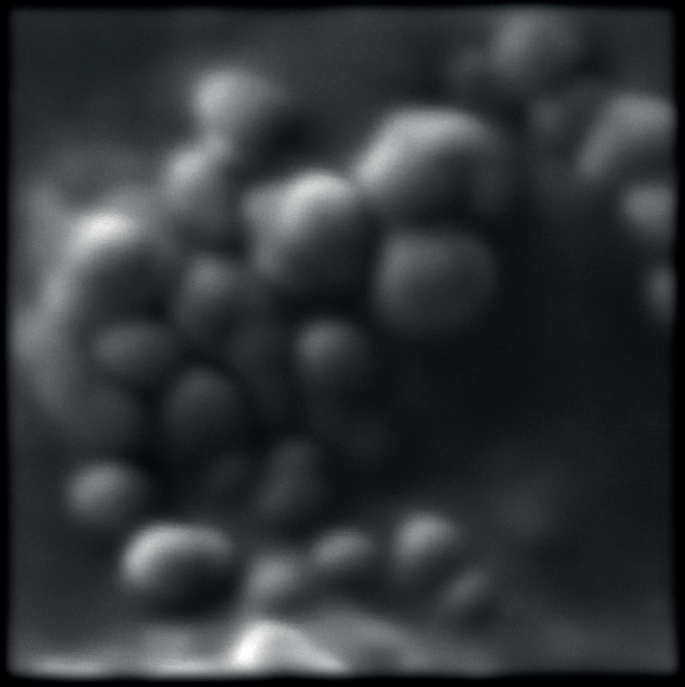

Rui Miguel Abrantes Pinho

SOFT CONTACT LENSES WITH IMPROVED OXYGEN CAPACITY

Masters Dissertation in Biomedical Engineering

September 2015

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UNIVERSIDADE DE COIMBRA

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FCTUC FACULDADE DE CIÊNCIAS
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UNIVERSIDADE DE COIMBRA

Rui Miguel Abrantes Pinho

Soft contact lenses with improved oxygen capacity

Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Engenharia Biomédica

Supervisor: Dr. Hermínio C. de Sousa
Co-supervisor: Dra. Mara M. Braga

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At the end of this stage I would like to express gratitude to everyone that contributed directly or indirectly to the accomplishment of this work.

To everyone,

Thank you!

Resumo

A oxigenação apropriada em tecidos biológicos é um elemento fundamental à sua sobrevivência. Mais concretamente, quando se tratam de tecidos oculares, a hipóxia tem sido uma questão extremamente importante na área médica e científica, podendo por vezes originar doenças graves e irreversíveis, como a cegueira.

Um dos principais motivos para o aparecimento ao longo do tempo da hipóxia ocular prende-se com a utilização de lentes de contacto no dia a dia. Devido à ausência de vascularização da córnea, grande parte do oxigénio absorvido pelo olho é captado diretamente do ar atmosférico, sendo este transporte limitado durante a utilização de lentes de contacto. Para resolver este problema, algumas soluções já foram desenvolvidas, como gotas, pomadas e lentes com ligeiros melhoramentos de permeabilidade ao oxigénio. No entanto, nenhuma dessas soluções é ainda ideal, uma vez que não permitem que pacientes com baixa oxigenação ocular utilizem lentes de contacto sem correrem sérios riscos de danificar a visão.

Este projeto interdisciplinar visa resolver este problema, desenvolvendo de raiz um novo tipo lentes de contacto gelatinosas com elevada capacidade de oxigenação, não só para os utilizadores de lentes em geral, mas também para pacientes com doenças específicas relacionadas com o défice de oxigénio, tais como o síndrome do "olho seco" ou queratite bacteriana. Com recurso a várias técnicas laboratoriais, uma solução inovadora foi desenvolvida, utilizando biomateriais para sorção, armazenamento e libertação de O_2.

Ao incorporar esta solução inovadora nas lentes aquando da sua polimerização, a lente final permitirá uma elevada captura de O_2 da atmosfera, mantendo um ambiente altamente oxigenado junto à córnea.

Os resultados deste estudo revelam-se coerentes com a ideia inicialmente proposta. Apesar de certos resultados necessitarem de ser aprimorados, existem evidências de que as lentes finais possuem propriedades de oxigenação substancialmente melhoradas em relação a lentes de contacto convencionais.

Palavras-chave: Lentes de contacto; Hipóxia; Permeabilidade; Solubilidade; Transportadores de oxigénio.

Abstract

Proper oxygenation on biological tissues is fundamental to their survival. More specifically, when it comes to ocular tissues, hypoxia has been an extremely important subject on both medical and scientific areas, occasionally leading to serious and irreversible diseases, such as blindness.

A major cause for the appearance of ocular hypoxia over time relates to the daily use of contact lenses. Due to the avascularity of the cornea, much of the oxygen absorbed by the eye is captured directly from the atmosphere, being this transport limited while using contact lenses. To solve this problem, some solutions have already been developed, such as droplets, ointments and lenses with slight oxygen permeability enhancements. However, none of those solutions is ideal, since they still don't allow patients with low ocular oxygenation to use contact lenses without running serious risks of damaging their eyesight.

This interdisciplinary project aims to solve this problem by developing, from scratch, a new type of soft contact lenses (SCLs) with high oxygenation capacity, not only for contact lens users in general, but also patients with specific diseases related to oxygen deficit, such as the "dry eye" syndrome or bacterial keratitis. Using various laboratory techniques, an innovative solution was developed, using biomaterials for sorption, storage and release of O_2.

By incorporating this innovative solution on the lenses during their polymerization, the final lenses will allow an elevated O_2 capture from the atmosphere, keeping a highly oxygenated environment near the cornea.

The results of this study turn out to be consistent with the originally proposed idea. Although certain results need to be improved, there is evidence that the final lenses have substantially improved oxygenation properties when compared to conventional contact lenses.

Keywords: Contact Lenses; Hypoxia; Permeability; Solubility; Oxygen Carriers.

List of abbreviations and acronyms

DLS	*Dynamic Light Scattering*
EGDMA	*Ethylene glycol dimethacrylate*
HCl	*Hydrochloric acid*
HEMA	*2-hydroxyethyl methacrylate*
NaCl	*Sodium Chloride*
NaOH	*Sodium Hydroxide*
PDI	*Polydispersity Index*
PFC	*Perfluorocarbon*
PTFE	*Polytetrafluoroethylene*
RGPs	*Rigid Gas Permeable lenses*
SCLs	*Soft Contact Lenses*
SEM	*Scanning Electron Microscopy*
TEOS	*Tetraethyl Orthosilicate*

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Goals and Motivations

Contact lenses are medical devices immensely used worldwide. Given that, even the slightest improvement to one of their properties can constitute a major scientific advance, solving common problems for those who use them on a daily basis.

One vital parameter for ocular health is the presence of a well-oxygenated eye surface. Despite all advances in this field, one of the main issues with all current contact lenses is the dramatic reduction of corneal oxygenation, leading to a number of serious problems, which in extreme cases may induce permanent eye damage or even blindness. This is the reason why researchers spend so much time studying new oxygenation possibilities while wearing contact lenses.

The main objective of this investigation involves the development of soft hydrogel contact lenses that are able to outperform current solutions in terms of sorption, storage and release of oxygen (better oxygen permeability), without compromising vital parameters, such as flexibility or surface properties, which strongly affect wearing comfort. Solving oxygenation issues could not only resolve hypoxia-related issues, but also introduce contact lenses to a vast amount of new users.

After researching the best possible methods of increasing oxygenation properties in various biomedical environments, the first objective consists in the development of a state-of-the-art method to produce an efficient oxygen sorption, storage and release solution to be used as a gas carrier for this specific application. This method combines perfluorocarbon nano and microemulsion techniques with sol-gel methods for silica production. Silica-PFC nano and microcapsules will be developed, where the PFC emulsion is encapsulated with a silica shell upon the formation of the capsule.

On the next step, a basis recipe for the production of the hydrogel SCLs has to be thoroughly developed. The lenses have to be fully transparent, flexible and hydrophilic. They also need to have the right shape (rounded, low thickness).

After developing and optimizing both SCLs and silica-PFC capsules, the integration of both concepts will take place. Several lenses with different concentrations of these innovative capsules will be produced. An extensive characterization process is executed to

prove the efficiency of the silica-PFC capsules, both in aqueous medium and inside the contact lenses, comparing results with current solutions.

Finally, the main conclusions, contributions and limitations to this work will be discussed and presented.

This dissertation is organized in IV main chapters:

In **Chapter I**, a small introduction to the importance of ocular oxygenation is presented. The need of new solutions to improve ocular oxygenation is evidenced, with focus on current solutions, not only ophthalmological, but also other biomedical applications. The main proposes of this study and its objectives are elucidated.

In **Chapter II**, all the experimental procedure regarding the development of soft contact lenses and innovative silica-PFC capsules is described.

Chapter III consists of the presentation of all the results and its discussion.

Finally, **Chapter IV** emerges from the presentation of the main conclusions, contributions and limitations of this work, as well as some suggestions for future investigations.

Chapter I – Introduction

In this chapter, we seek to identify some concepts that have been developed over the years regarding ocular health, as well as analyse some of the most important studies related with the topic of contact lenses and oxygenation.
To do this, in a first stage, we must define what are contact lenses and identify their main problems and limitations, as well as how they are characterized. Next, it is crucial to present an explanation of some problems associated with low ocular oxygenation. Some relevant studies will be described to eventually comprehend the need of an innovative creation to solve oxygenation issues on contact lenses.

Contact Lenses

Contact lenses are thin and transparent lenses placed directly on the surface of human eyes. They are usually utilized to correct human vision (for problems such as myopia/hyperopia, astigmatism and presbyopia). Contact lenses are often chosen over other alternatives because of aesthetical reasons. As an advantage to glasses, contact lenses aren't influenced by some external factors such as raindrops and sweat, leaving the optical path always clear. There are different types of lenses on the market, specified to various types of users. Different materials are used to produce each lens, depending on a number of properties such as oxygen permeability, type of visual distortion to solve, flexibility, surface wettability, price, among others. Therefore, each type of lens will have its own individual characteristics.
Hydrogel Soft Contact Lenses (SCLs) are made from soft and flexible materials, called hydrogels. Compared to other types of lenses, these are the most comfortable for most users because of their smoothness, caused by the high content of water. Therefore, this is the type of lenses that is most broadly used among all [2]. As the name suggests, hydrogel lenses are hydrophilic. The oxygenation properties of these lenses depend a lot on their water content: the higher the amount of water, the bigger will be the oxygen permeability. Also, because of its hydrophilicity, unlike rigid gas permeable lenses (described below), these lenses have great adhesion properties to the human eye.

Rigid Gas Permeable lenses (RGPs) are, as the name indicates, rigid and not flexible. They are usually produced from a hard hydrophobic plastic material (fluorosilicone acrylate for example). Because the human eye has a wet surface, these hydrophobic lenses have surface adhesion issues [32]. This means that the RGP lenses will often move out of the correct position and even pop out with relative ease. Although their oxygen permeability is, in general, slightly higher than hydrogel SCLs, these are not the ideal type of contact lenses for most users for comfort reasons.

Additionally, there are others types such as hybrid lenses (rigid gas permeable center optical zone surrounded by a peripheral soft zone) and silicone/hydrogel lenses. The latter were brought to the market in the beginning of the 21st century. Silicone was used because it allows more oxygen permeability than water. However, these lenses are generally more expensive than non-silicone lenses, more rigid and hydrophobic (causing a harder adaptation process) and may attract more lipid deposits, causing discomfort and blurry vision [25].

Not all patients react to each individual type of lenses in the same way. Therefore, it is fundamental that every patient consults his/her eye practitioner upon choosing the type of contact lens to use. Hydrogel SCLs are still the most utilized lenses globally. The most common element in those SCLs is the monomer HEMA (2-hydroxyethil methacrylate) [20].

One essential parameter to distinguish various types of contact lenses is their oxygen transmissibility (Dk/t), since a lot of users experience signs of discomfort caused by dryness during the wearing period. Oxygen transmissibility is obtained by calculating the ratio between the O_2 permeability of each lens (Dk, in Fatt units) and its thickness (in cm). The O_2 permeability is the ability of the lens to transport oxygen to the eye via diffusion. Typical Dk values for most contact lenses are between 25 and 50. As mentioned by Adrien Bruce (2003) [7], there is no standard requirement for O_2 transmissibility for a conventional contact lens. However, it is known [15] that the use of lenses with low oxygen permeability may lead to hypoxia in the eye. For such, it is essential to ensure that the oxygen permeability is maintained as high as possible during the synthesis of a contact lens.

Numerous attempts to bring the best oxygenation properties to the human eye have been developed, resulting on the different types of lenses known today in the market (described above). All the described types of contact lenses use different materials with different oxygen permeability values. The methods used today to improve oxygen permeability on

contact lenses are the selection of various types comonomers and the variation on the degree of cross-linking. However, all of the current existing products suffer from the same problem: the oxygenation properties are still not acceptable for a lot of consumers. Using any type of contact lens for extended periods of time may severely damage eyesight.

Need for oxygen

The average oxygen uptake rate of the human cornea is between 1 and 10 µl of oxygen/cm^2/h [9,11]. The ability of the cornea to retain both its transparency and healthiness, vital for visual function, highly depends on its capacity to obtain O_2. The cornea is an important eye component that protects the intraocular contents. For such, it is essential that the cornea remains transparent, allowing the formation of an image on the retina. Consequently, any opacity caused by a disease or trauma will disperse the light and degrade the image [37]. It's well known that the human cornea has no blood vessels, so it's not the blood that supplies oxygen and nutrients to their cells [1]. The main supply of corneal oxygen is through direct diffusion with the atmosphere. Consequently, while wearing contact lenses, because they cover the corneal surface, the O_2 diffusion will be drastically reduced. This lack of oxygenation (hypoxia) leads to loss of corneal transparency (due to swelling), inflammatory reactions, corneal infection and an extended number of pathologies. One of the most frequent pathologies is *Keratoconjunctivitis Sicca*, commonly known as the "dry eye" syndrome. It is normally caused by inadequate tear production. The prevalence of this disease usually increases in advanced ages. Still, considering only adults over 50 years old living in the USA, approximately 3,2 million women and 1,68 million men are affected [34,35]. Severe ocular hypoxia can cause permanent eye damage and, in extreme cases, even blindness [7]. This is one major reason why many research teams spend so much time studying and perfecting new ways of improving contact lenses.

Since none of the contact lenses today on the market has ideal oxygenation properties for certain consumers, the development of a new type of lens that provides a substantial increase of the oxygen flow to the human eye might represent a notable breakthrough in ocular health throughout world.

Current solutions for ocular hypoxia

Nearly 90% of current solutions for the inclusion of substances (O_2, drugs or others) in ocular tissues consist of topical applications (solutions, ointments or suspensions) [5,22,33]. Considering the case of "dry eye" syndrome, eye drops are typically used to provide eye lubrication, replacing the role of natural tears. These applications are non-invasive, simple to apply and have an acceptable cost. However, when an eye drop is placed on the ocular surface, only a greatly reduced portion of the substance penetrates the cornea and reaches intraocular tissues [5,33]. Moreover, these solutions provoke excessive concentrations when applied to the eye and very low concentrations when not used (i.e. not a gradual release). Furthermore, they are also just palliative solutions, i.e. they only reduce the symptoms (irritation, photophobia, etc.) that eventually return some time later.

Up to date, there have been very few investigations when it comes to including substances into contact lenses to achieve better oxygenation values. One study regarding scleral lenses has already been reported [31]. On these lenses, the proposed idea was to inject an emulsion with high O_2 solubility (previously bubbled with pure O_2) from time to time on the lenses for improved oxygen capacity. Thus, this solution was not ideal for the global market.

Despite the absence of frequent studies with contact lenses, lots of recent studies have been reported where some substances are used improve oxygen properties in other biomedical applications. Some of those studies are described over the next sections.

Perfluorocarbon emulsions

PFCs (Perfluorocarbons) are a family of compounds derived from a hydrocarbon in which the hydrogen (H) atoms are replaced by fluorine (F) atoms. Considering the high electronegativity of the fluorine, the formed carbon-fluorine bond is very strong, thus making PFCs chemically stable and also biologically inert [29]. Because of their weak intermolecular interactions, these compounds also have high gas solubility, when compared to other substances, such as water or blood [4,13]. However, because PFCs are immiscible with water, a specific surfactant selection has to be picked to make these substances usable as PFC-in-Water microemulsions.

