Novel approaches for production of membranes for virus removal

BACHELORARBEIT

VON

ANDRÉS MARTÍNEZ

Fachhochschule Münster
University of Applied Sciences
Fachbereich Chemieingenieurwesen

Universidad Pontificia Bolivariana
Facultad de Ingeniería Química

Die Abschlussarbeit wurde durchgeführt bei

Gambro Dialysatoren GmbH.
Research department
Ermelesstraße 76
72379 Hechingen

Firmenbetreuer: Dr.-Ing. Szymon Dutczak

Diese Abschlussarbeit wurde dem Prüfungsausschuss des Fachbereiches
Chemieingenieurwesen vorlegt am:

_____

Hiermit versichere ich, dass ich diese Arbeit selbstständig angefertigt habe und
die benutzten Hilfsmittel/Quellen angegeben worden sind.

_____
(Unterschrift)   Vorname / Nachname

Fachhochschule
Münster University of
Applied Sciences

Content

1. Introduction

1.1. The company

Gambro GmbH is a medical technology company funded in Lund, Sweden in 1964 by Swedish industrialist Holger Crafoord after meeting in 1961 with Nils Alwall, considered to be the "father of extracorporeal blood treatment" and one of the inventors of the world's first artificial kidney.

In 1973 the first plant outside Sweden, in Hechingen, Germany was opened. This is nowadays the company's biggest facility, employing over 1300 workers in two separate departments, production and research-and-development. As a whole, the company employs over 7500 workers worldwide, with presence in over 100 countries and a chronic dialysis share of approximately 25% of the world's market, approximately 350.000 chronic renal patients per year. The remaining market share is owned by Fresenius, Baxter and NxStage among others.

Figure 1. Gambro administrative offices. [a]

1.2. The project

1.2.1. Immunoglobulin G

Immunoglobulin G is a protein complex involved in the immune system response to pathogens (e.g. virus, bacteria, fungi) via a myriad of different action mechanisms, among which can be found:

- Binding to pathogens in order to signal them for phagocyte consumption and allowing the negatively charged cellular membrane of the phagocyte to approach the also negatively charged pathogen cellular membrane in order to carry out the phagocytic binding between both cells.
- The immobilization of pathogens by binding and agglutination.
- Binding and neutralization of toxins
- Binding to virions in the cytoplasm and enabling the destruction of them by proteolysis in the proteasome.

Immunoglobulin G (IgG) is the most numerous immunoglobulin (Ig) found in human blood plasma, accounting for approximately 75% of the total Ig, the remaining 25% is composed of IgA, IgE, IgD and IgM. This makes it the most abundant antibody isotype in circulation.

Industrial production of IgG is carried out using bioreactors along with genetically modified micro-organisms or by processing human or animal blood.

Given the unavoidable biologic nature of the process and the fact that IgG is a pharmaceutical product, special care must be given to guarantee the compatibility and innocuousness of the product in the future use. Such care is stressed by governmental controls and limit values on pharmaceutical products.

Among the most important parameters regarding the safety of IgG obtained via biotechnological processes is the presence of viruses in the finished product. This is especially critical since a pathogen contamination of IgG, a product itself used to improve immune response against infections or immunodeficiencies can create foreseeable undesirable consequences when applied to an already compromised patient.

1.2.2. Virus clearing

Virus clearing of the desired product can be achieved in two ways, virus inactivation or virus removal. The goal of virus inactivation is to either destroy the viruses or render them innocuous; this means that even after a successful virus inactivation process, components of the viruses can still be found in the final product. This can be achieved using thermal or chemical procedures, which either denature virus proteins or strip lipid-coated viruses from this protective structure. Even though relatively simple to carry out, the thermal or chemical stress caused by these procedures will invariably affect the desired product. This means, the use of chemical or thermal procedures to inactivate viruses, can only be used when the desired objective product itself is more resistant to said processes than the virus.

Virus removal is based on the physical separation of the virus from the desired product. Using nanofiltration as a mean to achieve this separation has the advantage of not exposing the components to thermal or chemical stress, since the separation principle is based on a size difference between the virus and the product.

1.2.3. Separation principle

Viruses have diverse range of sizes, from filoviruses reaching a length of 1400nm, to parvoviruses with a diameter between 18 and 26 nm. The desired product, immunoglobulin, has a size of approximately 10 nm. A virus separation process is considered successful if the 99% of the viruses are removed from the initial solution. A membrane with an average pore size of 20 nm is held to be ideal to reach such separation percentage. Given the small difference in size between the immunoglobulin and the smallest viruses, pore size homogeneity is essential to guarantee the easy passage of the protein and the successful retention of the viruses.

2. Theoretical background

2.1. Monolith characteristics

A monolith is in essence a single porous polymeric structure. This structure is obtained through a polymerization reaction of a monomer and a crosslinker. Besides the "building blocks" of the polymer, a porogen mixture and an initiator are needed in order to carry out the reaction.

The porogen mixture often consists of 2 substances, although porogen mixtures consisting of only one substance are also widely reported in the literature [7] [9] [11] [12]. The main characteristic of the porogens is their ability to dissolve the unreacted monomer and crosslinker, and their inability to dissolve the polymeric chains of the monolith itself once the reaction has started to take place. Each porogenic substance has a different capacity to dissolve the polymeric chains; this property allows the determination of the final pore profile of the monolith by changing the amount and nature of the porogenic substance [1]. As the polymerization reaction takes place and the polymer chain grows, the porogens will not be able to dissolve the newly formed structure and the polymeric chain will precipitate, creating a web of material and a homogeneous network of pores and channels.

The last component of the monolithic solution is the initiator which provides the free radicals in order to start the polymerization reaction. Since the homogenous nature of the pore-profile of the monolith depends on the fact that the monolithic solution itself (including the initiator) is also homogeneous, the initiator can only start the reaction when desired and not as result of its mere addition into the solution. This is achieved using initiators activated by either high temperatures or irradiation to achieve free radical polymerization.

An interesting characteristic of monoliths is their homogeneous porosity, which can vary from a macro- to microporous profile. This property, along with the relative simplicity with which the reaction can be started and maintained, has made them popular for use in HPLC (High Performance Liquid Chromatography), electrochromatography, immunoaffinity chromatography, capillary

chromatography/electrochromatography, etc [3] [4] [5] [6]. The monolithic structure used in the previous applications is usually obtained through in-situ polymerization columns. Another interesting property of monoliths comes from the fact that the monolithic solution, being a liquid, adopts the shape of the container where it is being stored. This allows the polymerization of monoliths into specific and complex shapes. This is of special interest in miniaturized analytical systems where, e.g. a long monolith column can be polymerized in a small area using a "zigzag" arrangement [2], forming a "lab on a chip".

Given these properties, it is hypothesized that it is possible to carry out a monolith polymerization inside the pores of a pre-existing membrane, yielding a homogeneous porous structure capable of performing a virus separation procedure using the principle of size exclusion. In order to achieve this final structure two essential steps are identified, the first one being the impregnation of the monolithic solution inside the pores of the pre-existing membrane, and the second one being the actual polymerization reaction once the solution is in the pores.

2.2. Monolith preparation

2.2.1. Monolithic solution

A monolithic solution is composed of: a monomer, a crosslinker, a porogenic mixture and an initiator. A wide variety of monoliths can be polymerized and have been reported in the literature, ranging from hydrophobic to hydrophilic, from macro- to nanoporous, cationic, anionic, and neutral. [4]

The process of selecting a monolith and its subsequent monolithic solution relies heavily on the use of pre-existing combinations reported in the literature due to the high number of possible components and their combinations. Modifying an existing combination or developing an entirely new one still remains a matter of trial and error. [17] [26]

Theory suggests that a lower monomer to crosslinker (M/C) content decreases pore size [3] and enhances homogeneity, the effect of modifying the ratio

between the porogens (P_1/P_2) depends on the nature of each porogen regarding the selected monomer and crosslinker. In our standard monolithic solution, dodecanol acts as a microporogen [6], however it can act as a macroporogen if a different monomer and crosslinker mixture is selected [24] [16]. The effect of changing the ratio between the building blocks and the porogenic mixture (M+C)/(P_1+P_2) depends on the selected chemicals as well. According to Buchmeiser et al "'Good' polymer solvents usually serve as microporogens and 'poor' polymer solvents as macroporogens." [16]

2.2.2. Monomer and crosslinker

Given the fact that a majority of chemical and especially fermentative processes are carried out in aqueous phases, a hydrophilic nature of the final structure is desired in order to guarantee a smooth protein passage and good flow through the final membrane without necessarily employing high pressures. This is achieved using hydrophilic "building blocks", such as hydroxyethyl-methacrylate (HEMA) as monomer and pentaerythritol-triacrylate (PETA) as crosslinker (Fig. 2 and 3). This monomer and crosslinker selection yields a neutral hydrophilic monolith [6].
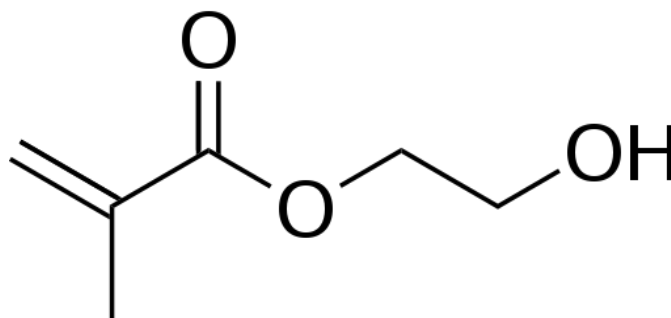

Figure 2. Hydroxyethyl-methacrylate
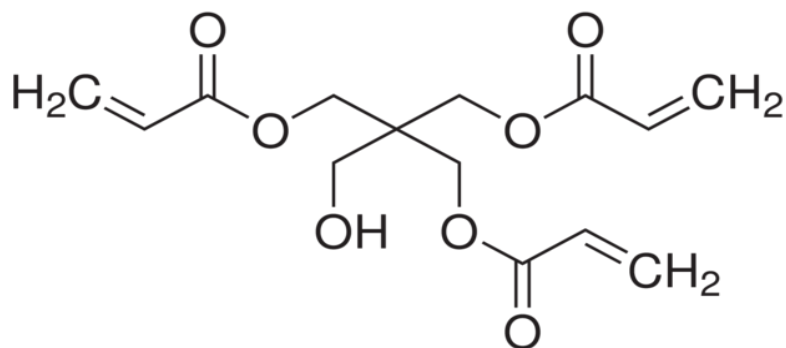
Figure 3. Pentaerythritol-triacrylate

Besides the aforementioned mixture of monomer and crosslinker, a wide range of monomers can be found in the literature, the most commonly reported are those belonging to the methacrylate and acrylate chemical family [2]. Among crosslinkers the possibilities are equally as wide, with reports of ethylene dimethacrylate (EDMA) [1], divinylbenzene (DVB) and trimethyloylpropane trimethacrylate (TRIM) being used as crosslinking components [2].

2.2.3. Porogens

Several possible porogenic mixtures have been reported in the literature.

Porogen 1	Porogen 2	Ref.
DMSO	2-Heptanol	[16]
DMSO	Dodecanol	[4] [16]
Toluene	Dodecanol	[10] [11] [3] [4] [16]
Toluene	Octa, Nona, Deca, Undeca, Dodecanol	[12]
Toluene	Propanol	[15]
Cyclohexanol	Dodecanol	[11] [1] [3] [15] [16]
Cyclohexanol	Ethylene Glycol	[4]
Cyclohexanol	1,4-Butanediol	[3]
Low-% Methanol	Water	[4]

Propanol	1,4-Butanediol	*[3] [4] [14]*
Dimethylformamide	1,4-Butanediol	*[4]*
Isoamyl alcohol	1,4-Butanediol	*[15]*
Methanol	Tetrahydrofuran	*[4] [10] [16]*
Mesytilene	Nonanol, Decanol	*[12]*
Methanol	Dodecanol	*[10]*
Propanol	Formamide	*[13]*
Tert-Butanol	1,4-Butanediol	*[8]*
Decanol	Tetrahydrofuran	*[5] [13] [15] [16]*
Heptane	Dodecanol	*[11]*
Polyethyleneglycol	Decanol	*[15]*
Dicloromethane	Dodecanol	*[15]*
Water		*[15]*
Methanol		*[4] [10]*
Iso-butanol		*[10]*
Tetrahydrofuran		*[10]*
Formamide		*[15]*
Heptanol, Octanol, Nonanol		*[12]*
Dodecanol		*[11] [16]*
Heptane		*[11]*
Toluene		*[4] [10] [11]*
Decanol		*[4] [10] [12]*
Mesytilene		*[12]*
Acetonitrile		*[7] [9] [10]*
Polyethylene glycol		*[2] [4] [15]*
Super critical carbon dioxide (scCO2)		*[15]*

Table 1. Porogenic mixtures reported in the literature.

For the HEMA-co-PETA monolith, a porogenic solution of Cyclohexanol and Dodecanol is often used [6]; this combination is especially used in methacrylate-

based monoliths [3]. Besides the ability of dissolving both the monomer and crosslinker, this mixture has the advantage of being relatively safe to use in comparison to other porogenic mixtures (e.g. Dimethylformamide, methanol, formamide, etc.) and not dissolving other polymers in the final product, (e.g. the finished filter polycarbonate casing). The only considerable disadvantage of this mixture is the fact that dodecanol solidifies at room temperature and it is often necessary to heat it before the monolithic solution can be prepared.

2.2.4.   Initiators

The commonly used initiators fall into two categories; photoinitiators and thermal initiators. The preferred ones are the photoinitiators since they're relatively simpler to use than thermal initiators, which require a homogeneous and often elevated [5] [12] temperature profile along the reaction volume. This might be of relative ease to achieve in a laboratory scale by submerging the system in a glycerin or water bath, unfortunately once a higher-volume and continuous process is desired, this arrangement becomes unusable and a new procedure involving thermal initiators is not only logistically difficult but also energy-intensive. The most widely used thermal initiator reported in the literature is azobisisobutyronitrile (AIBN) [5] [12]. Even more, when using thermal initiators, the often elevated temperatures cause the porogens to evaporate during the polymerization, producing a dense surface on top of the monolith.

On the other hand, employing photoinitiators only requires the use of an irradiation source with the adequate wavelength depending on each specific initiator and sufficient irradiation time of the solution. The scaling up of a process involving photoinitiators from a laboratory scale to a continuous one can be easily achieved. An essential advantage of photoinitiators over thermal initiators is the required reaction time, a polymerization using thermal initiators can last from 1.5h to more than 20h depending on the mixture itself and the reaction temperature [5], while empirical data shows that a monolithic solution

polymerized via photoinitiators can be completely cured using the irradiation from a small UV-lamp in less than 2 minutes.

Figure 4. UV-lamp employed for the polymerization (Uvahand 250, Dr. Hönle AG, Münich)

2.2.4.1. Photoinitiators

In the relevant literature, three general groups of photoinitiators have been reported:

2.2.4.1.1. Hydroxyphenylketones:

The most used photoinitiator during this project belongs to this family; 2-hydroxy-2-methylpropiphenone (Fig. 5).
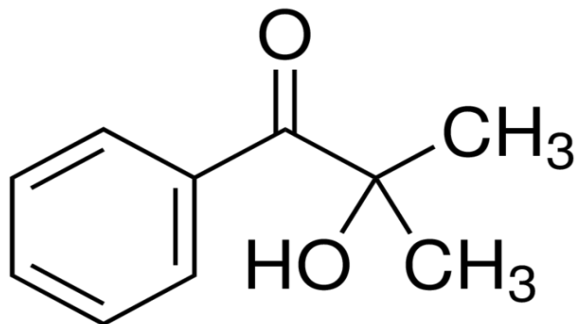
Figure 5. 2-Hydroxy-2-methylpropiophenone (Darocur 1173)

This kind of photoinitiator, even though less reactive than aminophenylketones or phosphine oxides [21] [22] [23] and more prone to oxygen inhibition [21] [22] [23] has the advantage of not reacting under visible light and not causing unwanted polymerizations in the solution reservoir (stopping the process, "trapping" the fiber and effectively spoiling the monolithic solution). A second advantage of this photoinitiator family is their liquid state at ambient conditions, making them easy to employ and dissolve in the monolithic solution.

In order to compensate for the reduced reactivity, a longer irradiation time is necessary; and to prevent any eventual oxygen inhibition, an inert atmosphere is preferred. The difference in monolith polymerization between being irradiated in an inert atmosphere and an atmosphere containing oxygen is considerable [21].

It was observed that even though a sudden polymerization under visible light does not occur during production of a monolith batch (i.e. approximately 5 hours of visible light exposure); the monolithic solution does polymerize if left exposed to the visible light in a closed test tube for a longer time (48 hours). The absorption spectra of 2-hydroxy-2-methylpropiophenone can be seen in Fig 6. [c]
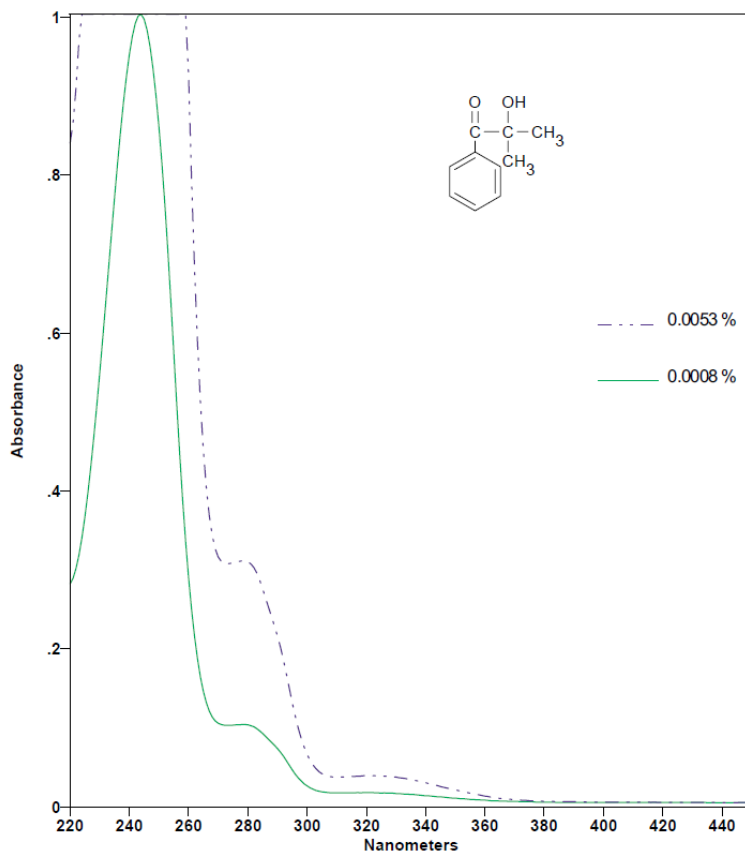
Figure 6. UV-Spectra of 2-Hydroxy-2-methylpropiophenone [c]

2.2.4.1.2. Aminophenylketones

Common aminophenylketone photoinitiators [21] are 2-benzyl-2-(dimethylamino)-4'-morpholinobutyrophenone and 2-methyl-4'-(methylthio)-2-morpholinopropiophenone (Figs. 7 and 8).
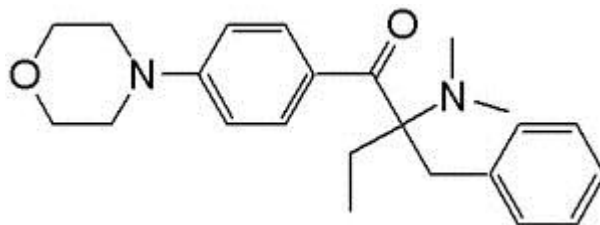

Figure 7. 2-Benzyl-2-(dimethylamino)-4'-morpholinobutyrophenone (Irgacure 369, Ciba specialty chemicals)
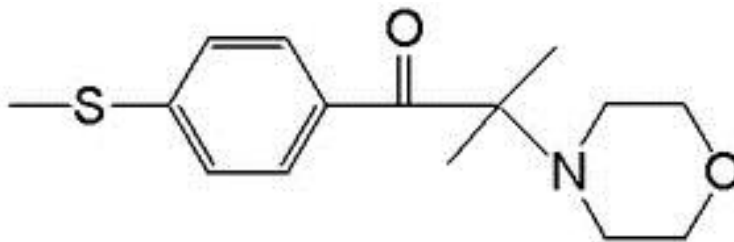
Figure 8. 2-Methyl-4'-(methylthio)-2-Morpholinopropiophenone (Irgacure 907, Ciba specialty chemicals)

Aminophenylketones are similar to hydroxyphenylketones, the presence of an amino group that acts as a hydrogen donor on the molecule grants a better resistance against competitive reactions by consuming the oxygen dissolved in the solution [23] and preventing the formation of radical-consuming oxides.

Unfortunately, the higher efficiency [21] of this photoinitiator family, means they are more likely to polymerize under visible light (Fig. 9), and during normal polymerizations batches (i.e. approximately 5 hours of visible light exposure) polymerized regions can be observed inside the solution reservoir

This tendency towards sudden polymerization, and its powder form which causes a necessity to first dissolve the photoinitiator, make the use of this photoinitiator family not as straightforward and easy to employ in comparison to the hydroxyphenylketones.

However, the reactivity difference between reactions carried out in inert or oxygen-containing atmospheres is small [21], offering the possibility not to employ an inert atmosphere during the polymerization and subsequently simplifying the overall process.

During this project, guaranteeing an inert atmosphere was considered easier and simpler than dealing with unexpected polymerization under visible light and the obstacles of using a powder-based photoinitiator.
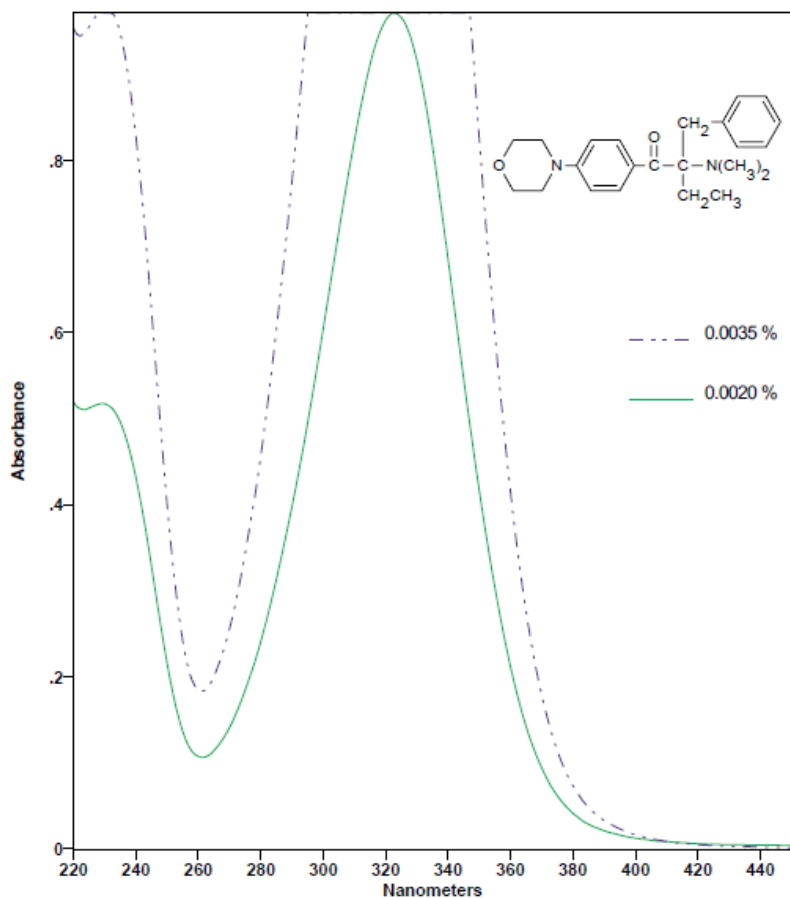
Figure 9. UV-Spectra of 2-Benzyl-2-(dimethylamino)-4'-morpholinobutyrophenone. (Irgacure 369, Ciba specialty chemicals) [c]

2.2.4.1.3.   Phosphine oxides

A common phosphine oxide photoinitiator [21] is diphenyl(2,4,6 trimethylbenzoyl)phosphine oxide (Fig. 10).
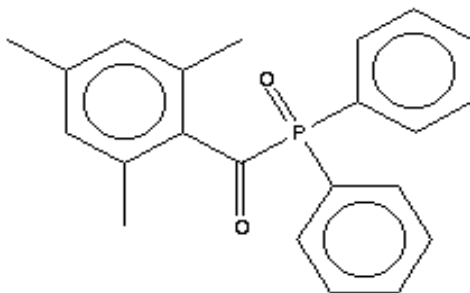
Figure 10. diphenyl(2,4,6 trimethylbenzoyl)phosphine oxide (Lucirin TPO, BASF)

Similarly to the Aminophenylketones, phosphine oxides are not as sensible to oxygen inhibition as the hydroxyphenylketones. They however, present the same obstacles as aminophenylketones; sudden polymerization under visible light [d] and a powder-based presentation.
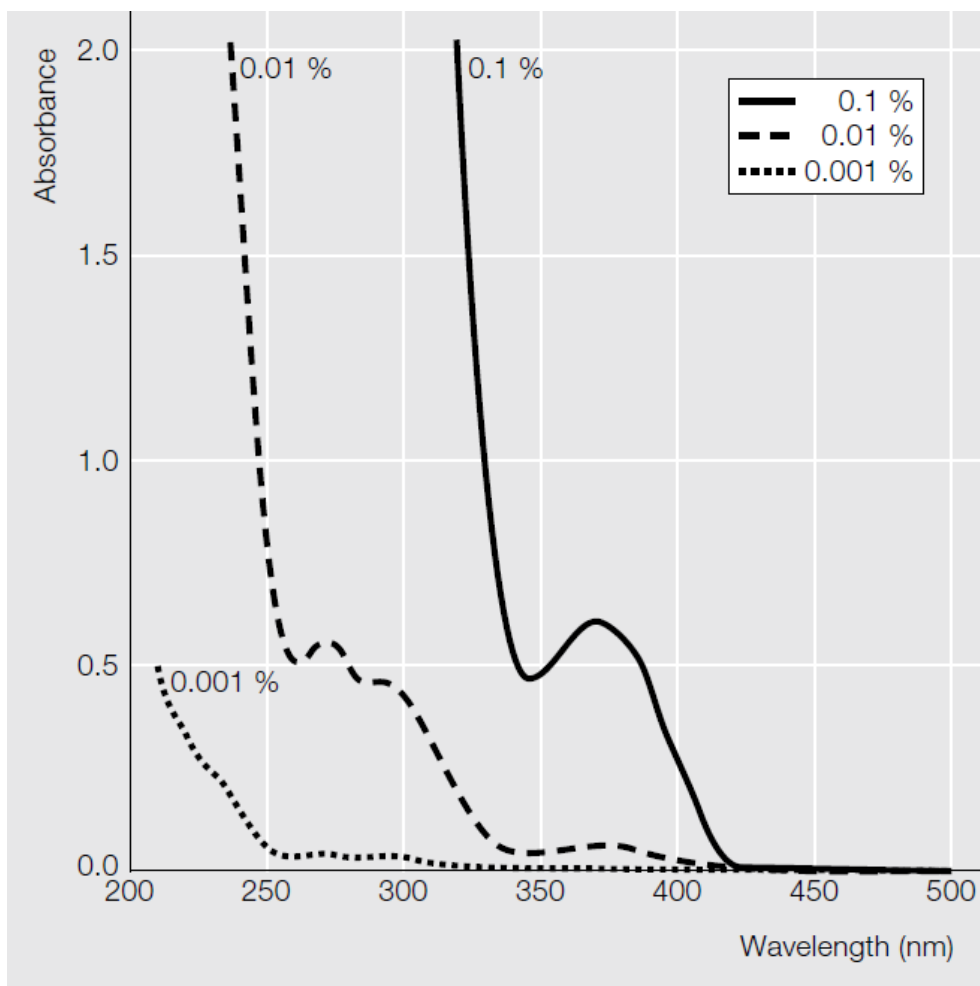
Figure 11. diphenyl(2,4,6 trimethylbenzoyl)phosphine oxide in ethanol (Lucirin TPO, BASF) [d]

