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NEXT

REPORT ON NEW SOLUTIONS FOR PHARMACEUTICAL PACKAGING

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A CASE STUDY BY
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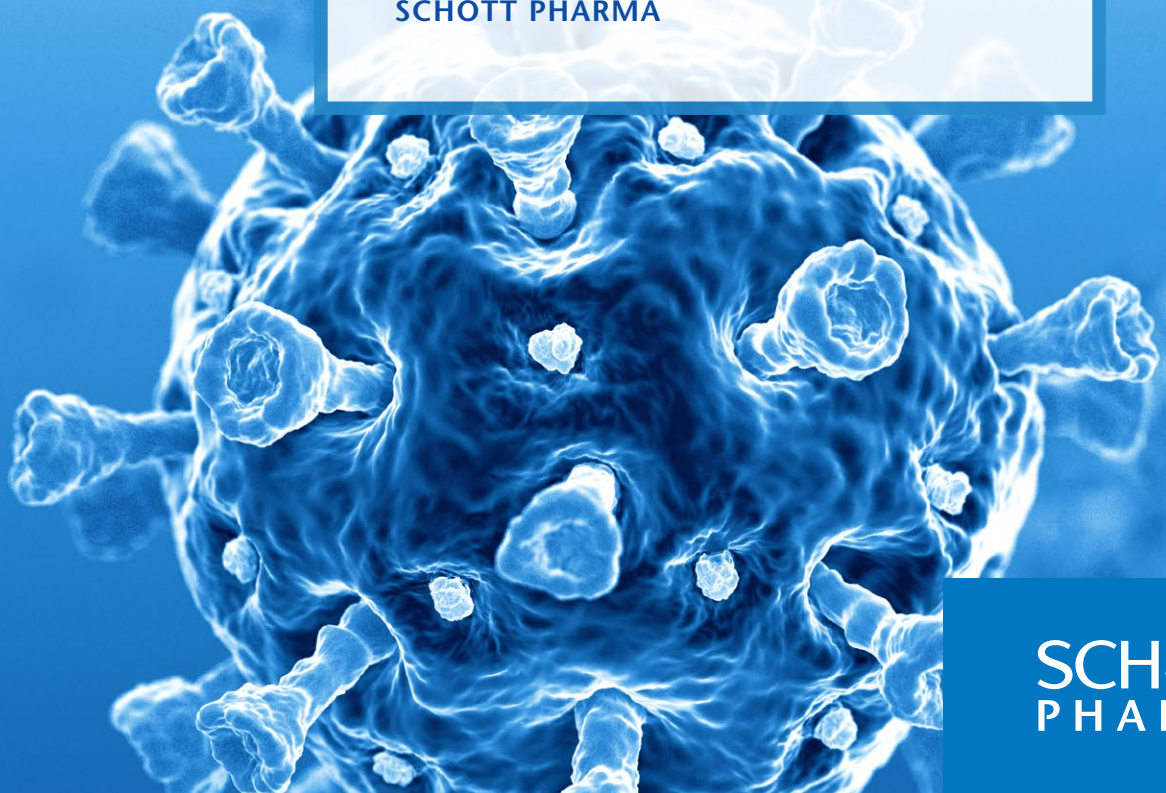


SCHOTT PHARMA

KEY CONSIDERATIONS IN SELECTING A POLYMER PREFILLED SYRINGE FOR LOW-TEMPERATURE mRNA VACCINES

A case study by Tom van Ginneken, Head of Global Product Management – Polymer Solutions, SCHOTT Pharma

During a pandemic state e.g. COVID-19, the most important goal is to get as many people vaccinated in the shortest time possible. Vaccination centers, with their highly efficient approach, were a paramount step to reach this goal. Multi-dose vials allowed for fast availability of vaccines and trained health care workers prepared the syringes around the clock to support the high vaccination rate.

With that in mind, most public health experts currently agree that the COVID-virus is here to stay. We are shifting from a pandemic state into an endemic one, which means that we will likely see a periodic re-vaccination such as a yearly booster shot for high-risk patients, similar to the flu shot.

This will drive a few changes to the vaccination approach. First, vaccination will likely happen more at a general practitioner or local pharmacy. Most developed countries have a vaccination rate of 50-80 % of the entire population with some countries reaching even a 90 % level for adults. The once so important vaccination centers are therefore disappearing because there is simply less need for efficient mass vaccination at a later stage.

Second, the current multi-dose vial requires multiple drug preparation steps, and once opened, the shelf life of the vial is limited to a couple of hours. If the physician doesn't have enough patients for a vaccination in this timeslot, the remaining drug in the vial has to be discarded. Therefore, we will see a shift towards single dose devices such as prefilled syringes (PFS).

Moving from a vial into a prefillable syringe is not an easy task and raises many technical questions. Certainly this is the case for mRNA based vaccines, which seem to be the preferred technology in the race to commercialize COVID vaccines. These mRNA vaccines are highly sensitive and require low temperature storage conditions which make them even more challenging to offer as a prefilled syringe.

In this article we will be addressing those technical challenges and provide supporting data to make this transition from vials to prefilled syringes for vaccines as easy as possible.