A microemulsion is a stable and optically clear liquid phase. Microemulsions have been used numerous times over years as they can be employed in a large variety of chemical

applications [10,21,23]. To induce the formation of an emulsion, a source of energy has to be used. A proven method to minimize emulsion droplet sizes is by using ultrasound sonication [24].

Upon an emulsion formation, two liquid phases are formed, dispersed and continuous. In a sentence, it is a fluid system in which liquid droplets are dispersed in a liquid [17]. However, emulsions have a number of stability issues like coalescence, flocculation, and Ostwald ripening because the involved liquids are immiscible [16]. This is the reason why generally, emulsions tend to revert back to their original state over time. To avoid that setback, an emulsifier is normally used. The most common types of emulsifiers are the surface active agents (surfactants). These compounds are capable of reducing the interfacial tension between two liquids, stabilizing the emulsion.

Because of their great oxygen solubility, numerous studies have been developed over the years, which have evidenced the efficiency of PFC (perfluorocarbon) emulsions when used as oxygen carriers [14,19,28]. In the biomedical field, PFCs have been previously tested in a number of applications, such as contrast agents and temporary blood substitutes [14,36]. However, while it is proved that PFC emulsions have strong oxygen carrying credentials, a novel method has to be developed to optimize the use of these substances in hydrophilic soft contact lenses.

Mesoporous silica nano and microcapsules

Mesoporous silica is an optically transparent, biocompatible (and biodegradable) material with strong mechanical stability. Besides, since silica is an inorganic material, it is chemically much more stable than isolated perfluorocarbon emulsions when used in an aqueous medium. For those and other reasons, this material has already been used for several therapeutic and diagnostic areas [8,38].

To induce the formation of mesoporous silica, one appropriate technique is the sol-gel method. As the name suggests, this method involves the gradual transformation from a “sol” phase into a “gel” phase. “Sol” is a terminology normally used to describe a solution of very small solid colloidal particles that are dispersed in a liquid. A “gel” is the resulting system formed by a solid porous tridimensional structure [6]. The precursors, initiator compounds for this transformation, undergo two main reaction steps: hydrolysis and

condensation. One of the most common precursors for silica production is tetraethyl orthosilicate (TEOS).

The hydrolysis is a reaction where, by consuming a water molecule, a hydroxyl ion (OH^-) becomes attached to the silicon atom of the precursor. After the hydrolysis, the precursors are soluble in water and the condensation process begins, forming linear SiO_2 chains in a tridimensional structure. Typically, these reactions happen in an acid or alkaline medium, which catalyze process [6].

The main advantages of the sol-gel method is its simplicity but also the fact that it doesn't require high temperatures, allowing a number of components to be incorporated without any issues. Several parameters such as the relative composition of co-precursors, pH, temperature, stirring times/speeds, among others, strongly affect the end results [6].

The innovative solution we propose

Hydrophilic SCLs, as mentioned before, have one major component, which is water. The incorporation of nano and microemulsions in hydrophilic contact lenses is, therefore, very unlikely to be successful because those emulsions would easily leach out of the final hydrogel. Because both PFC emulsions and mesoporous silica have proven to be great biomaterials with excellent individual properties over a variety of biomedical applications, merging both concepts could result in a key invention in the field of science and medicine.

This leads to the groundbreaking solution we propose: by combining PFC nano and microemulsion techniques with sol-gel methods for silica production, a novel method will be developed to allow the formation of silica-PFC nano and microcapsules to be used as stable gas carriers for ophthalmic applications. While producing the PFC emulsions, this innovative method would allow the formation of a mesoporous silica (SiO_2) shell over the PFC cores, forming silica-shell capsules. Developing an oxygen carrier consisted of a PFC emulsion surrounded by a silica shell makes it much more likely that the final product is widely more stable and efficient over long periods of time. Also, with a silica shell, the inclusion of PFCs in contact lenses would possibly be much more efficient: since the PFC is retained within the capsule cores, the probability of leaching out of a solid silica capsule is much lower.

On this novel approach, one of the 3 surfactants to be used will act both as an emulsion stabilizer and a silica precursor. After the hydrolysis, all of the utilized surfactants have hydrophilic heads, increasing the overall emulsion stability. TEOS is also included as a silica precursor. Therefore, after adding the pre-hydrolyzed TEOS to the solution with the PFC emulsion, the co-condensation process will and the silica-shell starts to develop over time.

Since these novel silica-PFC capsules are believed to have great oxygen carrying properties, by integrating them into a SCL, their oxygen permeability could rise considerably, allowing a much higher corneal oxygenation in comparison with current commercial lenses. This way, the user could, theoretically, utilize contact lenses over extended periods without worrying about eye dryness/redness or any other disease associated with lack of O_2. This procedure wouldn't require any additional concerns apart from using the lenses on a day-to-day basis.

To the best of our knowledge, the integration of silica capsules into contact lenses for increased oxygenation properties has not yet been reported. These silica-PFC capsules were studied and developed inside our research team and for legal reasons its formulation cannot yet be fully revealed

Characterization of contact lenses

Throughout the development of contact lenses, an extensive characterization process has to be performed to prove ensure optimal final properties. This characterization involves numerous laboratory techniques and tests that will be presented on the following sections.

Besides oxygen permeability, there are other major components that influence functional contact lenses properties: flexibility, surface properties and transparency/transmittance.

To determine the surface properties of contact lenses, the most adequate and utilized method is to perform a contact angle measurement [30]. This parameter quantifies the wettability of the surface by measuring how a contact lens reacts to a specific solvent (of known properties). If the solvent is water, the measured contact angle gives information about the hydrophilicity/hydrophobicity of each lens. The most utilized method is the sessile drop method, where a droplet of a specific volume is placed on top of each lens and high-resolution cameras capture and analyze the contact angle. Contact lenses with contact

angles lower than 90º are considered hydrophilic, while hydrophobic water repelling lenses (such as RGPs) present values over 90º [30]. Because the eye surface is wet, the finest adhesion properties to the cornea are only possible with hydrophilic contact lenses.

Transmittance tests are also fundamental to approve commercial contact lenses. Because they are medical devices used to improve human vision, it is absolutely essential that they are fully transparent, i.e. they fully transmit the incident visible light to the eye. Transmittance can be defined as the fraction of incident light that is transmitted through a sample, without attenuation. Transmittance values are read using a spectrophotometer.

Chapter II – Experimental

In this chapter, the main materials and methods used to achieve the proposed objectives are described. Three different stages of reactions occurred, regarding the development of the silica-PFC capsules (1), the synthesis of various contact lenses without capsules (2) and finally, the synthesis of SCLs loaded with silica-PFC capsules (3). Initially, all used reagents are displayed. The experimental procedure for each stage is then explained. Finally, all of the characterization tests are listed.

II.1. Materials

Silica-PFC nano and microcapsules

Table 1 – List of the main reagents used on the silica-PFC nano and microcapsules.

Reagent	M_w (g/mol)	Density $_{at\ 25^oC}$ (g/ml)	T_m (ºC)	T_b (ºC)	Distributor
S1 a)	a)	a)	a)	a)	a)
S2 a)	a)	a)	a)	a)	a)
S3 a)	a)	a)	a)	a)	a)
PFC a)	a)	a)	a)	a)	a)
TEOS	208,3	0,789 (20ºC)	-	169	Gelest
Bi-distilled water	18,02	0,9982	0	100	-
HCl	36,46	1,19	-35	57	Panreac Quimica SA
NaOH	40	-	318	-	Eka (Akzo Nobel Company)
Organic salt a)	a)	a)	a)	a)	a)

a) Not to be disclosed.

For the emulsion formation, each one of the surfactants presents different head/tail hydrophilic, lipophilic and fluorophilic characters. In addition, S2 plays a double role, acting both as an emulsion stabilizer and a silica precursor.

Hydrogel SCLs

Table 2 – List of the main reagents used to synthesize of the hydrophilic SCLs

Monomer	M_w (g/mol)	Density $_{at\ 25^oC}$ (g/ml)	T_m (^{o}C)	T_b (^{o}C)	Presence of Inhibitor	Distributor
HEMA	130,1	1,073	-	67	≤50 ppm of monoethyl ether hydroquinone	Sigma-Aldrich
Cross-linker						
EGDMA	198,2	1,05	-	98-100	90-100 ppm of monoethyl ether hydroquinone	Sigma-Aldrich
Initiator						
Irgacure® 2959	224.3	-	86,5-89,5		-	Ciba Specialty Chemicals

II.2. Synthesis and processing of Silica-PFC nano and microcapsules

In order to produce the oxygen loading capsules, an extensive experimental procedure was fully developed and executed. The first step consists on preparing a surfactant solution, obtained by mixing three surfactants (S1, S2 and S3) into an aqueous solution, using bi-distilled water. Two different types of reactions (6 and 7) were conducted with different relative compositions between these 3 surfactants. These experiments were repeated a number of times (Exp#6x and Exp#7x, where x is indicates each individual reaction) to guarantee reproducibility and optimize results. The relative molar compositions for this surfactants varies between the following ranges:

S1: 30-90%, S2: 5-50%, S3: 5-50%.

Due to industrial property issues, these values will not be disclosed. After this aqueous solution is stirred for several minutes, PFC is added. After applying ultrasonic sonication, an emulsion is formed, from which the silica capsules will be formed. To ensure small emulsion droplets, continuous ultrasound sonication is then applied (for predetermined sonication time and amplitude). Once again and due to industrial property issues, these values will not be disclosed.

The final capsules are formed once the silica formation starts around the emulsion droplets. For such, a pre-hydrolyzed silica precursor solution, TEOS (which is initially immiscible with water) has to be prepared using an acid catalyzed sol-gel method. In a small vial, bi-distilled water is added and its pH is lowered with an aqueous HCl solution. Due to industrial property issues, these values will not be disclosed. Then TEOS is added. Stirring this acid solution for several minutes creates a pre-hydrolyzed TEOS solution.

After its formation, the emulsion is encapsulated by mesoporous silica shell by using an aqueous buffered sol/gel method. This reaction step increases the emulsion stability and once again, using a low pH catalyzes it. After adding the pre-hydrolyzed TEOS solution, a fixed amount of an organic salt (not to be disclosed) is inserted. This organic salt is used to stabilize the particles. Finally, with a few droplets of a NaOH aqueous solution, the pH will raise up to near neutral pH values. After stabilizing pH at neutral levels, the condensation process will begin upon stirring the final solution for a predetermined period of time, leading to the formation of the final silica-PFC capsules.

Following the stirring of the final solution, the next step is the dialysis. This step is very important to remove the excess surfactant in the solution. The whole solution is transferred to 2 dialysis bags (Spectra/Por®4 Dialysis Membrane, MWCO: 12-14 kD). These bags were closed with Spectra/Por® closures (75 mm of width). The dialysis, which occurs for 5-10 days, begins upon placing the membranes into a recipient containing up to 3 liters of dH_2O and a magnetic stirrer. Stirring is performed at 200-500 rpm. It is important to replace the water every 2-3 hours during the day, letting stay overnight, to optimize this process.

Once the dialysis is complete, the suspension inside each dialysis membrane is carefully divided into 4 Falcon tubes (Corning® CentriStar® 430829). After that, centrifugation (Z366, Hermle) takes place to purify the final suspension, removing as much excess surfactant as possible. For initial reactions Exp#6 and Exp#7, centrifugation was performed at 8000 rpm for 10 minutes. After that, the supernatant liquid was removed and bi-distilled water was added to the falcon tubes to fill the removed volumes. Vortex stirring was then applied for 2 minutes. Finally another centrifugation took place. This cycle was repeated three times. For every other reaction, the followed procedure was slightly different. Firstly, centrifugation was performed for 5 minutes at 5000 rpm. This step was then repeated for another 5 minutes. Right after, one last centrifugation was executed, this

time at 8000 rpm. Next, the supernatant liquid is removed and bi-distilled water was added to the falcon tubes, in the same way as before. Finally, the suspension solutions were detached from the Falcon walls with a spatula and vortex stirring occurred for 20-30 seconds. For all reactions, all of the final suspensions inside the falcon tubes are then sealed with Parafilm M® and stored in the fridge for later analysis.

II.3. Synthesis and processing of Hydrogel SCLs

To produce the basis for the SCLs, the first part consists of the synthesis of a highly hydrophilic cross-linked polymer, which is then cut into circular lenses with an appropriate tool. The produced hydrogel is based on 2-hydroxyethyl methacrylate (HEMA). Since the synthesis of hydrogel SCLs results from a polymer cross-linking, the oxygen permeability of the prepared lenses will depend on the inner spacing between the polymer chains. To ensure maximum oxygen permeability, it was therefore important to select a cross-linker with the longest possible chain in order to obtain the largest possible amount of space within the polymer matrix. The higher the space between chains in the cross-linked polymer, the greater the uptake of water in the hydrophilic polymer. There is a mathematical relation between the percentage of water present in the lens and its Dk/t [7]. Thus, a higher proportion of water (or any other suspension solution with efficient gas carriers) in the polymer will likely lead to a higher O_2 permeability because this parameter increases with the amount of water in the medium. This leads to the conclusion that, for a high oxygenation, the synthesized SCLs should contain as much water content as possible. A high water amount also allows these lenses to have the desired flexibility and transmissibility.

The utilized monomers are 2-hydroxyethyl methacrylate (HEMA) and ethylene glycol dimethacrylate (EGDMA) as a cross-linker. These monomers contain inhibitors that may influence polymerization reproducibility. Thus, before starting the reaction, these inhibitors must be removed in order to achieve the most accurate results. The applied method consists of chromatography using Pasteur pipettes. To do so, the inhibited monomers were passed downward through vertical glass columns (Pasteur pipettes) containing approximately 1 cm of silica (sand), followed by 2 cm of alumina and another 1

cm of silica (in that order). Various columns were prepared to wash higher amounts of monomer faster. Approximately 5 ml of monomer was passed through each column.

Several approaches were carried out to synthesize contact lenses. Numerous experiments were developed involving the use of different types of monomer concentrations, initiators, polymerization methods, molds and others. More detailed information is described in chapter III. After optimizing results of the conducted experiments, the final SCLs have the following composition: 2,7 ml of HEMA, 2 ml of bi-distilled water, 40 µl of EGDMA and 4 mg of Irgacure 2959® (UV initiator).

Once the mixture (without initiator) was prepared in a glass vial, it was stirred for 10 minutes until the monomers were dissolved and homogenized. The mixture was then degassed by ultrasounds (Elma S30H, Elmasonic). This treatment was performed for 2 minutes at room temperature. Afterwards, the initiator was dissolved and once again the solution was stirred (15 minutes) to completely dissolve all the components. Then, the solution was injected into the mold. This mold is made with 2 square glass plates, separated by two acetate sheets (inside the glass plates) and silicone film in the middle (1 mm thickness), cut in "U" shape (figure below). Once prepared, this mold is sealed with duct tape and binder clips. Because the initiator works under UV light, the final step consists in applying UV-B light (Opsytec irradiation chamber BS-02, mode OS) for 24 hours.

Once the polymerization occurred for 24h in the UV chamber, the mold is carefully opened and the hydrogel is detached. Using an appropriate tool, several lenses are cut (with diameters ranging from 11 to 15 mm) and stored in a labeled container with bi-distilled water.

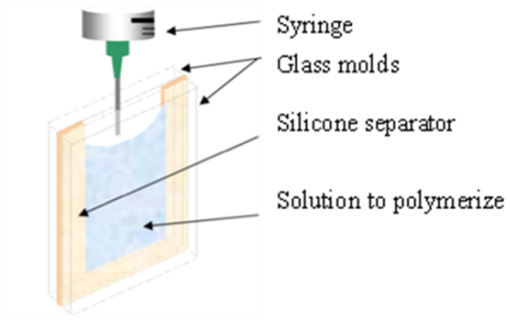


Figure 1 - Utilized mold for the production of the SCLs (adapted from reference [27]).

II.4. Synthesis and processing of SCLs loaded with silica-PFC capsules

To combine the silica-PFC capsules with the contact lenses, new SCLs had to be synthesized. Because the final lenses are made from a hydrogel, by inserting a small portion of the suspension solution onto the initial monomer mixture, the capsules will be included in the polymer matrix after the polymerization.

As mentioned before, results showed that the SCLs with the best final properties had the following composition: 2,7 ml of HEMA, 2 ml of bi-distilled water, 40 µl of EGDMA and 4 mg of Irgacure 2959®.