2.2.5.   Polymerization reaction

When the photoinitiator in the monolithic solution is irradiated, a reactive intermediate is formed. This intermediate can be reverted into the original photoinitiator molecule when in presence of dissolved oxygen. The molecule can

also undergo scission, which yields two free radicals molecules, each capable of starting a chain polymerization. However, in order to initiate the chain reaction, the radicals must first leave the solvent cage and find an acrylate double bond. The rate of the cage recombination reaction, that is, the reaction caused by the immobility of the radical molecules as result of the solvent cage effect, heavily depends on the viscosity of the solution.
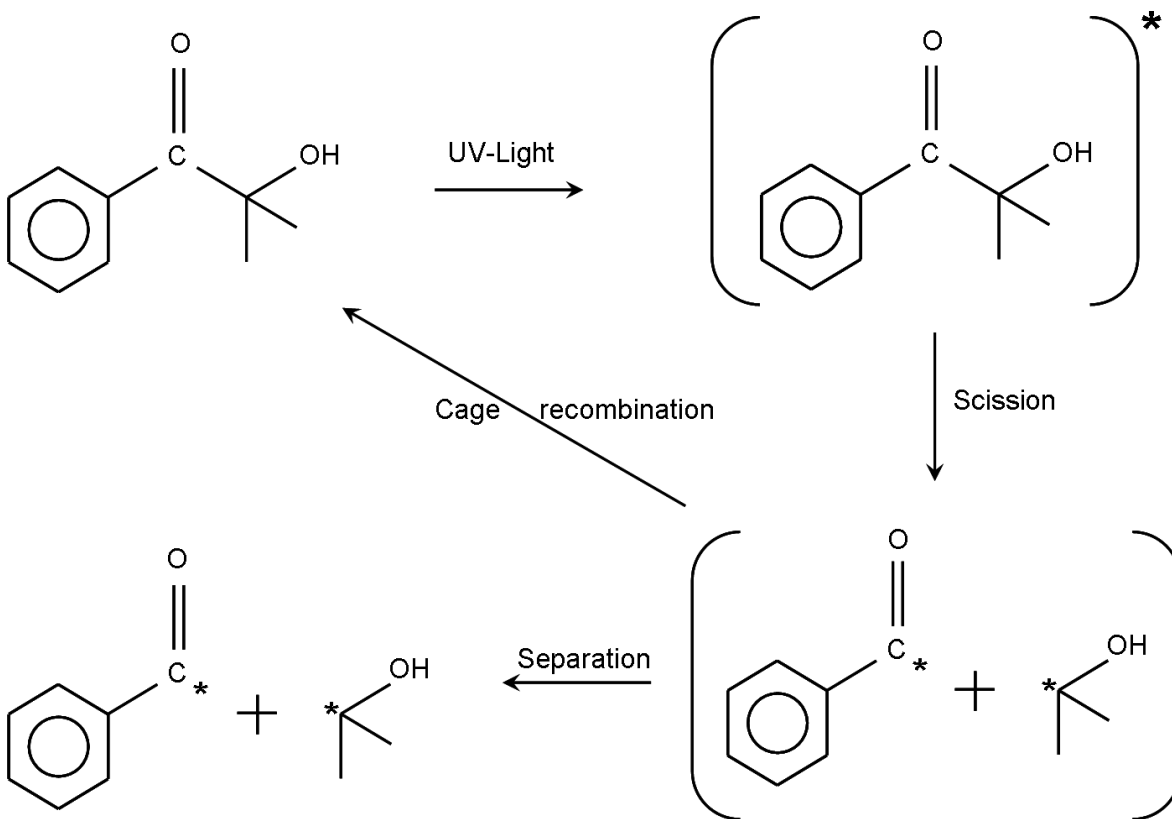
Scheme 1. Free radical formation

Once the radicals leave the solvent cage and separate from each other, the free radical polymerization reaction can take place. Besides double acrylate bonds, the radicals can also react with the dissolved oxygen in the mixture, thus creating peroxyl radicals, which do not interact with the acrylate bonds, effectively consuming and wasting the free radicals.

Peroxyl radicals (non-reactive towards acrylate double bonds)

Free radical Polymerization

Polymeric radicals

Premature termination

O₂

ROOH

Polymerization reaction propagation

Poly(Hydroxyethylmethacrylate-co-Pentaerythritoltriacrylate)
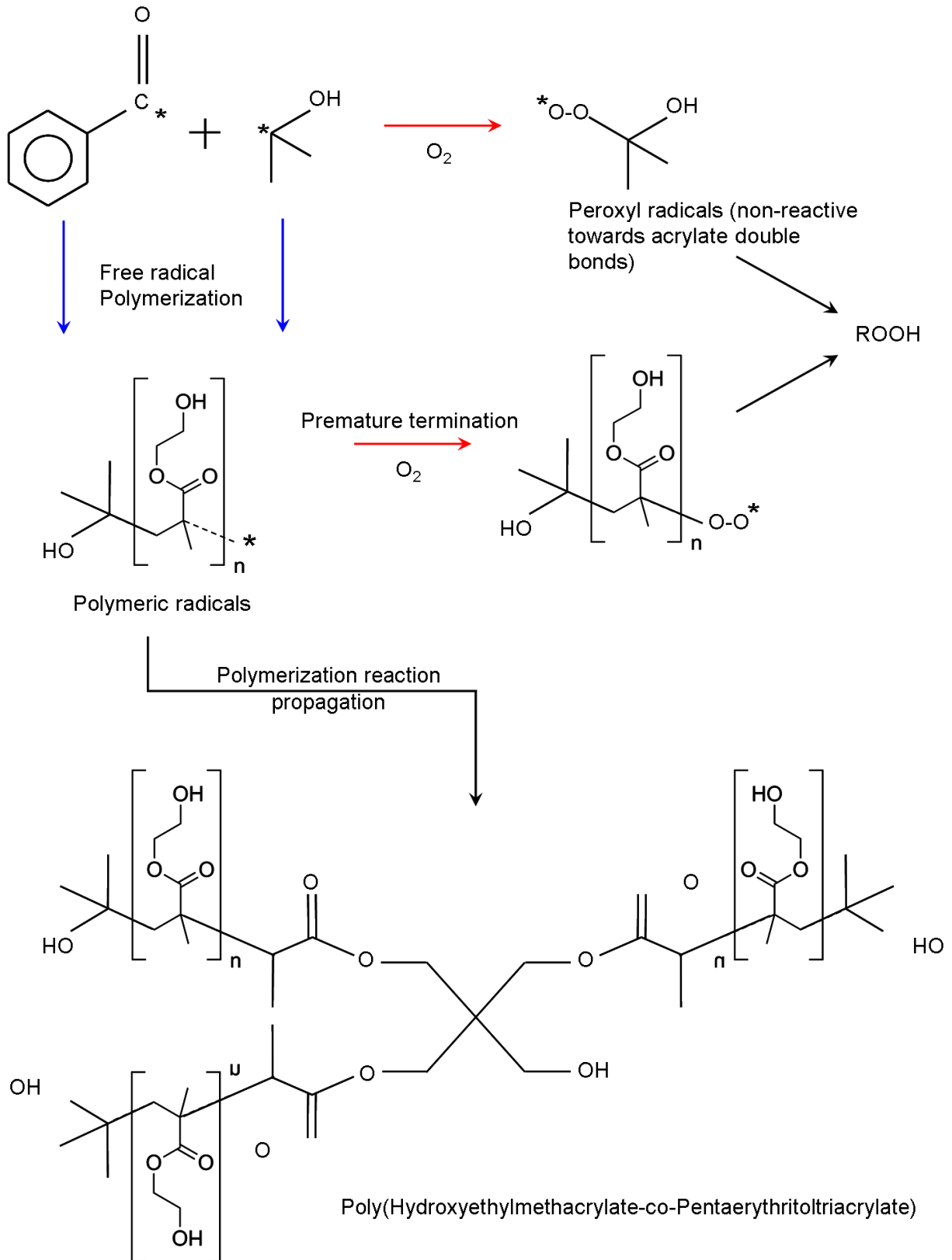
Scheme 2. Polymerization reaction

Even when a radical has reacted with an acrylate and has started the polymerization reaction, the polymeric chain itself can react with dissolved oxygen, prematurely ending the reaction and shortening the final chain. This is in contrast to the usual reaction termination caused by the growing chains contacting and reacting with each other.

In both circumstances where an oxygen molecule interacts with a free radical, a peroxyl radical is formed. Such radicals eventually find a hydrogen donor and become non-reactive. [21] [22] [23]

2.2.5.1.   Oxygen inhibition

Oxygen inhibition is a significant obstacle for a successful polymerization. It not only diminishes the reaction speed, but also lowers the maximum polymerization degree of the final structure [22] [23]. The use of additives to prevent oxygen inhibition has been previously explored. [23]

2.2.5.1.1.   Tertiary amines additives

The superior UV-curing capabilities of aminophenylketone photoinitiators are arguably caused by the tertiary amine group located in the molecule and its chain peroxidation capacity [23]. Thus, employing tertiary amine additives together with the usual hydroxyphenylketone photoinitiator yields a better oxygen-inhibition resistance. Such resistance path is as follows:

$$R^* + O_2 \rightarrow RO_2^*$$
$$RO_2^* + DH \rightarrow RO_2H + D^*$$
$$D^* + O_2 \rightarrow DO_2$$
$$DO_2 + DH \rightarrow DO_2H + D^*$$
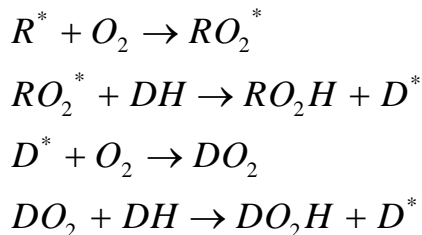
Scheme 3. Amino group preventing oxygen inhibition

The molecule containing the amino group (simplified as DH) serves as an effective hydrogen donor and improves the polymerization velocity of reactions carried out in air. However, some degree of oxygen inhibition inevitably remains and the reaction speed is still lower than under inert conditions. [23]

3. Experimental part

Hydroxyethyl-methacrylate, pentaerythritol triacrylate, 2-Hydroxy-2-methylpropiohenone and 2-Benzyl-2-(dimethylamino)-4'-morpholinobutyrophenone were obtained from Sigma-Aldrich. Cyclohexanol, dodecanol and ethanol were obtained from Merck. Human IgG was obtained from Octapharma GmbH, the gold solution was bought from British Biocell international and the Uvahand 250 UV-lamp was obtained from Dr. Hönler AG.

3.1. Monolithic solution

Different component proportions of the monolithic solution were polymerized in order to change the monolith's properties, specifically its pore size profile. Three solution parameters were regarded when defining the new monolithic solutions;

- Ratio of monomer to crosslinker (M/C).
- Ratio of the building blocks (monomer and crosslinker) to the porogens. $(M+C/P_1+P_2)$.
- Ratio of Dodecanol to Cyclohexanol (P_1/P_2).

Variation #	1	2	3	4	5	6	7	8
M/C	2,31	2,33	1,16	1,53	1,30	2,32	2,33	2,15
(M+C)/(P1+P2)	1,18	0,67	1,20	0,75	0,85	0,85	1,00	0,85
P1/P2	2	2	2	2	2	2	2	2

Variation #	9	10	11	12	13	14	15
M/C	2	1,85	1,85	2	1,85	9	9
(M+C)/(P1+P2)	0,92	0,85	0,92	1,02	1,02	0,67	0,44
P1/P2	2	2	2	2	2	4	4

Table 2. Variations of the standard monolithic solution regarding solution parameters

Weight percentage of the different solution variations can be easily derived

Variation #	1	2	3	4	5	6	7	8
HEMA, %	37,76	28	29,29	26	26	32,06	35,00	31,32
PETA, %	16,33	12	25,25	17	20	13,83	15,00	14,57
Cyclohexanol, %	15,31	20	15,15	19	18	18,04	16,67	18,04
Dodecanol, %	30,61	40	30,30	38	36	36,07	33,33	36,07

Variation #	9	10	11	12	13	14	15
HEMA, %	31,91	29,79	31,07	33,63	32,74	36	27,69
PETA, %	15,95	16,10	16,79	16,81	17,70	4	3,08
Cyclohexanol, %	17,38	18,04	17,38	16,52	16,52	12	13,85
Dodecanol, %	34,76	36,07	34,76	33,04	33,04	48	55,38

Table 3. Variations of the standard monolithic solution in weight percentages

Photoinitiator concentration was 1% relative to the total weight of the solution unless stated otherwise. Besides the standard solution employing dodecanol and cyclohexanol, polyethylene-glycol was employed as a substitute for dodecanol, unfortunately only a dense transparent monolith could be obtained, even after modifying the components percentages.

The monolithic solution was prepared in amounts ranging from 5 to 40 g, depending on the number of polymerizations to be carried out and the method to be used. After mixing the components in a beaker the solution was agitated during at least 10 minutes and then purged with nitrogen for 30 minutes in order to deoxygenate the mixture and prevent polymerization inhibition. During the entire process of solution preparation, special care was given to prevent any possible influence of outside irradiation on the solution. This was achieved by tightly wrapping the beaker containing the solution, usually with aluminum foil. This step is essential when a mixture with photoinitiators that react under visible light is used since they start to polymerize the solution if the beaker is not light-tight contained. This sudden polymerization can happen in any step of the process and bring the whole procedure to a halt.

3.2.   Fibers employed

Numerous types of fibers with varying materials, thickness, LP, dimensions and pre-treatments were used, however the most employed and representative were the polypropylene PF 1000N/2000N fibers and polyethersulfone Mikro-H-033 fibers (Table 4).

	PF 1000N / 2000N	MIKRO-H-033
Wall thickness (μm)	150	50
Inner diameter (μm)	330	320
Material	Polypropylene (PP)	Polyethersulfone (PES)
LP (10^{-4} cm.bar^{-1}.s^{-1})	Ca. 2000	Ca. 1000

Table 4. Basic properties of the hollow fibers used as a support of monolithic membranes.

3.2.1.   Polypropylene (PF 1000N and PF 2000N)

These fibers have relatively thick walls (Fig. 12) and are made from a polymer with low UV-absorbing properties (Fig. 13).
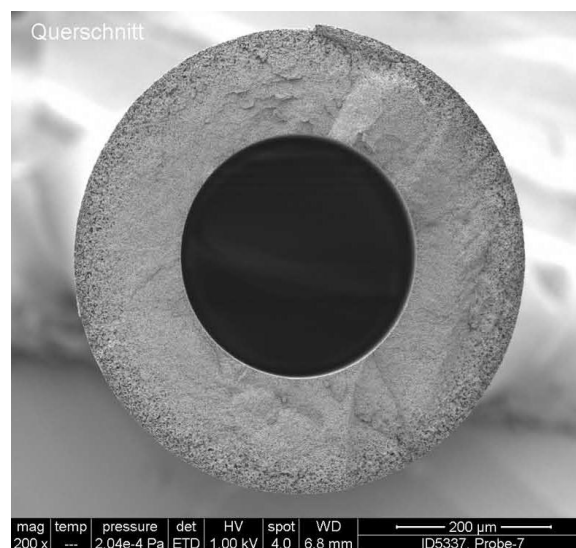

Figure 12. Unmodified polypropylene PF 2000N fiber

Gambro does not produce polypropylene hollow membranes; in consequence the fibers were acquired from Membrana GmbH. The fibers are contained in plasmapheresis filters PF 1000N and PF 2000N. The fibers can only be obtained by cutting the filters open and removing each individual fiber as needed.
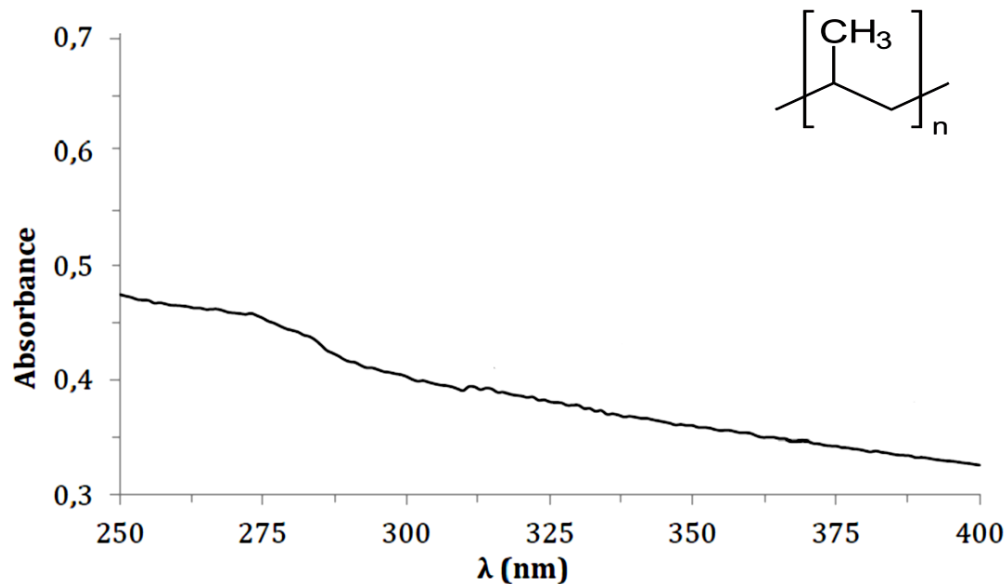

Figure 13. Polypropylene UV-Spectra and repeating unit [28]

The only difference between PF 1000N and PF 2000N is the filter length (see Fig. 14) and consequently, the length of each individual fiber.

Figure 14. Unmodified polypropylene PF 2000N fiber

3.2.2. Polyethersulfone (MIKRO-H-033)

The MIKRO-H-033 (20/10/05 lot: VVU-PL-231 "Spule 6") fibers have almost the same internal diameter and a smaller wall thickness (Fig. 15) in comparison to the PF 2000N fibers. The material of the membrane, polyethersulfone, contains aromatic structures (Fig. 16) that absorb UV-radiation under 308 nm.

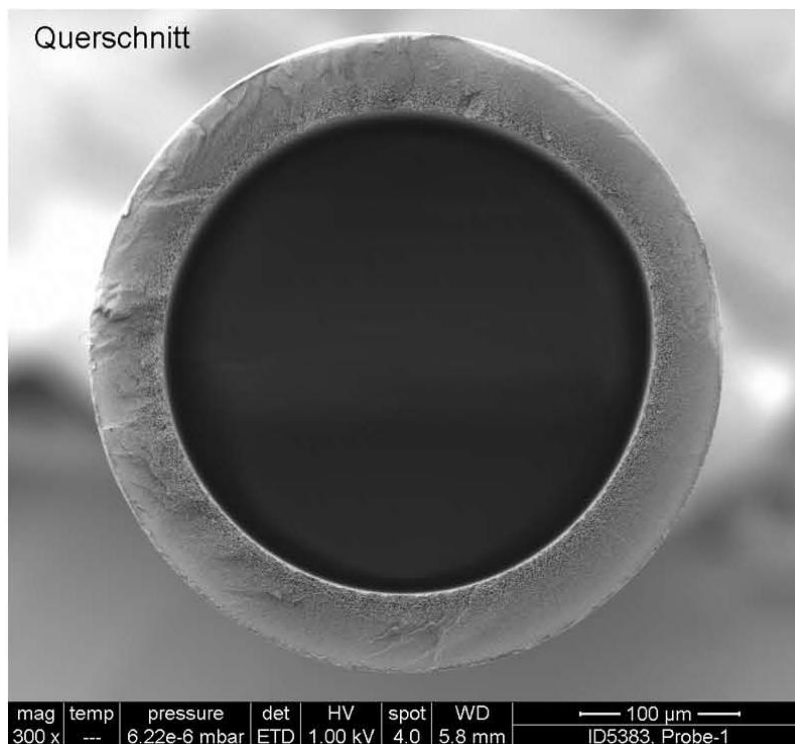*Figure 15. Unmodified MIKRO-H-033 fiber.*

Polyethersulfone fibers are spun by Gambro, which guarantees an ample supply and variety of work material. PES-fiber spools with different properties such as convective permeability (LP), inner diameter, wall thickness and pore size are readily available.

It is also possible to spin new PES fibers with desired parameters according to any eventual needs
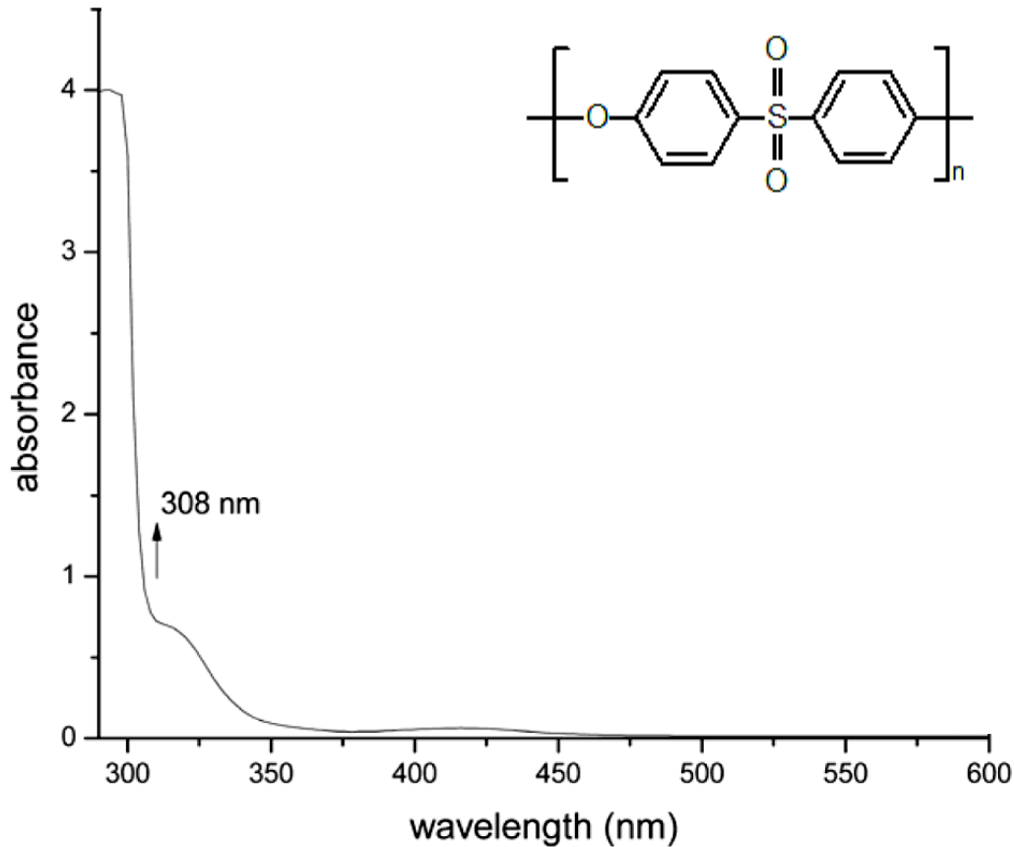
Figure 16. Polyethersulfone UV-Spectra and repeating unit [29]

3.3. Hollow fiber polymerization methods

Three different methods of delivering the monolithic solution into the membrane walls and then carrying out the polymerization were used.

3.3.1. First method: Single fibers polymerization

The first method consisted of sealing both ends of several short individual fibers with UV-glue, then laying them on a polyethylene (PE) sheet, using a dropper to impregnate them with the monolithic solution, covering them with another polyethylene sheet as to prevent oxygen diffusion from the atmosphere and finally irradiating them using a UV-lamp for at least 5 minutes.

The employed fibers for this method were: PES with a wall thickness of 50 μm, and PF 1000N 150 μm fibers.

3.3.2. Second method: Hand-bundle polymerization

Given the difficulty to handle single fibers, an aiding structure is needed in order to modify them. This is achieved by grouping a determined number of fibers together, tying them, placing small plastic cylinders at both ends, and finally filling the empty spaces between fibers and the plastic cylinders with polyurethane (PU), such tools are called hand-bundles and enable an easy manipulation of the fibers without having to touch them (Fig 17 and Scheme 5).
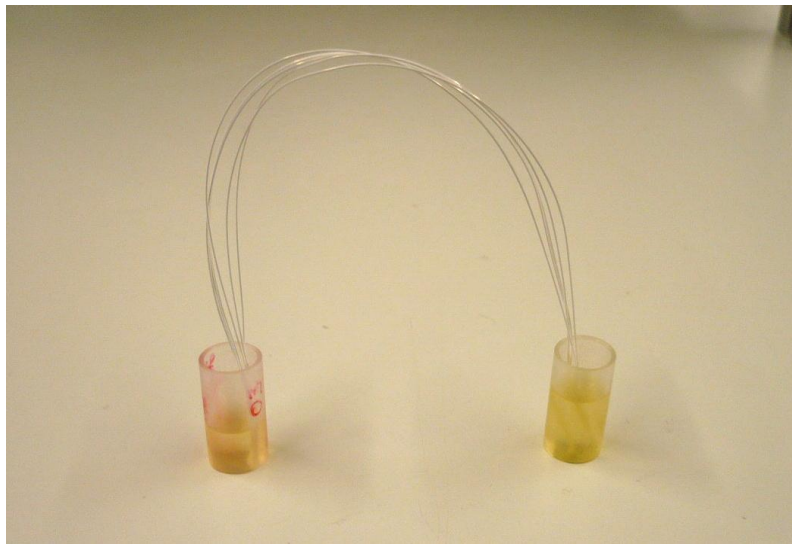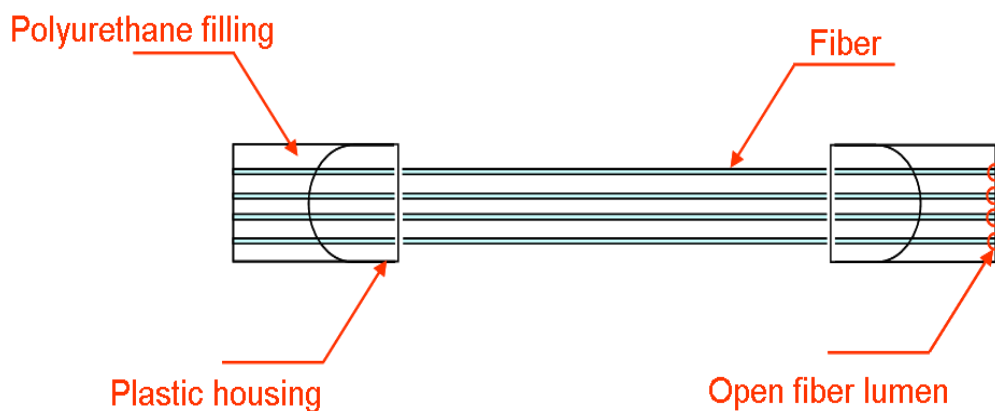

Figure 17. 5-fiber unmodified hand-bundle.

