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Influence of free-radical functionalization on non-woven filtering media for medical application

Tesi di laurea sperimentale

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Abstract

Nowadays the leukodepletion is one of the most important processes done on the blood in order to reduce the risk of transfusion diseases. It can be performed through different techniques but the most popular one is the filtration due to its simplicity and efficiency. This work aims at improving the current commercial GVS product, by developing a new filter based on Fenton-type reaction to cross-link a hydrogel on to the base material. The GVS Group is one of the world's leading manufacturers of filters and components for applications in the healthcare, life sciences, automotive, appliance, safety, and commercial and industrial filtration. Founded in 1979, GVS has been focusing on innovation in its products range and production processes and has been growing its research and development activities to satisfy the ever-growing need for the membrane filters by producing innovative products.

The filters for leukodepletion are preferably made through the melt flow technique resulting in a non-woven tissue; the functionalization should increase the stability of the filter restricting the extraction of substances to minimum amount when in contact with blood. Through the modification the filters can acquire new properties including wettability, surface charge and good resistance to the extractions. The most important for leukodepletion is the surface charge due to the nature of the filtration process. All the modified samples results have been compared to the commercial product.

Three different polymers (A, B and C) have been studied for the filter modifications. From the obtained results two modification conditions, obtained using Polymer B and Polymer C respectively, could be suitable for the substitution of the commercial product; further tests will be necessary in order to confirm the possibility of this substitution.

Table of contents

Chapter 1 Literature review

Chapter 2 Materials and methods

Chapter 3 Results and discussion

Chapter 1

Literature review

1.1 Blood and its components

The blood is normally formed by 55% of plasma and 45% of formed elements (44% of erythrocytes and 1% of buffy coat). The plasma is the liquid part that maintains the fluid balance of the blood and carries water and nutrients to the tissues of the body. It is composed by water (solvent, about 91%), proteins (about 7%) and electrolytes (about 2%). The plasma can be used as a source of the serum removing the fibrinogen (4% of total plasma and key substance for the coagulation process) and other proteins involved in blood clotting. Plasma is donated to people who suffer haemophilia, leukaemia and severe burns or with immunodeficiency. *Figure 1* shows the percentage of the three main blood components:

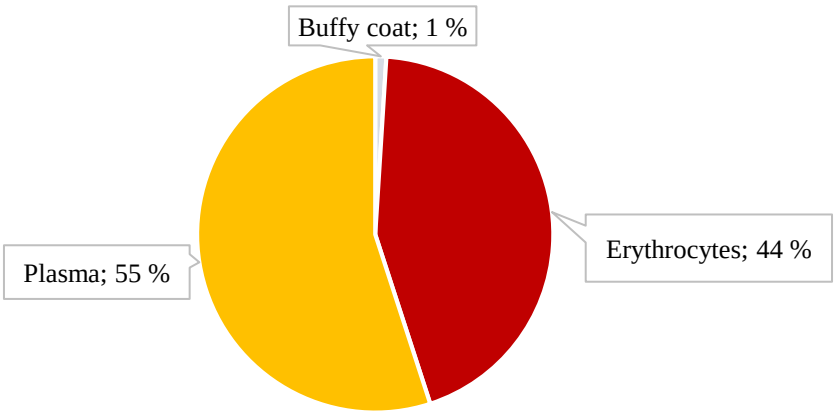


Figure 1 - Blood components percentage (v/v %)

Erythrocytes (red blood cells) are the second major part of the blood, are constituted by disks of 7 µm of diameter and 2 µm of thickness and are responsible for the oxygen transport in the body due to the presence of haemoglobin. They are produced in the red bone marrow and are eliminated at the end of their life in the milt. The haemolysis is the breaking process of erythrocytes and can cause very serious diseases. Haematocrit is the percentage of whole blood volume that is formed by erythrocytes and is a value that must be monitored.

The third part of blood is the buffy coat, formed by leukocytes (white blood cells) and thrombocytes (platelets) responsible respectively for immune response and blood clotting. Platelets are relatively small, 2-4 µm in diameter, and are numerous, more or less 150000 per µL of blood; they are released by megakaryocytes during their lifespan.

The leukocytes are of main interest for this work and will be studied in depth, they are between 4000-10000 per mm^3 and are the only formed elements that are complete cells, because they possess a nucleus and cytoplasmic organelles. They have a high mobility and can pass through capillaries and tissues through a process known as emigration [1,2].

1.1.1 Types of leukocytes:

There are many types of leukocytes that can be divided into two major categories: granulocytes (contains granules within the cytoplasm) and agranulocytes [1,2]:

- The granulocytes include
    - Neutrophils that are 50-70% of total leukocyte count, are 10-12 μm in diameter and are chemically neutral;
    - Eosinophils that are 2-4% of total leukocyte count and are 10-12 μm in diameter (they can be detected using eosin);
    - Basophils that are <1% of total leukocyte count and 8-10 μm of diameter.

- The agranulocytes are formed by
    - Lymphocytes, subdivided in natural killer cells, B cells and T cells, are the 20-30% of total leukocyte count and are from 6 to 17 μm in diameter;
    - Monocytes are the 2-8% of total leukocyte count and 12-20 μm in diameter (the largest leukocyte size).

The total charge of a leukocyte solution is slightly electrically negative, which, with the diameter distribution, are the most important characteristics for their separation from the blood. Some problems related to the leukocytes are the leukopenia and the leucocytosis that are respectively the small production of leukocytes and the excessive production of leukocytes. Leukocytes are also susceptible to cancer like leukaemia and lymphoma. Lifetime of this type of blood cells is short, typically hours or days [2].

To summarize the information about blood components and leukocytes the following *Figure 2* is shown:

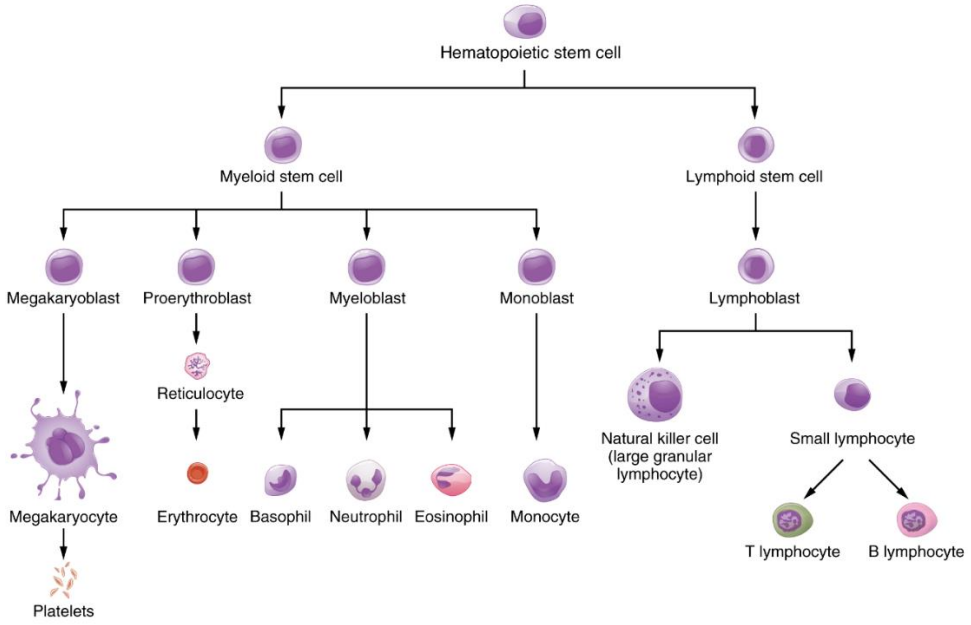


Figure 2 - Origin of the different blood components: platelets, erythrocytes and leukocytes [3]

1.2 Problems during blood transfer

Nowadays blood transfer is one of the most common process in hospital and due to its importance it has to be done using fixed steps carried out in some hours after the blood sampling. Every component (blood cells, plasma, leukocytes and platelets) obtained by the transfusion must be tracked so that, if there are problems with a product, other products from the same batch can be detected. Normally the whole blood is firstly centrifuged at high speed (about 3500 rpm): in this way, red blood cells and plasma are separated. If the centrifugation speed of this first step is higher, the platelets will be already associated to the leukocytes, otherwise the plasma has to be centrifuged twice in order to obtain the platelets, that can be used as clogging agents in patient who have coagulation problems. Subsequently the bags with the blood cells are submitted to a filtration to remove the white blood cells [4]. All these processes must be taken in the shortest possible time because of the low stability of the obtained components at room temperature. In addition to these steps, the blood must be separated in its four types: 0, A, B, AB and divided basing on Rh positive or negative. Depending on their main origin and on the way they are achieved, the blood components (hemoderivatives) can be classified as shown by *Table 1*:

Cellular components	Plasma component	Plasma derivatives
Red cell concentrate (RBC)	Fresh frozen plasma	Albumin 5% and 25%
Red cell concentrate Leukocytes-reduced	Single donor plasma	Plasma protein fractions
Platelet concentrate (PC)	Cryoprecipitate	Factor VIII concentrate
Platelet concentrate Leukocytes-reduced	Cryopoor plasma	Immunoglobulins
Platelet apheresis		Fibrinogen
Granulocytes apheresis		Other coagulation factors

Table 1 - Various blood components that can be separated and transfused [5]

Some very dangerous rare reactions can occur after blood transfusion if controls and procedures are not done in the correct way:

- *Acute immune hemolytic reaction*: the transfused red blood cells are attacked by the immune system of the patient due to the non-compatibility between the transfused blood and the patient.
- *Delayed hemolytic reaction*: similar to the precedent but it takes relatively long time to occur.
- *Graft-versus-host disease*: transfused white blood cells attack the bone marrow usually in a fatal way.

These cited effects and others can be evident in short time or in the days after the blood transfer.

1.3 Leukodepletion

The leukodepletion is the process of removal of the white blood cells and is done in order to eliminate, in post-operation time and during blood transfusion, unwanted effects like [6,8]:

- Viral re-activation in the receiving patient;
- Acute rejection in immunosuppressed patient;
- Non-hemolytic febrile transfusion reactions (NHFTR);

- Transmission of different microorganisms like viruses and bacteria;
- Formation of micro-aggregates (clumps of degenerating leukocytes, platelets and fibrin).

This process can be done using five techniques: differential centrifugation, sedimentation, cell washing, freezing and thawing or filtration [6,7].

- *Differential centrifugation*: method that makes a non-complete separation with some problems like loss of red blood cells, long process time, and high residue number of leukocytes. The blood is separated into the three fractions: red blood cell concentrate, cell-free plasma/leukocyte and platelet-rich layer.
- *Sedimentation*: is done through aggregation ("*rouleaux formation*") of the red blood cells performed by addition of agents that promote this process; after sedimentation the supernatant part and the buffy coat can be removed. This type of leukodepletion removes more than 80% of leukocytes with low loss of red blood cells, has a small cost compared to other techniques and is independent from specialized machines. However, this technique is no more used because it is labour-intensive, it needs long time and there is the possibility of bacterial contamination.
- *Cell-washing*: red blood cell washing combines differential centrifugation and continuous dilution of the cells using isotonic saline solution. The removal of the cells is based on their different density; the main problem of this method is the low difference between the cell density so that we have a consistent loss of red blood cells, in addition to long work time and high cost. On the other hand, the biggest advantage of this technique is the removal of more than 95% of the plasma with a reduction of virus transmission risk.
- *Freezing and thawing*: developed originally for the long-term preservation of red blood cells, protected by the addition of a cryo-protective agent. Ice crystals, that break the cell membranes during thawing, are formed in the leukocytes that are removed in a second stage with a washing step. More than 95% of leukocytes can be removed with a low red blood cells loss (less than 10%). However, this technique has many problems like the presence of residual leukocyte fragments in the red blood cell concentrates, the requirement for expensive facilities and the short expiration period due to open-system handling.

- *Filtration*: the function of the filters is based on the differences in deformability and adhesiveness between different cells. Nowadays this method is the most commonly used because it is fast, simple, effective (leukocytes removal can be more than 99.9%) and does not require expensive equipment. This technique can be improved by optimising the leukodepletion, the blood cell recovery, the process time and the cost. Since development of materials for leukodepletion is a topic of this thesis, filtration will be described more in detail in the next paragraphs.

1.3.1 Leukodepletion by filtration

There are three different approaches for the filtration: bedside, in laboratory, and pre-storage. In the bedside technique the filtration is done near the bed of the patient and a filter is inserted in the line of administration of the transfused blood, while in the pre-storage technique the blood is filtered directly during the withdrawal (so before the separation from the plasma components by centrifugation). On the other hand, the filtration in laboratory allows to use a better methodology and to have better quality products. Important factors that influence the performance of the filters are [8]:

- Temperature of filtration;
- Flow rate through the filter;
- Adhesion of the leukocytes to the filtering media;
- Amount of platelets and presence/absence of hemoglobine S.

From a research carried out on the internet and on literature reviews, the modern leukodepletion filters consist of a nonwoven tissue. This type of tissue, modified in the right way, allows to have a large adsorptive surface area with minimal channelling and bypass, low required dwell time, high interaction with leukocytes, low retention volume and hydrophilicity.

In *Figure 3* the nonwoven poly(butylene terephthalate) (PBT, chemical structure shown in *Figure 4)* is shown, which is one of the most common filtering media and is used as base material for the modifications done during this work. A complete leukocyte filter is formed by layers of modified nonwoven tissue and is packed into the filter housing as shown in

Figure 5: the filter is usually placed 50-80 cm below the blood bag, while the filtered blood is collected in another bag below the filtering media. It is important not to have a "*dead space*" inside the filter (space where the filter and the blood do not get in touch) in order to have the maximum contact between the filaments of the filter and the blood. Looking at the structure of the filtering system, there should be a tight seal between the media and the housing so that the upstream and downstream sides of the filter are not separated and the blood flows around the filtering media. It is also important to minimize the chance that the residual unfiltered blood enters the post-filtration component.

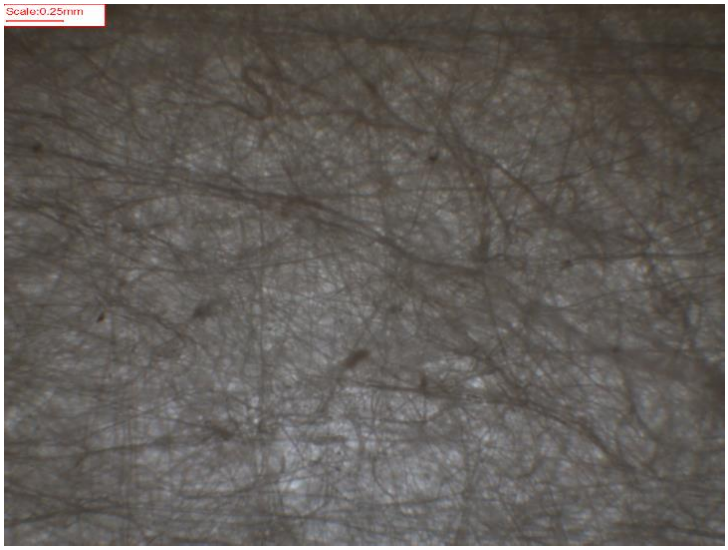


Figure 3 - Optical microscopy image of a typical PBT nonwoven tissue

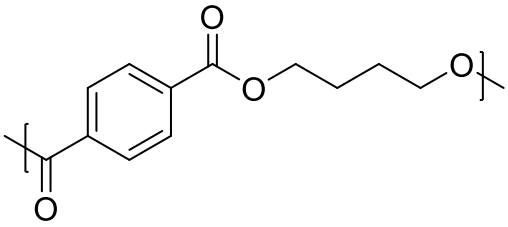


Figure 4 – Chemical structure of PBT

The nonwoven PBT, used in this work project, is formed through a technique called melt-flow where the thermoplastic polymer is melted and forced to pass across an extruder that consist of a wide number of little nozzles; the filaments are then blown to a collector with hot air when they are still melted to extend them and make them thinner. The obtained

tissue does not have a defined fabric mesh because of the randomness with which the filaments are blown to the surface.

Figure 5 - Typical system for leukocyte filtration

During the design of a new filter, a parameter that has to be taken into account is the "*critical shear force*" [6], defined as the shear force above which the cells do not remain attached to a biomaterial. This depends on the filtering rate and it is demonstrated, as shown in *Figure 6*, that a nonwoven tissue, like PBT, has a higher critical shear force (so that a higher flow rate can be used during the filtrations). Using the mass of fibres, leukodepletion must be accomplished at lower rate in order to have an acceptable efficiency.

Another important parameter is the hydrophilicity of the filter; this has to be sufficiently hydrophilic in order to permit a good contact between the blood and the media and to allow a flow through the small interstitial spaces present in the filter. A better hydrophilicity increases the leukodepletion efficiency.

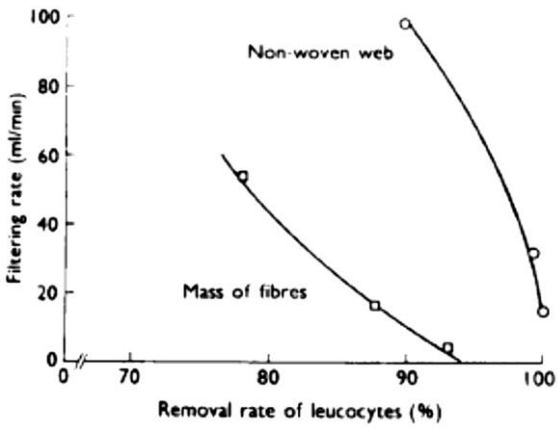


Figure 6 - Effect of filtration rate on the removal of leukocytes by filtration [7]

1.3.2 Mechanism of leukocyte retention

The filtration of leukocytes can be regarded as depth filtration process, where the retention of particles is not restricted to the filter surface; the typical filtration processes include blocking (A), bridging (B), interception (C) and adhesion (D) [6]. Simple examples of these four mechanisms are shown in *Figure 7*.

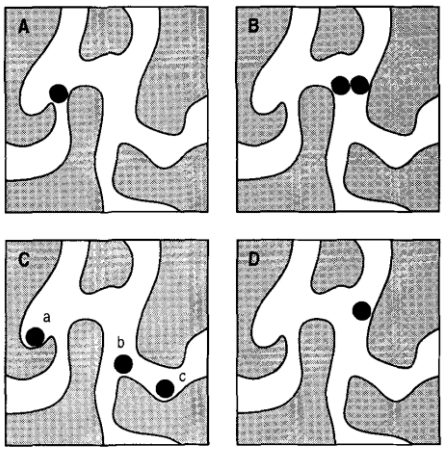


Figure 7 - Schematic diagrams of elementary mechanisms of particle retention in depth filters [6]

When adhesion is the predominant mechanism, as happens often in non-woven filters, blocking, bridging and interception are less important. In this way, we can distinguish two different filtrations: mechanical (sieving) and physiochemical (adhesion). For particles

between 1 and 30 µm both occur. This work focuses on the cell adhesion, trying to find out a way to increase this phenomenon.

Here there's a short description about the characteristics of the cell sieving and the cell adhesion [6,7]:

- *Cell sieving*: mechanical process in which pore size and pore branching have the most important effect. When filtered through a filter with 3-8 µm pores, the pore plugging (obstruction of the pores) can occur. It is important that hemolysis, due to shear stress, does not happen and this depends on the pore size distribution (parameter that has to be found out when a new material is studied) and the applied pressure (for leukodepletion this is connected to the height of the filtering system). *Figure 8* shows the dependence of the hemolysis on the filter pore diameter and the pressure differential.

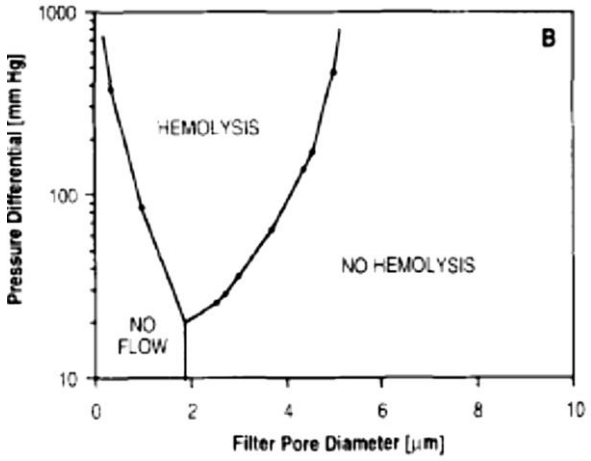


Figure 8 - Effect of filter pore size and pressure differential on the hemolysis thresholds [6]

- *Cell adhesion*: it is the most important factor in leukodepletion and depends principally on surface characteristics like composition, charge, wettability, microstructure and morphology. Other phenomena like protein adsorption and platelet adhesion can occur. Molecules that promote the contact and the adhesion are called adhesion molecules and belong to at least three different families of proteins: integrins, selectins and immunoglobulin gene superfamily. The introduction of specific chemical groups on the filter surface can change not only the surface chemistry of the filter but also the physical properties such as charge, wettability and microstructure. The most important property that will be

investigated in this thesis is the surface charge. Important for the cell adhesion will be the diameter distribution of the filtering media filaments. This is directly related to the available area for the interactions between the filter and the particles and, by consequence, to the available area for the modification of the filter. *Table 2* shows the classification of retention in the physical and biological categories.

Physical	**Biological**
Barrier retention	Cell adhesion
Surface phenomena	Cell-cell interactions
Charge density	

Table 2 - Physical and biological retention by filters [7]

1.3.3 Influence of surface charge on leukocytes filtration

As previously mentioned, leukocytes have a net negative surface charge caused by the presence of the anionic groups such as phosphate, sialate and carboxylate groups on the cell membranes. In order to obtain a good leukodepletion, the surface of the filter can be modified using different techniques so that it becomes positively or negatively charged; both charges can be used to separate the leukocytes from the blood. In fact, the positive charge promotes the cell adhesion, while the negative charge promotes the cell separation due to the repulsion between the filter and the leukocytes [6].

1.4 Modification techniques of the filtering media

In order to perform the leukodepletion, the filter has to be modified in the right way introducing new functional groups on the surface. Below the most important techniques used for surface modifications are described, with particular attention to the methodology used in this work [9]:

- *Ozone:* used to introduce peroxy groups onto the substrate surface; then monomers, like acrylamide or acrylic acid, are graft-polymerized by a near-UV induced technique. The ozone pre-treatment increases the yield of the modification although the UV irradiation is necessary for the grafting (addition of macromolecular segments on a polymeric surface).
- *γ-rays*: carried out by the following methods:
    - simultaneous irradiation and grafting through in situ formed free radicals;
    - grafting through peroxide groups introduced by pre-irradiation;
    - grafting initiated by trapped radicals formed by pre-irradiation.
- *Electron beams:* the filtering media, as done with the ozone, can be pre-treated with electron beam irradiation in order to facilitate the following graft polymerization.
- *Glow discharge*: low-temperature plasma can be applied to modify surface properties of the filtering media; it is a difficult process to control because of the relatively wide energy range (0.5-5 eV) of the glow discharge used. This generates a high number of charged and neutral molecular fragments. Also UV, vacuum UV, electron beams and ozone treatments can be used combined to glow discharge if there is oxygen or air. Plasma polymerization deposits a crosslinked thin polymeric layer on the substrate surface, while plasma treatment causes intensive oxidation on the surface region of the substrate.
- *Corona discharge*: corona treatment is by far simpler than glow discharge treatment because the former technique does not require evacuation of discharge system. However, it can result in more damage of the substrate polymers due to the high frequencies used and the discharge conditions that are more difficult to control than those of plasma treatment. After the corona treatment, the grafting is performed at proper conditions depending on the type of polymer used.
- *Fenton-type reaction*: exploits the formation of free radicals near the surface of the filtering media to obtain a cross-linked structure through the polymer chains. A hydrogel-coated material is obtained: the coating is due to free radical macromolecular grafting-crosslinking that causes the bond between the chemicals used and the surface.