This time, to keep the same final mixture volume, the 2 ml of bi-distilled water are divided between 2 components: bi-distilled water and the final suspension solutions with the silica capsules (from experiments 6, 6a, 6b, 7, 7a and 7b). Two different formulations were prepared. Initially, a 50/50 split was made, using 1 ml of bi-distilled water and 1 ml of silica-PFC capsules. Then, a new synthesis was performed with the whole 2 ml of suspension solution, without any bi-distilled water added.

II.5. Physical/Chemical/Functional characterization tests

After the development of several experiments for the silica-PFC capsules, a characterization process took place. After understanding some of the most important properties of these capsules, the synthesis and characterization of several SCLs with and without silica-PFC capsules began. Over the next sections, some of the applied techniques will be explained. Detailed results from all these tests and its critical analysis are described in chapter III**.**

Particle Size measurements (capsules) – This extensive study was performed to measure an important parameter of the silica-PFC capsules, its particle size. The distribution of the particle size was analyzed by a noninvasive laser scattering technique known as Dynamic Light Scattering (DLS). This technique measures the diffusion of particles moving under Brownian motion and converts this diffusion to a size distribution. The utilized equipment was ZetaSizer Nano (Malvern), commonly used for measuring particle size in suspension solutions on which the size of colloids is smaller than 1 µm [40].

This equipment also allows zeta Potential measurements. The intensity of the zeta potential indicates the degree of electrostatic attraction/repulsion between suspension particles. If a suspension solution of capsules has a zeta potential over ± 30 mV, this means that repulsion between particles exceeds attractive forces, therefore, resisting flocculation/aggregation. As previously reported, the development of nano-sized silica particles is hard to achieve without noticing the tendency of these particles to agglomerate [3]. Therefore, we should expect not only low zeta Potential values, but also a large range of particle sizes. In this case, the zeta Potential should probably be under ±30 mV, meaning that the suspensions have a high polydispersity index (PDI) is very likely to form agglomerates [40].

Several analyses were conducted in different days, including the study of reaction kinetics for experiments 6c, 7c and 6d. All results are analyzed in chapter III.

Although understanding of all properties of the initial emulsion is very important, no initial studies have been made for the determination of critical micelle concentration (CMC), as well as kinetic studies of emulsion growth.

Optical Microscopy (capsules) – Following the quantitative results from the technique described above, optical microscopy was the first visual characterization test performed regarding the silica-PFC capsules, to observe if the capsules were being formed. The optical microscope (Olympus BH2) had a camera attached, allowing it to register some photographs of the visualized structures.

Scanning Electron Microscopy (capsules, SCLs and SCLs with capsules) – To have more detailed results than optical microscopy, the Scanning Electron Microscopy (SEM) was used to visually confirm the average particle sizes of the produced silica-PFC capsules in all conduced experiments, as well as revealing their morphology.

A scanning electron microscope works by scanning a focused electron beam over a surface. The interaction between the sample and the electron beam produces various signals that can be used to study the surface morphology of each sample [39]. The utilized equipment, a Scanning Transmission Electron Microscope (STEM), was provided by Instituto Pedro Nunes (IPN, Coimbra). After applying vacuum for 10 minutes, the samples (experiments 6, 6a, 6b, 7, 7a and 7b) were observed at various magnifications.

A second analysis was made a few weeks later to compare SCLs with and without capsules. This additional test was made to check if the silica-PFC capsules were retained within the polymeric chains. SCLs. Two samples were compared: SCLs with and without capsules.

Various samples were also prepared for with Transmission Electron Microscopy (TEM). This method is used to ensure that the final nano-sized particles were in fact capsules with a thin silica shell (i.e. not massive particles). Carbon grids (200 mesh) were used. To prepare the samples, a small droplet of suspension solution from a specific experiment was applied over the TEM grid, held by a forceps (Dumont 0202-N5-PO). A petri dish was used to cover the grids while the droplet was absorbed to avoid seeing dust particles in the microscope. After the sample preparation, the analysis took place. Despite all efforts, nothing relevant was visualized. Although the TEM analysis was not repeated, in future investigations, the use of cryo-electron microscopy (cryo-SEM and cryo-TEM) should allow even better results, because the sample preparation would involve freezing the liquid samples, preserving most of the original properties of the suspension solutions as well as providing samples that are more resistant to evaporation.

Water swelling (SCLs) – This was one of the very first characterization tests conduced to the synthesized hydrogel SCLs without the silica-PFC capsules. Two different SCLs (stored in glass vials with bi-distilled water) were initially weighted. After that, they were placed in an oven with controlled temperature, at 30ºC. The loss of mass (evaporated water) was then measured over time.

Contact angle (SCLs and SCLs with capsules) – Contact angle is a key property on commercial contact lenses as it deeply influences the wearing comfort. The measurements were performed at room temperature, in an OCA20 (Dataphysics Instruments, Filderstadt, Germany) apparatus, applying the sessile drop method with a 10 µl droplet volume, using bi-distilled water. This method has been used before to measure surface properties on contact lenses [30,32]. Several SCLs with and without silica-PFC capsules were compared.

Transmittance (SCLs and SCLs with capsules) – The transmittance values of several SCLs, placed on the inner wall of a quartz cell, were recorded three times for each sample

in the 400–800 nm range using a Jasco V530 spectrophotometer. Average values were determined and compared with commercial contact lenses.

SDT/TGA (capsules, SCLs and SCLs with capsules) – Because the final silica-PFC capsules are presented in a suspension solution, no one can assure that all collected samples (of a specific volume) have the same concentration of capsules. This means that, to study the influence of the capsules in the produced lenses, we have to be sure that silica-based materials are present on every SCL loaded with capsules. For such, an analysis with a calorimetric device (Q200 MDSC, TA Instruments) was performed to measure the amount of inorganic materials present SCLs with and without silica-PFC capsules. Results were then compared to another preliminary test, where an aliquot of suspension solution from various experiments was taken to an oven (at controlled temperature) to measure the remaining mass of capsules after a few days.

CO_2 loading/release (capsules, SCLs and SCLs with capsules) – In contact lenses, it is extremely important to study the oxygen transportation capacity of each individual sample. However, preliminary tests with bubbling CO_2 were performed in an aqueous medium prior to testing the samples with oxygen, because at equivalent temperatures (20-30ºC), the solubility of CO_2 in water is 30 to 40 times higher than the solubility of O_2 in water [18]. This means that, for visual tests where temperature and pressure are stabilized, results are much more clear (more bubbles are released) while using CO_2. Moreover, the utilized PFC is permeable to certain gases such as carbon dioxide or nitric oxide as well as other bioactive substances, so this was a preliminary test used to guide future studies.

O_2 solubility (capsules, SCLs and SCLs with capsules) – Following the CO_2 loading/release tests, a new test was made. This time, pure oxygen was loaded to each sample and the release kinetics was studied over time with measurable results. Just 20 ml of water was used in falcon tubes (instead of 40 ml in CO_2 tests) in order to saturate all of the samples faster and more efficiently. After 1h of bubbling pure O_2 into bi-distilled water (always keeping the same flow), the O_2 probe (Consort Z621) was inserted into the falcon tube and sealed with Parafilm M®. The oxygen concentration of each sample (in ppm) was measured after 1 hour of stabilization time, in order to equilibrate O_2 concentration between liquid and gaseous medium. While this step occurs, the sample to be analyzed is

degassed with an ultrasonic cleaner (Elma S30H, Elmasonic). After this stabilization time and sample degassing, each sample was quickly inserted into the falcon tube to avoid big fluctuations on O_2 concentration. Once finished, values were registered over time (1 hour). Multiple assays were implemented for each sample until reproducible results were obtained.

Chapter III – Results and discussion

Throughout this chapter we will proceed to the presentation and interpretation of the main results based on the experimental procedure mentioned above, using the methods described in the previous chapter.

III.1. Characterization of the Silica-PFC capsules

Particle Size measurements – The particle size of the final capsules is essential to determine if they can be incorporated into ophthalmic applications. The utilized samples for this characterization test were solutions from various experiments (6, 6a, 6b, 6c, 6d, 7, 7a 7b and 7c) prepared in eppendorf tubes (100 µl of suspension solution with the silica-PFC capsules + 1 ml of bi-distilled water). The utilized dispersant was water. After using 120 seconds of initial stabilization time (at 25ºC) on a first trial, this value was set 60 seconds at for all next measurements.

As mentioned before, reactions starting with the same number (6 or 7) have the same experimental procedure, while the following character indicates each individual experiment. Thus, on ideal conditions, similar results should appear for those equivalent samples. In practical terms, nearly all of the analyzed samples presented a bimodal particle size distribution (2 high intensity peaks corresponding to reasonably different sizes). Therefore, the produced results presented below were divided into two main sections: sizes varying from 0 to 600 nm are labeled as peak 1, while sizes of 2000 nm and above are labeled as peak 2.

To analyze the particle size of each sample, initially it is necessary to apply ultrasound sonication to disaggregate as much agglomerates as possible. After optimization (appendix A.I), this procedure was performed for 2 minutes and 30 seconds. This procedure was applied because we believe that some of the biggest sizes (peak 2) are caused by a number of factors, including the presence of big agglomerates. After appropriate sample preparation in glass cuvettes (PCS1115), the main results of multiple measurements are summarized on the figure below:

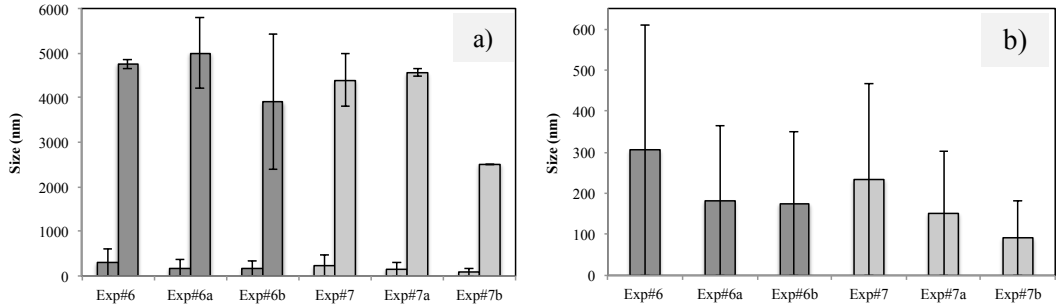


Figure 2 – Particle size results for reactions 6, 6a, 6b (■) and 7, 7a, 7b (□): a) Peak 1 and peak 2 average and standard deviation; b) Detailed peak 1 average and standard deviation for all reactions.

From the figure above, it is clear that all the analyzed reactions produced particles with a large variety of particle sizes. Between individual measurements of the same sample, the size differences were sometimes too high to reach any conclusions about the conditions of each separate reaction. Because of that concern, the software frequently showed a message stating that the polydispersity index (PDI) was too high (0,7 and 1) and the utilized samples maybe weren't suitable to this particle size test.

One possible explanation for these high variances may be the rapid deposition of the silica-PFC capsules on the bottom of the eppendorf tubes, because PFC has a high molecular weight. This causes the reduction on the number of measured capsules, leading to much more sensitive data.

Another possibility is that there may have been leftovers of surfactant S2 (silica precursor) that didn't bind to the PFC upon the formation of the initial emulsion. The resulting effect is that, if this surfactant is dispersed in an aqueous medium, it may have induced the formation of solid silica particles in that medium. Those solid particles may grow at a several rates and are also subject to aggregation, which could have influenced particle size measurements.

Also, as stated before, the development of nano-sized silica particles is hard to achieve in a linear size distribution [3]. The rapid development of agglomerates, even after applying ultrasound sonication, is possibly one of the main reasons of the variability of these results.

However, we believe that, by far, the most important factor that determined these results derives from the unavoidable properties of the initial PFC emulsion. Since the silica shell grows around the emulsion droplets (increasing final size no more than 5-10%), the final size of the silica-PFC capsules is highly dependent from the size of the droplets of the initial PFC emulsion. Consequently, if the initial emulsion was formed with large size

dispersion, all the capsules will also present similar results. As this is an investigation project, some variables were slightly modified over time to optimize final results. On the experiments described above, some difficulties with the ultrasound sonication may have provoked highly discrepant values between experiments. For Exp#6, various types of ultrasonic processors were tested unsuccessfully during the reaction, meaning that the formation of a microemulsion (with a cloudy appearance) wasn't visually observed for those devices. Once emulsification was visually observed at naked eye, that specific reaction continued. Also, on all experiments, the isolation and cooling methods during this step have varied slightly from experiment to experiment, avoiding possible evaporation of the PFC. Every little modification to the initial droplet sizes of the PFC emulsion will therefore define most of the final properties of the final capsules.

Finally, the slight variation between the various experiments may be explained not only by all the reasons presented before, but also by the presence of multiple variables present on each reaction: a minor pH variation at just one of several phases coupled with a minimal difference on hydrolysis time or room temperature could have slightly increased the error between measurements. However, because it is believed that the initial emulsion influences final results the most, more studies have to be developed in future investigations regarding the emulsification process, including emulsion growth trials, as well as the analysis of the critical micelle concentration (CMC).

Even so, with this data we could prove that all reactions were originating nano-sized silica-PFC capsules with sizes, in a lot of times, lower than 100 nm, which isn't an easy result to achieve. After a profound analysis of the measured results from different days, a comparative summary of all reactions was developed:

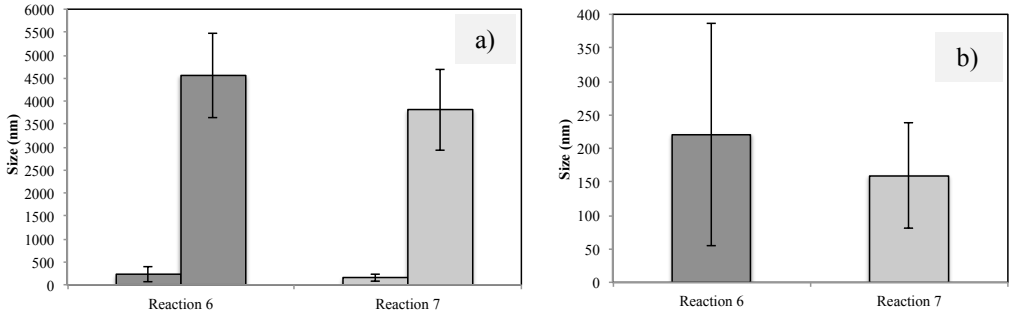


Figure 3 – Overall average of size results for silica-PFC capsules from reactions 6 (■) and 7 (■):
a) Peak 1 and peak 2 average and standard deviation; b) Detailed peak 1 average and standard deviation.

Based on all results from above, we cannot yet have clear conclusions when it comes to differentiating reactions 6 and 7. The high diversity of measured data doesn't allow clear conclusions when it comes to the influence of the relative surfactant concentrations (only difference between reactions 6 and 7) in the final particle size. To better understand these results, the same reactions (Exp#6, Exp#6a, Exp#6b, Exp#7, Exp#7a, Exp#7b) were analyzed with Scanning Electron Microscopy just 3 days later.

The zeta potential values were also recorded for the same samples studied above. The results of that analysis are presented below:

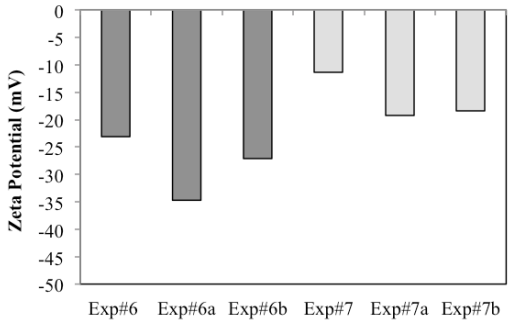


Figure 4 – Registered zeta potential for silica-PFC capsules from reactions 6 (■) and 7 (□).

All of the samples registered, on average, zeta Potential values between -35 and -11 mV. Every recorded value is negative because silica particles always have negative surface charge. These results prove that the silica capsules were effectively being formed around the PFC emulsion. To consider an analyzed suspension as stable, it needs to have a zeta potential of at least ± 30 mV [40]. This means that the stability of the colloidal dispersion wasn't particularly high for any reaction. In other words, these results support the idea that the initial emulsion has a very reduced resistance to flocculation, forming particles with very disperse sizes. Although Exp#6a presented slightly improved values, it is clear from results above that each individual measurement was always inevitably affected by the all factors stated above. Despite all error sources, these values are somewhat expected when handling particles produced by nano and microemulsion techniques and can be used to justify the nonlinearity of sizes during the emulsion formation.