Scheme 4. 4-fiber hand-bundle schematic

A similar structure named minimodule can also be produced, the difference between both tools lies in the fibers being covered by a plastic casing in the minimodules (Fig. 18), while being exposed in the hand-bundles. However, minimodule construction requires a minimum fiber length which was not met by the fibers used at the time (polypropylene fibers from PF 1000N).

The hand-bundles usually contained between 5 and 10 fibers. Both Mikro-H-033 and PF 1000N fibers were employed.

Figure 18. 7-fiber minimodule.

3.3.2.1. Impregnation

The first step of the polymerization is to guarantee that monolithic solution is actually inside the membrane pores. This is done by injecting the monolithic solution through one end of the hand-bundle with a syringe. The other end can be temporarily blocked in order to force all the injected solution to pass radially through the walls of the fiber; however this is not strictly necessary as the monolithic solution readily diffuses and flows out of the membrane walls of most fibers, even when the other end is opened (Scheme 5).
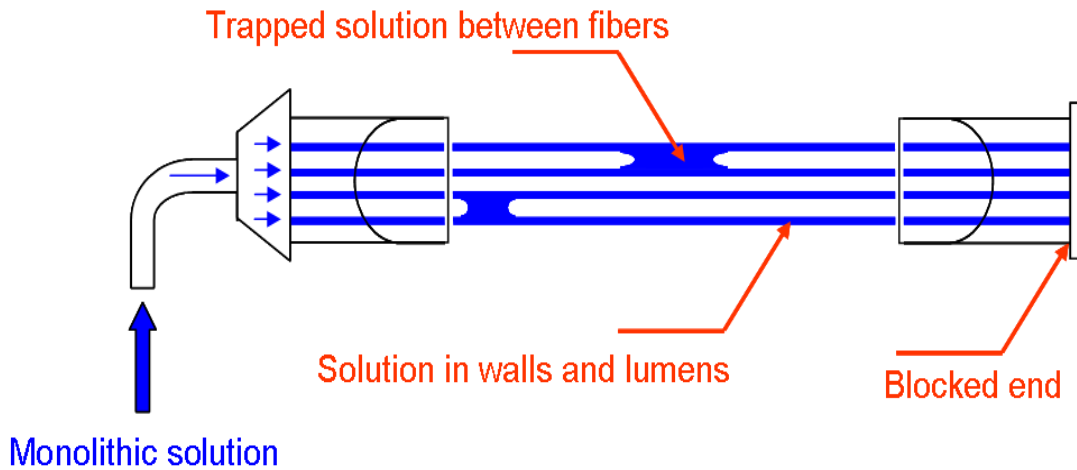
Scheme 5. Scheme of 4-fiber hand-bundle impregnation.

3.3.2.2.   Lumen clearing (nitrogen flushing)

After the impregnation step, monolithic solution can be found in the lumen, in the walls and on the surface of the fibers. If the polymerization reaction were to take place after the impregnation step, lumen blockage by the monolith would render the final product useless.

In order to clear the lumen of the fibers from monolithic solution, a controlled nitrogen flow was employed (Scheme 6).
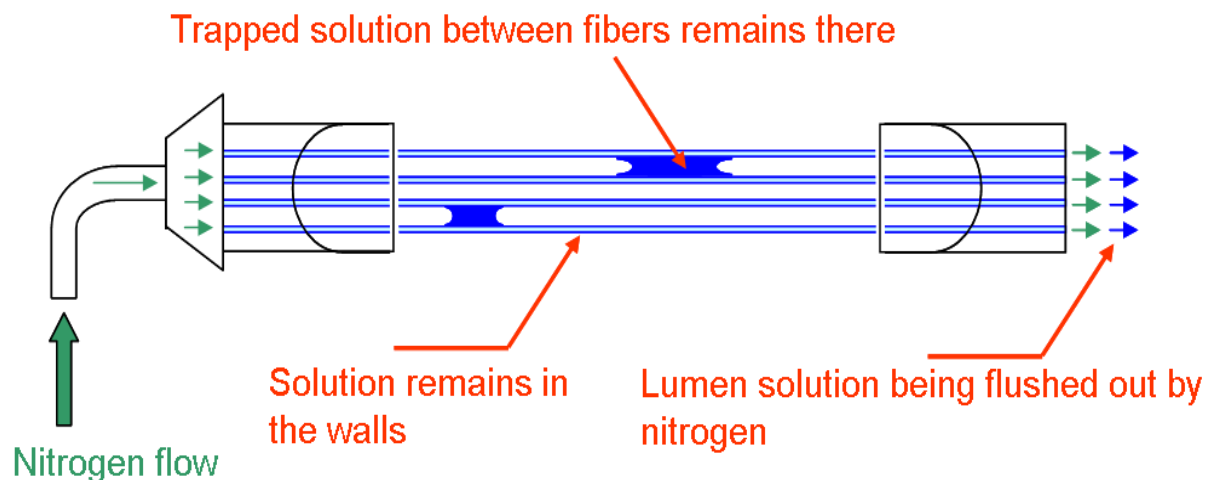
Scheme 6. Scheme of a 4-fiber hand-bundle flushing.

Two approaches were carried out, one employing a steady nitrogen flow during the polymerization reaction and the second one using a short burst of nitrogen during 10-15 seconds after the impregnation phase, and stopping the flow just before the reaction

3.3.2.3. Polymerization reaction (UV-irradiation)

Once the monolithic solution is in the walls of the membrane and none of it can be found in the lumens, the impregnated fibers are placed under UV-irradiation and left to polymerize during 10 minutes. When polymerizing a bundle with a high number of fibers, it is desired to periodically rotate the bundle to achieve a homogeneous irradiation along all the fibers and guarantee that the reaction effectively takes place in them.

The bundles are placed between 5cm and 20cm from the Uvahand 250 lamp. When using stronger irradiation sources, the influence of temperature must be taken into account, especially when using UV-chambers, which can even go so far as to cause the melting of the irradiated fiber. The vast majority of the polymerizations carried out using the second method were carried out in the presence of air.

3.3.2.4. Polyurethane re-sealing

After each polymerization, the hand-bundles were re-sealed with polyurethane to eliminate the influence of any weak points next to the PU surface.

This was done by pouring a small amount of PU into the remaining volume inside the plastic cylinders of the hand-bundle (Scheme 7).
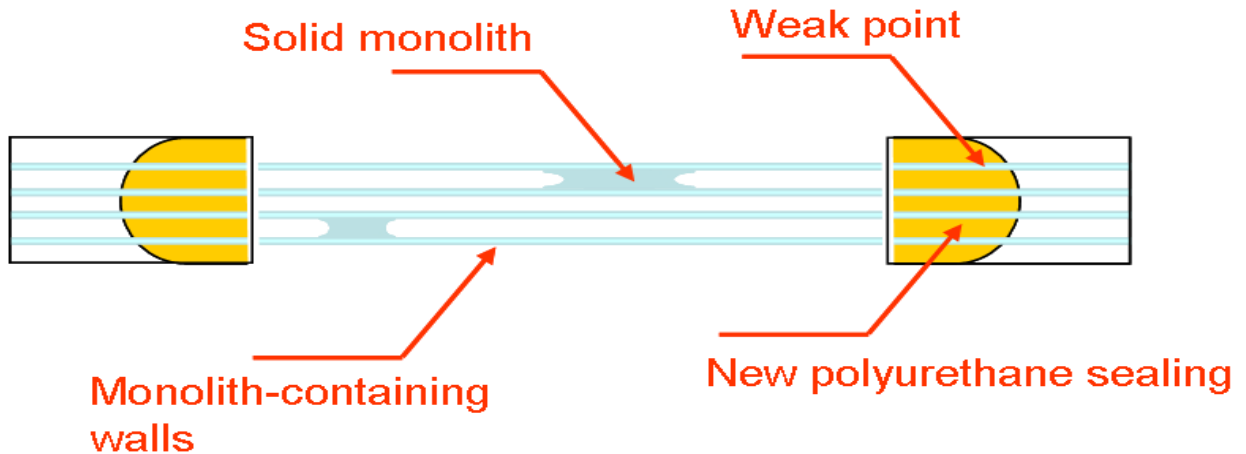
Scheme 7. Re-sealed 4-fiber hand-bundle schematic

A monolith-modified hand bundle can be observed in Fig. 20.

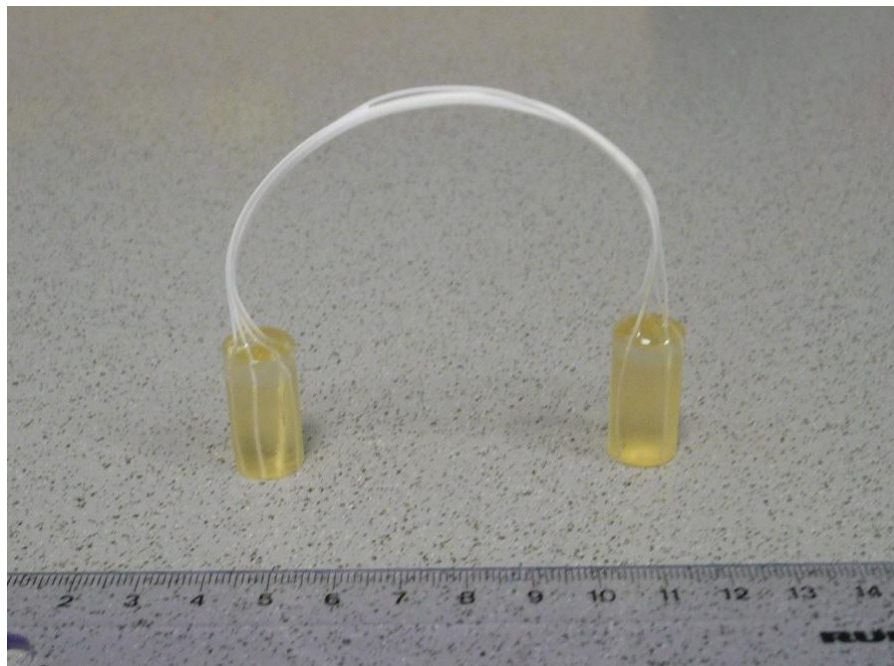*Figure 20. Modified and re-sealed 4-fiber polypropylene hand-bundle.*

During the entire second method process it is very important to use gloves and protective elements since in steps like impregnation and flushing, it is practically impossible not to have any kind of solution spill. If the flushing nitrogen flow is too high the hand-bundle can start to fluctuate (akin to a plastic hose with an

excessively high water flow) and might even be shot across the room. Attention must also be given during the irradiation step, since any solution patch receiving UV-rays will polymerize, including solution droplets on clothing, surfaces or laboratory elements. Once polymerized, the monolith is extremely hard to remove.

3.3.2.5.   Inert atmosphere

All the steps up to the UV-irradiation were the same. A medium-sized glass shell was place upside down and fitted with the end of a hose connected to a nitrogen source. A high flow was employed in order to provide an atmosphere as inert as possible. The UV-lamp was placed above the glass, and the permeated and flushed bundle inside it. A reference bundle was polymerized simultaneously outside the shell for posterior comparison of results.

3.3.3.   Third method: Continuous on-line polymerization

The employed fibers during the second method readily absorb the solution in its walls. It was proposed that monolithic solution could be absorbed from outside in with the same easiness with which the solution moves from inside out during the impregnation with a syringe. Along with this principle, a reaction atmosphere as inert as possible was regarded as an essential requirement for the success of a continuous method. Just like in the second polymerization method, the steps of the process can be divided in several distinct "sub-processes"; fiber source, impregnation, reaction and winding.

3.3.3.1.   Host fiber source

A continuous modification process is only possible with a continuous source of unmodified fiber. Because of this, only PES-fibers were available and used for this method. The main source was a MIKRO-H-033 spool (Fig. 21).

Figure 21. MIKRO-H-033 PES spool

The fiber spool is manually unwound since the fibers have a slight tendency to
stick to each other, which can cause them to break if left to unwind without
supervision. Special care must also be given to the unwound fiber since it tends
to curl into itself and get stuck in subsequent sections of the set-up (Fig. 22),
causing the fiber to break.
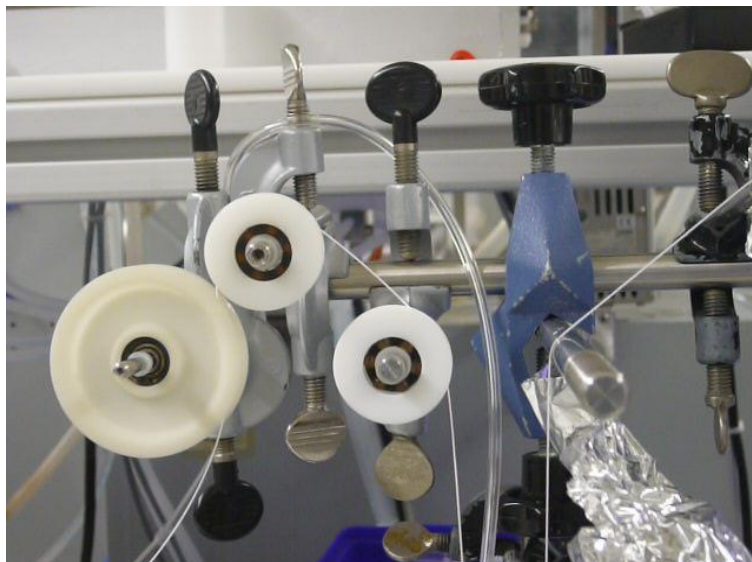
Figure 22. Rollers between fiber source and impregnation.

3.3.3.2.   Impregnation

In this method the fiber is the moving element, allowing the solution to be stationary. Having the solution remain stationary allows a continuous agitation and nitrogen purging, something which was not possible with previous methods since the solution was removed from the purging prior to its appliance on the fibers, weather it was done with a dropper or a syringe. Removing the solution from the nitrogen purge permits oxygen from the air to re-diffuse into the solution. It is also possible to heat the monolithic solution and keep its temperature constant. The solution usage is also notably lower and the process itself much more tidy and easy to handle.

The set-up inside the solution beaker is similar to Fig. 23, however instead of a roller wheel; a metallic cylinder is used as the surface where the fiber changes direction
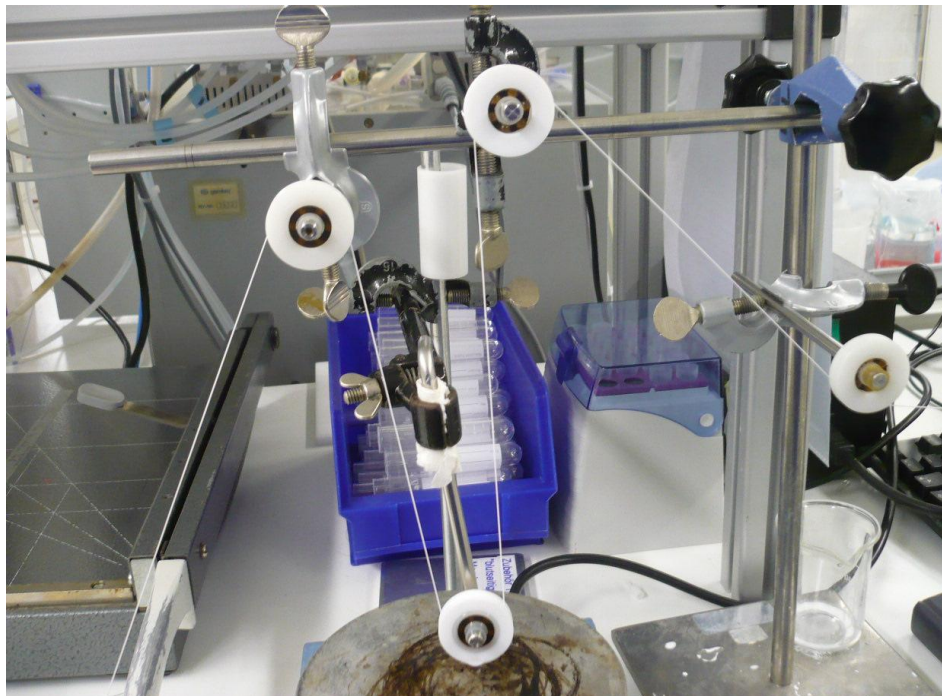
Figure 23. Impregnation set-up

The nitrogen flow was arranged so that the bubbles ascending through the solution had no contact with the fiber leaving the fluid (Scheme 8). Employing too high nitrogen flows or agitation RPM's can interfere with the membrane wetting and should be avoided.

The beaker itself is covered in aluminum foil in order to block any possible "stray" UV-rays (Fig. 24). The impregnation time can be set by modifying the winding speed and/or the solution level.
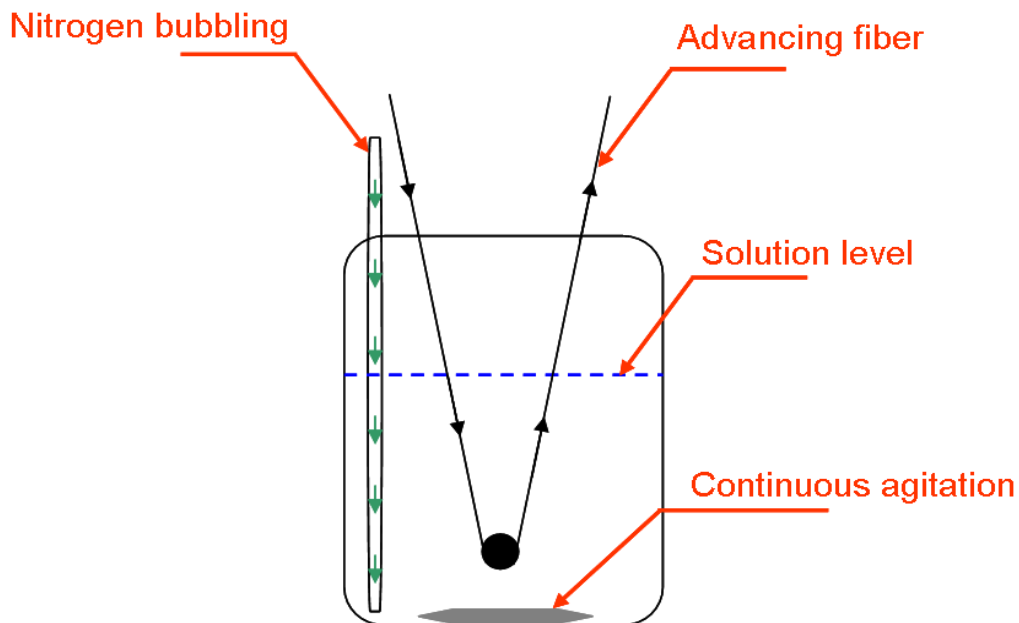
Scheme 8. Impregnation reservoir schematic

After the fiber impregnation, two turning points with 45° angle changes were used to direct the fiber into the reaction chamber instead of only one 90° abrupt turning point (Fig. 24), this is done to decrease the mechanical stress on the fiber when its path is changed.
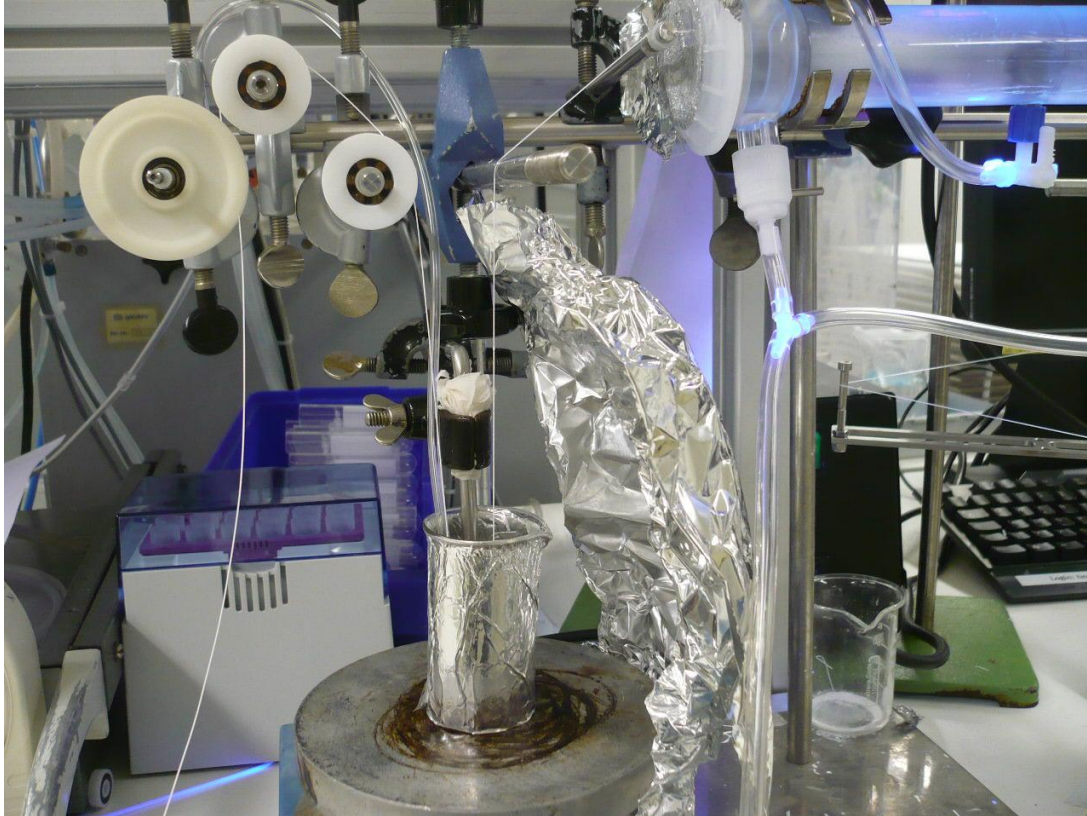
Figure 24. Impregnation reservoir.

3.3.3.3. Polymerization reaction

Once the monolithic solution is present inside the fiber walls, it is directed into the reaction chamber. Since the fiber is continuously moving forward, the irradiation time with a stationary UV-source can not be directly set like in the previous two methods. The time, however, is the result of the ratio between the fiber velocity and the irradiated length. As consequence, a long chamber able to provide a modified reaction atmosphere was desired. This was accomplished by using an empty filter body (Fig. 25) with a hole drilled in the middle, and both ends partially sealed with aluminum foil (Fig. 26).
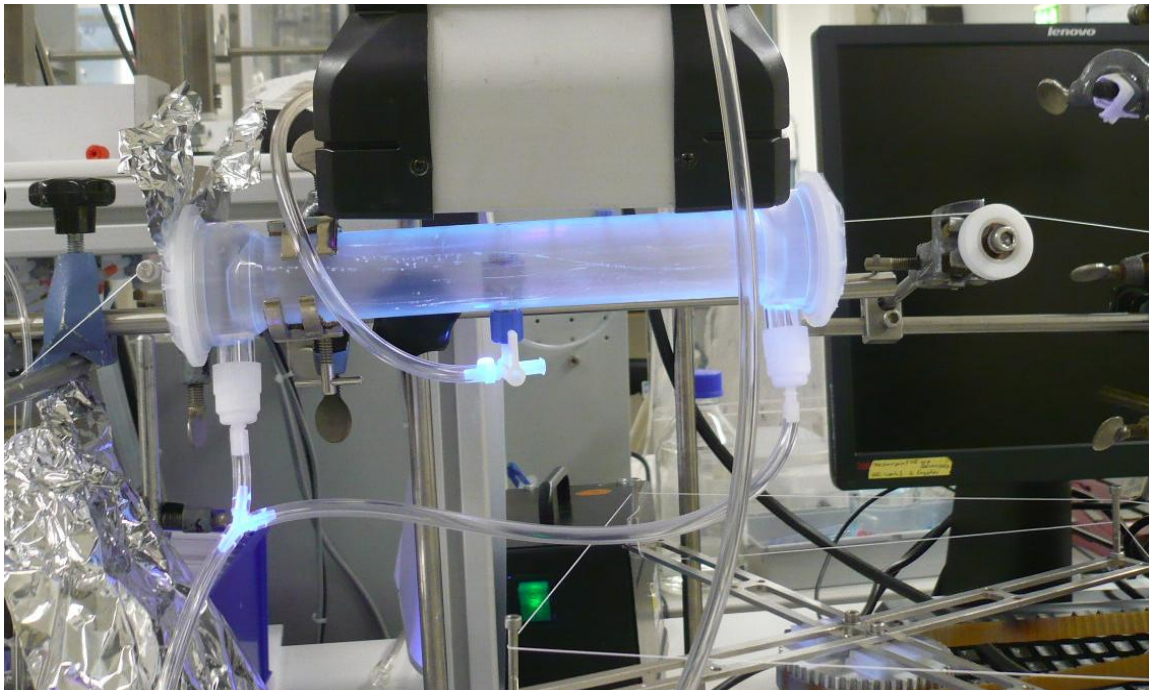
Figure 25. Reaction chamber.