Despite all these methods, the one applied to this work project is the Fenton-type reaction that it is being explained in depth in the following chapter.

1.5 Aim of the thesis

The aim of this thesis is to improve a current commercial GVS product, by using the Fenton-type reaction in order to create a cross-linked structure on the base material. This structure forms covalent bonds with the filter filaments, which should result in a more stable structure with better performance increasing the stability of the filter restricting the extraction of substances to minimum amount when in contact with blood. Whereas the current commercial product consists of functionalised layer obtained by simple dip-coating of PBT, and therefore is unstable and lacks any covalent bonding. By using the right reagent amounts, through the Fenton-type reaction some new properties can be given to the PBT filters including water wettability (hydrophilicity), high surface charge and good resistance to the extractions. Between these properties the surface charge is the most important due to its influence on the adhesion of molecules on the filter.

In this work, three different polymers have been used to modify the non-woven PBT; Polymer A and Polymer B, both characterized by amino groups, and Polymer C, characterized by acidic groups. These functional groups are responsible for the surface charge of the filter, after its modification through the formation of the cross-linked structure. The modified samples have been analysed using the techniques explained in Chapter 2 in order to check the influence of the modification on the filtering media. The characteristics of the new modified samples have been compared to the commercial product ones in order to check if its substitution is possible.

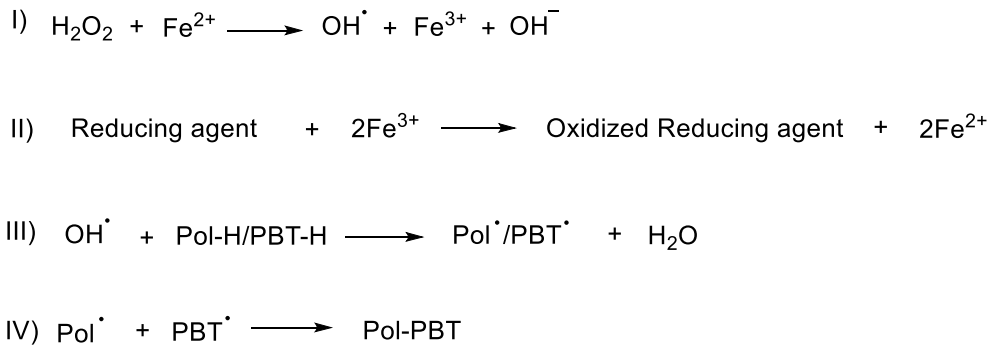
Chapter 2

Materials and methods

2.1 Fenton-type reaction

The filtering media can be modified through a free-radical polymerization exploiting the Fenton reaction that carries out the formation of free-radicals in solution: these are used for a macromolecular grafting-crosslinking where bonds like bridge are made among the molecules of the filter material. The chemicals that take part in this reaction are the following [10,11]:

- Cross-linker: is the branching and anchoring agent.
- Polymer/monomer: different polymers/monomers capable of forming a hydrogel structure can be used depending on the desired properties. They have to dissolve in the solvent used, be able to form radicals and react with the cross-linker bringing to the formation of the bridges between the filter filaments.
- Hydrogen peroxide (H_2O_2): initial source of radicals produced in the redox reaction with Fe^{2+} ions (see point I of *Scheme 1*). The OH• radicals formed in this way will react as shown in point III of *Scheme 1* to form the other radicals.
- Ferrous chloride ($FeCl_2$): it is the activator of the reaction, due to its capability to react with the H_2O_2 generating free radicals (see point I of *Scheme 1*). Also other salts can be used, but $FeCl_2$ is preferred because it is already used in industrial scale.
- Reducing agent: antioxidant and a good electron donor, have to preserve the Fe^{2+} from the oxidation to Fe^{3+} before the beginning of the reaction and reacting itself with the Fe^{3+} reducing the iron to Fe^{2+} (see point II of *Scheme 1*).
- Solvent 1 and solvent 2: have to dissolve well the reagents. Solvent 1 has to wet well the filtering media too.
- Filtering media: PBT is used in this thesis for the reaction, but also other materials can be used. It has to react forming radicals as shown in point III of *Scheme 1* and making the cross-linked system with the polymer/monomer radicals (see point IV of *Scheme 1*).



Scheme 1 Reactions that take place in the Fenton-type reaction

Two different solutions are prepared: the first one in solvent 1 contains the cross-linker and H_2O_2, while the second one in solvent 2 contains the $FeCl_2$, the reducing agent and the polymer. Using two different solutions the reagents are separated, limiting, as far as possible, the reaction space to the interfacial surface and increasing the process effectiveness. The filter is firstly placed inside the first solution: in this way, the solvent 1 permits the entrance of the cross-linker and H_2O_2 inside the pores of the filtering media (in our case PBT, which is hydrophobic). After a certain period, chosen according to literature studies and experience, the filter is placed inside the second solution as fast as possible in order to prevent the diffusion of the cross-linker and H_2O_2 outside the pores of the filter and the evaporation of the solvent 1.

In the second solution the reaction takes place and, after this step, the filtering media is dried and washed with a solution of sodium dodecyl sulphate (SDS solution) and lastly with water in order to eliminate residual chemicals.

Referring on precedent studies and on the research work done in this project the effects of the reagent concentration can be listed [10,11]:

- Concentration of Fe^{2+}: the ferrous chloride amount influences the number of free radicals at the interface; more Fe^{2+} corresponds to a higher cross-linking degree and to thinner layers. Normally the amount of hydrogel formed increases with the amount of $FeCl_2$ used but, after a certain value and depending on the other reagents, it reaches a plateau.
- Concentration of H_2O_2: with the addition of more hydrogen peroxide a thinner and stronger coating with a higher equilibrium-swelling ratio (ESR) is obtained. The ESR is also connected to the amount of water that the sample can absorb. It

influences the amount of radicals in the solution: in the presence of more radicals the modified layer is thinner.

- Polymer/monomer: the polymer amount influences the cross-linking degree; more polymer corresponds to more cross-link but, at the same time, due to the presence of the polymer in the solution and due to its high steric hindrance depending on its molecular weight, less Fe^{2+} can diffuse at the interface and by consequence less radicals are near that.
- Cross-linker: if not present, the reaction forms an instable structure; increasing the cross-linker amount, the coating becomes stronger and smooth influencing the hydrogel cross-linking density. However, if it is too high the coating becomes weak and can be fragile. As seen for Fe^{2+}, after a certain amount of cross-linker, the hydrogel properties are not influenced anymore.

In this work we tried to reach a hydrogel amount sufficient for having enough surface charge in order to obtain filters with good cell adhesion properties. Furthermore, the polymer used to form the hydrogel system has to ensure wettability of the filtering media without changing its colour, that has to stay white to guarantee a good market appeal.

Different polymers have been studied: Polymer A, Polymer B and Polymer C. These are compared to the unmodified PBT and the commercial product.

2.1.1 Unmodified PBT

The unmodified PBT is a non-woven tissue prepared by the melt flow technique. It guarantees [12]:

- Resistance at high temperature;
- Yield resistance, also at high temperature, with small coefficient of thermal expansion;
- Better rigidity and hardness, compared to other types of thermoplastic polymers;
- Resistance to abrasion;
- Dimensional stability;
- Resistance to atmospheric agents.

The unmodified PBT is used for the modification with hydrophilic polymers due to its capability to take part in the Fenton-type reaction and it will be analysed to have a comparison before and after modification.

2.1.2 Commercial product

It is a commercial product used for leukodepletion and is made through the coating of non-woven PBT with a polymeric solution characterized by amino groups. This yellowish solution guarantees a treatment that improves the moisture management, handle and comfort characteristics of all type of fibres. It does not colour the surface of the filtering media that, after the treatment, demonstrates a silky soft feel touch. However, it has to be substituted by new type of products due to the embrittlement of the coating that it forms on the filter surface.

2.1.3 Polymer A

Polymer A contains primary amino groups attached to the end of a polyether backbone. It consists in a polyetheramine bearing two primary amino groups and is water soluble. The two amino groups should guarantee a surface charge to the filtering media and a good reactivity in the Fenton-type reaction. At the same time the amino groups are responsible for the surface charge, so it will be important to control the final hydrogel degree in order to prevent a loss of the charge. Polymer A is a water solution and, before using it, must be heated to make it more homogeneous. Its relatively low stability at room temperature can represent a problem but this product is however studied to have a good comparison with the other polymers used.

2.1.4 Polymer B

Polymer B is an hyperbranched poly(ethyleneimine). In contrast with Polymer A, Polymer B contains also secondary and tertiary amines that will influence the final charge of the filter due to their ability to bind H^+ ions, thus releasing free OH^- ions in the solution. It is

a cationic polymeric water solution with high charge density, high molecular weight and a pH that is significantly basic. It is stable at environmental conditions and remains transparent also at lower temperatures, without aggregating. Due to its stability and its higher charge, Polymer B could be considered as a substitute to the current treatment.

2.1.5 Polymer C

The polymer C is sold as a powder and is characterized by free -OH groups. It can be cross-linked with an appropriate cross-linker by the Fenton-type reaction. The charge of the modified filter is negative and depends on the free -OH groups that have not reacted with the cross-linker.

2.2 Analysis of membrane surface before and after modification

In order to define the properties of the filtering media used in this work project, different analysis are done before and after the modifications: measurements of contact angle, porometry, water flow rate and air flow rate, scanning electron microscopy (SEM) and optical microscopy imagining, UV-vis spectrometry, Fourier Transform Infrared Spectroscopy - Attenuated Total Reflection (FTIR-ATR), thermogravimetric analysis (TGA) and extractable tests with Amicon cell (according to ISO 1135-4 [17]).

2.2.1 Contact angle

This analysis is performed using the Dataphysics OCA 15EC contact angle. Dynamic sessile drop technique is used, where the droplet is expelled by a syringe and dropped on the filtering media. Then the contact angle variation is registered by the camera installed on the instrument and is read by the software. The wetting property plays an important role in many industrial processes, such as oil recovery, lubrication, liquid coating, printing and spray quenching. The wettability studies usually involve the measurement of contact angles, which is related to the wetting degree when a solid and a liquid interact. The contact angle can be measured using different liquids with different surface tensions; in this work

project the water is used and the measurement gives the variation of water contact angle as a function of time. Contact angles above 90° correspond to bad wettability, if water is used the material is defined hydrophobic. Contact angles below 90° correspond to good wettability, if water is used the material is defined hydrophilic (see *Figure 10*). A small contact angle is observed when the liquid spreads to the surface, while a large contact angle is observed when the liquid beads on the surface. Apart from the chemical properties, the contact angle measurement is influenced by the morphology of the surface: a smooth surface increases the wettability of the material because the drop can expand more easily, while a rough surface, due to its characteristic irregularity, has the opposite effect [13].

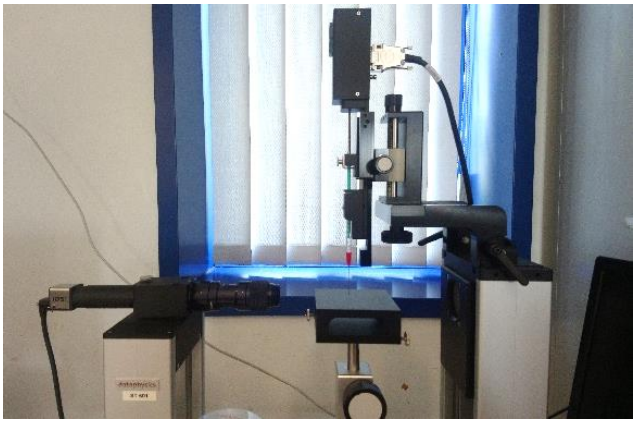


Figure 9 - Contact angle instrument

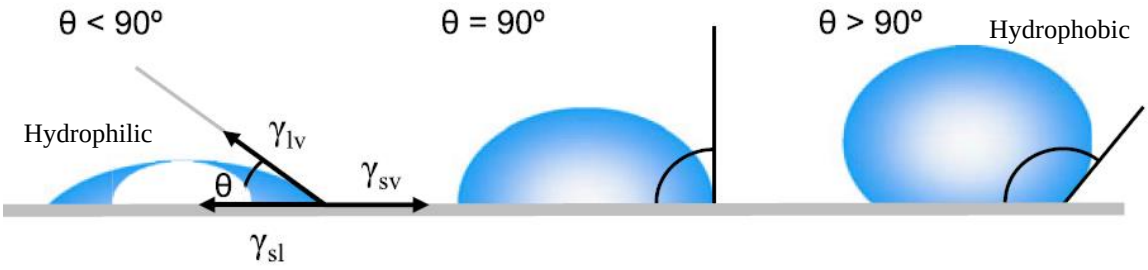


Figure 10 - Examples of contact angles formed sessile liquid drops on a smooth homogenous solid surface (γ are the interfacial tensions) [13]

2.2.2 Water flow rate (W.F.R.) and air flow (A.F.)

Air flow and water flow rate are evaluated in order to understand how much the filtering media is open and how simply the air or water pass through it at a certain pressure. A sample of a standard area of 3.7 cm^2 has to be cut and placed inside the appropriate holder for the analysis. Then for the air flow measurement the pressure is increased until the desired value and the air flow is measured on the gauge. On the other side the water flow rate measurement is carried out in a gravimetric way collecting the water for a certain time and then weighting it. The values obtained for air flow rate will be expressed in L/min while the ones obtained for water flow rate will be expressed in mL/(min cm^2 bar). These values influence the productivity of the filtration process because, normally, the higher are the values, the shorter is the time requested for the process. Every sample should be analysed with both water flow and air flow systems: the flow for modified samples should not significantly differ from the values obtained for PBT. If there are differences, a more complex porometry analysis should be performed. Furthermore, a constancy of the obtained values is index of a good homogeneity of the filtering media.

2.2.3 Capillary flow porometry

For porometry measurements, Quantachrome porometer 3G zh is used. These measurements are important to evaluate the rate of the filtration processes governed by the permeability, which is connected to the pore diameter distribution and to the number of pores. The sample is firstly wetted up using the Porofil wetting fluid, a liquid with a low surface tension that can wet all the sample; then the filtering media is placed into the holder and is tested in the following way: an inert gas is made to flow in the thickness direction of the sample while the pressure is slowly increased. Increasing the air flow pressure, the little pores are freed until the sample is completely dried and the pressure returns to zero. Then a second measurement starts to test the dried sample [14]. At the end, the software of the instrument provides the pore size distribution of the filtering media and a graph similar to the one shown in *Figure 11*. From the graph, the following information can be obtained:

- Bubble point: pressure at which the biggest pore is freed from the wetting liquid;
- Wet curve: obtained after wetting the filtering media. A part of the wet curve matches with the dry curve when all the water is expelled from the filter;
- Dry curve: obtained after the wet curve when all the liquid is expelled from the filtering media. It gives information about the dependence of flow rate on the pressure applied;
- Half-dry curve: extrapolated from the dry and wet curves, the half-dry curve is calculated by dividing the flow values with respect to the applied pressure by 2;
- Mean flow pressure: pressure at which the flow of the half-dry curve and the one of wet curve are the same. It corresponds to the pore size at which the 50% of the total gas flow can be accounted.

The porometry test is executed on unmodified PBT to characterize the raw material, while it is not performed on modified samples because there are not differences for air flow and water flow rates between the modified and unmodified PBT. In fact, the flow rate is directly connected to the results obtained with the capillary flow porometry (as can be seen in *Figure 11*) and if there are not differences between the air flow and water flow rate measurements, then there's no need of porometry analysis.

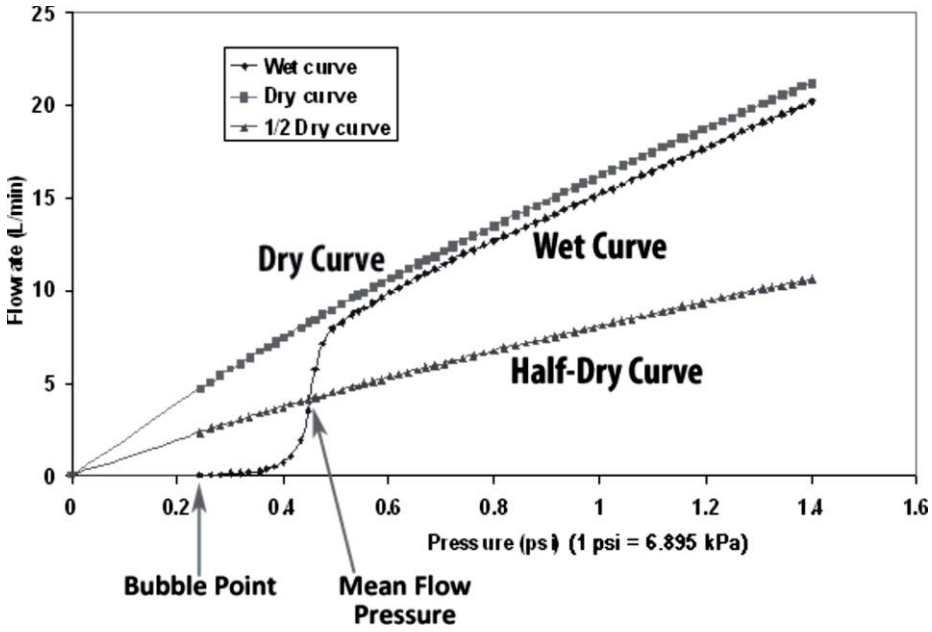


Figure 11 - Typical trend for porometry analysis chart [14]

2.2.4 Optical microscopy

For taking the images with optical microscopy an Exacta Optech instrument is used, fitted with a digital camera from Optika Microscopes Italy. The images are used to evaluate the efficiency of the modification as well as the influence of the process parameters on morphology of the cross-linked structure. Both unmodified and modified PBT are analysed at different magnification checking both top and bottom surfaces of the samples to see if there are differences between them.

2.2.5 SEM (Scanning electron microscopy) analysis

SEM analysis is used for unmodified PBT and some modified PBT, after optical microscopy images are taken and is performed using a COXEM EM-30 instrument. Samples are analysed from both the sides and in different areas after coating with a layer of gold; that process, that is called metallization, is carried out in order to increase the surface electro-conductivity and therefore the resolution of the images. The samples should not be coated with a too big layer due to the risk of covering some little details. SEM images are also used to evaluate the distribution of filament diameters of the unmodified PBT, factor that influences the area of the modification and by consequence the adhesion area: different images from the PBT samples are captured and the fibres diameters are measured. Finally mean values and distribution are calculated.

2.2.6 UV-Vis spectroscopy

The UV-Vis instrument is used to indirectly evaluate the surface charge of the modified filtering media, due to the importance of the charge on the filtration of blood components. The methyl orange binding test (MO test) and toluidine blue binding test (TB test) have been adapted basing on the literature [15] and [16] (the complete procedures for MO and TB test are in Appendix 1 and Appendix 2 respectively).

MO test is applied when amino groups are introduced on the filtering media and it evaluates the positive charge of the modified filter; on the other hand, TB test is applied

when Polymer C is used to modify the filtering media and it evaluates the negative charge of the modified filter. The absorbance peak of the methyl orange is observed at 466 nm while the one of toluidine blue is observed at 630 nm. The calibration curves are obtained by measurements at different concentrations where the absorbance values are connected to the concentration values. Then the feed solution needed for the test is filtered using a syringe filter system and the residual concentration of methyl orange/toluidine blue in the filter is calculated by difference between the initial solution concentration and the solutions collected removing the dyes reversibly stacked in the filter. MO and TB in excess on the filter are removed doing some washing steps with water and a final washing step with respectively a K_2CO_3 solution and an acetic acid solution. The UV-Vis used in this work is a SHIMADZU UV-1800.

2.2.7 Fourier Transform Infrared Spectroscopy - Attenuated Total Reflection (FTIR-ATR) analysis

FTIR-ATR analysis is performed in the wavenumber between 400 and 4000 cm^{-1} using a Bruker ALPHA instrument. The analysis enables the identification of characteristic bonds for functional groups located on the filter surface: in this way, the characterization of the unmodified PBT and the modified PBT filters is done checking if the instrument detects new bonds that are connected to the peak wavenumber (cm^{-1}) in the spectra. The instrument lends itself well to the analysis of filters due to the possibility to directly analyse the filter sample without specific preparation.

2.2.8 Thermogravimetric analysis (TGA)

In order to evaluate the thermal stability of the filtering media, thermogravimetric analysis (TGA) is done using the TA Instruments SDT Q600. In this way, the weight loss percentage can be followed increasing the temperature and important parameters like the maximum weight loss speed and the steps of weight loss can be evaluated. We decided to analyse the samples using the nitrogen atmosphere with the temperature increasing rate of 10°C/min and from room temperature to 600°C with a final isothermal in air for 20 min.

2.2.9 Extractable test (according to UNI EN ISO1135-4 [17])

This test consists of different analysis and is done in order to evaluate the presence of extracted substances in a liquid phase. The filtering media is placed inside the Amicon cell (see *Figure 12*) with the top side facing upwards; then the cell is filled with 200 mL of double filtered water (DFW) and, after setting the stirring speed at the desired value, the filter coating is challenged for extraction for 2 hours.

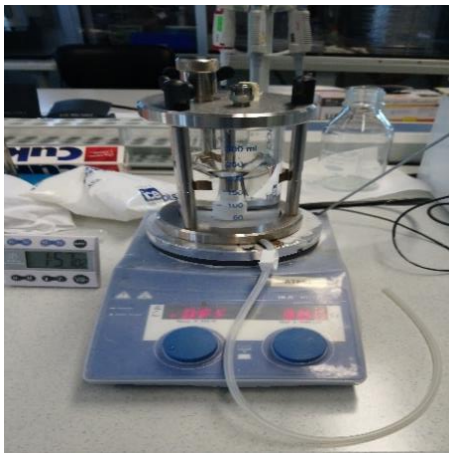


Figure 12 - Amicon cell for the test of extractable

After obtaining the extracted solutions, basing on the UNI EN ISO 1135-4 [17], tests are performed on the solutions. If the obtained values are below the limit suggested for the medical applications, the filtering media can be considered suitable for this use. The performed tests are:

- Test for reducing substances, to check if the filtering media leaves in the water some reducing agents;
- Test for non-volatile residue, to check how much material pass from the filtering media to the water;
- Test for titration acidity-alkalinity, to check how the pH varies after the extraction;
- pH measurements, to confirm the precedent test;
- Test for absorbance UV-Vis at 250-320 nm to detect absorbing products.