STERILITY BARRIER CAN BE CONTROLLED WITH THE RIGHT PFS COMPONENTS AND FILL- AND FINISHING PARAMETERS

Before arriving at the patient, a mRNA vaccine in PFS is frozen and undergoes one or more air transport cycles. These two steps can influence plunger movement due to freeze and underpressure. Once the plunger movement exceeds the distance between the first and the last sealing lip, it is defined as breaching the sterility barrier. It is therefore extremely important to understand this phenomenon and the factors that affect plunger movement.

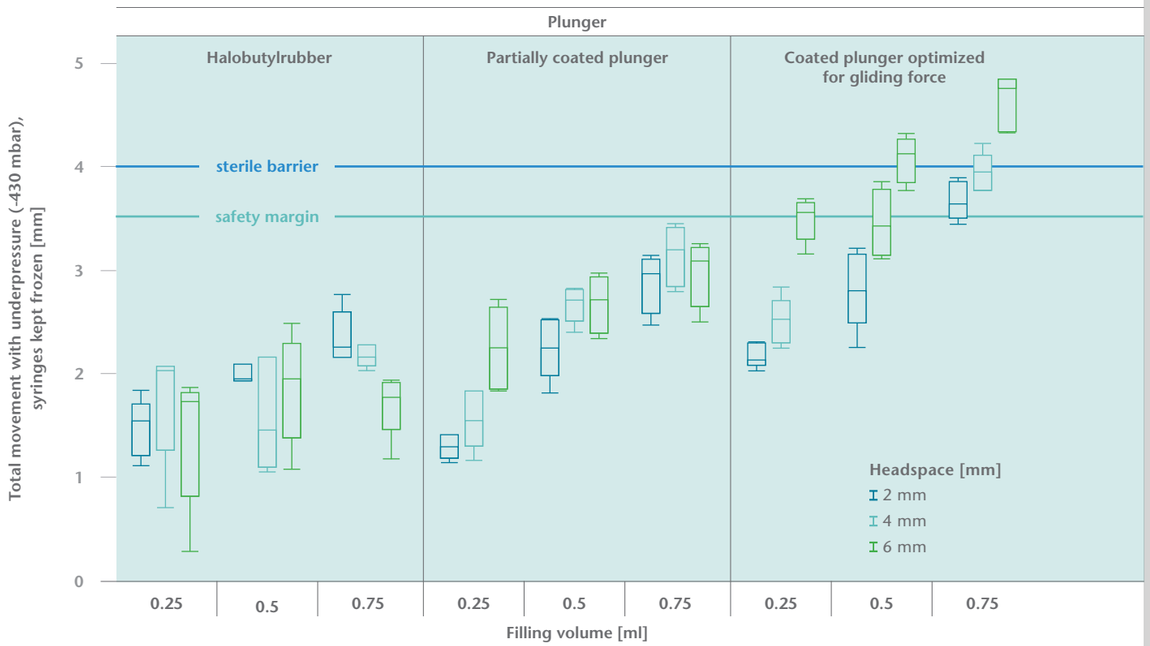
SCHOTT Pharma performed a design of experiments with different plunger materials, filling volumes, headspace sizes, filling medium, and freezing temperatures on SCHOTT TOPPAC® 1ml lg Cyclic Olefin Copolymer (COC) syringes to evaluate the impact of those variables. The maximal plunger movement was determined for the filled syringes that were frozen for 12 hours with one or multiple freezing cycles and at under pressure simulating worst case air transport in an non-pressurized aircraft.

Chart 1 shows that 3 factors have a significant influence on plunger movement:

– **Filling volume:** the bigger the filling volume the more plunger movement. More filling volume will expand more during freezing causing the plunger to move more.

– **Headspace:** the bigger the headspace the more plunger movement. Two factors are in play here: first, during freezing the air will contract in volume and therefore force the plunger to move towards the cone. The other force in play is the underpressure of the air transport simulation. This force will push the plunger towards the flange. Overall, the resulting force is pushing the plunger towards the flange.

CHART 1:
Maximal plunger movement (in mm) during -20°C and at an underpressure of 430 mBar



– **Plunger type and material:** the lower the plunger break loose and gliding properties are, the more the plunger will move. There are multiple plunger factors that could influence the gliding properties including: geometry, compression set, overlap with barrel, low friction coatings and sterilization mode. It is important to understand each parameter in order to select the right plunger and material for the applications.

Another critical point is that the design of the plunger has an impact on the sterile barrier. The distance between the first and last sealing lip is not identical for all plungers, which might affect the choice of plunger as well.

Chart 2 is showing the statistical significance of all parameters which were tested in the design of experiments. All parameters with a P-value of smaller 0.05 are deemed to have a significant influence on the plunger movement. The smaller the P-value, the bigger the influence of this parameter on the plunger movement.

