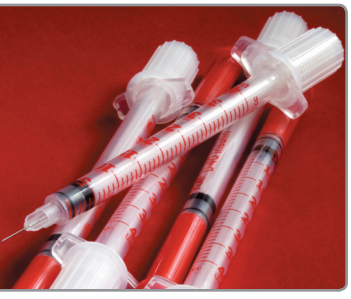

A Study of Leachable Silicone Oil in Simulated Biopharmaceutical Formulations

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Leachable silicone oil may have an effect on large-molecule APIs, making it important to establish a robust analytical method to detect and quantify the substance.

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Biopharmaceutical products are becoming the driving force of the pharmaceutical industry. The primary route of administration for biopharmaceutical products is by injection, and the commonly used container/closure systems use glass vials with rubber stoppers and prefilled syringes.

Silicone oil has been widely used to coat the components of container/closure systems for biopharmaceutical products, including syringe barrels and plungers for prefilled syringes and stoppers for glass vials (1). The drug product formulations typically are in direct contact with the silicone oil coating over long periods of time; there is a general concern that the silicone oil may leach into the drug product formulations, which may affect the drug product's purity and efficacy (2, 3, 4).

Unlike small-molecule pharmaceutical products, leachable silicone oil may affect the conformation of the large-molecule APIs of biopharmaceutical products, which can cause protein denaturation and, in the long term, can lead to protein aggregation (3). Protein aggregates can result in a loss of protein biological activity and may induce immunogenic effects (4) when injected into the human body. Therefore, it is important to evaluate leachable silicone oil for biopharmaceutical products.

There are different methods for analyzing silicone oil that, in general, fall into two categories: one is based on the polymeric nature of silicone oil, using a gel permeation chromatography column to separate silicone oil from the drug product ingredients. Silicone oil molecules typically do not contain a chromophore, so the commonly used ultraviolet detector is not suitable. The detectors typically used for silicone oil analysis are refractive index detector, evaporative light scattering detector, charged aerosol detector, etc. The second category of methods is based on silica-specific techniques, such as atomic absorption spectroscopy, inductively coupled plasma–atomic emission spectroscopy , also referred to as inductively coupled plasma–opti-

cal emission spectrometry (ICP–OES), and inductively coupled plasma–mass spectrometry (ICP–MS). In these methods, organic solvents such as xylenes, toluene, and others are used to dissolve and separate the silicone oil from any inorganic silica.

The objectives of this study were to:

- Evaluate an ICP–OES method for the analysis of leachable silicone oil amounts in simulated biopharmaceutical formulations
- Quantify silicone oil in typical pharmaceutical formulations (5) and evaluate the impact of commonly used ingredients on the amount of leachable silicone oil.

In this study, an ICP–OES method was developed to quantify the amount of leachable silicone oil. Leachable silicone oil in aqueous biopharmaceutical formulations was extracted with an organic solvent, either with liquid-liquid extraction or solid-phase extraction, and the organic solution was analyzed directly with ICP–OES. Method performance such as method sensitivity, linearity, non-interference, relative response factors of different grades of silicone oil, and method accuracy were evaluated.

The study was followed by an evaluation of the leachable silicone oil amount in various simulated biopharmaceutical formulations stored in silicone-coated pre-fillable syringes. Formulations of simple phosphate buffers—and those containing co-solvents, bulking agents, chelating agents, and surfactants—and with different pH levels were added to the pre-fillable syringes and stored at 5 °C, 25 °C, and 40 °C for a period of time and then analyzed for leachable silicone oil amounts. The impact of pH, co-solvent, surfactant, chelating agent, and bulking agents as well as storage temperatures on the amount of leachable silicone oil were investigated. Surfactant was found to be the most important factor affecting the amount of leachable silicone oil. Co-solvent, pH, and temperature also affected leachable silicone oil amount, while bulking agents, chelating agents, and buffer did not have a significant impact on the leachable silicone oil amount. Overall leachable silicone oil represented a small portion of the coated silicone oil. Up to 2.1 µg/mL or 4.2 µg/syringe of leachable silicone oil was observed, which represented less than 2% of the total coated silicone oil.

