Silicone oil (SiO) and fluorosilicone oil (FSiO) are used in difficult cases of retinal detachment surgery. Unidentified low-molecular-weight components (LMWC) and residual catalysts in SiO and FSiO have been implicated in the adverse reactions of the oils in the eye. The authors analyzed LMWC of SiO and FSiO using a gas chromatograph (GC) equipped with a 6-ft x 2-mm column packed with 3% SP-2250 and a flame-ionization detector. By commercially available standards and a homologous series plot, MD3M to MD23M (linear LMWC) and D4 to D30 (cyclic LMWC) were positively identified in commercial-grade 1000-centistokes (cs) SiO. Commercial-grade 12,500-cs SiO contained GC-detectable LMWC (up to MD2gM and D30) at higher concentrations than commercial-grade 1000 cs SiO, although the weight percent of acetone-extractable LMWC (including those larger than MD2gM and D30) was less in the former than in the latter. The GC-detectable LMWC in most medical-grade SiO were less than those in commercial-grade SiO. Tetramethylammonium siloxano-late (a residual catalyst) and tributylphosphine oxide (a heat-decomposition product of a polymerization catalyst) were tentatively identified in commercial- and medical-grade 12,500-cs SiO, respectively. Commercial-grade 1000- and 10,000-cs FSiO also contained LMWC, including F3 and/or F4 (cyclic LMWC). To eliminate LMWC from the oils, the authors developed a solvent fractionation method using acetone for SiO and hexane for FSiO. After continuous solvent extraction of SiO for 2 weeks and of FSiO for 3 weeks, all measurable LMWC were eliminated from the oils.
monomer$^{(1)}$ + end-capping$^{(2)}$ + catalyst$^{(3)}$ $\rightarrow$ MD$_{m}$M$^{(3)}$ + D$_{n}$

Key:
(1) Monomer = D$_3$, D$_4$, or D$_5$.
(2) End-capping: MM or MD$_{2M}$.
(3) Catalyst: potassium hydroxide, potassium siloxanolate, tetrabutylphosphonium hydroxide, tetrabutylphosphonium siloxanolate, tetramethylammonium siloxanolate, etc.
(4) Heat: 70° to 110°C in the case of tetramethylammonium siloxanolate.
(5) MD$_{2M}$: mixture of linear species (m $\geq$ 0).
(6) D$_n$: mixture of cyclic species (n $\geq$ 3).

**Fig. 1.** Polymerization of silicone oil (SiO): high-molecular-weight SiO is usually produced by anionic open-ring polymerization of cyclic monomers.5-6 High-molecular-weight fluorosilicone oil also is polymerized by a similar process.7

Study,4 were obtained from US Dow Corning (Midland, MI). Medical-grade 1000- and 12,500-cs SiO (JNDC-360) was obtained from Japan Dow Corning (Yamakita, Kanagawa). Medical-grade 5000-centipoises (cps) SiO (AM-5000), which is clinically used in Europe, was obtained from Adatomed GmbH (München, FRG). Origins, lot numbers, and abbreviations of SiO and FSiO are given in Table 1.

Hexamethyldisiloxane (MM), octamethyltrisiloxane (MD$_{3}$), decamethyltetrasiloxane (MD$_{2}$M), hexamethyldicyclosiloxane (D$_{3}$), octamethycyclocsiloxane (D$_{4}$), decamethycyclopentasiloxane (D$_{5}$), a mixture of polymethyl-3,3,3-trifluoropropylcyclosiloxanes (trimethyl-3,3,3-trifluoropropylcyclosiloxane (F$_{3}$) and tetramethyl-3,3,3-trifluoropropylcyclosiloxane (F$_{4}$), and tetramethylammonium siloxanolate (1.5-2.0% nitrogen as end-capped polydimethylsiloxane) were obtained from Petrarch Systems. Pure polydimethylcyclosiloxanes (D$_{6}$, D$_{9}$, and D$_{12}$), a mixture of polydimethylcyclosiloxanes (D$_{12}$, D$_{15}$, D$_{18}$, and D$_{21}$) and pure low-molecular-weight polydimethylsiloxanes (MD$_{3}$M, MD$_{7}$M, MD$_{11}$M, MD$_{15}$M, MD$_{19}$M, and MD$_{23}$M) were kindly supplied by Ohio Valley Specialty Chemical (Marietta, OH).

Tributylphosphine oxide was obtained from Aldrich (Milwaukee, WI). Hexane (97% n-hexane; Baker Analyzed HPLC Reagent), and benzene (Baker Analyzed Reagent) were obtained from Doe & Ingalls.
(Medford, MA). Acetone (Spectraanalyzed) and ethanol (HPLC grade, 4.5% methanol and 4.9% isopropanol) were obtained from Fisher (Pittsburgh, PA).