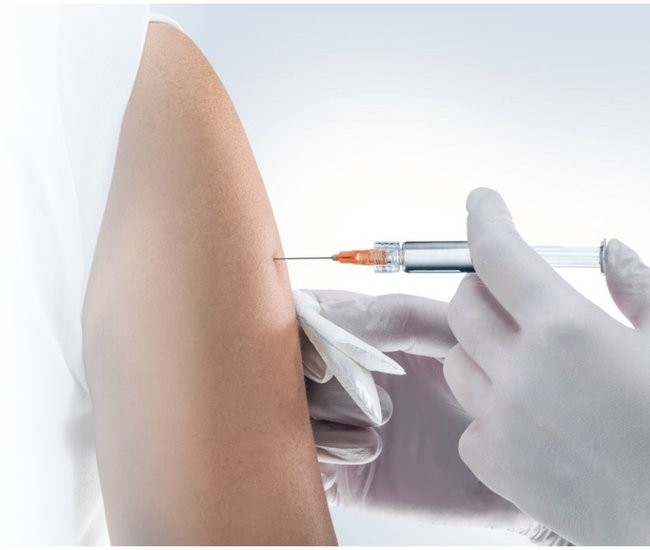


CHART 2:
Statistical analysis on impact plunger movement (for frozen syringes and at underpressure)

SOURE	LOG WORTH	P VALUE
Plunger	42.398	0.00000
Filling volume [ml]	28.099	0.00000
Headspace [mm]*Plunger	11.749	0.00000
Headspace [mm]	8.350	0.00000
Filling volume [ml]*Plunger	5.056	0.00000
Filling volume [ml]* Headspace [mm]	2.017	0.00961
Filling volume [ml]* Headspace [mm]*Plunger	1.057	0.08777

CHART 3:
Maximum plunger movement for different drug solutions.
Comparison between Placebo and WFI

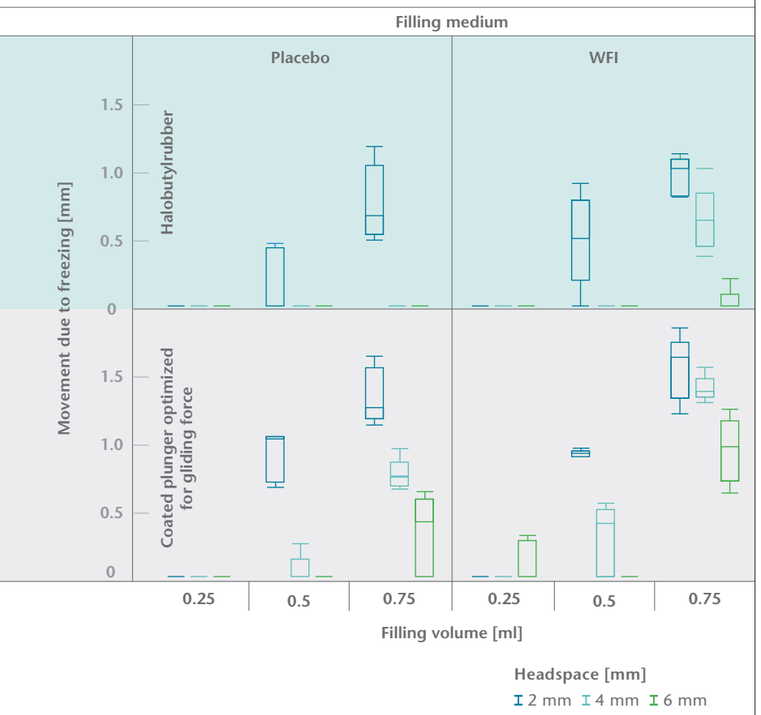
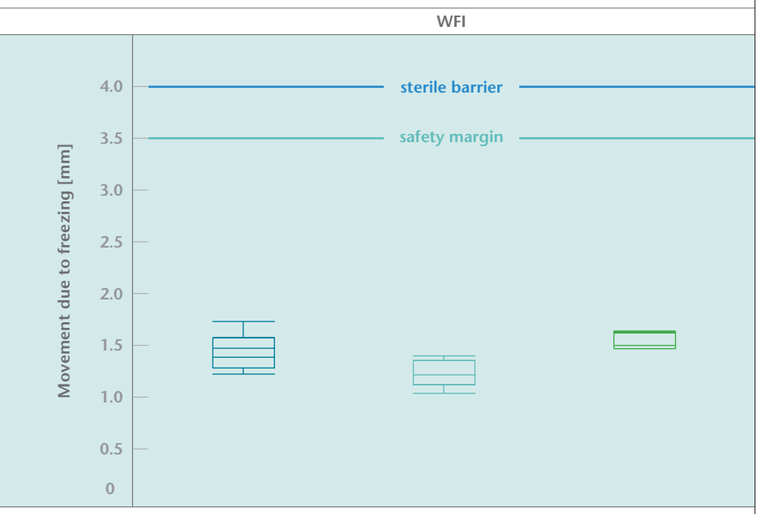


CHART 4:
Maximum plunger movement at different freezing temperatures



Two other parameter have been investigated, but didn't show any significant impact on the plunger movement:

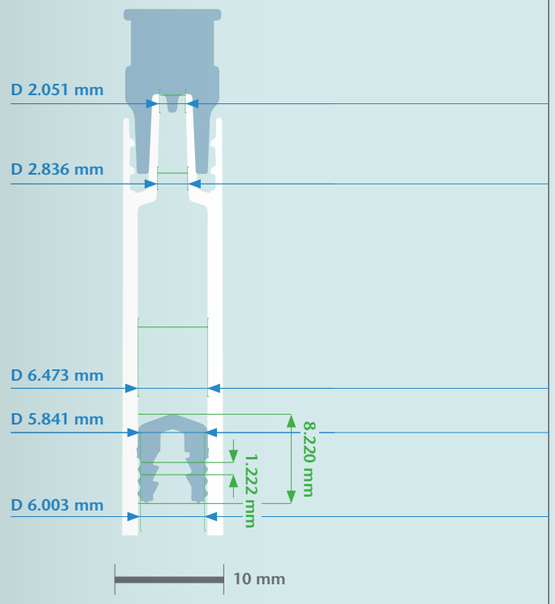
– **Drug solution:** water for Injection (WFI) was compared with a mRNA placebo solution (8.7 % sucrose solution) with no significant impact on plunger movement, which means that the results of this study executed on WFI should be comparable with drug solutions.