The study design

Silicone-oil coated pre-fillable syringes (Becton Dickinson) were used for the test system for this study. The total amount of silicone oil coating the inside of the pre-fillable syringes was determined by extracting the syringes with xylenes, followed by analyzing the extraction solution by ICP–OES. Xylenes is a strong solvent for silicone oil and extracts out all coated silicone oil in the pre-fillable syringes. The amount of silicone oil in the pre-fillable syringes was determined to be 302 µg/syringe.

The standard used for quantitation was a silicone oil (Sigma Aldrich) with a viscosity of 350 cSt and 100% purity.

The simulated biopharmaceutical formulations selected for the study included simple phosphate buffers with varying concentrations of propylene glycol (co-solvent), polysorbate 80 (surfactant), ethylenediaminetetraacetic acid (EDTA) (chelating agent), various sugars (bulking agents), and sodium chloride. A total of 15 different formulations were used in this study, as summarized in **Table I**.

The solutions of simulated biopharmaceutical formulations were added to the pre-fillable syringes, 2 mL per syringe, and the syringes were then stored in chambers at 5 °C, 25 °C, and 40 °C. The syringes were pulled from the chambers after 30 days, and the contents were transferred to silicone oil-free glass contain-

Table I: Simulated biopharmaceutical formulations for leachable silicone oil study.

Formulation number	Formulation	Buffer 20 mM	Bulking agent	Stabilizer	Tonicity modifier	Chelating agent	Surfactant	Co-solvent (propylene glycol)
1	Phosphate buffer	pH 6.8 Phosphate						
2	Buffer with co-solvent	pH 6.8 Phosphate						1%
3								2%
4								5%
5								10%
6	Chelating agent	pH 6.8 Phosphate	7% Sucrose	Sucrose	150 mM NaCl	0.1 mM EDTA		
7		pH 6.8 Phosphate	7% Sucrose	Sucrose	150 mM NaCl	0.5 mM EDTA		
8	Surfactant	pH 6.8 Phosphate	7% Sucrose	Sucrose	150 mM NaCl	0.1 mM EDTA	0.05% Tween 80	
9		pH 6.8 Phosphate	7% Sucrose	Sucrose	150 mM NaCl	0.1 mM EDTA	0.1% Tween 80	
10		pH 6.8 Phosphate	7% Sucrose	Sucrose	150 mM NaCl	0.1 mM EDTA	0.5% Tween 80	
11		pH 6.8 Phosphate	7% Sucrose	Sucrose	150 mM NaCl	0.1 mM EDTA	1.0% Tween 80	
12	pH	pH 5.0	7% Sucrose	Sucrose	150 mM NaCl	0.1mM EDTA	1.0% Tween 80	
13		pH 8.2	7% Sucrose	Sucrose	150 mM NaCl	0.1 mM EDTA	1.0% Tween 80	
14	Bulking agent	pH 6.8 Phosphate	7% Mannitol		150 mM NaCl	0.1 mM EDTA		
15		pH 6.8 Phosphate	7% Trehalose	Trehalose	150 mM NaCl	0.1 mM EDTA		

ers, then analyzed for leachable silicone oil using the ICP–OES method described in **Table II**.

Prior to ICP–OES analysis, the leachable silicone oil in the aqueous formulation solutions was extracted with an organic solvent, xylene, to avoid interference from inorganic silica. Inorganic silica was likely to be present in the aqueous formulations after the formulations were stored in the glass syringes for a month. Liquid-liquid extraction and solid-phase extraction were used to extract the leachable silicone oil from the aqueous formulation solutions.

The liquid-liquid extraction procedures were used for all formulations with no surfactant. Equal volumes of formulation solution and xylene were used for the liquid/liquid extraction. The xylene solution was then used for ICP–OES analysis.

For formulations with surfactant, liquid-liquid extraction with xylene caused excessive emulsion and made it difficult to separate the organic layer from the aqueous layer. Therefore, a solid-phase extraction method was used. A Bond Elut Plexa (Agilent, Part #12259506), with a styrene-divinyl benzene copolymer, was used for extraction. One milliliter of formulation solution was eluted through each column under ambient conditions and dried for one hour under a vacuum of 15–20 mmHg. The columns were eluted with three separate 5-mL aliquots of dichloromethane (DCM) under ambient conditions, which were concentrated to near dryness under nitrogen flow. One milliliter of xylene was added into the residue and used for ICP–OES analysis.

