Investigation of the emulsification of silicone oil used in vitrectomy in the presence of hydrophilic ophthalmic media

Summary of Ph.D. Thesis

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List of abbreviations

AH	aqueous humor
AIDS	acquired immune deficiency syndrome
BCVA	best corrected visual acuity
BSS	balanced salt solution
C3F8	perfluoropropane
ILM	internal limiting membrane
IOFB	intraocular foreign body
PDMS	polydimethylsiloxanes
PPV	pars plana vitrectomy
PVR	proliferative vitreoretinopathy
SD	standard deviation
VB	porcine vitreous
Zave	average droplet size

1. Introduction

Silicone oil endotamponade has frequently been applied in the last decades since its introduction by Armaly and Cibis in 1962, providing internal tamponade by intravitreal injection. Both Machemer and Scott believed that silicone oil could dissect preretinal membranes and work against retinal traction as a stand-alone therapeutic agent. Using Scott's technique to dissect membranes with an intravitreal injection of silicone oil without vitrectomy, others also reported good results. Due to later development of pars plana vitrectomy by Machemer, silicone oil use became even more widespread, particularly for complicated retinal detachments.

In 1994, a form of silicone oil was approved by the FDA for the treatment of AIDS-related disorder, complicated retinal detachment related to cytomegalovirus retinitis. In 1997, the FDA approved a commercial formulation of silicone oil, Silikon 1000 (Alcon, Fort Worth, TX), for use as a prolonged retinal tamponade in selected cases of complicated retinal detachment.

After implantation, silicone oil forms a spherical bubble in the vitreous cavity, but some residual vitreous can remain at the vitreous base, especially in phakic patients. Intraocular silicone oil is in contact with the residual vitreous, and aqueous humor secreted by the ciliary body. Silicone oil, however, may undergo emulsification in 4–72 % of the cases and lead to vision-threatening complications affecting nearly all ocular structures. Complications may include corneal decompensation, band keratopathy, acute and chronic changes in intraocular pressure, lens opacities, epiretinal membrane, retinopathy, optic neuropathy, and extraocular extension (such as migration into the optic nerve, chiasm and even into the cerebral ventricular system). Silicone oil may infiltrate the optic nerve and even the subarachnoid space, too.

The emulsification of silicone oil depends on the interfacial tension between the oil and the hydrophilic phases (aqueous, vitreous or balanced salt solution (BSS)), which means decreased interfacial tension results in increasing emulsification tendency. The time course and level of emulsification are rather variable, but it was established to occur within the first year and mainly after the 5^{th} postoperative month. Besides the physicochemical characteristics of silicone oils, biological factors such as biological environment and mechanical effects can influence the development of emulsification. On the other hand, based on literature information, until now there is no data on how the complex ocular biological media can influence emulsification.

2. Literature background

2.1 Pars plana vitrectomy

Pars plana vitrectomy (PPV) as a technique has revolutionized retinal surgery since its advent in 1970. This innovation allowed the treatment of previously blinding retinal diseases. PPV is a technique in vitreoretinal surgery that enables access to the posterior segment for treating pathologies such as rhegmatogenous retinal detachment, macular hole, epiretinal membranes, diabetes-related vitreous hemorrhage or tractional retinal detachment, endophthalmitis and ocular trauma in a controlled, closed system.

2.2 Endotamponades

During vitrectomy, the vitreous is removed. Since the vitreous is not able to regenerate or replenish, the vitreous has to be replaced by some kind of vitreous substitute. Artificial vitreous tamponades are applied in order to re-establish intraocular volume and provide a temporary or permanent tamponade to the retina after vitrectomy. They also play an important role in separating membranes adherent to the retina, manipulating retinal detachments, and mechanically flattening detached retinas.

Nowadays vitreous tamponades are composed of gases (e.g., air and perfluorocarbon gases) and liquids (e.g., silicone oil and perfluorocarbon liquids). Gases provide a short- to mid-term and silicone oil provides a long-standing endotamponade.

2.3 Silicone oils and their emulsification

Silicone oils for ophthalmic use are composed of polydimethylsiloxanes (PDMS), which are synthetic polymers. They are highly hydrophobic materials, thus they result in high interfacial tension with water, which makes them enable to use as endotamponade. Emulsification is a clinically significant complication of the usage of silicone oils as tamponade. The mechanism of their emulsification is still not completely known. It is hypothesized that small oil droplets come off the main oil bubble. The initiator of this process can be shear forces induced by saccadic eye movements. The small dispersed droplets can be stabilized by various endogenous substitutes (different blood components) and the small droplets can migrate to different parts of the eye or the human body.