– **Freezing temperature:** 20°C, -50°C and -80°C were compared to investigate impact on plunger movement. No significant differences were seen. The density – temperature curve of ice shows that with decreasing temperate, the density of ice is increasing, indicating that the lower the temperature the less volume the ice will have. Because filling volume is the second most significant factor to plunger movement, right after plunger type, we can expect no significant changes in plunger movement at different freezing temperatures.

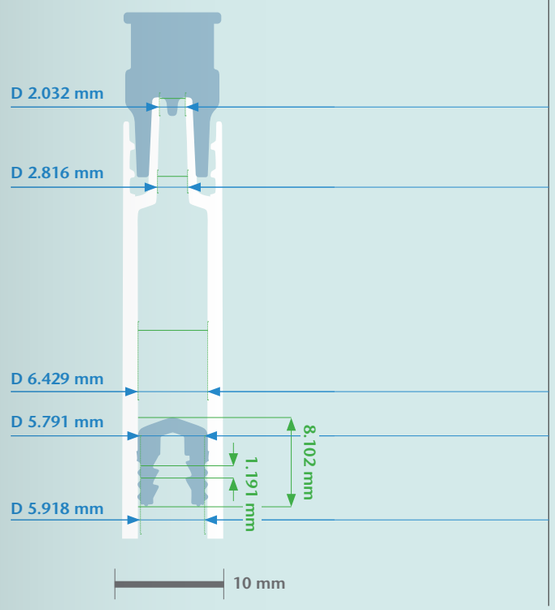
This study's recommendations are:
– Reduce headspace as much as possible (≤4mm)
– Lower fill volume (≤0.5mL)
– Choose a plunger with a higher break loose and gliding force

Overall, the key observation is that plunger movement can be controlled, and breaching sterility can be avoided by choosing the right primary packaging and filling parameters: filling volume, headspace size, and plunger type and material.

Before freezing without plunger rod



Frozen at -80°C without plunger rod



CONTAINER CLOSURE INTEGRITY (CCI) IS MAINTAINED AT TEMPERATURES DOWN TO -100°C

A prefilled syringe is a complex system comprising of multiple components: a closure for the cone side, a plunger and of course, the syringe barrel. There is a broad range of materials for those components, Chlorobutyl elastomers, Bromobutyl elastomers, and various coatings used on the syringe barrel and plunger. These materials all have different thermal expansion coefficients, meaning that they will shrink at different rates when frozen. This is extremely important for CCI because reduced sealing overlap between plunger and barrel will drastically increase the risk of CCI issues.

Elaborating on the plunger as an example: the plunger has two functions: keeping the drug stable by providing a barrier against leakage and microbial ingress as well as proper functionality during drug administration. There is a certain compromise between good sealing properties and as-low-as-possible gliding forces. For mRNA specific cold chain conditions, the plunger and the barrel will start shrinking, each at their respective material property rates. The COC material shrinks at a similar rate as the rubber components. This can be recognized in Figure 1: the inner diameter of the COC syringe shrinks roughly 0.044mm while the rubber plunger outer diameter shrinks with 0.050mm, 0.047mm and 0.040mm for the 3 respective sealing lips (fig 2). These absolute shrinkage values are comparable. Therefore even at -100°C, no CCI breach with a COC syringe is seen. This story looks completely different with other primary packaging materials such as glass. The thermal expansion coefficient for type I glass is at least a factor 10 smaller than COC or the rubber components. The glass container will shrink at a much lower rate than the rubber plunger, reducing the sealing overlap between plunger and syringe barrel and ultimately could be a potential risk for leakage or microbial ingress.