Among the different casings types, the one with a length slightly bigger than the UV-light length was selected. This allowed the UV-source to be placed directly on the body of the casing without wasting irradiation length. The drilled hole in the case center was fitted with a gas disperser, which diffuses a gas stream into a 120° cone (Scheme 9). This was done after noticing that using a direct gas flow through the drilled hole (i.e. simply connecting the orifice to a hose and letting the gas flow) caused the fiber to vibrate and touch the walls of the casing, thus causing solution loss and in some cases causing the fiber to become stuck to the case wall when being irradiated. As complement to the central gas flow, additional gas inputs were fitted at each end using the pre-existing filtrate ports. These complementary nitrogen streams were considerably higher than the central one. Ultimately, aluminum sheets were glued to each end of the case (where the fiber-bundle would be located), and small holes, through which the fiber goes, were cut (Fig. 26).
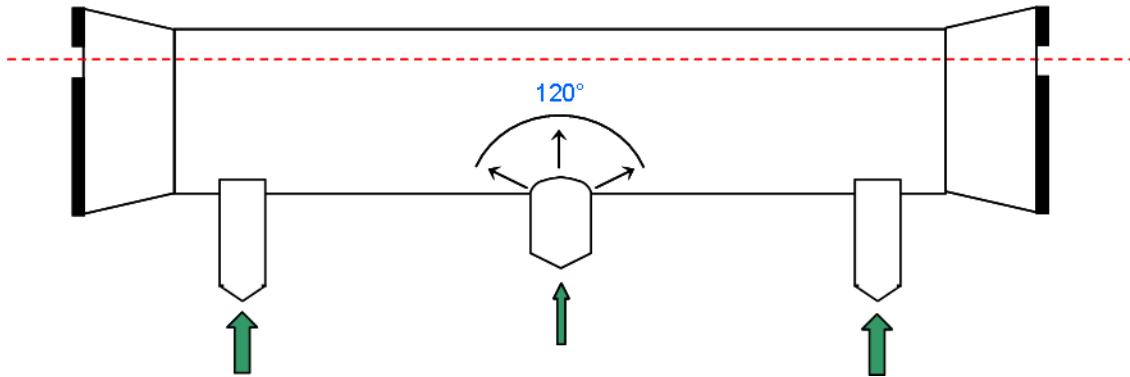
Scheme 9. Reaction chamber schematic

The fiber should move freely through the reaction chamber without touching it at any point. Critical points are the entry and the section just above the central gas diffuser. When entering the chamber the fiber contains unreacted monolithic solution, if the fiber touches any surface while being irradiated, a portion of the solution can flow out the fiber, polymerize and stick to said surface. If this happens and the winding motor is powered on, the fiber will break.

Figure 26. Reaction chamber end, sealed with aluminum-foil, the fiber moves through the opening.

If high nitrogen flows are being employed, the fiber can vibrate, touch the chamber walls and polymerize there, effectively sticking to the walls and breaking.

The UV-lamp is set as close as possible to the chamber, maximizing the amount of UV-rays irradiating the fiber and consequently increasing the reaction rate.

With the employed reaction times, once the fiber reaches the end of the reaction chamber, the fiber is not significantly wet; however it still contains the porogens from the monolithic solution since these do not react. This has the advantage of making the fiber not as brittle as if it were totally dry, enabling the posterior winding process.

3.3.3.4.   Winding

After a successful polymerization, the fiber must still be gathered and organized in a way such that the later minimodule or hand-bundle production is simple to carry out. The winding section not only comprises the gathering section, but also the mechanism that pulls the fiber and regulates its velocity. This section is composed of the gathering "wheel" and the motor (a small blood pump).

A roller wheel is placed close to the reaction chamber end (Fig. 27); this is not only necessary for directing the fiber, but it also provides stability and helps prevent the fiber from vibrating. Two other wheels are placed in order to direct the modified fiber into the gathering wheel (Fig. 27). Ideally, abrupt changes are to be avoided, however if thick fibers are being used, there is a higher degree of flexibility.
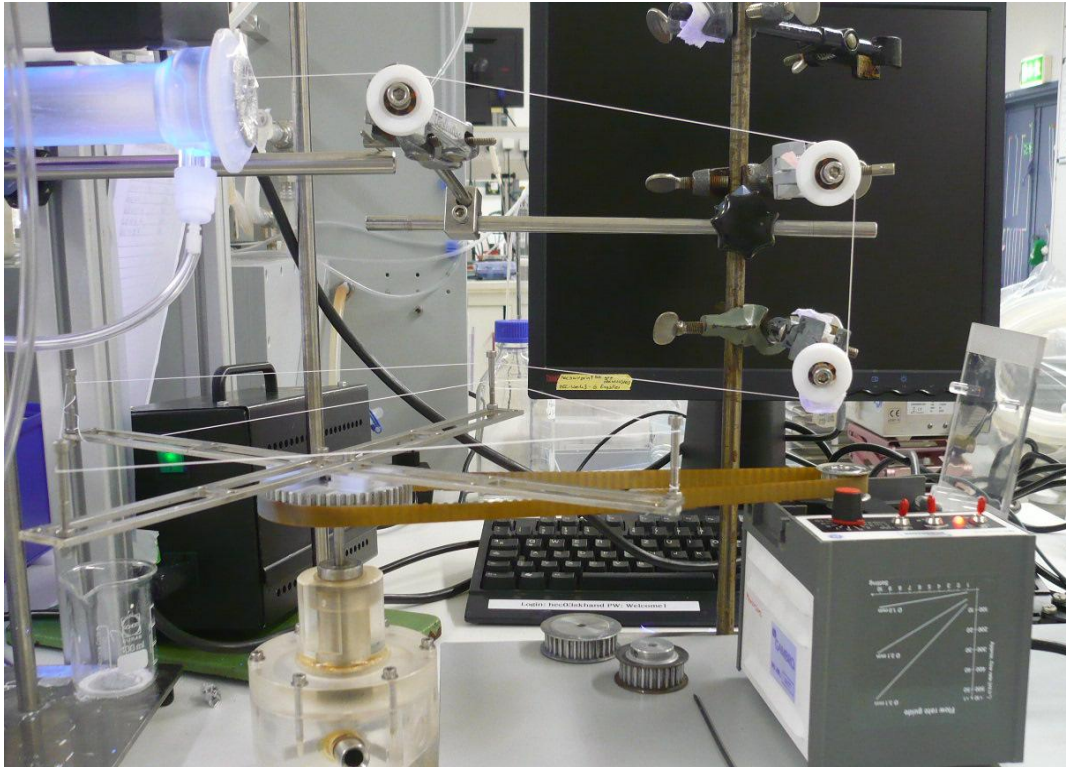
Figure 27. Winding section.

The gathering wheel was built joining two metallic arms and fitting them to a gear unit. On the end of each arm a single screw was positioned. When the wheel rotates, the fiber is gathered by the screws, forming a regular quadrilateral. Each side is made of the modified fiber and the screws are the corners. The length of the sides is ideal for the later minimodule production. The wheel gear is connected to another gear attached to the blood pump.

The complete third method process can be seen in Scheme 10 and Figure 28.
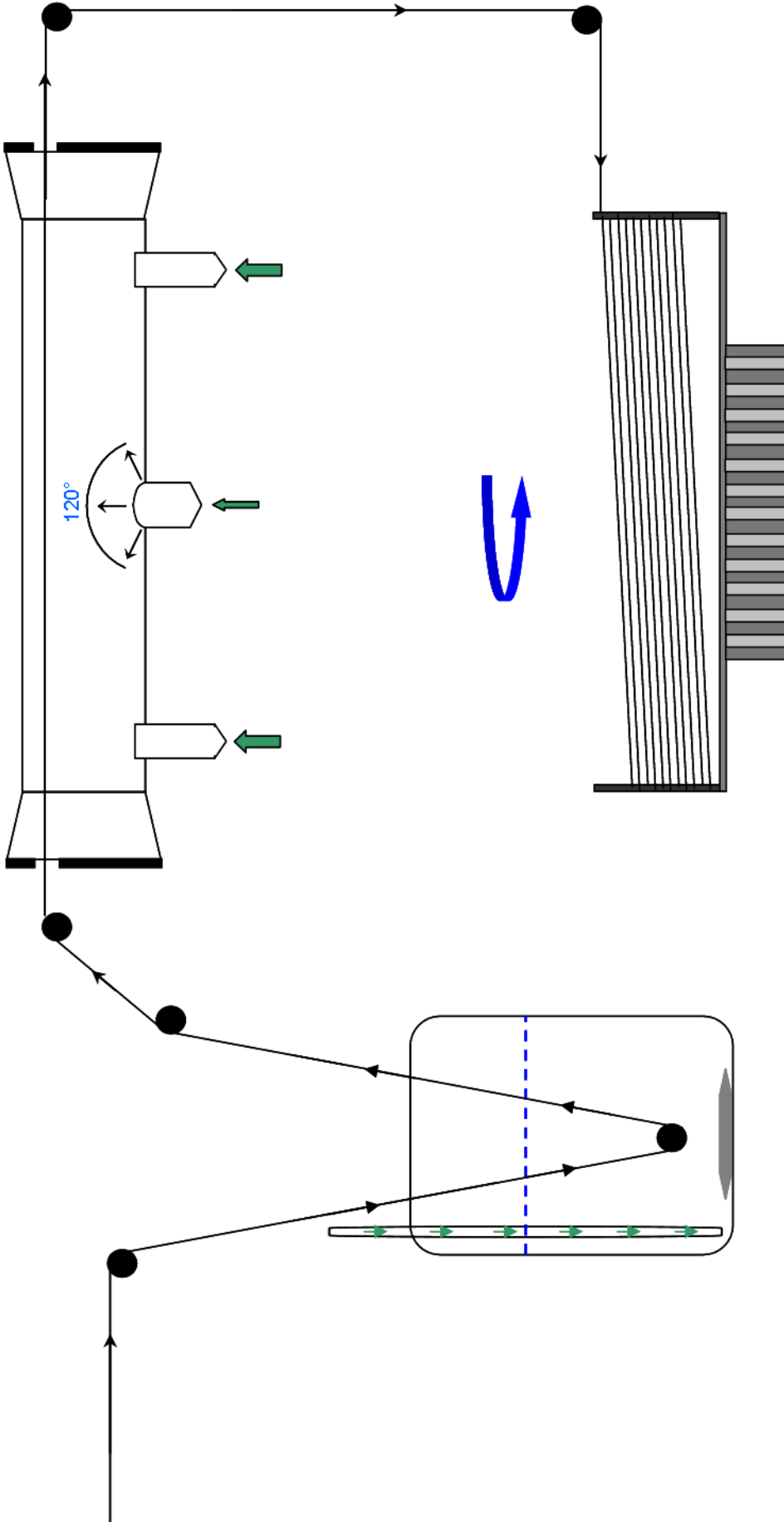
Scheme 10. Third method schematic

Figure 28. Third method laboratory montage

3.3.3.4.1. Motor

The motor used for the winding of the fiber must be capable of giving a smooth performance at very low RPM's. These low RPM's are required for having satisfactory reaction times. If the motor stutters, the fiber impregnation will not be smooth enough and solution droplets will form along the fiber, concentrating the solution in some areas and leaving other with no significant monolith presence. To counter this obstacle, a reduction gear 1:3 was employed. This meant that higher rotating velocities could be set on the pump, eliminating the stuttering, while at the same time having the slow winding speed required for a successful polymerization.

Generally speaking, the continuous process requires a couple minutes to reach stationary state since a portion of the monolithic solution carried in the walls of the fiber continuously flows into the cylinders between the impregnation section and the reaction chamber. A good practice is to use a dropper and wet such cylinders with a small quantity of monolithic solution, not only to reach the stationary state faster, but to act as a lubricant and facilitate the movement of the fiber. The portion of the fiber that is modified during the non-stationary state should be discarded.

3.3.3.5.   Multiple polymerizations on the same fiber

The influence of repeated modifications on the same fiber was explored. The method consisted of gathering the modified fiber, washing it, leaving it to dry in air and composing a new mini-spool from it. The fiber was then directed through the entire process in a similar manner to the first modification. Since the modified fiber is more brittle than the unmodified one, even more care must be given during the second modification.

Another double-polymerization experiment consisted of performing the third method as usual, then producing a minimodule with the modified fibers and

subsequently performing a modified second method on them. The second polymerization in the minimodule was carried out under a nitrogen atmosphere by attaching a nitrogen stream into one of the filtrate openings.

3.4. Cleaning and storing

After the polymerization has taken place; monolith, unreacted monomer and crosslinker, and porogens can be found in and on the fibers. In order to carry out further experiments and test the fiber, these impurities must be removed. The fastest way is to employ a syringe or a pump and inject ethanol into the bundle, first with the remaining end open and afterwards forcing the solution through the walls. Though fast and effective, this approach is time intensive given the fact that several bundles are polymerized in each experiment. A simpler approach is to submerge the bundles in a 50% ethanol-water bath overnight, renewing the solution when needed. Turbidity is observed in the bath and can be used as gross indicator of the amount of impurities in the fibers.

Even after the fibers are considered clean, it is a good practice to keep them wet by submerging them in a water bath. This prevents them from breaking during later handling. They also become easy to separate from each other when they are wet.

3.5. Characterization and evaluation of the monolith properties

Once the fiber has been cleaned, its filtrating and sieving parameters must be determined.

3.5.1. Convective permeability (LP-test)

The convective permeability coefficient (LP) describes the ease with which water can flow through a membrane's wall. It is defined as:

$$LP = \frac{V}{A * P * t} [=] 10^{-4} \frac{bar}{cm * s}$$

Where V = water volume

       A = area of the membrane in contact with the water

       P = Pressure difference

       t = time needed for the water volume V to flow through the area A with a

       P backpressure.

In the specific case of a hollow-fiber hand-bundle, the formula can be expressed:

$$LP = \frac{V}{\pi * d * L * n * P * t} [=] 10^{-4} \frac{bar}{cm * s}$$

Where d = inner diameter of a single fiber

     L = exposed length of the fiber

     n = number of fibers in the bundle

The higher the LP-coefficient of a membrane, the easier it is for water to flow through its wall. In most cases, a high LP is desired, as this means that the membrane will require a low backpressure (and subsequently, a low energy demand) to achieve the desired flow through its wall.

The permeability is a function highly dependant of the nature of the membrane material (i.e. hydrophobic or hydrophilic) and the pore profile in the walls. Being a parameter dependant on the porosity and hydrophilicity, and also being relatively simple to measure, make the LP ideal as a preliminary tool to determine whether a polymerization has taken place in the membrane and to estimate the extent of the polymerization. Through a simple comparison between the permeability of the membrane before and after modification, it can be concluded if the polymerization was successful or not. Also, by comparing the LP of two

membranes with different monolithic formulas in their walls it can be determined which one has a smaller pore size and/or lower porosity.

A direct relation between low LP and good filtrating properties was observed. As a rule of thumb, membranes with an LP higher than 10 have poor separating capacities, and membranes with LP below 5, show the best results in the gold particle retention test.

The permeability test can be carried out either in the automated LP measurement set up located in the clean room, or manually. When employing the automatic LP measurement, the hand-bundle with the modified fibers must be submerged in an UO-water bath 30 minutes beforehand. Once this time has passed, the hand-bundle is attached to the machine in a way such that all the fibers are entirely submerged and present no tension. A first phase called "leak test" is carried out to find any leakage or ruptures along the fibers or the PU-fillings. When the hand-bundle has such an imperfection, air bubbles can be observed coming from it. Once the fiber is considered leak free, the actual permeability test is started. A certain water volume is forced through the wall of the fibers. The machine measures and registers the time the water volume, and the pressure at which the impregnation took place. This data, along with the information of each hand-bundle (number of fibers, length and internal diameter) allows the automatic calculation of the LP. The test is usually performed three times with the final LP-coefficient being the arithmetic average.

A manual method can also be employed. In this method, the water flowing through the fibers is set manually, and the pressure monitored until stable. The manual method is used when the hand-bundles are too short and can't be attached to the LP-machine, or when half hand-bundles are used (Fig. 29).

3.5.2.   Gold particle retention test (GPRT)

The use of a virus suspension to evaluate the filtrating capacity of the membrane is not only an economical but also a logistical challenge. Producing, handling and analyzing the virus load of a solution require specific and stringent care in order

to maintain conditions such as salinity, pH, temperature, etc. in a predetermined range.

A colloidal solution of gold particles is used as replacement of actual viruses. The gold particles have a mean diameter of approximately 20 nm. 10 ml of this solution is prepared by adding 20,3 mg of polyoxyethylene β-naphthyl ether and 122,7 mg of poly(sodium 4-styrenesulfonate) to 10 ml of Plano gold solution. Once added, an ultrasonic bad is needed in order to completely dissolve the substances and obtain a readily usable solution. Submerging the solution in the bath for 15 min is usually enough to achieve this.

The gold retention test takes place in a "dead-end" configuration. One end of a hand bundle or a minimodule is connected to a pump, and at first, the solution is pumped through the lumen with the other end remaining open. This is done to flush out air bubbles and water from the lumens. Once the fibers are considered to contain only the gold solution, the open end is closed, which forces the solution to flow through the walls. Using a hand-bundle with an inner surface of $10 cm^2$, 4 filtrate samples of 1ml each are collected and analyzed in the spectrophotometer employing a wavelength of 530nm. The first sample is called "Fraktion I", the second "Fraktion II", and so forth. The gold particle removal rate is defined as;

$$GPR = Log\left(\frac{Absorption\,Original\,Solution}{Abosorption\;filtrate}\right)$$

A virus removal filter is considered to be adequate when 99% of the viruses are removed from the original solution. Employing Beer's law, the objective GPR can be obtained from the objective concentration.

$$GPR_{OBJ} = Log\left(\frac{100\%}{1\%}\right)$$
$$GPR_{OBJ} = 2$$

Using a logarithmic parameter permits a better appreciation and comparison of different filters, since like in any separation or purification process, once a percentage near 90% has been reached, any further separation is harder to achieve than the previous one (e.g. improving separation from 90% to 95% is considerably harder than doing so from 50 to 55%).

Given the short length of some polypropylene fibers, it was sometimes necessary to perform a GPRT with a "half"-bundle, that is, a bundle with only one open end (Fig. 29). In this configuration, it was of extreme importance to submerge the bundle in an ethanol/water 50% solution in order to get rid of air bubbles inside the lumen or walls

Figure 29. Half hand-bundle undergoing a dead-end GPRT.

3.5.3.   IgG-test

The filter must have a pore size small enough as to prevent the passage of viruses, but big enough to allow the protein to pass through its wall. This is checked by employing a human Immunoglobulin G solution from Octapharma GmbH in a dead-end configuration similar to that of the GPRT. The fibers are first rinsed using a PBS-buffer solution, not only cleaning the fiber, but also removing

air bubbles from the lumens. Using a minimodule with a total fiber internal surface of 10 cm^2, requires a flow of 2ml/min of the buffer solution for as long as necessary to wet the fibers and displace any air bubbles. Once this is achieved, the open end is closed and the cleaning is performed in a "dead-end" configuration using a lower flow of 0,7ml/min during 10 minutes. After the cleaning is finished, the closed end is opened and the PBS solution is replaced by the IgG solution (concentration 2,5g/l). The pump is set once again to 2ml/min until the new IgG solution has displaced the remnants of the PBS solution and any air bubbles that might have come into the system during the change of the solutions. The open end is closed and flow is set to 0,7 ml/min. The test is performed during 150 minutes, gathering 1ml filtrate samples every 15 minutes. Moreover, the pressure will also be noted and registered each time a sample is taken. A filter is considered to pass the test when 100% of the IgG is allowed to pass through.

4. Results and discussion

4.1.   Fibers

4.1.1.   Polypropylene (PF 1000N and PF 2000N)

Polypropylene PF 1000N and PF 2000N fibers are the most easy to handle and modify. Their relatively thick walls grant them extra structural strength while also providing a bigger volume for the monolith to occupy and endure oxygen inhibition of the outmost regions while still having enough volume to obtain uninhibited monolith in the inner regions. The UV-absorption spectra of polypropylene (Fig. 13) near the main photoinitiator's (2-hydroxy-2-methylpropiophenone) absorption wavelength, 240 nm (Fig. 6), means a total UV interference from the fiber is unlikely since the fiber's absorbance is low (ca. 0,47 at 250 nm) and employing a higher photoinitiator content could surpass such obstacle. Since polypropylene shows such relative low UV-absorption along a wide wavelength range (Fig. 13), PF 1000N/2000N fibers are ideal to use with photoinitiators with different absorption profiles.

While fibers obtained this way may suffice during the laboratory experiments, it is impossible to incorporate them into a continuous process. The previous factors make the polypropylene fibers ideal for small batch polymerizations and especially adequate for experiments when evidence of phenomena or basic principles is desired.

4.1.2.   Polyethersulfone (MIKRO-H-033)

The MIKRO-H-033 fibers have almost the same internal diameter and a smaller wall thickness (Fig.12 and Fig. 15) when compared to the PF 2000N fibers. Both factors combined result in a fiber ideal from a production-cost point of view, though weak when being handled and modified. A second consequence of the thinner wall thickness is that the monolith has less volume where it can be polymerized and oxygen inhibition has a bigger influence than in the polypropylene fibers, resulting in a higher susceptibility to both impregnation and

reaction problems than the polypropylene fibers. The material of the membrane contains aromatic structures (Fig. 16) that can interfere with the monolith polymerization by absorbing UV-radiation. Evidence of this is the graft functionalization PES undergoes when exposed to UV-light in adequate conditions [20]. However with the UV-lamps and photoinitiators employed in this project, no significant UV blockage or absorption by the fibers has been observed.

4.2. First method: single fiber polymerization

The results with this method were generally poor as a consequence of the inevitable oxygen diffusion into the fibers while they were being impregnated one by one and the subsequent inhibition caused by it. Another obstacle was the fact that when the second PE sheet was laid on top, monolithic solution from the fibers would flow towards the sheets, sticking to them and creating patches of monolith causing several fibers to stick to each other and to the sheets. No monolith was observed in the polyethersulfone fibers.

However the first hint towards the basic feasibility of the process principle was obtained when a blocked polypropylene fiber was observed (Fig. 30 and Fig. 31), a blocked lumen implied that the UV-rays could penetrate the fiber and cause a polymerization. Based on this result it was thought that the main obstacle laid on the impregnation of the solution into the wall, and as such, the second method was arranged with guaranteeing the impregnation step in mind. When fibers with large diameter (i.e. PF 1000N fibers) were used, the wetting of the fibers could be observed by the naked eye and thus the quality of the impregnation could be easily determined. The same observation was not possible with the smaller PES-fibers, so after the process was finished, the exact problematic point of the procedure could not be unquestionably identified.

Since polyethersulfone exhibits a considerable absorption at wavelengths under 308 nm (Fig. 16) and the fibers have thin walls, the exact reason the monolith

polymerization was unsuccessful could not be identified. The problem could lie on the fiber impregnation, the monolith reaction, or both.
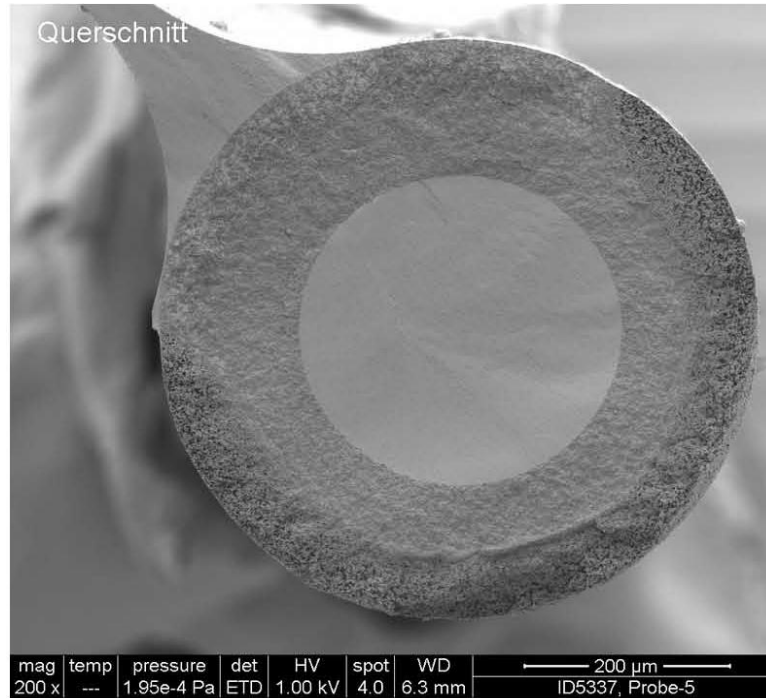

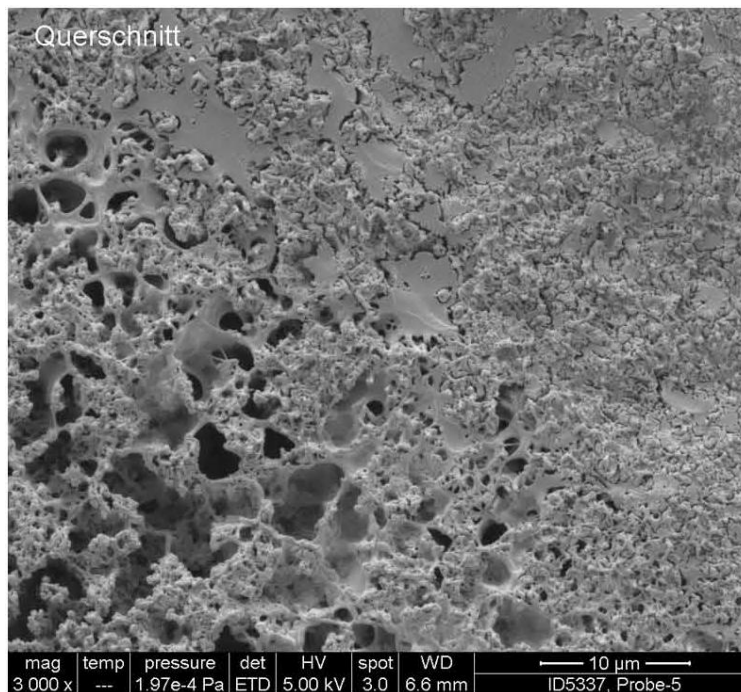*Figure 30. Monolith inside the walls and lumen of a polypropylene fiber.*

Figure 31. Detail of the monolith-containing wall of a polypropylene fiber

However, the fact that monolith was effectively polymerized in the pores of the polypropylene fiber (Fig. 31) wall meant that the basic premises of the project were correct. The monolith properties were not evaluated because of the lumen being blocked after the majority of the successful polymerizations in the polypropylene fibers (Fig. 32), and the fact that each single fiber had to be handled individually, which meant the process was slow and the reproducibility low.
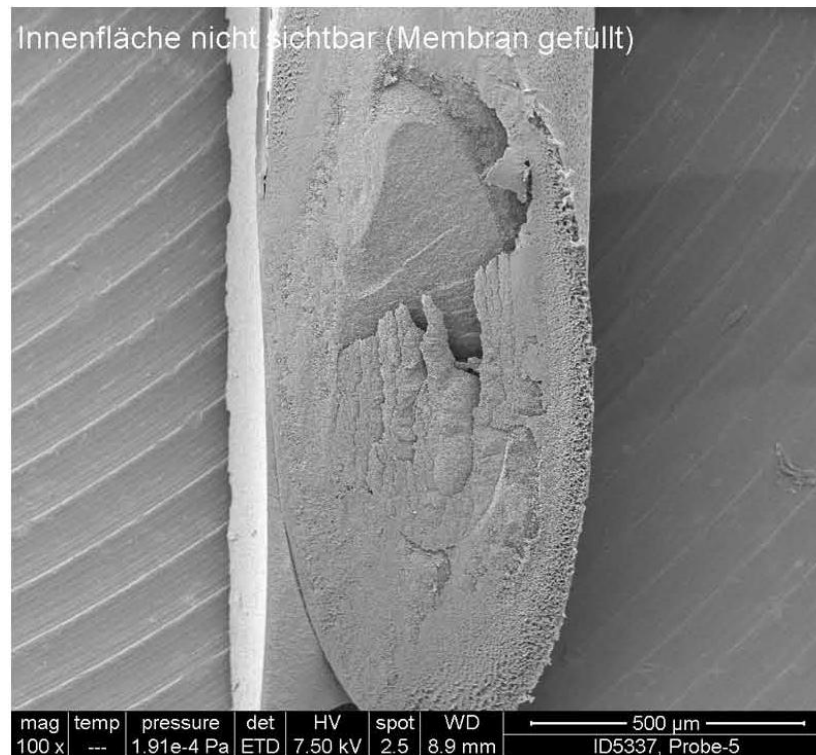

Figure 32. Monolith inside the walls and lumen of a polypropylene fiber.