Gas-Chromatographic Analysis

A Perkin-Elmer 3920 gas chromatograph (GC) (Buck, Norwalk, CT) equipped with a flame-ionization detector (FID) and a SP4100 integrator (Spectra-Physics, San Jose, CA) was used. A 6-ft X 2-mm glass column was packed with 1.7 g of 3% SP-2250 on 80/100 mesh Supelcoport (Supelco, Bellfonte, PA). Ultrasep-R (Alltech Associates, Deerfield, IL) was used as GC septa. The temperature program was 80°C for 4 min, then 8°C/min to 280°C, held at 280°C. Temperatures of injector and detector were 290° and 300°C, respectively. The carrier gas was nitrogen (flow rate, 26 ml/min). It was passed through a molecular sieve/silica gel trap and an oxygen trap (Buck). Pressure to the flow controller was maintained at 78 psi. Pressures of hydrogen and air to the FID were 20 and 50 psi, respectively.

Before injecting the sample into the GC, 2 ml each of SiO and acetone were placed in a test tube, shaken by a Vortex-Genie (Scientific Industries, Springfield, MA) at speed control 10 for 1 min, and centrifuged for 5 min at room temperature (21° ± 1°C). Then 2 µl of the solvent layer was injected into the GC with a Hamilton syringe (10 µl, fixed needle 701N) using the plug injection technique.13 The LMWC of FSiO were analyzed by the same procedure as SiO, except hexane was used as the extraction solvent.

We first identified the peaks of 1000-cs PS-S by the technique of peak coincidence with the aid of LMWC standards ("spiking")14 using the temperature program described above. In the GC (isothermal condition) of linear and cyclic LMWC of SiO, the linear correlation between the log retention volume (ln VR) and the number of silicon atoms has been reported.15,16 Therefore, the peaks of the LMWC, of which standards were not obtained, were identified using a homologous series plot.17 Briefly, the retention volume (VR) of each standard of linear and cyclic LMWC was determined by GC at 80°, 120°, 160°, 200°, 240°, and 280°C. The ln VR of the standards then was plotted against the number of silicon atoms, and the slope of the line of the linear and cyclic LMWC standards was separately calculated by regression analysis at the different temperatures. Acetone extract of 1000-cs PS-S also was measured by GC under the above isothermal conditions, and the unknown peaks of 1000-cs PS-S were identified by the relation to the lines obtained by regression analysis. The peaks in the chromatograms of the other SiO samples were identified similarly. We also spiked 1000- and 10,000-cs PS-F using a mixture of F3 and F4.

Because the response of FID is different among LMWC of SiO,5,13 the weight response factors (RFi5) of the LMWC standards (MDM, MD2M, MD3M, MD6M, MD9M, MD12M, D4, D5, D6, D9, and D12) were determined using the temperature program. Benzene was used as the reference standard, and the weight response factor (RF std) of benzene was assigned as 1.00.13

When the base line of the chromatogram was noisy, the peak areas were determined by cutting out the photocopied peak and weighing the paper on a balance (Mettler H54, Caley & Whitmore, Somerville, MA). Before analysis of the oil samples, the contamination of the injection port or the column was checked by injecting the pure organic solvents into the GC. Detector sensitivity was checked frequently by injecting 1.7 mg/ml of cholesterol in decane/toluene (50:50, v/v).

Fractionation of SiO and FSiO by Continuous Solvent Extraction

Commercial-grade SiO (1000- and 12,500-cs PS-S) was fractionated with acetone for 2 weeks, and commercial-grade FSiO (1000- and 10,000-cs PS-F) was fractionated with hexane for 3 weeks using a continuous extraction apparatus (250 ml, liquid/liquid type...
for use with solvents lighter than water, Kontes, Vineland, NJ). About ten granules of Chemware TFE Boiling Stones (Teflon; Fisher) were added to the boiling flask (500 ml, Pyrex; Corning, Corning, NY).

When the solvent fractionation was finished, the supernatant layer in the extractor was separated from the oil layer by decantation and added to the contents of the boiling flask. Using a rotatory evaporator (Rinco, Greenville, IL), the solvent in the boiling flask was evaporated. The solvent dissolved in the fractionated oils also was evaporated. (In this report, the fraction of the oils from which LMWC has been eliminated by the solvent fractionation is referred to as fractionated oils.)

The fractionated oils were shaken vigorously with an equal amount of distilled water in a separatory funnel, then separated from the water. After this procedure was repeated three times, the oils were heated under vacuum (~0.5 mm Hg) with a stirrer in a SiO bath at 110°C for 2 hr and at 150°C for an additional 2 hr, mixed with approximately 20 mg/ml of activated charcoal (Nuchar C-190-N; Eastman Kodak, Rochester, NY), and filtered twice (Whatman filter paper No. 1, then No. 2; Whatman, Maidstone, England).

Kinematic viscosity of the fractionated oils and the solvent-extractable LMWC of the oils was determined at 25°C using glass capillary viscometers. A Cannon-Manning Semi-Micro Viscometer (size 400 and 450; Cannon, State College, PA) was used for the oils with viscosity < 3000 cs and a Cannon-Fenske Routine Viscometer (size 600; International Research Glassware, Kenilworth, NJ) for the oils with viscosity ≥ 3000 cs. Weight percent (Wt %) of the fractionated oils and the solvent-extractable LMWC of the oils to the total oils was determined using a balance (Mettler P1000, Caley & Whitmore).