FIGURE 1:
CT scan of identical COC syringe systems at different temperatures

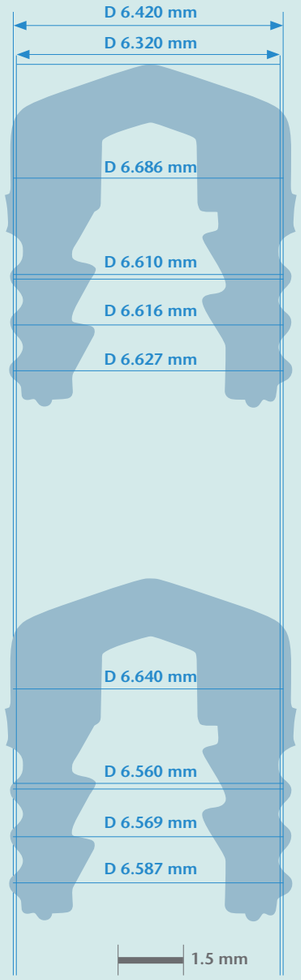
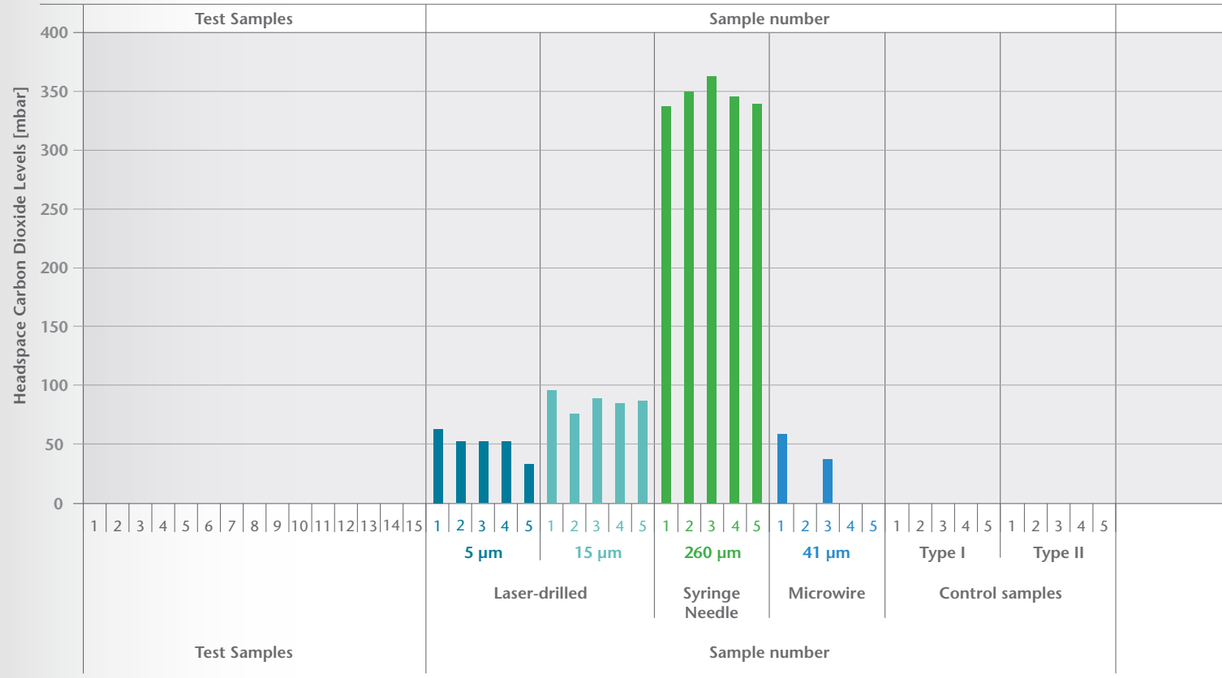


FIGURE 2:
CT scan of identical plunger in an uncompressed state (not in the syringe)

This reduced sealing overlap hypothesis was investigated further by executing an external headspace study. 15 empty COC syringes were stored for 24 hours on dry ice. Temperatures can fluctuate and reach -100°C during dry ice storage. The syringes were tested with qualified Lighthouse Instruments FMS-Carbon Dioxide headspace analyzer (Model FMS-CO2). Different positive control samples (laser drilled holes in plunger, a needle or a microwire bypassing the sealing lips of the plunger) were added to the test regimen to confirm the test method. The cone closure and the plunger were also individually tested to locate the source of leakage if there was one.

None of the 15 samples showed signs of carbon dioxide ingress. The data clearly indicate that even at an extremely low temperature of -100°C, the COC syringe system maintains CCI.

CHART 5:
Headspace carbon dioxide levels after 24 hours on dry ice



All 15 samples showed no sign of CO2 ingress. **This novel information clearly shows that even at an extremely low temperature, at -100°C, the COC syringe system maintains CCI.**

POTENTIAL DRUG INTERACTION CAN BE REDUCED WITH A CROSS-LINKED SILICONIZATION

A standard prefillable syringe has a lubrication layer to allow the plunger to move inside the barrel. There are many different technologies of lubrication but the most common one is sprayed-on silicone-oil. The silicone oil used here is normally not toxic for the human body, but an abundance of silicone oil can create sub-visible particles that could react with the drug and lower its efficacy.

One initial evaluation is the determination of leachable free-silicone amount, which is the free silicone oil that could interact with the drug substance. This value gives an indication on the potential risk of drug – lubrication interaction. Two lubrication technologies will be compared: the standard silicone-oil spraying and a cross-linked technology.

For each variation 5 syringes were filled with water for injection (WFI) and either went through 3 freeze – thawing cycles at -20°C or were stored at 5°C. The extract from 5 samples was pooled and analyzed by graphite furnace atomic absorption for free silicone oil. This test method has a detection limit of 0.2 mg/L.