After the analysis of all the suspensions listed above, the next studied reaction for the production of silica capsules was Exp#6c. For this experiment, the particle size measurements were made as the reaction occurred. This way, the goal was to analyze, over time, the kinetics for the formation of the capsules. To ensure reproducibility of the results

for Exp#6c, all collected samples at selected times were frozen right after its particle size measurement. On the next day, a new measurement with those frozen samples was performed for comparison purposes. Theoretically, by reducing environment temperature below 0ºC, the reaction should stop or slow down considerably. However, due to a number of experimental factors, individual results may vary.

After the study of reaction kinetics for the Exp#6c, a new study was performed using Exp#7c. Because reaction 7 uses different surfactant proportions from reaction 6, the results could be a bit different, hence this new analysis. To ensure reproducibility of these last kinetic studies, a final analysis was made with Exp#6d. For both Exp#7c and Exp#6d, two software measurements were performed in sequence over the same period of time. Besides that, as before, all the utilized parameters always remained the same.

A comparative analysis over time between the 3 reactions is described on the next sections. Detailed results about initial emulsion dispersions are available in appendix A.I.

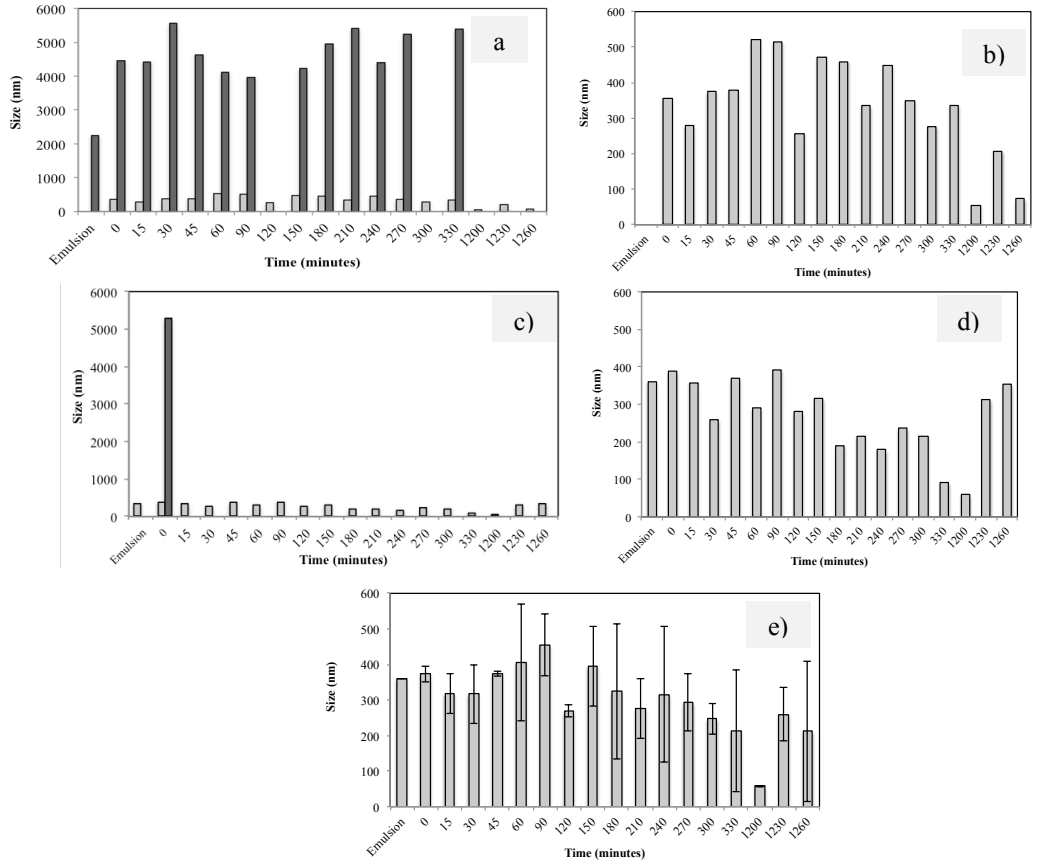


Figure 5 – Reaction kinetics for Exp#6c: a) Peak 1 (□) and Peak 2 (■) values throughout time (1st measurement); b) Peak 1 values throughout time (1st measurement); c) Peak 1 (□) and Peak 2 (■) values throughout time (2nd measurement - after freezing samples); d) Peak 1 values throughout time (2nd measurement - after freezing samples); e) Average of all peak 1 values throughout time.

Starting with Exp#6c, all of the first measured results presented sizes over 1000 nm (peak 2), just like previous results. By comparison, the second measurement with the frozen samples presented just one bimodal dispersion. The most likely reason for this occurrence is the detection of fewer agglomerates on the second measurement. After resting the samples in the freezer for one day, the heaviest agglomerates may have decanted, forming even bigger groups. On the next day, before measuring the particles sizes, 2 minutes and 30 seconds of continuous sonication was applied. This time might not have been enough to fully disaggregate those big agglomerates. The consequence is that, during initial stabilization time, bigger agglomerates will suffer sedimentation faster than small isolated particles, because of their higher mass. This sedimentation will then lead to the detection of less aggregates and more individual capsules, leading to the disappearance of peak 2.

With a detailed analysis of peak 1 values, some conclusions can be listed. As before, it is clear that the variation of some results over time is sometimes higher than the difference between frozen/non-frozen samples at any given moment. Although these differences cannot be ignored, the average size of most detected particles was always in the same range, between 150 and 400 nm. In general, we can also state that a stable growth of the size was not observed over time. The reason why this growth was not visualized for this reaction is that the silica shell only adds a very small fraction of the size over the initial emulsion droplets. Considering that, if (hypothetically) a stable growth occurred over time (from 50 to 400 nm, as an example), this would not mean that the silica shell was being formed around the PFC; that would mean that the formed capsules were suffering from increasing coalescence or aggregation over time.

Also, as stated before, the initial emulsion size plays a major role in determining the final properties of the silica-PFC capsules. In this case, since the emulsion already presented a huge dispersion of sizes, it is natural that the size of the capsules over time varied between a large array of values. Nonetheless, these results have given suggestions that some of the final capsules from this experiment were produced with sizes lesser than 70 nm, which is a remarkable achievement.

After the presentation of Exp#6c, results from Exp#7c are displayed next:

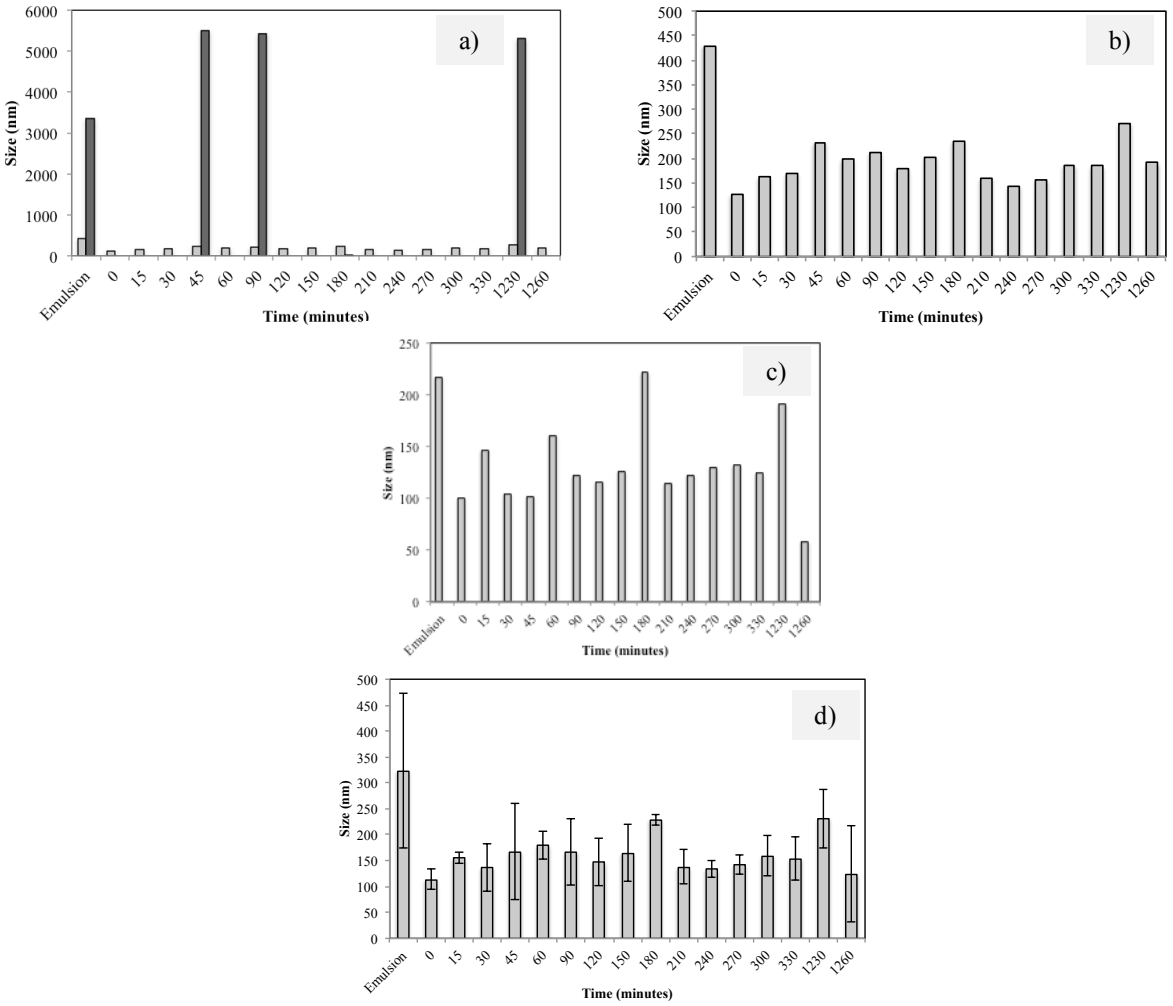


Figure 6 – Reaction kinetics for Exp#7c: a) Peak 1 (□) and peak 2 (■) values throughout time (1st measurement); b) Peak 1 values throughout time (1st measurement); c) Reaction kinetics for Exp#6c: Peak 1 values throughout time (2nd measurement - no peak 2 was detected); d) Average of all peak 1 values throughout time.

Unlike Exp#6c, the second measurement for this reaction (as well as Exp#6d) was performed right after the first one, via software. However, because of the resting time between these two measurements, the effect from sedimentation of agglomerates was also present. This explains the fact that this time, no peak 2 values were detected on the second measurement. By comparing peak 1 on both measurements, the distribution of measured sizes was a little bit narrower (on average, from 50 to 250 nm) than before (with Exp#6c). This is probably due to the fact that, for each time step, the very same individual sample was always used, ensuring a better reproducibility of results. However, as clarified earlier, since the initial emulsion presented a very large measurement error, these values may not

be representative of the average particle sizes of the silica-PFC capsules stored in the falcon tubes. Even so, we have indications that the final capsules from Exp#7c might have sizes under 50 nm, which is a very good outcome.

Finally, the results for Exp#6d are detailed below:

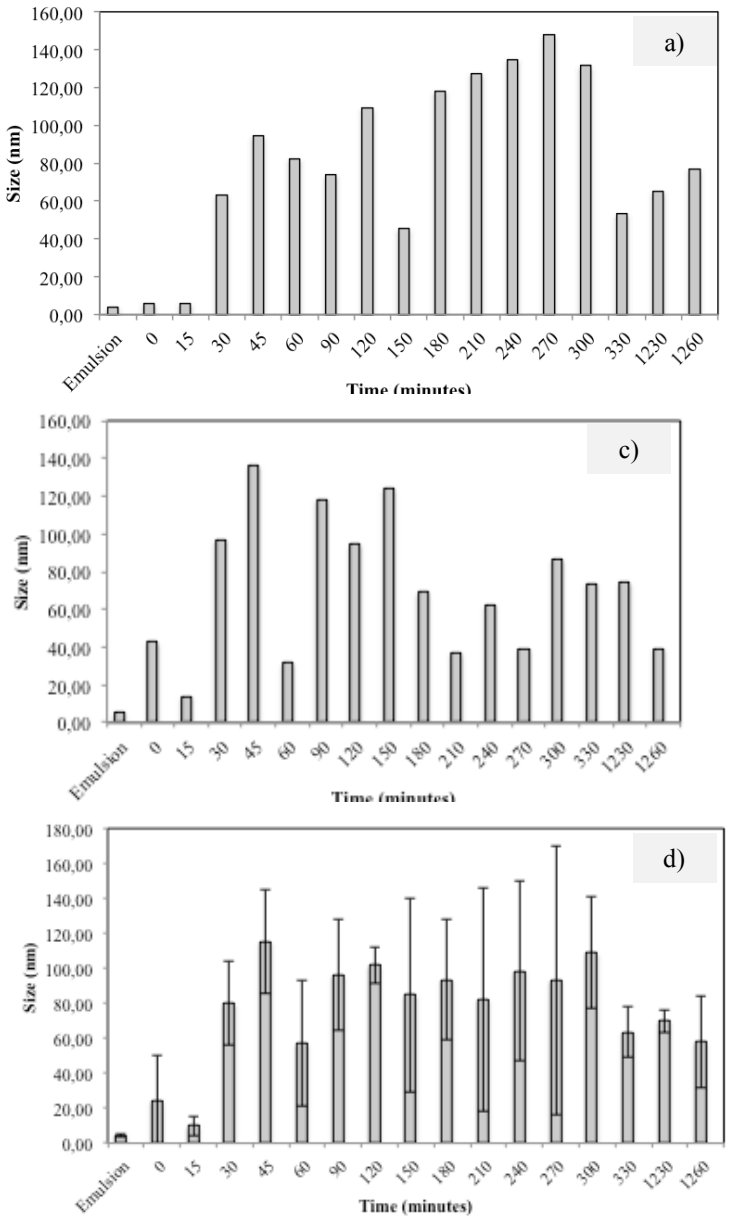


Figure 7 – Reaction kinetics for Exp#6d: a) Peak 1 values throughout time (1st measurement – no peak 2 was detected); b) Peak 1 values throughout time (2nd measurement – no peak 2 was detected); c) Average of all values throughout time.

Unlike reaction Exp#6c and Exp#7c, Exp#6d didn't present any peak 2 whatsoever. The main reason for this absence is that, over time, with increased understanding of some

problems originating the initial emulsion, ultrasound sonication technique was further improved, as well as the sample preparation. On every initial trial (before the study of reaction kinetics), the discrepancies between measured values were much higher than with these last tests. With an increase of experience, better isolation and cooling methods were continuously developed. Moreover, the time control was pin-sharp and the sample collection method was more careful. All these small modifications add up to produce improved final emulsion properties, as well as particle size measurement reproducibility. In this case, with all those improvements, the initial emulsion had the smallest size and variability among all (appendix A.I). The consequence is that the average size of the final silica-PFC capsules was also lower, ranging from 35 nm to approximately 100 nm. These results are in line with Exp#6c and Exp#7c, where the final capsules presented sizes varying from under 50 to over 200 nm. Of course, due to the reasons previously stated, the average size of the final capsules will always inevitably follow a statistic distribution and some experimental errors will always affect the final data. To ensure the veracity of all the quantitative data from this analysis, optical and electron microscopy were used as a visual confirmation.

For a more detailed view of the 3 reactions over time, a comparative analysis of all 3 reactions is displayed below:

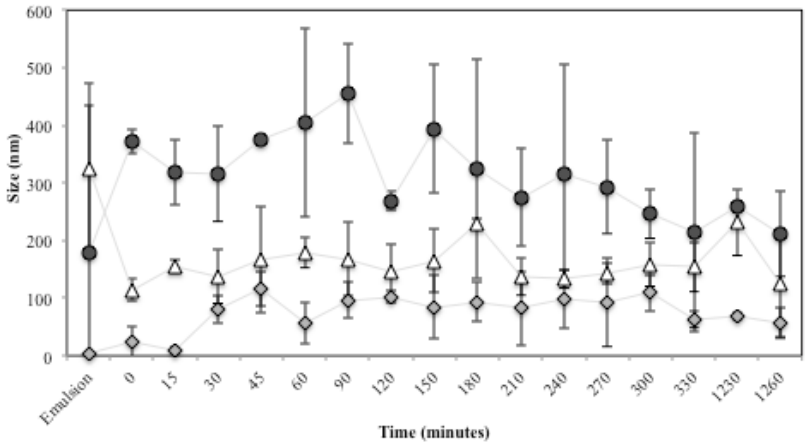


Figure 8 – Reaction kinetics for Exp#6c (●), Exp#7c (△) and Exp#6d (◇): Comparison of peak 1 average values throughout time.