Fundamentally, the first method fulfilled its purpose of checking the basic feasibility and principles of the project.

4.3. Second method: hand-bundle polymerization

This method was a considerable improvement and had several advantages over the previous one:

- Several fibers could be polymerized at a time
- Clear lumens
- Less direct handling of the fibers.
- Simple to clean, use and measure.

Similar results to that of the first method were observed. Monolith-containing PP fibers had few problems during the polymerizations and had a reliable monolith presence while monolith-containing PES fibers were hard to obtain and fragile. Hand-bundles of PP fibers modified through this method were extensively used and were the main resource to test the different monolithic solution formulations. Several observations were made during each step of the process

4.3.1. Impregnation

A portion of the monolithic solution will remain trapped between the fibers because of its surface tension. This effectively limits the amount of fibers that can be grouped and modified in a hand-bundle, since in future steps of the process, the trapped solution can re-diffuse into the fiber's lumen, causing big blobs of monolith along the fibers or stick the fibers one with another. Using 5 fibers per bundle allows a relatively low amount of solution to be contained between the fibers and in most cases, guarantees that all fibers are free of monolith inside their lumens and are easy to separate from each other after the polymerization is finished. Good results can be obtained with bundles containing up to 10 fibers. If more fibers per bundle are used, there will be too much solution trapped between the fibers and these will become blocked during later steps. Even if the solution between fibers does not go back into the lumens, it will polymerize and form a single structure from which the fibers are not able to be separated.

4.3.2. Nitrogen flushing

Even though the use of a continuous flow of nitrogen effectively cleared the lumens, it also had an undesirable effect on the reproducibility of the modified

bundles, since employing such flow for extended periods of time could cause porogen evaporation, changing the basic composition of the monolith, and introducing a new factor in the process whose influence was hard to account for.

When using short nitrogen bursts it was slightly harder to clear the lumen of all the fibers than when using a continuous flow. However, short bursts are less likely to change the chemical composition of the monolithic solution.

As with the previous phase, using a lower number of fibers per bundle guarantees an easier handling and a more reliable final product, mainly because lower nitrogen flows are required. When low flow is used in a bundle with a high number of fibers, it was observed that only a portion of the fibers were cleared and it was necessary to increase the nitrogen flow, which enhanced the possibility of porogen evaporation.

4.3.3. Polyurethane re-sealing

An important phenomenon was observed on the polymerized hand-bundles. The fiber region that could touch either the PU-filling or the plastic housing was identified as weak point regarding its filtrating capacity during later tests. This is arguably caused by a poorer presence of the monolith in this region in comparison to the main body of the fiber. This is hypothesized to be caused by the loss of monolithic solution from the membrane walls caused by the contact of the fibers with the PU filling and the plastic housing. An experiment made to confirm this theory was performing an LP-test of a polymerized bundle, and then re-sealing both ends of the bundle where the fiber comes into contact with the housing or the PU filling, and then carrying out the LP measurement again. In all cases, the LP after re-sealing was considerably lower. After the experiment, PU-resealing became standard practice. It was also interesting to observe these "weak points" after a gold retention test (GPRT). After a GPRT was performed on a hand-bundle that was not re-sealed, a deep purple coloration could be observed on the weak points (the gold solution has a purple colour). This was in all cases accompanied by poor GPRT results. Once the hand-bundles were

resealed and the weak points covered, the filtrating results were in all cases improved.

4.3.4. REM-Pictures

Polypropylene fibers usually underwent a successful modification and had a reliable monolith presence in its walls and free lumens (Fig. 33). Such reliability depended on the number of fibers in the modified hand-bundle. Using more than 10 fibers in a hand-bundle usually resulted in a majority of them having their lumens blocked.
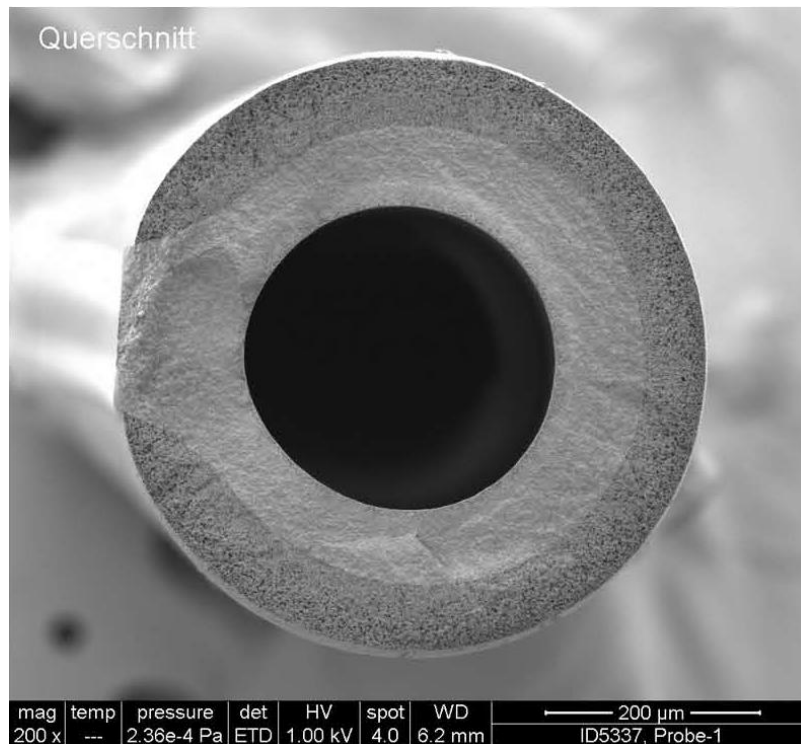

Figure 33. Polypropylene fiber with monolith in its wall and a clear lumen.

The monolith in the PP-fibers had a very defined and regular diameter (Fig. 34)
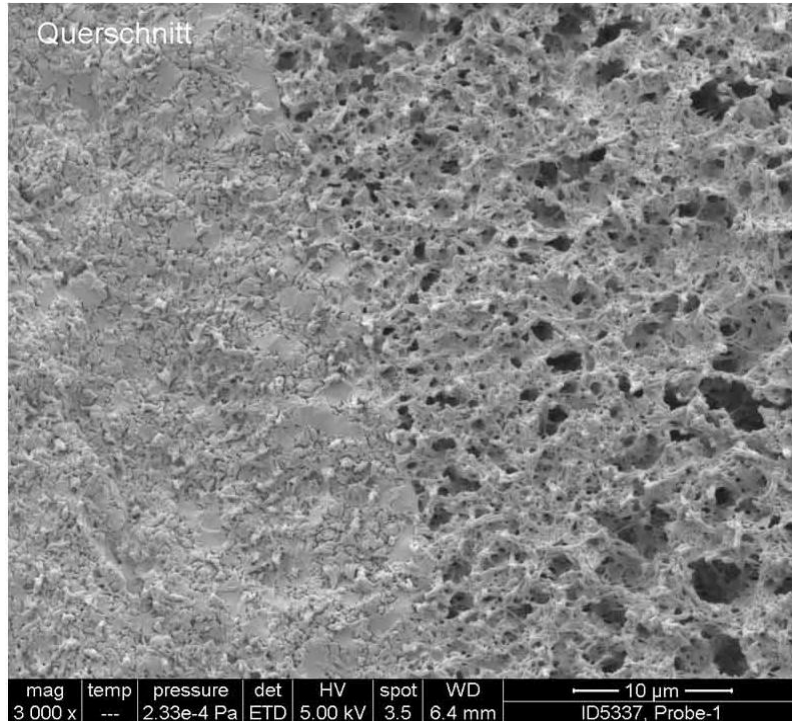
Figure 34. Wall of a monolith-containing polypropylene fiber.

Polymerizing the monolith in the walls of polyethersulfone fibers was still an unreliable matter, having both empty lumens and walls as the most common result (Fig. 35).
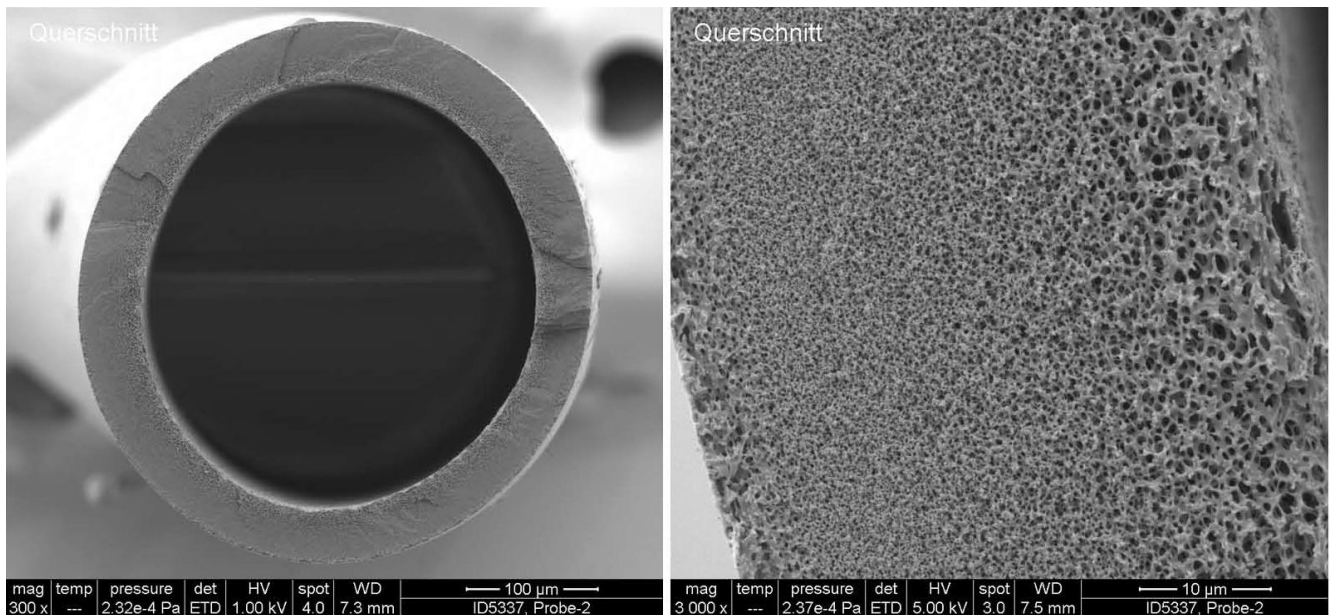

Figure 35. Polyethersulfone fiber after an unsuccessful modification

The second most common outcome was the presence of monolith inside the lumen, but not in the wall (Fig. 36).
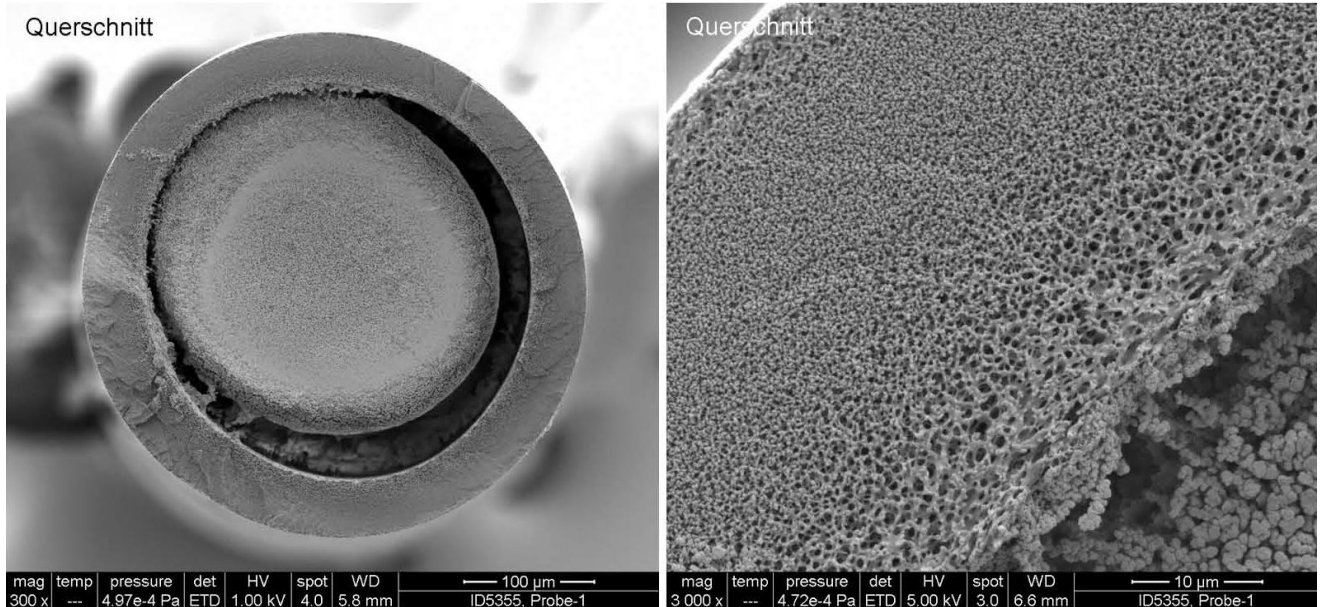

Figure 36. Blocked polyethersulfone fiber after an unsuccessful modification

The presence of a polymerized monolith inside the lumen, indicated that an UV-blockage caused by the fiber did not take place since there was enough radiation to reach and polymerize the solution in the lumen of the fiber, which logically meant that the rays were able to travel first through the walls of the membrane. The fact that the reaction phase was supposedly not the problem, strongly suggested that the obstacle lied in the impregnation phase at this point.

However, PES fibers with monolith both inside the wall and the lumen were observed (Fig. 37). This meant that both impregnation into the wall, and the polymerization reaction inside the fiber were in fact possible, and at this point an unknown disruptive phenomenon yet to be explained was preventing the monolith from polymerizing in the PES-fibers.
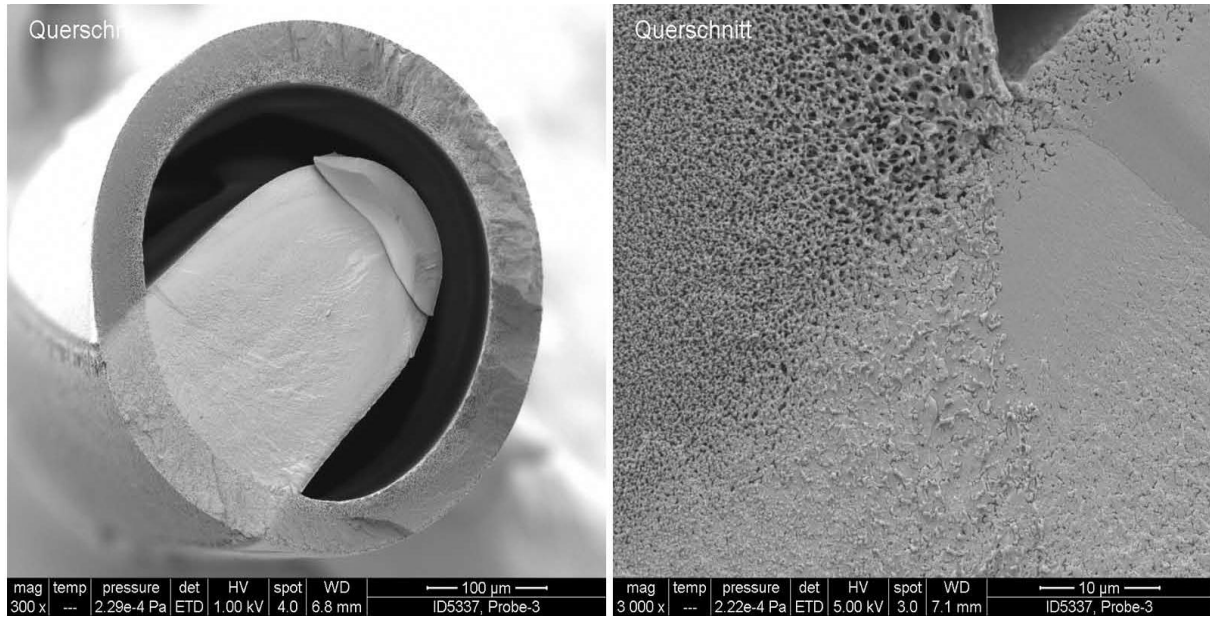
Figure 37. Blocked polyethersulfone fiber after an unsuccessful modification

4.3.5. Inert atmosphere

Up to this point the oxygen inhibition was thought to be in its majority avoided with the 30-minute long bubbling of the monolithic solution. This assumption along with the difficulty of creating an inert atmosphere where the handling and irradiation of hand-bundles are possible, were the reasons for the lack of use of an inert atmosphere. However an experiment to determine the influence of atmospheric oxygen on the polymerization and confirm the previous assumption was carried out.

Modifying two hand-bundles in identical conditions besides the reaction atmosphere shed light on the phenomenon affecting the monolith polymerization. The walls of the PES-fiber modified in air had a poor monolith (Fig. 38), while the presence of monolith in the fiber modified in nitrogen was remarkably better (Fig. 39)
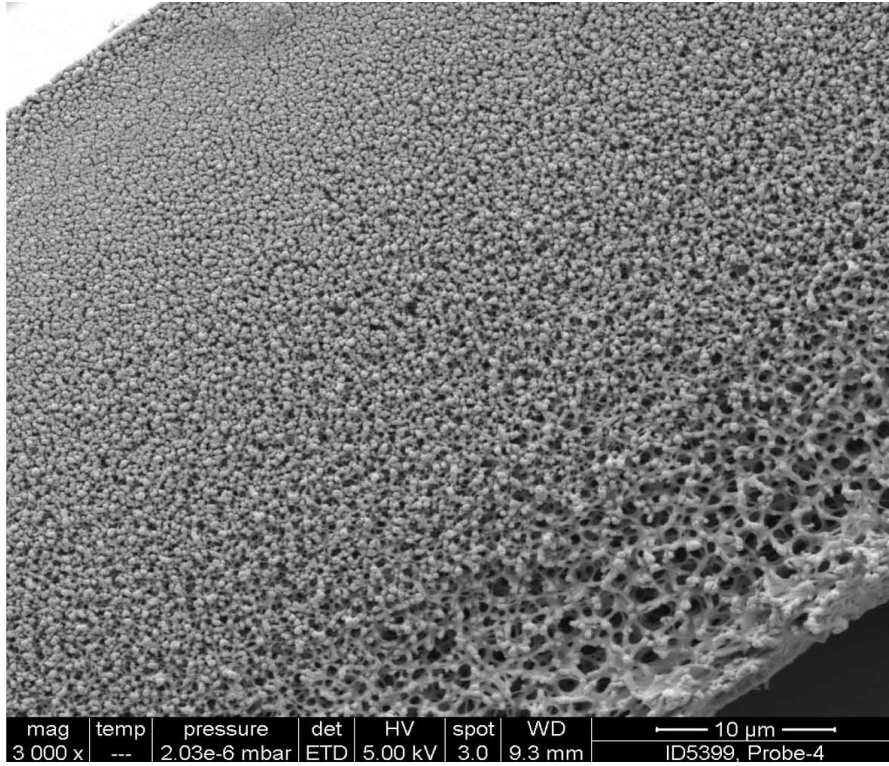
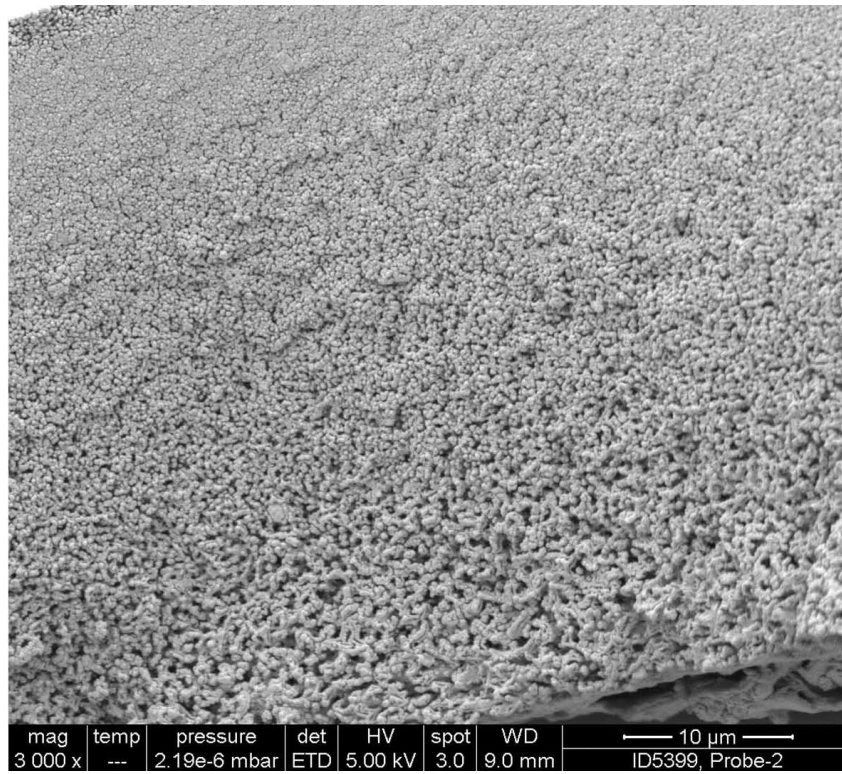*Figure 37. MIKRO-H-033 fiber modified with V12, 3% PI in air*

Figure 38. MIKRO-H-033 fiber modified with V12, 3% PI in nitrogen atmosphere

Based on the previous results, the atmospheric-oxygen inhibition hypothesis was proposed.

4.3.6. LP and GPRT

A strong relation between LP-values and GPRT values were observed. In most cases a low LP accurately predicted good gold retention values.

It was decided that polypropylene hand-bundles of 5 fibers each were the best carriers to test the properties of the monolith inside their pores. Even though easy to handle and evaluate, the reproducibility was still relatively low since the process was still very manual.

The monolithic solutions with the best properties are shown in Table 5.

	PP(5)-V6	PP(5)-V9	PP(5)-V12	PP(5)-V14	PP(5)-V12	PP(5)-V12	PP(5)-V12
HEMA	32,06 %	31,9%	33,63%	36%	33,63%	33,62%	33,62%
PETA	13,83 %	16,0%	16,81%	4%	16,81%	16,82%	16,82%
Cyclohexanol	18,04 %	17,4%	16,52%	12%	16,52%	16,52%	16,52%
Dodecanol	36,07 %	34,8%	33,04%	48%	33,04%	33,04%	33,04%
2-Hydroxy-2 methylpropio phenone	1%	1%	1%	1%	1%	1%	1%
LP-Value (10^{-4} $cm.bar^{-1}.s^{-1}$)	10,02	7,08	3,82	7,44	7,45	1,99	4,72

Table 5. LP-coefficients from several polymerization batches.

Such variation can be observed regarding the monolithic solution variation 12 (V12), with LP's ranging from 2 to over 7.

After the LP-coefficient was obtained, the hand-bundles were subjected to a gold particle retention test.

Since the GPRT is standardized to hand-bundles or minimodules with an internal surface of 10 cm^2, and the employed 5-fiber hand-bundles had usually a surface around 5cm^2, the first 1ml sample usually regarded as Fraction I corresponded in

reality to Fraction I and Fraction II mixed together. And the second 1 ml sample regarded as Fraction II amounted to Fraction III and Fraction IV mixed together (Table 6).

	PP(5)-V6	PP(5)-V9	PP(5)-V12	PP(5)-V14	PP(5)-V12	PP(5)-V12	PP(5)-V12
LP-Value (10^{-4} cm.bar^{-1}.s^{-1})	10,02	7,08	3,82	7,44	7,45	1,99	4,72
Separation percentage	52,1%	62,8%	53,8%	49,9%	63,9%	90,0%	92,8%
GPR of Fraction I	0,32	0,43	0,335	0,3	0,442	0,998	1,14
GPR of Fraction II	0,25	0,38	0,306	0,16	0,25	0,93	0,93

Table 6. GPRT values.

A rule of thumb was soon discovered: hand-bundles with LP's over 10 had unsatisfactory retention values in all cases and hand-bundles with LP's below 10 had the best results.

The monolithic solution V12 had the best retention results. The retention value of 93% confirmed the potential of this technology and opened the door for the search of a continuous way of polymerizing monoliths inside fibers through a third and final method. This solution provided a monolith whose pores were small enough to retain a considerable amount of gold particles, however the monolith polymerization in the fiber walls was still not reliable enough as to obtain significant data from IgG-tests.

It was also reported by prior interns working previously on the same project that the chosen monolith (HEMA-co-PETA) never presented any kind of IgG-absorption phenomena.

