of one side of each small plate. Always siliconizing and testing the same side of the manufactured glass thus minimized error related to variation in glass surface characteristics. Next, the plates were finished to eliminate sharp edges. Finally, the plate surfaces were cleaned using Isopropyl alcohol before being packaged and shipped.
4.2 Curved Glass Cartridge Sections
As a further effort to correlate silicone layer characteristics to frictional performance, curved glass sections were laser-cut out of emptied 3.0mL cartridges. Figure 29 is a picture of glass sections prepared by a supplier who first performed a feasibility study to determine cutting process parameters.

Before cutting the glass, the cartridges were appropriately labeled and tested with RapID Interferometry to collect baked-in silicone layer height data (see 5.4 for information regarding the test method). Because only a portion of the glass cylinder is recovered from the cutting process, it was critical to work with the supplier to define section dimensions, location, and orientation. Without appropriate definition and labeling, it would not be possible to correlate silicone layer height versus friction performance for specific locations on the inner surface of the original glass cartridge. After testing the silicone layer height with RapID, the cartridges were sectioned by a supplier who followed the process described in Table 10.

Table 10 - Steps to Manufacture Curved Sections from Glass Cartridges

<table>
<thead>
<tr>
<th>Step</th>
<th>Name of Step</th>
<th>Description</th>
</tr>
</thead>
</table>

Page 43 of 92
1 Remove Cap End of Cartridge | Score end of cartridge with hard metal disc and break off end from body of cartridge
2 Section Cartridge | Induce thermal local strain in the longitudinal axis with a CO₂ laser. Cool the interface with active air-cooling to increase the tension. Following the induction of stress, cracks occur along the predetermined point. Rotate the tube 180° and repeat the process.
3 Clean | Lightly blow out the half-shells with recycled clean compressed air to remove any glass splinters on the surface. Maximum 1bar pressure.
4 Package | Individually pack half-shells in plastic film.

With a low thermal conductivity (1.2 W-m⁻¹K⁻¹) and short laser processing time, it is assumed that the sectioning process only affected the siliconized glass in the vicinity of the cut. It is also assumed that the cleaning step did not substantially disturb the silicone layer. Frictional testing with the tribometer performed thereafter was conducted in the trough of the curved section away from the cut.

### 4.3 3D-Printed Siliconization Masks for Glass Plates

In order to control the area of the glass plate siliconized, 3D-printed masks were conceptualized using Computer-Aided Design (CAD) software and manufactured. See 5.1 for details of 3D-printing process. A rendering of a basic spraying mask is shown in Figure 30.

![Figure 30 - Rendering of Glass Plate Siliconization Mask to Control Spray Area](image)

Additional mask designs were developed to block certain areas from being siliconized and create artificial “dry-spots” whose size can be tested for significance with respect to friction. Definition of maximum acceptable dry-spot size could be a useful manufacturing requirement for monitoring the siliconization process. Previous studies have shown that suboptimal siliconization processes can leave some areas of siliconized cartridges with substantially less silicone, resulting in increased friction forces.²³
4.4 3D-Printed Jigs for Curved Glass Sections
The 3D printer was also used to quickly manufacture simple jigs to support the curved glass sections, which were tested using the linear tribometer. See 5.1 for details of 3D-printing process. The curved glass section was held to the jig using two-part epoxy and thus the jigs were single use. The step feature of the jig allowed for assembly to the reciprocating module of the tribometer using thumbscrews. Figure 32 is a picture of a 3D-printed jig supporting a curved glass section for testing.

4.5 Silicone Emulsion
Dow Corning 365, 35% Dimethicone NF Emulsion, also known as Polydimethylsiloxane or PDMS for short, is commonly used in siliconization as a hydrophobic lubricant for medical devices and glassware. Water for Injection (WFI) is added to dilute the emulsion down to a few percent by volume before it is sprayed into the cartridges prior to baking and insulin filling.
Previous Sanofi experimentation and external studies have already shown that the percentage silicone emulsion is a statistically significant factor with respect to relevant performance criteria such as break-loose and gliding forces, which are related to insulin injection time. Holding all other factors constants, increasing the percentage emulsion in theory and practice increases the amount of silicone deposited on the glass. Thus, within a certain range, it is intuitive that this is significant. The interest in the emulsion as a potential variable in the study was not to confirm its significance, but rather the emulsion dilution percentage is a possible vehicle to achieve different silicone layers for testing.

In the end, it was found to be feasible to vary the siliconization spraying parameters such as flow rate to achieve different silicon layer thicknesses for testing. Thus, the percentage silicone in the emulsion was not a design factor in the study. A 5% volumetric emulsion, diluted in the factory at Sanofi, was used for all testing. It was mixed using a magnetic stirrer for at least 1 hour before siliconization. Figure 33 is a picture of the silicone emulsion.

![Silicone Emulsion Diluted to 5% by Volume and Mixed Prior to Testing](image)

### 4.6 Tribological Rubber Test Tips

Two different rubber components were selected for friction testing with the tribometer: one of the rubber stoppers used in the actual glass cartridge application, and a rubber sphere, which is a typical geometry for tribological testing. In practice, a variety of elastomeric stoppers are assembled and used in glass cartridges. Generally, stopper designs vary in terms of material, features (such as number of ribs), and dimensions. Some glass cartridges even have multiple fluid chambers and stoppers, enabling mixing of fluids during injection.

During insulin injection, the stopper glides through the inside of the glass cartridge and extrudes the drug product. Prior to assembly in the cartridges, the stoppers are procured through one of the two following channels: (1) purchased, siliconized, and sterilized, or (2) purchased pre-siliconized (called “Ready-to-Sterilize” or RTS) and sterilized. Figure 34 shows gray siliconized rubber stopper used in testing and is followed by Table 11 describing the typical properties.
In addition to one of the actual rubber stoppers, rubber spheres were employed. Rubber spheres were procured from Spherotech GmbH (Fulda, Germany). The spheres were selected to have a Shore-A hardness rating similar to that of the stoppers used in the application, and a diameter suitable for integration to the tribometer sensor per recommendation from Fraunhofer. The rubber spheres were not siliconized as that was thought to introduce unnecessary variation to the testing. Figure 35 and Figure 36 show the rubber spheres used in testing.
The different tribological test tips employed have pros and cons. On one hand, the rubber sphere is a typical geometry for tribological testing. A sphere is easier to install to a tribological sensor given its symmetry. Furthermore, the shape deforms more consistently under load. Thus testing with the rubber sphere was expected to produce more repeatable results and serve to better differentiate the performance of siliconized glass surfaces. However the sphere is a deviation from reality: it has a different geometry than rubber stoppers used in glass cartridges and is unsiliconized. On the other hand, the stopper presents its own challenges. Although it is a component used in the actual application, it is not as reliably installed to the tribological sensor (though radially symmetric, the stopper is not transversely symmetric and could be installed at an improper angle). Given these realities, different results are expected when using the different rubber components in the tribological testing. For a given normal load applied during a tribological test, differences in test tip geometry and material properties will result in different contact areas and pressures, to which friction may be dependent.