As demonstrated before, the improvement of initial reaction conditions over time (from Exp#6c to Exp#7c to Exp#6d, respectively) allowed the formation of more concise emulsions with narrower size variations, which, over time, lead to the formation of smaller

and more consistent capsules. This is visible above by analyzing the variations of the capsule size over time for the each reaction. Exp#6c presented variations of average values higher than 238 nm, considering just peak 1. In comparison, Exp#7c varied less than half (115 nm). Exp#6d varied even less: no second peaks were ever observed and the maximum difference of peak 1 average values was just 105 nm. Because of the big variability of results, once again no conclusions can be made in terms of distinguishing reactions 6 and 7.

In resume, it was proven that, for nearly all experiments, nano-sized particles were being produced with final sizes as low as 35 nm. Although the existence of a large array of sizes cannot be unnoticed, more profound studies should be made on future investigations regarding kinetic properties of the formation of the initial emulsion.

Optical Microscopy – After the particle size measurements, this first visual test was performed. The best collected images are displayed below:

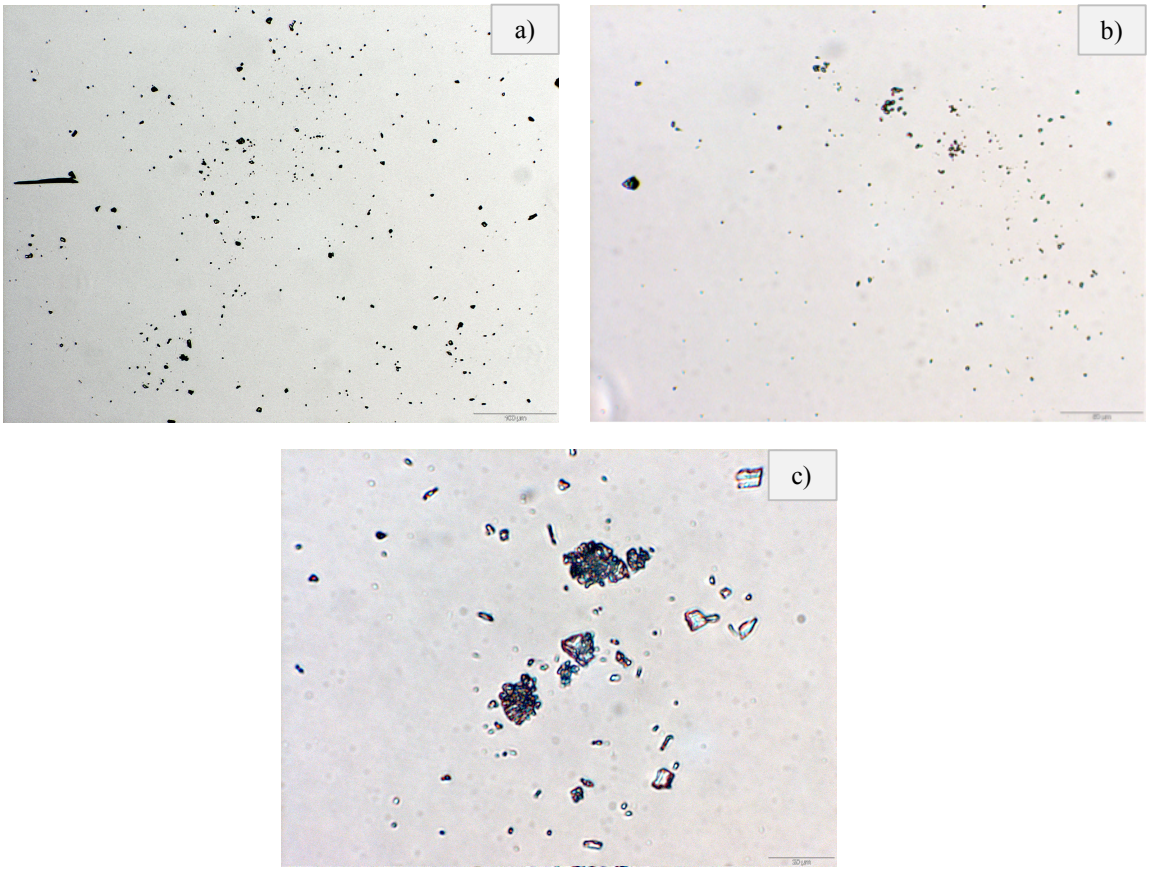
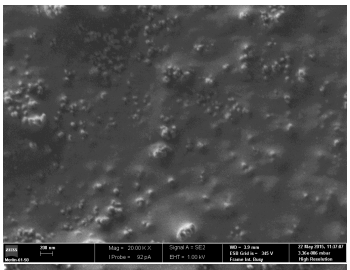
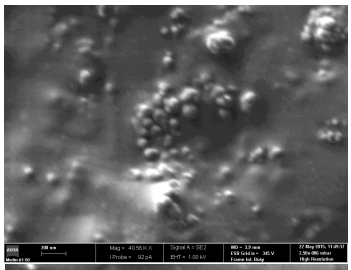
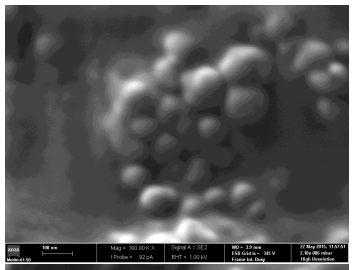
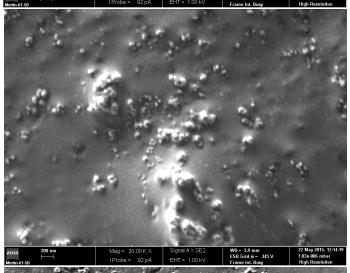
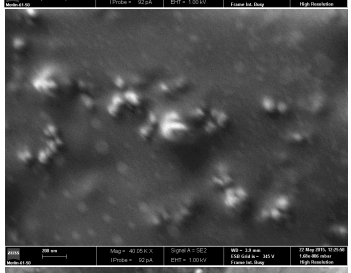
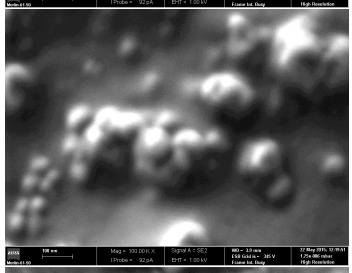
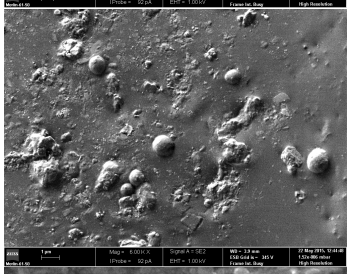
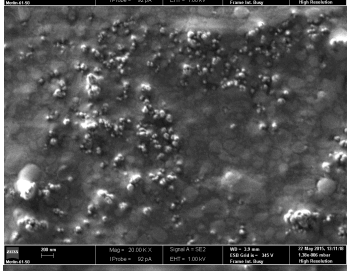
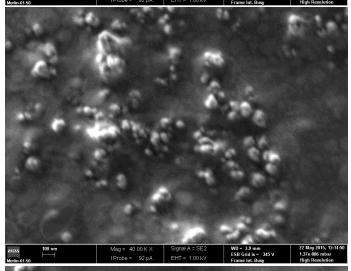
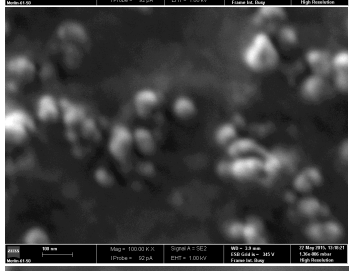
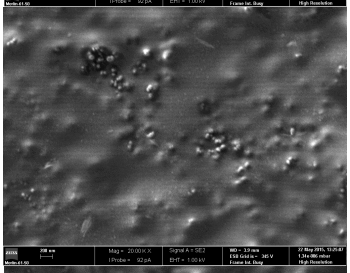
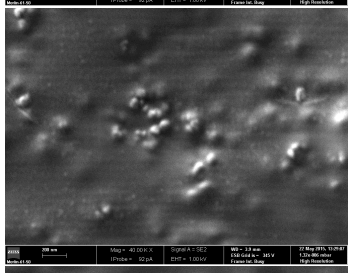
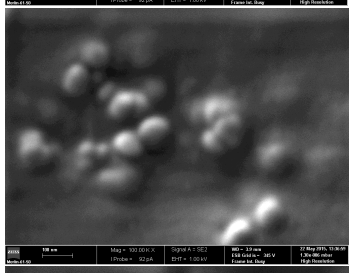
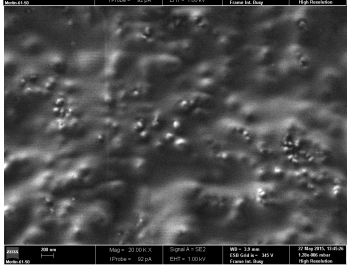
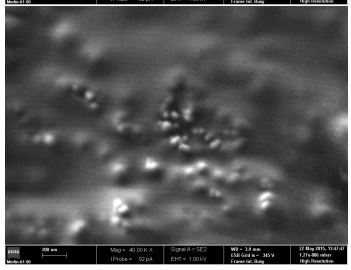
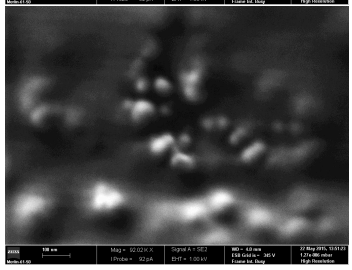


Figure 9 – Optical Microscopy Analysis: a) Exp#6 using 10x magnification; b) Exp#7b using 20x magnification; c) Exp#7 using 40x magnification.

With this visual analysis, the first main conclusion is that the prepared reactions (experiments 6, 6a, 6b, 7, 7a and 7b) were able to produce extremely small particles with rounded shapes, believed to be the silica-PFC capsules. Many agglomerates were also detected. As stated before, the presence of compounds with much bigger sizes when compared to each individual silica-PFC capsule may have strongly influenced dynamic light scattering measurements, supporting previous results.
The size of each individual silica-PFC capsule couldn't be identified with this technique, as it was too low to be clearly visualized with the utilized microscope (even with the maximum amplification). For further analysis, electron microscopy was used.

Scanning Electron Microscopy – Samples were prepared by drying a small droplet of a specific suspension solution in a conductive carbon disk. The droplet evaporated at room temperature for several hours, leaving the dry samples on the SEM support. After the appropriate sample preparation, each individual sample was visualized independently. The best images are displayed in the table below:

Table 3 –SEM Analysis for the suspension solutions with the silica-PFC capsules: comparison between suspension solutions at different magnifications.

	20 000x	40 000x	100 000x
Exp#6			
Exp#6a			
Exp#6b 6000x instead of 20000x			
Exp#7			
Exp#7a			
Exp#7b			

Silica is known to have some electrical conductivity, making the produced nano capsules very sensible. Therefore, gold coating was not applied. Although some of the collected images are not very sharp, they consisted of a hugely important qualitative proof of the quantitative data obtained from the particle size analysis tests.

Upon observing all samples at different magnifications, a general conclusion is that all experiments produced spherical capsules with sizes ranging, on average from 50 to 100 nm. Additionally, as mentioned before, since the reaction leading to the formation of silica-PFC capsules involves the formation of a silica shell over nano and microemulsions, a distribution of various sizes was observed. Finally, because of the utilized method of sample preparation, the suspension solutions dried over time, therefore causing a decrease on the suspension volume over time. Hence, this method alone is likely to induce the formation of a lot of agglomerates caused by the surface tension properties of the suspension droplet. This agglomeration phenomenon was observed for all of the analyzed experiments. Still, considering how small and sensitive the utilized samples were, we could state that the dispersion of capsules over the SEM supports was very decent.

Because of the individual conditions of each reaction, some specific conclusions can be listed. One of the best-captured images was recorded with Exp#6. At 100000x of magnification, lots of capsules with sizes varying from 60 nm to over 100 nm were observed in detail.

Exp#6a also showed remarkable results, with some visualized capsules under 40 nm (visible on the lower left corner with 100000x of magnification). Yet, right beside those extremely small particles, the frequent amount of agglomerates form groups with sizes over 300 nm. These numbers are in agreement with previous particle size measurements, which lead to the conclusion that a very specific particle size control is very hard to achieve with silica-PFC capsules developed with this method (merging emulsion techniques with sol-gel method for silica production).

Upon observing the sample regarding Exp#6b, some very large particles (over 1 μm) were detected on certain regions. Some of those particles also didn't have rounded shape. The most probable motive for this singularity is that, on this specific experiment, a higher amount of silica precursor was used, which didn't bind to the PFC emulsion. Being dispersed in the aqueous medium, that precursor may have lead to the formation of these solid particles. Despite the presence of these particles, Exp#6b also presented similarly

sized capsules to other reactions.

The results of observing experiments 7, 7a and 7b at the same magnifications were extremely similar to samples for Exp#6 and Exp#6a: lots of capsules with sphere-like shapes with sizes ranging from 50 to 100 nm. Some visualized agglomerates with much higher sizes were also detected. Also, just like the results for Exp#6a, upon observing Exp#7b at 100000 x of magnification, we could detect particles with sizes around 40-50 nm.

Overall, it was proven that all visualized capsules were in the same size range of the results from previous particle size analysis. Also, the frequent aggregation between small particles, which, added up, form agglomerates of considerable sizes, can explain previous discrepant values. Another conclusion is that no notable difference was identified when comparing reaction 6 with reaction 7. Therefore, just as previously, this analysis suggested that a slight variation on the relative surfactant concentrations doesn't strongly modify the final capsule's morphology and size. Finally, although some the images with higher magnifications were slightly blurry, we can conclude that this scanning electron microscopy analysis was crucial to better prove and confirm the individual size of each silica-PFC capsules, as well as their overall morphology.

III.2. Characterization of the SCLs with and without capsules

More than 50 different experiments with different concentrations of each component were developed to optimize the optimal final properties of the HEMA-based lenses (transparency, flexibility, among others). Some of those experiments were performed using high temperatures instead of UV light. The utilized the thermal initiator for those reactions was 2,2'-azobis(2-methylpropionitrile) (AIBN). However, because the silica-PFC capsules could potentially be damaged with high temperatures, UV polymerization was chosen. A number of different molds, such as various types of petri dishes, a PTFE well board (10 and 12 mm wells) and others were utilized until the desired consistency was reached. Also, several UV chambers were tested, as well as different reaction times, stirring speeds, among others. Some of the most interesting reactions are described in the table below:

Table 4 – Some of the conducted experiments for the hydrogel SCLs.

Date	HEMA (ml)	EGDMA (µl)	Bi-distilled water (ml)	Initiator (mg)	Polymerization method	Mold	Final results
27th Nov	5,4	82	-	AIBN 8	Memmert oven (70ºC) for 16h	Glass petri dish (50 mm)	Polymerization occurred but the formed hydrogel was too rigid and not very transparent.
10th Feb	1,35	55,7	1	Irgacure® 2959 6,94	UV chamber BS-02 for 4h	PTFE well board	Reticulation occurred but the amount of solution was not enough fill the wells and make thin lenses. Ring shaped hydrogels obtained.
12th Feb	2,7	111,4	2	AIBN 6,3	Memmert oven (70ºC) for 27h	PTFE well board	Thin SCLs were obtained. Good transparency and shape but still too rigid.
10th Mar	2,7	20	2	Irgacure® 2959 4,16	UV chamber BS-02 for 24h	Glass mold silicone & acetate sheets	Much better results than before: Lenses were very homogeneous, transparent and flexible.
10th Mar	5,4	40	-	Irgacure® 2959 12,32	UV chamber BS-02 for 24h	Glass mold silicone & acetate sheets	Very transparent film, yet a bit rigid because no water was added. After submerging it in bi-distilled water, flexibility increased significantly.
17th Mar	2,7	40	2	Irgacure® 2959 3,95	UV chamber BS-02 for 24h	Glass mold silicone & acetate sheets	Final SCLs were very thin, flexible, transparent and consistent. Smooth surface on both sides.
24th Mar	2,7	40	2	Irgacure® 2959 4,16	UV chamber BS-02 for 24h	Glass mold silicone & acetate sheets	Same recipe as last experiment, to prove the concept. Results looked exactly the same.