There are several factors that can promote or prevent silicone oil emulsification after retinal detachment repair, including proteins, surfactants, contaminants, and shear forces. However, the duration of tamponade remains the most significant one. After emulsification has occurred, keratopathy and glaucoma are the most common complications. The emulsification and migration of

silicone oil can also affect the retina, the optic nerve, and it may even migrate into the brain. The minimalization of the residence time of silicone oil in the eye is the most important factor in reducing its complications.

3. Aims

The use of silicone oils in vitreoretinal surgery, especially in complicated cases, is essential nowadays too. I present its importance and difficulty through my case reports.

The experimental aims of my Ph.D. work were to develop an *in vitro* model for the complex investigation of the phenomenon of silicone oil emulsification in the presence of potential ophthalmic hydrophilic phases obtained from porcine eyes.

The aims were as follows:

1. Measurement of the emulsification ability of the biological media (aqueous humor, vitreous) and BSS.

   The purpose was to investigate the potential media (hydrophilic phase) and silicone oil and their mixture/emulsions by means of:

   - surface tension,
   - zeta potential,
   - microscopic, and
   - rheological measurements.

2. Evaluation of the emulsification effect of the vitreous.

   The plan was to analyze the formation and stability of the emulsion formed with the vitreous and silicone oils, therefore:

   - zeta potential,
   - macroscopic investigations were performed, and
   - the stability of the emulsions was followed.

4. Application of silicone oils in vitreoretinal surgery: Case studies

4.1 Case study 1

17-year-old male patient with myopia in both eyes reported with one week long visual disturbance on the left eye. Retinal detachment was noted (Figure 1). Scleral buckling surgery was performed. On postoperative day two PVR formation accelerated, and new retinal tears were found.

Additional vitrectomy was performed with repositioning the buckle and peeling of the ILM, additional endolaser treatment and ultimately silicone oil implantation. Best corrected visual acuity (BCVA) at postoperative 1 month is 0.3, retina is attached, retinal breaks are closed (Figure 1).

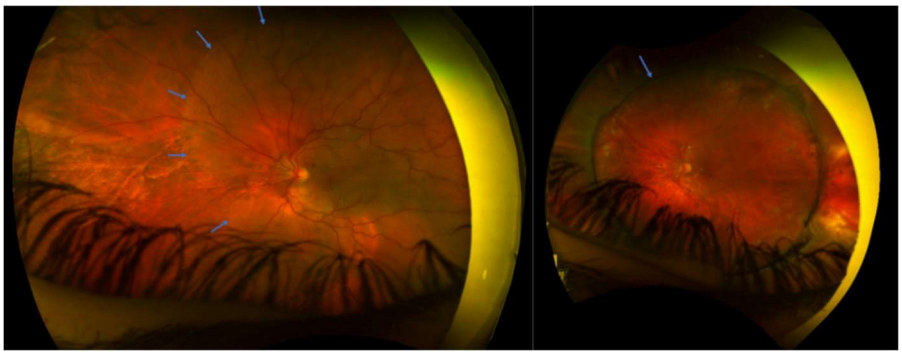

Figure 1 Retinal detachment (left image) and one month later (right image); retina is attached, retinal breaks are closed (blue arrow: indentation of the buckle).

4.2 Case study 2

A 39-year-old female patient suffered perforating injury with intraocular foreign body (IOFB) as a bystander of a household accident. She presented with a 5 mm lacerated corneoscleral wound with iris prolapse, incipient traumatic cataract, vitreous haemorrhage and penetrating wound in the superotemporal part of the macula. Due to the inability of proper posterior reconstruction, surgery was terminated without detachment of the posterior vitreous with spontaneous closure to be expected. With frequent follow-up, retinal detachment occurred by postoperative day 20. Reoperation was scheduled with removal of the traumatic cataract in the bag implantation of intraocular lenses and peeling of both the posterior vitreous face and the ILM was performed via repeated vitrectomy. Spontaneous scleral wound closure was confirmed and focal retinectomy, diathermy and endolaser treatment were performed. Retinal reattachment was secured with additional endolaser burns and silicone oil (Figure 2).