The unmodified PBT must be prewetted in order to ensure that all the sample is effectively submitted to the analysis. Six different extracts are obtained at different conditions:

- S1: with the filtering media placed in Amicon cell at room temperature, at 300 rpm and for 2 hours without a preheating.
- S2: with the filtering media placed in Amicon cell at 37°C, at 300 rpm and for 2 hours without a preheating.
- S3: with the filtering media placed in Amicon cell at room temperature, at 300 rpm and for 2 hours with 10 minutes of preheating at 60°C.
- S4: with the filtering media placed in Amicon cell at 37°C, at 300 rpm and for 2 hours with 10 minutes of preheating at 60°C.
- S5: with the filtering media placed in Amicon cell at room temperature, at 300 rpm and for 2 hours with 10 minutes of preheating at 80°C.
- S6: with the filtering media placed in Amicon cell at 37°C, at 300 rpm and for 2 hours with 10 minutes of preheating at 80°C.

Test at 37°C are done to simulate physiological body temperature while preheating at different temperatures simulates the filter modification conditions.

To evaluate the behaviour of the modified PBT samples, they are tested with the extractable test following the conditions applied for S1. In case of good results, the extractable test should be extended also to the 37°C as per S2 in order to understand the behaviour at physiological temperature. It is obvious that this results of conformability with medical applications should be confirmed by filtration test performed on blood samples. For the complete procedure of the extractable test check Appendix 3.

Chapter 3

Results and discussion

3.1 Characterization of unmodified PBT

The unmodified PBT filter is analysed in order to understand the main characteristics of this material and to compare them to the ones obtained for the modified samples. The filter is characterized using optical microscopy, scanning electron microscopy (SEM), capillary flow porometry followed by water flow rate (W.F.R.) and air flow (A.F.) measurements, contact angle (C.A.) measurements and the extractable test.

3.1.1 Optical microscopy and SEM analysis

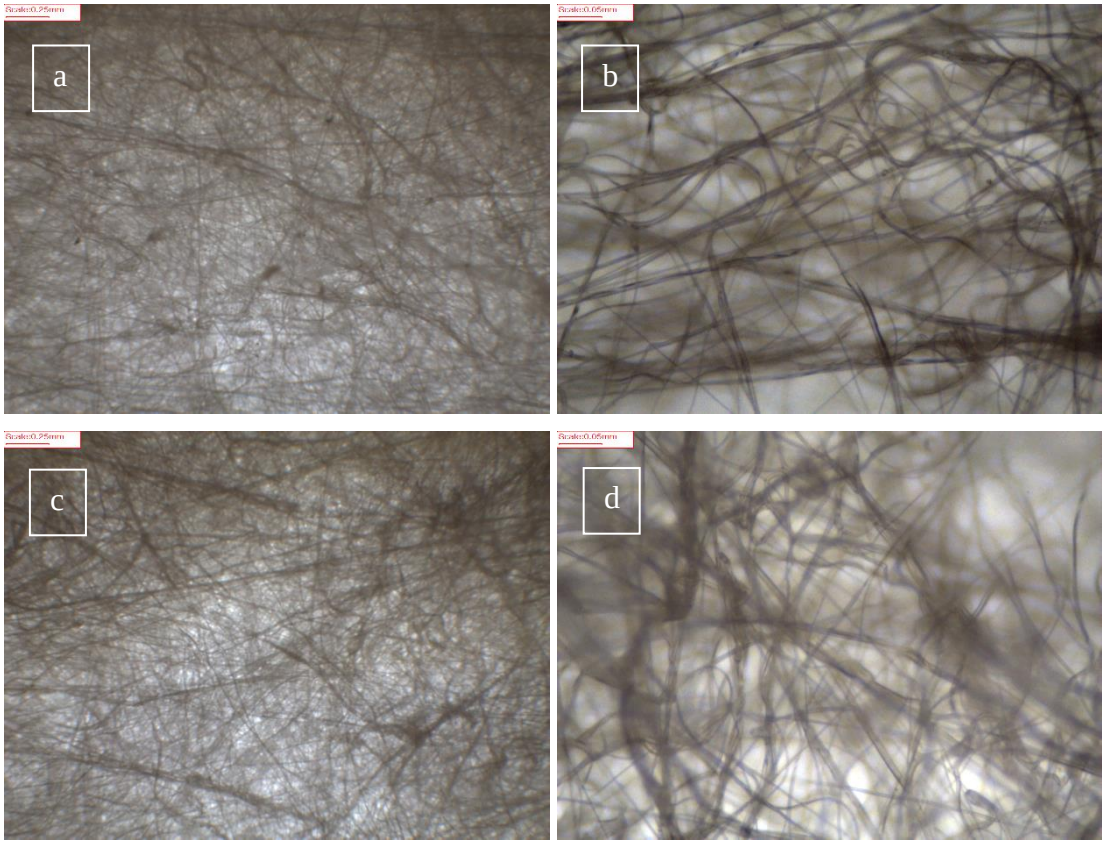


Figure 13 - Optical microscopy images for the unmodified PBT: top (a-b) and bottom (c-d)

The optical microscopy images show the morphology of the filters; this instrument gives a better overview than the SEM because wide images of the sample can be taken. As expected, the filaments of the sample are variable and inhomogeneous, with big spaces between them, and there are not differences from top to bottom of the same filtering media.

The SEM is used to measure the filament diameter distribution: ten images are taken from a sample and, using the software provided with the SEM instrument, more than 230 diameter values are obtained. It is important to underline that filament diameter and not pore diameter is analysed considering that the property that we want to study is the adhesion, which does not depend on the pore diameter, and the type of filtration mechanism. A typical SEM image for unmodified PBT is shown in *Figure 14*:

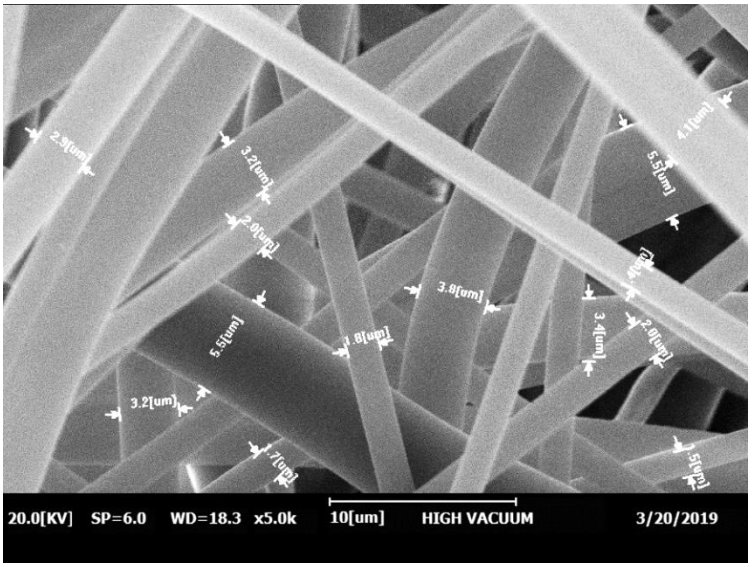


Figure 14 - Example of SEM image for unmodified PBT (with diameter measurements)

The obtained result is shown in the following graph:

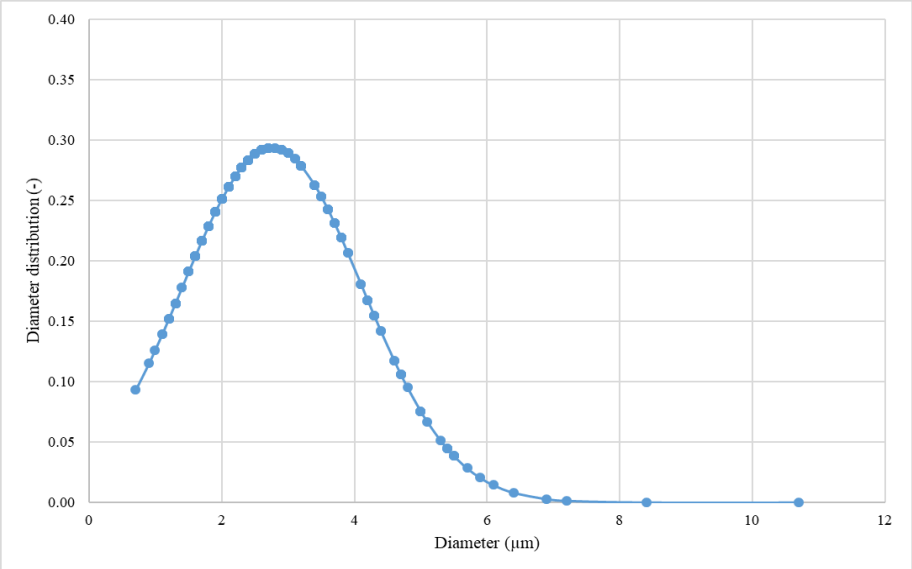


Figure 15 - Filament diameter distribution for unmodified PBT

The average filament diameter value is 2.76 µm with a standard deviation of 1.36 µm. As can be seen in the graph the distribution is wide, what that is expected considering that the PBT is produced using the melt flow technique that has not a good control of the filament diameter. However, the majority of the values are distributed in a small range around the average value.

3.1.2 Porometry measurement, Water Flow Rate and Air Flow Rate analysis

As can be seen in *Figure 16*, that summarizes the three porometry measurements done for unmodified PBT samples with area of 3.14 cm^2, the mean flow pore size is around 18 µm, with maximum of 21 µm and minimum of 14 µm, without noticeable differences between the three curves/measurements. This analysis, in addition to the SEM used to calculate the filament size distribution, allows us to characterize the fibrous structure of the material.

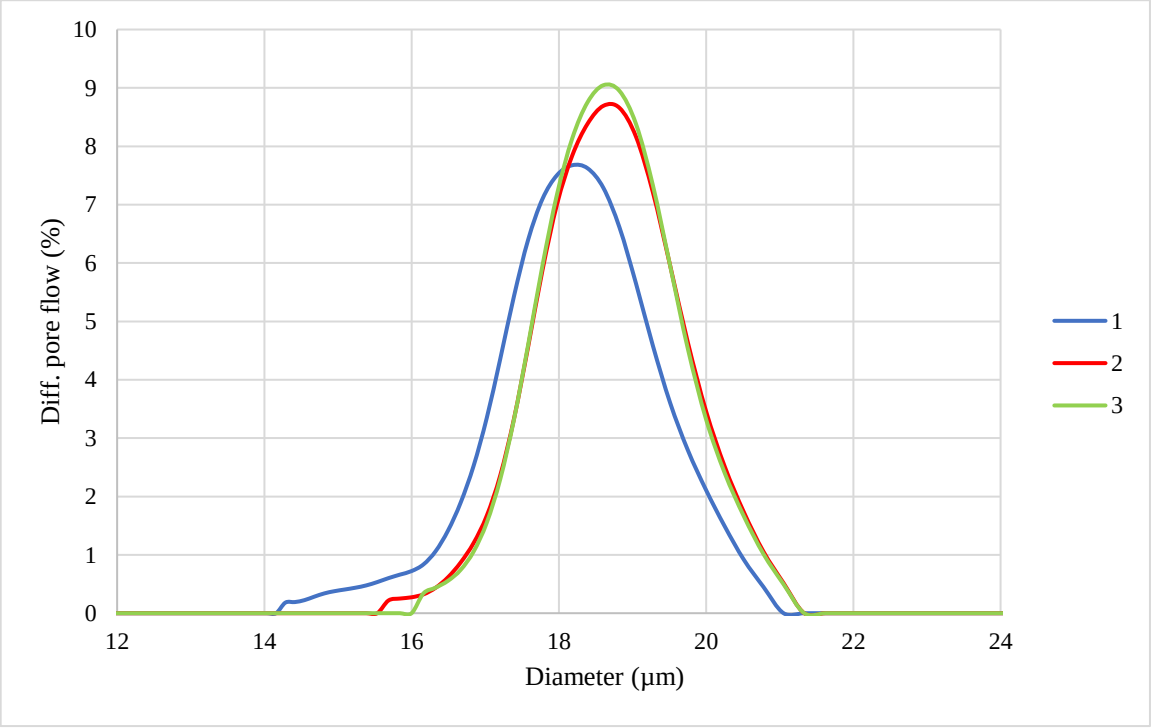


Figure 16 - Porometry measurements for unmodified PBT

Differences on the results can be ascribed to the sample heterogeneity. The porometry measurement allows us also to obtain a graph for the variation of air flow increasing the ΔP applied as shown in *Figure 17*:

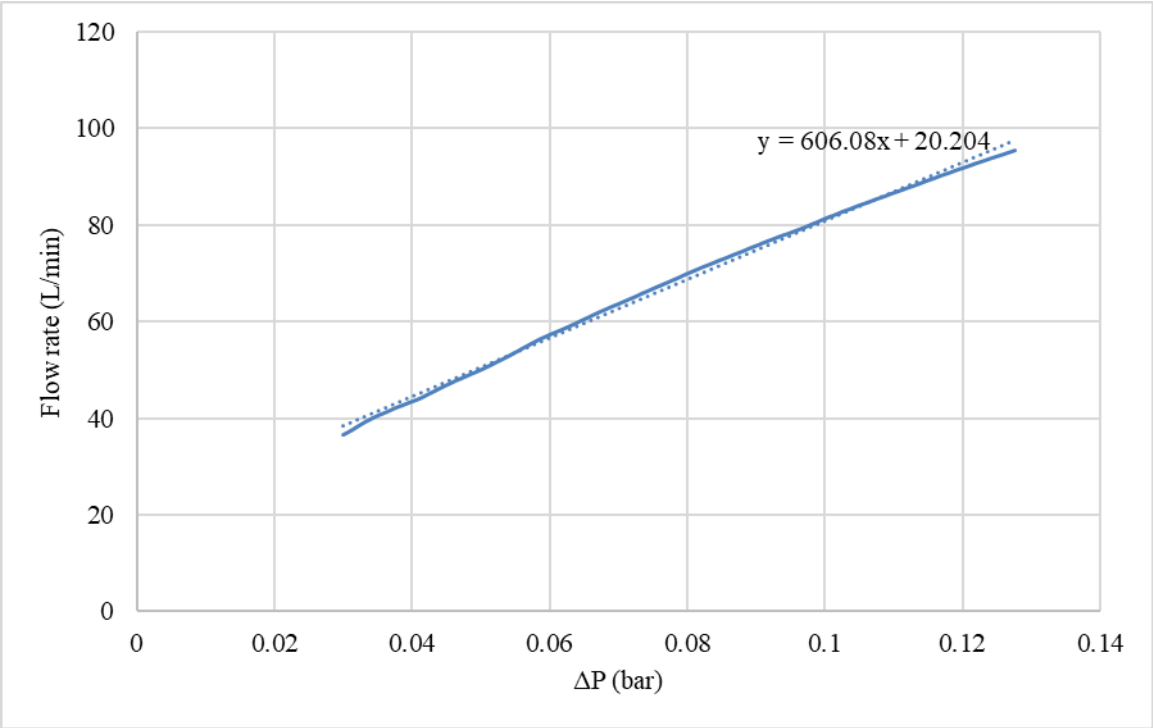


Figure 17 – Flow rate function of ΔP (the equation in the graph represents the trend line)

Furthermore, the air flow and water flow rate are measured on samples with area of 3.7 cm^2, as explained in Chapter 2.2.2: air flow is always measured as explained in chapter 2.2.2; at 0.1 bar and for unmodified PBT is 74.7 ± 0.3 L/min, while water flow rate, measured at 0.2 bar, is 581.1 ± 13.5 mL/(min cm^2 bar).

In order to measure the water flow rate of unmodified PBT, which is hydrophobic, it has to be wetted with isopropyl alcohol (IPA) before performing the measurement so that the water can wet the entire sample during the analysis.

3.1.3 Contact angle analysis

The contact angle analysis measured using the sessile drop technique is done to understand the behaviour of the unmodified PBT, which is expected to be hydrophobic. The degree of hydrophobicity is confirmed by the obtained contact angle values above 90°, reported in *Figure 18*. Both top and bottom filter surfaces are studied with no significant observed differences between them (the data are reported with standard deviation). After 2 minutes of analysis, the values can be considered constant and the test is stopped. As shown by *Figure 18*, the PBT is strongly hydrophobic with a C.A. value near 130°. It

will be interesting seeing the differences after the modification with the hydrophilic polymers.

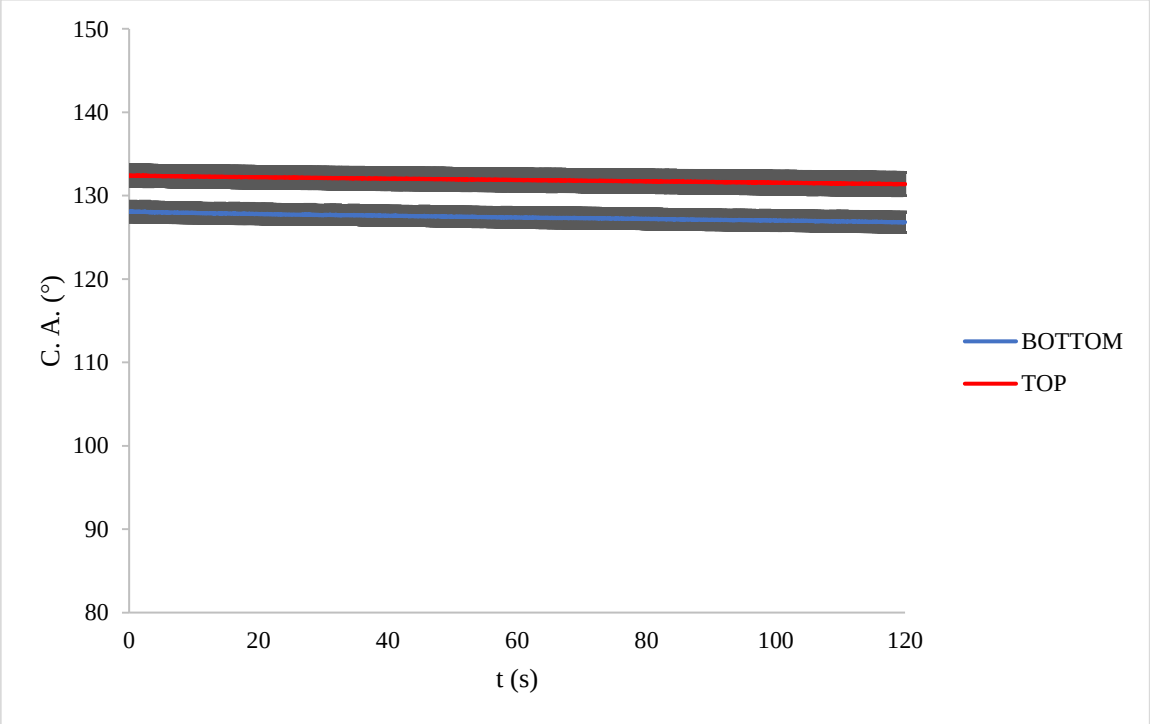


Figure 18 - Contact angle measurements for top and bottom surfaces of unmodified PBT (the grey shade around the lines is due to the standard deviation)

3.1.4 FTIR-ATR analysis

The spectrum in *Figure 19* shows the FTIR-ATR analysis results:

- Peak at 1708 cm^{-1}: corresponds to the C=O group of the carbonyl group;
- Peaks from 1000 to 1300 cm^{-1}: correspond to the O-C-O bonds (symmetric and asymmetric stretching);
- Peaks at 724 cm^{-1} and between 800 and 900 cm^{-1}: correspond to the aromatic ring.

All the recognized peaks correspond to the PBT structure.

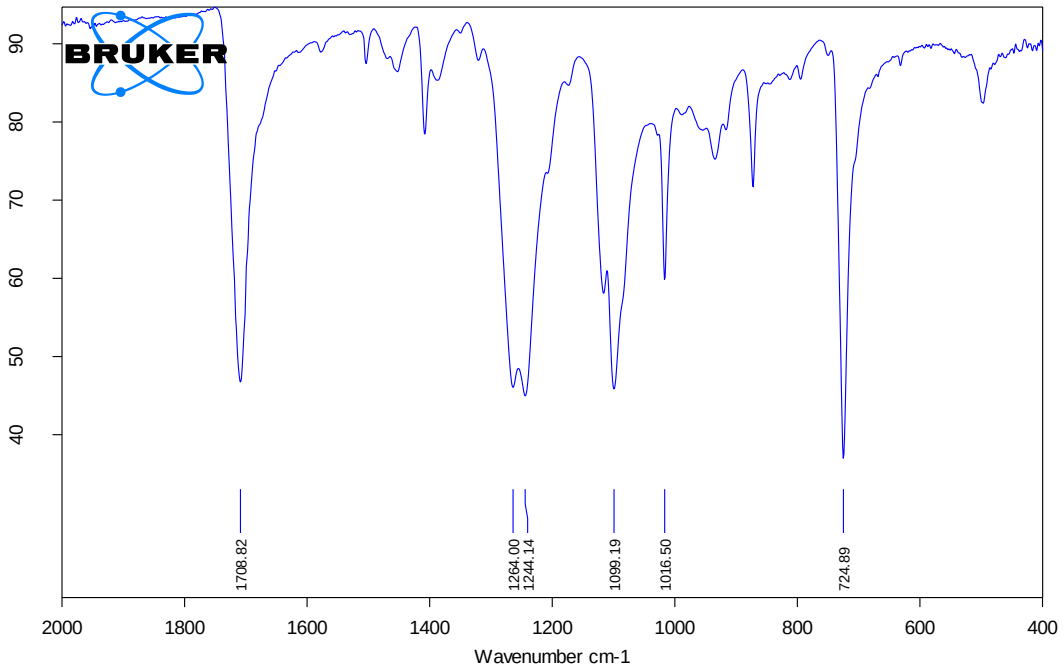


Figure 19 - ATR spectrum for unmodified PBT

3.1.5 Extractable test (chapter 2.2.9 and Appendix 3 for a furthered description of the method)

- Test for reducing substances:

  $KMnO_4$, H_2SO_4 and KI are added to 10 mL of the extracted solutions as explained in Appendix 3 and they are titrated with Na_2SO_3 in the presence of starch as indicator. The following table introduces the results of the test for reducing substances where S0 sample is double filtered water, DFW, used for the extractions and is used as a reference for all the analysis of the extractable test:

Sample	Na_2SO_3 (mL)		Average (mL)	Sn – S0 (mL)	Extraction temperature	Preheating
S0	19.00	19.50	19.25	-	-	-
S1	19.25	19.45	19.35	0.10	RT	-
S2	19.50	19.15	19.32	0.07	37°C	-
S3	19.00	19.20	19.10	-0.15	RT	60°C
S4	19.35	19.40	19.38	0.13	37°C	60°C
S5	19.45	19.35	19.40	0.15	RT	80°C
S6	19.30	19.40	19.35	0.10	37°C	80°C

Table 3 - Values for the reducing substances test obtained for unmodified PBT

From the values in *Table 3* the following bar charts are derived:

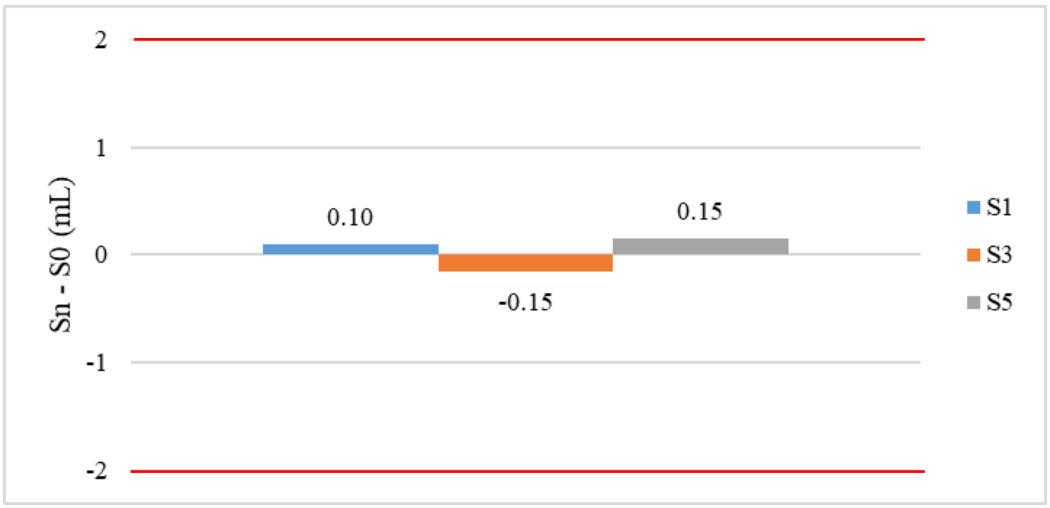


Figure 20 - Reducing substances test for unmodified PBT extractions at RT

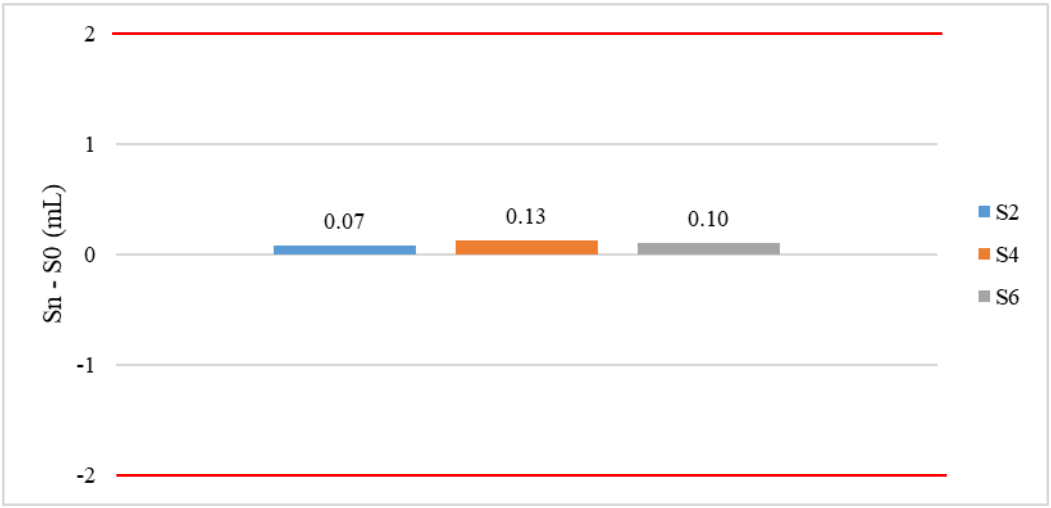


Figure 21 - Reducing substances test for unmodified PBT extractions at 37°C

The limit value for this test is (Sn – S0) = ± 2 mL. For this reason, the test can be considered passed. The only particular situation is for S3 sample where the value obtained is negative probably because something has been absorbed from the filter during the extraction.

- <u>Test for non-volatile residue:</u>

  Non-volatile test is relevant to understand the stability of the filtering material during a filtration process and it is important to confirm that the filter does not

release residues. 50 mL of the extracted solutions are placed in a wide beaker and are evaporated; then the residue in the beaker is weighted and the values shown in *Table 4* are obtained.

Sample	Mass gained (mg)	Difference (Sn-S0) (mg)	Extraction temperature	Preheating
S0	3.2	-	-	-
S1	7.8	4.6	RT	-
S2	4.3	1.1	37°C	-
S3	7.5	4.3	RT	60°C
S4	7.1	3.9	37°C	60°C
S5	4.8	1.6	RT	80°C
S6	8.0	4.8	37°C	80°C

Table 4 - Values for the non-volatile residue test obtained for unmodified PBT

In the bar chart below, both the tests at RT (S1, S3, S5) and at 37°C (S2, S4, S6) are shown: they seem to have an opposite trend with respect to each other but all the values remain below the limit of 5 mg, so that the test can be considered passed.

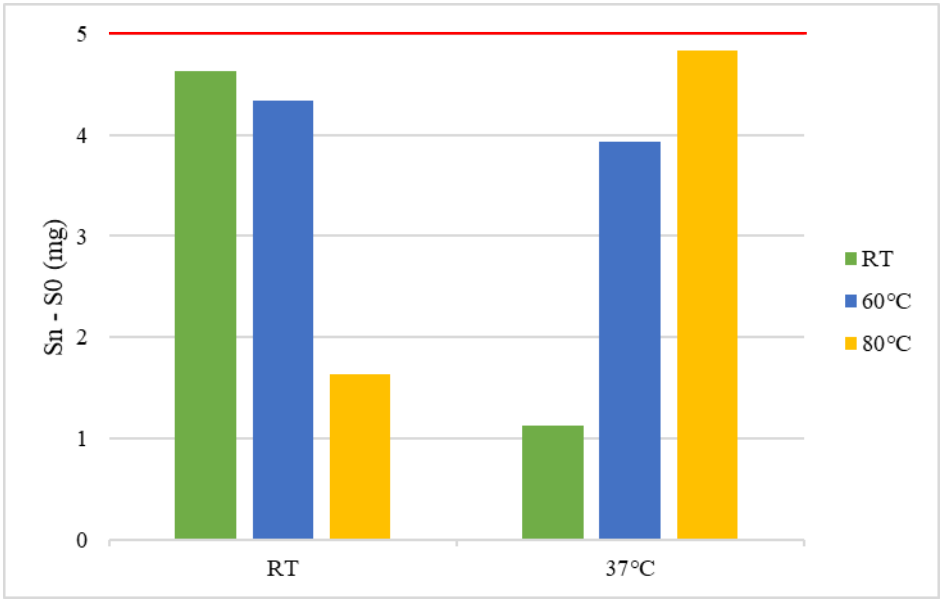


Figure 22 - Non-volatile residue for unmodified PBT extracts

- Test for titration acidity-alkalinity:

  In order to check how the extraction influences the pH of the DFW used, a titration test has to be done. For this reason, the Toshiro indicator is added to the extracted

solutions that become pale green. Therefore, they are titrated with acidic solution until a greyish colour appears. The volume values used for the titrations are shown in the following table and plotted in *Figure 23* and *Figure 24*:

Sample	HCl (mL)		Average (ml)	Sn –S0 (mL)	Extraction temperature	Preheating
S0	4.30	4.30	4.30	-	-	-
S1	4.15	4.30	4.22	-0.08	RT	-
S2	4.10	4.10	4.10	-0.20	37°C	-
S3	4.35	4.15	4.25	-0.05	RT	60°C
S4	4.45	4.40	4.43	0.13	37°C	60°C
S5	4.30	4.40	4.35	0.05	RT	80°C
S6	4.20	4.10	4.15	-0.15	37°C	80°C

Table 5 - Values for the acidity-alkalinity test obtained for unmodified PBT

There are no significant differences between the values that are all below the limit of ±1 mL so that the test can be considered successful. Some values are negative and some positive but the differences are small.

Figure 23 – Acidity-alkalinity test for PBT extractions at RT

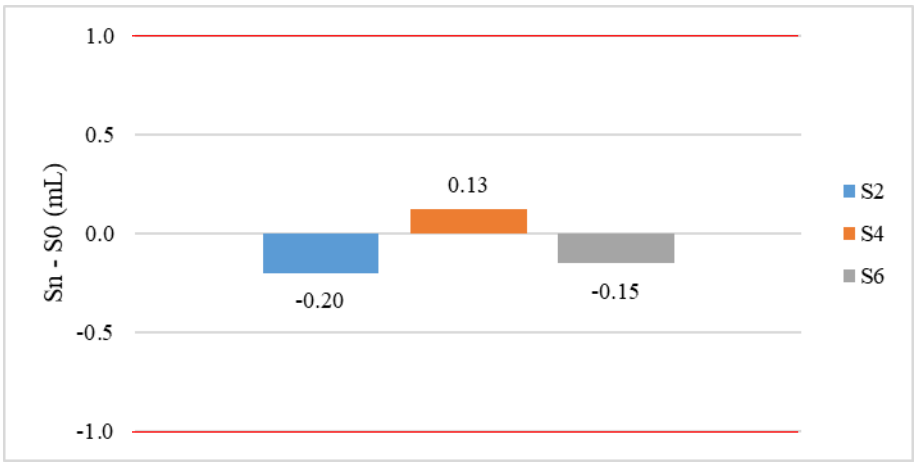


Figure 24 - Acidity-alkalinity test for PBT extractions at 37°C

- pH measurements:

  Used to confirm the result of acidity-alkalinity test, the pH values, obtained using the GLP-21 Crison pH-meter, are shown in the following table and bar charts:

Sample	pH	St. dev.	Extraction temperature	Preheating
S0	7.33	0.02	-	-
S1	7.27	0.06	RT	-
S2	7.25	0.03	37°C	-
S3	7.23	0.04	RT	60°C
S4	7.31	0.05	37°C	60°C
S5	7.36	0.04	RT	80°C
S6	7.38	0.04	37°C	80°C

Table 6 - Values for the acidity-alkalinity test obtained for unmodified PBT

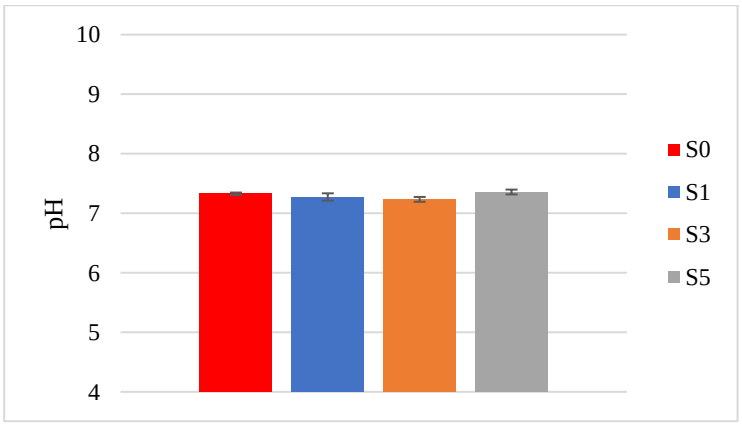


Figure 25 – pH measurements of unmodified PBT extracts at RT

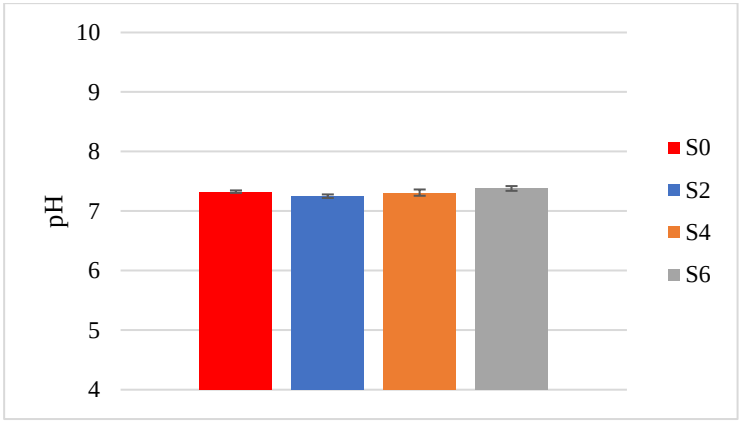


Figure 26 – pH measurements of unmodified PBT extracts at 37°C

The values are practically the same as the blank solution; this confirms the result of the acidity-alkalinity test where only slight differences can be seen.

- Test for absorbance (UV-Vis):

Few mL of the extracts are placed in cuvettes for testing them with the UV-Vis spectrophotometer SHIMADZU UV-1800 between 250 and 320 nm. The two graphs in *Figure 27 and 28* show that the test is successful because all the values are below the limit of 0.1; so, the filter does not practically release in the solution substances that absorb between 250 and 320 nm.

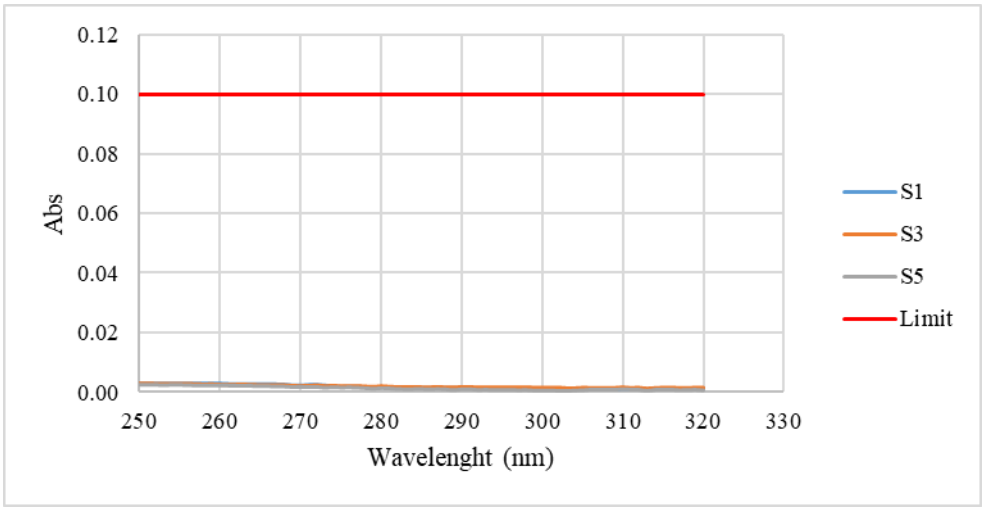


Figure 27 – UV-Vis test for unmodified PBT for extractions at RT

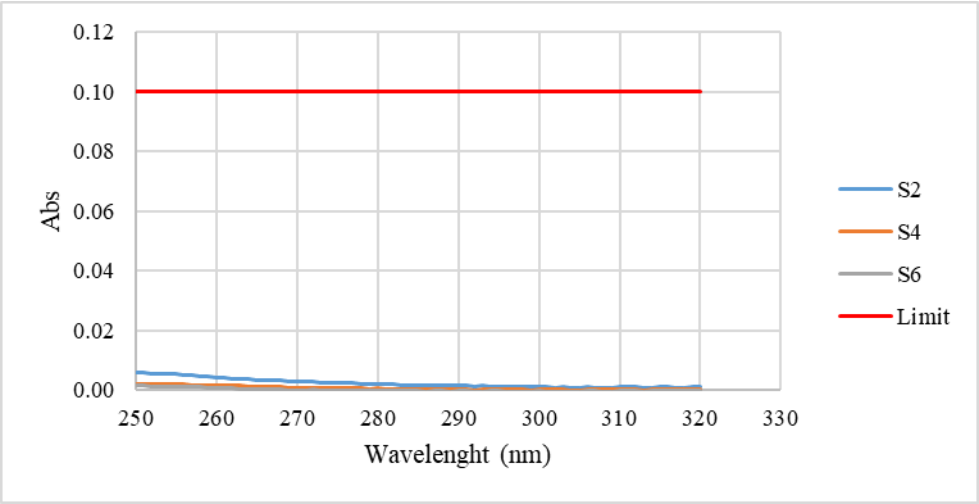


Figure 28 - UV-Vis test for unmodified PBT for extractions at 37°C

In conclusion, every test is successful: all the values are below the corresponding limits and are all comparable and similar to the values obtained for S0 sample (DFW). This underlines that the different temperatures used for the tests do not significantly influence the extractions and that the unmodified PBT does not release enough substances in solution to exceed the limits imposed by ISO 1135-4 [17]. For this reason, the extractable test will be performed on the modified samples only at the conditions used for S1 sample.

3.2 Characterization of modified PBT

Three different hydrophilic polymers are used for the modification of PBT; the modified filters are then analysed with the techniques described in Chapter 2.2 in an analogue way to the unmodified PBT. Their main characteristics are compared to the ones of the unmodified PBT and the commercial product, which properties are described along the following chapters. The first polymer used for the modification is the Polymer A, so its results will be the first analysed.

3.2.1 Commercial product

A commercially available product is formed through the coating of the PBT filter with a water polymeric solution (with free amino groups) where no covalent bonding takes place

between the filaments of PBT and the polymer. The final product is highly hydrophilic, with homogeneous coating and has a stable positive charge. This filter is already used for manufacturing of filtering systems for leukodepletion. The biggest problem is that the coating of the fibres is not stable and there is the risk that some of the substances from the coating are extracted.

It is used as a comparison for the modifications done in this project, with the final target to substitute it with a better product, and the values obtained in the study will be reported as reference in the following chapters.

3.2.2 PBT modified with Polymer A

Polymer A is used to deeply understand the Fenton-type reaction (see Chapter 2.1) and to check how the properties of the filter change, varying the amount of reactants. Seven types of modifications of PBT disks, with area equal to 18.1 cm^2, are performed using the reagent amounts reported in the following table:

	Polymer A (% m/v)	**Cross-linker (% v/v)**	**$FeCl_2$ (% m/v)**	**Reducing agent (% m/v)**	**H_2O_2 (% v/v)**
A-1	0.1	1.25	0.1	1.0	fixed
A-2	0.5	1.25	0.1	1.0	fixed
A-3	1.0	1.25	0.1	1.0	fixed
A-4	0.1	2.5	0.1	1.0	fixed
A-5	0.5	2.5	0.1	1.0	fixed
A-6	1.0	2.5	0.1	1.0	fixed
A-7	10.0	5.0	0.1	1.0	fixed

Table 7 - Reactants amount for the modifications with Polymer A

The final colour of the modified samples is white but sometimes some violet shades can appear (this is probably due to the inadequacy of the washing process for that samples). The sample A-7, due to the high reactants concentration, shows brownish and white zones, due to the inhomogeneity of the modification (brownish zones correspond to a higher functionalization or presence of reaction residues). Furthermore, at concentrations used for A-7, solution 2 ($FeCl_2$, reducing agent and water-soluble polymer) assumes a violet colour, that is assumed to be due to the formation of a complex between iron ions and amino groups [18].

- Weight differences:

  The weight difference between modified and unmodified samples was studied; surprisingly, it has been found out that the reaction with the Polymer A is not fully controllable. In fact, the weight gained does not follow the expectations and does not increase with the amount of the reactants. In two situations, A-2 and A-3, the weight decreases. This can happen because the reaction could take place more in the solution or near the interface of the sample than inside the sample bringing to an erosion of the PBT surface. Furthermore, the weight difference is not constant: the data resulting from the standard deviation reported in *Figure 29* show that filters modified with the same amount of reactants have differences in weight change.

  It seems also that the amount of cross-linker is important because, decreasing it, the process becomes less controllable and the standard deviation increases; by consequence, the situations that show the best controllability are the A-4, A-5, A-6 and A-7.

  In the following bar chart is shown the dependence of the weight gained during the modification process using the different amounts of Polymer A and cross-linker reported in *Table 7*.

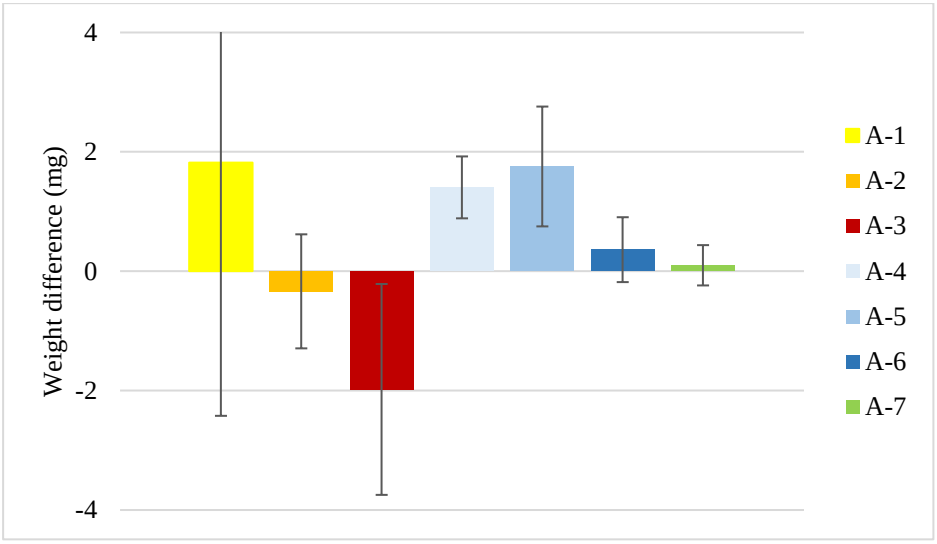


Figure 29 – Weight difference before and after modification for samples modified with Polymer A

- Contact angle analysis:

  In general, there are no big differences between the samples modified with Polymer A; if compared to the unmodified PBT, which was hydrophobic, the material obtained by the modification is strongly hydrophilic: the water drops disappear in more or less 80-90 ms with some small differences between the samples. On the other hand, if compared to the commercial product, the hydrophilicity is similar.

  For the samples modified with 2.5% of cross-linker there are no differences between top and bottom. They maintain a strong hydrophilicity also after the surface is purposely ruined and the fibres are ravelled.

  The following contact angle images are an example of the vanishing of the water droplets:

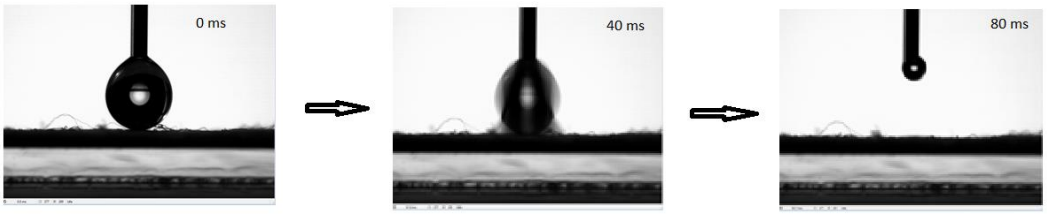


Figure 30 - Images of the contact angle analysis for sample A-5: the absorption of the water drop is too fast and no contact angle value can be taken during the analysis: the first image shows also how the water attracts the filaments of the filter

Analysing the samples modified with 1.25% of cross-linker, sometimes some hydrophobic zones appear, the drop remains stable for some seconds and then,

helped by application of a slight pressure, it is absorbed by the filter; maybe the external part is less hydrophilic than the internal part and the modification is less regular than for samples with 2.5% cross-linker. They lose a little bit of hydrophilicity after that the surface is purposely ruined and the fibres are ravelled, showing a lower stability of the hydrogel formed if compared to the samples modified with 2.5% of cross-linker. Furthermore, in the case of 0.1% Polymer A amount, due to the low polymer concentration, there are more hydrophobic zones. In the sample A-7 the drop disappears in different ways depending on the spots: in brown spots the areas of the drops are smaller but the drops are absorbed faster, while in the white spots the areas of the drops are wider but they are absorbed slower. A higher presence of cross-linked structure in the brownish zones can cause this different behaviour: in fact, if the cross-linking degree is too high, it can obstruct the pores and the water drop cannot enter inside the filter and remains on the surface.

