In vitro emulsification assessment of new silicone oils

Albert Caramoy, Sabine Schröder, Sascha Fauser, and Bernd Kirchhof

University of Cologne, Center of Ophthalmology, Department of Vitreo-Retinal Surgery, 50924 Cologne, Germany

Corresponding author:
Dr. med. Albert Caramoy
University of Cologne
Center of Ophthalmology
Department of Vitreo-Retinal Surgery
Kerpener Str. 62
50924 Cologne
Germany
Tel: 0049 (0) 221 478-4308
Fax: 0049 (0) 221 478-3526
E-Mail: acaramoy@yahoo.co.uk

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ABSTRACT

Purpose: To investigate whether the emulsification of conventional silicone oils can be reduced by adding small amounts of silicone molecules of very long chain length.

Methods: Siluron 1000, Siluron 2000, Siluron 5000, Acri.Sil-Oi® 5000, Oxane® 5700, Densiron 68 LV, Densiron 68, and Densiron 68 HV (0.5 ml) were each tested along with either plasma or serum (0.5 ml) in a glass cuvette. Emulsification was induced by sonication and documented by photography. The total area of emulsified oil was assessed using the ImageJ software.

Results: The addition of small amounts of very long-chain silicone molecules significantly reduced the emulsification for 1000 cSt silicone oil (Siluron 2000) and for 1000 cSt silicone oil with admixture of F6H8 (Densiron 68 HV).

Conclusion: New low-viscosity silicone oils show reduced emulsification similar to 5000 cSt oils. In future, it may be possible to avoid using 5000 cSt oils. The findings may foster silicone oil surgery in general and particularly the application of small-incision techniques.
INTRODUCTION

A major problem inherent in the use of silicone oil as vitreous tamponades is emulsification.\textsuperscript{1} Emulsification can generate oil droplets that may cause secondary glaucoma, keratopathy, and subjective disturbances even after the silicone oil is removed.\textsuperscript{2-5}

The emulsification of silicone oil is influenced by many factors including viscosity and interface tension.\textsuperscript{6} Highly viscous silicone oil tends to be more stable in terms of emulsification than less viscous oil.\textsuperscript{7} Consequently, surgeons – at least in Europe – prefer high-viscosity silicone oils (5000 cSt), accepting extended filling and removal times. On the other hand, less viscous silicone oils allow better handling when using small gauge instruments (25 or 23 gauge). It is therefore desirable to develop silicone oils that are resistant against emulsification and of low viscosity.

Silicone oils are made up of polymers and hence show the characteristics of non-Newtonian fluids. This means that the viscosity changes along with the shear rate. The shear viscosity does not fully explain the emulsification tendency, however. By mixing a small amount of very long-chain silicone molecules into common silicone oil, the extensional viscosity may be increased while more or less maintaining the shear viscosity.\textsuperscript{8-10} The addition of very long-chain silicone molecules increases the resistance to extensional deformation and therefore increasing its extensional viscosity. This increases the resistance to emulsification.\textsuperscript{11}

In this study, using an \textit{in vitro} test system, we compared the emulsification of new silicone oils that contain very long-chain silicone molecules (Siluron 2000 and Densiron 68 HV) and various commonly used silicone oils (Siluron 1000, Siluron 5000, Densiron 68 LV, Densiron 68, AcriSil-Oi\textsuperscript{®} 5000 and Oxane\textsuperscript{®} 5700).
MATERIAL AND METHODS

The different silicone oils were assessed using a modification of an *in vitro* model originally developed by Savion et al. 1996.\textsuperscript{12} In this model, oil droplets that are similar in diameter to those in patient eyes are generated. Two different established emulsifiers were used, serum and plasma.\textsuperscript{12}

*Emulsifier preparation*

Blood samples were taken in compliance with the Declaration of Helsinki; informed consent was obtained. The samples were taken from a young healthy male individual on one day. Blood samples were taken using Sarstedt tubes (S-Monovette\textsuperscript{®}, Sarstedt) filled with EDTA (for plasma samples) or with gel platelets (for serum samples). The blood samples were centrifuged at 1000 g for 10 minutes at 4°C. The supernatant was carefully collected (not disturbing the buffy coat) and stored at -80°C until use.