4.4. Third method: continuous polymerization

4.4.1. Impregnation

When first implementing the method it was feared that if the fiber was left submerged in the solution for too long, the fluid would eventually reach the lumen and block the fiber. Different experiments with long impregnation times were carried out in order to find this time. The only instance where the lumen got blocked by the solution was when the fiber had a rupture or a leak not necessarily only in the submerged region, but also somewhere along the unsubmerged portion of the fiber. The reason behind this fact is that the solution readily diffuses into the membrane walls as a consequence of the fluid's surface tension. The impregnation is regulated by the capillary forces and hydrophilic properties of the fiber, which along with the fiber porosity and pore size influence its LP. In fibers with high LP the impregnation time is not as significant as in fibers with low LP, even with impregnation times of a couple seconds the impregnation of fibers such as MIKRO-H-033 (LP ca. 1000) is satisfactory. On the other hand, fibers with low LP's present poor impregnation results even with long impregnation times. After leaving the solution bath, each time the fiber comes in contact with a surface a small quantity of solution is lost. This happens for two reasons. The first one is when the fiber has its course changed, in order to change the path direction the fiber is pressed against a metal cylinder (Scheme 11), bends on it and adopts its new direction. The side facing the metal surface lightly shrinks while the side facing away lightly extends, causing a small quantity of the solution to flow out. This effect is compounded when the change of path is abrupt; the motor pulling the fiber causes it to not only bend on a side, but also to be deformed. The influence of this factor depends on the strength of the fiber, the surface of the element upon which the fiber is bending and the angle change. The effect is easily observable when fibers with a wall thickness of 50 μm are subjected to an angle change of 90°. Thicker fibers can better endure the deformation forces.
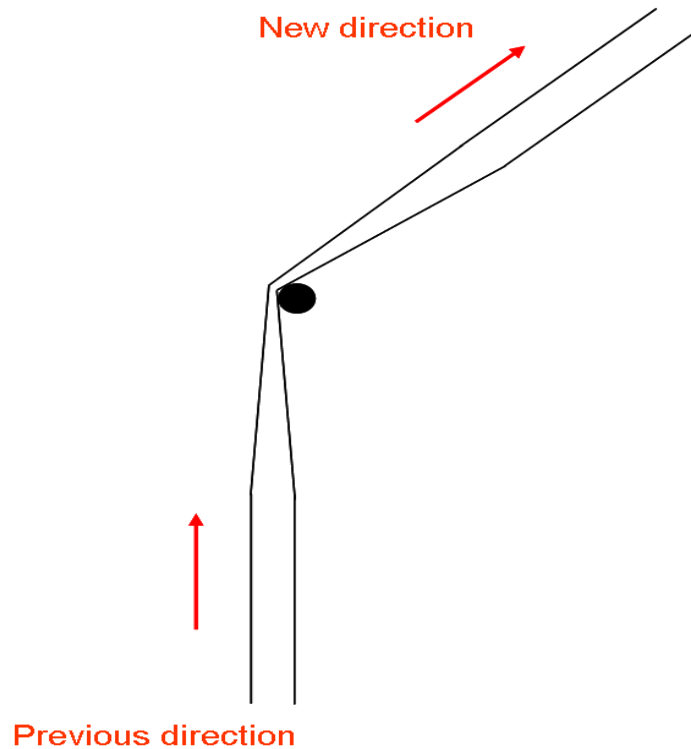
Scheme 11. Fiber deformation

The second cause of solution being lost is the natural tendency of the solution to flow towards and wet the surfaces with which it comes in contact. This is the reason behind the decision of employing cylinders as turning points instead of roller wheels. The latter have a ridge in the middle in which the fiber goes; this means the fiber would be in contact with 3 surfaces (bottom and sides of the ridge) instead of just one (cylinder surface) and more solution would be lost.

4.4.2. Photoinitiator content

Using V12 monolithic solutions with different photoinitiator contents of 1% and 5%, PES-fibers were continuously modified in air maintaining all other variables equal, the reaction time comprised approximately 15 seconds in air. After collecting and washing the fibers, REM-pictures were taken.

Monolith polymerization using a solution with 1% photoinitiator content has no appreciable monolith inside its walls (Fig. 39 and Fig. 40). This agrees with the previous results.

As expected, the fibers carrying a higher photoinitiator percentage contain an observable degree of monolith polymerization in the form of a white ring (Fig. 41). However, the reaction is not uniform and some regions have a poor monolith presence (Fig. 42).
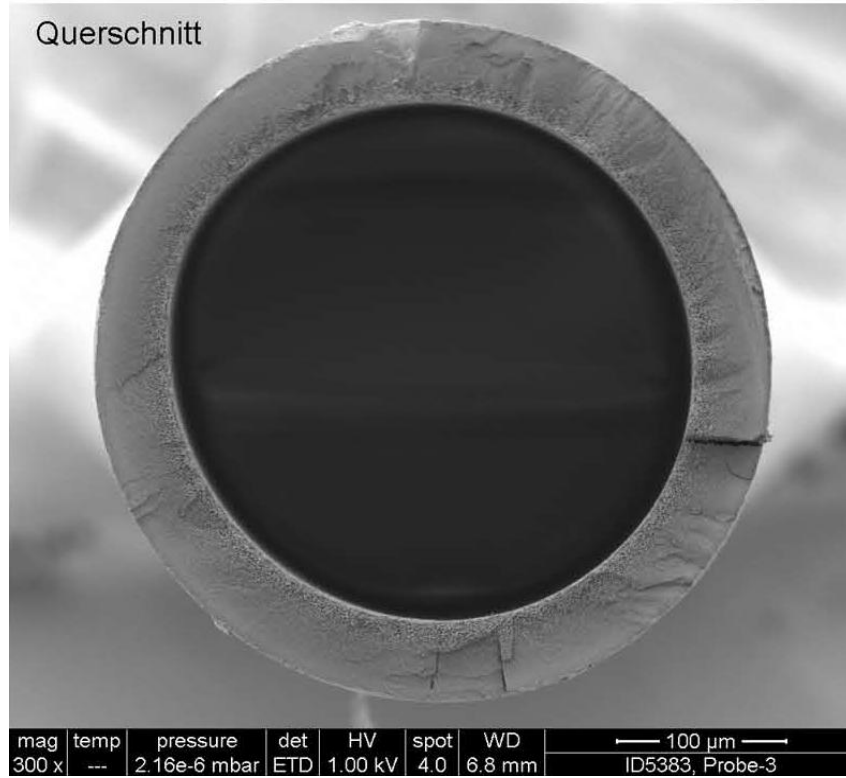
Figure 39. PES fiber cross-section modified with a monolithic solution containing 1% PI.
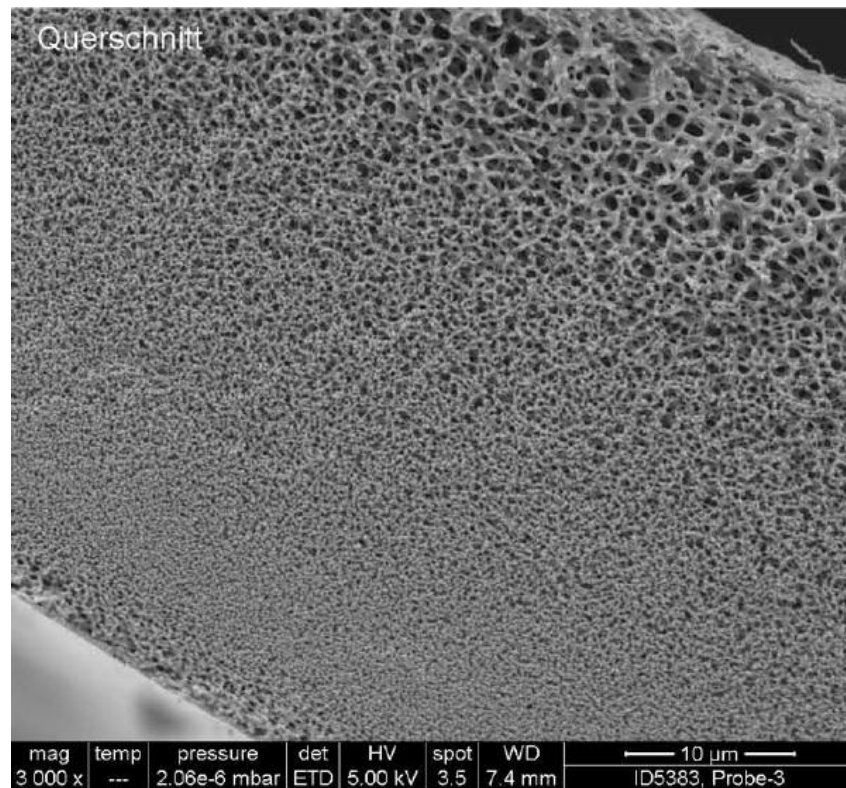

Figure 40. PES fiber wall modified with a monolithic solution containing 1% PI.

mag	temp	pressure	det	HV	spot	WD	⊢— 100 µm —⊣
300 x	---	2.03e-6 mbar	ETD	1.00 kV	4.0	9.2 mm	ID5399, Probe-3
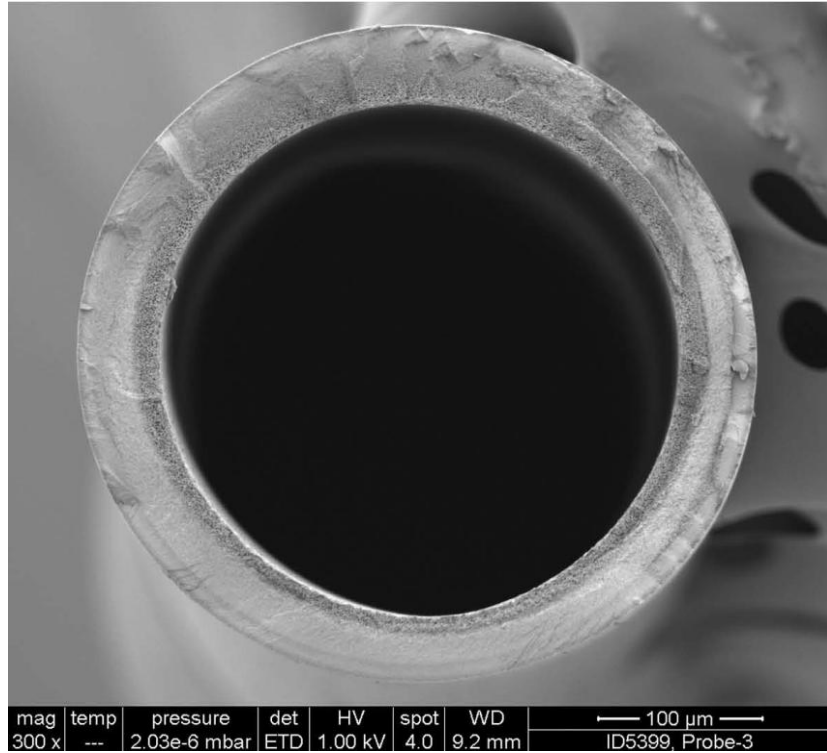
Figure 41. PES fiber modified with a monolithic solution containing 5% PI.

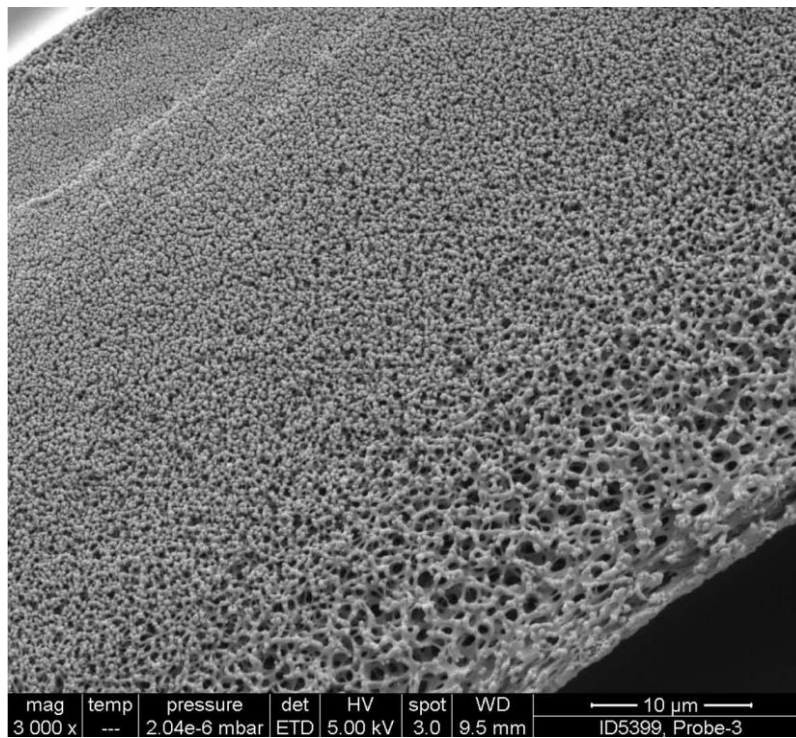| mag	temp	pressure	det	HV	spot	WD	⊢— 10 µm —⊣
3 000 x	---	2.04e-6 mbar	ETD	5.00 kV	3.0	9.5 mm	ID5399, Probe-3

Figure 42. Poor monolith presence in a PES fiber modified with a monolithic solution containing
5% PI.

4.4.3. Atmosphere

The absence of monolith in some regions of the fiber (Fig. 41 and Fig. 42) can be explained by the fact that both previous experiments were carried out in air.

After using a nitrogen flow to displace the oxygen in the reaction chamber, the polymerization using the V12 monolithic solution with a PI content of 5% was carried out again. After gathering and cleaning the fiber, REM-pictures were taken (Fig. 43 and Fig. 44).

The fiber in Fig. 43 was the first polyethersulfone fiber in which a consistent monolith along the entire wall circumference was observed. The use of a high photoinitiator content and nitrogen atmosphere presented a breakthrough, especially considering the poor PES-fibers previous results. REM-pictures of the wall of this fiber were also taken (Fig. 44).

A considerably better monolith presence was obtained and no regions lacking monolith were observed. However, when comparing the monolith profile with previous ones from the second method (polypropylene fibers), an inwards diminishing monolith "density" was noted, indicating only partial polymerization of the monolith. Since the use of nitrogen guaranteed an almost-inert atmosphere, the problem was considered to lie on a too short reaction time.
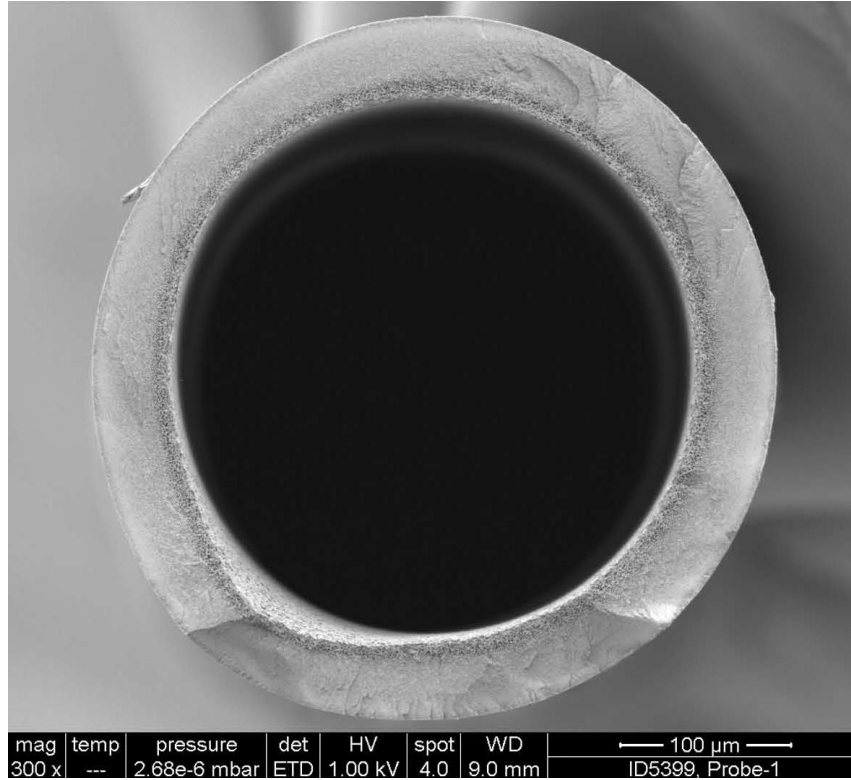
mag	temp	pressure	det	HV	spot	WD	⸺ 100 µm ⸺
300 x	---	2.68e-6 mbar	ETD	1.00 kV	4.0	9.0 mm	ID5399, Probe-1

Figure 43. PES fiber modified with a monolithic solution containing 5% PI in nitrogen.

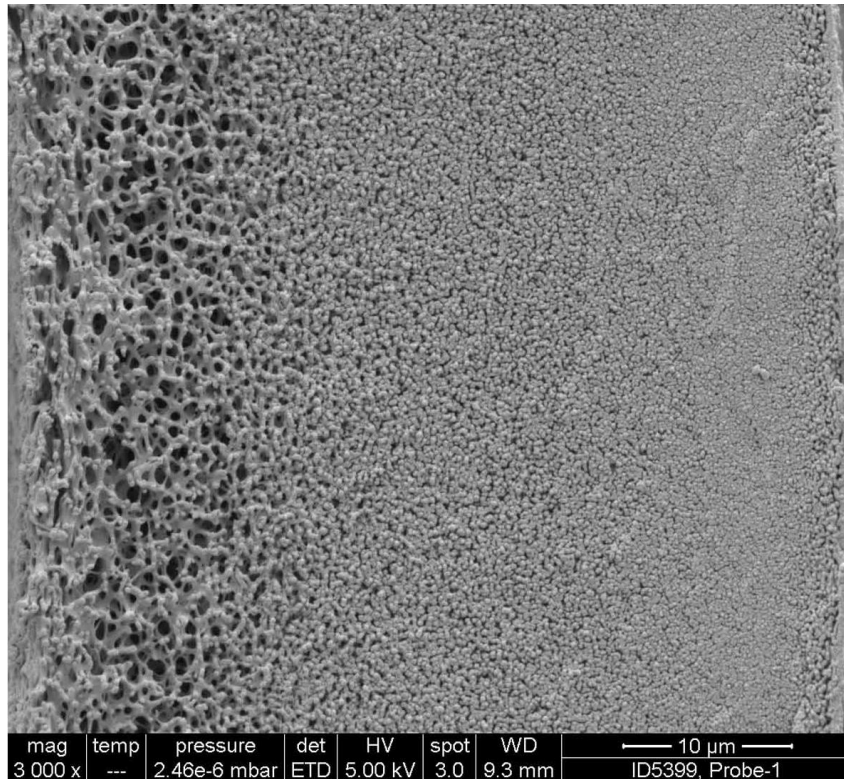| mag	temp	pressure	det	HV	spot	WD	⸺ 10 µm ⸺
3 000 x	---	2.46e-6 mbar	ETD	5.00 kV	3.0	9.3 mm	ID5399, Probe-1

Figure 44. PES fiber modified with a monolithic solution containing 5% PI in nitrogen.

4.4.4. Reaction time

Different reaction times were set while the other variables were kept the same. The reaction time itself was limited by the ability of the pump to perform without "jumping" or stuttering at low velocities. In order to guarantee a continuous smooth winding velocity, gear exchanges were employed for reaction times over 20 seconds. All the reactions were carried out under a nitrogen atmosphere, using a V12 monolithic solution with a 5% photoinitiator content based on the total solution weight and employing PES-fibers. After gathering and cleaning, REM-pictures of the cross-sections of both fibers and walls were taken.

The different reaction times were 15, 20, 35 and 65 seconds (Figs. 45 to 52).

The influence of the reaction time on the modified fibers can be seen by comparing their wall REM-pictures (Fig. 53).

Longer reaction times have two visible effects:

- A denser monolith caused by a more complete polymerization reaction.

- More monolith-occupied fiber volume when compared to shorter reactions.

A more thorough reaction with longer times was to be expected. However, the extended monolith presence was not as easily predicted. To understand the reason behind this phenomenon, it must be taken into account that the lumen of the fiber contains oxygen, which is continuously consumed by the free radicals in the solution. In contrast to polymerizations carried out in air, the oxygen concentration inside the fibers is not constant and steadily diminishes while it's being consumed. In shorter reaction times the amount of oxygen in the lumen is enough to inhibit a considerable monolith layer (Fig. 46). The longer the irradiation is maintained, the more oxygen will be consumed, diminishing both its concentration inside the lumen and its concentration profile in the fiber. With a long enough reaction time, all the oxygen inside the fiber will be consumed by the free radicals, which will then start to react with the acrylate double bonds. This means that previously inhibited regions will start to gradually polymerize (Fig. 52) and the monolith appears to advance towards the lumen (Fig. 53).
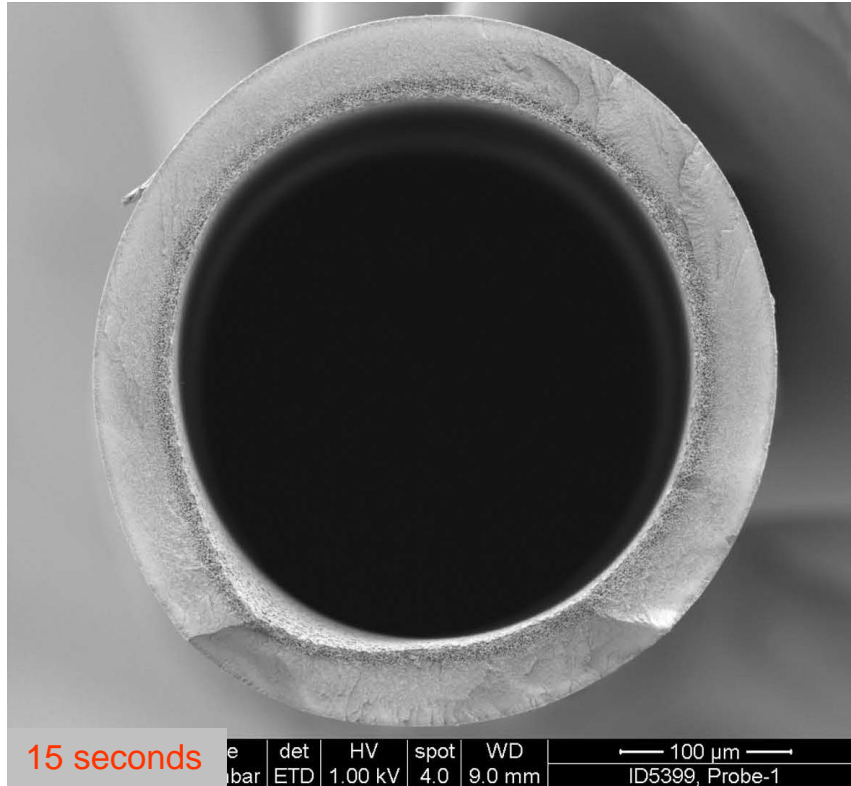
Figure 45. Perpendicular REM. 15 seconds long reaction time. N2 atmosphere, V12(5%) solution.
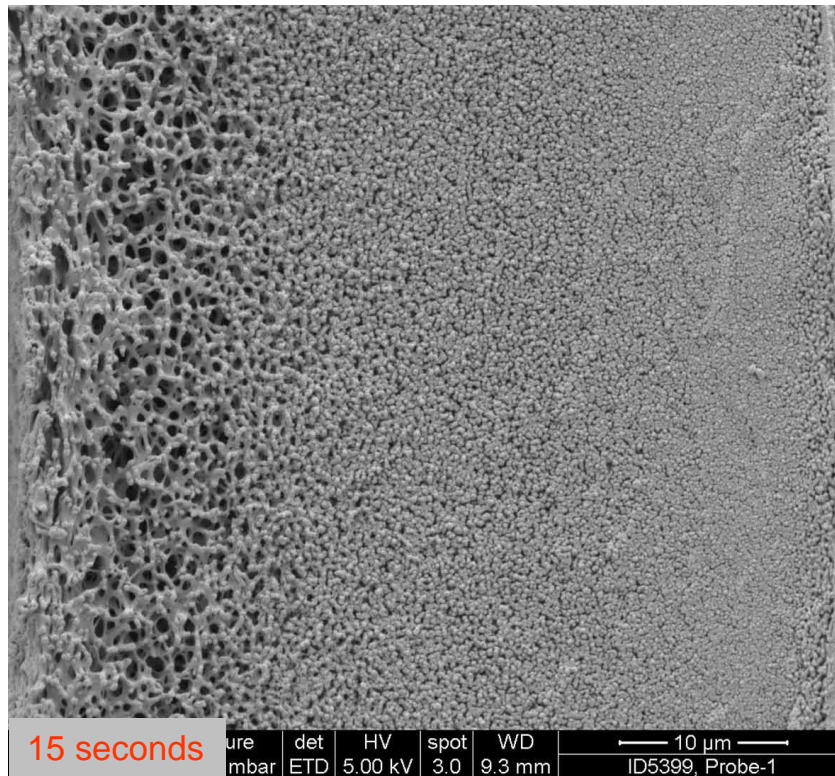

Figure 46. Wall REM. 15 seconds long reaction time. N2 atmosphere, V12(5%) solution.

20 seconds

| det | HV | spot | WD | 100 μm |
| ETD | 1.00 kV | 4.0 | 8.8 mm | ID5399, Probe-6 |

Figure 47. Perpendicular REM. 20 seconds long reaction time. N2 atmosphere, V12(5%) solution.

20 seconds

| det | HV | spot | WD | 10 μm |
| ETD | 5.00 kV | 3.0 | 9.0 mm | ID5399, Probe-6 |

Figure 48. Wall REM. 20 seconds long reaction time. N2 atmosphere, V12(5%) solution.
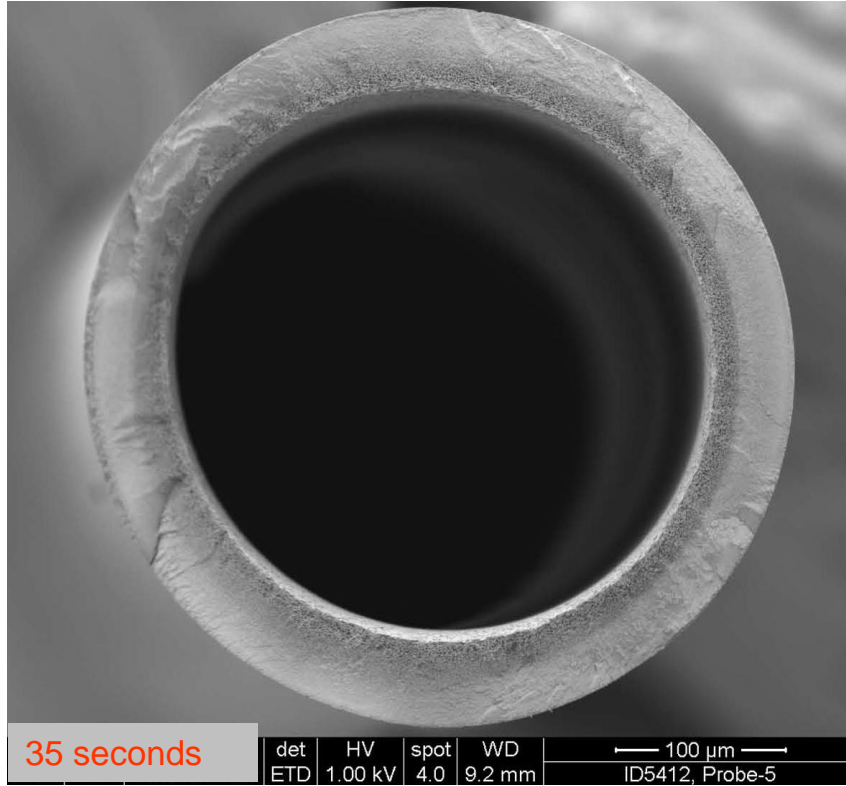
Figure 49. Perpendicular REM. 35 seconds long reaction time. N2 atmosphere, V12(5%) solution.