Refractive index (nD), density, and surface tension of 1000- and 12,500-cs PS-S and 1000- and 10,000-cs PS-F were determined before and after fractionation. The nD was measured at 25°C using a Valentine Precision Refractometer Model 350 A (Vista, CA). Density was measured at room temperature using a Moore-Van Slyke Specific Gravity Bottle (capacity, 2 ml; Fisher) and a balance (Mettler H54). Surface tension was measured at room temperature by the method previously described.

To determine the recovery of LMWC from SiO by our extraction procedure for the GC analysis, 0.01, 0.1, or 1 mg/ml each of linear and cyclic LMWC standards (D6, MD6M, D9, MD9M, D12, and MD12M) were mixed together with fractionated 1000-cs PS-S. Then 2 ml of the oil was extracted with 2 ml of acetone at room temperature, and the concentrations of the LMWC standards in the acetone were measured. The recovery of the LMWC standards from SiO was calculated according to the following equation:

\[
\text{Recovery of each LMWC standard} = \frac{\text{concentration of the standard in acetone} \times 100}{\text{concentration of the standard in fractionated 1000-cs PS-S} \times 100}.
\]

Elimination of LMWC from SiO and FSiO by Heating

Because heating of SiO and FSiO is sometimes used to eliminate LMWC from the oils, we examined the effect of the heating on the elimination of LMWC. After 10 ml of commercial-grade SiO (1000- and 12,500-cs PS-S) and FSiO (1000- and 10,000-cs PS-F) were placed in separate beakers (capacity, 50 ml), they were heated in an air-circulating oven at 200°C for 24 hr. Then the oils were analyzed by GC and compared with those before heating. The specimens of SiO and FSiO were weighed using a balance (Mettler H54) before and after heating, and the weight loss (%) by heating was calculated. These experiments were done in duplicate.

Analysis of Nitrogen, Phosphorus, and Potassium

Medical-grade SiO (1000- and 12,500-cs USDC-360), commercial-grade SiO (1000- and 12,500-cs PS-S before and after fractionation), and commercial-grade FSiO (1000- and 10,000-cs PS-F before and after fractionation) were sent to an outside contractor (Galbraith, Knoxville, TN) for the analyses of nitrogen, phosphorus, and potassium, which are the characteristic components of possible residual catalysts in the oils (Fig. 1). Nitrogen was measured by the Kjeldahl method. Phosphorus was determined by colorimetry using the molybdenum blue method. In the positive control, phosphorus also was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) using a Perkin-Elmer ICP 6000 (Norwalk, CT). In both analyses of phosphorus, the oils were decomposed by wet digestion using a mixture of sodium molybdate, nitric acid, sulfuric acid, and perchloric acid before measurements. Atomic absorption spectroscopy was used for the determination of potassium. We mixed 10 mg of tetramethylammonium siloxanolate (30-40 ppm of nitrogen) and 6.80 mg of tributylphosphine oxide (190 ppm phosphorus) in 5 g of fractionated 1000-cs PS-S separately, and used this for positive controls. Each sample was analyzed once.

Fluorescence Spectroscopic Analysis

Using a fluorescence spectrophotometer (Perkin-Elmer MPF-4), we measured the fluorescence of medical-grade SiO (1000- and 12,500-cs USDC-360),...
commercial-grade SiO (1000- and 12,500-cs PS-S), and commercial-grade FSiO (1000- and 10,000-cs PS-F). These oils were extracted with an equal amount of ethanol, and the fluorescence in the ethanol was measured.

**Results**

**GC Analysis**

By spiking with commercially available standards, we positively identified linear LMWC (MD₄M, MD₅M, MD₆M, MD₁₀M, and MD₁₂M) and cyclic LMWC (D₄, D₅, D₆, D₁₂, D₁₅, D₁₈, and D₂₁) in 1000-cs PS-S. Because of elution on the solvent front, the detection limits of D₄ and D₅ were only 0.1 and 0.01 mg/ml, respectively. The detection limit of LMWC larger than D₅ was 0.001 mg/ml. The MM and D₃ were not separated from the solvent front in our GC analysis, and MDM and MD₂M were not detected in 1000-cs PS-S. Pyrolysis of LMWC standards was not observed.

Figure 4 shows a homologous series plot of linear and cyclic LMWC of SiO at different temperatures. We found a high correlation between the In Vᵣ of LMWC and the number of silicon atoms at all temperature ranges measured (coefficient of determination > 0.983). The Vᵣ of linear LMWC was larger than that of cyclic LMWC of the same number of silicon atoms. The slope of the line obtained by regression analysis decreased with an increase of temperature. This may be explained by the partition coefficient of LMWC between stationary phase and carrier-gas phase, which decreases with an increase of column temperature because of the increment of the vapor pressure of LMWC.²²

As shown in Figure 3, RFᵣ of LMWC of SiO increased with an increase of molecular weight of LMWC, indicating the response of FID to LMWC decreased with an increase of molecular weight of LMWC. The values of RFᵣ were an average of six measurements. (Larger aberrations in the values of RFᵣ for MDM, MD₂M, D₄ and D₅ compared with those of the other standards probably were caused by their close proximity to the solvent front.)