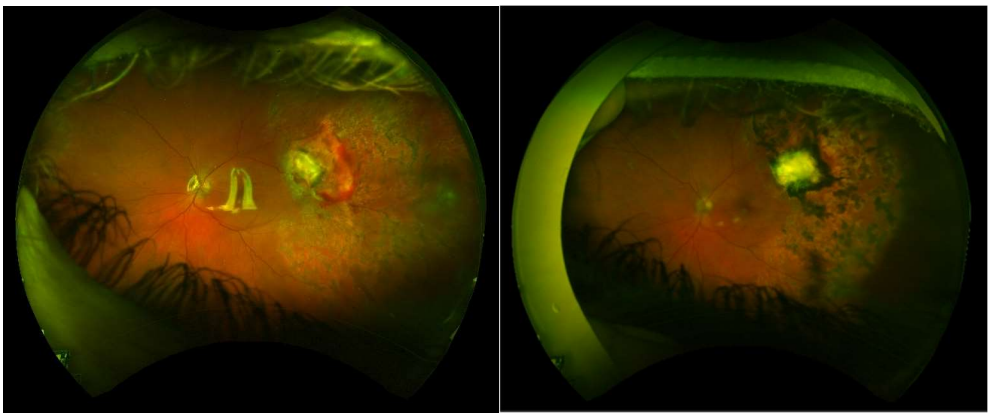

Figure 2 Retinal reattachment is secured with additional endolaser burns and silicone oil (left image); Silicone oil is removed 7 months after surgery (right image).

4.3 Case study 3

A 78-year-old man underwent combined phacoemulsification and vitrectomy with perfluoropropane (C3F8) gas tamponade due to temporal macula off retinal detachment in 2017. Total retinal redetachment was seen three months postoperatively with retinal tear wide open resulting from PVR. Repeated surgery was performed with silicone oil implantation. Persistent lower temporal peripheral detachment remained, the posterior pole showed anatomical restitution. During the Covid-19 lockdown, the patient failed to report for follow-up for a year. In June 2021, the patient presented with a history of decreasing vision in the previous 6 months. Visual acuity was down to light perception with intraocular pressure exceeding 50 mmHg and loss of corneal transparency induced by emulsified silicone oil present in the anterior chamber (Figure 3).

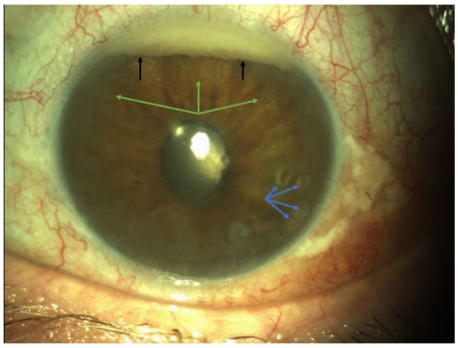

Figure 3 Emulsified silicone oil covering the iris (green arrow) and forming 'inverse hypopyon' (creaming of the emulsified oil droplets) in the anterior chamber (black arrow) accompanying corneal oedema and bullous keratopathy (blue arrow).

4.4 Summary of the case studies

Silicone oils are widely used in vitreoretinal surgery, mainly in complex cases. As can be seen from the clinical case studies presented, their use is problem-free in a significant percentage of cases, but complications also occur. It should also be noted that there is no widely accepted alternative to silicone oils to date, so their use in vitreoretinal surgery is necessary. In my Ph.D. work, I aimed to investigate factors that can be associated with the phenomenon of emulsification during the use of silicone oils, and the elimination of which may reduce the development of complications. Accordingly, an *in vitro* study design was compiled, the details of which are discussed in the next part of the dissertation.

5. *In vitro* experiments: Materials and Methods

5.1 Materials

Original silicone oil Oxane 1300 (Bausch&Lomb GmbH, Germany) (SiO) and potential hydrophilic and lipophilic phases of the emulsions were investigated separately and in a mixture or

emulsions. For the hydrophilic phase of the emulsions balanced salt solution (BSS, Alcon Laboratories, Inc., USA) and biological aqueous phases such as porcine aqueous humor (AH) and porcine vitreous (VB) were used. Porcine eyes were freshly obtained from a slaughterhouse (Pick Szeged Zrt., Szeged, Hungary). Mixtures of the vitreous and BSS were made to model *in vivo* vitrectomized (and silicone oil filled) eyes with residual vitreous. Silicone oil and the hydrophilic phases were mixed with a Vortex stirrer for 5 min. The emulsions/mixtures were measured immediately after preparation. Each emulsion was prepared three times.