	Time	Free silicone [mg/L] 3x frozen at –20°C and thawed	Free silicone compared to standard SCHOTT TOPPAC®	Free silicone [mg/L] stored at 5°C	Free silicone compared to standard SCHOTT TOPPAC®
SCHOTT TOPPAC® cross-linked siliconization Standard cross-linked silicone	0d	0.23	N/A	< 0.2	N/A
SCHOTT TOPPAC® sprayed siliconization Sprayed on DC360, 0.55 mg/barrel	0d	5.6	24 TIMES	1.09	5 TIMES

TABLE 1:
Leachable free silicone quantities of two siliconization technologies

Table 1 shows clear differences between sprayed on silicone oil and cross-linked silicone. Even for the reference samples which were stored at 5°C, the leachable silicone amount for sprayed on silicone was at least 5 times higher. These results were even more pronounced at lower temperatures. After 3 cycles of freezing and thawing at -20°C, the leachable silicone quantities increase for both siliconization technologies, but sprayed silicone is much more affected. 24 times more free silicone oil was observed for the syringe siliconized with sprayed on siliconization.

The second consideration regarding siliconization is the amount of sub-visible particles. These sub-visible particles could react with the drug and negatively impact its efficacy.

In this study, 4 different siliconization technologies were compared to evaluate siliconization impact on sub-visible particles: cross-linked siliconization, the same siliconization mixture used for cross-linked siliconization but without the activation of the cross-linking process, silicone oil, and finally silicone free syringes were used as a reference.

Sub-visible particles are determined via the method described in USP <788> via light obscuration.

Syringes were filled with particle free WFI and stored at different storage conditions: room temperature, refrigerated temperatures 2-8°C, -20°C and also at elevated temperatures 40°C. The syringes were stored for three timepoints 12 hours, 31 days and 121 days.

The chart 6 provides 4 key messages:

1. Storage at freezing conditions are the worst case for sub-visible particles. The freezing and thawing cycle shows significantly higher sub-visible particles for all siliconization technologies.

2. Longer storage conditions tend to slightly increase sub-visible particles.

3. There is a good probability that the particles observed are coming from the siliconization process because the silicone free syringes are showing almost no sub-visible particles.

4. The siliconization technology has a significant impact on the sub-visible particle burden.

Two examples have been provided where the siliconization technology has significant different outcomes that could potentially impact drug stability. It is therefore important to consider the impact of siliconization technologies on a specific drug application. The results collected at -20°C are likely applicable for lower temperatures down to -100°C as it is the thermodynamic phase transition (crystallization) effect of the freezing process that is putting stress on the lubrication layer, and not the temperature itself.

This study recommends cross-linked siliconization technology for mRNA applications as it provides superior results in leachable silicone free quantities and sub-visible particle burden. This cross-linked siliconization process is standard for the SCHOTT TOPPAC® COC syringe portfolio. The immobilized lubrication layer not only provides great drug stability properties, but also stable gliding performance at different storage conditions, which will be discussed further in the next chapter.

This study recommends cross-linked siliconization technology for mRNA applications as it provides superior results in leachable silicone-free quantities and sub-visible particle burden.

CHART 6:
Influence of storage conditions and siliconization technologies on sub-visible particle count

SYRINGE FUNCTIONALITY IS NOT IMPACTED WITH LOW TEMPERATURE STORAGE

Controlling the plunger movement and having a sterile syringe system that maintains CCI is necessary for a robust system, but the syringe also needs to perform well after these low temperature storage conditions. If the low temperatures increases the probability for breakage or the likelihood that a plunger doesn't move after freezing, then the syringe system would be useless. Therefore SCHOTT Pharma performed studies to compare polymer syringe functionality after freezing with room temperature stored syringes.

Syringes were filled with WFI and went through 3 cycles of freezing to -20°C and -50°C and thawing to room temperature. The break loose and gliding forces were tested at room temperature.