The contamination of the injection port was eliminated by injecting organic solvents several times or by increasing the injection port temperature for several days. The sensitivity of FID, measured by the response to cholesterol, did not change. However, the hydrogen flame was occasionally extinguished by a build-up of combustion products (probably silicon dioxide) that clogged the detector nozzle.

In the GC of commercial-grade 1000-cs SiO (Fig. 5), main peaks and satellite peaks appeared alternatively. By spiking and by using our homologous series plot (Fig. 4), we determined the satellite peaks to be linear LMWC (MD₃M to MD₂₃M) and the main peaks to be cyclic LMWC (D₄ to D₃₀). Commercial-grade 12,500-cs SiO (Fig. 6A) contained GC-detectable LMWC (up to MD₂₈M and D₃₀) at higher concentrations than commercial-grade 1000-cs SiO. (Molecular weights of MD₂₈M and D₃₀ were 2234 and 2220 daltons, respectively.)

Figure 4. The weight response factor (RFᵣ) of low-molecular-weight components (LMWC) of silicone oil (SiO) for flame ionization detector relative to benzene (RFstd = 1.00): RFᵣ of each LMWC standard is plotted against the number of silicon atoms. RFᵣ = Astd/Ai × Wᵣ/Wstd × RFstd, where Astd, Ai, Wstd and Wᵣ are the peak area of benzene and LMWC of SiO, and the weight of benzene and LMWC of SiO, respectively.¹³

At least 17 and 26 LMWC were detected by GC in commercial-grade 1000-cs FSiO (Fig. 6C) and 10,000-cs FSiO (Fig. 6E), respectively. Using a mixture of F₃ and F₄, we identified the first peak of these oils as F₃ and/or F₄ (Figs. 6C, 6E). (Separation of the peaks of F₃ and F₄ could not be achieved with our system.) The amounts of GC-detectable LMWC in 10,000-cs PS-F (Fig. 6E) were more than those in 1000-cs PS-F (Fig. 6C).

The GC-detectable LMWC in most medical-grade SiO were less than those in commercial-grade SiO. Both AM-5000 (Fig. 7B) and 1000-cs USDC-360 (Fig. 8A) contained the least concentrations of LMWC among all SiO products we analyzed. Particularly in 1000-cs USDC-360, linear LMWC smaller than MD₁₂M and cyclic LMWC from D₈ to D₁₀ were less than detection limits. The USDC-Q7 (Fig. 8B), which is clinically used in The Silicone Study,⁴ contained relatively high concentrations of LMWC. Although 1000-cs JNDC-360 (Fig. 8C) contained much larger amounts of LMWC (especially linear species) than 1000-cs USDC-360 (Fig. 8A), the concentra-
Fig. 5. Gas chromatogram of commercial grade 1000-cs silicone oil: each peak is identified by spiking with standards\(^1\) and a homologous series plot.\(^1\) The concentrations\(^*\) of MD\(_9\)M and D\(_{12}\) in this sample are 0.006 and 0.044 mg/ml, respectively. *Concentrations in the acetone extract.

Concentrations of LMWC in 12,500-cs JNDC-360 (Fig. 9D) were similar to those in 12,500-cs USDC-360 (Fig. 9A). Except 1000-cs JNDC-360 (Fig. 8C), commercial- and medical-grade SiO contained more cyclic LMWC than linear LMWC.

Fractionation of SiO and FSiO by Continuous Solvent Extraction

The efficacy of our solvent fractionation method can be seen by the before and after chromatograms of

Fig. 6. Gas chromatogram of commercial grade 12,500-cs silicone oil (PS-S) and 1000- and 10,000-cs fluorosilicone oil (PS-F) before and after fractionation. (A) 12,500-cs PS-S before fractionation; (B) 12,500-cs PS-S after fractionation; (C) 1000-cs PS-F before fractionation; (D) 1000-cs PS-F after fractionation; (E) 10,000-cs PS-F before fractionation; (F) 10,000-cs PS-F after fractionation. *F\(_3\) and/or F\(_4\).

1000- and 12,500-cs PS-S and 1000- and 10,000-cs PS-F (Figs. 5–7), showing that all measurable peaks of LMWC were eliminated from the fractionated oils. After the solvent fractionation of SiO and FSiO, the contents in the boiling flask separated into two phases. The top layer was solvent rich, and the bottom layer was rich in LMWC of the oils.