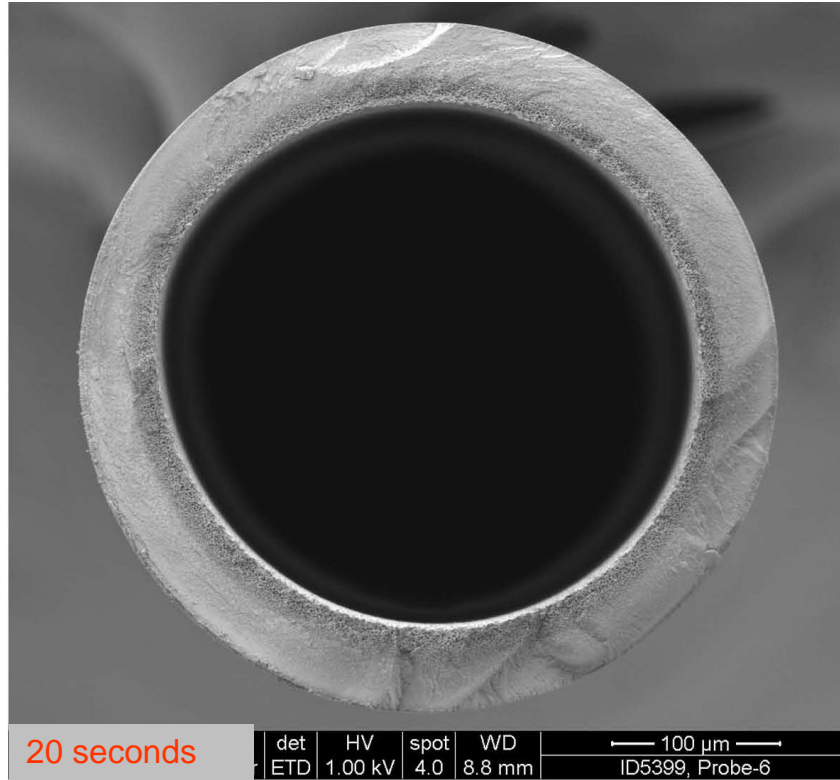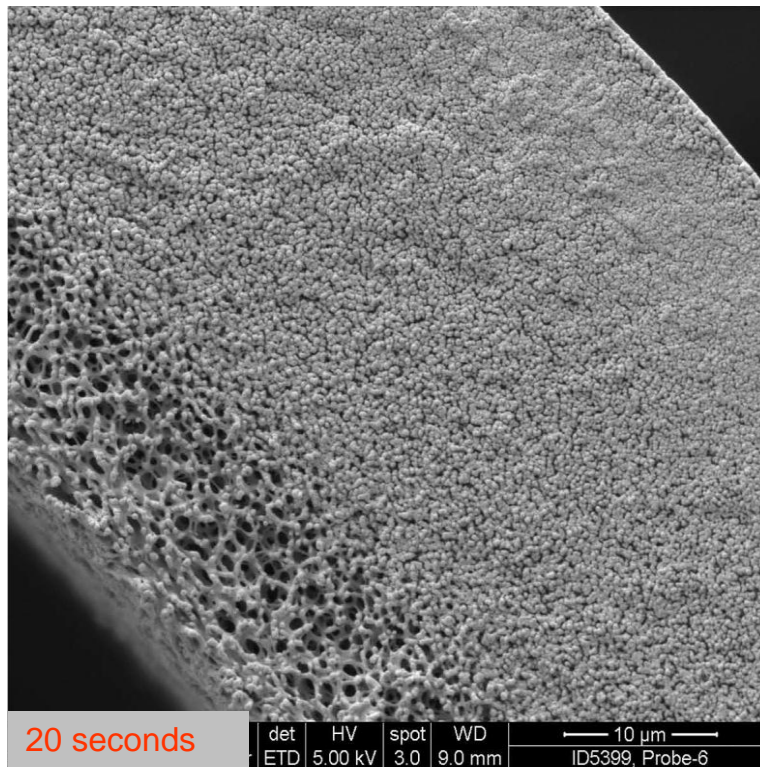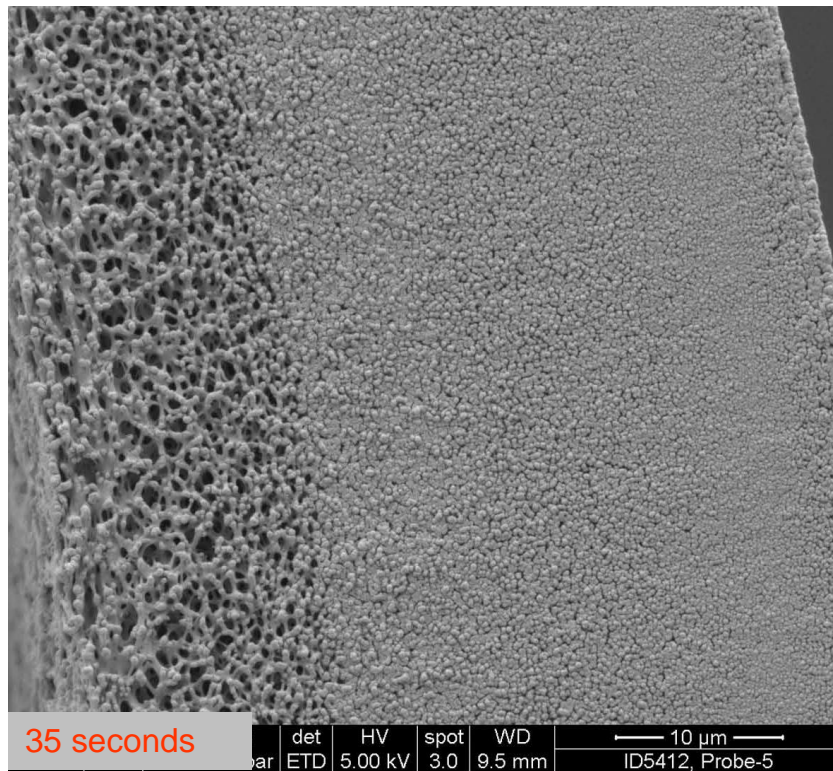*Figure 50. Wall REM. 35 seconds long reaction time. N2 atmosphere, V12(5%) solution.*

65 seconds

| det | HV | spot | WD | 100 μm |
| ETD | 1.00 kV | 4.0 | 6.3 mm | ID5418, Probe-4 |

Figure 51. Perpendicular REM. 65 seconds. N2 atmosphere, V12(5%) solution.

.

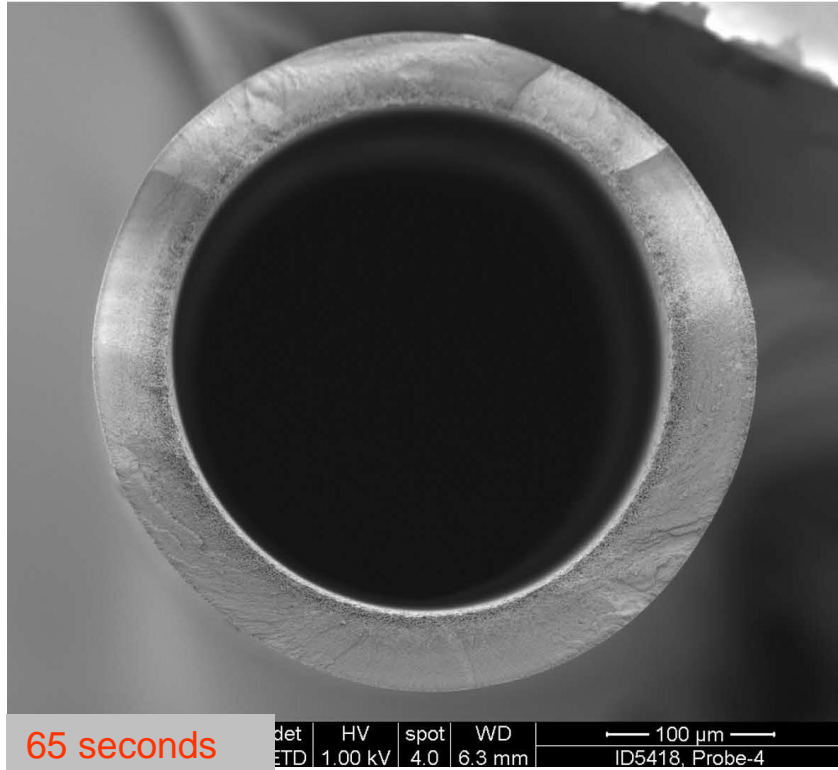65 seconds

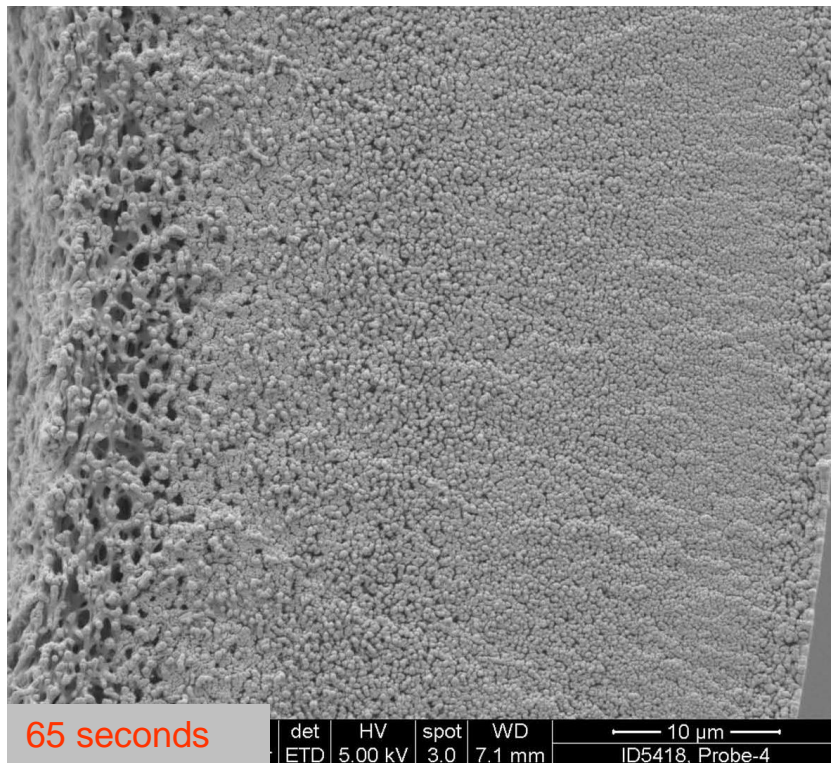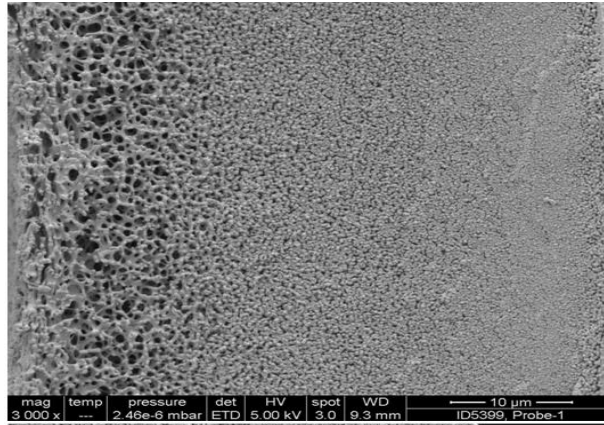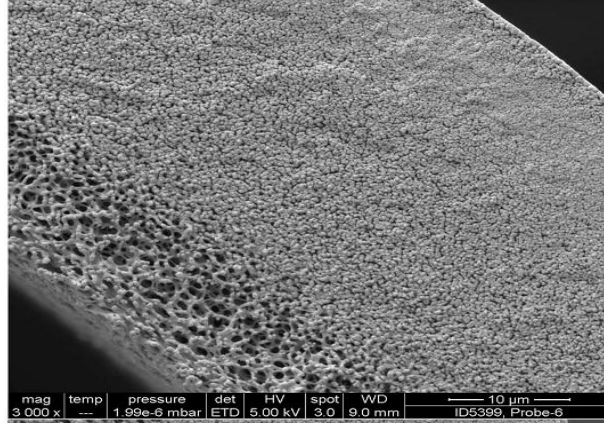| det | HV | spot | WD | 10 μm |
| ETD | 5.00 kV | 3.0 | 7.1 mm | ID5418, Probe-4 |

Figure 52. Wall REM. 65 seconds long reaction time. N2 atmosphere, V12(5%) solution.
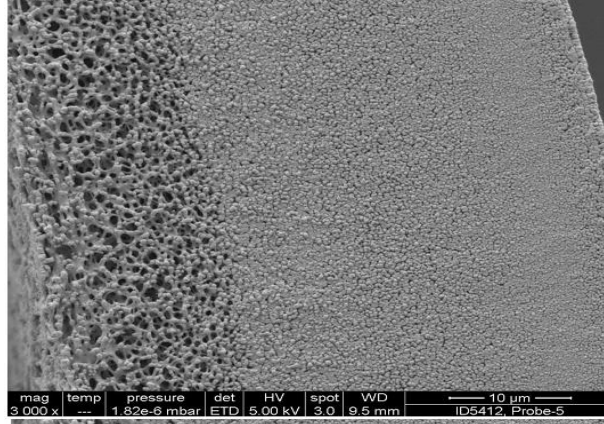
15 seconds

20 seconds

35 seconds

65 seconds
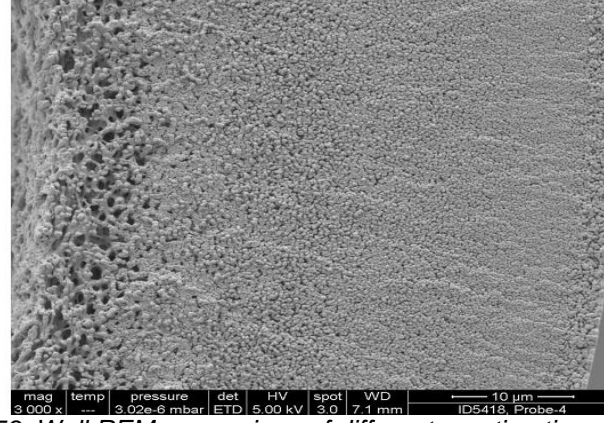
Figure 53. Wall REM comparison of different reaction times.

4.4.5. Patterns after polymerization

After the fiber has been gathered, a quick way to assess the quality of the polymerization is to observe the fiber against a backlight. After the polymerization, the porogens remain unreacted inside the fiber. The regions where there is a lower quantity of porogens (and arguably a lower quantity of monolith) look opaque in comparison to the regions with a higher content of porogens (and arguably higher monolith content). This patterning becomes obvious when high winding velocities are used or extreme motor stuttering happens. The droplets caused by high RPM's concentrate the solution in a periodic way (Scheme 12), yielding a very identifiable pattern after polymerization (Scheme 13).

Scheme 12. Droplet pattern after inadequate impregnation

Scheme 13. Polymerization pattern after reaction.

4.4.6. Macro effects

Even though a successful monolith polymerization on the PES-fibers was achieved, the LP values and gold retention results obtained from them were not only unsatisfactory but also seemed to vary between reproductions of identical batches. This variation was determined to be caused by macro effects like: motor stuttering; influence of agitation and nitrogen bubbling in the solution reservoir during the impregnation step; the drainage of solution during path changes between the impregnation step and the reaction chamber; uneven radiation between the fiber surface facing the UV-lamp and the one facing away; and

gravity and surface tension affecting the solution distribution along the fiber. The observation of the patterns further indicated towards the existence of such macro disturbances in comparison to the micro phenomena that had been process-determinant until this point (oxygen inhibition and reaction kinetics). Because these effects caused some fiber regions to contain a better monolith presence than others, the LP and gold retention values were not determined by the monolith properties, but by an average between the intrinsic monolith properties and the uniformity of monolith along the fiber. In general the LP of the PES-fibers after modification was found to be between 30 and 100, this again indicated a successful modification, yet not satisfactory enough like those observed in the polypropylene fibers from the second method (with LP values generally around or below 10). Given the previous reasons, individual values for each minimodule are not reported since they are not trusted to truthfully indicate the properties of the monolith

The variability along the fiber length caused by the macro influences is not only observed in the varying presence of monolith inside the fiber, but also on the occasional polymerization on the outer fiber surface. This can be attributed to the effect of gravity on the solution, as well on its tendency to form drops along the fiber.

4.4.7. Double polymerization on the same fiber

The same PES-fiber was subjected to two monolith modifications. Each process was done with a V12(5%) solution, under nitrogen atmosphere, with no roller wheels, no nitrogen bubbling in the solution reservoir, and a reaction time of 65 seconds. Between the modifications, the fiber was washed with ethanol, water and left to dry in air. After cleaning the fiber for a second time, REM-pictures were taken (Figs. 54 and 55)

mag	temp	pressure	det	HV	spot	WD	
300 x	---	3.27e-6 mbar	ETD	1.00 kV	4.0	5.9 mm	━━━ 100 μm ━━━ ID5418, Probe-7
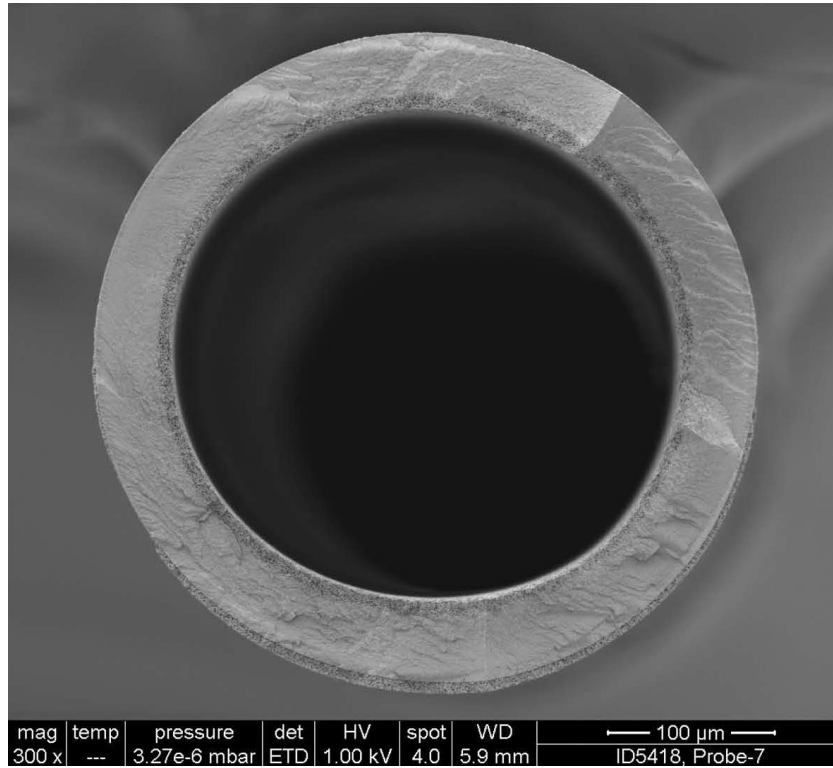
Figure 54. Perpendicular REM. PES-fiber, double polymerization (65 s). N2 atm., V12(5%)

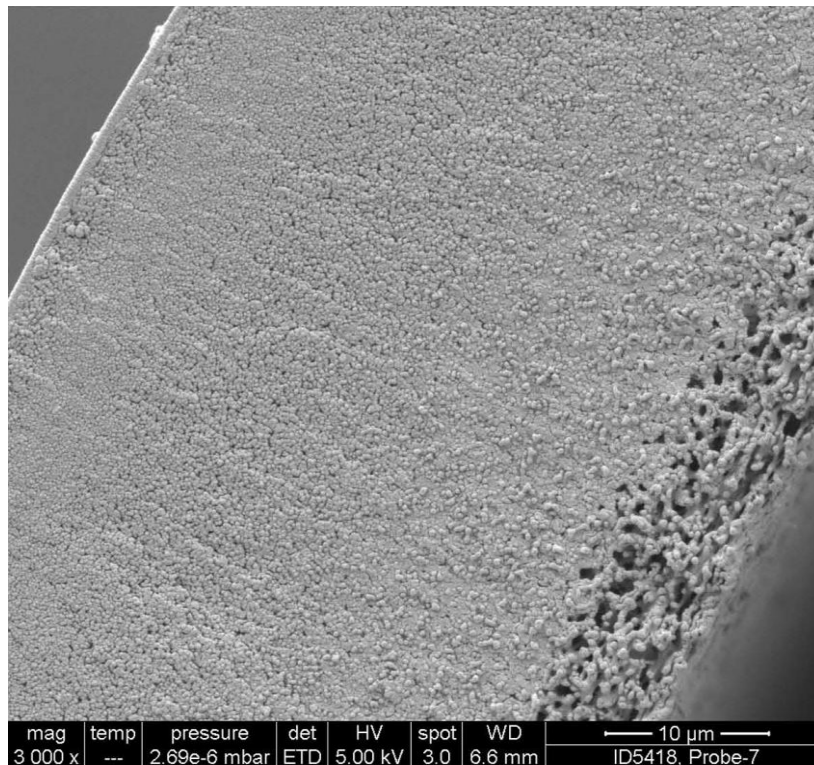| mag	temp	pressure	det	HV	spot	WD	
3 000 x	---	2.69e-6 mbar	ETD	5.00 kV	3.0	6.6 mm	━━━ 10 μm ━━━ ID5418, Probe-7

Figure 55. Wall REM. PES-fiber, double polymerization (65 s). N2 atm., V12(5%)

The monolith presence is better and more homogeneous than in the previous configurations. Two distinct identical batches (double polymerized) were prepared to test the reproducibility and the predicted improvement of the properties given the solution "re-filling" and re-polymerization in the regions where the monolith wasn't successfully polymerized during the first run.

Double polymerization batches had similar results between themselves in contrast to the high variance of the properties of fibers modified only once.

	(Double polymerization) PES-V12(5%).N_2.(65s reaction)	(Double polymerization) PES-V12(5%).N_2.(65s reaction)
Employed solution	V12 in both modifications	V12 in both modifications
LP-Value (10^{-4} cm.bar^{-1}.s^{-1})	13	11,05
Separation percentage	43,3%	26,1%
GPR of Fraction I	0,247	0,131
GPR of Fraction II	0,086	0,044

Table 7. GPRT values of PES-fibers polymerized twice.

After the second polymerization the fiber does not seem to be much more brittle than after the first one and no presence of outer monolith layer can be observed. The thin monolith layer observed in the bottom region of Fig. 54 can be attributed to the usual gravity and surface tension effects.

Another double polymerization experiment was carried out, it consisted in modifying PES-fibers through the third method as usual, cleaning them, and producing minimodules from them. The minimodules were then subjected to a process similar to the second method (hand-bundle modification) with the variation of connecting a nitrogen stream to the minimodule filtrate openings to form an inert reaction atmosphere. A syringe was used to inject the solution in the fibers and nitrogen bursts were used to clear the lumens. The minimodules exhibited LP's near 2 and gold retentions up to 94%. The reason behind such good results is that the regions where the fiber had a poor monolith presence as

result of macro effects during the continuous polymerization were polymerized during the minimodule modification.

An IgG-test was carried out after the gold retention test. The pressure rapidly increased over the 4 bar limit, after which the pump was stopped, the flow reduced to 0.15 ml/min, and then restarted. Even with such a low flow, the pressure increased after 25 minutes over the 4 bar limit and the procedure was stopped. This means that the V12 monolithic solution builds pores too small for the passage of the IgG and/or is not hydrophilic enough and its modification is necessary.

4.4.8. Reaction with the polyurethane filling

Once the fibers have been modified and gathered, the usual procedure is to directly make a hand-bundle or a minimodule by potting the fibers using polyurethane. An interesting interaction between the fibers and the PU filling was observed. The fibers seemed to slowly slide out from the ends of the minimodule, going even so far as to totally exit the PU-seal and leave a channel in the polyurethane where the fiber used to be. The phenomenon can be explained when the polyurethane reaction is regarded. Usually the urethane (also known as carbamate) bonds are created when an isocyanate reacts with a polyol (Scheme 13). The presence of two or more alcohol groups in the polyol molecule is crucial for the polymerization reaction. If the polyol has at least two -OH groups the polymerization can propagate, when molecules with a higher number of hydroxyl groups are used, the crosslinking reactions start taking place. The minimodule production process includes pouring such stoichiometric mix of isocyanates and polyols on the fibers.
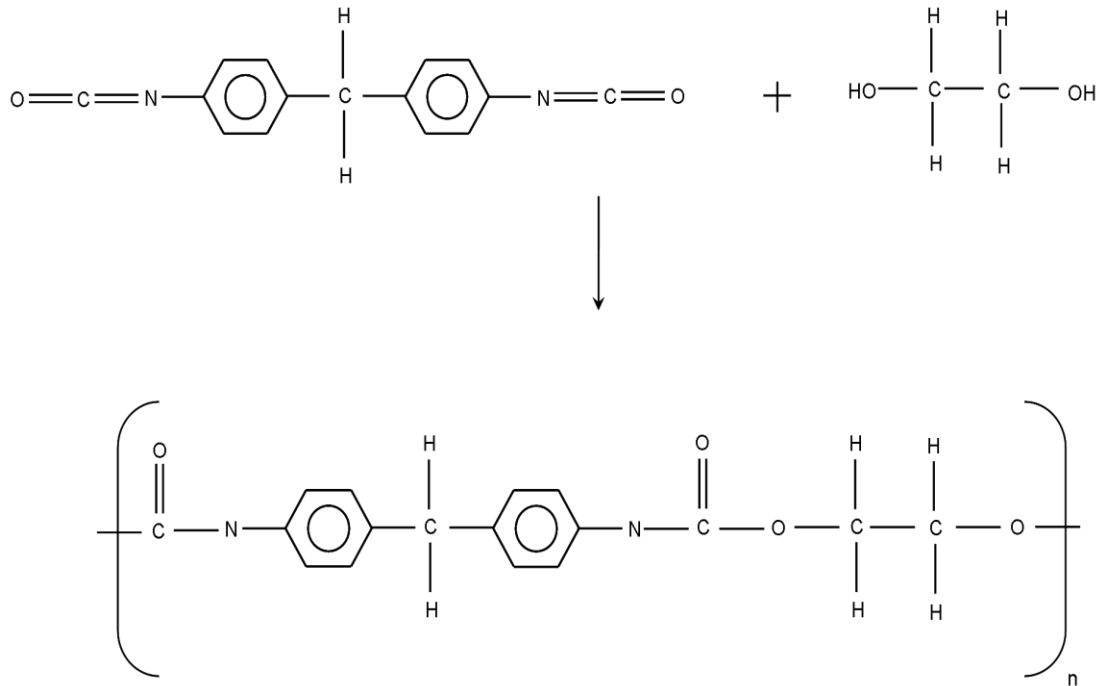
Scheme 13. Polyurethane reaction.

