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1. INTRODUCTION

The main motivation of the study is the possibility to build a sensor based on cellulose. The world is living a change, the companies are now considering the need to use renewable resources instead of fossil materials. I always have been an enthusiastic about materials and biomaterials that can be used in biomedical applications. When I unified these two interests, I find cellulose as a possible solution to environmental and circularity problems. The polyvalence and the many applications of this material can be massive.

In the biomedical field, electrodes are one of the sensors most used to acquire bio-signals [4]. There are plenty of biomedical processes to obtain body constants that require an electrode for bio-signals acquisition. Hospitals nowadays use hundreds of electrodes every day. The currently commercial electrodes are not reusable and are built with plastics that are non-biodegradable materials. Then the alternative would be to a biodegradable environmentally friendly electrode that can help to achieve the sustainable and environmental global objectives.

Another motivation to perform this study is that an electrode, is a device that I am very acquainted. I used some electrodes doing some university projects and I know how to use them and the principal requirements to build a functional one. The project is related to my degree and I think that will contribute to my formative dimension and to improve in a field where I am not an expert, such as the biomaterials for medical application.

This project started with two weeks stay Åbo Akademi University in Turku, Finland. There I was working in the Laboratory of Natural Materials Technology, a research group led by Dr. Chunlin Xu. During the period, I earned the knowledge that I have used to develop my project here at the University of Girona.

2. PREVIOUS CONCEPTS

2.1. CELLULOSE AND NANOCELLULOSE

2.1.1. CELLULOSE AS A RAW MATERIAL

Cellulose is a molecule found in the cellular structure of almost all plants, even marine algae also contain cellulose. This compound offers strength and structure to a plant's cell walls. It is made up of carbon, hydrogen and oxygen, and it is considered to be the most

abundant organic (based on carbon) compound on earth as it conforms the major part of its biomass. It is found as a structural component in wood, annual plants, algae, certain fungi, tunicates, and some bacteria. Furthermore, it is a renewable and inexhaustible material which has become a primary product of several fields in industry. This polysaccharide is also very important in nature as a protection element of the vegetal cellular wall. [2]

Figure 1. Wood as a source of cellulose

Recently the cellulose has become one of the most interesting biomaterials for developing medical and electronic devices. This happened due to its applications and the incredible mechanical properties. The most significant one is the rich hydroxyl groups for modification that we can find in the fibres. This provides the biomaterial with a big polyvalence and gives plenty of possibilities to explore. Also, it is significant the fact that it is 100% environmentally friendly. The combination of all the features makes plenty of possibilities to explore around cellulose.

Nanocellulose fibres are obtained from wood-derivate fibre (pulp) that has been defibrated to the nano level using different possibles techniques. Nowadays, it is one of the most advanced biomass materials. The material is extracted from plants and creates a low environmental impact in production and disposal. The structure of the material shown in Figure 2.

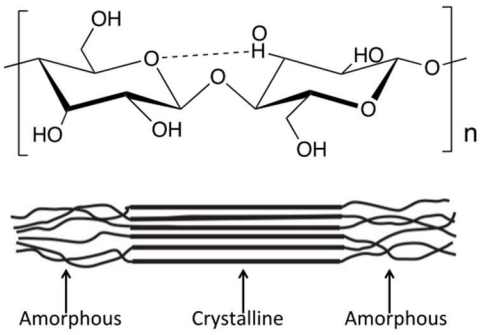


Figure 2. The picture shows the structure of cellulose. [2]

2.1.2. NANOCELLULOSES

Cellulose has highly ordered regions with crystalline structure and amorphous regions. The crystalline parts can provide the structure of stiffness and strength and the amorphous ones contribute to the flexibility of the material. Nanocellulose fibres are built the cellulose polymer chains, where the size of the fibres can be 3-100 nm of size in diameter and 1-4 mm in length. [2] Nanocellulose structure has various hydroxyls groups that produce strong hydrogen bonding.

CELLULOSE NANOFIBRES (CNF)

The obtention of the cellulose nanofibers can be done with several methods. Usually there is a pre-treatment to the fibre, because the mechanical process of separating the celluloses chains in nanocellulose fibres is very demanding in energetic conditions.

There are several pre-treatments to apply, the most important are the enzymatic hydrolysis and the chemical pre-treatment. Enzymatic hydrolysis neutral charged fibre. One very common pre-treatment is TEMPO oxidation, that will be the method used in this project, this method provides the fibre with surface charge. Finally, it is also possible to use mechanical pre-treatments, there are different machines (refiners) that apply pressure to the fibre to separate and fibrillate the cellulose chains. [6]

After the pre-treatments, there is one final step called homogenization, where the cellulose chains opens and go from the micrometric range to the nanometric range.

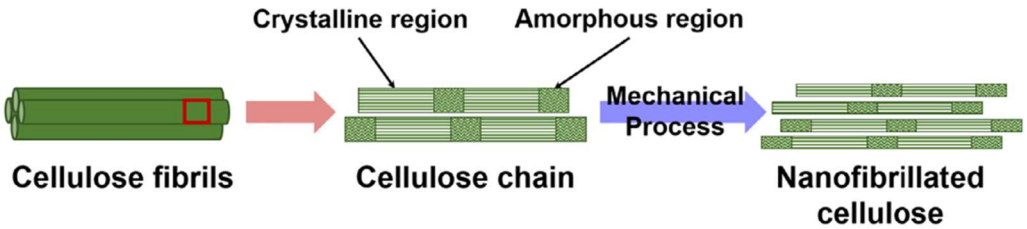


Figure 3. Schematic of nanofibrillated cellulose which can be extracted from cellulose chains using mechanical process to cleavage the fibre into nanometre size in diameter.[2]

CELLULOSE NANOCRISTALS (CNC)

Another type of nanocellulose is the cellulose nanocrystals. For the extraction of nanocrystalline cellulose the option is to apply acid hydrolysis. This acid hydrolysis uses different acids or an enzymatic process. Acid hydrolysis gives us a low efficiency and a high production of sub-products. The reaction will need of an elevated energy quantity. Enzymatic hydrolysis with less energy can give a pure product of cellulose. [2]

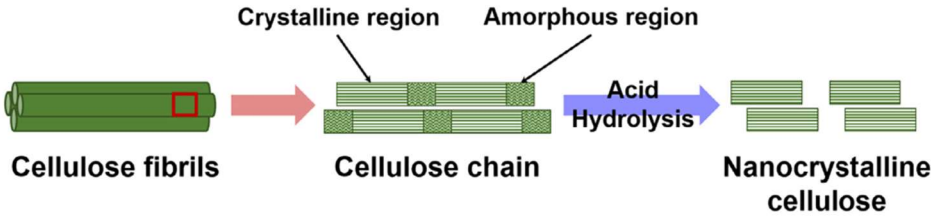


Figure 4. Schematic of nanocrystalline cellulose which can be extracted from cellulose chains using acid hydrolysed amorphous region and left only crystalline region. [2]

BACTERIAL CELLULOSE (BC)

Other possibility to obtain CNF is using bacterial cellulose (BC). This can be done using bacteria. These bacteria will consume a solution based on low molecular weight sugars and will leave a coat of nanocellulose (NC). This NC has the same chemical composition as the other two kinds. The type of the bacteria used for this process is the Gluconacetobacter [10]. A short explanation on how do the synthesis of BC in Figure 5.

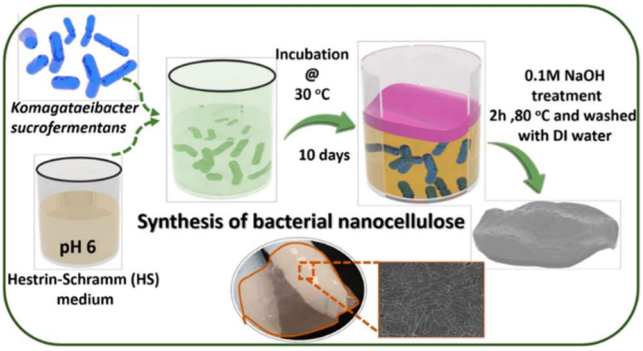


Figure 5. Fabrication example of BC [10]

This BC has established to be a remarkably versatile biomaterial and can be used in wide variety of applied scientific endeavours, especially for medical devices. With the increased interest in tissue-engineered products the BC due to its unique nanostructure and properties is a candidate in numerous medical applications.[10]

2.1.3. NANOCELLULOSE FEATURES

CNF depending on the pre-treatment and the characterization of the fibres have different features.

In general, the most fascinating properties that I can stand out are lightweight, an incredible elastic modulus, show an incredible thermal expansion, presents a high barrier property, has a very especial behave in water spaces and can apply some pre-treatments that give facilities to polymerize the compounds that made easily useful in lots of applications. [2] Moreover, the most remarkable features are that it is a renewable raw material, biodegradable and biocompatible.

2.1.4. TEMPO OXIDATION

In this project TEMPO oxidation was used as pre-treatment, which differs from the Neutral one on the characteristic surface charge that brings to the fibre. Also, it is possible using the same pre-treatment to have different results. The main point is to decide how much oxidated must be the fibre. This will result on different mechanical properties, surface charge and the length and width of the fibre. The results will be evaluated in the future characterizations.

An oxidation happens when a substance comes into contact with oxygen or another oxidizing substance. The oxidizing agent takes electrons from that other substance. Therefore, an oxidizing agent must gain electrons. [6]

The TEMPO oxidation uses NaClO as oxidizing agent. This TEMPO oxidation causes that the primary hydroxyls of the fibres react with the TEMPO solution and oxidizes the group alcohol to an acid group [6]. Depending on the quantity of NaClO the fibres will be more or less oxidated. Also, the chains break in smaller parts when this happens. From these previous concepts, I will explain the experimentation process that I followed to do my Tempo CNF. The TEMPO substitution of the Alcohol group on primary carbon for an acid shown in Figure 6.