5.2 Methods

Silicone oils and aqueous phases were analyzed by means of surface tension measurements by OCA 20 instrument (Dataphysics Instruments GmbH, Filderstadt, Germany) using the pendant drop method.

For the macroscopic observations, the hydrophilic phases of the emulsions were dyed with 0.001 % methyl-blue (Sigma-Aldrich GmbH, Germany). The emulsions were photographed, and the clear oil droplet could be seen in the thin layer of the emulsion sample without any magnification.

For the microscopic observations, the hydrophilic phases of the emulsions were stained with 0.001% Fluorescein sodium (Sigma-Aldrich GmbH, Germany). Microscopic images were made by a Leica DMB6 microscope (Biomarker GmBH, Hungary) in fluorescence mode.

The zeta potential of the *in vitro* emulsion was measured by Zetasizer Nano ZS (Malvern Instrument, UK) with electrophoretic light scattering. Emulsion droplets are surrounded by an electric double layer and stabilized with a given zeta potential (ζ, mV). An emulsion with a high absolute value of zeta potential is more stable in comparison to that with lower zeta potential absolute values.

The viscosity of silicone oil and *in vitro* formed emulsions/mixtures was measured by a Physica MCR 101 rheometer (Anton Paar, Austria). The viscosity of the samples was evaluated. The experiments were carried out in triplicate. With this method, emulsions with 2:8 oil and aqueous phase ratio were investigated.

Unpaired t-test was performed using GraphPad Prism (GraphPad Software Inc., USA). A level of $p \leq 0.05$ was taken as significant, $p \leq 0.01$ as very significant, and $p \leq 0.001$ as highly significant.

6. Results and discussion

6.1 Comparison of the different ophthalmic media with different methods

All ophthalmic hydrophilic substances had significantly lower surface tension than that of BSS. Aqueous humor had the lowest surface tension (54.99 ± 1.98 mN/m), which was followed by the value of the vitreous (61.16 ± 1.30 mN/m), while BSS had the highest value (72.62 ± 0.08 mN/m) (Figure 4). The surface tension of silicone oil was 20.64 ± 0.29, to which aqueous humor presented the closest value, indicating the lowest interfacial tension in the case of the aqueous humor and silicone oil mixture. If interfacial tension is low, emulsification occurs more easily, smaller mechanical energy is needed for the formation of droplets.

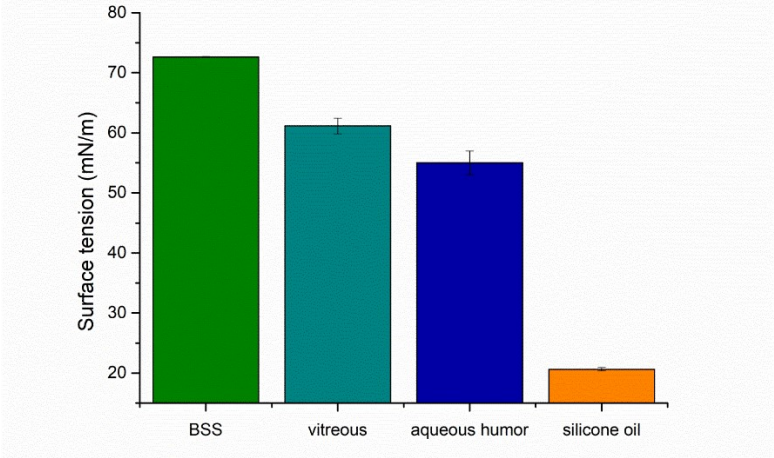

Figure 4 Surface tension of the aqueous phases and silicone oil.

For easy distinction of hydrophilic and lipophilic phases, a fluorescent dye was applied during the microscopic measurements. Green fluorescein sodium can dye the aqueous phases of the emulsions, while silicone oil can be seen in black color in the pictures. A few large and many small black oil droplets can be observed in the green aqueous phase in the microscopic images (Figure 5 A, B and C) when a high amount of aqueous phase (80%) was applied. At this oil:water ratio an oil-in-water type (o/w) emulsion was formed.