We tried continuous water extraction of fractionated 1000- and 12,500-cs PS-S and 1000- and

Fig. 7. Comparison of gas chromatogram of fractionated silicone oil (A) fractionated 1000-cs PS-S with that of clinically used silicone oil; (B) AM-5000; (C) 1000-cs USDC-360. Fractionated 1000-cs PS-S does not have any detectable LMWC, which are still measurable with flame ionization detection in AM-5000 and 1000-cs USDC-360. These chromatograms are the highest amplification and the drift of the base line is column bleed, which appears at a column temperature above 240°C.
Fig. 8. Gas chromatogram of medical grade 1000-cs silicone oil (A) USDC-360; (B) USDC-Q7; (C) JNDC-360; (D) a mixture* of 100-cs USDC-360 and 12,500-cs JNDC-360. (A) 1000-cs USDC-360 contains much less LMWC than (C) 1000-cs JNDC-360. (B) USDC-Q7 contains relatively high concentrations of LMWC. (C) 1000-cs JNDC-360 has a gas chromatogram similar to that of the mixture of (D) 100-cs USDC-360 and 12,500-cs JNDC-360. *The volume ratio of 100-cs USDC-360 and 12,500-cs JNDC-360 in this mixture is 48:52, and the measured viscosity is 2290 cs.

Fig. 9. Gas chromatogram of medical grade 12,500-cs silicone oil (A) USDC-360 without spike; (B) USDC-360 with spike; (C) USDC-360 purified by activated charcoal; (D) JNDC-360. By spiking, one of the peaks (*) in (A, B) 12,500-cs USDC-360 are tentatively identified as tributylphosphine oxide. This peak is not detected in (C) 12,500-cs USDC-360 purified by activated charcoal, and in (D) 12,500-cs JNDC-360. The size of the peak (*) in the chromatogram of (A) 12,500-cs USDC-360 corresponds to 0.11 mg/ml of tributylphosphine oxide.

The LMWC of FSiO, of which retention time (tR) was shorter than 15 min (168°), also were almost entirely eliminated by this heating period (Fig. 11). The weight loss of 1000- and 12,500-cs PS-S and 1000- and 10,000-cs PS-F by the heating was 0.24, 1.17, 0.80, and 0.55%, respectively. Heating of the oils (10 ml) in scintillation vials (capacity, 20 ml) was less effective in eliminating LMWC from the oils than that in beakers (capacity, 50 ml), probably because the surface area of the oils in the former was smaller than that in the latter.

Analysis of Residual Catalysts

High-molecular-weight SiO and FSiO, which are clinically used, are probably produced by anionic open ring polymerization of cyclic monomers (Fig. 1).5-7 Low-molecular-weight oils usually are produced by cationic open ring polymerization of cyclic monomers, or polycondensation of hydrolyzed dimethyldichlorosilane.5,6 Because catalysts for the anionic polymerization of the oils are not volatile,23,24 they cannot be detected by GC directly.

The presence of a basic catalyst, such as tetramethylammonium siloxanolate, was suspected in 12,500-cs PS-S by the following experiments. When the organic solvents in the boiling flask were evaporated after the solvent fractionation, the residues in the boiling flask obtained from 1000-cs PS-S and 1000- and 10,000-cs PS-F before fractionation was 1341, 12,221, 980, and 11,043 cs, respectively. The measured viscosity of 1000- and 12,500-cs PS-S and 1000- and 10,000-cs PS-F before fractionation was 1341, 12,221, 980, and 11,043 cs, respectively.

The recovery of the LMWC standards (D6, MD6M, D9, MD9M, D12, and MD12M) from fractionated SiO by a single extraction with an equal amount of acetone was ~30% in the concentration range from 0.01-1 mg/ml of each standard (Table 4). The value of recovery was an average of two measurements.

Elimination of LMWC from SiO and FSiO by Heating

After 1000- and 12,500-cs PS-S were heated in beakers at 200°C for 24 hr, LMWC of the oils smaller than D7, became less than detection limits of GC-FID. Larger LMWC still remained in the oils, although the peak sizes decreased. (The peaks of 12,500-cs PS-S decreased more than those of 1000-cs PS-S.) (Fig. 10).
Table 2. Weight percent (Wt %, at 21° ± 1°C) and viscosity (at 25°C) of fractionated silicone (PS-S) and fluorosilicone (PS-F) oils and low-molecular-weight components (LMWC)* of the oils

<table>
<thead>
<tr>
<th>Oil</th>
<th>Fractionated oils</th>
<th>LMWC*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wt %</td>
<td>Viscosity (cs)</td>
</tr>
<tr>
<td>1000-cs PS-S</td>
<td>69.4 ± 13.6</td>
<td>2722 ± 95</td>
</tr>
<tr>
<td>12,500-cs PS-S</td>
<td>88.1 ± 16.6</td>
<td>16,395 ± 1435</td>
</tr>
<tr>
<td>1000-cs PS-F</td>
<td>77.9 ± 4.5</td>
<td>1556 ± 103</td>
</tr>
<tr>
<td>10,000-cs PS-F</td>
<td>87.5 ± 3.3</td>
<td>14,212 ± 1446</td>
</tr>
</tbody>
</table>

* Solvent-extractable LMWC: this fraction is larger than that of gas-chromatography-detectable LMWC.