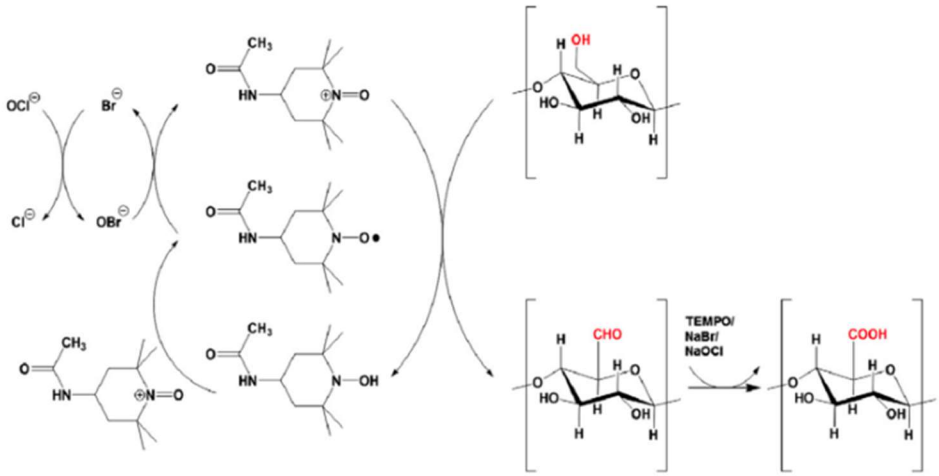


Figure 6. TEMPO substitution of the Alcohol group on primary carbon for an acid [6]

2.2. HYDROGEL DEFINITION

Hydrogel products constitute a group of polymeric materials, the hydrophilic structure of which renders them capable of holding large amounts of water in their three-dimensional networks [7]. Hydrogel structure example in Figure 7.

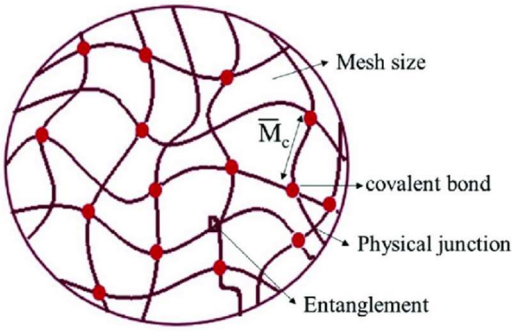


Figure 7. Hydrogel structure [8]

Hydrogels are defined as a water-swollen and cross-linked polymeric networks. This fact makes it easy to combine the polymeric chains with different monomers that can change the features of the hydrogel making the material easy to be personalized.

The ability of the hydrogel to absorb the water arises from hydrophilic functional groups attached to the polymeric backbone, while their resistance to dissolution arises from cross-links between network chains.

The latest works with the hydrogels involve the technology of the 3D printing as these hydrogels are very accurate to attach the main needed points to produce bio-inks.

Cross-linking concept is the process of forming covalent bonds or relatively short sequences of chemical bonds to join two polymer or monomer chains together [7]. One example of cross-linking in Figure 8.

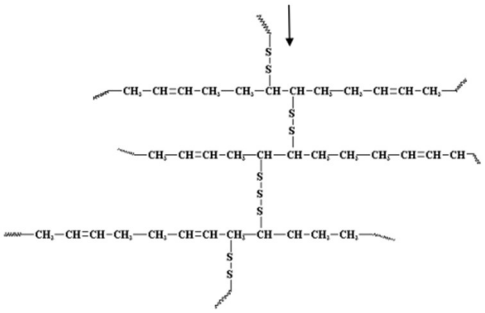


Figure 8. Cross-linking example [7]

Depending on the nature of the polymer, different techniques may be used to cause cross-linking. The cross-link happens when the monomers polymerize or by covalent bonding between polymeric chains. There are different methods to produce this phenomenon.

- Chemical conjunction.
- Temperature.
- Irradiation.
- Mechanical methods.

2.3. BIO-SIGNALS

2.3.1. DEFINTION

A bio-signal can be defined as a physiological phenomenon. This phenomenon can be measured and monitored. The number of different bio-signals is huge [12]. There are many ways to classify the bio-signals.

- Intrinsic/Extrinsic to body
- Static/Dynamic
- Origin

Intrinsic bio-signals are those that exist without any excitation from outside and are always present in Human body. One example is ECG that is induced by electrical heart muscle excitation.

Extrinsic or induced ones are artificially induced. The main difference between intrinsic and extrinsic is that extrinsic bio-signals only occur when the human body is exposed to excitation. The signal decay in the time that there is no artificial induction. Plethysmography is an example of an artificial current induced in the tissue.

Dynamic bio signals are the ones that show big changes during the time, heart rate is an example. Static bio signal is the opposite of dynamic ones. Static bio-signals may show little changes during the time and the changes may show slow variations during the time.

The origin is a kind of the description of the type of signal. For example, there are electric, magnetic, mechanic, optic, acoustic, chemical, and thermal bio-signals.

Also, bio signals can be classified as active and passive, the active is the bio-signals that the energy comes from the patient, the passive is when the energy for the measurement is from an external source.

2.3.2. ELECTROCARDIOGRAM (ECG)

The electrocardiogram (ECG) is the most widely used diagnostic tool in today's health care environment. ECG provides information and assessment of cardiac rhythm, ischemic changes, and other information for early prediction and rapid treatment of acute myocardial infarction and coronary event [1].

ECG signal is spectrum recording of the heart bioelectricity. The basic mechanism of this bioelectricity is the energy consuming cell membrane ion pumps polarizing a cell, the action potential is generated. The depolarization will generate a current flow in extracellular volume, this will result in another measurable biopotential. A pump is a molecular device, embedded in the cell membrane, capable of generating current across the membrane, and thus electro-genic [1].

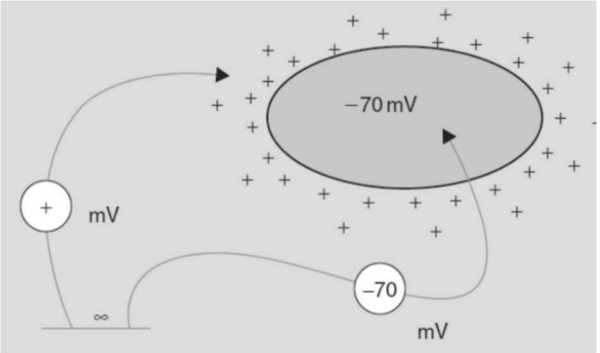


Figure 9. The polarized excitable cell [1].

The heart signal is produced by the action of polarization and depolarization of the cardiac tissue. This movement is the contraction and descontraction of the cardiac tissue, also known in the whole tissue as Systole and Diastole, are produced by the activity of sodium-potassium pumps of the cells that exchange ions depending on the concentrations.

Figure 10. Na+ - K+ ATP pump embedded in a cell membrane [1]

The cell in resting state has excessive potassium inside and excessive sodium outside. This state is named polarized state. When the muscle contracts sodium moves into the cell and potassium moves out. This movement finishes with the state of depolarized cell. This exchange can be done by the use of the transmembrane proteins located shown in the Figure 10. It is in this depolarization and polarization that the cell generates the bio-signal generating a detectable biopotential.

The shape detected named as PQRST wave, in Figure 11, can be divided in four different stages.

- P waves indicate the depolarization of the atria.
- P-R segments indicate the depolarization of the ventricle.
- QRS indicates the depolarization of ventricles.
- T and U indicate repolarization of ventricular cells.

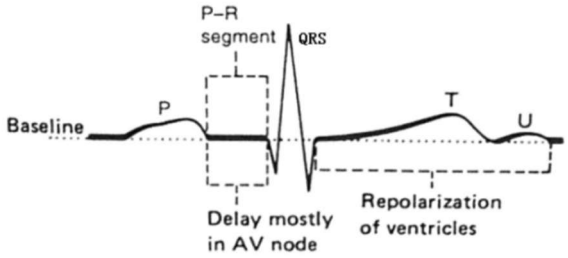


Figure 11. Components of typical electrocardiogram [1]

An ECG electrode is a sensor to transduce ionic current in the skin into electron current in metallic wire connected to the ECG machine [1].

THE IMPEDANCE OF THE HUMAN SKIN

For acquiring the current of the bio-signal, the current should pass through the body electrolyte and human skin to reach the electrode.

The skin cells are organized in layers, these layers are in the form of gap junctions, as shown in Figure 12. The transmembrane admittance depends both on the type of cell junctions and to what extent the epithelium is shunted by channels or specialized organs [1].

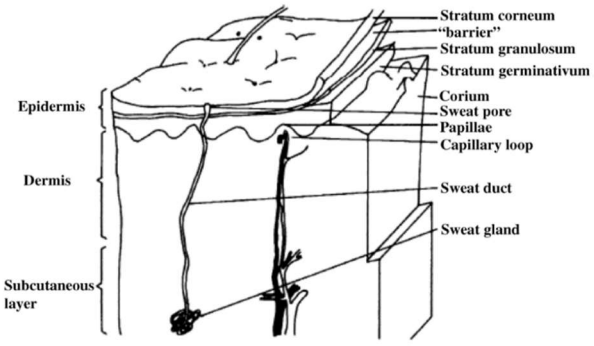


Figure 12. Cross-section view of skin structure [1]

A wet-gel application in the position that the electrodes are going to be placed usually is common in ECG procedures. This is because the use of a gel decreases the skin impedance. The hydration in skin is very important. The human skin conductivity in Figure 13.

Tissue	σ(S/m) 1 Hz–10 kHz	σ(S/m) ca 1 MHz
Human skin, dry	10^{-7}	10^{-4}
Human skin, wet	10^{-5}	10^{-4}

Figure 13. Skin conductivity [1]

HALF-CELL POTENTIAL

This potential is the result of the charge layers. This is produced when the electrolyte is placed in a chemical solution, then cations and anions redistribute and cause a potential difference known as half-cell potential. This is a static feature of the electrodes.