Water swelling (SCLs) – This test was performed with both hydrophilic SCL types synthesized on March 10th. One of these lenses didn't have water in its original formulation, just monomers and initiator. The other one had the original water amount (2 ml) that was used before. The quantity of water that each lens can swell was measured for both types of lenses. Initially, the hydrophilic lenses were weighed. After that, the loss of mass throughout time was measured by placing the lenses in an oven at 30ºC. Over time, the water evaporates until a stable mass value is achieved. The percentage of water on each lens is calculated by the following quotient:

$$\text{Water content}_{\text{each lens}}(\%) = \frac{\text{Initial mass} - \text{Final mass}}{\text{Initial mass}} \times 100$$

Knowing that the density of water is approximately 999,7 mg/ml, the initial volume of bi-distilled water in each lens (in microliters) is obtained the following way:

$$\text{Amount of Water}_{\text{each lens}}\ (\mu\text{l}) = \frac{\text{Loss of mass (mg)}}{\rho_{\text{water}}(\frac{\text{mg}}{\text{ml}})} \times 1000$$

Results were collected over a few days. The average water content of each lens was 49% for the lenses with water in its initial solution and 45% for the lenses without any water added. These preliminary results evidenced that there is a large possibility of increasing oxygenation properties on these hydrophilic lenses by replacing the full amount (2 ml) of bi-distilled water by certain concentrations of suspension solutions with the silica-PFC capsules.

Scanning Electron Microscopy – SEM was also used to compare lenses without capsules with lenses loaded with capsules (from Exp#6). The purpose of this test was to check if the capsules were being preserved inside the polymer matrix. Comparative images between lenses with/without silica-PFC capsules were obtained. Two of the best images are displayed below:

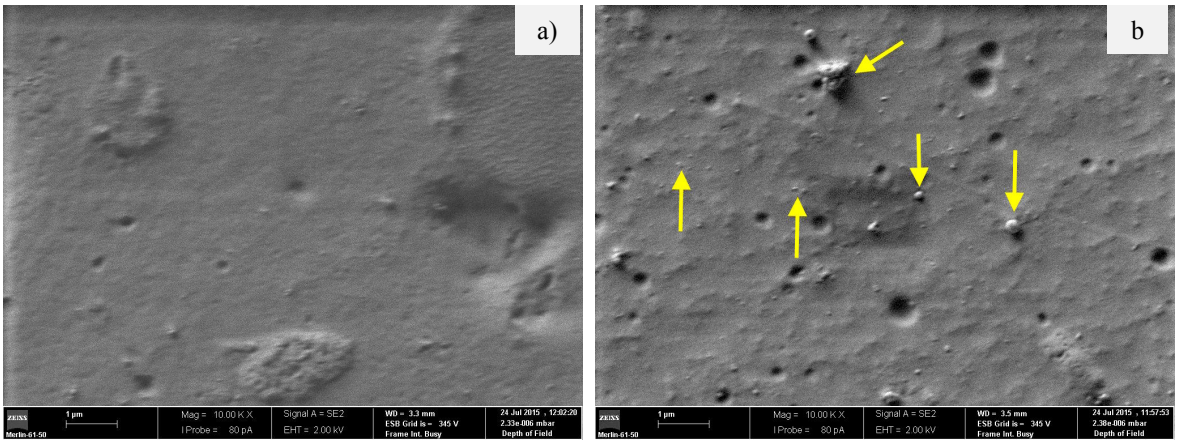


Figure 10 – SEM microscopy for SCLs (10000x magnification): a) SCLs without silica-PFC; b) SCLs loaded with silica-PFC capsules from Exp#6.

The collected images clarify that the SCLs loaded with silica-PFC capsules contained very small (from 50 to 75 nm) and circular particles (arrows pointing up on the image). Some other capsules had slightly bigger sizes. Once again, this is probably caused by the unavoidable size dispersion of the initial emulsion. On the picture, two of those capsules had sizes around 220 and 350 nm (vertical arrows pointing down). Also, an agglomerate of capsules is visible, with a size over 700 nm (marked with a diagonal arrow).

These visualized images were very similar to the ones seen before with the suspension solutions. Since the same type of structures was observed on the SCLs, the main

conclusion is that the visualized particles were indeed the silica-PFC capsules and that they were successfully immobilized in the polymeric chain of the lenses. Consequently, we can conclude that this analysis in in line with previous tests (particle size and SEM for suspension solutions).

Regarding the SCLs without capsules, as expected, nothing relevant was observed other than the polymer itself.

Contact angle – Because the synthesized lenses are highly hydrophilic, the initial amount of water on the surface of each lens was hard to control. Thus, this test was repeated 6 times for each type of lens. The results of this analysis are presented below:

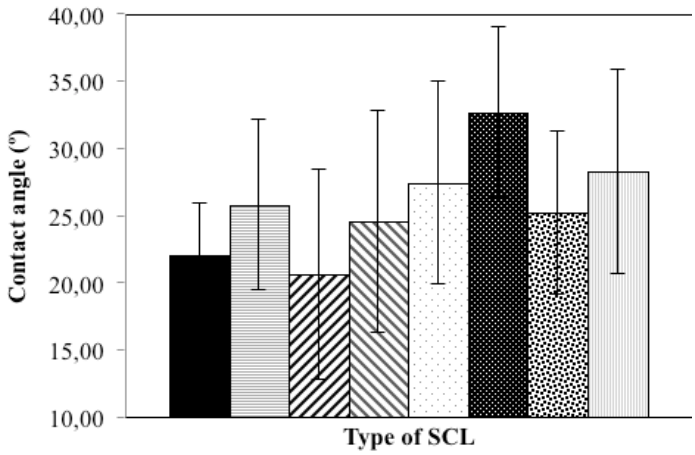


Figure 11 – Contact angle analysis: average values and standard deviation (error bars) for each type of lens: SCLs without silica capsules (■), SCLs + Exp#6 (▣), SCLs + Exp#6a (▨), SCLs + Exp#6b (▧), SCLs + Exp#7 (□), SCLs + Exp#7a (■), SCLs + Exp#7b (▩), SCLs + 2ml Exp#7b (▥).

As shown above, on average, all the synthesized lenses have contact angles ranging from 20º to 30º, depending on how wet the surface was when the measurement was performed. From this analysis we can conclude that the incorporation of silica-PFC capsules in each lens does not change its contact angle. This is due to the fact that the silica-PFC capsules that constitute this system are stored inside the hydrogel matrix, not on the surface. Therefore, this does not alter the surface wettability properties of the original SCLs.

The measurement of contact angle of some of the most used contact lenses in the market has previously been reported. by Read at al. (2011). Results are described on the figure below [30]:

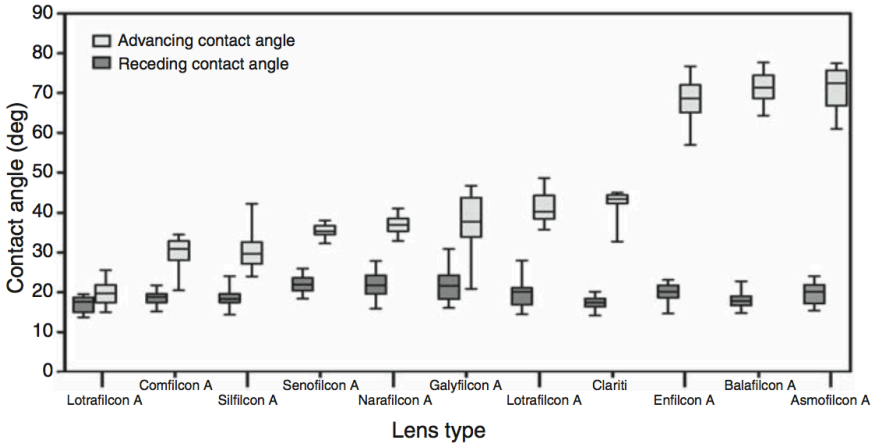


Figure 12 – Contact angle values for several commercial lenses (adapted from reference [30]).

As shown above, average values for most contact lenses are near 20-40º. Comparing these numbers with the obtained results from the SCLs we synthesized, we can conclude that the surface properties of our contact lenses are exceptional: in most cases, the novel SCLs loaded with silica-PFC capsules presented a more hydrophilic surface than current alternatives on the market. Lower contact angles allow better surface adhesion properties as well as a superior wearing comfort.

Transmittance – This analysis was performed to evaluate the influence of the suspension solution on the transparency/transmittance of light through each lens, using 3 equivalent SCLs of each type. For comparison purposes, values at 600 nm (visible light) are displayed. Results were clear, as seen from the figure below:

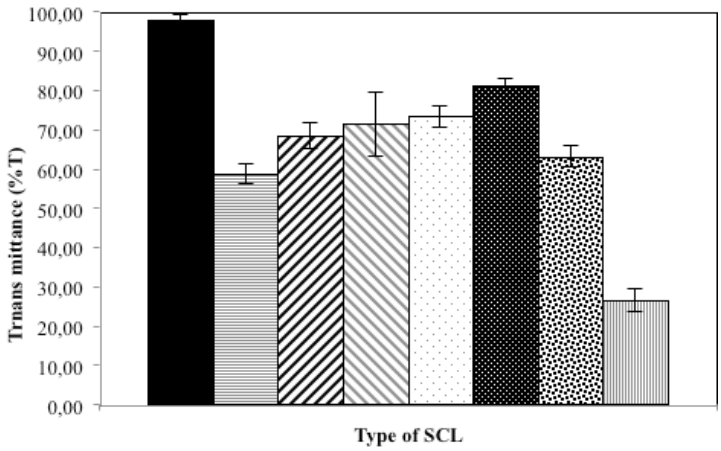


Figure 13 – Transmittance analysis: average values and standard deviation (error bars) for each type of lens: SCLs without silica capsules (■), SCLs + Exp#6 (▣), SCLs + Exp#6a (▨), SCLs + Exp#6b (▧), SCLs + Exp#7 (□), SCLs + Exp#7a (■), SCLs + Exp#7b (▩), SCLs + 2ml Exp#7b (▥).

Different quantities of silica capsules (distributed homogeneously) were incorporated into the lenses, as explained previously (II.4). Even though the silica-PFC capsules are nano-sized particles, those particles are still not fully transparent. The natural side effect is the reduction of the overall lens transmittance at all wavelengths. For the hydrogel SCLs without silica capsules, the average transmittance was 97,97%. This value decreased to 59-81% on lenses incorporating 1 ml of suspension solution containing the silica-PFC capsules. With the SCLs containing 2 ml of suspension solution, the transmittance values dropped to just 26,53% at the same wavelength, suggesting that the concentration of capsules was probably too high.

Transmittance measurements in several commercial contact lenses have already been reported [26]:

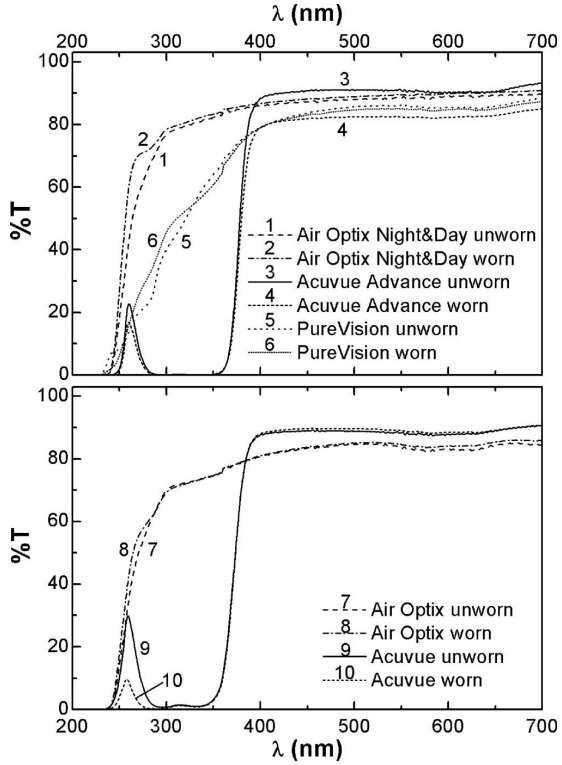


Figure 14 – Transmittance values on various types of commercial contact lenses (adapted from reference [26]).

As seen from the image above, average commercial lenses [26] have transmittances higher than 80% when exposed to visible light. In order to develop an improved new type of SCL, it has to be ensured that its transmittance is near those numbers.

Reading values at 600 nm, it was noticed that when the utilized concentration of capsules is too high, transmittance values drop too much, as expected. However, some developed SCLs with 1 ml of capsules presented very similar transmittance figures, when compared

to final commercial products. This proves the idea that it is possible to include nano-sized silica-PFC capsules in contact lenses, without deeply affecting one of the main properties of a contact lens, it's transmittance.

SDT/TGA (capsules, SCLs and SCLs with capsules) – This test was designed to ensure that the novel lenses loaded with capsules have indeed interesting oxygenation properties due to the existence of silica-based materials. Because silica (SiO_2) is a material that can withstand temperatures over 600ºC without losing its mechanical properties, various types of SCLs with and without capsules were compared in this thermal analysis (samples of 10-15 mg were prepared). After setting the initial stabilization temperature at 25ºC, the heating process occurred at 10ºC per minute up to 600ºC. At that temperature, all of the water and polymeric materials are decomposed, as well as the perflurocarbon. Therefore, most residues at 600ºC should only come from the most resistant inorganic materials, in this specific case silica from the silica-PFC capsules. Various measurements were made to ensure the credibility of results. The average values of final residues are displayed below:

Table 5 – SDT/TGA analysis: average and standard deviation of residues for all SCLs.

Sample	Average of residues (%)	St. Deviation (%)
SCL without capsules	1,19	0,04
SCL + Exp#6A	2,07	0,02
SCL + Exp#6B	1,29	0,49
SCL + Exp#7B	1,60	0,16

For the full combustion of the samples, an O_2 atmosphere should be applied. However, because this equipment utilizes an atmosphere of Nitrogen, the decomposition of the lenses is never 100% effective. Hence, as expected, some residual mass was still detected on the samples from the lenses without capsules. Those leftovers are due to the formation of secondary compounds during the decomposition of the polymeric material. Because these compounds are formed in every type of lens, to better compare individual samples, the residues from SCLs with capsules were subtracted by the residues from the SCLs without capsules. That difference corresponds to the inorganic mass of silica capsules, which didn't decompose at 600ºC. The average and standard deviation of that difference for each SCL was calculated. Results are displayed below:

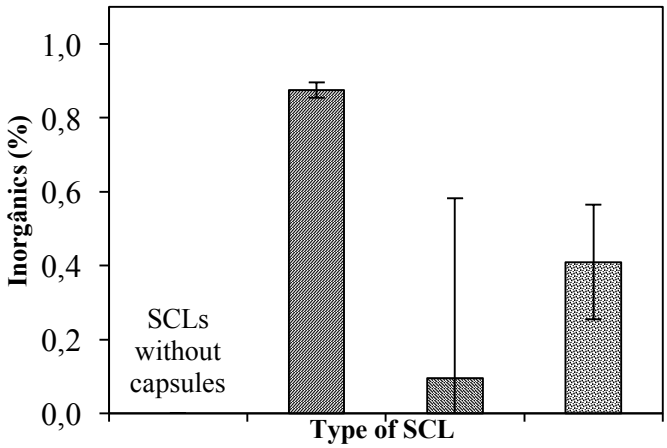


Figure 15 – SDT/TGA analysis: average values and standard deviation (error bars) for the inorganic mass for each type of lens: SCLs + Exp#6a (▨), SCLs + Exp#6b (▧) and SCLs + Exp#7b (▩).

By examining the loss of mass over the heating period, we have a confirmation that the SCLs loaded with capsules had a higher residual mass than SCLs without capsules. This differences, which correspond to the concentration of inorganic mass (silica capsules) varied for each sample. The percentage of inorganics (in mass) corresponds to the concentration (in grams per each 100 ml) of capsules in the initial suspension solutions. Therefore, considering that the distribution of silica-PFC capsules was perfectly homogenous throughout the final hydrogel, we can calculate the concentration of capsules produced per milliliter on each reaction. The measured values showed that Exp#6a had 0,88 ± 0,02 % of silica, which converts to 8,8 ± 0,2 mg of silica per each milliliter of suspension solution. For Exp#7b, this value was 0,41 ± 0,16 % of silica (4,1 ± 1,6 mg/ml). Results for Exp#6b returned 0,10 ± 0,49 %, which translates to 1,0 ± 4,9 mg/ml of silica. As seen from collected data, this last value has a standard deviation higher than the measurement itself. This was probably due to the sample preparation: the utilized polymer fragments (from SCL loaded with capsules from Exp#6b) for this measurement may not have had a homogeneous distribution of capsules.