Silicone oil compositions

The compositions of the various silicone oils are summarized in table 1

**Table 1. Compositions of various silicone oils**

| Silicone oil | Composition                                           |
|--------------|-------------------------------------------------------|
| Siluron 1000 | PMDS 1000 mPas (MW 36,760 g/mol)                      |
| Siluron 2000 | 5% HMW (423K PMDS) + 95% Siluron 1000                 |
| Siluron 5000 | PMDS 5000 mPas (MW 65,025 g/mol)                      |
| Acri.Sil-Ol\textsuperscript{®} 5000 | PMDS 5000 mPas                                     |
| Oxane\textsuperscript{®} 5700 | PMDS 5700 mPas                                     |
| Densiron 68 LV | 30.5% SFA + 69.5% Siluron 1000 | |
| Densiron 68  | 30.5% SFA + 69.5% Siluron 5000                       |
| Densiron 68 HV | 30.5% SFA + 69.5% of (10% Sil 2,500,000 + 90% Siluron 1000) |
Emulsifier (0.5 ml) was placed in a glass cuvette with a flat base (inner dimensions: 4 × 10 × 40 mm). Silicone oil (0.5 ml) was added carefully to allow phase separation. The whole glass cuvette was then at 5000 g centrifuged to eliminate air bubbles. Since the emulsification procedures occur in the border between the two phases, no air bubbles were involved. The following silicone oils were used: Siluron 1000 (1000 mPas, Lot SIL1 170708), Siluron 2000 (2000 mPas, Lot SIL2 120808), Siluron 5000 (5000 mPas, Lot SIL5 191007), Acri.Sil-Ol® 5000 (5000 mPas, Lot 3282), Oxane® 5700 (5700 mPas, Lot 31908), Densiron 68 LV (300 mPas, Lot D68-LV 120207), Densiron 68 (1400 mPas, Lot D68 101207), or Densiron 68 HV (1300 mPas). Siluron 1000, Siluron 2000, Siluron 5000, Densiron 68 LV, Densiron 68, and Densiron 68 HV were provided by Fluoron GmbH, Neu-Ulm, Germany. Acri.Sil-Ol® 5000 was obtained from Acri.Tec GmbH (Hennigsdorf, Germany), and Oxane® 5700 was obtained from Bausch & Lomb Surgical (Berlin, Germany).

The test was performed in duplicate for each oil type and for each emulsifier. For the negative control, the emulsifier was replaced with distilled water. The glass cuvette was held in the center of a sonication device (Sonorex TK 30, Bandelin). The water in the sonication device was kept at 20 to 24°C. Sonication was performed for 3 minutes to induce emulsification. Thereafter, the glass cuvette was centrifuged at 5000 g for 30 minutes.

Assessment of the emulsification

The emulsification was assessed every 10 minutes after centrifugation. The cuvette was photographed together with a ruler on its side, and the amount of emulsified oil was then measured on the photograph using the ImageJ software (National Institutes
for Health, Bethesda, Maryland). The ratio of emulsified silicone oil was determined by comparing to the whole area of the aqueous phase, emulsified oil, and non-emulsified oil. The emulsification area in percent was calculated. Emulsification area (\%) = (area of emulsified oil / total area) × 100. Total area = area of aqueous area + area of emulsified oil + area of non-emulsified oil. Each measurement for each sample (n = 2) was done 10 times. The mean ratio was calculated from all 10 measurements. All data were calculated from the emulsification area after 30 minutes of centrifugation.

Statistical methods

For statistical analysis, a t-test was performed using the NCSS (number cruncher statistical system) software (version 2004). The means of all 10 measurements were used. P-values < 0.05 were considered to be statistically significant. All values are shown as means ± standard deviation.