For knowing the half-cell potential is using a reference electrode that will be the one that has to be defined as zero potential. Some half-cell potentials of the most common electrode materials in Figure 14.

Reduction reaction	$E^0(V)$
$Al^{3+} + 3e^- \rightarrow Al$	−1.662
$Zn^{2+} + 2e^- \rightarrow Zn$	−0.762
$Cr^{3+} + 3e^- \rightarrow Cr$	−0.744
$Fe^{2+} + 2e^- \rightarrow Fe$	−0.447
$Cd^{2+} + 2e^- \rightarrow Cd$	−0.403
$Ni^{2+} + 2e^- \rightarrow Ni$	−0.257
$Pb^{2+} + 2e^- \rightarrow Pb$	−0.126
$2H^+ + 2e^- \rightarrow H_2$	0.000
$AgCl + e^- \rightarrow Ag + Cl^-$	+0.222
$Hg_2Cl_2 + 2e^- \rightarrow 2Hg + 2Cl^-$	+0.268
$Cu^{2+} + 2e^- \rightarrow Cu$	+0.342
$Cu^+ + e^- \rightarrow Cu$	+0.521
$Ag^+ + e^- \rightarrow Ag$	+0.780
$Au^{3+} + 3e^- \rightarrow Au$	+1.498
$Au^+ + e^- \rightarrow Au$	+1.692

Figure 14. Half-cell potential for common electrode material [1]

Also, this half-cell potential can be considered like an offset in the signal. The skin, when using wet electrodes behaves as shown in the equivalent electric circuit of Figure 15.

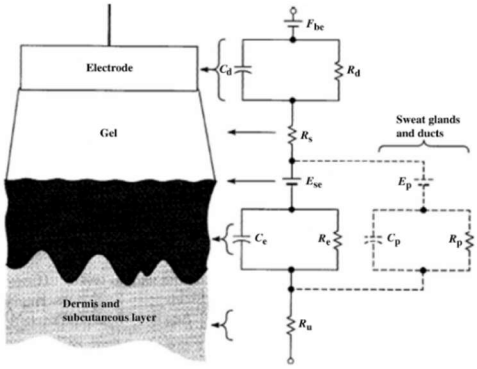


Figure 15. Equivalent electric circuit. [4]

If the electrode is a classical wet-electrode the gel usually is part of the electrode. This gives the possibility of working with just one part instead of working with some gel in the skin and an external electrode.

The bad part of working with wet-electrodes, is that the gel pad loses adhesivity and consequently can produce a decrease on how consistent is the electrical measure of the bio signal.

2.3.3. ELECTRODE FEATURES AND REQUIREMENTS

Figure 16 shows the different parts of a classical electrode. The image is extracted from a book about medical instrumentation [4]. This book explains the main features that the different biomedical objects need to have good functionality.

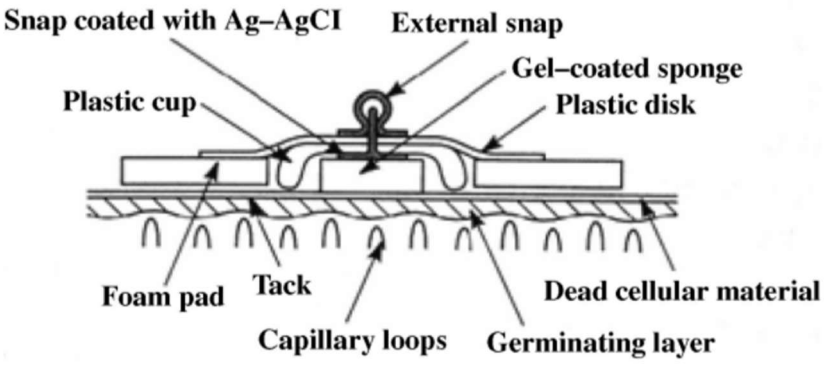


Figure 16. Commercial electrode schematic [4]

Foremost I will relate the different parts that are explained in Figure 16.

- Foam pad. This pad is a circular torus. The main functions of this pad are to give some adhesion to the human skin and make comfortable the place of the electrode. Also, must have biocompatible materials in order to don't produce allergy or problems related to the contact between the electrode and the skin.
- Gel-coated sponge. Here is placed the Ag/AgCl coating. The main function will be acquiring the bio-signal and move the bio-signal to the external snap, that will connect the electrode with ECG machine.
- Plastic disk. This will join all the parts of the electrode. Must be flexible.

As it is possible to see in the description of the different parts, all of them have plastic components. As, it is explained in the commercial applications there are lots of plastics and this fact makes necessary a biodegradable electrode application.

As in the most of the biomedical applications an electrode has some compulsory standards before its clinical application. Now I am going to explain the main points of one of the standards used in this field. ANSI/AAMI EC12:2000. This standard is established for the regulation of disposable electrodes used for diagnostic ECG.

- ELECTRICAL
    - AC impedance. None of individual pair impedances shall exceed 2kOhms.
    - DC offset voltage. After a 1-minute stabilization period a pair of electrodes connected gel to gel shall not exhibit an offset voltage greater than 100mV.
    - Combined offset instability and internal noise. After a 1-minute stabilization period, a pair of electrodes connected gel-to-gel shall not generate a voltage greater than 150 uV peak-to-peak in the pass band.
    - Bias current tolerance. The observed DC offset change across a pair of electrodes connected gel-to-gel shall not exceed 100mV when the electrode pair is subjected to a continuous 200nA DC current over the period recommended by the manufacturer for the clinical use of the electrodes. In no case shall this period be less than 8 hours.
- SAFETY REQUIREMENTS

  - Biological response. The device shall be biocompatible. Requires an evaluation of the possible cytotoxicity, skin-irritation and either skin sensitization or intra-cutaneous reactivity.
  - Pre-attached lead wire safety. Electrodes with pre-attached lead wires shall be constructed in such a manner that the lead wire connector used to mate with the instrument boot cable cannot contact ground or a possibly hazardous potential.

- ADHESIVE PERFORMANCE
  - An adhesive in a short-term resting ECG must maintain contact with the body for a period of time of 5 min to 30 min.
  - Long term analysis may require enough time to be on the patient for a period depending upon the hospital protocol.

3. STATE OF THE ART

There are lots of applications for the CNF, I will only talk about the new medical applications. Also, I must say that there are lots of studies that are improving the knowledge of the fibre treatment and the results that can have, the study of the kinetic of the reaction and the efficient production of the fibres are the fields that are living a huge development. There are different pre-treatments that can be applied to the pulp [6].

Different articles are developing electrodes for ECG using conductive elements. There are some studies that use textile-structured electrodes, that mixed with some conductive elements can perform as an electrode in ECG [1]. There are also reviews that are working with conductive elements mixed with CNF. The conclusions are that single cellulose-based systems may not be applicable to these applications [11]. Also, there are studies that combining cellulose and CNF with conductive elements, have achieved enough good electrical features to perform as bio-sensors [14].

There is a big interest on developing bio inks. This bio inks can be very useful in medical field, as shown in Figure 17. The main interest, is that the production of bio inks combined

with the advance in 3D cell printing can become a huge advance for developing functional organic tissue [9].

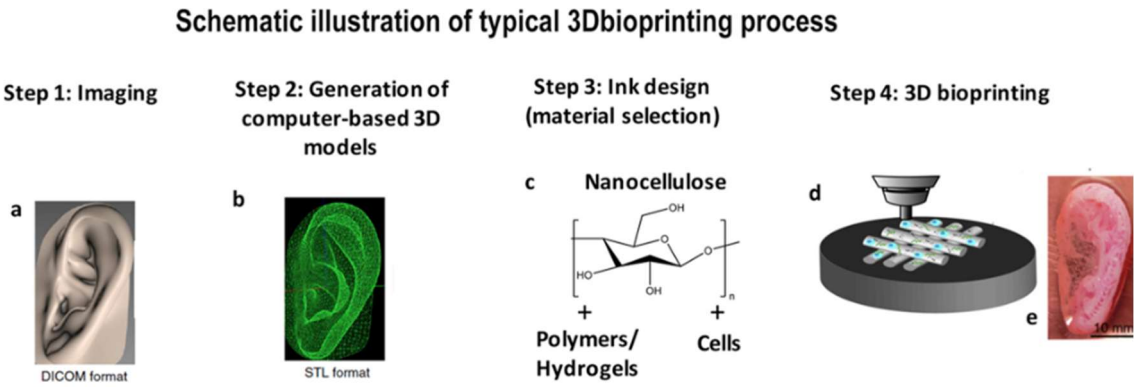


Figure 17. Example of CNF implementation as a bio ink [9]

Also, the production of hydrogels is very related with the 3D Cell culture. The hydrogels due to their good structure, can perform really well as an extracellular matrix. [10]

One of the most advanced techniques is BNC, that can be used in medical issues as wounds and scorches, very related with drug delivery systems. Also, there are applications on the medical implants. The unique characteristic of being pure cellulose biomaterial is very helpful in medical field due to there is no immune rejection in the human body [10].

4. HYPOTHESES AND OBJECTIVES

4.1. RESEARCH QÜESTION

The research question of the Project is the following one: Is it possible to elaborate CNF fibres and combine it with conductive agents to give the material the properties to work as a sensor in ECG applications?

4.2. HYPOTHESE

My hypothesis is that: It is possible to produce CNF fibres that can have the conductivity characteristics needed to perform in catching ECG bio signal.

4.3. OBJECTIVE

The general objective is to produce conductive nanocellulose to be used in sensing bio signals, specifically in ECG applications.

Then, there are specifics objectives, for example, applying to the fibre a mechanical pre-treatment in combination with the TEMPO oxidation. Also, applying TEMPO oxidation as unique chemical pre-treatment to the fibres. Another example is preparing different samples in hydrogel state and in membrane or paper form. Also, applying characterizations to all the samples in order to have a complete information to discuss about the results with a complete information.