5 Project Methods

5.1 3D Printing for Siliconization Masks and Test Jigs

3D printing was used to produce masks designed used to control siliconization spraying area on glass plates. Jigs were also similarly produced to support the curved glass sections tested using the linear tribometer. 3D printing enabled a fail-fast iterative design approach and allowed for rapid production of low quantities of components with suitable dimensional accuracy. A ZPrinter 650 from ZCorp (3D Systems) was employed and uses a fine powder composite as raw material. A picture of the machine is shown below in Figure 37, followed by select technical specifications in Table 12.41.
3D-printed parts were finished following printing. After allowing the parts to dry and set, they were sanded with fine-grit sandpaper to smooth the surfaces. Compressed air was used to blow off residual powder and dust. A light coating of clear spray paint was applied to seal the material and smooth surfaces in order to prevent any residual powder from the 3D printing process from contaminating siliconized glass surfaces. Finally the critical mask dimensions were verified using calibrated calipers.

5.2 Siliconization of Glass Plates
Several methods were considered to siliconize the glass plates including modification of existing siliconization systems and development of a custom system. Existing systems were developed specifically for cylindrical systems (notably cartridges and syringes) and no feasible modification was found, having discussed options internally at Sanofi and externally with current suppliers of production siliconization machines. Other research groups investigating glass syringe siliconization previously worked with third parties to develop custom solutions but limited time precluded this option. In parallel, alternative methods were investigated including companies catering to a variety of industries offering equipment for characterizing precision coatings.
Rubröder Group (Bendorf, Germany), a distributor for Sono-Tek Corporation (Milton, New York, USA), presented a solution. Sono-Tek developed the “ExactaCoat Ultrasonic Coating System”, which can be finely tuned to spray ultrathin coatings of various fluids. While this is indisputably a different spray process than used in production siliconization of glassware, the goal was to investigate the characteristics of an optimal siliconized glass surface, and the ExactaCoat system offered an accurate and repeatable process for that purpose. Moreover, production processes were not suitable for flat substrates. Below in Figure 38 is a picture of the ExactaCoat machine at Rubröder in Bendorf, Germany.

Rubröder first performed a study and demonstrated the compatibility of silicone emulsion with the ExactaCoat system process when operating at 120 KHz and 2 Watts. Those parameters are fluid specific and were found to be appropriate for the emulsion. Further feasibility testing led to the determination of the process parameters summarized in Table 13.

**Table 13 - Ultrasonic Siliconization Spray Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silicone Emulsion %</td>
<td>5%</td>
<td>-</td>
<td>Percent emulsion (by volume)</td>
</tr>
<tr>
<td>Emulsion Density</td>
<td>998</td>
<td>mg/ml</td>
<td>Calculated separately</td>
</tr>
<tr>
<td>Ultrasonic Power</td>
<td>2</td>
<td>W</td>
<td>Per Rubröder feasibility study</td>
</tr>
<tr>
<td>Ultrasonic Frequency</td>
<td>120</td>
<td>KHz</td>
<td>Per Rubröder feasibility study</td>
</tr>
<tr>
<td>Temperature</td>
<td>-</td>
<td>-</td>
<td>Room temperature</td>
</tr>
<tr>
<td>Nozzle Height</td>
<td>7</td>
<td>cm</td>
<td>Observed to affect coating structure. Varied until coating appeared consistent per visual inspection.</td>
</tr>
<tr>
<td>Air pressure</td>
<td>50</td>
<td>mbar</td>
<td>Observed to affect coating structure. Varied until coating appeared consistent per visual inspection.</td>
</tr>
<tr>
<td>Travel Velocity</td>
<td>5</td>
<td>cm/sec</td>
<td>Affects deposited amount; held constant</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Spray Width Width</th>
<th>2 cm</th>
<th>Spray width on plate</th>
<th>Deposition Density</th>
<th>Variable mg/cm²</th>
<th>Determined to be nominal deposition density for siliconized 1.5mL cartridges; calculated separately</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfer Efficiency</td>
<td>80%</td>
<td>Per Rubröder feasibility/experience</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flow Rate</td>
<td>Variable ml/min</td>
<td>Calculated based on target coating deposition density</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The nominal “deposition density” for spraying the flat plates was determined through calculation using target production spray amount and inner surface area for siliconized 1.5mL cartridges.

With a conversion between target coverage and set flow rate on the machine, an array of layer densities (spray amounts) was achieved. Figure 39 is a basic schematic of the glass plate siliconization showing plate dimensions (24 x 75mm), siliconization area achieved through use of 3D-printed masks (X*Y mm²), and target thickness (Z) achieved through varying flow rate (deposition density).

![Figure 39 - Schematic of Glass Plate and Siliconization](image)

### 5.3 Glass Baking Processes

Glass cartridges that are siliconized with emulsion go through a baking process for sterilization and to achieve an ultrathin coating. After baking, cartridges are filled with the drug product. This process is compared to that of prefilled syringes, which tend to be siliconized with oil (instead of emulsion) before being filled with the drug product. Prefilled syringes, which are not baked, have a silicone layer that is substantially thicker than that of baked cartridges, details of which are summarized in Table 14. See Section 4.5 for details of the silicone emulsion used for testing.

<table>
<thead>
<tr>
<th>Comparison of Prefilled Syringes to Glass Cartridges</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Table 14 - Comparison of Prefilled Syringes to Glass Cartridges</strong></td>
</tr>
<tr>
<td><strong>Siliconization Fluid</strong></td>
</tr>
<tr>
<td>Silicone Oil</td>
</tr>
<tr>
<td><strong>Baking Process?</strong></td>
</tr>
<tr>
<td><strong>Resulting Silicone Layer Thickness</strong></td>
</tr>
</tbody>
</table>

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During baking, cartridges travel on a slow moving conveyor belt through a heating tunnel before arriving at cartridge assembly (rubber stopper, cap) and filling stages. Baking conditions, also discussed in Section 2.1.3, are sufficient for depyrogenation and substantial evaporation of the silicone emulsion’s water content. If there are downstream issues in the production process, cartridges may remain in the baking tunnel for several hours. After a maximum delay, the parts must be scrapped. Practically for production, a longer allowable baking time is thus preferable to accommodate delays and reduce scrap.