The measurement of the quantity of capsules on each suspension solution had already been previously tested in an initial trial. This trial consisted in collecting a 1 ml sample of each suspension solution (experiments 6, 6a, 6b, 7, 7a and 7b were analyzed) and measuring the amount of silica in that volume. The utilized method involved various steps. Initially, vortex stirring was applied to the falcon tubes (where the suspensions were stored) for 20-30 seconds. Then, a 1 ml sample was collected and placed in an eppendorf tube (1,5 ml).

After weighing all eppendorf tubes (empty and filled with suspension solutions), they were centrifuged for 2 minutes at 5000 rpm. Finally, the supernatant liquid (800 µl) was removed. With the lids open, the eppendorf tubes with aliquots of all experiments were then placed in an oven at 32ºC. Their loss of mass was measured over time.

The results of these preliminary measurements for equivalent samples (Falcon tube I for experiments 6a, 6b and 7b) were the following: Exp#6a presented a concentration of 3,28 mg/ml. This value is slightly lower than the one measured with the thermal analysis, yet in the same size range. Exp#6b showed a similar concentration of 3,96 mg/ml, which is within the interval of the values for this thermal analysis (1 ± 4,9 mg/ml). Finally, Exp#7b showed a slightly lower amount of capsules: just 0,83 mg/ml, versus 4,1 ± 1,6 mg/ml presented before. These values are, once again slightly different, but in the same size range.

It is essential to notice that these last numbers were collected by weighing the falcon tubes in a semi micro balance (Sartorius CPA225D), which has a much lower measuring precision then SDT/TGA techniques. The SDT/TGA tests provide much more detailed, precise and trustworthy information. Nonetheless, combining all data from the two techniques proves that the overall utilized concentration of capsules was always in the range of 1-10 mg/ml for all tested experiments.

CO_2 loading/release (capsules, SCLs and SCLs with capsules) - Numerous CO_2 loading/release tests in an aqueous medium were performed in different conditions to visually investigate if the formed mesoporous capsules were loading gas bubbles in their cores. Firstly, the suspension solutions were analyzed independently. After that, various assays were executed evolving the contact lenses (with and without the silica capsules). To guarantee comparative results, the flow rate of CO_2 that was passed through each sample was the same. This was achieved by loading for two equivalent samples side by side, at the same time.

On the first test, 40 ml of bi-distilled water were added to 3 glass vials. Then, a solution of capsules from Exp#6 (500 µl) was added to one of those vials. The final samples were the following: samples 1 and 2: bi-distilled water (40 ml); sample 3: bi-distilled water (40 ml) + suspension solution from Exp#6 (500 µl). Sample 1 was untreated. The samples 2 and 3 were exposed to bubbling CO_2 for 12 minutes, saturating the samples with this gas. After

putting the 3 vials side by side in an ultrasonic cleaner (Elma S30H, Elmasonic) and applying ultrasound sonication, the release rate of the CO_2 in all 3 samples was observed. Sample 1 didn't release any CO_2 bubbles, which makes sense because the utilized water was not loaded with any gas. Sample 2 released a few CO_2 bubbles for around 30 minutes. However, sample 3 (with the silica-PFC capsules) released a considerably increased amount of gas bubbles, when compared to sample 2 (also for about 30 minutes). While previous analyses proved that the silica-PFC capsules were being produced with the appropriate size and shape, this preliminary test provided some clues that the formed capsules were indeed working as efficient gas carriers.

A number of tests were also made to compare the efficiency of SCLs with and without the silica-PFC capsules. One initial trial is included in the appendix A.II. This time, two different types samples were examined side by side: sample 1 included degassed bi-distilled water (40 ml) + SCLs without capsules, while sample 2 included the same degassed bi-distilled water (40 ml) but the utilized SCLs were loaded with silica-PFC capsules. All lenses had 11 mm of diameter. While loading (this time for over 90 minutes), the SCLs were immobilized in the middle of the glass vials so that the distribution of CO_2 was homogeneous (figure below).

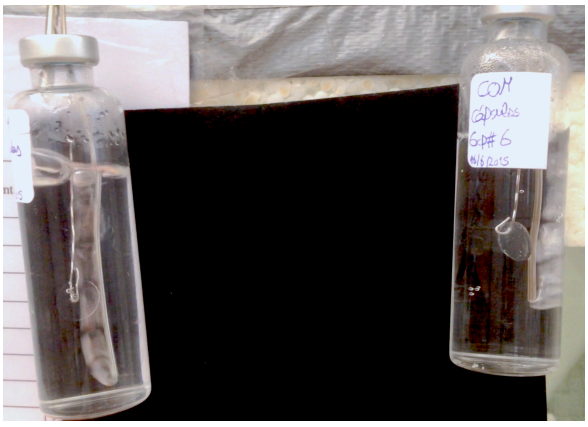


Figure 16 – SCLs with and without capsules while CO_2 is being loaded.

The release of CO_2 was observed by drowning lenses in newly degassed water. Almost no gas was released from SCLs without capsules. However, an increased amount of bubbles appeared to be coming out of the SCLs loaded with capsules. The size of the visual bubbles was so small, they were barely visible at naked eye (magnified image below). This phenomenon might be explained by the fact that the absorbed CO_2 may have been stored

inside the nano-sized silica-PFC capsules. Thus, during degassing, the formed bubbles may be too small to be easily identified visually. However, by comparing the release profile between SCLs with/without capsules side by side, several release tests strongly suggested that the contact lenses that included the CO_2 loaded capsules stored (and released) a higher amount of CO_2 than the standard SCLs.

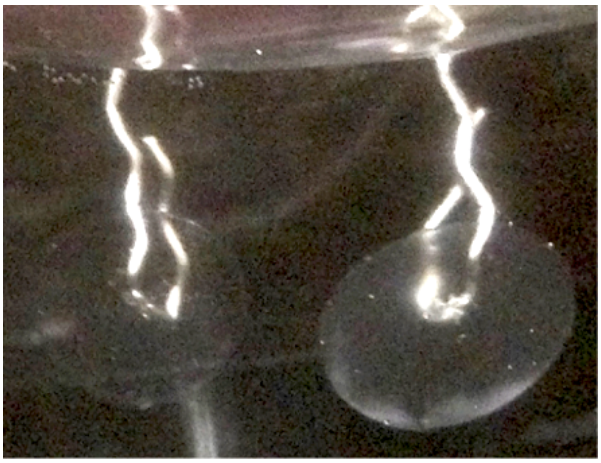


Figure 17 – SCLs with (on the right) and without (on the left) capsules while degassing.

Following the same methodology as before, two last tests were performed with 2 types of SCLs loaded with silica-PFC capsules: the first sample had SCLs loaded with 1 ml of suspension solution from Exp#6a; the second sample had SCLs loaded with 2 ml of suspension solution from Exp#7b. Theoretically, because the lenses with 2 ml of suspension solution have twice the concentration of silica-PFC capsules, they should load and release twice the amount of gas in their interior. To achieve the best possible results, this last CO_2 loading lasted 120 minutes. As before, very little bubbles were released on both types of SCLs, with a slight increase on the SCLs with higher concentration of capsules.

Although the duration of these preliminary CO_2 release tests was always under 1 hour, the natural release profile of the SCLs with capsules may last several hours. Even so, this analysis helped to confirm that the SCLs with capsules always had superior efficiency in terms of storing and releasing gas in their interior, when compared to equivalent hydrogel SCLs without capsules. This strongly supports the initial hypothesis that the combination of PFC microemulsions in aqueous medium with a silica shell actually produces efficient gas carriers for various biomedical applications.

O_2 solubility (capsules, SCLs and SCLs with capsules) - This test was implemented not only to study the amount of stored oxygen of each sample in a specific time interval, but also the rate of O_2 transportation of between the saturated bi-distilled water and each individual sample. Initially, 20 ml of bi-distilled water was bubbled with pure O_2 for 1 hour in falcon tubes (Corning® CentriStar® 430829). Next, the O_2 probe was inserted inside the falcon tube and the system was isolated with Parafilm M®. The probe was immersed in the water for 1 hour with the falcon tube as well isolated as possible to equilibrate the concentration of oxygen between the aqueous and gaseous medium. This step is very important because, on the 5-10 first trials (without this critical step), a lot of fluctuations were observed in the first minutes, affecting final results significantly. After 1h of stabilization time, the measurements at specific times (controlled with a chronometer) began.

The first analyzed samples were suspension solutions with the silica-PFC capsules from Exp#6a. Because previous results for SCLs loaded with 1 ml of Exp#6a suggested a bigger amount of inorganics (which are believed to come from the silica-PFC capsules) on that reaction, a new and higher concentrated suspension solution was utilized by centrifugation (5000 rpm for 5 minutes). After a new thermal analysis, it was concluded that this new sample had a concentration of inorganics of 10,5 mg/ml. 2 assays were made with this concentrated solution with 300 µl samples.

Initially, the first comparison test was between this aqueous solution of capsules (300 µl of suspension solution + 20 ml of water saturated with O_2) and the same 20 ml of saturated bi-distilled water without any added compound. By adding a certain amount of silica-PFC capsules (previously degassed with ultrasound sonication) onto O_2-loaded water, because of their physical and chemical properties, they should capture the oxygen from the water over time. Resuming, this test will then measure the transportation (concentration) of oxygen over time from the water to the silica-PFC capsules. Those results will then be compared to the standard sample, the same O_2 loaded bi-distilled water without any solution added.

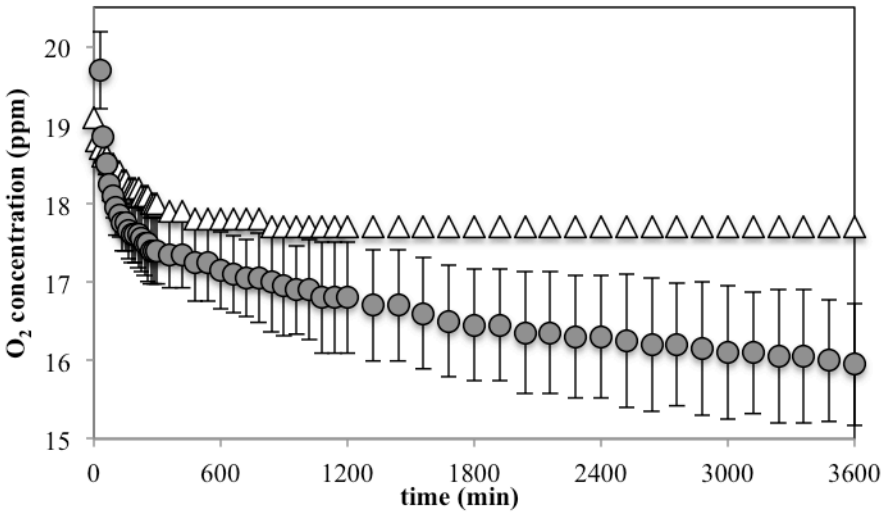


Figure 18 – O_2 solubility analysis: comparison between a solution of silica-PFC capsules from Exp#6a (●) and a bi-distilled water (△).

From the results above, it is clear that the solution of capsules has decreased oxygen concentration on the aqueous medium a lot more, which is a first proof of concept of this study. Although measurement errors cannot be ignored, there is a clear difference in the loss of oxygen over time between these two samples. The overall loss of oxygen from the bi-distilled water on both samples can be calculated by subtracting the final measurement (after 1 hour) with the first stable value. This stability time varied from sample to sample according to a number of factors, but in this case it was about 30 seconds on both cases. From the measured data, the silica-PFC capsules have absorbed approximately 3,75 ppm of O_2 from the oxygen-loaded water. In comparison, the sample with only bi-distilled water lost 1,4 ppm over 1 hour. The 1,4 ppm loss over time may have been caused by small isolation leaks, which may have caused some of the oxygen in the water to escape to the atmosphere.

There is a suggestion that the capture of oxygen from the silica-PFC capsules would continue even after 1 hour at a similar rate. After 20 minutes (1200 seconds) of measurement, the decrease of O_2 concentration over time followed a continuous linear function where the slope is approximately $\frac{16{,}8-15{,}95\ (\text{ppm})}{60-20\ (\text{minutes})} = 0{,}02125$ ppm/min. This means that, after one hour of measuring the O_2 sorption, the solution of capsules would probably continue to absorb oxygen at a rate of 1,275 ppm per hour. Oppositely, the sample that only contained bi-distilled water appears to have stabilized its final O_2 concentration of

17,7 ppm. After just 14 minutes (840 seconds), this value remained unchanged till the end of the measurement.

This very slow oxygen transport (only a few ppm decrease) may explain previous preliminary results from CO_2 tests, where only a small amount of very little bubbles were observed over several minutes. If the degassing test was extended for several hours, the bubbles would probably be still coming out of the gas-saturated samples.

On another trial, another new type of sample was compared with the solution of capsules stated above. This time, an aqueous solution of SBA-15 (mesoporous silica) was used. By being mesoporous, SBA-15 is known by its ability to physically adsorb gases to its surface [12]. This solution was prepared by adding 54 mg of SBA-15 into 2 ml of bi-distilled water. As before, that solution was added to the 20 ml of bi-distilled saturated with O_2 after 1 hour of stabilization time with the measuring probe.

Like previously, the measured O_2 concentration corresponds to the amount of gas that is captured by the SBA-15 sample in the aqueous medium. The comparative result from this test and the previous sample of silica-PFC capsules is shown in the image below:

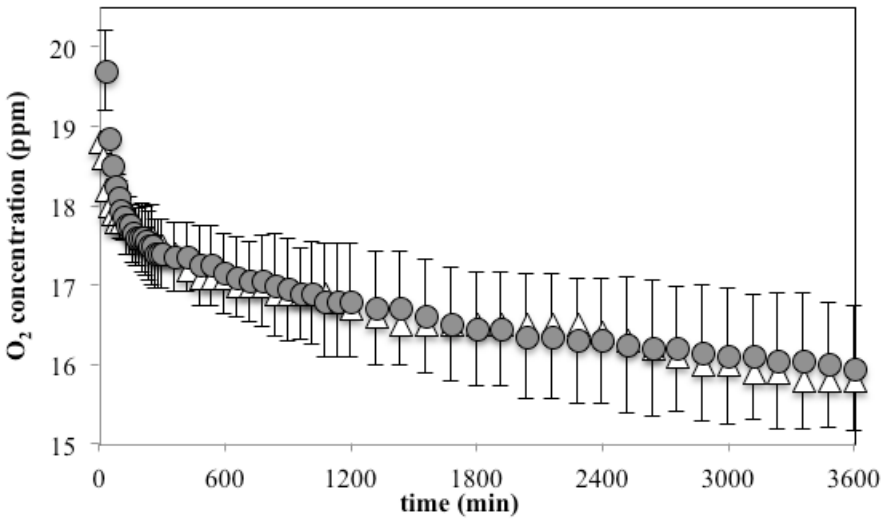


Figure 19 – O_2 solubility analysis: comparison between a solution of silica-PFC capsules from Exp#6a (●) and a solution of SBA-15 (△).

As before, the sorption of oxygen from both samples can be calculated subtracting the final value with the first stable one. In this case, the O_2 absorbed by the silica-PFC capsules was, as mentioned before, 3,75 ppm. The amount of oxygen adsorbed by the SBA-15 solution was 3 ppm. However, from the image above, we can see that, considering the measurement

errors, the differences are maybe too close to call. Therefore, with these results we can conclude that, during 1 hour, the prepared solution of silica-PFC capsules captured a similar amount of oxygen to the prepared solution of SBA-15.

Although the mesoporous silica, as stated before, has great oxygen adsorption properties, these values should be under the sorption capacity of the silica-PFC capsules. After studying numerous possibilities for these results, we have come to a number of factors that might justify them.