5. MATERIALS AND METHODS

5.1. MATERIALS

During the experimentation the same initial pulp was used. The reference of the pulp is softwood sulphite Domsjö, extracted from Swedish forests. The main feature of the pulp is that has a high cellulose fibrils content. This feature is very important because the experiment works completely different using other pulp.

Also, different chemical reactive from Sigma- Aldrich were used. List below:

- TEMPO radical: 2,2,6,6-tetramethyl-1-piperidinyloxy. $C_9H_{18}NO$
- NaClO: Sodium hypochlorite
- NaOH: Sodium hydroxide
- HCl: Hydrochloric acid
- NaBr: Sodium bromide
- PEDOT:PSS PT-2: poly(3,4-ethylenedioxythiophene) polystyrene sulfonate. From Heraeus company.
- PEDOT:PSS PH-1000: poly(3,4-ethylenedioxythiophene) polystyrene sulfonate. From Heraeus company.
- PolyDADMAC: Poly(dimethyldiallylammonium chloride)
- CuED: Copper(II)-ethylenediamine complex. $C_4H_{16}CuN_4(OH)_2$
- Methyl blue. $C_{37}H_{27}N_3Na_2O_9S_3$
- pH buffer.

5.2. METHODS

5.2.1. NANOCELLULOSE PREPARATION

5.2.2. MECHANICAL PRE-TREATMENT

The mechanical pre-treatment applied to some samples was a refining (PFI). The main goal of the PFI was trying to open the fibre and make easier the procedure of the oxidation. The disadvantage was that also cut the fibre.

The program of the refiner was programmed to 4.500 revolutions. Different values were tested in order to know how was the effect of the program to the pulp. This fact has no interest because it is better to have different fibres length to work with, making a differentiation in every sample.

The use of the machine was very easy. First of all, put the pulp that was prepared before in the walls of the PFI and then introduce the gear in the space of the gear and then start the program.

5.2.3. TEMPO REACTION

The first part was to select the pulp that was used to prepare CNF. In this case, three small pieces of softwood sulphite Domsjö were taken in order to determine the humidity level that was in the paper. In this process, a thermobalance was used to dry the piece of paper and determine the consistency. This concept named consistency, is the dry mass taking into account the water of the fibre, the way of calculate the consistency in Equation 1.

$$consistency = \frac{dry\ weigh}{wet\ weig} \qquad Eq.1$$

The resulting percentage was the humidity or water content present in the pulp. Three pieces were taken, and the average was calculated to determine the exact quantity needed to achieve 30 grams of dry pulp.

In the next step, the pulp was broken into small pieces of paper. Afterward, 1.5 litres of distilled water were added. The main objective is to ensure that all the broken pulp gets wet.

A stirrer was used to agitate the solution. A gripper was employed to remove any pollutants before proceeding to shake the pulp and water mixture. The pulp was placed in the disintegrator for half an hour, as shown in Figure 18. This method was done until the solution appears mostly white. The stirring process will then continue.

Figure 18. Disintegrator

Now the TEMPO reaction explanation.

Firstly, different solutions were prepared for the Tempo reaction. The first solution prepared was a NaOH 0.5 M solution. A 500mL water solution was carefully prepared, considering the exothermic nature of this reaction. Na was slowly added to the solution while carefully shaking, with the addition of water to facilitate the combination of Na with water.

The other solution was the TEMPO solution. In this case, a 1.5L solution was prepared, dissolving 3g of NaBr and 0.48g of TEMPO. The solution was divided into two bottles. Homogenizing the solution involved using a ultrasounds bath to ensure a uniform solution. Alternatively, heating the water to 20 degrees and stirring the solution can also be effective.

Once the solutions were prepared, the experiment starts. Prior to that, a preliminary setup is required, which involves using a structure as shown in the following picture. This structure is crucial as it allows a controlled addition of NaOH and NaClO to the reaction at specific intervals during the initial minutes.

Additionally, an instrument for pH measurement was necessary, and it required from a calibration. In this case, the pH working value was around 10.5, so the instrument was calibrated using pH 7 and pH 10 solutions.

Once the instrument was calibrated, it was incorporated into the experimental setup, as depicted in Figure 19, to facilitate the measurement process.

Figure 19. Complete structure used for the TEMPO reaction

First, both TEMPO solutions were poured into the pulp with water. The solution's colour changed to a mixture of green and yellow due to the presence of TEMPO.

A quantity of 61.02 mL of NaClO was placed in one of the burettes for this particular example, as it corresponds to a TEMPO 5. The calculations of Equation 2 provided below, justify this quantity:

$$30\ g\ of\ dry\ fiber\ \times \frac{5mmol}{1g\ fiber} \times \frac{1mol}{1000mmol} \times \frac{74g\ NaClO}{1\ mol} \times \frac{100g}{15g\ NaClO} \times \frac{1ml}{1{,}22g} = 61{,}02\ mlNaClO \quad \text{Eq 2}$$

The specific mmol x fibre in the equation depended on the oxidation degree desired in the fibre. Also, adjustments may be necessary based on the concentration and density of the NaClO that was used.

To optimize the substitution of radicals on primary carbons, it was important to raise the solution's pH to approximately 10.5. NaOH was used for this purpose. Initially, the reaction was quite active, resulting in significant pH fluctuations. The first minutes, close monitoring was required, and the quantities of NaOH and NaClO added to the solution were adjusted carefully. Then, when the reaction becomes stable, pH measurements were taken every five minutes until the pH level stabilized or the reaction colour turned white, indicating complete consumption of TEMPO.

To increase the performance of the reaction, consider the following tips:

- Working in a controlled temperature room, preferably around 20 degrees Celsius.
- Adding HCl to the NaClO can help in the moment of initiate the reaction, particularly for TEMPO reactions with high oxidation levels. HCl was added until

the pH of NaClO reached 10.5. In the case that this is not used the initiation time can be of 20 minutes in high oxidation levels.

After that, the reaction was filtered to extract only the pulp. A suitable filter, with porosity that retains cellulose fibres, was chosen for this process.

The filtration was performed using a vacuum filtration setup with an Erlenmeyer flask. Using a water pump facilitated and expedited the filtration process. Shown in Figure 20 the structure of the filtration setup.

Figure 20. Filtration set up.

The filtration step can be also performed by using a Kitasato filter montage.

The Kitasato structure has two parts. The Erlenmeyer flask with the filter and tweezer that is used to fix the structure and the void pump that helped in the filtering step.

The filtering process started using approximately 10 litres of water. This method was useful to purify the pulp, as the TEMPO reaction generates significant waste. The conductivity of the filtered water was monitored during the process. Once the conductivity of the filtered water goes below 5 us/cm, was enough cleaning to the fibre. Through various tests conducted, it has been determined that approximately 10 litres of water were approximately the quantity required for a TEMPO 5 reaction.

Ensuring that the fibre was clean had a huge importance, as the project involves using the fibre in biomedical applications and the TEMPO reagent can potentially be skin-toxic. This step must be executed carefully. When this conductivity goes below the indicated

will mean that there are not waste of the TEMPO and the fibre will be clean from pollution that the TEMPO reaction can produce.

Conductimeter image shown in Figure 21.

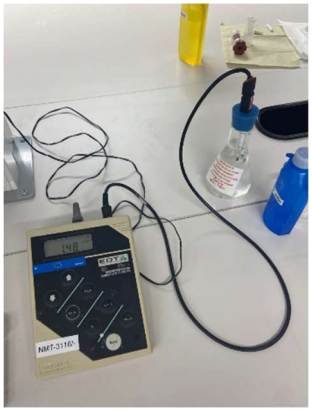


Figure 21. Conductimeter

It is important to choose in the right porosity of the filter. Taking in consideration the size of the fibre that can be expected. This is important for avoiding a loss of the fibre and also, to make faster the filtration step. It is recommended to look for standard values of the width and length of the different TEMPO oxidations with similar pulps.

Every 30 minutes clean with pressurized water the filter. This is because when the pulp is cleaned for 3rd time the fibres every time has more water absorbance and the pulp has more viscosity. This makes that the filter gets stacked and makes the filtration slowly.

The result of the filtration shown in Figure 22.

Figure 22. Fibres when are filtrated.

When the filtration is over the next step was the homogenization. This step is critical, because it transforms the fibres in the characteristic CNF fibres.

The first step was measuring the dry weight of the sample. As in the first step of the process when the dry weight of the paper was calculated, three samples were taken in order to do have the highest precision on the value. It was very important to know exactly at what consistency the sample was.

Depending on the aim of the project, the concentration of the fibres can variate but never should be more than 2% of cellulose dry mass. In this case, the concentration chosen was 1%.

It is very important to do not choose a high concentration; this can make the homogenizer bog down due to the concentration of fibres.

5.2.4. HOMOGENIZATION

The homogenizer is a machine that works applying pressure, causing the fibres to pass through a small hole and resulting in the separation of microfibers into nanofibers. It is crucial to notify that excessive pressure, relative to the current fibre width and length, can cause the machine to become stuck or result in the breakage of an internal component. Therefore, it is essential to gradually increase the pressure while ensuring that the fibre can withstand the applied pressure.

Figure 23. Homogenizer

The separation of fibres leads to an increased quantity of fibres in the dispersion, consequently transforming the solution into a hydrogel.

To begin, the measure that was taken to ensure that the machine was free from contamination was cleaning the system with distilled water. After that, the solution was poured into the machine without applying additional pressure. The time required for the complete processing cycle of the solution was recorded. This time was crucial for determining the duration of the maximum pressure phase. In this case, 1 cycle took 90 seconds, so the machine operated at maximum pressure for 15 minutes. This was calculated in order to apply 10 cycles at maximum pressure to the sample.

The pressure was gradually increasing every 3 cycles, ensuring that the process was running properly. If any issues were detected, the pressure was reduced.

The outcome was a hydrogel. The viscosity of the hydrogel variates depending on factors such as concentration, pre-treatment of the pulp, and the type of pulp chosen for the pre-treatment process. As shown in Figure 24, the final result was this hydrogel.