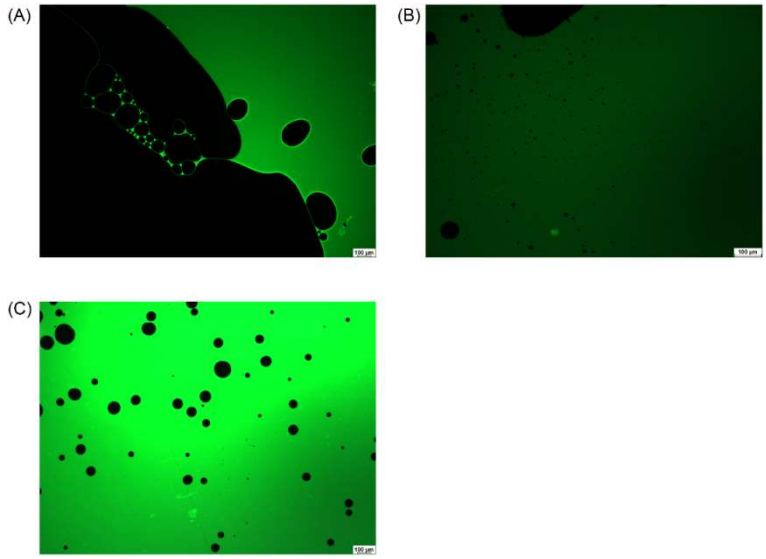

Figure 5 Microscopic pictures of emulsions containing BSS (A), VB (B) or AH (C) at 2:8 oil and aqueous phase ratio.

Zetasizer was used to measure droplet size from nanometer to several micrometers using dynamic light scattering, and zeta potential using electrophoretic light scattering. In our work the droplet size and the zeta potentials of emulsions containing 20 % of silicone oil were only measured (Figure 6), since a larger percent of oil could not be measured by this method.

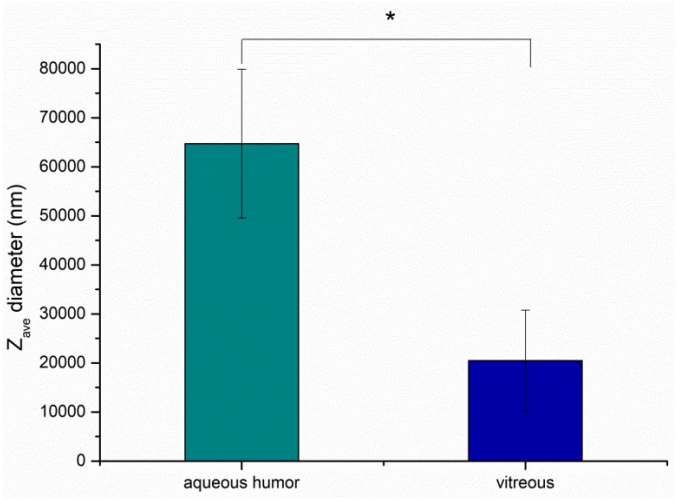

Figure 6 Diameter of the emulsion droplets at 2:8 oil and aqueous phase ratio ($p \leq 0.05$).*

The average droplet size (Zave) of emulsions containing VB was significantly smaller (about 20 μm) ($p = 0.018$), while that of emulsions containing AH was bigger (over 60 μm).

The absolute zeta potential of the emulsion with VB was remarkably higher than that of AH (Figure 7). The zeta potential value can indicate the formation of an electric double layer surrounding the oil droplets stabilizing the emulsion droplets. High negative or positive potential results in the repulsion of droplets, decreasing the tendency of droplets to aggregate or coagulate. A higher absolute zeta potential value indicates higher stability for the dispersed (emulsion) system, thus the phases will not separate and return to the original state. In this measurement, similarly to the microscopic investigation, smaller oil droplets were present in the case of VB, furthermore the absolute Zeta potential of the emulsions containing VB was also higher compared with emulsions containing AH (Figure 7). These statements can mean that the residual vitreous body, which can form an interface with the silicone oil droplet, has more emulsification potential, and it can be the starting point of further emulsification. On the other hand, the formed oil droplets are more stable in the vitreous (indicated by the higher absolute Zeta potential value), predicting that the oil droplet will remain and will not fuse with the continuous oil droplet later.