Glass plates siliconized at Rubröder were transported and baked at Sanofi in Frankfurt in a production-grade baking tunnel. Glass was baked at 320°C for a duration of 30 minutes, unless otherwise noted. The transportation of glass between siliconization and baking is a deviation from typical production processes described above, in which glass cartridges are baked directly after siliconization. Other baking solutions were also considered for the glass plates but did not offer the proper ventilation for the evaporated water content. Additionally, Rubröder’s siliconization equipment was not transportable to be setup in Frankfurt.

Baking parameters are potentially interesting design parameters with respect to silicone layer performance. Typical baking conditions result in a permanent bond between the PDMS chains in the silicone emulsion and glass surface that cannot be removed even with use of solvent. On top of the chemically bonded layer, some of the silicone remains as a loose free layer. A hypothesis is that higher temperatures or longer baking times can be expected to affect the ratio of baked versus free layers of silicone, which could impact the coefficient of friction. In spite of the interest in testing the baking parameters, limitations in baking tunnel availability and access resulted in only baking at nominal conditions, unless otherwise indicated.

5.4 Silicone Layer Height Measurement: Interferometry

A Layer Explorer UT 2.0 (LE UT 2.0) from RapID (Berlin, Germany) was used to measure the baked-in silicone layer on the inner surface of emptied glass cartridges. The LE UT 2.0 operates in two modes: UT (ultra thin) Interferometry and BI Reflectometry. The UT Interferometry mode is particularly relevant for thin baked-in silicone layers and is typically used to measure layer thickness and distribution in the 20-100nm range. Above 80nm, the machine relies on the Reflectometry mode. Below in Figure 40 is a picture illustrating the modes of the LE UT 2.0 machine and is followed by Table 15 containing select specifications.
The LE UT 2.0 is currently best suited for testing in a laboratory setting. Although the Layer Explorer's inspection modes are non-destructive, only emptied cartridges are tested. Furthermore, the cited test time of 1 minute is not fast enough to keep up with production speed over many hundred per minute. Testing is relatively simple given the level of automation within a single test. Once calibrated for the type of cartridge being tested and specifying the required inputs (geometry, material properties like index of refraction, etc.) a test can be executed. Silicone layer thicknesses are measured at a set number of points along a line, such as 100 points. Then, the cartridge automatically rotates to begin to test the next line. Typically around 10 lines of data from around the cartridge are collected but up to 36 may be specified. Test time increases with the number of points per lines and number of lines. Currently there is no automation to change out the cartridge and cartridges must be manually placed before a test is run.

### 5.5 Silicone Layer Height Measurement: 3D-LSM
A Keyence Violet Laser Color 3D Laser Scanning Microscope (3D LSM), model VK-9710, was used to investigate and measure the baked in silicone layer. Below in Figure 41 is a picture of the microscope from Keyence, followed by Table 16 containing select technical specifications.

![3D Laser Scanning Microscope](image)

**Table 16 - 3D LSM Select Technical Specifications**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Objective lens magnification</td>
<td>10x, 20x, 50x, 150x</td>
</tr>
<tr>
<td>Height measurement resolution</td>
<td>1 nanometer</td>
</tr>
<tr>
<td>Height measurement repeatability σ</td>
<td>14 nanometer</td>
</tr>
<tr>
<td>Width measurement resolution</td>
<td>1 nanometer</td>
</tr>
<tr>
<td>Width measurement repeatability 3σ</td>
<td>20 nanometer</td>
</tr>
</tbody>
</table>

In order to measure the layer thickness, a needle was first used to produce a narrow scratch through the baked-in silicone. Figure 42 is a picture of the needles used to create the scratch.

![Needles](image)
Funke had success with this method for baked-in silicone layer thickness measurement with accuracy down to 10nm\textsuperscript{31}. Only light force was required to produce the scratch and the glass substrate was inspected under magnification to verify that only the silicone layer was displaced. Scratching the silicone plowed through the layer and built up two banks, one on either side of the scratch. 10x magnification was first used to identify the scratched silicone layer. 50x magnification was then used to focus on a region large enough to capture the scratch and areas beyond the banks of built-up silicone material. After the laser was used to scan and measure the surface, post-processing software was used to manually draw a line perpendicular across the scratch creating a relative height profile. A height difference between the bare glass (silicone layer scratched away) and the silicone layer was then calculated. This process is illustrated below in Figure 43. The virtual line drawn through the scratch with the software to create the profile is shown in the upper left, and the resulting step profile is in the bottom.
The method proved to be challenging to reliably implement in practice. It was observed that the glass substrate was itself not perfectly flat and it was necessary to pre-process the layer height data to compensate for slight curvature in the otherwise flat glass. Inspecting siliconized surfaces sprayed at different target amount or deposition densities, there were clearly differences in realized thicknesses. However, within a sample’s siliconized surface, there was significant variation in the layer thickness. It was thus challenging to state that a given sample had a particular layer thickness, while the calculated difference between the bare glass (due to the scratch) and silicone layer was very sensitive to the position and orientation of the profile drawn across the scratch with Keyence’s software. In an attempt to better characterize an average layer height, samples were scratched in different locations and profile measurements were repeated. Furthermore, although calibrated, a 44nm layer height reference was procured from John P. Kummer GmbH (Augsburg, Germany) to check microscope accuracy in the under-100nm range.

Already a destructive method, Funke further describes how this method is cumbersome for measuring silicone layer heights on the inner surfaces of cartridges or syringes. The glass must first be broken into pieces to access the inner surface. If the broken glass pieces are mixed up, it then becomes difficult to associate layer height of an individual piece with the original cartridge location.

5.6 Silicone Layer Characterization: XPS
In collaboration with the Fraunhofer Center for Microtribology, X-Ray Photoelectron Spectroscopy (XPS), also known as Electron Spectroscopy Chemical Analysis (ESCA) was used as another method to investigate the baked-in silicone layer. According to Evans Analytical Group, XPS Analysis is used “to determine quantitative atomic composition and chemistry” 35. A sample of siliconized and baked flat glass was irradiated with monochromatic x-rays, resulting in the release of photoelectrons characteristic of the elements in the sample. It was thus possible to characterize the surface chemistry. Further, by sputtering away the surface, it was possible to characterize the silicone layer chemistry as a function of depth until the underlying glass substrate was reached.