Figure 24. Hydrogel at 0.6%.

5.2.5. CONDUCTIVE NANOCELLULOSE PREPARATION

The preparation of the hydrogel was a combination between the protocol that was followed to prepare a conductive nanopaper and one own idea.

The desired dry mass for the sample needs to be determined. In this case, the sample was prepared with 0.5 g of dry mass. The use of a small quantity of mass is preferred to minimize fibre loss and facilitate testing in smaller samples, which can increase the efficiency of the process. For this example, a proportion of 50% CNF and 50% PEDOT: PSS was used. However, different proportions were tested in next trials. The calculations in Equation 3 and Equation 4.

$$0.5 \times {}^{50}/_{100} = 0.25\ \text{g CNF} \quad \text{Eq. 3}$$

$$0.5 \times {}^{50}/_{100} = 0.25\ \text{g PEDOT: PSS} \quad \text{Eq. 4}$$

Depending on the consistency of the fibre and the consistency of the PEDOT: PSS, different quantities of both samples were added. It was important to note that the quantities calculated in Equation 3 and Equation 4 refer to dry content, whereas the amounts that were added to the solutions have to correspond to wet content.

To ensure a homogeneous mixture of both samples, the CNF was diluted at a concentration of 0.2%, while the PEDOT: PSS was diluted at 0.5%.

To achieve homogeneity, a stirrer was used for 15 minutes for each sample. Later, an ultrasound machine was used to remove any bubbles present in the solution. These bubbles increase the difficulty of the adhesion of the PEDOT. The ultrasound machine was set to an amplitude of 60% and operated for 5 minutes, followed by a 2-minute waiting period, and after that, 5 more minutes of sonication.

To homogenize the PEDOT: PSS solution, a stirrer was used for 10 minutes. This ensured that the solution achieved a uniform consistency. Then for mixing both samples the PEDOT: PSS solution was poured into the CNF solution. It was important to pour PEDOT: PSS and not in the inverse way. Then the solution was stirring for 1 hour.

At this point, there were two options. The first option was to pour the solution through the KitaSato filter, resulting in the production of a conductive nanopaper. The second option was to transform the solution into a hydrogel form. To achieve the hydrogel state, it was necessary to remove water from the solution. The chosen method for water removal was the Rotavapor.

Before starting, it was important to consider whether the action of the Rotavapor would have any impact on the polymer chains of PEDOT: PSS. Usually, many of these polymers are sensitive to heat or light. However, in this particular case, such concerns do not arise, allowing the process to continue without issues [16].

By knowing the quantity of water present in the solution, an approximation was made and knowing that the CNF fibres in a concentration of 0.5% should become a hydrogel, it

was possible to know the quantity of water needed to remove from the solution. The objective was to remove enough water to increase the sample's concentration to around 0.4%. This was because during the experimentation time was notified, that taking off too much water could burn the sample. This issue, theoretically should not appear but in the practical field there were some problems related.

To dry the hydrogel, it was placed in petri dishes and left to dry for a period of three to four days. It was important to monitor the drying process daily to determine the level of dryness. Once it reached the desired point, a small sample was taken from the hydrogel and its consistency was calculated using a thermobalance.

5.2.6. FIBRE CHARACTERIZATION

All the procedures that are explained are from the BIAMATEC folders.

FIBER MORPHOLOGY (MORFI)

The main objective of MORFI characterization was giving some information of the width and length of the fibre. Also, gave other some useful information such as finite fibres.

This was a simple characterization; the main problem was putting to many fibres in each beaker. If the quantity of fibres exceeds the limit of 25 mg/litre could be difficult for the system to read the samples. The objective was having a clear image with all the fibres separated, this guaranteed that the software could work in the proper way.

First, it was necessary to prepare carefully all the solutions. The solution contained the fibre to analyse and 800ml of water. Then, also put two beakers with water in order to clean the camera. It was necessary to program the iterations. This was done by describing the pathing that the camera.

When, the iterations were prepared just start the program and the analyse was completed.

CATIONIC DEMAND

The procedure of the cationic demand gave information on the amount of cationic polymer required to neutralize the charge of the suspension per given amount of stock

solution. This characterization combined with the carboxyl content gave a complete analysis of the oxidation grade of the fibre.

First, if the sample had a big cationic demand (samples as TEMPO), the sample prepared had to be of 0,01 of dry mass. Then, 25 ml of poli-DADMAC and distilled water were added until 40 grams. The next step was in a centrifuge glass, put the same weight that there was in the first solution. Then, put both samples in the centrifuge in opposite holes. The next step was centrifuge at 10.000 rpm twenty minutes. The resulting sample was what was ready to be analysed. This task was done in the Mütek.

10 ml of the overflowing solution were added to the Mütek. In the screen must show a positive voltage bigger than 200 mV. If this was not like this, repeat the sample and more poli-DADMAC would be added. If everything was fine, add volume of PES-Na to the Mütek, the best way was adding little volumes (1ml). The objective now was calculating the volume needed of PES-Na to neutralize the voltage. Finally, by using the Equation 5, the Cationic Demand was calculated.

$$D.C. = \frac{\left(C_{Poly-DADMAC} \cdot V_{Poly-DADMAC}\right) - \left(C_{PES-NA} \cdot V_{PES-NA\ GASTAT} * F.\ Dilució\right)}{P_{sec\ pot\ centrifuga}} \qquad \text{Eq.5}$$

$C_{poly\text{-}DADMAC}$: Concentration of Poli-DADMAC (usually 0.001N)

$V_{poly\text{-}DADMAC}$: Volume of poly-DADMAC that has been added.

$C_{PES\text{-}NA}$: Concentration of PES-Na (0.001N)

VPES-NA: volume of PES-Na needed to neutralize the voltage.

POLYMERIZATION DEGREE

The viscosity procedure was used to know the polymerization degree of the fibre.

In 100ml test tubes some solutions as the shown in the Table 1 were prepared.

Table 1: Amount of fiber, H_2O and CuED used for the viscosity test.

Fibra seca mostra	H_2O	CuED
0 g (Blanc)	Fins a 25 g	25 ml
0,2 g	Fins a 25 g	25 ml
0,125 g	Fins a 25 g	25 ml
0,08 g	Fins a 25 g	25 ml

It was important to calculate the consistency of the fibre to have the exact dry weight that was in the table. The next step is leaving the samples one week for having the sample dissolved.

The next step is open the thermostatic bath and using the Ostwald viscosimeter measure the time. Then the process was done three times to have a mean.

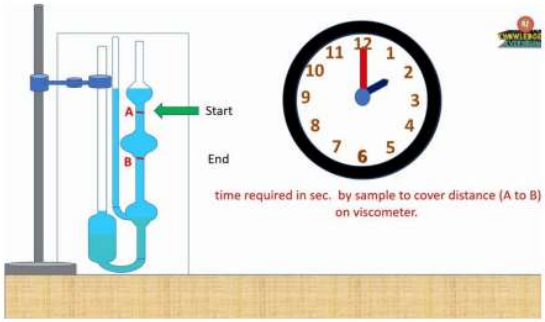


Figure 25. How calculates the time.

The time and the quantity of dry mass was used in one excel file that is in the folders of BIAMATEC. This excel gave the value of polymerization degree by dividing the dry mass and the molecular weight of the monomer of cellulose.

CARBOXYL CONTENT

The main objective of the Carboxyl content was knowing the level of oxidation of the fibre. This procedure gave some useful information to know how good were the TEMPO reactions related to the oxidation of the fibre. The procedure said the quantity of the COOH groups.

For preparing the calibration curve, a stock solution of methylblue with a concentration of 50 mg/l. 3,33 ml methylblue (300 mg/l) were added into a beaker and then 16,67 ml of distilled water were added also.

The calibration was prepared using the stock solution according the Table 2.

Table 2: Stock solutions used for in the calibration curves for the carboxyl content test.

V_{stock} (ml)	V_{total} (ml)	Conc (mg/l)
0,4	10,00 ml	2
0,8		4
1,2		6
1,6		8

2,0		10
2,4		12
2,8		14

For the sample preparation, 1-3 mg of dry mass for oxidized fiber or 5 mg dry mass for non-oxidized fiber were weighted in a tube. 5,000 ml buffer and 5,000 ml methyl blue were added with a micropipette. The tube was shaked and left to incubate for two hours. The tubes were placed in the centrifuge with 4650 revolutions for 20 minutes. 200 μl of the sample was added to the solution into a cuvette and then 2,3 ml HCl 0,1 M were also added.

The absorbance of the calibration curve samples and the samples were measured in the spectrophotometer at λ 664 nm.

MECHANICAL TENSILE TEST

The mechanical tensile test gave the information of the mechanical properties of the fibres.

The first step was cutting the samples. Before this the sample was conditioned in the temperature chamber. Then the samples were cut as shown in Figure 26.

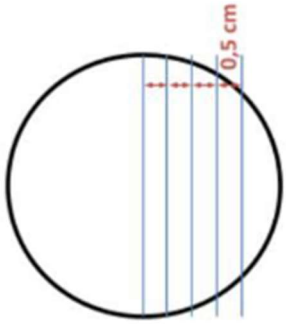


Figure 26. Example of cut samples for mechanical characterization.

The main reason for cutting the samples like this was that the Kitasato filter that it was used to form the samples was not as big as the paper former available in the lab. So, the best option was to adapt the test and cut little samples in order to have different samples for each paper.

Then for the tensile test it was needed to know the thickness of the sample, that was recorded with a micrometre. Then for doing the tensile test it was necessary to adjust the distance between both machine tweezers and recover the tweezers with an insulating tape to avoid the possibility of breaking the sample before. The montage shown in Figure 27.