- Optical microscopy:

  Analysing the modified samples with the optical microscopy we can see the cross-linked structure; in fact, new polished threads, which were not observed in the unmodified PBT, appear in the images; this is more evident for the extreme situation (A-7) which images are shown in *Figure 31*:

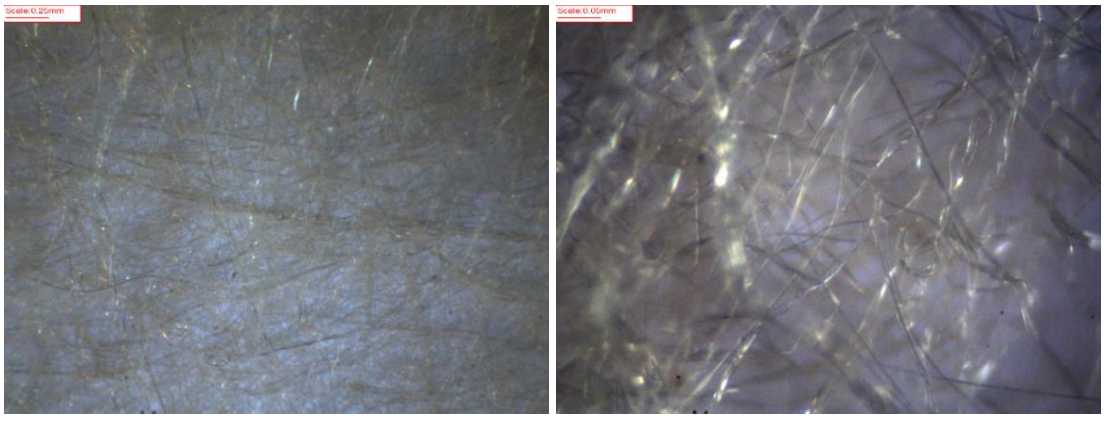


Figure 31 - Cross-linked structure in sample A-7 at different magnification

Almost all the samples give this type of images; obviously, depending on the reactant amounts, the cross-linking degree varies but the variation is not always relevant when the amount differences are small. However, more reactants normally

correspond to a high cross-linking degree; indeed analysing the sample A-7, it has been seen that the brownish zones correspond to a high cross-linking degree.

For samples A-2 and A-3, where the weight difference before and after modification was negative some polished threads that correspond to the cross-linked structure can be noticed on the surface but not in the depth of the material, confirming that the reaction took place more near the surface than inside the filter disk.

There is also a huge difference in the cross-linked structure morphology in the sample A-4 where some polymeric particles can be seen over the filaments of PBT. This allows us to think that the relative amount of the reactants used for the modification is not appropriate for creating a worthy coating of the fibres (optical microscopy images of system A-4 are shown in *Figure 32*).

If compared to the commercial samples, which images are shown in *Figure 33*, it can be seen how the commercial product consists only in a coating of the filter surface because is not possible to observe the polished threads or particles that can be seen for sample A-7 and A-4 (*Figure 31* and *32* respectively).

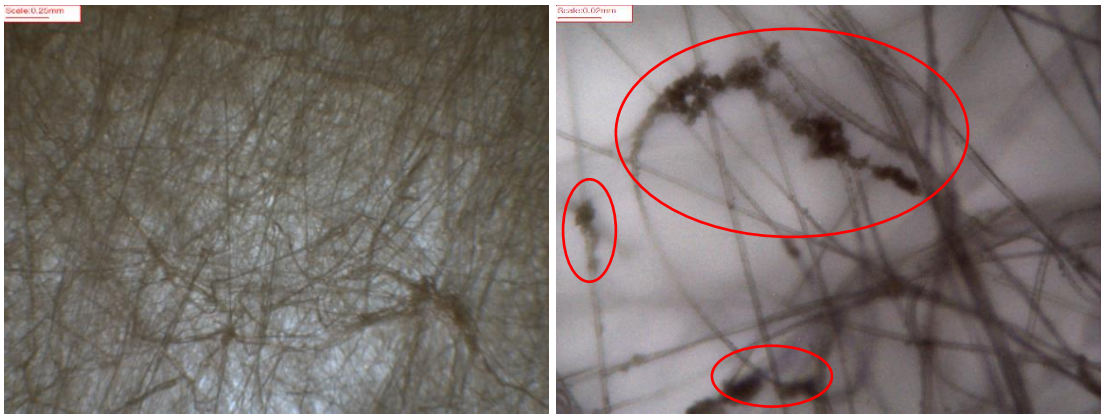


Figure 32 - Optical microscopy images for sample A-4: little particles are present instead of the cross-linked structure

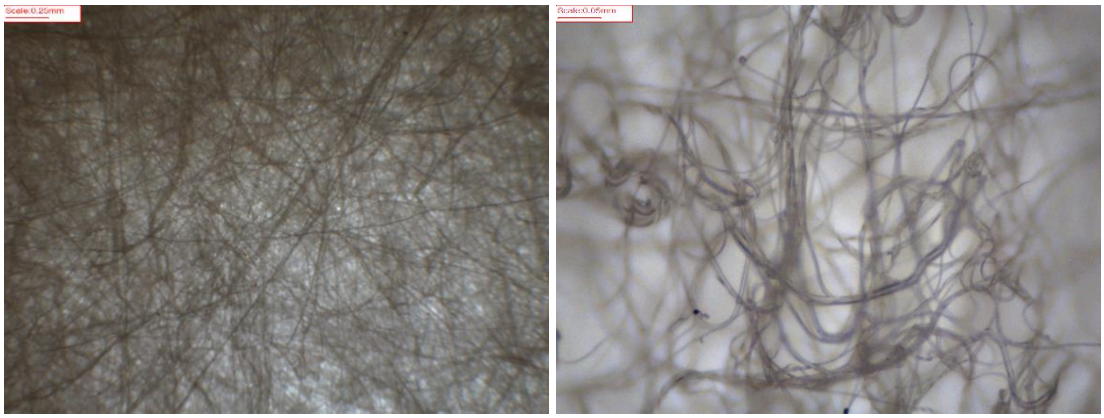


Figure 33 - Optical microscopy images for the commercial product: no polished threads or particles can be observed in the images

- FTIR-ATR analysis:

  ATR analysis is done only for two Polymer A functionalised samples (A-4 and A-6), in order to see if there are any differences in the spectra, comparing the results obtained with unmodified PBT. As can be seen in *Figure 34*, there are no clear differences between the spectra, probably due to the low concentration of the reactants.

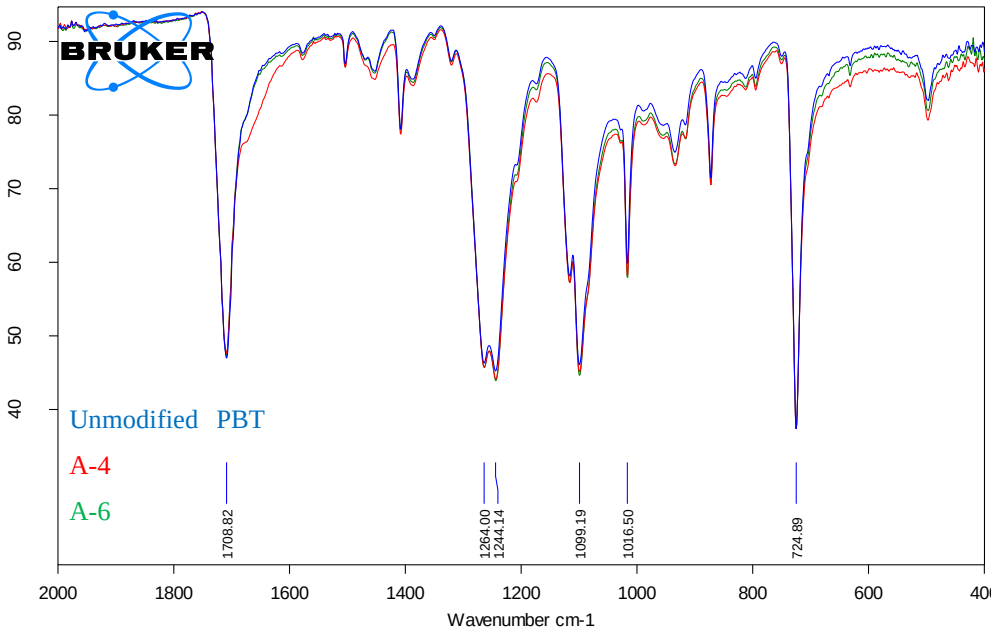


Figure 34 - ATR spectrum for samples of unmodified PBT (blue), A-4 (red), and A-6 (green)

- Air flow rate analysis:

  Sample of 3.7 cm^2 are tested at $\Delta P = 0.1$ bar. From the results obtained, shown in the bar charts below, no considerable differences between the unmodified and the modified samples can be noticed, so can be stated that the modification with Polymer A does not block the pores of the filters. In general, the air flow rate value is between 74-78 L/min, whereas for unmodified PBT and commercial sample it was 74.7 ± 0.3 and 75.6 ± 0.4 L/min respectively.

  Both top and bottom surfaces are analysed in order to understand if there are differences in the free-radical polymerization due to the better access that a surface could have to the reagents. Since the values are more or less the same, we can say that there are no differences between top and bottom and that both the surfaces are modified with the same result as regards the air flow measurements. Also using the maximum tested amount of polymer and cross-linker as done for the A-7 modification, the air flow does not considerably change: in fact, for sample A-7, the obtained values are 73-74 L/min, just below the values obtained for the other samples.

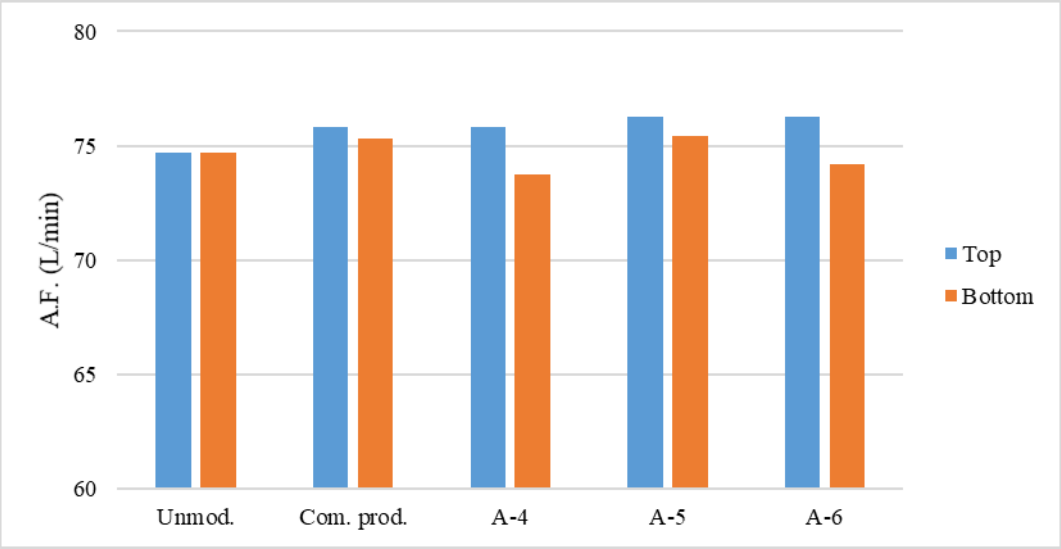


Figure 35 – Air flow rate measurements for Polymer A functionalised samples with 2,5% cross-linker

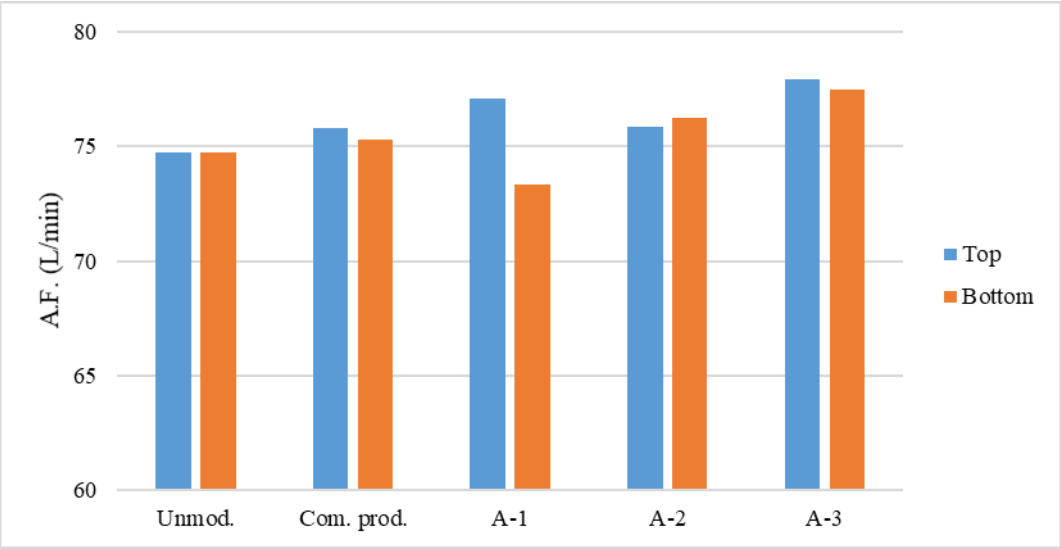


Figure 36 – Air flow rate measurements for Polymer A functionalised samples with 1.25% cross-linker

- Water flow rate analysis:

  Since the values obtained for the air flow rate analysis were all similar, it has been decided to analyse only the samples A-1, A-6 and A-7 to check if the significant change of the reagent amounts influences the water flow rate. As expected, the values of water flow rate (shown in *Table 8*) do not vary considerably: also using the highest Polymer A and cross-linker concentrations tested (sample A-7), the obtained value of the water flow rate is equal to the unmodified PBT one.

	Unmodified	**Commercial product**	**A-1**	**A-6**	**A-7**
W.F.R. (mL/min cm^2 bar)	581.1	608.1	573.0	559.8	581.1

Table 8 - Water flow rate values for Polymer A modified samples A-1, A-6 and A-7

- Methyl orange test (MO test) for charge analysis:

  The methyl orange test is the key test to define the surface charge of the filter, important for the adhesion capacity of the filtering media. As shown in *Figure 37*, for the samples with cross-linker amount of 1.25% the obtained results have a trend. Increasing the amount of Polymer A, the methyl orange residue in the filter, after washing steps with water and the K_2CO_3 solution, increases as expected. This

means that increasing the amount of functionalising polymer there are more free amino groups capable of binding the methyl orange. On the other side, using a higher amount of cross-linker (2.5%), the quantity of free amino groups on the surface decreases most probably due to the higher cross-linking degree, so the residual concentration in the filter is less. Considering the A-4 sample, it should have a value more or less similar to A-5 but this does not happen. The reason for this behaviour could be connected to the morphology of the cross-linked structure. In fact, as seen using the optical microscopy, A-4 sample has a structure with a different morphology (little particles instead of cross-linked threads): this can cause a higher presence of free amino groups with consequent higher surface charge and higher possibility to bind the MO molecules.

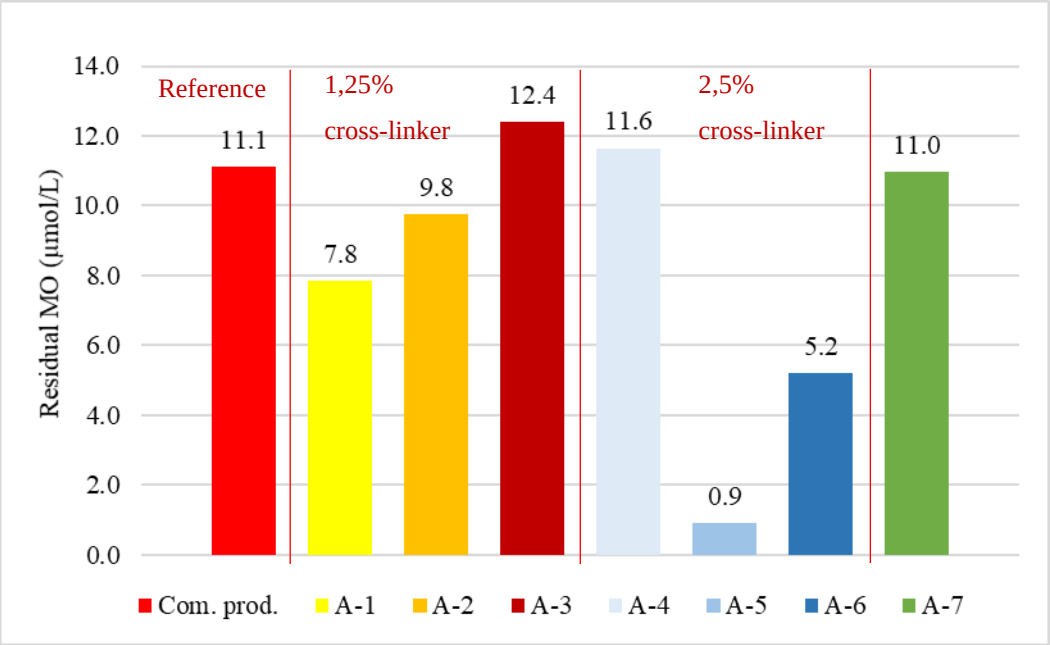


Figure 37 - MO test for Polymer A modified samples

Comparing the values to the one obtained for the commercial sample (11.1 µmol/L) the values that are more similar are the ones from A-3, A-4 and A-7.

The presence of amino groups could be detected also using a ninhydrin solution that should react with the amino groups of the modified PBT making the solution purple. Placing a piece of modified filter into a 1 M of ninhydrin solution and heating at 80°C, the solution becomes blue. The problem is that heating at 80 °C is not suitable for the membrane and the cross-linked structure that could be damaged:

for this reason, considering that at low temperatures the reaction, due to the low concentration of amino groups, does not take place, the test has been abandoned and only the methyl orange test has been performed.

3.2.3 PBT modified with Polymer B

Polymer B is another interesting possibility for the modification of the non-woven PBT due to its stability at room temperature without special precautions and the presence of many primary, secondary and tertiary amino groups. Based on literature studies and on the knowledge obtained during the analysis of the Polymer A functionalised samples, the modification of PBT disks with area 18.1 cm^2 are performed using the four concentrations listed in *Table 9*:

	Polymer B (% m/v)	**Cross-linker (% v/v)**	**$FeCl_2$ (% m/v)**	**Reducing agent (% m/v)**	**H_2O_2 (% v/v)**
B-1	0.5	2.5	0.1	1.0	fixed
B-2	1.0	2.5	0.1	1.0	fixed
B-3	5.0	2.5	0.1	1.0	fixed
B-4	10.0	5.0	0.1	1.0	fixed

Table 9 - Amounts of reactants used for modifications with Polymer B

Looking at the colour of the solutions during the modification process, the solution 2 (the one containing the Polymer B, the reducing agent and $FeCl_2$) becomes blue also at the lowest concentration (B-1) and the colour does not disappear anymore. Probably, due to the higher concentration of amino groups of Polymer B, compared to Polymer A (for which the solution became blue only at high concentration), a strong complex is formed with the Iron ions [18]. *Figure 38* shows the colour of the organic solution when the three reagents are mixed:

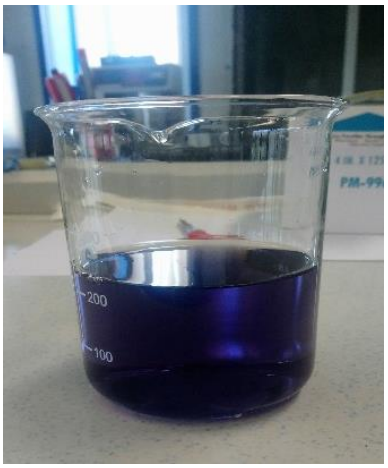


Figure 38 – Colour of solution 2 with Polymer B compound

This has an effect also on the colour of the final product that, mostly for B-2, B-3 and B-4 samples, is pale brownish as shown in *Figure 39*. This could be a problem for a future commercial use of the final product.

B-1 B-2 B-3 B-4

Figure 39 - Colour of the filtering disks modified with Polymer B

In order to understand this behaviour, modifications with a bigger area of filtering media will be performed at different concentrations of $FeCl_2$, which will be discussed in the next paragraphs of this thesis.

- Weight differences:

  As shown in *Figure 40*, unlike the Polymer A samples, an increase of the polymer amount gives a higher final weight. In fact, using 10% of Polymer B increases the mass by 16.90 mg, approximately 10 times more than using 0.5% of Polymer B. Based on the weight results, the Fenton-type reaction with the Polymer B is controllable and reproducible. Also checking the standard deviation, it can be said that weight gained for the same concentrations is more or less always constant

showing a good process controllability (differently to what happened with the samples modified with Polymer A). Due to the high concentrations used, the modification for samples B-4 is more difficult to control.

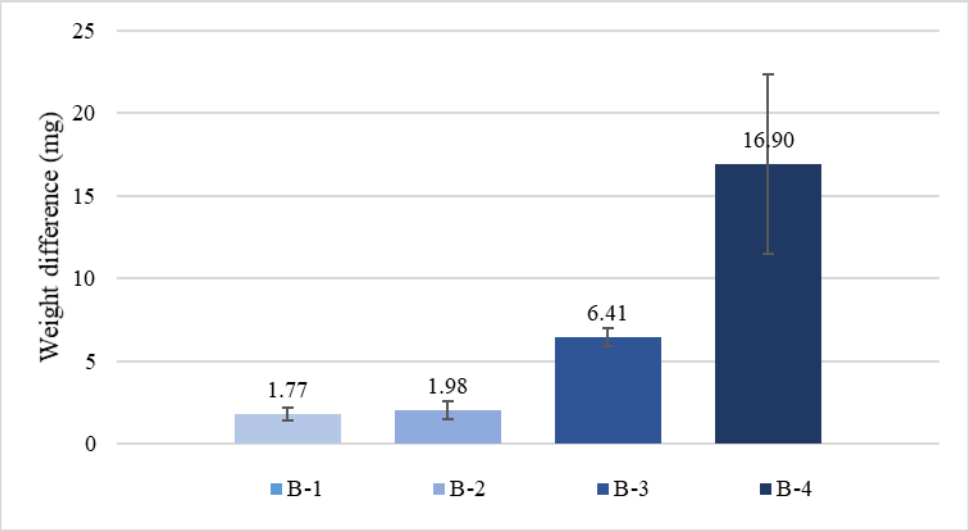


Figure 40 – Weight difference before and after modification for samples modified with Polymer B

- Swelling test:

The swelling test is performed in order to analyse the capacity of the hydrogel structure to hold water, property that is directly related to the cross-linking degree. The dry samples are weight and immersed in water for 24 hours; then, after removing the excess water on the samples surface, they are weighed for the second time.