Evaluation of the ICP–OES method

To evaluate the ICP–OES method as a means to analyze leachable silicone oil in simulated biopharmaceutical formulations, this study looked at the following factors: the relative response factor of silicone oils with different molecular weights, method sensitivity, method non-interference, and linearity.

Relative response factor. Usually, leachable silicone oil quantitation will need to use a silicone oil standard of different molecular weight and molecular-weight

Table II. Inductively coupled plasma/optical emission spectrometry (ICP-OES) method conditions.

Instrument	Thermo iCAP 6500 Duo		
Plasma view	Axial		
Analyst	Si (251.611 nm)		
Plasma	Radio frequency power	1200 W	
	Gas flow	Auxiliary (Ar)	1.0 L/min
		Nebulizer (Ar)	0.90 L/min
		Additional gas (20% 02, 80% Ar)	0.125 L/min
		Purge	Normal
Nebulizer	PFA–ST microflow, 20 µL/min		
Injector	2.0 mm inner diameter		
Spray chamber	Quartz		
Peristaltic pump	Flush rate		10 rpm
	Sample flush time		120 seconds
	Pump stabilization time		15 seconds
	Analysis pump rate		10 rpm
	Diluent rinse		15 seconds
Sample options	Analysis mode		Precision
	Repeats		3

distribution than leachable silicone oil. For accurate quantitation of leachable silicone oil, the silicone oil standard and the leachable silicone oil must have the same response factor.

There are several reasons why the molecular weight and molecular weight distribution of the leachable silicone oil and silicone oil standards need to be different:

- There are different grades (e.g., silicone oil of different average molecular weight) of silicone oil used for the coating of container/closure components. The end-user of the prefilled syringes may not necessarily know the exact grade of silicone oil used for their products.
- The molecular weight and molecular-weight distribution of the leachable portion of silicone oil may not be the same as those coated on the container/closure components. For example, the high-molecular-weight portion silicone oil may not leach out the same way as the low-molecular-weight portion silicone oil.
- The components of the container/closure systems may be coated with different grades of silicone oil. For example, the syringe barrel and plunger of a prefilled syringe may be coated with two different grades of silicone oil. Therefore, the leachable silicone oil may be a mixture of the two grades of silicone oil.

To use one silicone oil standard to quantitate leachable silicone oil of different average molecular weight and molecular-weight distribution, the response factor of the silicone oil of different average molecular weight and molecular-weight distribution must be the same or the relative response factor must be known. To evaluate the relative response factor of different silicone oils, five silicone oil standards with viscosity ranging from 50 cSt to 1000 cSt prepared at 10 ppm in xylene solution were analyzed for determining the relative response factors against the standard silicone oil of cSt 350.

In addition, volatile cyclic oligomers of silicone oil—hexamethylcyclo-trisiloxane (D3), octamethyl-cyclotetrasiloxane (D4), and decamethyl-cyclopentasiloxane (D5)—also were evaluated for their relative response factors against the silicone oil standard. The results are summarized in **Table III**.

The data indicate that the ICP–OES response factor of the silicone oil of different molecular weights were

Table III. Relative response factors of silicone oil of different molecular weight.

Silicone oil viscosity(cSt)	Average molecular weight*	Relative response factor
Plasma view	3800	0.99
Analyst	5970	0.97
350 cSt	13,700	0.99
500 cSt	17,300	0.99
1000 cSt	28,000	0.99
D3 (hexamethylcyclotrisiloxane)	222	0.72
D4 (octamethylcyclotetrasiloxane)	296	0.42
D5 (demethylcyclopentasiloxane)	370	0.36

*The average molecular weight data are from *Viscosity Correlation to Molecular Weight for Clearco PSF Fluids* (6). The exact molecular weights of the silicone oil used in this study may be slightly different; the molecular weights are included for information purposes.

Table IV. Non-interference results.

Formulations	5 °C	25 °C	40 °C
1	0.035	0.031	0.026
2	0.022	0.009	0.007
3	0.009	-0.004	-0.003
4	0.028	0.024	0.018
5	0.011	0.016	0.012
6	0.004	-0.012	-0.006
7	-0.006	-0.005	-0.008
8	0.015	0.022	0.017
9	-0.025	-0.062	-0.089
14	-0.007	-0.004	-0.004
15	0.015	0.026	0.025

virtually the same and were independent of the viscosity (e.g., the average molecular weight and molecular-weight distribution). Therefore, a silicone oil standard of one molecular weight and molecular weight distribution can be used for the quantitation of leachable silicone oil of different average molecular weight and molecular weight distribution.