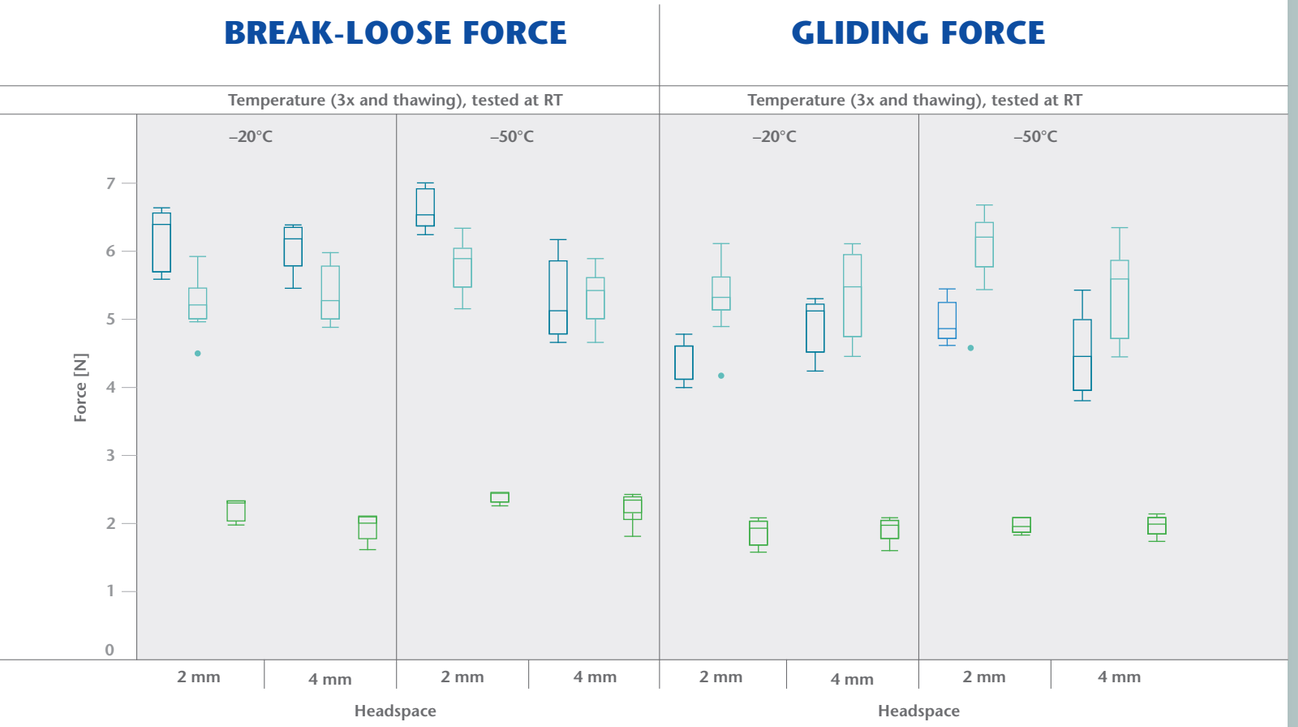


CHART 7:
Influence of temperature and plunger types on break-loose and gliding forces

	–20°C	–50°C	–80°C
Optical properties	■	■	■
Stress cracking	■	■	■
Breaking-Cracks	■	■	■
Luer Lock Adapter damage	■	■	■

■ No significant changes versus syringes stored at room temperature

TABLE 2:
Impact of temperature on syringe functionality

Chart 7 shows the results of that study:

– There is a limited difference between break loose forces and gliding forces. Due to the stickiness effect of the rubber, break loose forces tend to increase over time. In this application, because of the plunger movement during freezing and thawing, the thawing process already forces the plunger to move and therefore little increase in break loose force is observed.

– There is no significant difference observed between storage temperatures. The -20°C and -50°C break loose and gliding forces are comparable with room temperature stored syringes. It seems the freezing process doesn't have a significant impact on the forces needed to perform the injection. This means that injection forces are expected to be comparable to room temperature stored syringes even at -100°C.

– Headspace didn't seem to have an impact on the forces.

– Significant impact of plunger type can be observed, which was expected. Different types of plungers have their own gliding profile and characteristics. It is not surprising that the plunger developed with improved gliding properties performs the best.

Another risk of extreme low storage temperatures is a change in PFS optical properties or breakage.

Table 2 gives an overview of the most important functionalities tested at different temperatures compared to syringes stored at room temperature. No significant changes were noticed.

LIPID NANO PARTICLES (LNP'S) ARE JUST AS STABLE IN POLYMER SYRINGES AS THEY ARE IN GLASS VIALS

Injecting unprotected mRNA into the body would not lead to the desired therapeutic outcome. mRNA is extremely delicate and enzymes in our body would attack and destroy the mRNA before it could reach the required cells. LNP's are a lipid layer that fully encapsulates the mRNA thereby protect the mRNA and allow it to travel to the dedicated cells to perform its therapeutic function. The stability of these LNP's is paramount to maintain the stability and proper function of the mRNA.

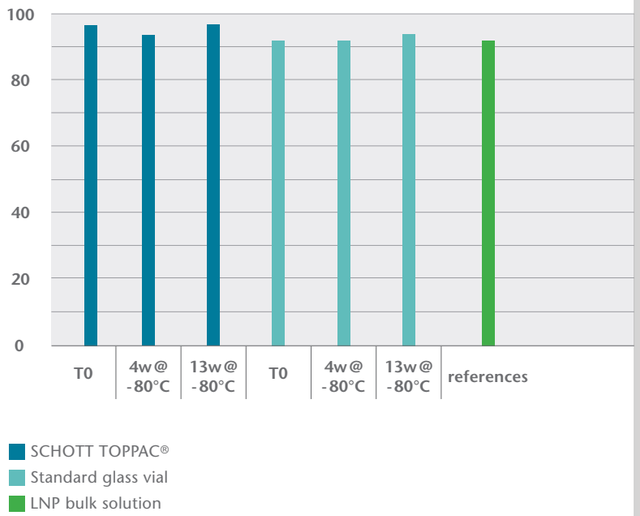
Three analytic methods have been chosen to characterize the syringe compatibility with LNP's:

1. **LNP particle size**
2. **Polydispersity index**
3. **LNP adsorption**

1.

LNP particle size is measured with a dynamic light scattering technique from Zetasizer. Tests were executed in collaboration with IPBW (Institut für Pharmazeutische und Biomedizinische Wissenschaft) at the university of Mainz. SCHOTT TOPPAC® syringes are compared with glass vials after storage at -80°C. No significant difference was observed between LNP size between solution stored in a glass vial or a polymer syringe.

CHART 8:
Diameter in nm



2.