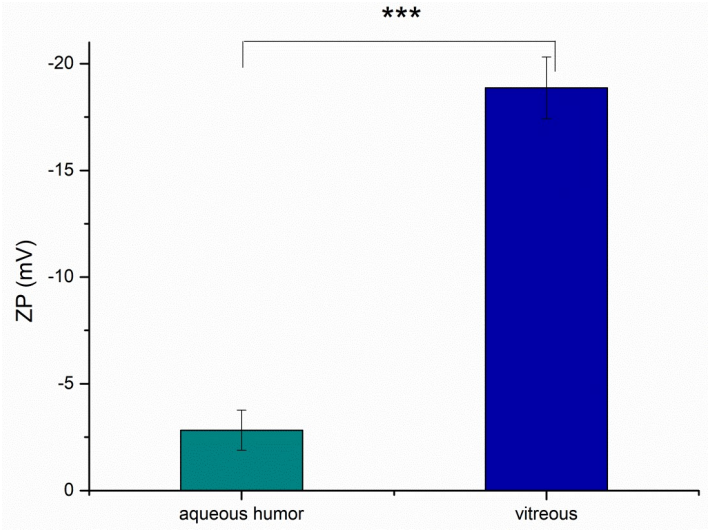

*Figure 7 Zeta potential of the emulsions at 2:8 oil and aqueous phase ratio (*** $p \leq 0.001$).*

The viscosity of the original oil, Oxane 1300, was measured to be 1313.3 ± 5.8 mPa·s (mean ± SD). Emulsions were prepared on the basis of the "Materials" section, where the oil concentration was 20 %, while the aqueous phase (AH or VB) concentration was 80 %.

In the case of emulsions containing AH, the viscosity values were lower than those of Oxane 1300, and the standard deviation (SD) was very high. Contrarily, the emulsions containing VB revealed elevated viscosity values with moderate standard deviation (Table 1). When a nearly stable

w/o emulsion is formed, the emulsified droplets increase the viscosity of the continuous phase, in our case this phase is silicone oil. Considering the viscosity data of the formulated *in vitro* w/o emulsions, mixtures with AH exhibited varying viscosity data indicating an unstable system, while the viscosity of the emulsion containing VB showed an elevated value, which predicts a real, stable emulsion formation. The latter suggests the VB droplets are more stable in the continuous silicone phase.

Table 1 Viscosity value of Oxane 1300 and the emulsions in 2:8 oil and aqueous phase ratio.

	Viscosity (mPa·s)				
	Sample 1	Sample 2	Sample 3	Mean	SD
Oxane 1300	1310	1320	1310	1313.3	5.8
Emulsion with AH	1000	702	65	589.0	477.6
Emulsion with VB	1490	1540	1320	1450.0	115.3

6.2 Evaluation of the emulsification effect of the vitreous

In order to analyze the effect of the vitreous on emulsification, the surface tension of the vitreous and the vitreous diluted with BSS was measured. In composites of the vitreous and BSS, we could observe a remarkable fall in surface tension between 1 %and 10 % vitreous concentrations. Surface tension decreased further when adding more vitreous, but remarkable changes could not be observed between 50 % and 100 % vitreous contents (Figure 8).

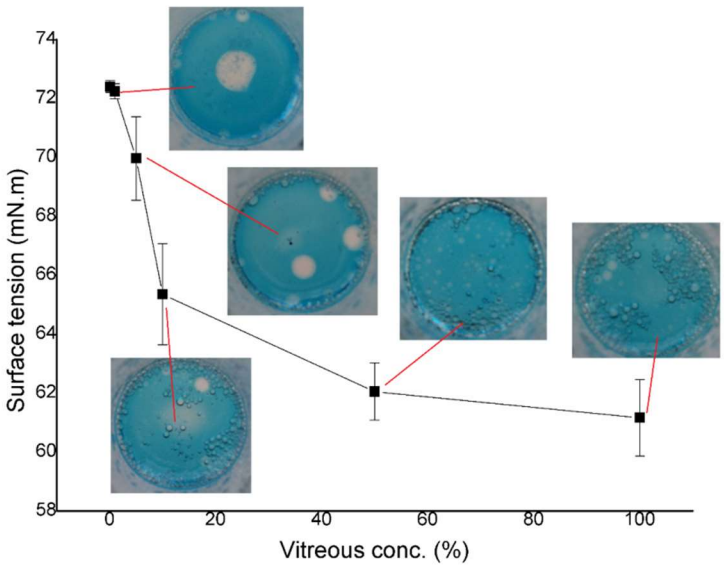

Figure 8 Surface tension of the vitreous-BSS composites with increasing the vitreous concentration, and the picture of the emulsion containing 10 % oil.

The emulsification ability of the diluted vitreous was also investigated. The vitreous and its dilutions were mixed with silicone oil. In the range of 0.01-10 % vitreous concentration in the aqueous phase, a remarkable change can be observed in the emulsification ability. More and more and smaller

and smaller emulsified oil droplets can be seen with increasing vitreous concentration in the aqueous phase, while over 10% vitreous concentration, the number of emulsified droplets did not increase remarkably, as can be observed in the photos of the emulsion (Figure 8). This finding predicts that dilution up to 10 times does not change surface tension remarkably, thus from this point of view the emulsification ability of the vitreous can be retained even after 10-fold dilutions.