With a known sputtering rate (e.g. 2nm per minute) it is theoretically possible to determine layer thickness when the chemistry of the coating is different than that of the substrate. In the case of the flat siliconized glass tested, there was for example Boron and Aluminum present in the glass (see Table 8) but not the silicone. Even trace amounts are detectable by XPS. Unfortunately the exact sputtering rate of the baked-in silicone layer was unknown. Thus at this time, beyond surface chemistry, it would only be possible to ascertain relative thickness. In the future, a baked-in silicone layer with known thickness could be potentially tested with XPS in order to first determine the sputtering rate. Thereafter, further samples could be very accurately measured for layer chemistry versus thickness. It may even be possible to investigate differences in the baked-in versus free silicone layers.

5.7 Silicone Layer Performance: Linear Tribometer
In order to assess the silicone layer performance, a linear tribometer was utilized. Test methods were developed in collaboration with the Fraunhofer Institute’s Center for Microtribology (Pfinztal, Germany). Figure 44 is a picture of the machine used and is followed by Table 17 containing certain specifications43.
Different rubber test tips were attached to the force sensor for testing. Because superglue was used to affix the test tip to the sensor platen, it was not feasible to change the test tip in between every test run. Although glue with five-minute cure time was used, the test tip was allowed a minimum of 30 minutes to cure to be sure it was set. For future testing, an adapter was envisioned to enable the ability to quickly change test tips in order to use, for example, a fresh test tip for each experimental run. Below in Figure 45 is a picture of the gray rubber stopper (1.5mL cartridge size) glued to the force sensor. For scale, the rubber stopper is approximately 6mm in length.
In addition to the gray rubber stopper, a small, unsiliconized rubber sphere was also used for some testing. As was mentioned in Section 4.6, the sphere is typical test tip geometry as the contact surface is more predictable and repeatable in between tests. Below in Figure 46 is a picture of the rubber sphere affixed to the force sensor.
Once the rubber test tip was attached to the force sensor, the sensor was then installed in the tribometer. When siliconized glass plates were tested, they were held onto the testing table using two thumb screws. Using the computer application, inputs of normal force \( (F_N) \), linear velocity \( (V) \), test distance, and number of cycles were given. For the condition illustrated below in Figure 47, to achieve an effective stopper velocity to the right \(+X\) , the table to which the plate was attached would move to the left \(-X\) . The variable \( T_S \) refers to the nominal baked-in silicone layer height that would have been previously measured. An optical sensor measured deflection of a small vertical plate that was correlated to friction force based on the geometry of the force sensor. Because the normal force \( (F_N) \) was specified and the friction force \( (F_F) \) was measured, the coefficient of friction \( (\mu) \) could be calculated using . A test would start with application of the specified normal force as the rubber test tip was pushed into the glass substrate. Then the table would move at the specified velocity. For one cycle, the table would move back and forth. It was also possible to specify a half cycle, in which the table would move in the specified direction and then stop.

![Figure 47 - Friction Testing Siliconized Glass Plate with Tribometer](image)

For part of the study, curved glass sections, cut from 3.0mL cartridges, were tested with the tribometer as shown in Figure 48. Some sections were unsiliconized in order to measure baseline friction. Others were siliconized and baked in Sanofi’s production facility, measured for coating thickness, and tested. Only 1.5mL gray rubber stoppers were used for this testing. Given their smaller diameter, they would intentionally only contact a small portion of the larger 3.0mL cartridge inner surface. Rubber spheres were too small to contact the inner glass surface and were not used. Single-use fixtures were designed and manufactured using 3D printing to hold the curved glass sections. Two-part epoxy was used to glue the glass sections to the fixture, and a “step” feature of the jig allowed for the assembly to be installed onto the tribometer table using the thumbscrews.
Two tests were conducted per sample. After achieving the specified normal force, the table holding the jig and curved glass sample would move at the set velocity up to the travel distance of 10mm (+X). Then the machine head with the force sensor and rubber stopper would retract vertically away from the glass (+Z). After moving approximately 8mm (+X), the machine head would descend to again apply the set normal force and conduct the second test. In this way, two sections per curved glass sample were tested.

6 Test Results and Observations

6.1 Initial Glass Plate Test Results

In order to gain some experience with the materials and refine test methods, some initial testing with the linear tribometer was conducted in collaboration with the Fraunhofer. Early insights into the behaviors of factors on the friction coefficient were gained to inform planning of subsequent formal testing. For the initial testing, only glass plates were used because the curved glass sections were not yet available. The factors that were explored are summarized in Table 18 along with ranges tested. Initial testing did not follow a structured DOE plan so not all factor combinations were investigated. Reference section 5.7 for details of the linear tribometer test method.

<table>
<thead>
<tr>
<th>Category</th>
<th>Design Factor</th>
<th>Units</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass Type</td>
<td>A Glass Substrate</td>
<td>[-]</td>
<td>Flat Plates</td>
</tr>
<tr>
<td>Siliconization Parameters</td>
<td>B Dry Spot Size / Mask</td>
<td>[mm]</td>
<td>Unsiliconized plates also tested</td>
</tr>
<tr>
<td></td>
<td>C Silicone Emulsion %</td>
<td>[%]</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>D Spray Deposition Density</td>
<td>[mg/cm²]</td>
<td>Variable</td>
</tr>
</tbody>
</table>
The first tests were done with the rubber stopper against an unsiliconized plate to get a sense of baseline friction. Between five and ten cycles were tested to see how friction would change in time. It was acknowledged that in the application, a rubber stopper is pushed through the glass cartridge a single time in one direction. An initial test investigated the parameter of test distance (in one direction) between 1-10mm. Figure 49 summarizes the results. Individual points represent the average gliding friction of a given test cycle. It was observed that the shortest test distance of 1mm had the highest friction coefficient (highest horizontal line). From test observation it was believed that very short test distances resulted primarily in the static friction regime, especially because of the 6mm length of the rubber stopper used for this test. There was perhaps an outlier seen from the 6mm test distance (lowest horizontal line), which resulted in significantly lower friction.
The next test, testing the rubber sphere against an unsiliconized plate, investigated the parameter of velocity. The results shown in Figure 50 suggest higher velocities yield higher friction coefficients. This is consistent with expectations from the Strubeck curve (See Figure 15), though this result could be different for another range of velocities. It was also observed that initial cycles had slightly higher friction coefficients.
Figure 50 - Effect of Velocity on Friction Coefficient

The next test examined a range of normal forces applied at a constant velocity of 2.5mm/s. It was observed in Figure 51 that higher normal forces resulted in lower friction coefficients (though higher friction forces, which are not shown in the chart).

![Graph showing the effect of normal force on friction coefficient.]