The data also show that response factors for the volatile silicone oil oligomers were lower than the silicone oil standard. This indicates that a portion of the volatile cyclic siloxanes escaped prior to atomization because of their volatility and were not detected. Therefore, volatile cyclic siloxanes will not be accurately quantitated by ICP–OES (e.g., their amounts will be under-estimated).

Method sensitivity. The ICP–OES method did not have a response distinguishable from the background noise when silicone oil concentration was below 0.1 ppm. When increasing the silicone oil concentration above 0.1 ppm, the response gradually became more distinguishable from the noise. The noise level varied significantly after adequate buildup of carbon within the instrument detector during analysis, affecting instrument sensitivity and precision. For the purposes of this study, any response with a reading below 0.1 ppm was considered noise.

Silicone oil at a concentration of 0.5 ppm can be measured with good precision. Six measurements of 0.5 ppm silicone oil solution in xylene yielded responses as follows: 0.5205, 0.5176, 0.5283, 0.5293, 0.5240, and 0.5289. The percent relative standard deviation of the six measurements was 1.0%.

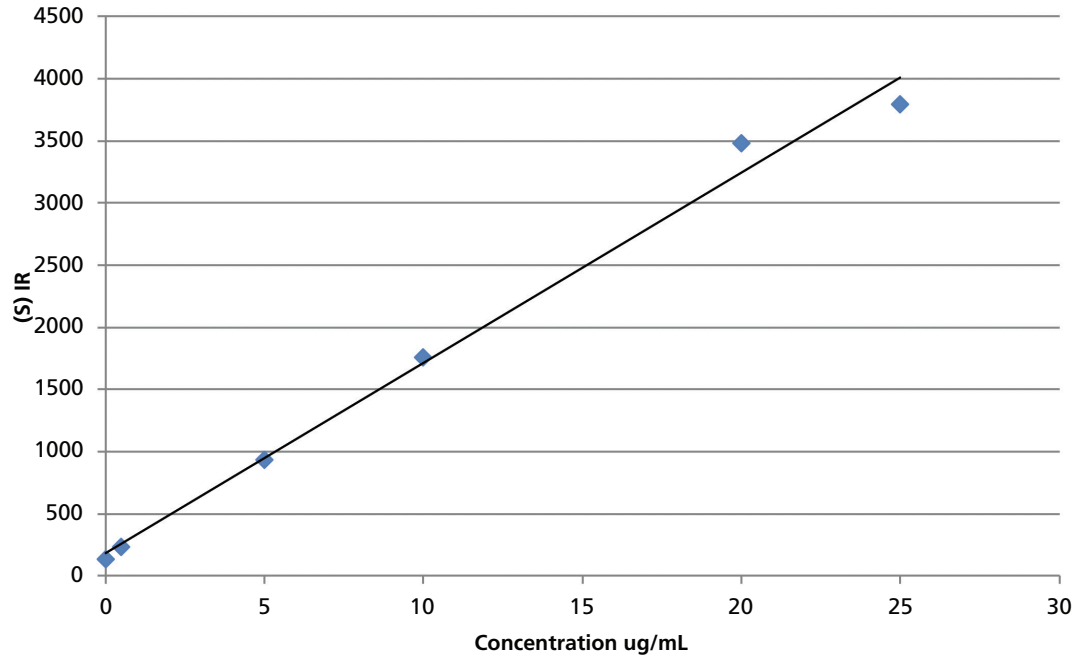
Method non-interference. Eleven of the 15 formulations were stored in silicone oil-free glass containers at 5 °C, 25 °C, and 40 °C for 30 days and were then analyzed by ICP–OES, with the data summarized in **Table IV**.

The data indicate that all the formulations stored in silicone oil-free glass containers after 30 days had ICP–OES responses below 0.1 ppm, the noise level of the ICP–OES method. This indicated there was no interference for the detection and quantitation of leachable silicone oil from the formulations.