The **polydispersity index (PDI)** gives an overview of the homogeneity of the particle size and therefore provides valuable feedback on the particle stability and/or potential agglomeration. The PDI is measured with a dynamic light scattering technique from Zetasizer. Tests were executed in collaboration with IPBW at the university of Mainz. SCHOTT TOPPAC® syringes are compared with glass vials after storage at -80°C. The lower the PDI, the lower the particle size distribution is, and therefore shows higher LNP stability. Values of 0.3 and below are most commonly deemed acceptable in practice for drug delivery applications using lipid-based carriers. No significant difference was observed between LNP PDI between solution stored in a glass vial or a polymer syringe.

CHART 9:
PDI in nm

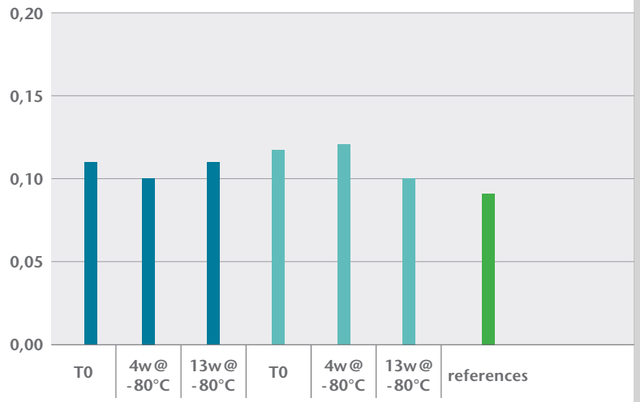
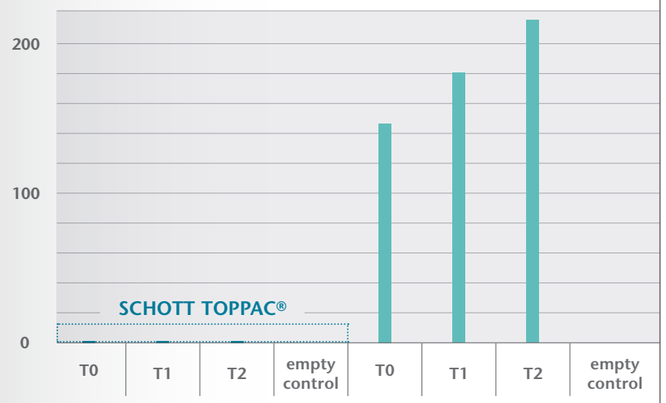


CHART 10:
Score (x10[12]) for lipid factor



Storage with LNPs
T0: initial (overnight, -80°C)
T1: 13 weeks, -80°C
T2: overnight, -80°C + 13 weeks 2-8°C

3.

LNP adsorption on the inside surface of the container can reduce the concentration of the active pharmaceutical ingredient. A lower adsorption level is desired, giving an indication of the chemical inertness of the container.

The inner surface of the containers are analyzed and deposits are identified using ToF-SIMS (Time-of-Flight Secondary Ion Mass Spectrometry) spectroscopy. Multivariate curve resolution (MCR) technique was applied to extract a pure component spectrum from a ToF-SIMS spectrum which contains mixed materials information. A pure component spectrum extracted by MCR is useful for selecting important fragment ions of each material in a sample. These pure spectra are called loadings and can be matched with signal patterns expected for the analyzed sample, e.g. silicone. To compare the different sample sets and various time points, MCR was conducted for all samples at once. For each loading (pure spectrum), this results in scores, which describe the occurrence of this loading in the recorded spectra. For easier comparison, the scores of one loading were normalized to the maximum score value and are shown in the stacked bar diagrams.

For all three aging and temperature points we see a clear lower adsorption for the SCHOTT TOPPAC® polymer syringes compared to glass vials.

These three analytic methods provide a robust indication on the stability of LNP based solutions stored in a polymer syringe. No significant difference was observed in particle size or polydispersity between a glass vial and a polymer syringe. Even no significant difference was seen between the reference sample and the syringe stored solutions. A lower LNP adsorption effect was noticed for polymer syringes compared to glass vials.

In conclusion, the requirements for mRNA-based vaccines were analyzed and SCHOTT TOPPAC® syringes were subjected to these conditions. The broad data package generated shows that these COC syringes are ideally suited for low temperature vaccine storage with the following major conclusions:

– **Plunger movement does not breach the PFS sterile barrier, but understanding the syringe and fill- and finishing parameters that have an impact helps to control this phenomenon.**

– **COC syringes can maintain container closure integrity down to -100°C because the thermal expansion coefficients of the different syringe components are similar.**

– **Cross-linked siliconization offers superior performance for leachable silicone oil quantities and sub-visible particles. This reduces the risk for any drug interaction and loss of efficacy.**

– **Normal syringe functionalities are maintained even at deep-cold storage.**

– **LNP stability in polymer syringes is comparable with glass vials**

For more information, please visit:
www.schott-pharma.com

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Tom Van Ginneken studied chemical engineering in Antwerp and added a MBA from the University of Sankt Gallen to his resume. After working in the chemical and pharmaceutical sector in Belgium for 3 years he joined SCHOTT in 2008. After different positions in the pharmaceutical product development department he became part of the product management team with focus on the SCHOTT TOPPAC® brand. In this role he works on the strategic orientation and innovation pipeline of the product group.

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