Figure 51 - Effect of Normal Force on Friction Coefficient

The test was run again, but with the rubber stopper against a siliconized plate. The results are summarized in Figure 52. Further evidence was found of higher normal forces resulting in lower friction coefficients. Interestingly, friction coefficients increased with increasing cycles. This was likely due to the stopper plowing away the silicone lubricant with each cycle, increasing friction.
The previous test was repeated, except using the unsiliconized rubber sphere. The results are summarized in Figure 53. A similar trend was observed, though apparently insufficient cycles were run to witness a leveling-off of increasing friction coefficients.
In this final initial test, shown below in Figure 54, the unsiliconized rubber sphere was tested against a siliconized plate under different velocities. Again, higher velocities yielded higher coefficients of friction. However, the range of friction coefficients (0.1-0.17) was significantly lower than was tested with the baseline, unsiliconized plate (0.4-0.7). This was specific evidence of the effect of unsiliconized or bare glass on friction.

![Friction Test: Sphere/Siliconized Plate @ F_N = 1N, 10mm Test Distance, Variable Velocity](image.png)

**Figure 54 - Effect of Velocity on Friction Coefficient**

Below in Table 19 is a summary of findings from the initial testing that was used to inform further test planning.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Distance</td>
<td>When testing with stopper, 1mm Test Distance yielded results only in static friction regime. 5mm Test Distance is also too short as results were mixed and also considering length of stopper is approximately 6mm. 10mm is preferred for further tests.</td>
</tr>
<tr>
<td>Number of Cycles</td>
<td>Number of cycles has strong impact on results. First cycle in test tended to have higher coefficient of friction. For unsiliconized glass results were consistent after 2-3 cycles. For siliconized glass results did not tend to level off until 10 cycles. Regardless, ½ cycle is more representative of the application because the rubber stopper glides only in one direction through the cartridge. Thus ½ cycle is preferred for further testing.</td>
</tr>
</tbody>
</table>
Test Tip

Velocity and Normal Force

Silicone Spray Amount / Baked-in Layer Height

Dry Spots (Spots free of Silicone)

Both have a strong impact on coefficient of friction and should continue to be explored.

Results of initial tests suggest slight impact and this should be explored further.

This was not explored directly in the initial testing but comparing test results of unsiliconized glass to siliconized glass, not surprisingly the coefficient of friction is significantly higher without silicone. Further testing should explore this more carefully.

### 6.2 Main Glass Plate DOE Results

Following the initial testing, a formal DOE was planned and executed. Glass plates were siliconized under three different conditions and baked at 320°C for 30 minutes. Due to constraints in production, it was not possible to investigate the baking parameters in the study. The three-factor, two-level full factorial DOE explored the factors of siliconization, normal force, and velocity ($2^3 = 8$ treatments). Three replicates were run so that analysis of variance (ANOVA) could be conducted. Testing was run first with the siliconized rubber stopper, and then repeated with the unsiliconized rubber sphere. All tests were randomized to mitigate the risk of uncontrolled time-dependent effects. Table 20 summarizes the variables and conditions tested.

<table>
<thead>
<tr>
<th>Table 20 - Factors Explored During Main Glass Plate Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Category</strong></td>
</tr>
<tr>
<td>Glass Type</td>
</tr>
<tr>
<td>Siliconization Parameters</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Baking Parameters</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Silicone Layer Performance</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
Below in Figure 55 is an example test result plotting the coefficient of friction (left vertical axis) and friction force (right vertical axis) versus test distance. A "break-loose" force was followed by a gliding regime. Average values were taken from each test run as outputs.

### Friction Testing with Sphere: Treatment 1, Replicate 1, Run 13

- **v=10mm/s; F_N=1200mN, 167% Spray Amount**

**Figure 55 - Typical Test Result for Rubber Sphere DOE: Friction Coefficient, Force vs. Test Distance**

Once all data were collected and entered into a table, a statistical package (Minitab 17) was used to model the results. First, the residuals (error between model prediction and actual data) were graphically analyzed as a check for model validity. It was observed that residuals followed a normal distribution. The graphical residuals analysis is shown in Figure 56.
Effects with P-Values less than 0.05 were considered significant. Figure 71 in the Appendix shows the ANOVA table output from Minitab. Normal force and velocity were found to be highly significant but when testing with the rubber sphere, the spray amount was found to be insignificant.

A Pareto chart of standardized effects (Figure 57) illustrated the relative magnitude of the effects on the output of average gliding friction coefficient. For this test, normal force and velocity had much stronger effects than the spray amount.
The main effects plot in Figure 58 illustrates the direction and magnitude of the main effects on the output of average gliding friction coefficient. This was directionally consistent with the initial test findings.

![Main Effects Plot for Avg Glide Friction Coefficient (Rubber Sphere)](image)

Testing was repeated using the siliconized rubber stopper. A typical test run is plotted and shown below in Figure 59. In this test, a brief break-loose force was followed by a gliding regime that was not as flat as that seen when testing the rubber sphere. This could be due to a difference in geometry. Again average values of friction coefficients were taken as outputs and used to model the effects of the inputs.
The residuals were graphically analyzed for any time trends and to verify the assumption of normality. The graphical analysis is shown below in Figure 60.

![Residual Plots for Avg Glide Friction Coefficient](image)
As was seen with the rubber sphere testing, both normal force and velocity were found to have statistically significant effects on friction. However, with the rubber stopper, spray amount was found to be highly significant. Because time permitted, an additional level of spray amount was added to the experiment. The nominal spray amount condition (100%) was tested to gain further insight into the shape of the friction curve for three total spray amounts. The ANOVA table for the rubber stopper DOE is shown in Figure 72 of the Appendix.

The main effects plot for average gliding friction coefficient (Figure 61) looks very similar to that from testing with the rubber sphere. However, the spray amount effects plot on the right-hand side looks different. Between 100% and 167% spray amounts, the relationship with friction is nearly flat at 0.125. However, friction at 33% spray amount is nearly double at approximately 0.24.

![Figure 61 - Main Effects Plot from Rubber Stopper DOE](image)

Figure 62 is an interaction plot for average gliding friction coefficient from the rubber stopper testing. One notable interaction between velocity and spray amount is in the lower right corner of the chart. While increasing velocity generally increases the friction coefficient, at lower spray amount (thinner silicone layers) the friction coefficient was found to be even more sensitive (greater slope) to changes in velocity.
Differences in results observed when testing the rubber sphere versus the rubber stopper were interesting but perhaps not surprising. One test tip was siliconized, while the other was not. Differences in geometry and surface conditions likely also played a role. This relates to discussion from Section 2.2.2. Table 21 summarizes the findings from the main glass plate DOE, from which a final iteration of testing was planned.