Linearity. Silicone oil solutions prepared in xylene solution at different concentrations (0.5 ppm to 25 ppm) were analyzed by ICP–OES, and the responses were plotted against the concentrations seen in **Figure 1**. The data showed a linear correlation of the ICP–OES responses with the silicone oil concentration. The correlation coefficient was 0.995.

Method recovery. The silicone oil was extracted into the organic solvent xylene prior to ICP–OES analysis to avoid possible interference from inorganic silica. A liquid-liquid extraction was used for all formulations with no surfactant to transfer the leachable silicone

Figure 1. Correlation of inductively coupled plasma/optical emission spectrometry (ICP-OES) responses vs. silicone oil concentration. (S) IR is standardized intensity ratio.



oil from the aqueous formulations into xylene. Equal volumes of the aqueous formulation and xylene were mixed, and the xylene layer was analyzed directly. Silicone oil recovery from the formulation was evaluated using Formulation 6 (20mM phosphate, pH 6.8, 7% sucrose, 150mM sodium chloride [NaCl], 0.1mM EDTA). The recovery data are shown in **Table V**. The data indicated that with liquid-liquid extraction procedures, leachable silicone oil can be recovered from the formulation matrixes and quantified.

For formulations with surfactant polysorbate 80, the liquid-liquid back extraction generated severe emulsions, which yielded low recovery of silicone oil. A different technique, solid-phase extraction, was used to transfer the leachable silicone oil for formulations with surfactant. Silicone oil recovery from the formulation was evaluated by using Formulation 11 (20mM phosphate, pH 6.8, 7% sucrose, 150mM NaCl, 0.5mM EDTA, 1% polysorbate 80), and the recovery data are shown in **Table VI**. The data indicated that with solid-phase extraction procedures, leachable silicone oil can be recovered from the formulation matrixes and quantified.

Determining leachable silicone amounts

Leachable silicone oil for formulations with no surfactant or co-solvent. The leachable silicone oil results for five formulations with no co-solvent or surfactants are summarized in **Table VII**.

The five formulations included simple phosphate buffer and formulations containing chelating agent (EDTA), tonicity modifier (NaCl), and different bulking agents (sucrose, mannitol, or trehalose). The amount of leachable silicone oil for all five formulations stored at the three different temperatures (5 °C, 25 °C, and 40 °C) was below the detection limit of 0.1 µg/mL; no leachable silicone oil was detected after 30 days. The primary reason for this was the low solubility of silicone oil in water. The addition of the chelating agent EDTA, tonicity modifier NaCl, or bulking agents (sucrose, mannitol, and trehalose) did not significantly

Table V. Recovery of spiked silicone oil in formulation with no polysorbate 80. Method performance evaluation-recovery test with formulation: 20mM phosphate, pH 6.8, 7% sucrose, 150mM NaCl, 0.1mM EDTA.

Replicates	Recovery %
1	92%
2	93%
3	93%

Table VI. Recovery of spiked silicone oil in formulation with polysorbate 80. Recovery test with formulation: 20mM phosphate, pH 6.8, 7% sucrose, 150mM NaCl, 0.5mM EDTA, 1% polysorbate 80.

Preparation Replicates	Recovery with Liquid/ Liquid Extraction Procedures	Recovery with Solid Phase Extraction Procedures
1	49	94
2	43	117
3	49	118

Table VII. Leachable silicone oil in formulations without co-solvent or surfactants.

Formulations	5 °C	25 °C	40 °C
1 (phosphate buffer)	0	0	0
6	0	0	0
7	0	0	0
14	0	0	0
15	0	0	0

enhance the low aqueous solubility of silicone oil for these formulations.

Leachable silicone oil for formulations with co-solvent. The leachable silicone oil analysis results for the formulations with propylene glycol as a co-solvent are summarized in **Table VIII**.

The data indicated there was detectable leachable silicone oil in all the formulations with propylene glycol as a co-solvent, but the overall leachable silicone oil amounts were low, even with 10% propylene glycol in the formulation. The amount of leachable silicone oil in the formulations after 30 days stored in the syringes at 5 °C, 25 °C, and 40 °C was still below 1 µg/mL, or below 2 µg/syringe. Considering there is more than 300 µg silicone oil coated on each syringe, only a very small portion of the coated silicone oil (less than 1%) leached into the formulations. The primary reason for this is the low solubility of silicone oil in water. The addition of the co-solvent propylene glycol only slightly enhanced the solubility of silicone oil for these formulations.