RESULTS

In the negative control using distilled water a small amount of oil droplets was detected after sonication; the oil droplets disappeared after centrifugation. After the emulsification procedure using silicone oils with an emulsifier three phases were seen in the glass cuvettes (Figure 1). An aqueous phase at the bottom, emulsified silicone oil in the middle, and non-emulsified silicone oil on top were seen for Siluron 1000, Siluron 2000, Siluron 5000, AcriSil-Ol \(^\text{®}\) 5000, and Oxane \(^\text{®}\) 5700. Heavier-than-water silicone oils showed non-emulsified silicone oil at the bottom, emulsified silicone oil in the middle, and an aqueous phase on top. During the sonication procedures the emulsified oil bubbles are created at the border between the aqueous and oil phase. No air bubbles were involved in this process. The emulsification area
was assessed every 10 minutes after centrifugation, no statistical difference was found among them. Therefore all data shown are using the data after 30 minutes of centrifugation.

Overall, the emulsification areas of the silicone oils tested were higher in the serum than in the plasma group (Table 2). The lowest emulsification areas were found for Siluron 2000 in the serum group (8.2%) and Densiron 68 HV in the plasma group (2.4%). A summary of the emulsification areas in both groups is presented in figure 2 and 3. The p-values of all groups are presented in figure 4. Overall no statistical significance was seen between AcriSil-Ol® 5000, Oxane® 5700, and Siluron 5000. Compared to other lighter than water silicone oils, Siluron 2000 has less emulsification, which is also statistically significant. Densiron 68 HV has also less emulsification compared to other heavier than water silicone oils. In the serum group, Densiron 68 HV has about the same emulsification as Siluron 5000 and Oxane® 5700.

Table 2. Emulsification area (%; mean ± SD) of silicone oils determined using an in vitro test

| Emulsifier | Siluron 1000 | Siluron 2000 | Siluron 5000 | AcriSil-Ol® 5000 | Oxane® 5700 | Densiron 68 LV | Densiron 68 | Densiron 68 HV |
|------------|--------------|--------------|--------------|------------------|-------------|----------------|-------------|--------------|
| Plasma     | 11.0 ± 1.7   | 6.5 ± 0.4    | 8.9 ± 2.0    | 8.9 ± 1.8        | 8.9 ± 1.6   | 46.3 ± 1.6     | 14.9 ± 5.7  | 2.4 ± 0.5    |
| Serum      | 16.4 ± 11.1  | 8.2 ± 1.6    | 10.0 ± 1.9   | 9.9 ± 0.8        | 9.6 ± 2.2   | 50.3 ± 2.0     | 26.7 ± 1.8  | 10.5 ± 0.7   |

DISCUSSION
In this study, we present a quantitative analysis of the emulsification of silicone oil. We modified the method originally developed by Savion et al.\textsuperscript{12} by using a glass cuvette that has a flat base, which allowed us also to measure heavier-than-water silicone oils. We also determined the area of the emulsified oils instead of the height, which increased the precision of the test.

Siluron 5000, Acri.Sil-Ol\textsuperscript{®} 5000, and Oxane\textsuperscript{®} 5700 are the tamponade agents most frequently used in Germany and are considered the standard silicone oils against which all other oils are tested. Using our in vitro model, there were no statistically significant differences between these three oils with regard to their emulsifications (Figure 4). As expected, a significantly higher emulsification was found for 1000 cSt silicone oil (Siluron 1000). This difference was more prominent when using serum as emulsifier rather than plasma.

The difference between serum and plasma is the content of thrombocytes. Serum lacks thrombocytes, while plasma does not. Overall a slight higher emulsification was seen, using serum instead of plasma. One explanation for this might be the activation of the thrombocytes in the serum tubes during the coagulation process. During this thrombocytes undergo granules secretion, various coagulation proteins are secreted, such as serotonin, platelet factor 4, platelet derived growth factor, fibrinogen, etc. These might serve as emulsifier as well, explaining the slight more emulsification rate in the serum group. The role of each proteins secreted in emulsification ist though still speculative.
Siluron 2000 (1000 cSt silicone oil plus long-chain silicone oil) emulsified significantly less than Siluron 1000 (1000 cSt conventional silicone oil), and even less than Siluron 5000, Acri.Sil-Ol® 5000, and Oxane® 5700 (Figure 3 and 4). This shows that the emulsification can be reduced by adding very long-chain silicone molecules. The shear viscosity of Siluron 1000 (1000 cSt) increased to 2000 cSt in case of Siluron 2000.