The stability of the emulsions formed from the vitreous or its dilutions was demonstrated with zeta potential measurements. In our measurements, the increase in the vitreous concentration increased the absolute value of zeta potential, and a linear relationship was found between the two factors (Figure 9). This finding means the presence of the vitreous increases the stability of the emulsion. Our vitreous containing emulsions showed very high absolute zeta potential values, the sample which contained 10% vitreous in the aqueous phase demonstrated a value of -25 mV, which can already provide good stability for the dispersed droplets, and at higher vitreous concentrations this value decreased continuously, indicating a higher absolute value and higher stability. This observation may mean that at 10-fold dilution the vitreous can emulsify silicone oil and the system remains stable.

The zeta potential measurements could not be performed over 20 % oil concentration, this was the upper limit of the measurements, and therefore, we used macroscopic observations.

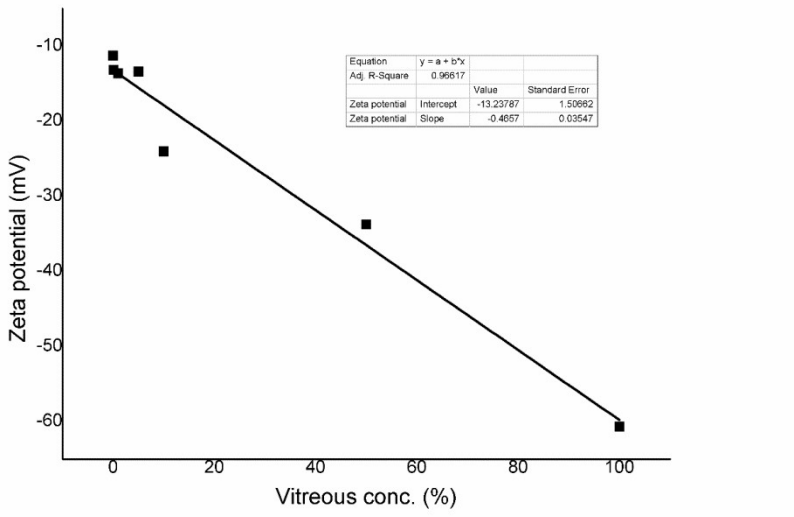

Figure 9 Zeta potential of emulsions containing 10 % silicone oil and 90 % vitreous-BSS composites with increasing the vitreous concentration.

Mixtures were prepared from silicone oil and BSS or vitreous in order to demonstrate the formation of emulsions (Figure 10). The mixtures containing BSS did not show an emulsion structure, the phases remained separated at 10-50 % oil concentrations. At higher oil contents, emulsified BSS droplets could be observed in the continuous oil phase (blue droplets in the transparent oil phase).

This phenomenon can be explained by the steric stabilization effect of the viscous oil phase. Using the vitreous as the aqueous phase, oil-in-water type emulsions were obtained up to 80 % of oil concentration (transparent oil droplets in the blue colored vitreous). At high oil contents, the vitreous formed droplets in the oil phase similarly to the BSS mixture with 90 % silicone oil, but the vitreous presented smaller droplets.