The equilibrium swelling ratio (eq.SR) is calculated with the following formula:

$$[\%]\ eq.SR = \frac{Weq - Wd}{Wd - W_0}\ x\ 100$$

Where: Weq = weight of the sample after 24 hours in water

Wd = dry weight of the sample after modification

W_0 = dry weight of the sample before the modification

For the Polymer A functionalised samples, due to the uncontrollability of the modification results, the swelling test was not performed.

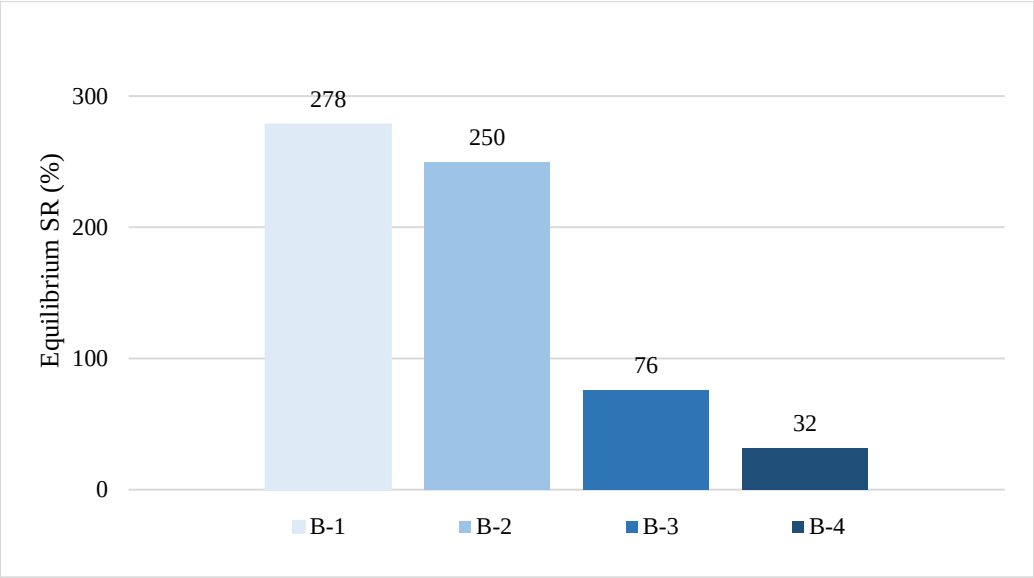


Figure 41 – Swelling test for samples modified with Polymer B

In *Figure 41*, it can be seen that with increasing the amount of Polymer B the equilibrium swelling ratio decreases. This is due to the reduction of the free volume between the hydrogel structure, which results in reduced degree of swelling. This explains the reason for the lowest eq.SR for B-4 sample, additionally SEM analysis confirms that the sample is completely stacked.

- <u>Contact angle analysis:</u>

  The contact angle measurements surprisingly show the irregularity of the modified disks: in fact, by changing the spot of the measurements, different behaviours can be detected; there are both hydrophilic and hydrophobic regions in the same filtering media. However, applying a gentle pressure the water is absorbed, showing that sometimes the modification happens inside the filter more than outside. On the other hand, increasing too much the amount of Polymer B the hydrophilicity decreases, meaning that the porosity of the filtering media decreases and there is not enough space for the water to easily enter into the pores. In some cases, the top of the filtering media can be more hydrophilic than the bottom. If compared to Polymer A functionalised samples and the commercial product, in the Polymer B ones the hydrophilicity is lower, but the rigidity of the filters is higher.

Figure 42 shows the different behaviours that can be observed on the same modified surface:

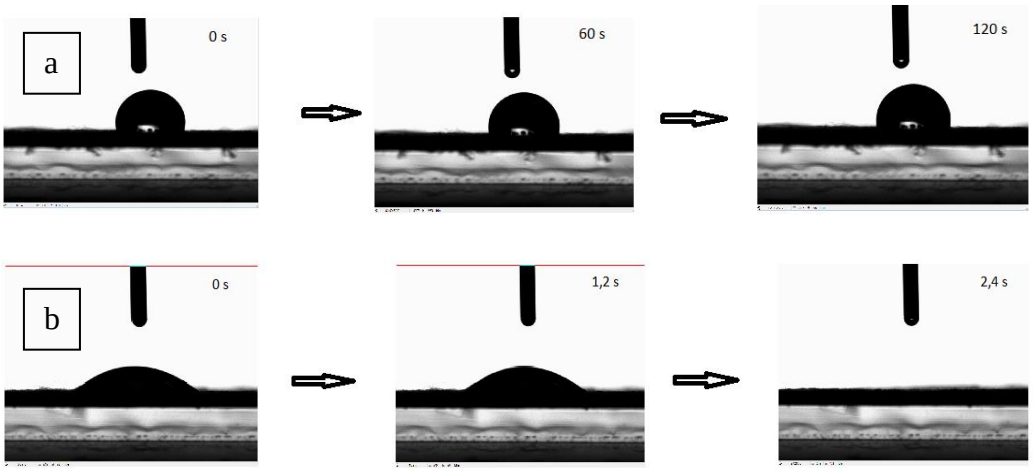


Figure 42 - Images taken during the contact angle analysis of sample B-2 show the different behaviours of the same surface: a) shows the hydrophobicity and b) the hydrophilicity of the same filtering media

- Optical microscopy:

The modification of the filters with Polymer B is evident, much more than for Polymer A functionalised samples. In fact, as shown in *Figure 43*, both little polymeric particles of Polymer B and long threads of the cross-linked structure are observed.

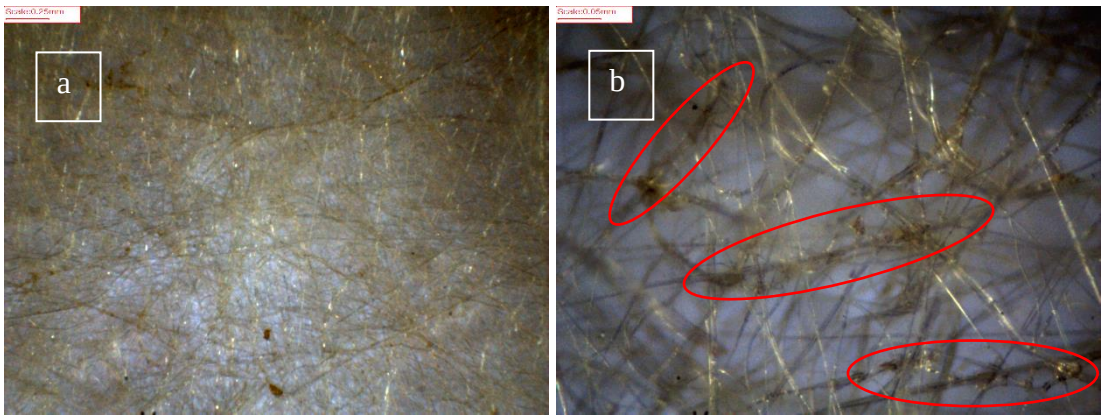


Figure 43 - Optical microscopy images for sample B-1

Comparing to the commercial product and Polymer A functionalised samples the images are completely different. By increasing the amount of water-soluble polymer and cross-linker, the filaments of PBT become completely covered by the hydrogel layer until the point where they are glued together (check the SEM images

in *Figure 44*). This is not optimal for the leukodepletion that is based on the depth filtration and that needs a wide free surface for the interaction between fibres and leukocytes.

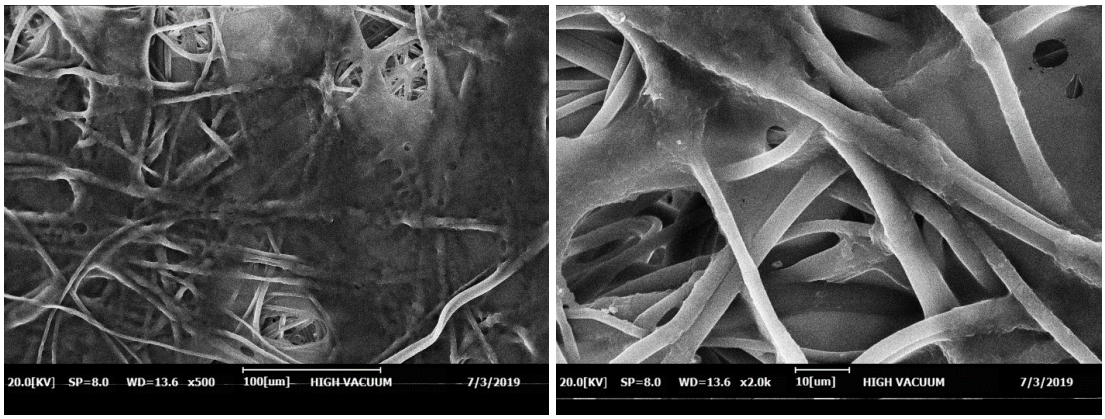


Figure 44 - SEM images for sample B-4: the pores are completely blocked and this influences also the contact angle measurements

It seems that both Polymer A and Polymer B can form little spheres of polymer but for the Polymer B they are more evident (a higher presence of the amino groups facilitates the formation of both the cross-linked structure and the particles as shown in *Figure 43-b*).

- <u>FTIR-ATR analysis:</u>

  As shown in *Figure 45*, we can see some differences between the unmodified PBT and the Polymer B functionalised samples. In fact, using a high amount of Polymer B (for example in B-4), in the spectrum some peaks become more strong, due to the high concentration of the functional groups on the modified surface, and at 1240 and 1600 cm^{-1} there are two little shoulders due to the higher concentration of the C-O and C=O bonds, derived probably from the cross-linker. Furthermore, an increment of the signal can be seen between 2700 and 3000 cm^{-1} due to an increase of the aliphatic fraction.

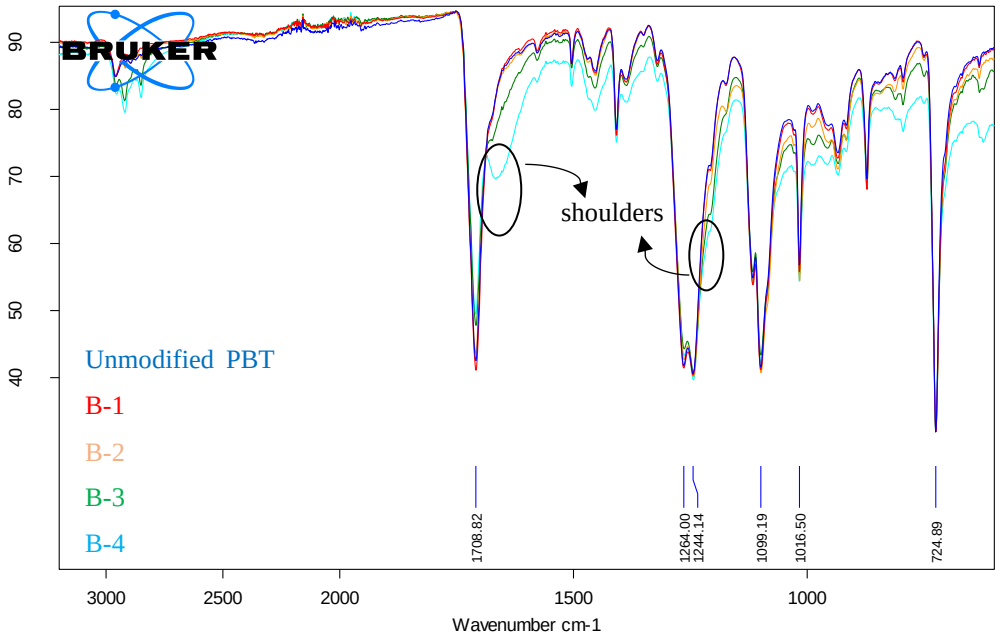


Figure 45 - ATR spectrum for samples: unmodified PBT (blue), B-1 (red), and B-2 (brown), B-3 (green) and B-4 (light blue)

- Air flow rate analysis:

Figure 46 shows the air flow results for Polymer B modified samples:

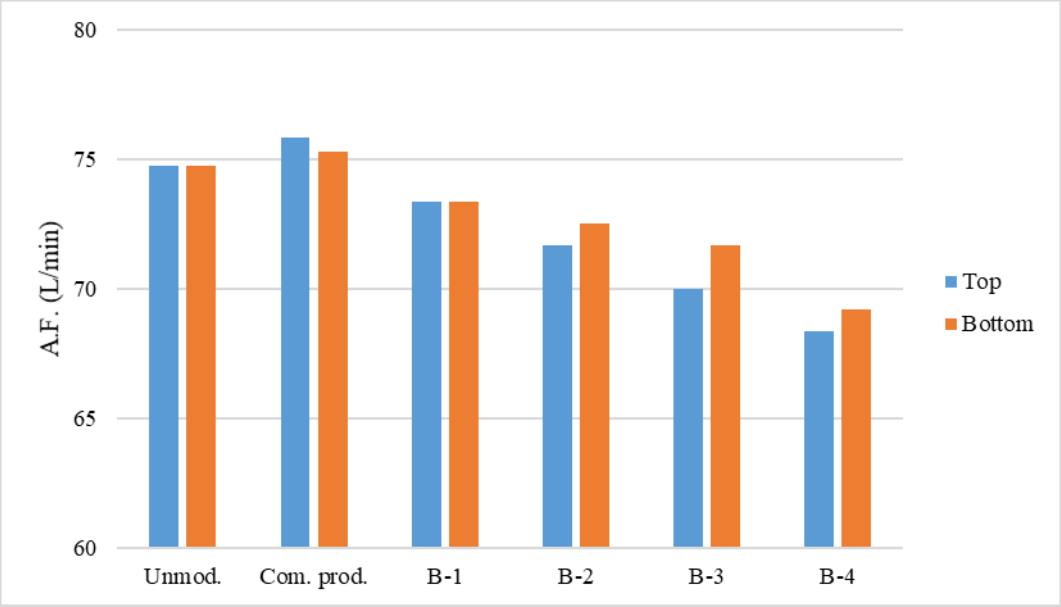


Figure 46 – Air flow measurements for sample modified with Polymer B

There's a little decrease of air flow value increasing the amount of Polymer B. This confirms that the cross-linked structure blocks the pores of the filtering media and

that, after increasing the reagents amount, the air flow rate decreases. This is a different behaviour if compared to the Polymer A modified samples where, increasing the amount of reagents, the air flow values did not change.

- Water flow rate analysis:

  Table 10 shows the results for the PBT modified with Polymer B. As expected and as happened for Polymer A modified samples too, there is not a significant variation of the obtained values that are comparable to the ones obtained for unmodified PBT, the commercial product and the PBT modified with Polymer A. The higher difference is with sample B-4, probably due to the stacked pores.

	Unmodified	**Commercial product**	**B-1**	**B-2**	**B-3**	**B-4**
W.F.R. (mL/min cm^2 bar)	581.1	608.1	540.5	554.1	581.1	527.0

Table 10 - Water flow rate analysis for sample modified with Polymer B

- Methyl orange test (MO test):

  As expected and shown in *Figure 47*, increasing the amount of Polymer B (see *Table 9*), the residual MO concentration in the filtering media increases. This trend is due to the rise of the free amino groups on the filter surface that cause an increase of the positive charge [15] and a better linkage with the MO molecules. Despite what happens for the filtering media modified with Polymer A, where the values are not always what expected (sample A-4), for Polymer B we have a clear trend. Furthermore, for the same amount of polymer, the residual MO concentration in the filter is higher when Polymer B is used.

  However, due to the inconstant contact angle results and due to the yellowish colour of the samples B-3 and B-4, it is not possible to use Polymer B concentrations above 5% for the filter modifications. For these reasons, the sample B-2 can be taken as a comparison for the substitution of commercial product.

  Another information that is obtained with this analysis is that the final washing step is more relevant on the commercial product sample than on the Polymer B ones; in fact the MO concentration removed in the commercial product is 3.9 µmol/L, while

for B-1 (modified with Polymer B) is 1.1 µmol/L. Basing on this data we can say that the bond between Polymer B and methyl orange is stronger than the one with polymer used to make the commercial product. This characteristic is important for the final application because a stronger bond between the MO and the filter could correspond also to a stronger bond between leukocytes and the filtering media.

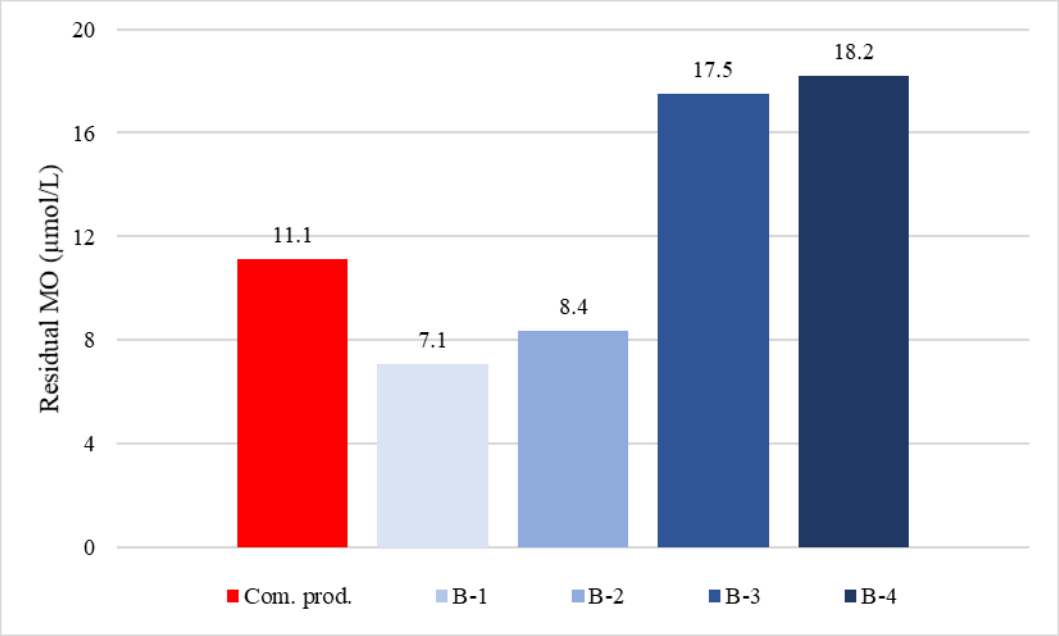


Figure 47 – MO test for Polymer B modified samples

Furthermore, as shown in *Figure 47*, it seems that, increasing above 5% the concentration of Polymer B used in the reaction, the effect on the binding capacity does not change a lot passing from 17.5 µmol/L for sample B-3 to 18.2 µmol/L for sample B-4. In these conditions the retention of the MO has probably reached a maximum concentration. In fact, washing the sample B-4 with the carbonate solution, more than 15 µmol/L are removed, so that the pores of the filter surface, as shown in *Figure 44* by the SEM, are clogged by the hydrogel and the methyl orange is not really bound to the surface but is only stacked on it.

Once we have understood the positive charge of the filter (compared also to the Polymer A samples), we can proceed trying to obtain Polymer B functionalised filters with a white colour. Assuming that the $FeCl_2$ or the complex that it forms with the amino groups are responsible of the yellowish colour, it has been tried to

decrease its concentration, but keeping the same properties obtained for sample B-2. Using 1% of Polymer B we decreased the concentration of $FeCl_2$ to 0.075% m/v and to 0.050% m/v (the amount of reducing agent diminishes by consequence). Diminishing too much the amount of $FeCl_2$ is not recommended due to the rapid decrease of the final hydrogel amount and equilibrium swelling ratio values [10,11].

3.2.4 Influence of reagent concentrations on colour of Polymer B modified PBT

To understand how much the colour of the modified samples depends on the $FeCl_2$, it has been decided to try reducing its amount. Other three modifications, performed on bigger rectangular PBT samples with area 120.3 cm^2, are carried out using the concentrations shown in *Table 11* in order to evidence the colour changes.

	Polymer B (% m/v)	**Cross-linker (% v/v)**	**$FeCl_2$ (% m/v)**	**Reducing agent (% m/v)**	**H_2O_2 (% v/v)**
B-5	0.5	1.25	0.05	0.5	fixed
B-6	0.5	1.25	0.075	0.75	fixed
B-7	0.5	1.25	0.1	1.0	fixed

Table 11 - Concentrations of the reagents used for modification with variable amount of $FeCl_2$

In order to obtain a whitish final colour, it has been tried to reduce the amount of Polymer B and cross-linker with a constant ratio of the two reagents (cross-linker : Polymer B = 2.5 : 1, the same ratio used for sample B-2). The colour of the modified disks is acceptable with a slight improvement when $FeCl_2$ concentration decreases. Based on weight differences before and after modification (*Figure 48*), we can see a clear dependence of the hydrogel amount on the % m/v of $FeCl_2$ in solution 2 (the one with polymer, reducing agent and $FeCl_2$).

Furthermore, by adding the $FeCl_2$ to the solution 2, only after the placement of the filtering media inside it, the final colour after the modification is whitish and acceptable. In any case, this way to add the $FeCl_2$ is not industrially feasible.

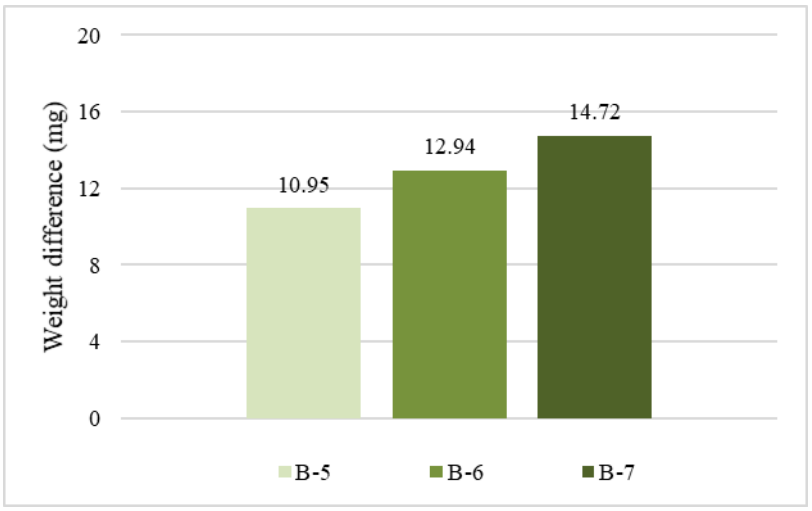


Figure 48 – Weight difference for Polymer B modified samples changing the $FeCl_2$ concentration

In order to understand how the $FeCl_2$ concentration influences the properties of the filtering media, the usual tests are performed.

Analysing the samples B-5, B-6 and B-7 with the techniques used for the other samples modified with Polymer B, no clear differences are observed. Only the wettability of the samples seems to be different, indeed diminishing the $FeCl_2$ amount as done for samples B-4 and B-5, the surface wettability decreases becoming less strong and homogeneous so that hydrophobic spots are observed. Furthermore, as happens for sample B-5, sometimes hydrophilic spots can be observed but the water drops are not completely absorbed by the filter. Analysing these drops with the contact angle instrument, the C.A. value remains stable for long time.