Table 3. Refractive index (nD, at 25°C), density (g/cm³, at 21° ± 1°C), and surface tension (dyne/cm, at 21° ± 1°C) of commercial grade silicone (PS-S) and fluorosilicone (PS-F) oils before and after fractionation

<table>
<thead>
<tr>
<th>Oil</th>
<th>nD</th>
<th>Density</th>
<th>Surface tension</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000-cs PS-S</td>
<td>Before</td>
<td>1.4039</td>
<td>0.966</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>1.4038</td>
<td>0.967</td>
</tr>
<tr>
<td>12,500-cs PS-S</td>
<td>Before</td>
<td>1.4039</td>
<td>0.970</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>1.4039</td>
<td>0.971</td>
</tr>
<tr>
<td>1000-cs PS-F</td>
<td>Before</td>
<td>1.3809</td>
<td>1.267</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>1.3812</td>
<td>1.282</td>
</tr>
<tr>
<td>10,000-cs PS-F</td>
<td>Before</td>
<td>1.3816</td>
<td>1.293</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>1.3817</td>
<td>1.293</td>
</tr>
</tbody>
</table>

Table 4. Recovery (%; at 21° ± 1°C) of several standards of low-molecular-weight components (LMWC) from fractionated 1000 cs silicone oil (PS-S) by a single extraction with an equal amount of acetone

<table>
<thead>
<tr>
<th>LMWC standards</th>
<th>Concentration of the standard in fractionated PS-S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.01 mg/ml</td>
</tr>
<tr>
<td>D₆</td>
<td>37.4</td>
</tr>
<tr>
<td>MD₆/M</td>
<td>28.8</td>
</tr>
<tr>
<td>D₉</td>
<td>32.2</td>
</tr>
<tr>
<td>MD₉/M</td>
<td>23.2</td>
</tr>
<tr>
<td>D₁₂</td>
<td>26.2</td>
</tr>
<tr>
<td>MD₁₂/M</td>
<td>22.6</td>
</tr>
<tr>
<td>Mean</td>
<td>28.4</td>
</tr>
</tbody>
</table>

152°-154°C and the bottom layer (nD, 1.40) were identified as diacetone alcohol (4-hydroxy-4-methyl-2-pentanone) and SiO, respectively. The IR spectrum of the top layer had the characteristic absorption bands of alcohol (O-H) stretching vibration at 3540 cm⁻¹ and ketone (C=O) stretching vibration at 1705 cm⁻¹. The remainder of the spectrum was also identical to diacetone alcohol, except a few bands possibly caused by impurities.

Diacetone alcohol is formed by base-catalyzed aldol condensation of acetone. When we refluxed acetone with a trace amount of tetramethylammonium siloxanolate, diacetone alcohol was produced. When 1000-cs PS-S and 1000- and 12,500-cs USDC-360 were extracted with acetone, diacetone alcohol was not formed in the boiling flask. Therefore, we suspect, among these oils, only 12,500-cs PS-S contained a residual amount of basic catalyst, which catalyzed the aldol condensation of acetone during the solvent fractionation.

The GC of 12,500-cs USDC-360 (Fig. 9A) showed one extra peak between D₁₁ and D₁₂, which had some tailing. (The Rₜ of D₁₁ and D₁₂ was 18.5 and 19.8 min, respectively.) By spiking (Fig. 9B), this peak was tentatively assigned as that of tributylphosphine oxide. The peak of tributylphosphine oxide standard had a similar tailing as the extra peak of 12,500-cs USDC-360, and the Rₜ became shorter with an increase of its amount. (The Rₜ of 0.01, 0.1, and 1 mg/ml of tributylphosphine oxide was 21.3, 18.8, and 17.4 min, respectively.) This phenomenon may be explained by nonlinear adsorption (Langmuir isotherm) of this compound on the stationary phase. By percolation through activated charcoal (Fig. 9C) or by continuous extraction with water, the extra peak of 12,500-cs USDC-360 disappeared. (Tributylphosphine oxide is water soluble.) In the GC of 12,500-cs JNDC-360 (Fig. 9D), this extra peak was not found.

Based on these results, we deduced that tetrabutylphosphonium hydroxide, tetrabutylphosphonium silanolate, or tetrabutylphosphonium siloxanolate was used as an anionic polymerization catalyst of 12,500-cs USDC-360 and that tributylphosphine oxide was produced by the heat decomposition of the catalyst in the factory. (Tributylphosphine oxide also has been reported to be used for the deactivation of the other catalysts [ie, potassium hydroxide] in the process of the anionic polymerization of SiO.)
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Analysis of Nitrogen, Phosphorus, and Potassium

As shown in Table 5, 12,500-cs PS-S (in which the presence of tetramethylammonium siloxanolate was suspected) contained the largest amount of nitrogen (31 ppm). After the fractionation of the oil, the amount of nitrogen was less than 1 ppm. The amount of phosphorus was the largest (16 ppm) in 12,500-cs USDC-360 (in which the presence of tributylphosphine oxide was suspected). Potassium in most samples was lower than the detection limit of atomic absorption analysis. Using the Kjeldahl method, we obtained the expected value of nitrogen in the positive control. The value of phosphorus in the positive control, which was determined by colorimetry, was slightly lower than we expected; however, a similar value was obtained by ICP-AES.