Table 21 - Summary of Results from Main Glass Plate DOE

<table>
<thead>
<tr>
<th>Factor</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Velocity and Normal Force</td>
<td>Further data supports theory and past test results that these factors alone have a significant impact on friction coefficient. More interesting were relative significance vs. silicone layer, and factor interactions.</td>
</tr>
</tbody>
</table>
| Silicone Baked-in Layer Height, Spray Amount | Results suggest thinner baked-in silicone layers result in increased friction coefficients and also variability.  
**Rubber sphere**: silicone layer found to play no role in friction in range of 33-167% Spray Amount. Thinner layers may yield different results.  
**Rubber stopper**: silicone layer thickness played only a minor role between 100-167% Spray Amount. At 33% Spray Amount the coefficient of friction was significantly higher, highlighting the role of the test tip material and contact area. |

6.3 Final Glass Plate DOE Results
Based on the findings from the Main Glass Plate DOE, additional testing was conducted to further investigate the relationship between siliconization amount (proxy for silicone layer height) and the coefficient of friction. For this testing, conducted with the siliconized rubber stopper, seven different siliconization amounts were tested, ranging from 0% to 100% (previously 33% to 167%). Baking conditions were the same as those from the Main DOE. Velocity and normal force were held constant and six replicates yielded a total of 42 runs. The average gliding friction was taken as output and modeled against spray amount. The residual plots in Figure 63 looked suspicious but are explainable.

Residual Plots for Avg Glide Friction Coefficient

The plot below in Figure 64 illustrates the exponential model that was fit to the data. The behavior at 0% siliconization explains the residuals. Because the same siliconized stopper was used for all tests, when testing against an unsiliconized plate (0% condition), the stopper’s coating wore off and friction increased with each test run. Had a new stopper been used for each test, this result likely would not have occurred, and it is thus believed to be an artifact and limitation of the method. Regardless, this result supports a hypothesis that above a certain amount of siliconization or coating thickness, no further reduction in friction is realized. This study found that the coefficient of friction above 40% siliconization was minimized and repeatable. The result also supports the notion that spray amount could be reduced from 100% to, for example, 60% +/- 20%. This could mitigate drug-silicone interaction issues previously discussed resulting from excess silicone.
The findings and implications from the final glass plate DOE assume a consistent application of silicone to the glass substrate. What if the glass substrate is not fully covered with silicone?

6.4 Glass Plate Dry Spot Test Results

In addition to the DOE, some supplemental tests were run to specifically look at the effect of “dry spots” or unsiliconized areas on the glass substrate. During the actual production siliconization process, if a nozzle were to misfire, it could result in unexpected coating coverage. Alternatively, foreign material or residue not removed by the cleaning process could theoretically result in dry spots. To investigate these types of issue, siliconization masks were used to create dry spot defects of particular dimensions. Glass plates subjected to these conditions were then tested with the linear tribometer to compare defect dimension to change in the friction coefficient. For these experiments, velocity, normal force, and siliconization condition were held constant. Two different dry spot sizes were tested. Only the rubber sphere was tested due to a limitation with the rubber stopper length relative of 6mm to the test distance of 10mm.

Figure 65 is a chart of friction coefficient and friction force versus test distance for the smaller defect size tested. This was a typical result for this test group. An increase in friction of approximately 5% was observed when the 1-mm dry spot was encountered.
For the larger dry spot size tested, illustrated in Figure 66, friction increased nearly 80% when a 5-mm dry spot was encountered. After passing the dry spot, friction returned to normal values.

Table 22 summarizes the results of the dry spot testing. The average increases in friction for small and large dry spots were 5.3% and 89.3%, respectively.
Table 22 - Dry Spot Testing Results Summary

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dry Spot Size (mm)</th>
<th>Average Gliding Friction Coefficient (mN)</th>
<th>Peak Friction Coefficient (mN)</th>
<th>Change in Friction Coefficient (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0.116</td>
<td>0.122</td>
<td>+5%</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0.118</td>
<td>0.122</td>
<td>+3%</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>0.120</td>
<td>0.130</td>
<td>+8%</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>0.126</td>
<td>0.224</td>
<td>+78%</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>0.132</td>
<td>0.240</td>
<td>+82%</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>0.124</td>
<td>0.258</td>
<td>+108%</td>
</tr>
</tbody>
</table>

The results suggest that dry spots are detectable and a potentially significant issue for manufacturers even if the average coating thickness is above the critical minimum. It is interesting to note that even with a large dry spot, friction did not increase to levels measured on completely bare glass. This may be because the rubber test tip plowed silicone forward in an auto-siliconizing mechanism.

The test method shows promise in the definition of acceptable dry spot size. Further work could investigate realistic spot sizes in glassware from production and testing of additional conditions (dry spot sizes and shapes, nominal silicone layer thicknesses, velocities, normal forces, etc.). A thicker nominal silicone layer, for example, could hypothetically diminish the effects of a dry spot.

6.5 Curved Glass Section Test Results

Curved glass sections, cut from production-siliconized cartridges, were tested using the linear tribometer to further investigate the relationship between the silicone layer and friction. Due to the challenge encountered of cutting the cartridge, only a handful of samples were procured and tested. As such, test results are not as statistically relevant as those from the structured DOE. However, the results provide additional insights into the friction performance associated with siliconized glass. Two 10mm tests were conducted per glass section. The average friction coefficient from each section was compared against the silicone layer height data that had been previously collected with the RapID machine. Generally, samples tested had layer heights between 73-120nm. An unsiliconized glass section was also tested using this method to get a sense of baseline friction.

Test results from sample S3 are illustrated below in Figure 67. The silicone layer thickness generally ranged from 60-100nm and the average gliding friction coefficients were consistent across both sections tested with 0.126 measured for section 1 and 0.117 for section 2. These values are similar to friction measured between the stopper and siliconized flat glass. This result suggests that the minimum siliconization condition was met, resulting in little change in friction.
Test results from sample S9, illustrated below in Figure 68, tell a different story. In this case, “dry spots” were identified in section 1. The purple-colored areas measured less than 20nm in silicone layer thickness. Consequently, the measured friction coefficient was indeed higher in section 1 (0.222) than section 2 (0.148), representing an increase of 50%. It cannot be concluded that similarly siliconized glass in a complete cartridge would behave similarly, though it does suggest provide additional evidence that dry spots can be identified in siliconized glass cartridges and may contribute to unexpectedly high friction conditions, resulting in adverse injection performance.