First, for comparative results, equal concentrations of SBA-15 and silica-PFC capsules should be used. In this case, according to the results from the SDT/TGA analysis, even with a much higher concentration of capsules than previous initial trials (more than 10 were made), only $10{,}5\ \left(\frac{\text{mg}}{\text{ml}}\right) \times 0{,}3\ (\text{ml}) = 3{,}15$ mg of capsules were present in the full volume of water. Therefore, the concentration of capsules was $\frac{3{,}15\ (\text{mg})}{20{,}3\ (\text{ml})} \cong 0{,}155$ mg/ml. Comparatively, on the utilized solution of SBA-15, 54 mg were dissolved in a total volume of 22 ml. This means that the concentration of SBA-15 in the aqueous medium was $\frac{54\ (\text{mg})}{22\ (\text{ml})} \cong 2{,}454$ mg/ml. This value is over 15 times higher than the concentration of silica-PFC capsules and might have been the most significant factor that stimulated such similar results.

Another factor that might have influenced the measured data is the temperature control. It is well known that oxygen solubility is strongly influenced by minimal variations in temperature [18]. This means that, for every single step regarding sample preparation and measurement, the temperatures must be clinically controlled for each individual test. This temperature control is so meaningful that even a slight temperature difference in the distilled water from the ultrasonic cleaner during sample degassing (prior to measurements) could influence measurement concentrations by a few decimals. Having a room with controlled temperature and pressure conditions is additionally very important to ensure the reproducibility of results in future investigations. On this individual case, while measuring O_2 concentrations with SBA-15, the variation in temperature over 1 hour was 1,5ºC. On the other side, while performing measurements with the silica-PFC suspension solutions (two different measurements were made), this temperature variation was, on one case 1ºC and the other, only 0,1ºC. This lack of reproducibility might justify some of the results.

Another possible source of measurement errors is the pressure control on various steps. This is extremely important since the equilibrium of O_2 solubility is only possible for very stable temperature and pressure conditions. The isolation method (using Parafilm M®) has proved not to be 100% effective since the sample with bi-distilled water has lost some residual O_2 during its measurement. Due to weak isolation, a lot of oxygen may have been lost to the atmosphere during the first hour of stabilization time, which may have strongly reduced the visible differences between analogue samples (in this case silica-PFC capsules and SBA-15).

Another source of possible errors is the uncertainty that the initial sample of bi-distilled water is completely saturated for every single measurement. On some of the first (discarded) trials, this parameter was not well defined, which resulted on measurements with very different initial O_2 concentration values. Due to increasing experimental experience, those differences were significantly less noticeable on the latest tests, but they still cannot be ignored.

Still, even considering all these possible factors, both materials (silica-PFC capsules and SBA-15) have demonstrated great O_2 sorption capacities because both of those samples, in just one hour, were able to noticeably reduce the oxygen concentration in the aqueous medium. Also, as previously noted, O_2 concentrations in the aqueous medium continuously kept dropping till the end of the measurement, suggesting that these values would continue to decrease over time. Only with perfected methods (better isolation, improved temperature control, among others) and longer trials is possible to reach more specific conclusions when it comes to analyzing the efficiency of the silica-PFC capsules in a suspension solution over time.

After testing the silica-PFC suspension solutions, new tests were conducted with the contact lenses with and without capsules. This test was performed to measure the O_2 sorption differences between normal hydrogel contact lenses and the innovative SCLs loaded with the silica-PFC capsules. Because the capsules are retained into the polymer matrix, results could be a bit different than comparing only suspension solutions in water.

Four SCLs of each type were used for each test to maximize the differences of the sorption potential of the SCLs.

For this test, it was decided to use the lenses with the highest concentration of capsules (2 ml of suspension solution from Exp#7b). Despite acknowledging that the transmittance

values for this type of lens are much lower when compared to other types of SCLs with capsules, these SCLs were still utilized in this test for the proof of concept. Besides, as expected, these lenses were the ones that appeared to have performed best on the preliminary CO_2 loading/release tests. Hence, on the following tests, various SCLs loaded with 2 ml of suspension solution from Exp#7b were compared to SCLs without capsules. The result of several measurements is summarized in the image below:

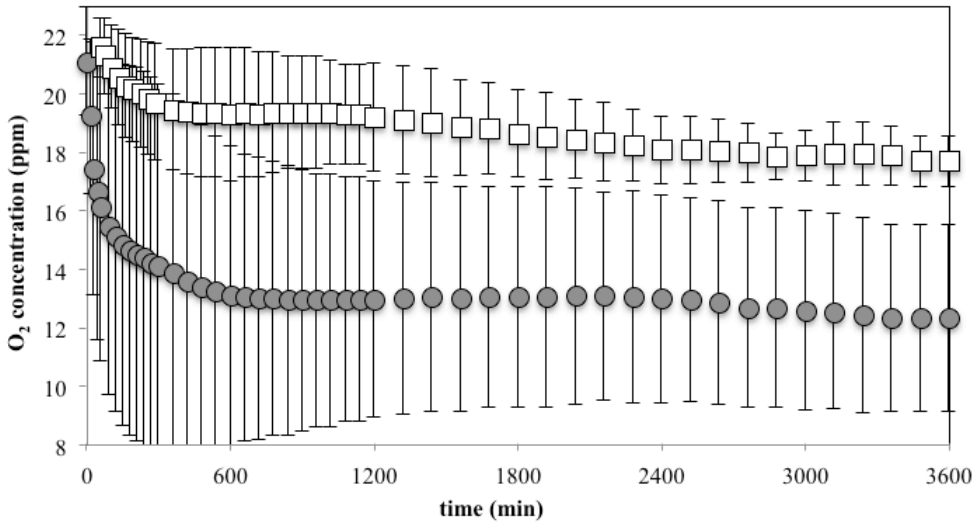


Figure 20 – O_2 solubility analysis: comparison between SCLs with 2 ml of silica-PFC capsules from Exp#7b (●) and SCLs without capsules (□).

The collected data evidences a clear difference: the SCLs with capsules have captured a much higher amount of oxygen from the saturated water than the SCLs without capsules. Although measurement errors were elevated, the average values from several measurements present obvious differences in the O_2 sorption capacity on both samples. In this case, the O_2 sorption from SCLs with capsules was approximately 8,35 ppm, a value that is curiously even higher than the initial suspension solution (above) itself, probably due to the fact that the amount of capsules inside the lenses were higher than on the suspension solution. Moreover, during the first instants it is visible that the rate at which the oxygen is captured is much higher on SCLs with capsules.

As before, there are a number of motives that might have caused the big variability of the results (resulting in higher measurement errors). As explained before, the first is the temperature. The temperature at which all assays were developed sometimes varied more than 2ºC between various assays. Besides, all the isolation issues stated above were also

present for all of the measurements with contact lenses. Furthermore, although all efforts have been made to develop SCLs with a fully homogenous dispersion of capsules (i.e., each individual lens has the same type and amount of capsules), this dispersion cannot be assured upon the formation of lenses, leading to less reproducible results. This is due to non-linearity on some characteristics of the final silica-PFC capsules, mainly controlled by the formation of the initial PFC emulsion, as explained in previous sections.

This leads to the conclusion that, although it was the first time we did this type of measurements with these innovative silica-PFC capsules, the registered results consist in just preliminary data. Since this is an investigation project, much still needs to be improved with this technique which, in future investigations, has to be repeated with more precise control over important parameters to achieve the most accurate results as possible.

Even so, from the results of these and other tests, there is strong evidence that the introduction of silica-PFC capsules in hydrogel contact lenses really provides substantially increased oxygenation properties, when compared to normal hydrogel SCLs. With perfected measurement parameters, future tests will continue to be developed to achieve more accurate and precise data to further prove this initial idea.

Chapter IV – Conclusions and future work

The initially proposed idea was to develop contact lenses with improved oxygenation properties, when compared to products already on the market. The utilized methodology involved the development and inclusion of silica-PFC capsules into hydrogel SCLs.

Based on all developed work, various individual goals were accomplished. Initially, various experiments regarding silica-PFC capsules were developed and perfected to reach the best properties upon their introduction in contact lenses. Then, a large amount of several types of contact lenses were synthesized. After many trials, the properties of the final lenses were in line with most products in the market (in terms of flexibility, water content, surface properties and transmittance). After proving that point, the merging of both concepts was achieved. Various types of contact lenses loaded with capsules were synthesized and extensively characterized.

Initial particle size measurements have shown that, despite the big variability of results, all reactions produced nano-sized capsules with relative reproducibility. Also, it was shown that some produced capsules had sizes lesser than 50 nm. The inclusion of a pre-determined concentration of such small particles in a hydrogel contact lens should allow increased oxygenation properties, without altering the transparency of those lenses.

With microscopy tests, there was visual confirmation of quantitative data from particle size results. It was proved that spherical nano-sized capsules were produced on all reactions. While observing contact lenses loaded with capsules, the same type of structures was visualized, confirming that the silica-PFC capsules were present inside the hydrogel SCLs.

On contact angle measurements, it was proven that the inclusion of these innovative silica-PFC capsules didn't affect the surface properties of the SCLs, just as expected. All of the produced lenses were highly hydrophilic, with contact angles always under 40º.

On the transmittance analysis, it was shown that introducing the suspension solution with the silica-PFC capsules into the SCLs causes a perceptual loss on the transmitted light. Therefore, if a high concentration of these innovative silica-PFC capsules is going to be implemented, new and improved methods have to be delineated in future investigations to produce lenses with excellent oxygenation properties without losing crystal clear transparency. Still, it was already proved that, by perfecting the concentration of capsules on a given lens, the transmittance values are very close to commercial products.

With preliminary CO_2 loading/release tests, only visual data was obtained. Nonetheless, we are already able to prove the efficiency of the silica-PFC capsules, when used as gas carriers.

The final characterization test consisted in measuring the amount of transported oxygen from saturated bi-distilled water to degassed samples over a 1h period. In other words, the O_2 sorption capacity of both suspension solutions and lenses loaded with silica-PFC capsules was studied. The measured data provided strong evidence that the presence of silica-PFC capsules influences the capacity of O_2 sorption in hydrogel contact lenses.

Although the proof of concept has been accomplished, some experimental parameters still need to be sharpened in future investigations to achieve more precise data. The formation of the initial emulsion is an essential reaction step that defines most properties of the final capsules. Therefore, kinetic studies of emulsion growth and critical micelle concentration (CMC) have to be developed for better understanding the size dispersion of the final capsules. To improve data from electron microscopy, two suggested methods are the use of cryo-SEM and cryo-TEM, which would likely allow more precise information about the thickness of the silica shell, as well as the actual dispersion of capsules, without as much agglomeration (caused mostly by the method of sample preparation). Finally, since contact lenses are medical devices used to correct human vision, they have to be subjected to a number of security procedures before being implemented to the global market. The most important step is the addition of a final coating to protect the eye from UV light. Also, to correct visual distortions, the final lenses have to be produced with an appropriate curvature (concave/convex).

Although a lot of experimental factors have limited some of the collected results, we can conclude that this study has already yielded some important advances in the field of science and ocular health.

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Appendices

A.I. Particle Size measurements

Initial sonication time trials – Starting with just 30 seconds of sonication, the first sample was analyzed. As represented from the figure below, because the results clearly showed a bimodal distribution, another minute of sonication was applied. Once again, the results weren't perfect, yet improved. With one more minute of sonication (totaling 2 minutes and 30 seconds), the blue curve below was obtained. With these results, it was decided that the appropriate sonication time before each analysis should be 2 minutes and 30 seconds for each sample, because it appeared to be the adequate time to disaggregate most of the formed capsules in the suspension solution. This initial sonication time was kept for every other analysis with this device.

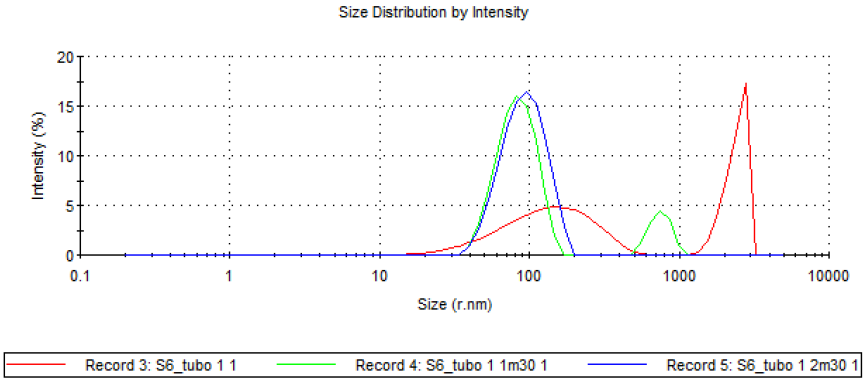
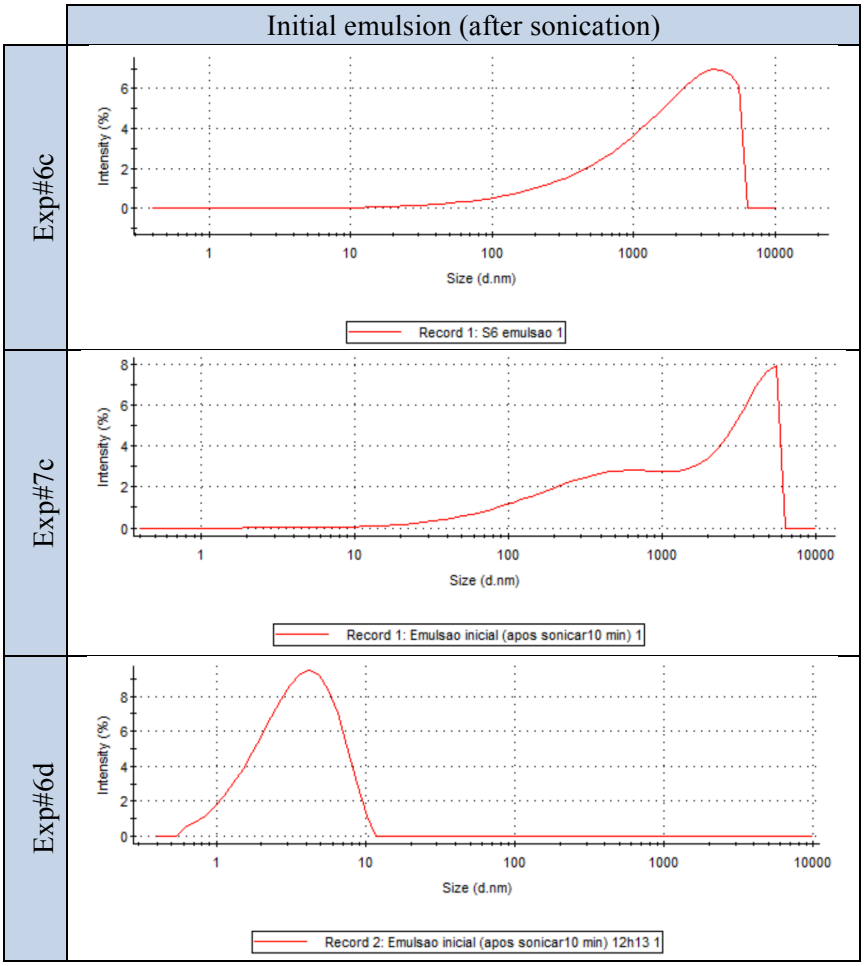


Figure A.I 1 – Zetasizer analysis 1: Sonication time test.

Table A.I 1 – Comparison of the size distribution for Exp#6c, Exp#7c and Exp#6d: Initial emulsion.



A.II. Initial CO_2 loading/release trials without degassed water

One of the first trials with CO_2 showed that the contact lens that included the gas loaded capsules appeared to have stored a higher amount of CO_2 than the normal contact lenses. However, results weren't conclusive because it was noticed that the visualized CO_2 bubbles came not only from the SCLs, but also the saturated water in which the lenses were loaded. This is clearly visible in the figure below.

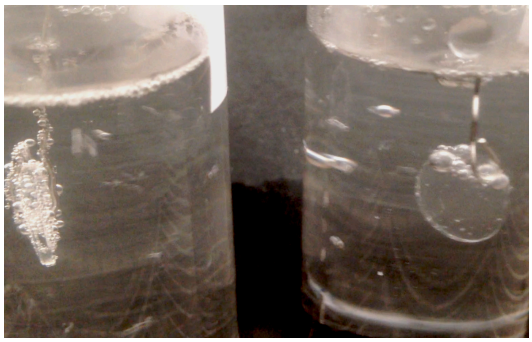


Figure A.II 1 – SCLs with (on the left) and without (on the right) capsules while degassing.