Table 5. Analysis of nitrogen, phosphorus, and potassium* (ppm) in silicone (USDC-360, PS-S) and fluorosilicone (PS-F) oils

<table>
<thead>
<tr>
<th>Oil</th>
<th>Nitrogen</th>
<th>Phosphorus</th>
<th>Potassium</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000-cs USDC-360</td>
<td>1</td>
<td>12</td>
<td>&lt;2</td>
</tr>
<tr>
<td>12,500-cs USDC-360</td>
<td>11</td>
<td>16</td>
<td>&lt;2</td>
</tr>
<tr>
<td>1000-cs PS-S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before fractionation</td>
<td>7</td>
<td>6</td>
<td>&lt;2</td>
</tr>
<tr>
<td>After fractionation</td>
<td>6</td>
<td>8</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Add nitrogen (30-40 ppm)</td>
<td>37</td>
<td>9</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Add phosphorus (190 ppm)</td>
<td>13</td>
<td>126 (148$)</td>
<td>&lt;2</td>
</tr>
<tr>
<td>12,500-cs PS-S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before fractionation</td>
<td>31</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>After fractionation</td>
<td>&lt;1</td>
<td>9</td>
<td>&lt;2</td>
</tr>
<tr>
<td>1000-cs PS-F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before fractionation</td>
<td>10</td>
<td>7</td>
<td>&lt;2</td>
</tr>
<tr>
<td>After fractionation</td>
<td>1</td>
<td>6</td>
<td>&lt;2</td>
</tr>
<tr>
<td>10,000-cs PS-F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before fractionation</td>
<td>&lt;1</td>
<td>8</td>
<td>&lt;2</td>
</tr>
<tr>
<td>After fractionation</td>
<td>&lt;1</td>
<td>8</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

* Nitrogen, phosphorus, and potassium are determined by Kjeldahl method, molybdenum blue method, and atomic absorption spectroscopy, respectively.
† Positive controls.
‡ Determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES).

Fluorescence Spectroscopic Analysis

Using fluorescence spectroscopy, we found that lot #44158 of 10,000-cs PS-F was fluorescent (excitation, 310 nm; emission, 368 nm). This fluorescence was eliminated from FSiO by continuous extraction with hexane. However, continuous extraction with water and percolation through activated charcoal or alumina (Acid and Basic, Brochman Activity I, 80–200 mesh; Fisher) was not effective in eliminating it from the oil. The cause of the fluorescence in this FSiO was not identified. Lot #90626 of 10,000-cs PS-F did not fluoresce. Fluorescence also was not found in 1000- and 12,500-cs USDC-360, 1000- and 12,500-cs PS-S, and 1000-cs PS-F.

Discussion

When the samples are introduced into the GC, they are first volatilized at the injection port, and carried as vapor through the column by the carrier gas. Therefore, when a compound is analyzed by GC, the compound must be volatile and stable at high temperature. The LMWC of SiO and FSiO have been shown to be volatile and measurable by GC without pyrolysis.5,29,30 However, high-molecular-weight SiO and FSiO are not volatile. Hence, when SiO and FSiO are injected directly into the GC, high-molecular-weight oils may accumulate in the injection port and then undergo pyrolysis, which will interfere with the analysis of LMWC of the oils. Some organic solvents...
such as acetone, ethanol, dioxane, and dihexyl adipate (partial solvents of SiO) do not dissolve high-molecular-weight SiO, but they do dissolve LMWC of the oil.\textsuperscript{8,13} Hexane, ethanol, and methanol are partial solvents of FSiO. To inject only LMWC of SiO and FSiO into the GC, we first extracted LMWC of the oils with the partial solvents (acetone for SiO and hexane for FSiO). Then we injected the solvent extracts into the GC.

Only a part of LMWC of the oils were extracted by a single extraction procedure using the partial solvents. However, the concentrations of LMWC in the solvents did not change, when the time of vortex-mixing was prolonged for 5 min or the test tube was kept for 1 day after centrifugation. Therefore, we believe that the approximate equilibrium concentrations of LMWC between the oils and the partial solvents occurred during the 1-min shaking time.

We assumed that the amounts of LMWC in the partial solvents correlate with the amounts in the oils. To test this assumption, we mixed some of the LMWC standards with fractionated SiO and extracted with acetone. The recovery of the LMWC standards from the oil was nearly constant in the concentration range of 0.01–1 mg/ml of each standard (Table 4). Therefore, we believe it is possible to compare the relative amounts of LMWC in the oils by obtaining the peak area of LMWC in the partial solvents after a single extraction at constant temperature.