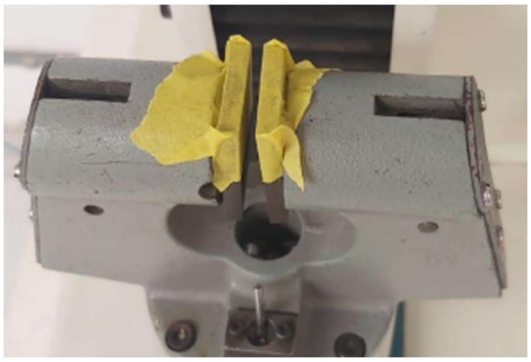


Figure 27. Tweezers with insulating tape.

The next step was introducing the thickness and width of the sample in the software and perform the tensile test.

CONDUCTIVITY MEASURE

This conductivity (σ) measure refers to the ability of the solution to carry an electrical current.

Conductivity measurement in hydrogels was done with a conductometer.

For measuring the conductivity, the main point was a good calibration. In my experience and with the machines that I used; the best was to calibrate every 10 measurements. The calibration procedure worked by using the standardized solutions. The probe was introduced in the hydrogel directly and the result was recorded.

Conductivity measurement in membranes was done with a multimeter.

For the measurement was used the resistance measure. The resistance of the paper was converted in resistivity and the resistivity in conductivity by using the Equation 6 and Equation 7.

Resistivity (ρ) = Resistance (R) * Area (A) / Length (L) Eq.6

Conductivity (σ) = 1/ Resistivity (ρ) Eq.7

6. RESULTS

From the cellulose nanofibers produced during my stay in Finland, I attach here the images of CNF in Transmission electron microscopy (TEM). The lack of this equipment here at the University of Girona forced that I could not do this characterization for my cellulose nanofibers produced in Girona. In Figure 28 there is an image from the TEM microscope, for cellulose nanofibers produced following the TEMPO 5 process. The image shows cellulose nanofibers in agreement to what is found in the literature, with average diameters in the range of 4 nm.

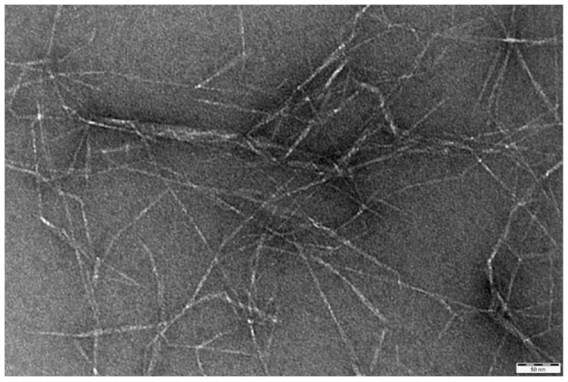


Figure 28. TEMPO 5 in TEM.

I prepared different samples, combining mechanical pre-treatment with TEMPO pre-treatment and others just with the TEMPO procedure. I list the different samples that I have prepared.

- TEMPO 1
- TEMPO 2,5
- TEMPO 5
- TEMPO 7,5
- TEMPO 10

All of these samples have been replicated, the first replica has mechanical pre-treatment plus TEMPO reaction, and the second one just with the TEMPO reaction. Knowing all the fibres I am going to explain the first characterization and the first results.

I used the MORFI in order to see the fibre length and width in all of the samples. This is because as explained in the literature [6] I know that some pre-treatments can cut the

fibre. If this happens, I would lose one of the differential factors of each sample. That is why I find out very important to have complete information about this. The next charts are from the samples of TEMPO 5 PFI, TEMPO 7,5 PFI and TEMPO 10 PFI. Also, result of the different TEMPOs done.

The MORFI characterization gives lots of information but I am only going to use the mean arithmetic length and mean arithmetic width, also for putting the images in perspective I am going to show the number of fibres analysed. That are the ones that I am most interested in.

Table 3. MORFI results of TEMPO samples.

TEMPO	LENGTH mean (µm)	WIDTH mean (µm)
0	1236,6	43,2
1	624,5	24,01
2,5	591,3	23,93
5	589,5	24,6
7,5	457,5	26,6
15	252,6	25,3

Table 4. MORFI results of TEMPO + mechanical pre-treatment samples.

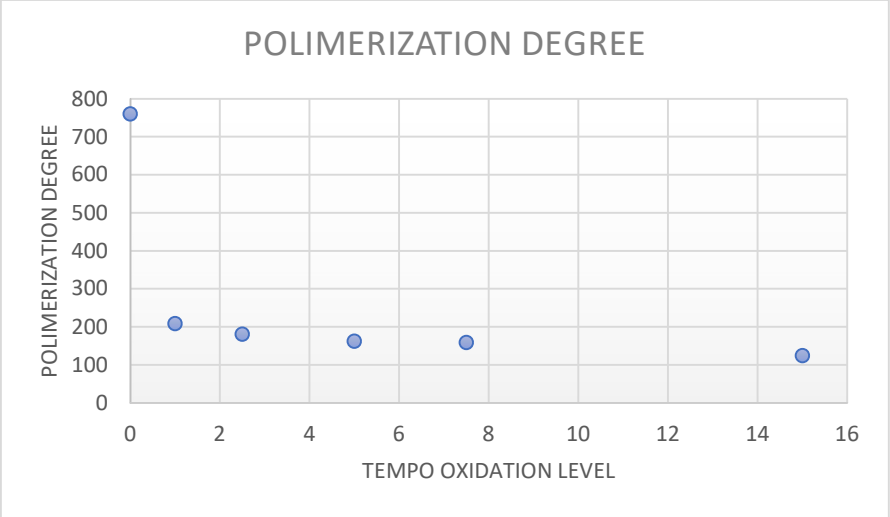
TEMPO	LENGTH mean (µm)	WIDTH mean (µm)
5	366,5	26,8
7,5	360,6	28,4
10	352,4	25,9

In Table 3 is shown that the TEMPO reaction chops the fibre and the length can variate a lot. The width is more regular and all the samples are in the same value. In Table 4 comparing results with Table 3 I notice that the length of the fibre is variating a lot and also, I see that the results from the fibres are very similar. As I want to have different

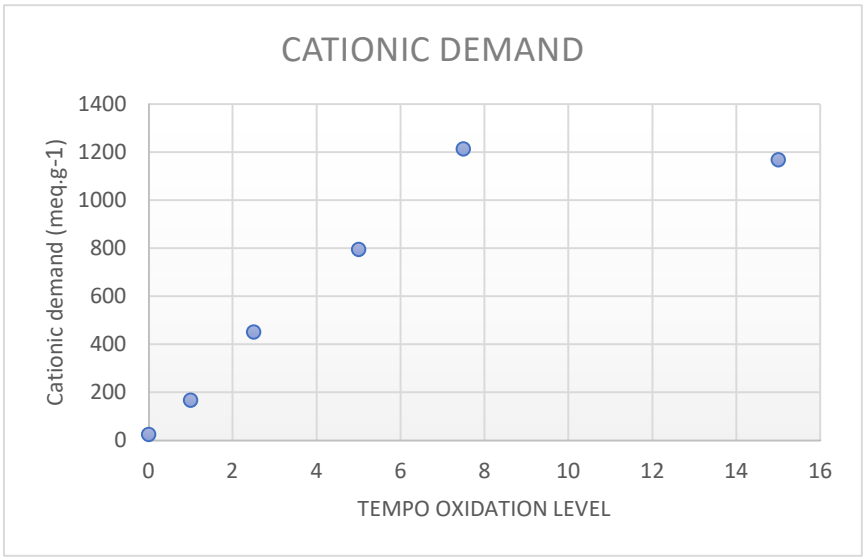
samples, I will discard the samples with mechanical pre-treatment because I see that the PFI cuts down the fibre a lot and the samples loses the differential factor of the fibre length. Also, in MORFI results I can see that the results are not consistent. As explained in Materials and Methods, there are 4 different beakers per sample, and the result of each beaker is different to each other. This also gives me information on how it is the PFI working.

My hypothesis is that the results are not good enough because I work at low revolutions (4500 rev). So, I think that the reproducibility and the results would be better if I apply more revolutions.

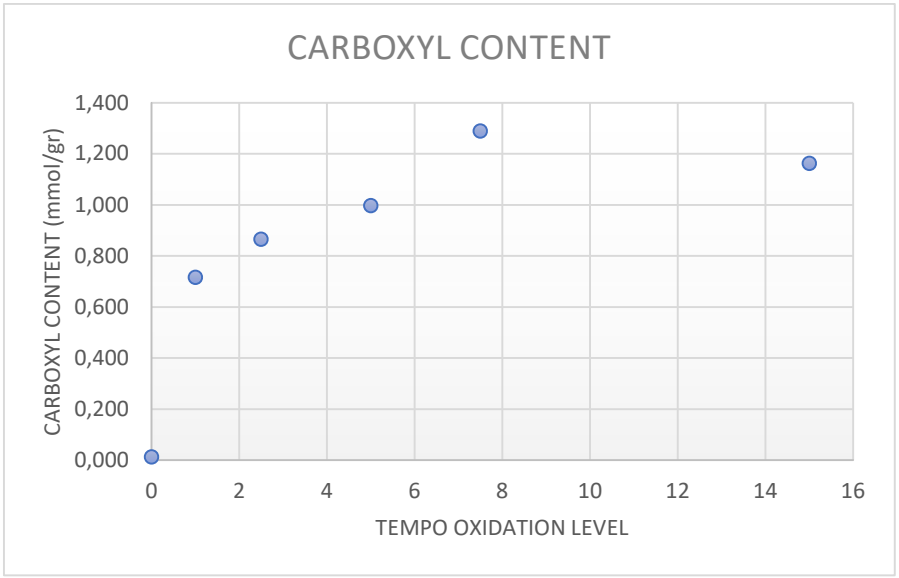
I prepared the other characterization to the samples that have only the TEMPO pre-treatment.



Graph 1. Polymerization degree.



Graph 2. Cationic demand.



Graph 3. Carboxyl content.

I can see that all the results are good except on the TEMPO 10. I can see that in this case the oxidation levels do not match with what is expected. That can be because the TEMPO 10 reaction takes lots of time and according to the literature [6], there is a point that the fibre gets degraded instead of being oxidated. For this reason, I discard the sample.