The polymerization of the polyurethane can not propagate if the isocyanates react with the dodecanol and the cyclohexanol present in the fibers instead of with the polyols. Since both porogens only have a single alcohol group, the reaction stops and can not propagate. A sticky liquid around the fibers is obtained. This substance does not harden and can not support the fibers. Such problem was not present in the previous method since the hand-bundle production was made with the unmodified fibers, and the porogens only came into contact with the polyurethane after it had completely harden, in other words, the isocyanate had already completely reacted.

This problem can be easily avoided with a prior washing of the modified fiber with ethanol or a mix of ethanol/water (50%), then removing the ethanol using a water bath, drying the fibers and then sending them to minimodule production.

4.5. Oxygen inhibition

It is clear that the presence of dissolved oxygen in the monolithic solution is an obstacle. The fact that the reaction atmosphere has a considerable influence on the monolith polymerization means that the diffusion of atmospheric oxygen into the reaction solution is not negligible and nitrogen purging of the solution alone is not enough to prevent oxygen inhibition

Thus, it is of utmost importance to not only de-oxygenate the monolithic solution while it is located in the reservoir, but also carry out the irradiation itself in an inert atmosphere (nitrogen or CO_2 atmosphere), and to shorten the exposed fiber length between both steps as much as possible. Leading the fiber through a tube containing an inert atmosphere can also be useful to prevent oxygen diffusion into the fiber between the impregnation and reaction steps. The relation between the oxygen diffusion and consumption determines the scale of polymerization inhibition.

The determinant phenomenon regarding oxygen inhibition is the continuous diffusion of atmospheric oxygen into the irradiated monolithic solution. This was both inferred from researching previous studies regarding UV-curing of resins [21] [22] [23] and from experimental results. In the literature, several studies regarding UV-polymerization of thin horizontal films in presence of air and CO_2 were reported.

The previously mentioned phenomenon of gas-liquid diffusion and the subsequent inhibition by the oxygen resulted in a top layer of unpolymerized and inhibited solution, on top of successfully polymerized resin. It can be inferred that this top inhibited layer was caused by the continuous consuming of free radicals by oxygen coming from the air. The oxygen diffusion and its consumption rate determine the thickness of the inhibited layer. For example, with all other variables remaining equal, if the diffusion rate of the oxygen dramatically increased, the inhibited layer thickness would increase as well, since any consumed oxygen (i.e. oxygen reacting with free radicals) is quickly replaced by newly diffused oxygen. On the other hand, if the oxygen consumption (i.e.

increased photoinitiator content or stronger UV-irradiation) dramatically increased, the oxygen would be immediately consumed at the gas-liquid boundary and the inhibition would dramatically decrease.

Applying this phenomenon to the hollow-fiber case, a similar layering effect can be expected (Fig. 56); an outer circular layer of inhibited polymerization, and an inner layer of polymerizing solution.
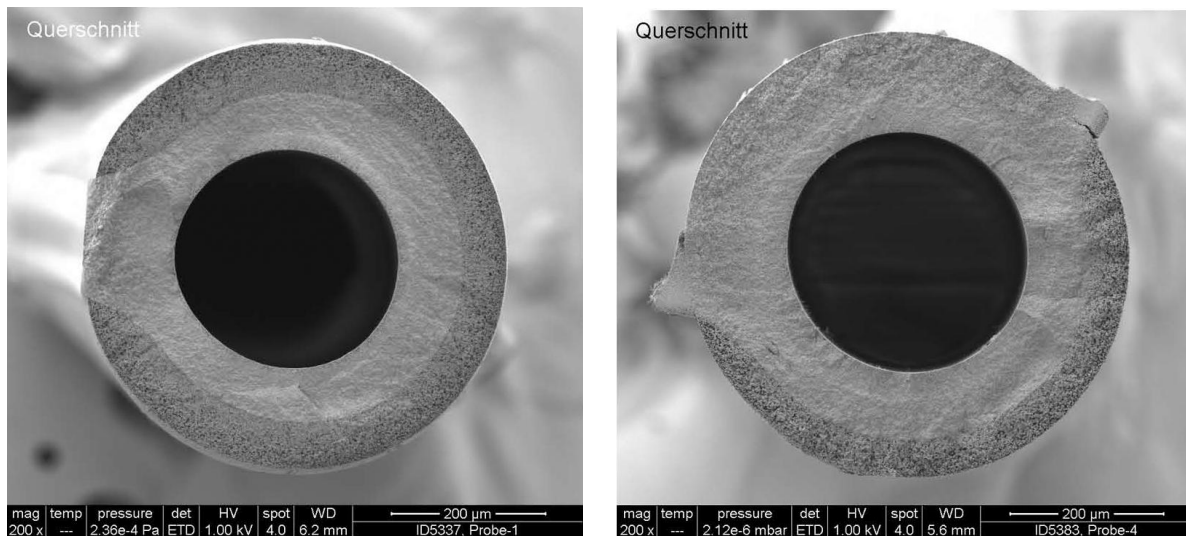

Figure 56. Layers in Polypropylene fibers after polymerization in air, 1% PI.

In Fig. 57, the vertical dotted line represents the gas-liquid boundary. The air can be assumed to have a constant oxygen concentration. The dark blue line represents the free radical formation rate (and consequently the oxygen reaction or consumption rate) across the monolithic solution contained in the fiber. Since the fiber is not thick enough to block UV-rays and its material does not significantly absorb UV-light at the photoinitiator's wavelength, the rate can be taken as a constant. The free radical formation rate is closely related to the oxygen consumption rate since the free radicals will preferentially react with O_2 than with acrylate bonds. The oxygen profile inside the fiber depends on the relation between the new molecules arriving from the boundary and the molecules that react with the free radicals

The oxygen concentration profile is indicated by the cyan line, the oxygen consumption is indicated by the deep blue line. The intersection between the oxygen profile and the horizontal dotted line indicates the point where the oxygen concentration is zero.
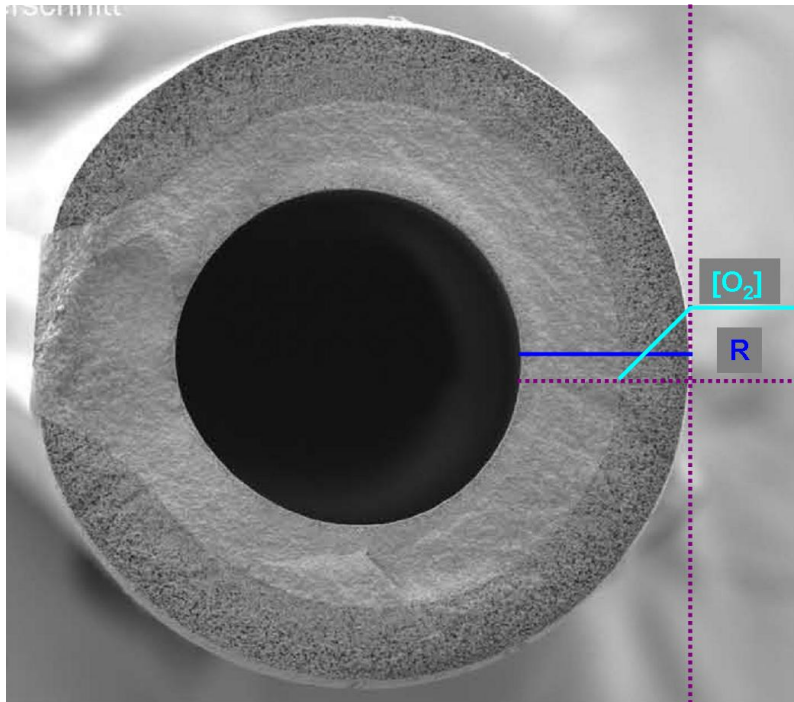

Figure 57. Graphical representation of the relation between oxygen diffusion and consumption.

At the point where the oxygen concentration profile reaches zero, the diffusion phenomenon can not sustain new reactions and all the molecules that have traveled up to this point have already been consumed.

Given the lack of oxygen molecules in the solution beyond this point, any free radicals formed in this region will react with the acrylate bonds and thus initiate the polymerization.

This layering appearance has been observed in all methods. The leftmost region in Fig. 57 does not present the usual monolith layering since at the time of the polymerization, this fiber region was resting on a surface, meaning it was not in contact with air, creating a boundary with no oxygen and allowing the formed free

radicals to only react with the acrylate bonds. This further proves the oxygen inhibition from air hypothesis.

A consequence of the previous hypothesis is the existence of an inhibited layer in every polymerization. Changing the process parameters change the thickness of such layer. A polymerization in air using a PI content of 1% is expected to have a relatively thick inhibited layer (Fig. 58).
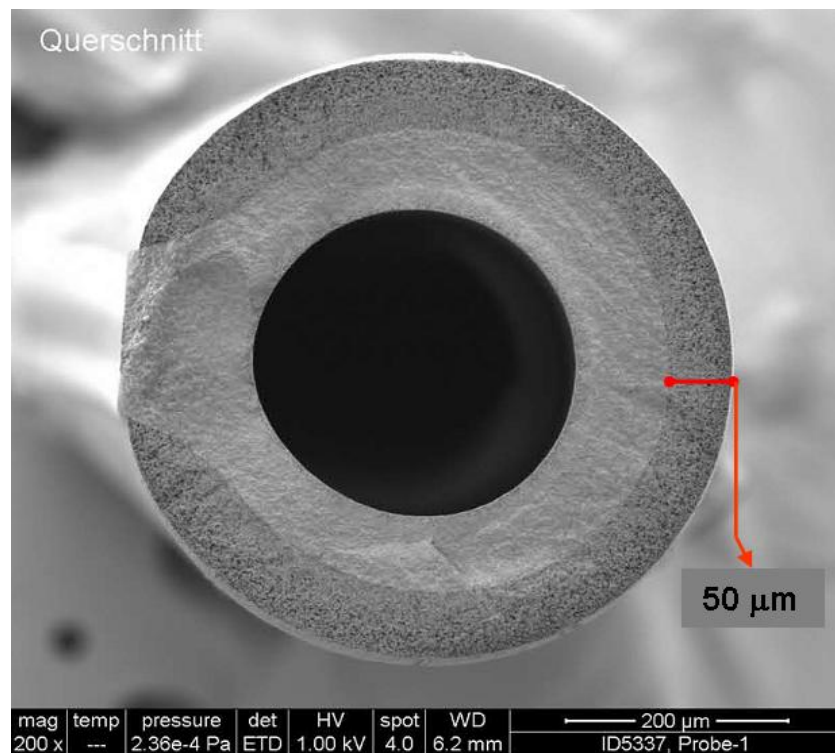

Figure 58. Inhibited layer thickness in a PP fiber modified in air, 1% PI.

This explains why no monolith was observed in the walls of PES-fibers polymerized in air during the second method; the oxygen inhibition created an inhibited layer thickness of 50 μm, the same thickness as the fiber wall (Fig. 59).
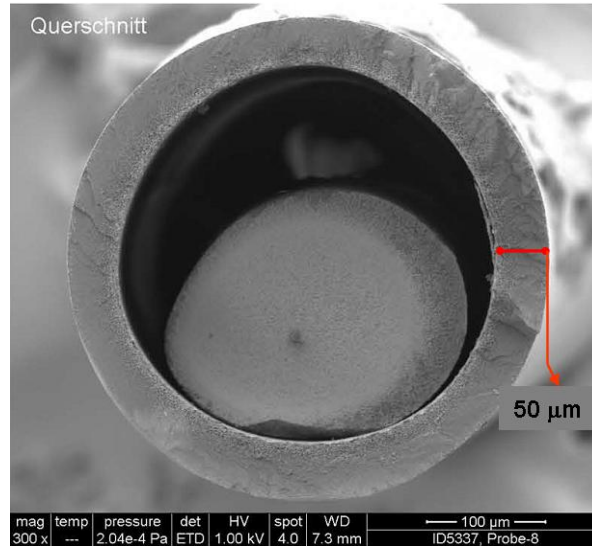
Figure 59. Inhibited layer thickness in PES-fiber modified in air, 1% PI

By changing the process parameters, the result of the oxygen diffusion-consumption balance also changes. A PES-fiber was modified using the third method with a 5% PI concentration and in a nitrogen atmosphere. The fiber itself was not flushed with nitrogen, which means the air contained inside the lumen never left the fiber and the inside the fiber contains oxygen; this explains why the inhibition propagates from the inside out. The change in the process parameters had as result a diminished inhibited layer thickness (Fig. 60).

Figure 60. Inhibited layer thickness in PES-fiber modified in nitrogen, 5% PI

5. Conclusions

- A HEMA-co-PETA monolith was successfully produced through UV-initiated free radical polymerization. The reaction took place in the walls of a supporting hollow fiber membrane without blocking its lumen.

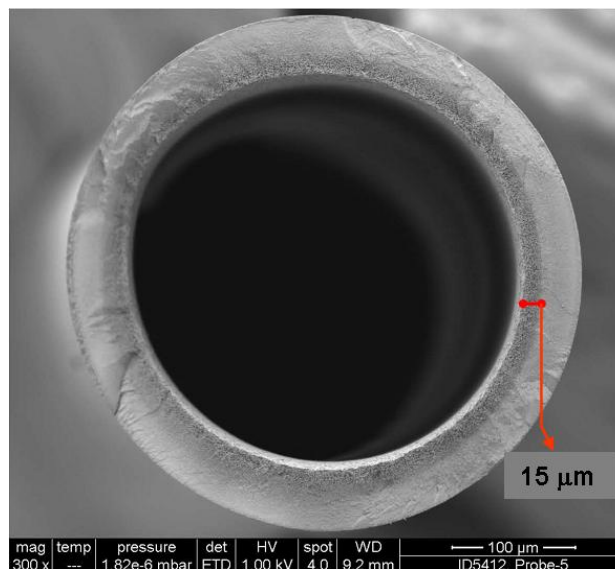- Oxygen competitive reactions were identified as the biggest obstacle for correct monolith polymerizations.

- Several measures for the prevention of oxygen inhibition were researched and subsequently some of them experimentally explored and implemented. Among such measures are: nitrogen purging of the monolithic solution, aminophenylketone photoinitiators, inert reaction atmosphere, higher photoinitiator content and tertiary amine additives.

- A monolithic solution (V12, HEMA: 33,63%, PETA: 16,81%, cyclohexanol: 16,52% and dodecanol: 33,04%, all %w/w) capable of forming a monolith with a gold-particle retention of over 90% was successfully determined and polymerized inside the 150 μm thick walls of a polypropylene hollow membrane without blocking its lumen.

- A monolithic solution (V12, HEMA: 33,63%, PETA: 16,81%, cyclohexanol: 16,52% and dodecanol: 33,04%, all %w/w) capable of forming a monolith with a gold-particle retention of over 93% was successfully polymerized inside the 50 μm thick walls of a polyethersulfone hollow membrane without blocking its lumen.

- The easy modification of the final monolith through simple changes of its forming monolithic solution was established and proved, paving the way for a technology with the potential of creating "personalized" membranes at a low cost and complexity.

- A continuous fiber modification process was developed. The process was built on the basis of the two basic steps of impregnation and reaction and continuously expanding and improving them.

- A continuous monolith modification process of polyethersulfone fibers with a wall thickness of 50μm was built and successfully operated.

- The oxygen inhibition influence was successfully prevented during the continuous process through the use of a reaction chamber with an inert atmosphere (N_2).

- The influence different process parameters such as: photoinitiator content, reaction atmosphere, reaction time and number of polymerizations had on the continuous modification method was investigated:

- Increasing the photoinitiator concentration from 1% to 5% favorably influenced the monolith polymerization.

- The use of an inert reaction atmosphere favorably influenced the monolith polymerization

- Employing longer reaction times in an inert atmosphere promoted a more complete polymerization and a smaller inhibited region. Longer reaction times in an oxygen-containing atmosphere would also promote a more complete polymerization, but would not affect the extent of the inhibited region.

- Micro factors such as oxygen inhibition and reaction kinetics and their influences over the process were identified and overcome. This shifted the control of the process over to macro factors, such as: the influence of the gravity and the surface tension on the homogeneous solution distribution along the fiber; the smoothness of the fiber-winding and the fiber deformation and subsequent solution drainage as result of abrupt path changes.

- A possibility to dramatically improve the final product through the use of multiple polymerizations on the same fiber was proposed and proved to be successful and relatively simple to implement. A Polyethersulfone fiber with actual virus removal capacities was obtained through this alternative double-polymerization process.

- The feasibility of a novel, cheap, simple and effective modifying process of pre-existing membranes through a monolith polymerization in the membrane walls was proved.

6.   Future steps and outlook

One of the most simple yet potentially significant changes in the process is the use of a polyethersulfone fiber with a thicker wall. Such thicker wall, along with all the previously explained and implemented conditions (nitrogen atmosphere, photoinitiator content, reaction time) would not only greatly improve the amount of monolith polymerized but would also diminish the susceptibility of the modification process to disruptive factors and would also increment the fiber resilience both during and after the modification process. A possible disadvantage of a thicker fiber can be a lower LP. If the fiber has a low LP, the impregnation step will be considerably harder to carry out.

The continuous process (Section 3.3.3.) explained in this project should and can be easily modified. Simple yet potentially important modifications include a change in the reaction chamber position. If the reaction is carried out in a vertical manner immediately after the impregnation step, the use of rollers or metal cylinders in order to change the path of the fiber can be avoided. This means getting rid of two possible process disruptions: solution drainage caused by the solution migrating to the surfaces on which it changes direction; and gravity, since its influence is transversally homogeneous on the fiber when it moves vertically. A second possible modification is to carry out two modification processes on the same fiber. The second polymerization can be done either on-line through an expansion of the set-up, or by simply gathering the modified fiber in a spool after its first polymerization, cleaning it and feeding it once again into the modification system.

An experimental procedure with great results was carried out. It consisted in modifying a batch of PES-fibers and potting them into a minimodule. The minimodule was cleaned with ethanol, water and left to dry. The minimodule was then subjected to a process similar to the second method; permeating the fibers with a syringe, flushing the lumens with nitrogen and then irradiating. However, the filtrate openings in the minimodule casing were used to continuously flush the inside of the minimodule with nitrogen and create an inert atmosphere. Gold-

retention results of the first minimodule obtained through this double-polymerization process are very promissory; retention of ca. 94% percent was obtained.

The difference in reactivity between the monolith contained inside the wall being directly irradiated and the wall facing away should be explored.

If such simple modifications do not yield satisfactory results, more elaborated changes can be performed, such as changing the impregnation system from a vertical one where the fiber is bent, to a long horizontal one, where the fiber continuously touches and absorbs the monolithic solution.

The use of new more reactive monomers, photoinitiators or additives should also be explored. However, it must be taken into account that introducing new components into the monolithic solution will change the micro effects (influence of oxygen inhibition and polymerization reaction kinetics) and modify the final monolith properties, causing a new series of monolith recipes trial to be necessary.

If on the other hand, the current monolith components are maintained, the monolithic solution V12, capable of more than 90% virus retention is an excellent starting point for the optimization and determination of the ideal monolithic solution for virus removal.

Once a reliable modification process able to yield a satisfactory amount of modified fiber is obtained, the monolith itself can be easily optimized through changes in its precursor solution and evaluated.

The scaling up of the pilot system should be straightforward since the major variables and their influence have been identified.

Bibliography

[a] http://www.acer-horb.de/show_img/gambro01.html (accessed on 24.10.12)

[b] www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Aldrich/General_Information/photoinitiators.pdf (accessed on 25.10.12)

[c] http://www.sigmaaldrich.com/etc/medialib/docs/Aldrich/General_Information/photoinitiators.Par.0001.File.tmp/photoinitiators.pdf (accessed on 10.01.13)

[d] http://www2.basf.us/rawmaterials/pdfs/ed1787e_TPOL.pdf (accessed on 18.01.13)

[1] Lämmerhofer, Michael. Macroporous monolithic chiral stationary phases for capillary electrochromatography: New chiral monomer derived from cinchona alkaloid with enhanced enantioselectivity. Electrophoresis 2003, 24, 2986–2999.

[2] Potter, Oscar G. Porous polymer monoliths for extraction: Diverse applications and platforms. Journal of Separation Science 2008, 31, 1881 – 1906

[3] Jian Lin. Preparation of polymethacrylate monolithic stationary phases having bonded octadecyl ligands and sulfonate groups: Electrochromatographic characterization and application to the separation of polar solutes for pressurized capillary electrochromatography. Journal of Chromatography A, 1169 (2007) 220–227

[4] Dilani N. Gunasena. Organic monoliths for hydrophilic interaction electrochromatography/chromatography and immunoaffinity chromatography. Electrophoresis 2012, 33, 251–261.

[5] Lukas Trojer. Monolithic poly(p-methylstyrene-co-1,2-bis(p-vinylphenyl)ethane) capillary columns as novel styrene stationary phases for biopolymer separation. Journal of Chromatography A, 1117 (2006) 56–66

[6] Xiaochun Wang. Preparation and evaluation of a neutral methacrylate-based monolithic column for hydrophilic interaction stationary phase by pressurized capillary electrochromatography. Journal of Chromatography A, 1216 (2009) 4611–4617

[7] Lokman Uzun. Cysteine functionalized poly(hydroxyethyl methacrylate) monolith for heavy metal removal. Colloids and Surfaces A: Physicochemical and Engineering Aspects 330 (2008) 161–167

[8] Zhendong Xu. Different alkyl dimethacrylate mediated stearyl methacrylate monoliths for improving separation efficiency of typical alkylbenzenes and proteins. Journal of Chromatography A, 1216 (2009) 3098–3106

[9] Abdus Salam, Mathias Ulbricht. Effect of Surface Modification on the synthesis of Pore-Filling Polymeric Monoliths in Microfiltration Membranes Made from Poly(propylene) and Poly(ethylene terephthalate). Macromolecular Materials and Engineer 2007, 292, 310–318

[10] Kun Liu. Highly crosslinked polymeric monoliths for reversed-phase capillary liquid chromatography of small molecules. Journal of Chromatography A, 1227 (2012) 96– 104

[11] E.S. Sinitsyna. Hydrophilic methacrylate monoliths as platforms for protein microarray. Polymer 52 (2011) 2132e2140.

[12] Alexander Korolev. Monolithic columns with optimized pore structure for molecular size-based separations of synthetic polymers. Journal of Separation Science 2012, 35, 957–963

[13] Karel Stulık. Monolithic organic polymeric columns for capillary liquid chromatography and electrochromatography. Journal of Chromatography B, 841 (2006) 79–87

[14] Arpita Bhattacharyya, Catherine M. Klapperich. Microfluidics-based extraction of viral RNA from infected mammalian cells for disposable molecular diagnostics. Sensors and Actuators B 129 (2008) 693–698

[15] Hiroshi Aoki. Polymer-based monolithic columns in capillary format tailored by using controlled in situ polymerization. Journal of Separation Science 2009, 32, 341 – 358.

[16] Michael R. Buchmeiser. Polymeric monolithic materials: Syntheses, properties, functionalization and applications. Polymer 48 (2007) 2187e2198.

[17] Yun Li. Preparation of polymeric monoliths by copolymerization of acrylate monomers with amine functionalities for anion-exchange capillary liquid chromatography of proteins. Journal of Chromatography A, 1216 (2009) 5525–5532.

[18] Alois Jungbauer, Rainer Hahn. Monoliths for fast bioseparation and bioconversion and their applications in biotechnology. Journal of Separation Science 2004, 27, 767–778.

[19] Tomoko Hongo-Hirasaki, Kazuhito Yamaguchi, Kouichirou Yanagida, Kazuo Okuyama. Removal of small viruses (parvovirus) from IgG solution by virus removal filter Planova®20N. Journal of Membrane Science 278 (2006) 3–9.

[20] Bernstein R, Antón E, Ulbricht M. UV-Photo Graft Functionalization of Polyethersulfone Membrane with Strong Polyelectrolyte Hydrogel and Its Application for Nanofiltration. Lehrstuhl für Technische Chemie II, Universität Duisburg-Essen , 45117 Essen, Germany.

[21] F. Masson, C. Decker, S. Andre, X. Andrieu. UV-curable formulations for UV-transparent optical fiber coatings I. Acrylic resins. Progress in Organic Coatings 49 (2004) 1–12.

[22] Katia Studer, Christian Decker, Erich Beck, Reinhold Schwalm. Overcoming oxygen inhibition in UV-curing of acrylate coatings by carbon dioxide inerting, Part I. Progress in Organic Coatings 48 (2003) 92–100.

[23] Katia Studer, Christian Decker, Erich Beck, Reinhold Schwalm. Overcoming oxygen inhibition in UV-curing of acrylate coatings by carbon dioxide inerting: Part II. Progress in Organic Coatings 48 (2003) 101–111.

[24] R. Bandari, B. Schlemmer, C. Elsner, W. Knolle, M.R. Buchmeiser. Monolithic supports prepared via electron beam curing. Leibniz-Institut für Oberflächenmodifizierung e.V. Biannual report 2006/2007..

[25] A. Yang, X.M. Tao, X.Y. Cheng. Prediction of fiber coating thickness via liquid-phase process. Journal of materials processing technology 202 (2008) 365–373.

[26] Frantisek Svec. There's more than one way to skin a cat: A wide selection of techniques used for the preparation of porous polymer monoliths. Journal of Chromatography A. 2010 February 5; 1217(6): 902–924.

[27] Sara Gillers, Christopher D. Atkinson, Aaron C. Bartoo, Madhumita Mahalanabis, Michael O. Boylan, John H. Schwartz, Catherine Klapperich, Satish K. Singh. Microscale sample preparation for PCR of C. difficile infected stool. Journal of Microbiological Methods 78 (2009) 203–207.

[28] Tero-Petril Ruoko. UV-light induced degradation of polypropylene and polystyrene – a spectroscopic and DSC study. Tampere University of Technology.

[29] Hedieh Pazokian ; Mahmoud Mollabashi ; Saeid Jelvani ; Jalal Barzin ; Shahryar Abolhoseini. Etching of a polyethersulfone film using a XeCl laser. Optical Engineering 50(8), 084301 (July 28, 2011)