After all GC-detectable LMWC (up to MD\textsubscript{28M} and D\textsubscript{30}) were eliminated from SiO by solvent fractionation, a part of the fractionated oil still dissolved in acetone, indicating that acetone-soluble LMWC contained low-molecular-weight species larger than MD\textsubscript{28M} and D\textsubscript{30}. Although 12,500-cs PS-S (Fig. 6A) contained more GC-detectable LMWC than 1000-cs PS-S (Fig. 5), acetone-extractable LMWC were less in 12,500-cs PS-S than in 1000-cs PS-S (Table 2). As PS-S, 10,000-cs PS-F (Fig. 6E) contained more GC-detectable LMWC than 1000-cs PS-F (Fig. 6C), while hexane-extractable LMWC were less in the former than in the latter (Table 2).

Not only commercial-grade SiO and FSiO but also 12,500-cs USDC-360 (Fig. 9A) contained more GC-detectable LMWC than 1000-cs USDC-360 (Fig. 8A). These results appear to indicate that the elimination of GC-detectable LMWC from the high-viscosity oils (12,500-cs SiO and 10,000-cs FSiO) is more difficult in the factoy than from the low-viscosity oils (1000-cs SiO and FSiO). However, 1000-cs JNDC-360 (Fig. 8C) contained much higher concentrations of the LMWC (especially linear species) than 12,500-cs JNDC-360 (Fig. 9D). It is known that SiO of two different viscosities is sometimes blended by the supplier to reach the required viscosity.\textsuperscript{8,11} Because the gas chromatogram of 1000-cs JNDC-360 (Fig. 8C) was similar to that of the mixture of 1000-cs USDC-360 and 12,500-cs JNDC-360 (Fig. 8D), it is our opinion that 1000-cs JNDC-360 may have been produced by a blending process. (100-cs USDC-360 contained considerable amounts of linear LMWC.)

Elimination of residual catalysts from high-viscosity SiO in the factory also appears to be more difficult than from low-viscosity oils, because tetramethylammonium siloxanolate and tributylphosphine oxide were tentatively identified only in 12,500-cs PS-S and USDC-360, respectively. The elemental analysis (using the Kjeldahl method) showed that 12,500-cs PS-S contained more than three times higher concentration of nitrogen than the other oils. This result supports our speculation that the residual basic catalyst in 12,500-cs PS-S was tetramethylammonium siloxanolate.

By colorimetric determination, a higher concentration of phosphorus was found in 12,500-cs USDC-360 compared with the other oils. Parel et al\textsuperscript{11} and Johnson et al\textsuperscript{12} previously analyzed the elements in clinically used SiO by atomic emission spectroscopy (or ICP-AES) and found phosphorus in the oils. In the first report, the values of phosphorus in the oils were extremely high (200–2200 ppm).\textsuperscript{11} However, in the second report, a much smaller amount of phosphorus was denoted without specified values (≤150 ppm).\textsuperscript{12} This range of values agrees with our results (Table 5). If the extra peak between D\textsubscript{11} and D\textsubscript{12} in the chromatogram of 12,500-cs USDC-360 (Fig. 9A) was tributylphosphine oxide, the peak size was consistent with the value of phosphorus which was determined by colorimetry.

Although SiO and FSiO are relatively stable compounds, they are subject to thermal attack at the siloxane bond (Si-O) and oxidative attack at the side group of the siloxane chain.\textsuperscript{31} Thermal attack at the siloxane bond converts the oils into a mixture of cyclic LMWC. This begins to occur around 400°C in a vacuum.\textsuperscript{32,33} However, oxidation of the side group of SiO, which produces formaldehyde and formic acid, begins to occur even below 200°C.\textsuperscript{31,34} Consequently, an environment free of oxygen will prevent the oxidative degradation of SiO, when the oil is heated for purification or sterilization before intraocular use.

Siloxane bonds also are cleaved by alcoholysis. Rearrangement of D\textsubscript{1} to D\textsubscript{2} in ethanol takes place at room temperature.\textsuperscript{35} In the case of FSiO, even high-molecular-weight oil is degraded by ethanol at room temperature to give linear and cyclic LMWC.\textsuperscript{35} Therefore, alcohol should not be used for purification of FSiO.\textsuperscript{18,36}

The LMWC and residual catalysts in SiO and FSiO have been implicated in the ocular toxicity of the oils.\textsuperscript{1,2,9–12} Kampik et al\textsuperscript{37} first indicated that one of
the causes of the variable frequency of the complications of intraocularly applied SiO among different research groups may be the varying physical and chemical properties of the oil they used. Because the experimental evidence of this assumption has not been reported as yet, in our opinion, the effect of LMWC and residual catalysts in SiO and FSiO on the ocular toxicity is still obscure. Currently, we are doing in vivo experiments on the tolerance of fractionated SiO and FSiO.

**Key words:** fluoro silicone oil, gas chromatography, retinal detachment surgery, silicone oil, solvent fractionation

**References**