Leachable silicone for formulations with surfactant. The leachable silicone oil analysis results for the formulations with polysorbate 80 as surfactant are summarized in **Table IX**.

The data indicated there was detectable leachable silicone oil in all the formulations with polysorbate 80 as a surfactant in the formulations. The amount of leachable silicone oil ranged from 0.2 µg/mL to approximately 2.0 µg/mL. The amounts of leachable silicone oil were more than those observed for all other formulations, including formulations with propylene glycol as a co-solvent, suggesting that among all the typical ingredients in the biopharmaceutical formulations, surfactant is the most significant ingredient that may enhance the silicone oil solubility in the formulation and thus cause more leaching of silicone oil.

Storage temperature affected the leachable silicone oil amounts, with the greatest leachable silicone oil amounts typically observed at 40 °C compared to 5 °C and 25 °C storage.

The greatest leachable silicone oil amount observed in formulations with polysorbate 80 as surfactant in this study was approximately 2 µg/mL, which is equivalent to 4 µg/syringe. Considering there was more than 300 µg silicone oil coated on each syringe, the leachable silicone represented less than 2% of the coated silicone oil. This means only a very small portion of the coated silicone oil leached into the formulations, even for those with surfactants.

Leachable silicone for formulations with different pH. The evaluation of pH impact on the leachable silicone oil amounts was performed with formulations with polysorbate 80 as a surfactant because the formulations

with surfactants had the highest leachable silicone oil amounts. The leachable silicone oil analysis results for the formulations with different pH are summarized in **Table X**.

The data show that the pH of the formulations had a significant impact on the amount of leachable silicone oil. The 8.2 pH formulation had significantly more leachable silicone oil than the 5.0 pH formulation. There may be several reasons for the pH impact on the leachable silicone oil amounts. First, the bonding between glass and silicone oil molecules is attributed to the cross linking of polydimethylsiloxane to silanol groups on the glass surface (7), including hydrogen bonding between glass silanol and electronegative oxygen of polydimethylsiloxane. A higher pH may weaken the hydrogen bonding and make the silicone oil more prone to leach into the formulation. Second, pH may affect the degradation of silicone oil, especially breakdown of the end group to trimethylsilanol. The exact cause of the pH effect on the amount of leachable silicone oil will require further study.

The data also indicated that storage temperature had significant impact on the amount of leachable silicone oil. For example, 40 °C storage samples typically had more leachable silicone oil compared to 5 °C and 25 °C, consistent with the results in previous sections.

Table VIII. Leachable silicone oil in formulations with co-solvent.

Formulations	Propylene glycol%	5 °C	25 °C	40 °C
1	0	0	0	0
2	1	0.3	0.4	0.5
3	2	0.4	0.2	0.1
4	5	0.6	0.2	0.9
5	10	0.3	0.7	0.8

Table IX. Leachable silicone oil in formulations with surfactant.

Formulations	Polysorbate 80%	5 °C	25 °C	40 °C
1	0	0	0	0
8	0.05	0.2	0.2	0.7
9	0.1	0.2	0.2	2.1
10	0.5	0.5	0.3	1.0
11	1.0	0.2	1.4	1.6

Table X. Leachable silicone oil in formulations of different pH.

Formulations	pH	5 °C	25 °C	40 °C
12	5.0	0	0.4	0.3
11	6.8	0.2	1.4	1.6
13	8.2	0.4	1.9	2.1

Conclusion

ICP–OES is a suitable technique for the analysis of leachable silicone oil in biopharmaceutical formulations. Leachable silicone oil in aqueous formulations requires further sample preparation to extract the leachable silicone oil from aqueous biopharmaceutical formulations into organic solvents by liquid/liquid extraction or solid-phase extraction.

There is a low risk of silicone oil leaching into a typical biopharmaceutical formulation as long as the formulation does not contain a co-solvent or surfactant. The risk increases if the formulation contains a co-solvent or surfactant. Surfactant is the most critical ingredient affecting the amount of leachable silicone oil, while formulation pH and storage temperature also have an impact. Overall, however, the amount of leachable silicone oil represents only a small portion of the total silicone oil coated on prefilled syringes.

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