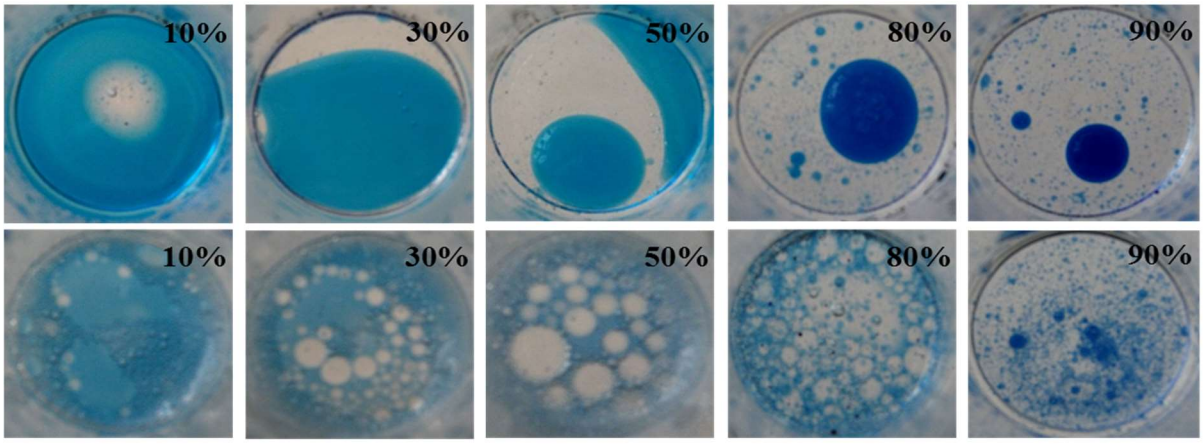

Figure 10 Macroscopic pictures of the formulation prepared from Oxane 1300 (transparent liquid) and from different aqueous media (color blue). In the top right corner of the figures the concentration of Oxane 1300 is shown. In row 1 BSS, and in row 2 vitreous as aqueous media.

Mixing the vitreous with silicone oil resulted in an emulsion system. The Figure 11 illustrates the stability of the emulsions formed, each system remained stable one week after the mixing process (Figure 11 2nd row) regardless of the oil concentration. The type of the emulsion and the appearance of the droplets (size and number) were the same after one week. This finding is in correlation with the zeta potential measurements, where we found that the electrokinetic potential around the droplet can ensure the long-term stability of the systems.

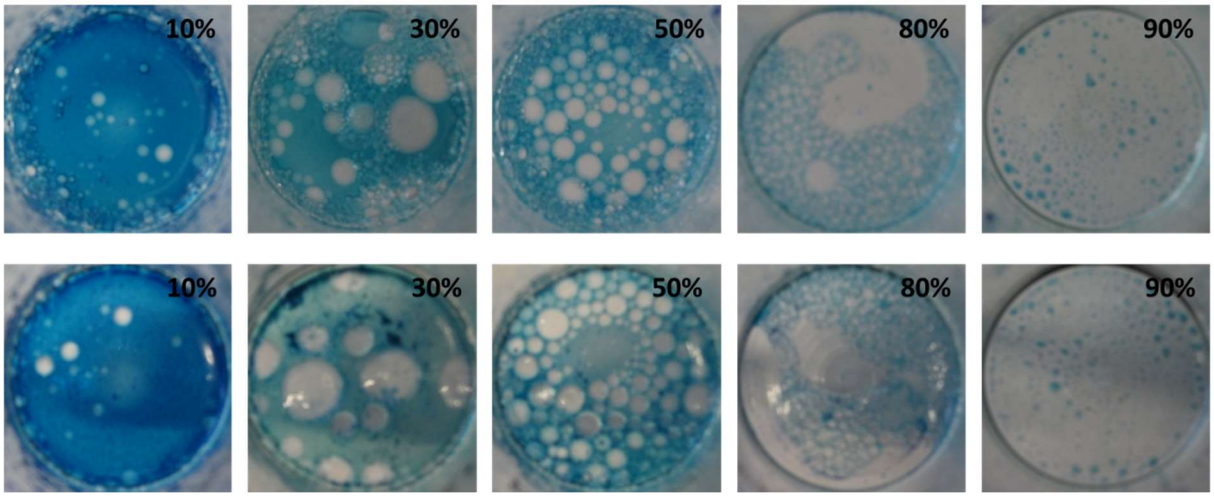

Figure 11 Macroscopic pictures of the formulation prepared from silicone oil (transparent liquid) and vitreous (color blue). In the top right corner of the figures the concentration of silicone oil is shown. In row 1 the state after mixing, and in row 2 the state 1 week after mixing.

7. Conclusion

The aim of my Ph.D. work was to investigate the emulsification process of silicone oil, used as endotamponade, in the presence of hydrophilic phases obtained from porcine eyes and BSS. Our new findings are as follows.

- The vitreous increases the risk of the emulsification of silicone oil.
- The vitreous has remarkable emulsification potential compared with aqueous humor and BSS.
- The vitreous retains its emulsification ability even in 10-fold dilution.
- The vitreous-silicone oil *in vitro* emulsions are very stable, which means that phases of the already formed emulsion will not separate over time. The emulsified silicone oil does not return to its original spherical form.
- Both emulsion types (w/o and o/w) can be developed depending on the silicone oil concentration.

The results of our *in-vitro* studies raise the possibility that the remaining vitreous after vitrectomy may have an emulsifying effect. In case of incomplete oil filling, the oil-water interface increases, which can further enhance the emulsification process.

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