Also, I want to discuss about the correlation between the results of the Cationic demand and the Carboxyl content. The tendency of the results is very similar, showing a high value on both Graphs 2 and Graph 3. This is really good because both characterizations are useful know about the oxidation level of the fibre and this correlation shows that the samples are performing as expected.

Now having the first characterization done, I will produce CNF in the homogenizer. These fibres will be the ones that I will combine with the conductive elements. Also, I discard the TEMPO 1 sample. The reason is that it is impossible to homogenize the fibre. The PANDA cannot defibrate the sample and gets stacked. I tried to decrease the concentration of the solution in order to pass the fibres, but all the attempts failed.

This first characterizations have been useful to discard the samples that are not significative and the ones that are not having good results. The samples for the final steps are the following ones.

- TEMPO 2,5
- TEMPO 5
- TEMPO 7,5

The different combinations that I will try are the following ones. First of all I will test with some hydrogels samples, then I will prepare some membranes in order to have different samples and values to compare.

- CNF + PEDOT: PSS PT-2
- CNF + PEDOT: PSS PH-1000

Both samples use the same protocol preparation. The main difference is the conductivity and the prize of the conductive elements. The PEDOT: PSS PT-2 is theoretically giving worse conductive results than PEDOT: PSS PH-1000 and the prize is lower. So, the first combinations that I will test will be with PEDOT: PSS PT-2 with the following proportions.

- CNF (100%)
- CNF (70%) + PEDOT: PSS PT-2 (30%)
- CNF (50%) + PEDOT: PSS PT-2 (50%)
- CNF (30%) + PEDOT: PSS PT-2 (70%)

All, the samples are at 0.4% of consistency for having a value at the same concentration in every sample. After preparing the samples, the results are the following ones:

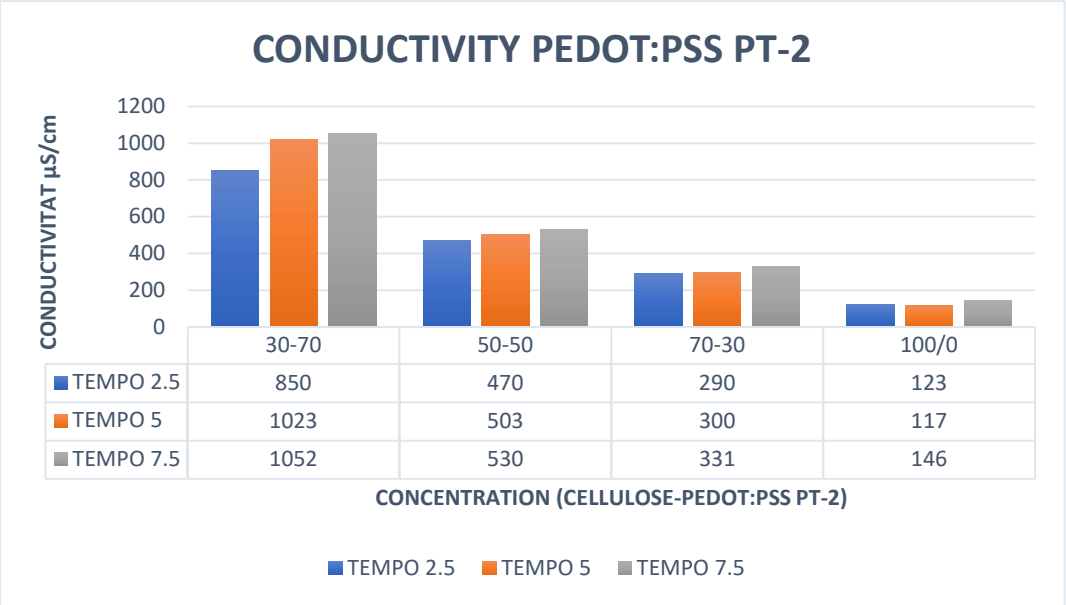


Chart 4. Conductivity measures results.

For having a reference, the standard conductivity of the water is in a range between 0-200 µS/cm [10].

And the image of the dried conductive hydrogel:

Figure 29. 70-30 / 50-50 / 30-70 (in order left to right) conductive hydrogels after 4 days drying by casting.

As shown in Figure 29, the hydrogel with 70-30 proportion has good consistency and behave like hydrogels. The ones that are at 30-70 are the ones with higher conductivity values but behave as liquid due to the little quantity of CNF. The best samples combining both characteristics are the 50-50 ones that behave as hydrogels but are more conductive than the 70-30 ones.

The results of the conductivity on the dry hydrogels are better than when the samples are at 0.4%. As I cannot know the consistency of every hydrogel because it is needed a good quantity of sample to know the consistency and the samples have low dry mass quantity, I cannot say that all the hydrogels have the same consistency, but I am going to show the results after 4 days of casting in the same room at the same temperature.

Table 5. Result of conductivity after 4 days of casting in PEDOT: PSS PT-2 + CNF.

PT-2	50-50	70-30
TEMPO 2,5	1039 µS/cm	445 µS/cm
TEMPO 5	1050 µS/cm	558 µS/cm
TEMPO 7.5	1718 µS/cm	1347 µS/cm

The results of Table 5 are way better than the ones presented before. This it can be because the hydrogel is more concentrated and, in both cases, there is a structure that combined with the conductive element increases the results.

According to the results I can see that the conductivity result of the hydrogel without any conductive element is lower than the ones that have PEDOT: PSS PT-2. This means that definitely the conductive element works and there is adhesion of the polymer to CNF.

As I can see in the tables of Chart 4, there is a relation between the quantity of PEDOT: PSS PT-2 and the conductivity. So, in conductivity terms the best samples are the ones that have more PEDOT: PSS PT-2. The bad part is that, there is not enough CNF fibre in this sample to become a hydrogel. As I want to work with the hydrogel, I will discard this samples. Now I will repeat the same procedure with PEDOT: PSS PH-1000 that according to the datasheet of the product should increase the conductivity.

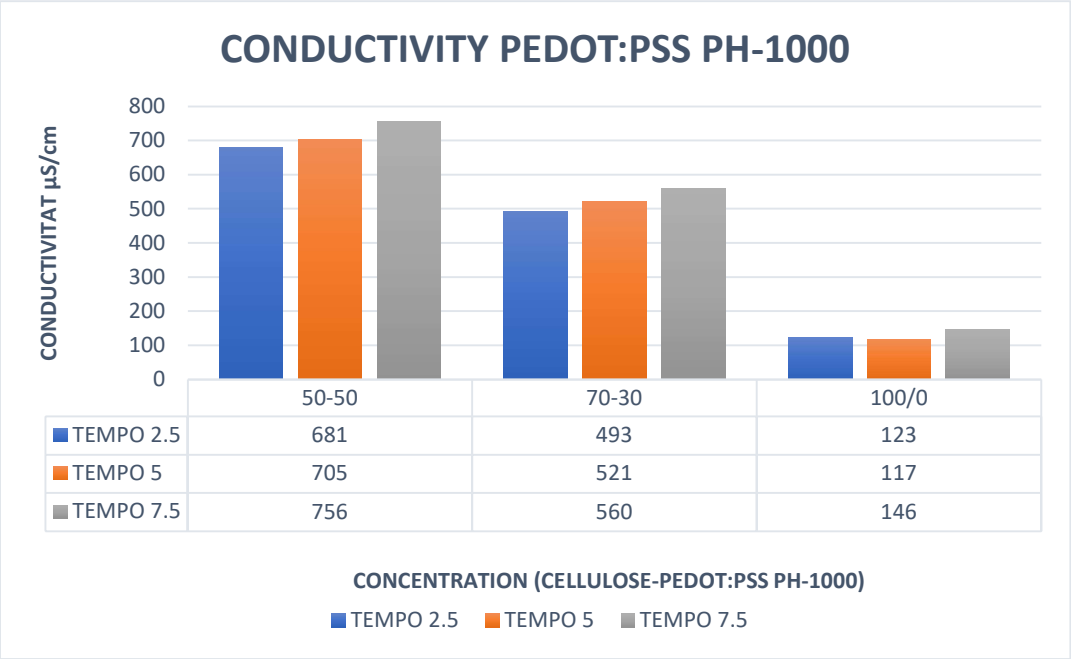


Chart 5. Conductivity measures results.

The results shown in Chart 5 are, as expected, better than the shown in Chart 4. The conductivity increases in a constant way in all the samples. This means that both can be used in the same way. This fact, makes me know that I can test with PEDOT: PSS PT-2 and figure out the results that I would obtain with PEDOT: PSS PH-1000.

Now I will prepare membranes with the same proportions that I used in the hydrogels with PEDOT: PSS PH-1000. The samples prepared are going to be filtered in the Kitasato structure. The paper grammage is 60g/m^2. The conductivity results in Table 6.

Table 6. Conductivity calculated in membranes.

FORMULATION	CONDUCTIVITY (S*m^{-1})
TEMPO 2.5 + PEDOT: PSS PH-1000+ 50/50	0,4786
TEMPO 5 + PEDOT: PSS PH-1000+ 50/50	0,3771
TEMPO 7.5 + PEDOT: PSS PH-1000+ 50/50	0,6137
TEMPO 7.5 + PEDOT: PSS PH-1000+ 70/30	0,2245
TEMPO 5 + PEDOT: PSS PH-1000+ 70/30	0,4129
TEMPO 7.5 + PEDOT: PSS PH-1000+ 70/30	0,4367

The results are low compared with the ones of the hydrogel. The appointment is that there is a difference on the material, because hydrogels contain water that is a conductor of electricity, the paper just has CNF plus conductive element. These papers have a grammage of 60g/m^2. According to the definition of conductivity, it increases when the quantity of conductive elements increases, so the difference between both samples is logic. Likewise, there is a variation in the conductivity results of the samples with less quantity of PEDOT: PSS PH-1000.