Most objections against heavier-than-water silicone oil are based on the higher emulsification of Densiron 68 (Siluron 5000 + F₆H₈).¹⁻¹³⁻¹⁵ We speculate that the addition of semifluorinated fluorocarbon not only renders silicone more hydrophilic but also makes it more susceptible to the splitting-off of small droplets into an aqueous environment. It is fortunate that the addition of long-chain silicone oil molecules (Densiron 68 HV) to Densiron 68 LV (Siluron 1000 + F₆H₈) significantly reduces emulsification compared to either Densiron 68 LV or Siluron 1000. When compared with the three 5000 cSt oils, Densiron 68 HV shows about the same emulsification in the serum group but shows a lower emulsification in the plasma group (Figure 4). The shear viscosities of Densiron 68 (1400 mPas), and Densiron 68 HV (1300 mPas) are rather similar. One may suggest therefore giving preference to Densiron 68 HV over Densiron 68; studies on retinal tolerance are pending, however.

The higher the probability of emulsification for the conventional silicone oils, the more pronounced was the lowering effect of adding long chains of silicone. This effect is best shown when comparing Densiron 68 LV (mixture of Siluron 1000 and F₆H₈) with Densiron 68 HV (mixture of Siluron 1000, F₆H₈, and very long-chain silicone
molecules). The emulsification of Densiron 68 HV is lower, and the difference to Densiron 68 LV is strikingly significant. We conclude that adding small amounts of very long-chain silicone oil molecules has a large influence on reducing the emulsification. The reason for this is the increased extensional viscosity of a non-Newtonian fluid, which determines the propensity for emulsification.

The *in vitro* results presented here require verification by future studies using an animal model. Still some difficulties using animal models for testing emulsification and toxicity of silicone oil must yet to be overcome. In *in vivo* model various amounts of emulsifiers or various intensity of eye movement in the animal eye might produce different amount of emulsification for the same silicone oil. Also no objective method is currently known for quantifying emulsification *in vivo*. In terms of toxicity some animal models, such as rabbits have been shown to be inadequate. This is because of the inability to induce complete posterior vitreous detachment, causing the retina to be protected from the silicone oil by the rest vitreous.16 Proper animal models for testing emulsification and toxicity must still to be developed. Nevertheless we show in this paper that the emulsification of silicone oils can be influenced by adding very long chain silicone oil. This *in vitro* model shown in this paper is even more important, since it shows under same amount of emulsifier and same energy used for inducing emulsification, the emulsifications of Siluron 2000 and Densiron 68 HV are less than other silicone oils. This is an important step to be shown before moving ahead to *in vivo* models. Also in an *in vivo* models different proportions of oil and emulsifiers would be expected.
In conclusion, using an *in vitro* technique, we quantified the emulsification of various silicone oils. The method presented in this study provides a very effective tool for the accurate determination of emulsification and comparison of different silicone oils. We also showed that, by adding long-chain silicone oil molecules, the emulsification of silicone oil can be reduced, as in the case of Siluron 2000 and Densiron 68 HV.
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FIGURE LEGENDS

Figure 1. Emulsification of various silicone oils using plasma as emulsifier

When testing lighter-than-water silicone oils, three phases can be seen: non-emulsified silicone oil (top), emulsified silicone oil (middle), and aqueous phase (bottom). When testing heavier-than-water silicone oils, the aqueous phase was on top, emulsified silicone oil in the middle, and non-emulsified oil at the bottom. Densiron 68 LV emulsifies easily and somewhat disperses, creating a hazy appearance, as compared with prior sonication.

Figure 2. Emulsification areas of various silicone oils using plasma as emulsifier

Figure 3. Emulsification areas of various silicone oils using serum as emulsifier

Figure 4. Probability level (p-values) of differences between the silicone oils tested using plasma and serum as emulsifiers

Non-significant values are shown in gray shaded boxes.