![Figure 67 - Curved Glass Section Test Result (Sample S3)](image_url)
The table below summarizes test results from all five runs, including the experiment run with an unsiliconized glass section. It is interesting to note that when the stopper ran over the dry spots in sample S9, friction did not increase to friction levels associated with the bare glass of sample NS4. This may be due to a self-siliconization mechanism as the stopper plows lubricant ahead.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Average Gliding Friction Coefficient – Test Section 1</th>
<th>Average Gliding Friction Coefficient – Test Section 2</th>
<th>Dry Spots Observed?</th>
</tr>
</thead>
<tbody>
<tr>
<td>S3</td>
<td>0.126</td>
<td>0.117</td>
<td>No</td>
</tr>
<tr>
<td>S4</td>
<td>0.123</td>
<td>0.114</td>
<td>No</td>
</tr>
<tr>
<td>S5</td>
<td>0.155</td>
<td>0.137</td>
<td>No</td>
</tr>
<tr>
<td>S9</td>
<td>0.222</td>
<td>0.148</td>
<td>Yes – in Test Section 1</td>
</tr>
<tr>
<td>NS4</td>
<td>0.693</td>
<td>0.472</td>
<td>NA – Sample was unsiliconized and tested as a baseline</td>
</tr>
</tbody>
</table>

6.6 XPS Test Results
Limited XPS testing with baked and siliconized flat glass revealed some basic insights. This technique can be used to identify materials present on the surface. For example, a peak in binding energy at 102.4 in Figure 69 is characteristic of PDMS, the material used to siliconize the glass. More interesting is the potential combination of the technique with sputtering.

![XPS Chart 1: Binding Energy](image)

When combined with sputtering, the atomic concentration of materials was plotted against time. As shown below in Figure 70, the concentrations of different elements changed with depth. Because the chemical compositions of the silicone coating and glass substrate were different, it was possible to identify when the substrate was reached. For example, only the borosilicate glass contains Boron and Aluminum. As those concentrations spike, it can be inferred that the coating has been sputtered away, leaving the glass substrate. If the input sputter rate is known, based on previous characterization of the coating, the combined technique can be used to accurately measure ultrathin coating thickness. Unfortunately, the sputter rate in this case was unknown and the data cannot be used in this fashion. However, if a future effort is made to first determine the sputter rate for baked-in silicone, it may prove to be a viable method.
6.7 3D-LSM Test Results

Table 24, Table 25, and Table 26 summarize the 3D laser-scanning microscope results. The method, as previously mentioned, proved challenging and subjective. The first set of data from the pre-testing was compared against measurements taken with a confocal microscope using 50x magnification at the Fraunhofer Institute. Ranges are given due to the variability observed. All values are shared for reference only, to provide a general sense of layer height. It should be noted that although all glass plates were siliconized with 5% silicone emulsion at baked at 320°C, the baking time was not consistent due to limitations encountered in the production facility. Pre-testing samples were baked for 2 hours and 20 minutes, while all other samples were baked for 30 minutes. Spray conditions are noted as a percentage of nominal for proprietary reasons. Finally, images of the 3D-LSM testing can be found in the Appendix.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Spray Condition</th>
<th>N</th>
<th>Average Height (nm)</th>
<th>Standard Deviation (nm)</th>
<th>Confocal Microscope, 50x (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>33%</td>
<td>2</td>
<td>11</td>
<td>1.4</td>
<td>5-20</td>
</tr>
</tbody>
</table>
### Table 25 - 3D LSM Results from Main Glass Plate Testing

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Spray Condition</th>
<th>N</th>
<th>Average Height (nm)</th>
<th>Standard Deviation (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-4</td>
<td>33%</td>
<td>3</td>
<td>35</td>
<td>17</td>
</tr>
<tr>
<td>9-4</td>
<td>100%</td>
<td>3</td>
<td>148</td>
<td>6</td>
</tr>
<tr>
<td>11-4</td>
<td>167%</td>
<td>3</td>
<td>278</td>
<td>12</td>
</tr>
</tbody>
</table>

### Table 26 - 3D LSM Results from Final Glass Plate Testing

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Spray Condition</th>
<th>N</th>
<th>Average Height (nm)</th>
<th>Standard Deviation (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-3</td>
<td>10%</td>
<td>3</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>19-1, 19-3</td>
<td>20%</td>
<td>3</td>
<td>19</td>
<td>6</td>
</tr>
<tr>
<td>18-3</td>
<td>40%</td>
<td>1</td>
<td>24</td>
<td>NA</td>
</tr>
<tr>
<td>17-1, 17-3</td>
<td>60%</td>
<td>4</td>
<td>75</td>
<td>27</td>
</tr>
<tr>
<td>16-1, 16-3</td>
<td>80%</td>
<td>4</td>
<td>122</td>
<td>24</td>
</tr>
<tr>
<td>15-1, 15-3</td>
<td>100%</td>
<td>6</td>
<td>194</td>
<td>58</td>
</tr>
</tbody>
</table>

---

7 **Recommendations and Conclusions**

7.1 **Key Findings**

Many articles and studies reviewed suggested that "inadequate" or "insufficient" glass siliconization could lead to poor mechanical performance, though "excessive" siliconization could also create problems. Reuter and Petersen claimed "the main objective in siliconization is to achieve the most homogenous possible coating"\(^{17}\).  

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While these generalizations are intuitive, they are too unspecific to enable process improvement. This project aimed to improve the definition of ideal baked-in glass siliconization in order to support future optimization and improve the injection performance of insulin-delivery devices.

The key findings include confirmation of significant interactions and factors of normal force and velocity with respect to friction. Additionally, baked-in siliconization amount was found to have an exponential relationship with the friction coefficient, suggesting that above a minimum amount, diminishing gains can be expected to reduce friction. Dry spots, through direct testing using the 3D-printed siliconization masks and indirect testing of the curved glass sections, were found to have a significant impact on friction, and should be avoided. This is consistent with the claim that “insufficient” siliconization could lead to poor mechanical performance, but now a method is proposed to define maximum dry spot defect size. Finally, use of a linear tribometer and other methods such as 3D-LSM proved to be useful tools in the characterization of baked-in glass siliconization performance.

7.2 Recommendations

The principal recommendations are to ensure that the baked-in glass siliconization amount exceeds the minimum threshold and that dry spots are eliminated. The specific minimum found in this study was 40% of the nominal siliconization amount. This amount should be verified for cartridges through confirmation testing. It is also suggested that production verifies both the amount sprayed as well as the amount achieved in order to catch defects such as clogged siliconization nozzles. While there does not exist a validated technology today to verify baked-in silicone coating thickness, it is advisable to pull samples and measure this on a regular basis for quality control. Production equipment settings, such as the dynamic spraying profile of diving nozzles, should be established to ensure dry spots do not occur.