In general, the results are good and comparing the measures of conductivity with the ones in the literature [14] that say that around 3 S/m is a good value for sensing bio signals, I consider that the 50-50 formulations are good enough to be considered as useful.

The last characterization is the mechanical tensile test. This will give information on the mechanical properties of the fibres used, that is useful for having a complete information of the material. The results are shown in Table 7. The samples that do not have CNF in the name are the ones without homogenizing.

Table 7. Nanofibres mechanical properties results.

	WIDTH (mm) mean	Thickness (µm) mean	SECTION (mm^2) mean	Cmax (MPa) mean	Young modulus (MPa) mean
TEMPO 2,5	5	0,245	1,2	5,82	1875,34
TEMPO 5	5	0,145	0,725	19,34	7661,10
TEMPO 7,5	5	0,102	0,523	45,68	14041,860
TEMPO 2.5 CNF	5	0,075	0,375	130,44	46358
TEMPO 5 CNF	5	0,07	0,225	201,99	58198
TEMPO 7,5 CNF	5	0,045	0,35	152,47	51903,8

7. DISCUSSION

7.1. LIMITATIONS

The objective of the project has been accomplished. The nano fibres of cellulose did work in combination with the conductive elements used. Despite the fact that the objective is achieved I think that there are some limitations related with the project. The first one is the lack of time, as I consider that this project is very ambitious and needs lots of hours and resources to have final satisfactory results. I consider that there are plenty of tasks to do in order to implement the hydrogels in a functional electrode. Also, the laboratory availability is a limitation as I have been working on a lab that there are other students working, this makes that the limited quantity of devices available sometimes make slower the process.

The main problem and limitation that I find in the project is the lack of previous information around bio sensing applications involving CNF. There are projects that work with CNF as substrate for electronics but in the literature, I have not found results on using the biomaterial to perform as a sensor. Some studies are related, using different synthetic materials and hybrid materials. The field is completely new and this motivates me but also gives me an extra limitation.

7.2. CONTRIBUTIONS TO THE OBJECTIVES OF SUSTAINABLE DEVELOPMENT (ODS)

This project fully meets the objectives 3 and 13 on the sustainable development. The objective number 3 talks about health and wellness, as the project is aiming to the fabrication of a medical device, I feel that this objective suits perfectly. Also, the objective number 13 is very much related with the project. The objective talks about working for the environment. I feel that also suits perfectly, because the main motivation of the study is changing the plastic-based electrodes and elaborate a sensor that can be biodegradable and can give the same functionality as the plastic ones.

8. CONCLUSIONS

Talking about the proposal objectives, the main goal is achieved. The main goal was to elaborate nano fibres of cellulose and combine the material with conductive elements in order to achieve the conductivity results to work as a bio-sensor but there are some points that where modified due to the limitations explained in the Chapter 7. The conductive elements used in the test are both PEDOT: PSS, but the graphene has not been tested. Also, I could not test as electrodes the samples, due to the lack of previous tests needed to perform such an important test that involves humans. Despite this fact I think that the main goal is achieved, but there is a lot of work to do in order to implement and perform the material in sensing in an ECG application. The conductivity results are good enough in the case of the hydrogel and not enough good in the case of the membranes [14] but the papers results are good enough to be useful and can be improved. In the case of the hydrogel the result is obvious because the samples contain water and the water is very conductive. The fibres obtention and characterization has been a success.

Using the project as a starting point in short term I think that some test with other conductive elements can be performed. There are also different formulations that are necessary to test, for example, formulations with two conductive elements can be interesting for trying to look for some synergies between conductive elements. Also, different electrical characterizations such as cyclic voltammetry. This characterization would give necessary information to complete the knowledge of the behaviour of the material working as a sensor. Likewise, a cross-linking method for having the hydrogel with a stronger structure is a very interesting way to advance.

Then in long term applications there are plenty of options, the most interesting in my opinion is trying to develop a bio-ink with the conductive hydrogel. This would give the possibility of fabricating the electrodes by printing. This bio-ink would make possible to work on a patient approach medicine, that is the future of the medical field. Also, some biodegradability and biocompatibility test are necessary for knowing if the material can be considered biodegradable.

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ANNEX

ANNEX A. PLANIFICACIÓ

STEP 1. PROJECT KNOWLEDGE

Duration: 2 weeks

- Searching information.

In this part I searched for some useful information in order to know if the topic was interesting to me. The main point was having some context to know what I was going to do and decide if this would be the topic of the project.

STEP 2. PROJECT SETUP

Duration: 2 weeks

- Define project objectives.
- Planification Finland visit.

In this part we were planning the visit to Finland at Abo Akademi. Also, we defined the different objectives and all the knowledge that I should earn travelling to Finland.

STEP 3. LEARNING METHODS

Duration: 2 weeks

- Understand the mechanical and chemical pre-treatments.
- Learning the different way of producing CNF.

In this part I travelled to Finland and I learned all the methods.

STEP 4. SAMPLE PREPARATION AND CHARACTERIZATION

Duration: 1 months

- Preparation of all the samples.
- Characterization of all the samples.

In this part I preparate all the samples used during the project and I did all the characterizations explained before.

STEP 5. TESTING WITH CONDUCTIVE ELEMENTS

Duration: 1 month.

- Test with conductive elements and choose what conductive element use.

STEP 6. FINAL SAMPLE PREPARATIONS AND CHARACTERIZATIONS.

Duration: 3 weeks.

- Final preparation of the samples and characterizations

STEP 7. DISCUSSION OF THE RESULTS

Duration: 3 weeks.

- Discussion of the results.
- Repetition of some wrong samples.

Here I repeated some samples and finished the whole characterizations.

The writing of the document has been done in every step. I preferred to work including to the document the information while I was working.

GANTT CHART

The development of the project expressed as Gantt chart is presented here below:

	WEEK NUMBER																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
STEP 1																				
STEP 2																				
STEP 3																				
STEP 4																				
STEP 5																				
STEP 6																				
STEP 7																				
WRITING																				

ANNEX C. PRESSUPOST

1. PRICE PER UNIT

   a. MATERIAL

DESCRIPTION	UNIT	PRICE/UNIT
PH-1000	g	0,23 €
PT-2	g	0,07 €
NaClO	mL	0,42 €
TEMPO	g	6,06 €
NaBr	g	0,40 €
NaOH	g	0,11 €
HCl	mL	3,75 €
CuED	mL	0,14€
Poli-DADMAC	mL	0,70 €
Methylblue	mL	1,28 €
Buffer	mL	0,3 €
Softwood sulphite Domsjö	g	0,79 €

   b. WORKING HOURS

DESCRIPTION	UNIT	PRICE/UNIT
Engineer	h	40,00 €
Chemical Lab Technician	h	20,00 €

2. FRAGMENTARY BUDGET

a. SAMPLE FABRICATION

DESCRIPTION	UNIT	QUANTITY	PRICE/UNIT	VALUE
PH-1000	g	140	0,23 €	32,2 €
PT-2	g	400	0,07 €	28 €
NaClO	mL	650	0,42 €	273 €
TEMPO	g	2,4	6,06 €	14,54 €
NaBr	g	30	0,40 €	12 €
NaOH	g	400	0,11 €	44 €
HCl	mL	200	3,75 €	750 €
Softwood sulphite Domsjö	g	400	0,79 €	316 €

b. SAMPLE CHARACTERIZATION

DESCRIPTION	UNIT	QUANTITY	PRICE/UNIT	VALUE
CuED	mL	500	0,14€	70 €
Poli-DADMAC	mL	400	0,70 €	280 €
Methylblue	mL	100	1,28 €	128 €
Buffer	mL	200	0,3 €	60 €

c. WORKING HOURS

DESCRIPTION	UNIT	QUANTITY	PRICE/UNIT	VALUE
Engineer	H	125	40,00 €	5000 €
Chemical Lab Technician	H	275	20,00 €	5500 €

3. TOTAL BUDGET

TASK	PRICE
SAMPLE FABRICATION	1469,74 €
SAMPLE CHARACTERIZATION	538 €
WORKING HOURS	10500 €

TOTAL PRICE	12507,74 €

The total budget distribution in terms of sample fabrication, sample characterization and working hours is presented in figure 30.

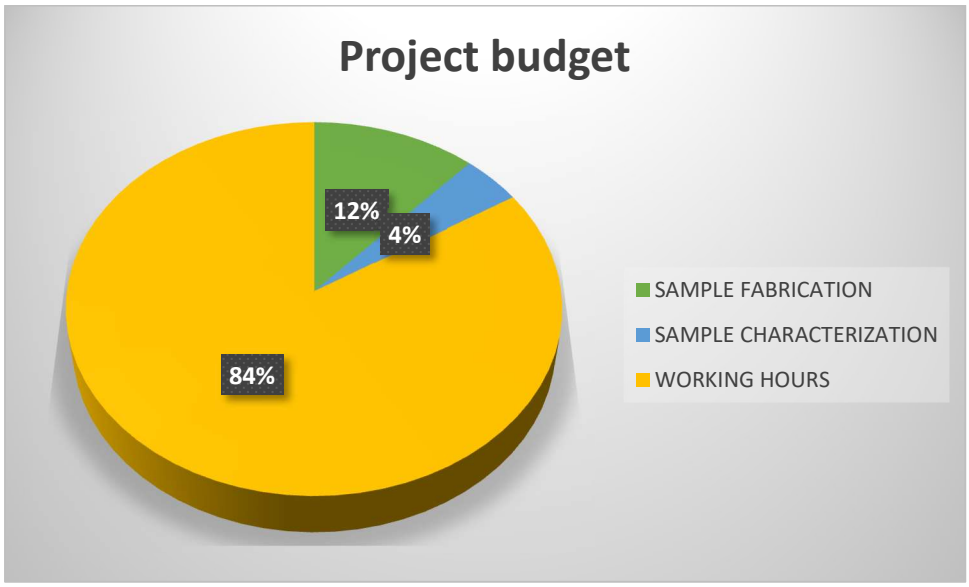

Figure 30: Summary chart of the total budget of the current project