To show the air flow and water flow rate homogeneity with the values of the samples from B-1 to B-4, the following *Tables 12* and *13* are displayed:

	Unmodified	Commercial product	B-5	B-6	B-7
A.F. (L/min)	74.7	75.3	77.5	78.3	76.7

Table 12 - Air flow rate measurements for PBT modified with different concentrations of $FeCl_2$

	Unmodified	Commercial product	B-5	B-6	B-7
W.F.R. (mL/min cm² bar)	581.1	608.1	527.0	541.9	538.3

Table 13 - Water flow rate measurements for PBT modified with different concentrations of $FeCl_2$

Looking at the MO test result, shown in *Figure 49*, it is recognisable a trend by changing the concentration of the ferrous chloride; in fact it is the activator of the reaction and, as underlined also by the bar chart in *Figure 48*, a decrease of the $FeCl_2$ amount corresponds to a reduction of the hydrogel structure in the filtering media.

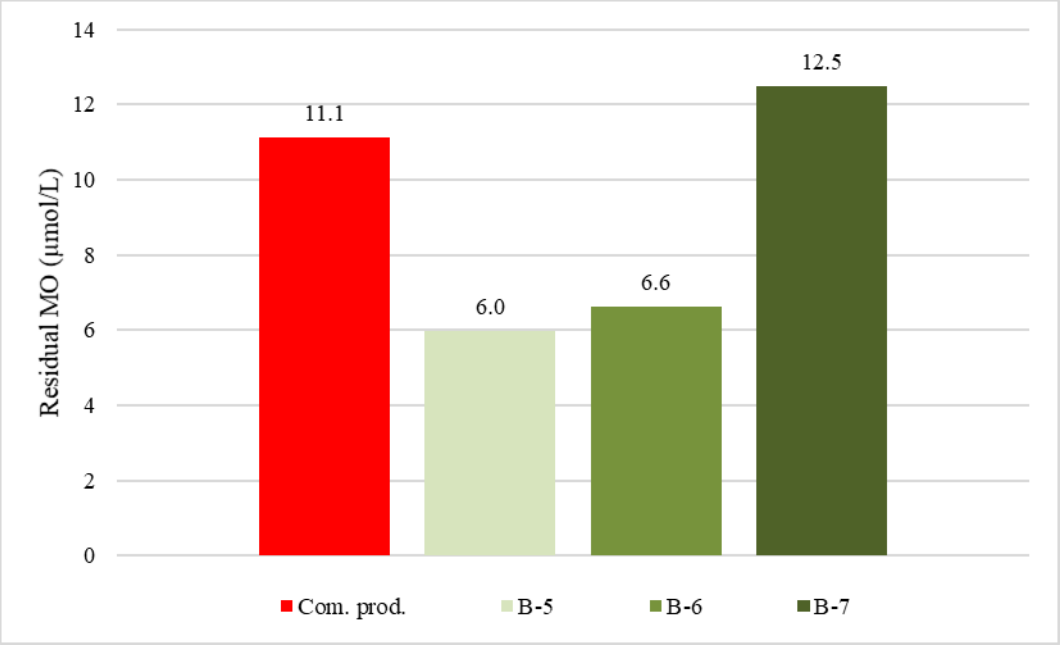


Figure 49 – MO test for Polymer B modified samples using different concentrations of $FeCl_2$

Figure 50 shows the colour of the filter B-7:

Figure 50 - Sample B-7: it has a good surface colour combined with good characteristics (the dark part was out of the SDS solution during the washing step so is not properly washed)

Since the sample B-7 shows the best characteristics (high MO residual value, whitish colour and homogeneous hydrophilic behaviour), considering every Polymer B sample analysed and using a quite low amount of reagents, it is the one chosen for the final MO test at pH = 7.4 and for the extractable test, respectively explained in chapters 3.2.5 and 3.2.6.

3.2.5 PBT modified with Polymer C

Being sure about the suitable concentrations to obtain the desired results, due to the quantity of test done and the results obtained by the modification with Polymer A and Polymer B, only one modification with Polymer C is done using the following concentrations:

	Polymer C (% m/v)	**Cross-linker (% v/v)**	**$FeCl_2$ (% m/v)**	**Reducing agent (% m/v)**	**H_2O_2 (% v/v)**
C-1	0.5	1.25	0.1	1.0	fixed

Table 14 - Concentrations of the reagents used for PBT modification with Polymer C

The colour of the filter after the modification is shown in *Figure 51*. It is whitish, except for the dark parts that have not been in contact with the SDS solution during the washing step of the filter modification.

Figure 51 - Sample C-1 (the dark part is not properly washed as happened for sample in Figure 50)

As expected, the tests performed on this sample show suitable results if compared to the one obtained for Polymer A and Polymer B modified samples:

- Weight difference:

  The weight of the sample increases by 16.11 mg after the modification, value similar to the ones obtained with sample B-7, modified with Polymer B (weight differences can be compared only when similar sample areas are used).

- Contact angle analysis:

  With homogeneous surface behaviour, contact angle test results are positive as the water drops are absorbed within 80-120 ms.

- Optical microscopy:

  In *Figure 52* it can be seen that the filaments of sample C-1 are coated and that the modification has happened; if compared to the sample B-7, that has the same reagents concentration, the images are similar.

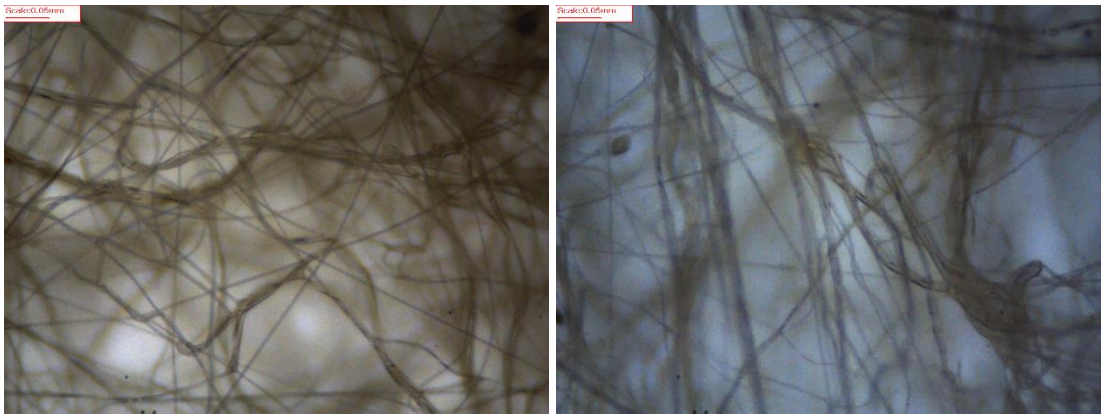


Figure 52 - Optical microscopy images for sample C-1 (left) and B-7 (right): no particular differences can be seen

- A.F. and W.F.R. analysis:

  Air flow rate and water flow rate values are comparable to the values obtained for the other functionalised samples: A.F. = 78.3 L/min and W.F.R. = 527.0 mL/(min cm^2 bar). Therefore, the modification with Polymer C using these concentrations does not stack the pores of the material.

- ATR analysis:

  Figure 53 shows the ATR analysis result:

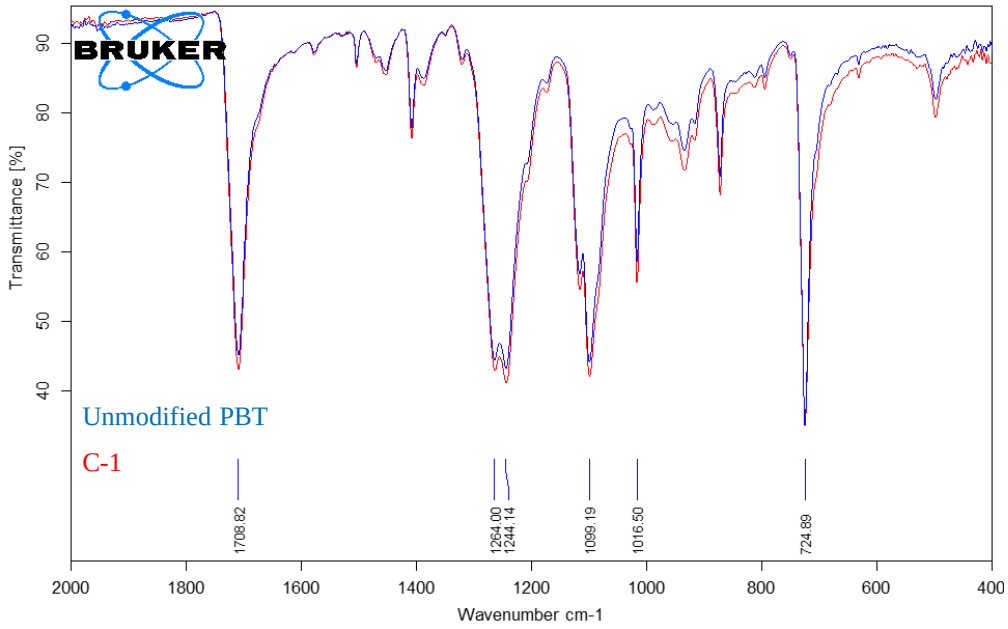


Figure 53 - ATR spectrum for sample C-1 (red) and unmodified PBT (blue)

The spectrum does not show differences between unmodified PBT and sample C-1. Probably, as happens for other samples too, the degree of modification is not enough to carry to a variation in the spectrum. Furthermore, this is also due to the similar characteristic groups of PBT and Polymer C, though the -OH groups of Polymer C should be visible in the spectrum.

- Toluidine blue test (TB test):

  MO test cannot be used for the sample C-1 due to the acidic functional groups; to determine the Polymer C amount on the surface the TB test has been performed (as explained in Chapter 2.2.6). The TB feed solution needed for the test is filtered using a syringe filter system and the residual concentration of toluidine blue in the filter is calculated by difference between the initial solution concentration and the solutions collected after removing the dye reversibly stacked in the filter. TB in excess on the filter is removed by some washing steps with water and a final washing step with an acetic acid solution. The result is shown in *Figure 54* (for the commercial product the TB test does not always give meaningful results so the residual concentration values are the ones obtained with the MO test).

  The data obtained are encouraging, particularly if the bars without the two washing steps are considered: the final steps with acetic acid (for sample C-1) removes a large amount of TB bound to the surface. This is positive; in fact, it leaves a good margin for better results in the blood filtration, if compared to the commercial product samples where the value obtained without the washing steps is sensibly lower. It means that the C-1 modified filter can have more interactions with the TB than the commercial product with MO.

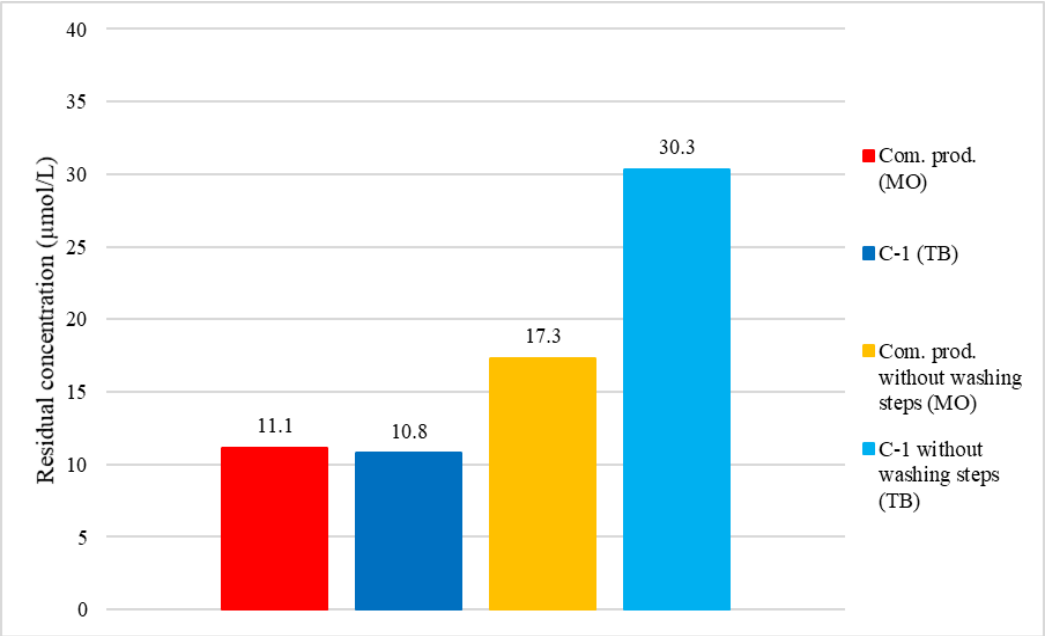


Figure 54 – TB test for sample C-1 compared to the MO binding test for commercial sample

Since the result obtained with sample C-1 is positive, it has been chosen to be compared with the sample B-7 using the filtration test at pH = 7.4 and the extractable test, explained in the two following chapters.

3.2.6 Methyl orange and toluidine blue tests at pH = 7.4 for samples B-7 and C-1

The filtration tests at pH = 7.4 are performed in order to study the dependence of the filtration on the pH (7.4 value simulates the physiological pH). The values reported in *Figure 55* are sensibly lower if compared to the one obtained at non-physiological pH, showing a dependence of the two cross-linked structures on the pH. This dependence was already suspected due to the chemical composition of the hydrogel, with amino groups for sample B-7 and acidic groups (-OH) for sample C-1, and it can decrease or increase the capacity of the filter to bond the MO and TB molecules. Both the values in *Figure 55* are significantly lower than the values obtained with the MO and TB test at acidic or basic pH, but they are higher if compared to the commercial product that has a value equal to zero.

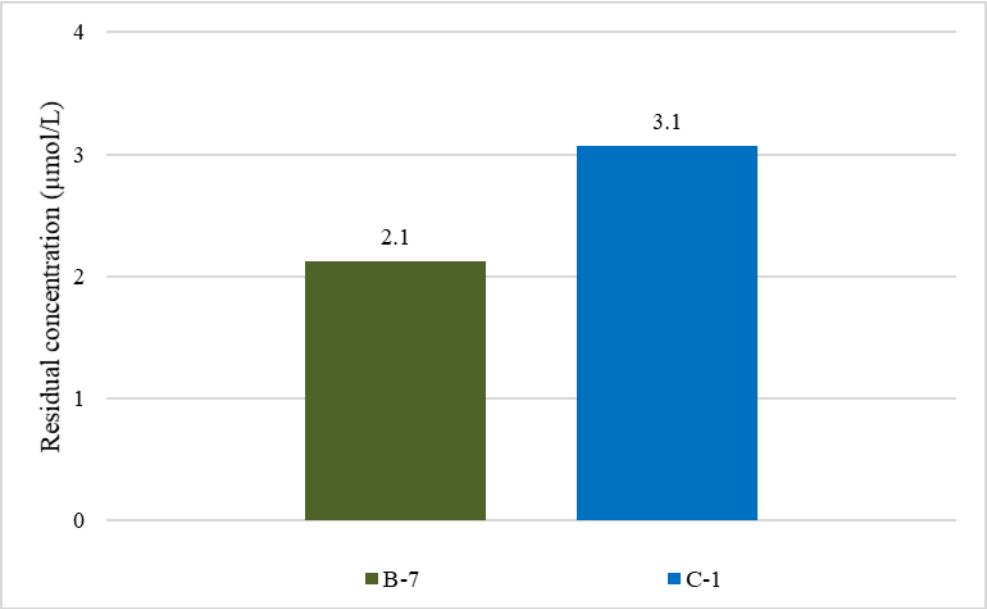


Figure 55 – Comparison between the residual concentration of MO and TB in filtering media B-7 and C-1 respectively for MO and TB test at pH = 7.4

3.2.7 Extractable tests for Polymer B modified PBT (sample B-7) and Polymer C modified PBT (sample C-1)

The same procedure described in Chapter 3.1.5 to test the unmodified PBT is employed to test the samples B-7 and C-1. The extractions are performed at room temperature (RT) without preheating the filtering media. The results are described in the following points:

- Test for reducing substances:

		Na_2SO_3 (mL)			Average (mL)	S1 – S0 (mL)
	S0	19.65	19.60	19.60	19.62	0
B-7	**S1**	19.20	19.10	19.40	19.23	-0.38
	S0	19.70	19.60	19.60	19.63	0
C-1	**S1**	19.60	19.45	19.50	19.52	-0.12

Table 15 - Values of the test for reducing substance for samples B-7 and C-1

Considering the limit value of (S1 –S0) = 2 mL, the test can be considered passed, with the values that are similar to the ones obtained for the unmodified PBT at the

same conditions. The negative values can be caused by absorption of reducing substances contained in DFW.

- Test for non-volatile residue:

		Mass gained (mg)	Difference (S1-S0) (mg)
	S0	6.5	0
B-7	**S1**	8.3	1.7
	S0	3.4	0
C-1	**S1**	6.3	2.9

Table 16 - Values of the non-volatile residue test for samples B-7 and C-1

The results are good for both the samples B-7 and C-1 that seems to lose less substance than the unmodified PBT (4.6 mg) tested at the same conditions. Probably the formation of the hydrogel on the filtering media surface makes it stronger and less material is transferred from the filter to the water.

- Test for titration acidity-alkalinity:

		HCl (mL)			Average (mL)	S1 – S0 (mL)
	S0	3.20	3.10	3.30	3.20	0
B-7	**S1**	2.95	3.10	3.10	3.05	-0.15
	S0	3.00	3.10	3.15	3.08	0
C-1	**S1**	3.10	3.15	3.12	3.13	0.05

Table 17 - Values of the test for acidity-alkalinity for samples B-7 and C-1

Also this test is passed with values that are considerably below the limit (1 mL) and the alkalinities of the solutions that are all similar among them. The negative values for sample B-7 means that the extracted solution is more acidic than the DFW and C-1 extract; probably the B-7 filter absorbs some basic compounds from the DFW, reducing its alkalinity.

- pH measurements:

		pH			Average	St. dev.
	S0	8.35	8.30	8.25	8.30	0.05
B-7	**S1**	8.21	8.17	8.18	8.19	0.02
	S0	8.58	8.56	8.54	8.56	0.02
C-1	**S1**	8.51	8.47	8.46	8.48	0.03

Table 18 - Values of the pH measurements for the samples B-7 and C-1

The pH measurements confirm the results obtained with the previous test; in fact, the values are all similar and uniform, establishing that no relevant differences are between the B-7 and C-1 samples in terms of basicity.

- Test for absorbance UV-Vis:

		λ	Abs
	S0	Baseline	0
B-1	**S1**	250	0.00131
	S0	Baseline	0
C-1	**S1**	250	0.00264

Table 19 - Values of the test for absorbance for the samples B-7 and C-1

The values reported in *Table 19* are plotted in the following graph:

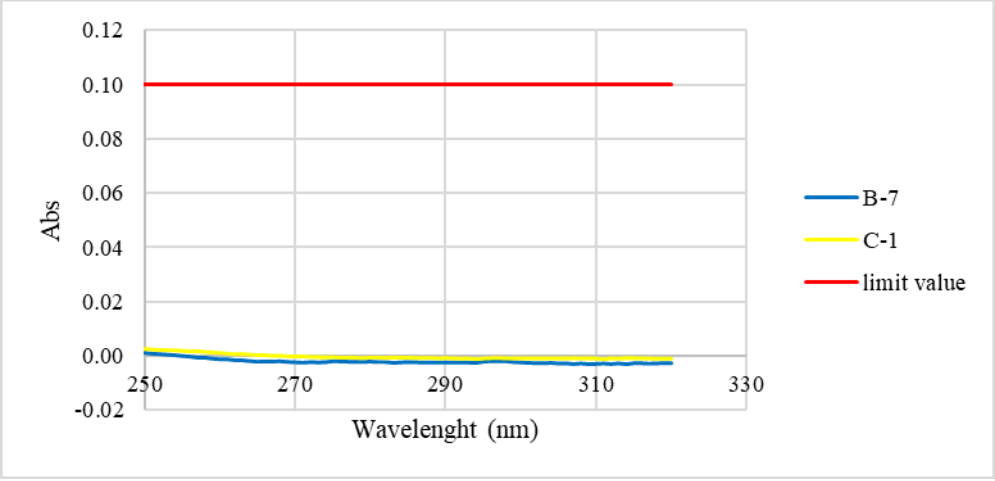


Figure 56 – UV-Vis test for extracts of samples B-7 and C-1

As shown by the graph above, also for this last test the values are inside the limit value (0.1).

In conclusion, the extractable tests are all positive with every result below the limit value. This means that both B-7 and C-1 filters can be used for medical applications.

3.3 TGA for commercial product, B-7 and C-1 samples

The two best samples B-7 and C-1 are lastly analysed using the TGA. The results are compared to the values obtained for the unmodified PBT and the commercial product. We decided to carry out this test in order to understand if the formation of the cross-linked structure brings to a different behaviour of the material at high temperatures.

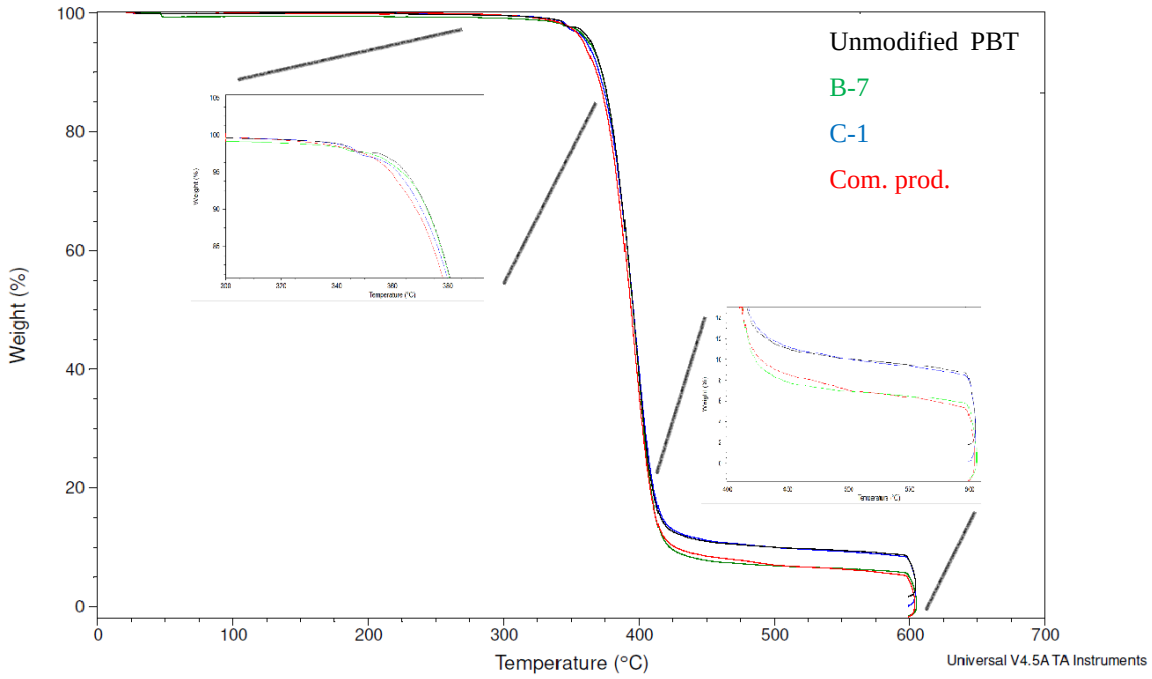


Figure 57 – TGA analysis for samples B-7 (green), C-1 (blue), commercial product (red) and unmodified PBT (black)

The analysis was firstly performed in air with a temperature ramp of 20°C/min. Because the generated curves were not decipherable, we decided to use different conditions; by consequence, the tests have been all carried out in N_2 atmosphere until 600°C with a temperature ramp of 10°C/min with a final isothermal step in air to burn all the residual

sample. As shown in the graph there are two degradation steps, one at 385°C and the second one at 600°C when the switch to air atmosphere is done. However, it is important that the material is stable until relative high temperature so that there are no problems during the modification of the filter and the expected medical applications. Only a slight difference between the samples is in the last 200 degrees after the first degradation step, but this is not important for the final use of the filtering media due to the low temperature at which they need to be used.