Although resource limitations did not allow for testing of silicone emulsion concentration as part of the study, this is a factor with potential to affect resultant coating performance. It is suggested that production consider standardizing the concentration across production lines. Further, care should be taken when diluting the emulsion to the target concentration. Variability in this process could diminish production’s ability to produce consistently performing product.

The rubber stopper is obviously a critical component for injection performance. Geometry and material properties directly impact normal force, which was found to be very significant with respect to friction. Improving consistency in all respects for the stopper (manufacturing, assembly, siliconization, storage) will help reduce variation in pen performance.

Another aspect that Sanofi can control is the rubber stopper siliconization process. Currently Sanofi employs a dual-sourcing strategy that was previously discussed. Sanofi should take care that both RTS and internally siliconized stoppers perform equivalently. While there are benefits to the supply chain with this approach, such as risk mitigation and development of internal knowhow, it highlights a tradeoff in terms of introducing variability to the stopper, a key player in the stopper-silicone-glass frictional interface. If any link in the supply chain breaks down, it could nullify efforts to optimize the glass siliconization processes.
Finally, it is highly recommended that production and engineering organizations continue a dialogue, share information, and work together to integrate production process development with engineering design. Engineers in the Medical Devices group should be aware of the role of the friction coefficient in design. They should also understand how friction could fluctuate under different conditions: under-siliconized glass; presence of dry spots of different dimensions; higher normal force resultant from dimensional tolerance of components such as large stopper and/or small glass cartridge.

7.3 Future Work
There are several areas for further exploration. Most importantly, experimental results should be confirmed or amended through testing of whole glass cartridges, as opposed to glass plates. The 40% minimum amount may take on a different value when testing glass cartridges. One hypothesis is that the rubber stopper plows the silicone lubricant forward in a glass cartridge, whereas it may spread the silicone outwards on a plate. This difference in mechanism may yield different friction results. To test this, a custom tribometer that could measure friction of siliconized surfaces inside cartridges, while eliminating factors that confound many existing test methods, could prove valuable. Precision rubber O-rings used in lieu of rubber stoppers could be employed to improve consistency when testing siliconized glass cartridges for optimization purposes.

Additional parameters could be investigated such as the effects of baking temperature and time. The impact of position within the baking tunnel (left, middle, right) could be verified. It would be suggested that future studies attempt to collocate siliconization and baking processes to simplify logistics. Also, only a single rubber stopper version was tested and others could be considered for future work. The impact of rubber stopper siliconization could prove very interesting.

Finally, the normal force range tested was based on the stopper-cartridge interference fit and relevant dimensional tolerance. It did not include the additional normal force occurring when the stopper is compressed between the injection drive mechanism (e.g. spring) from above and incompressible fluid from below. It may be possible to measure the resulting total normal force with contact pressure paper or through other means. Future efforts may want to consider this presumably higher normal force scenario.

7.4 Final Considerations
There are a couple of scenarios that would render these project findings obsolete. For example, new materials are emerging that eliminate the need for siliconization entirely. Silicone-free systems may be in the not-too-distant future of insulin primary packaging. In the meantime, costs associated with alternative materials or required testing to qualify such materials remain prohibitively high. Another scenario is that the cartridge becomes consolidated into the insulin pen – this would most certainly have an impact on production processes.

Regardless of future events impacting insulin primary containers or glass siliconization, the approaches proposed in this work can be broadly applied. Design of experiments and employment of new test methods to characterize production processes and define defects are timeless.
8 References


25. Hasenclever S. Charakterisierung einer 1,5 mL Ampulle zur Verwendung in einem federgetriebenen Injektionssystem. 2014.


9 Appendix

9.1 ANOVA Tables

The following ANOVA tables correspond to the analysis for the Rubber Sphere and Rubber Stopper DOE.

Source | F-Value | P-Value
------- | -------- |--------
Model | 0,000   | 0,000
Linear | 0,000   | 0,000
Normal Force (mN) | 0,000   | 0,000
Linear Velocity (mm/sec) | 0,000   | 0,000
Spray Amount | 0,000   | 0,000
2-Way Interactions | 0,000   | 0,000
Normal Force (mN)*Linear Velocity (mm/sec) | 0,000   | 0,000
Normal Force (mN)*Spray Amount | 0,023   | 0,053
Linear Velocity (mm/sec)*Spray Amount | 0,000   | 0,000
3-Way Interactions | 0,000   | 0,000
Normal Force (mN)*Linear Velocity (mm/sec)*Spray Amount | 0,000   | 0,000
Error | 0,006   | 0,006
Total | 0,006   | 0,006

Figure 71 - ANOVA Table from Rubber Sphere DOE

Source | F-Value | P-Value
------- | -------- |--------
Model | 0,000   | 0,000
Linear | 0,000   | 0,000
Normal Force (mN) | 0,000   | 0,000
Linear Velocity (mm/sec) | 0,000   | 0,000
Spray Amount | 0,000   | 0,000
2-Way Interactions | 0,000   | 0,000
Normal Force (mN)*Linear Velocity (mm/sec) | 0,023   | 0,053
Normal Force (mN)*Spray Amount | 0,000   | 0,000
Linear Velocity (mm/sec)*Spray Amount | 0,000   | 0,000
3-Way Interactions | 0,949   | 0,949
Normal Force (mN)*Linear Velocity (mm/sec)*Spray Amount | 0,949   | 0,949
Error | 0,949   | 0,949
Total | 0,949   | 0,949

Figure 72 - ANOVA Table from Rubber Stopper DOE
9.2 3D-LSM Images

This section contains images taken of siliconized and baked glass plates using the 3D laser-scanning microscope. Some samples were carefully scratched using a fine gage needle in order to conduct a layer height measurement.

Figure 73 - 33% Spray Amount, 50x Magnification, Sample 7-4 from Main Glass Plate Test Batch
Figure 74 - 100% Spray Amount, 50x Magnification, Sample 9-4 from Main Glass Plate Test Batch

Figure 75 - 167% Spray Amount, 50x Magnification, Sample 11-4 from Main Glass Plate Test Batch

Figure 76 - 100% Spray Amount, Sample 15-1 from Final Glass Plate Test Batch
Figure 77 - 80% Spray Amount, Sample 16-1 from Final Glass Plate Test Batch

Figure 78 - 60% Spray Amount, Sample 17-1 from Final Glass Plate Test Batch
Figure 79 - 40% Spray Amount, Sample 18-1 from Final Glass Plate Test Batch

Figure 80 - 20% Spray Amount, Sample 19-1 from Final Glass Plate Test Batch
Figure 81 - 10% Spray Amount, Sample 20-1 from Final Glass Plate Test Batch