Conclusions

The non-woven PBT modification through Fenton-type reaction has been studied thoroughly to understand the influence of free-radical polymerization on non-woven filtering media for medical application. The goal of the work was to improve and potentially replace an existing commercial product manufactured by simple dip-coating of non-woven PBT by a polymeric solution. The application of this type of filter is for leukodepletion. After the characterization of the non-woven unmodified hydrophobic PBT, three different water-soluble polymers have been studied for its functionalization.

Polymer A was the first water soluble polymer used to modify the PBT. Its molecular structure is characterized by two terminal amino groups, hence was used to impart positive surface charge along with high hydrophilicity, confirmed by the contact angle measurements. Influence of the cross-linker amount on the stability of the structure was studied which indicated that higher amounts of cross-linker during the modification process leads to a stable structure. However, this also decreased the free amino groups responsible for the surface charge of the filter. In order to study the influence of the modification on the surface charge, methyl orange binding test (MO test) was carried out which indicated higher positive charge if the residual MO on the membrane was high. At certain concentrations, Polymer A showed comparable positive charge with the commercial product. The air flow and water flow rate tests showed no change before and after modification suggesting the voids are not overly stacked with the hydrogel. However, the biggest problem with Polymer A was the storage as it solidifies at room temperature and has to be stored at particular conditions in order to prevent its damage.

The second water-soluble polymer used for the PBT modification is Polymer B, a hyperbranched poly(ethyleneimine) that should have a better reactivity and positive charge than Polymer A due to the higher free amino groups concentration. This is confirmed by the MO binding test performed that showed better results than Polymer A ones. However, the modified filters surface was not always homogeneous, showing inconstant contact angle results and a yellowish colour when Polymer B concentrations above 5% w/v were used. Consequently, the reagents concentration (with specific attention on $FeCl_2$) was reduced in order to eliminate the coloration and achieve a good wettability, combined with the optimal positive surface charge. By testing the modified filters, the filter B-7 has been

found out to be the best with good wettability, a positive charge comparable to the commercial product and good air flow and water flow rates. For these reasons, the sample B-7 has been tested with two further analysis: MO test at pH = 7.4 (physiological pH) and the extractable test. The first test showed a clear dependence of the residual MO concentration in the filter on the pH with a lower final value compared to the MO test at acidic pH, but still positive and higher than the one obtained from the commercial product. On the other hand, the extractable test showed good results with every value obtained below the limit imposed by the UNI EN ISO 1135-4 [17].

The last water-soluble polymer used for modifying the PBT was the Polymer C, characterized by acidic groups capable to impart negative surface charge to the filter. The concentrations used for the non-woven PBT modification were the same as B-7. Tests done on the sample modified with this polymer showed results similar to the B-7 ones, with a slight difference for the test for surface charge (TB/MO test) performed at physiological pH.

Indeed, both the Polymer B and the Polymer C modified samples seem to be suitable for the substitution of the commercial product. The most important properties obtained for samples B-7 and C-1 are the following:

- No storage problems for both the water-soluble polymers;
- Controllable reaction and product;
- Stable hydrogel structure (mostly for Polymer B modified samples);
- High hydrophilicity at low concentrations;
- High surface charge.

Appendix

Appendix 1: Method to test the positive charge of filtering media

Methyl orange is an acidic dye, which can interact and bind to the positive charges on the filter surface and which can be removed by using appropriate bases. By determining the amount of methyl orange bound with the filtering media, the quantity of positive charge can be determined by using UV-Vis spectrophotometer as explained in the procedure below (based on [15]).

I. Solutions preparation

Prepare the following solutions in analytical way; they will be used for calibration curves and for the analysis.

1) Solution 1: NaH_2PO_4 solution (0.5 M)

Compound	Mass	Concentration (M)
A. $NaH_2PO_4 \cdot H_2O$ (138 g/mol)	17.45 g	0.5 M of NaH_2PO_4 (in 250 mL double filtered water)

2) Solution 2: K_2CO_3 solution (0.1 M)

Compound	Mass	Concentration (M)
B. K_2CO_3 (138 g/mol)	0.70 g	0.1 M (in 50 mL double filtered water)

3) Preparation of:
   a. Solution 1 + methyl orange (solution 3.A) (0.01% w/v)

Compound	Mass	Concentration (% w/v)
C. Methyl orange (327 g/mol)	0.025 g	0.01% w/v (in 250 mL solution 1)

NOTE: check the pH of the solution: it should be ≈ 4.5. Only if you want to do the analysis at physiological pH (= 7.4) prepare the solution 3.A using phosphate buffer instead of solution 1.

b. Double filtered water + methyl orange (solution 3.B) (0.01% w/v)

Compound	Mass	Concentration (% w/v)
C. Methyl orange (327 g/mol)	0.02 g	0.01% w/v (in 200 mL double filtered water)

c. Solution 2 + methyl orange (solution 3.C) (0.01% w/v)

Compound	Mass	Concentration (M or w/v)
B. K_2CO_3 (138 g/mol)	2.76 g	0.1 M (in 200 mL double filtered water)
C. Methyl orange (327 g/mol)	0.02 g	0.01% w/v (in 200 mL double filtered water)

II. Experimental procedure

1) Sample preparation:
   a. Punch 25 mm diameter of the required sample by carefully selecting the part of the filtering media that has no defects.
   b. Make sure that samples are clean of dust particles, fingerprints, etc. before using.
2) Methyl orange binding:
   a. Place prepared filtrating media sample in the clean filter holder and close it tightly (as shown in the *Figure 58*).
   b. Suck in the clean syringe 5 mL of solution 3.A and ensure that there are not air bubbles in the syringe; if there are, point up the syringe (without the filtering media holder) and push out the air bubbles, making sure that there are still 5 mL in the syringe.

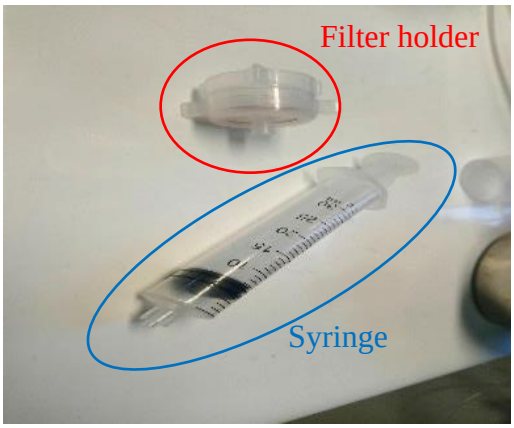


Figure 58 - Syringe filter set-up

c. Fix the syringe to the holder and push gently the 5 mL of solution 3.A through the filter.
d. Collect the filtered solution, mark it correctly and store it for further UV-Vis analysis.
e. A part of the filtered solution will remain in the space above the filter: remove the syringe and suck in air, then push gently the air through the filter to ensure that all the liquid passes through the filter and collect this as well.
f. To compare samples, it must be ensured that they are all in contact with the same quantity of solution 3.A.

3) Washing of unbound methyl orange:
   a. Rinse the filtering media used above for methyl orange binding with 20 mL of double filtered water (DFW) using the syringe filter set-up as above (for filtering media pore size < 1µm → 3 times; pore size > 1 µm → 6 times).
   b. Make sure to collect the double filtered water used in different containers, in order to further analyse it in UV-Vis spectrophotometer one by one.
4) Removal of bound methyl orange:
   a. Irrespective of how the sample looks on visual inspection (yellow → binding of methyl orange / white → no/less binding of methyl orange), filter 5 mL of the solution 2 using syringe filter set-up as explained in point II.2 to remove the methyl orange bound with the filtering media.
   b. If the colour of the filtering media is yellow/orange also after the use of solution 2, open the membrane holder and place the filtering media into the solution obtained in point II.4.a; then, shake the solution with the filtering

media for 2 minutes and proceed again with the filtration of the shaken solution using the same filter.

c. Collect the obtained solution as done in point II.2.d.

NOTE: Collected samples must be stored with correct labelling to further analyse them in UV-Vis according with the point II.2.d.

III. Obtaining of the calibration curves

Three calibration curves are obtained from the respective solutions 3.A, 3.B and 3.C by plotting **[Abs = f(conc.)]**. From the obtained curves the trend line needed to calculate the values of concentrations of the collected solutions can be extracted.

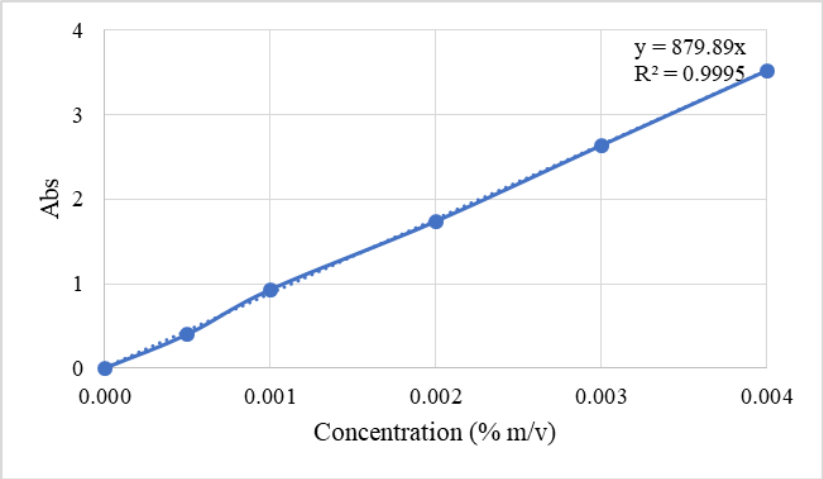


Figure 59 – Typical calibration curve (in this case for solution 3.A)

IV. Obtaining of concentration values of collected solutions

All the solutions collected in points II.2.d (methyl orange in NaH_2PO_4), II.3.b (6 water samples) and II.4.c (methyl orange in K_2CO_3) are analysed with the UV-Vis spectrometer using the corresponding blanks as shown below:

Solution	Blank / baseline
II.2.d	Solution 1
II.3.b	Double filtered water
II.4.c	Solution 2

Using the corresponding trend line equations obtained in point III it is possible to calculate the concentrations of the different solutions analysed above.
The residual concentration (μmol/L) in the filtering media can be obtained subtracting to the concentration of solution 3.A the concentration of every filtered solution.

NOTE: make sure to analyse also the feed solution 3.A using the solution 1 for blank/baseline.

Appendix 2: Method to test the negative charge of filtering media

Toluidine blue is a basic dye which can interact and bind to the negative charges on the filter surface and which can be removed by using appropriate acids. By determining the amount of toluidine blue bound with the filtering media, the quantity of negative charge can be determined by using UV-Vis spectrophotometer as explained in the procedure below (based on [16]; the toluidine blue binds 1:1 with the functional groups of Polymer C).

I. Solutions preparation

1) Solution 1: acetic acid solution (50% v/v)

Compound	Volume	Concentration (% v/v)
A. Acetic acid (60.05 g/mol)	25 mL	50% v/v (in 50 mL double filtered water)

2) Preparation of:

a. Double filtered water + toluidine blue at pH 10 (solution 2.A) (0.01% w/v)

Compound	Mass	Concentration (% w/v)
B. Toluidine blue (270.37 g/mol)	0.01 g	0.01% w/v (in 100 mL double filtered water)

NOTE: check the pH of the solution: it should be ≈ 6.5. Bring it to 10 using a NaOH diluted solution. Only if requested to do an analysis at physiological pH (= 7.4) the solution is prepared using phosphate buffer.

b. Double filtered water + toluidine blue at pH = 6.5 (solution 2.B) (0.01% w/v)

Compound	Mass	Concentration (% w/v)
B. Toluidine blue (270.37 g/mol)	0.025 g	0.01% w/v (in 250 mL double filtered water)

c. Solution 1 + toluidine blue (solution 2.C) (0.01% w/v)

Compound	Volume/ Mass	Concentration (% v/v or w/v)
A. Acetic acid (60.05 g/mol)	100 mL	50% v/v (in 200 mL double filtered water)
B. Toluidine blue (270.37 g/mol)	0.02 g	0.01% w/v (in 200 mL double filtered water)

II. Experimental procedure

1) Sample preparation:
   a. Punch 25 mm diameter of required sample by carefully selecting the part of the filtering media that has no defects.
   b. Make sure that samples are clean of dust particles, fingerprints, etc. before using.

2) Toluidine blue binding:
    a. Place prepared sample of the filtrating media in the clean membrane holder and close it tightly (as shown in the *Figure 58*).
    b. Suck in the clean syringe 5 mL of solution 2.A: ensure that there are not air bubbles in the syringe; if there are, point up the syringe (without the filtrating media holder) and push out the air bubbles, making sure that there are still 5 mL in the syringe.
    c. Fix the syringe to the holder and push gently the 5 mL of solution 2.A through the filter.
    d. Collect the filtered solution, mark it correctly and store it for further UV-Vis analysis.
    e. A part of the filtered solution will remain in the space above the filter: remove the syringe and suck in air, then push gently the air through the filter to ensure that all the liquid passes through the filter and collect this as well.
    f. To compare samples, it must be ensured that all the samples are in contact with the same quantity of solution 2.A.
3) Washing of unbound toluidine blue
    a. Rinse the filtrating media used above for toluidine blue binding with 20 mL of double filtered water (DFW) using the syringe filter set-up as above (for filtrating media pore size < 1μm → 3 times; pore size > 1 μm → 6 times).
    b. Make sure to collect the double filtered water used in different containers, in order to further analyse it in UV-Vis spectrophotometer one by one.
4) Removal of bound toluidine blue
    a. Irrespective of how the sample looks on visual inspection (blue → binding of toluidine blue / white → no/less binding of toluidine blue), filter 5 mL of the solution 2 using syringe filter set-up as explained in point II.2 to remove the toluidine blue bound with the filtering media.
    b. If the colour of the filtering media is blue also after the use of solution 1, open the membrane holder and place the filtering media into the solution obtained in point II.4.a; then, shake the solution with the filtering media for 2 minutes and proceed again with the filtration of the shaken solution using the same filter.
    c. Collect the solution obtained as done in point II.2.d.

NOTE: Collected samples must be stored with correct labelling to further analyse them in UV-Vis according with the point II.2.d.

III. Obtaining of the calibration curves

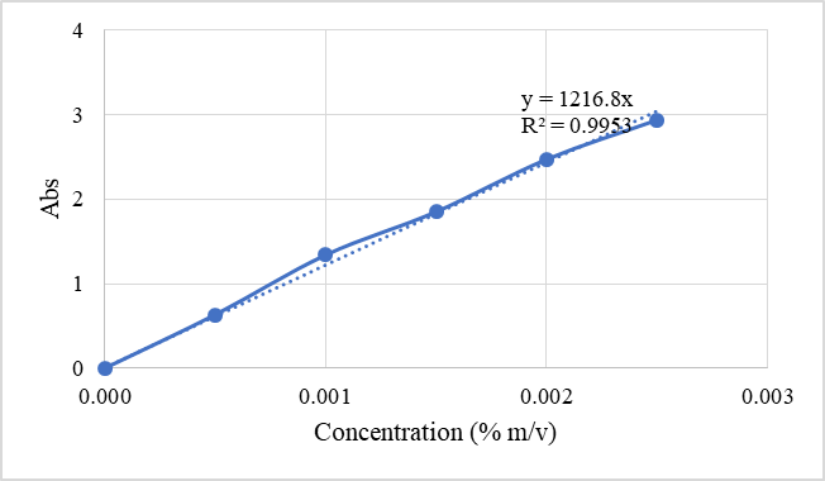


Figure 60 - Typical calibration curve (in this case it is for solution 2.B)

Two calibration curves are obtained from the respective solutions 2.B and 2.C by plotting **[Abs = f(conc.)]**. From the obtained curves the trend line needed to calculate the values of concentrations of the collected solutions can be extracted.

IV. Obtaining of concentration values of collected solutions

Analyse the solutions collected in points II.2.d (toluidine blue in double filtered water at pH = 10), II.3.b (6 water samples) and II.4.c (toluidine blue in acetic acid) using the UV-Vis spectrophotometer using the corresponding blanks as shown below:

Solution	Blank / baseline
II.2.d	Double filtered water
II.3.b	Double filtered water
II.4.c	Solution 1

Using the corresponding trend line equations obtained in point III calculate the concentrations of the different solutions analysed above. Obtain the residue concentration

(μmol/L) in the filtering media subtracting to the concentration of solution 2.A the concentration of every filtered solution.

NOTE: make sure to analyse also the feed solution 2.A using the double filtered water for blank/baseline.

Appendix 3: Procedure for the extractable test

I. Sample praparation

1) Cut the filtering media in order to have an area of 35.3 cm^2.
2) Place the filtering media in the hole of the metal plate and place the o-ring on it and assemble the Amicon cell following the instructions manual.

II. Experimental procedure

1) Fill the Amicon cell with 200 mL of double filter water using a funnel.
2) Turn on slowly the stir until reaching 300 rpm.
3) Leave the system and, after 2 hours, collect the water.
4) Repeat from points I.1 to II.3 for all the samples to test and mark the containers in a proper way in order to recognize them.

III. Test for exctractables for the collected samples (based on [21])

NOTE: When requested, as blank solution for the analysis below, use double filtered water (it has to be collected and stored from the same batch of the one used for the extractions)

blank solution (S0) = double filtered water

1) Test for reducing substances:
   a. Add 10 mL of solution collected in point II.3 to 10 mL of $KMnO_4$ 0.002 mol/L solution and 1 mL of H_2SO_4 1.0 mol/L solution.
   b. Agitate and allow to react for 15 minutes at room temperature.
   c. Add 5 drops of starch solution.
   d. Add 0.1 g of KI and titrate the solution against a $Na_2S_2O_3$ 0.005 mol/L solution, until it turns color in light brown.
   e. Repeat points from III.1.a to III.1.d for the blank solution.

f. Calculate the volume of $KMnO_4$ 0.002 mol/L solution consumed as the difference between the two titrations.

Iodide titration reactions:

1. $2\ KMnO_4 + 10\ KI + 8\ H_2SO_4 \rightarrow 2\ MnSO_4 + 5\ I_2 + K_2SO_4 + 8\ H_2O$
2. $5\ I_2 + 10\ Na_2S_2O_3 \rightarrow 10\ NaI + 5\ Na_2S_4O_6$

Knowing the total added volume and the concentration of $Na_2S_2O_3$ solution calculate the moles of $Na_2S_2O_3$ reacted in reaction 2 and, by consequence, the reacted moles of I_2 (1 mole of I_2 for 2 moles of $Na_2S_2O_3$); then calculate the moles and volume of $KMnO_4$ reacted in reaction 1 (2 moles of $KMnO_4$ for 5 moles of I_2).

2) Test for non-volatile residue:
   a. Weigh a wide base beaker and note the weight.
   b. Transfer 50 mL of solution 1 collected in point II.3 to the wide base beaker; placing it on a heating plate, evaporate the solution at a temperature just below the boiling point (ex. 95°C) until dryness is reached.
   c. Dry to constant mass at 105°C and, after placing the beaker in exicator for cooling it, weigh it.
   d. Repeat points from III.2.a to III.2.c for the blank solution.
   e. Express the difference between the residual mass obtained from the extracted solutions and the blank solution in mg:

$$m\ (s) - m\ (b) = mass\ of\ non\ volatile\ residue$$

   where: m (s) is the mass of dried solution and m (b) is the mass of dried blank solution.

3) Test for titration acidity-alkalinity:
   a. Add 0.1 mL of Toshiro indicator solution to 20 mL of exctracted solution in a titration flask.
   b. If the color of the resulting solution is violet, titrate it with NaOH = 0.01 mol/L until a greyish color appears.
   c. If the color of the resulting solution is green, titrate it with HCl = 0.01 mol/L until a greyish color appears.
   d. Calculate the acidity/alkalinity knowing the mL of NaOH/HCl used.
   e. Repeat points from III.3.a to III.3.d for blank solution.

4) pH:

   Using a pHmeter measure the pH of the extracted solutions and compare to the pH of the blank solution.

5) Test for absorbance (UV-Vis):

   Test the extracts with the UV-Vis specrtophotometer in the range of 250-320 nm after registering the baseline with the blank solution.

Appendix 4: Methodology for Fenton-type reaction

I. Cut the sample with a defined area (18.1 cm^2 for disks and 120.2 cm^2 for rectangular samples). In case of disks, up to six samples can be prepared.

II. Measure the weight and thickness of the samples before the modification.

NOTE: make sure to be able to recognize the samples easily in order to calculate the weight difference in the right way at the end of the experiment.

III. Preparation of solution 1

1) Place the cross-linker and H_2O_2 in a proper solvent (solvent 1) as per the required concentrations with total volume of 225 mL and mix well.
2) If disks are used for the modification, pour the solution 1 in six different containers and mark them correctly.

IV. Preparation of solution 2

1) Place the polymer, $FeCl_2$ and reducing agent in water as per the required concentrations with total volume of 225 ml and mix well.
2) If disks are used for the modification, pour the solution 2 in six different containers and mark them correctly.

V. Prepare 225 ml of SDS diluted solution for the washing step; then, if disks are used for the modification, place 40 mL of the prepared SDS diluted solution in 6 different beakers marking them correctly.

VI. Follow the steps below for the modification process:

a. Place the PBT disks one by one in the containers of solution 1 (filter 1 in the container 1, filter 2 in the container 2, and so on). If the rectangular sample is used, place it directly in the total solution 1.

b. Place the containers with the filters on the shaker for fixed time.
c. Transfer the filters from containers with solution 1 to containers with solution 2.
d. Place the containers on the shaker for fixed time.
e. Dry the filters in the oven at set temperature for sufficient fixed time.
f. Place the six filter disks in the containers of the SDS diluted solution for fixed time. If the rectangular sample is used, place it directly in the SDS diluted solution.
g. Place the six filter disks in six different beakers containing reverse osmosis water and place them on the shaker for fixed time. If the rectangular sample is used, place it directly in a beaker with enough RO water.

NOTE: if needed repeat points VI.f and VI.g in the same order for washing in a better way the samples; then proceed with point VI.h.

h. Dry the filters in the oven at set temperature for sufficient fixed time.

VII. Measure the weight and thickness of the filter disks after the modification, then proceed with other analysis.

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