الجمهورية الجزائرية الديمقراطية الشعبية
People's Democratic Republic of Algeria
وزارة التعليم العالي والبحث العلمي
Ministry of Higher Education and Scientific Research
جامعة عين تموشنت بلحاج بوشعيب
University –Ain Temouchent- Belhadj Bouchaib
Faculty of Science and Technology
Departement Of Chemistry

End of studies project
For the attainment of a Master's degree
Field: Material Science
Sector: chemistry
Specialty: Macromolecular Chemistry

Theme

# Extraction and characterization of biopolymer and study its effect in drug release

**Presented by:**

Miss. BENALI Hosna

**Before the jury composed of:**

| | | | |
|---|---|---|---|
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Academic year **2024/2025**

# Appreciation

We thank God, the Almighty who gave us the courage, strength, health and patience to carry out this end-of-study project.

We express our sincere thanks to my supervisor **Dr SEDIRI. K** , Conference maitre B at the University "Belhadj Bouchaib Ain Temouchent" for her unconditional support, her availability, her valuable advice, her expertise, her contribution to the project and the permanent help which she provided us.

My sincere thanks go to **Dr CHAIBI. W**, Conference maitre A at the University "Belhadj Bouchaib Ain Temouchent", for having done us the honor of chairing this jury. To **Dr RAMDANI. N**, Conference maitre B at the University"Belhadj Bouchaib Ain Temouchent". Thank you for agreeing to participate in order to evaluate and judge this modest work, please find here the expression of my sincere thanks.

We are also very grateful to the Head of the Department, **Pr BAILICHE. Z**, Professor at University of Ain Temouchent, for her great help in the smooth running of our research.

In recognition, we would like to express our sincere thanks to **Dr BENNABI. L** for helping us to realize the microbial study, for her encouragement and her constant presence throughout this work. We also thank **Dr FKIH. N** and **Pr AZZI. H** for the time it took them to do the characterization XRD and FTIR of our products.

We would also like to thank all the professors of the Department of Sciences of matter at the University –Ain Temouchent- BelhadjBouchaib,, without exception, who have supported us over the past five years ,for their quality teaching and the knowledge they gave us during our years of study. Their passion and dedication to research inspired and motivated us to pursue our own scientific questions.

We would particularly like to thank the laboratory staff: **Mrs. Bahria** , **Mrs. Wahiba** and **Mrs Karima** at the Chemistry Laboratory and **Mr. Ahmed** , **Mrs Choukria** at the Biology Laboratory at the "Belhadj Bouchaib- Ain Témouchent" University for their their Availability and their help and thank you again for your support and encouragement throughout our work Experimentation.

Finally, we would like to warmly express our thanks deep gratitude to everyone who contributed directly or indirectly to achieve it, because the project cannot do so be the fruit of one person.

A big thank you to all

# DEDICATION

قال تعالى: "وَقُلِ اعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ وَسَتُرَدُّونَ إِلَىٰ عَالِمِ الْغَيْبِ وَالشَّهَادَةِ فَيُنَبِّئُكُمْ بِمَا كُنْتُمْ تَعْمَلُونَ". سورة التوبة، الآية 105

Praise be to Allah, by whose grace the ends were achieved and we completed the road with His approval, neither the journey nor the road was short, but I did it, so praise be to Allah, who was pleased with us and the beginnings, and we reached the ends by His grace and generosity.

I dedicate the fruit of my graduation and my success in this field:

To, my eternal role model, my moral support, my source of joy and happiness, to the one who always sacrificed himself to see me succeed, to my dear father **DAHMAN**

To the most beautiful creatures God created on earth, the light of my days, the source of my efforts, words will not suffice to express the extent of my love and gratitude to you, the haven where I find comfort and security to you, my mother

To He was not lucky enough to share my joy, my steadfast support, my brother **Mokhtar**, to those whose presence makes me happy today, my brothers' **Chihab** and **Amin**, and my sister **Wafaa**. You are the pillars of my life, the stars that illuminate my path and my most precious possession; I wish you a future full of joy, happiness, success and serenity. Through this work, I express my feelings of brotherhood and love.

To my dear **grandmothers** and **grandfathers**, whose prayers have never left me throughout my life . I ask God to grant them health and well-being. I will never forget my **grandfather,** may God have mercy on him.

To all members of **my family**, young and old, thank you for always standing by me. To all who have been a help and support on this path, to loyal friends and companions of the years.

To **myself** who said I will get it and finally stand on the threshold of my graduation, reaping the fruits of my labor and raising my hat with pride.

To my supervisor **Mrs K. SEDIRI**

***Hosna***

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Introduction

# General Introduction

# General Introduction

Natural polymers, or biopolymers, are derived from renewable sources such as plants, food waste (e.g., banana and orange peels), and microorganisms. Their use can help reduce the environmental impact of non-biodegradable industrial waste. Among these, cellulose stands out as one of the most abundant and versatile biopolymers. Discovered by Anselme Payen in the 19th century, cellulose is a plant-derived polymer composed of repeating glucose units. It is found in large quantities in wood, cotton, and other plant fibers, and has historically played a significant role in the development of synthetic materials like cellulose nitrate, cellulose acetate **[1]**.

Cellulose is valued for its renewability, biodegradability, and favorable physical properties, such as low density, high porosity, and large surface area. These characteristics make it suitable for a wide range of applications including adsorption, oil/water separation, thermal insulation, and biomedical uses **[2, 3].**

Additionally, cellulose obtained from bacterial (BC) and plant (PC) sources has proven to be an excellent scaffold material for tissue regeneration, thanks to its biocompatibility, low toxicity, cost-effectiveness, and structural similarity to native tissues [4]. These advantages make natural cellulose a strong alternative to synthetic polymers in both industrial and medical applications, and the reason for its selection in this study **[5].**

The Alfa plant (*Stipa tenacissima*), a hardy perennial grass native to North Africa and parts of the Mediterranean, is a valuable natural source of cellulose. Traditionally used in paper production and handicrafts **[6]**, Alfa's high cellulose content and fibrous structure make it ideal for sustainable cellulose extraction. Through chemical or mechanical treatments, cellulose can be isolated from its stalks for use in various industrial and biomedical applications **[7].** Similarly, waste paper an abundant cellulose-rich material offers an eco-friendly and cost-effective alternative for cellulose recovery through recycling processes. Extracted cellulose from both Alfa plant and waste paper plays a crucial role in medical applications due to its biocompatibility, non-toxicity, and ability to form scaffolds for tissue engineering, wound dressings, and drug delivery systems. Additionally, its high surface area, porosity, and functionalizability make cellulose an effective adsorbent for removing dyes and other pollutants from wastewater, contributing to environmental sustainability and clean water technologies **[8, 9].**

In this work, cellulose was selected as the polysaccharide of interest, which will be extracted from Alfa plant "*Stipa tenacissima"*. Cellulose also will be extracted from wastepaper, and then will be modified structurally to obtain the Cellulose triacetate CTA using esterfication. The CTA will used as a polymeric matrix to prepare a pharmaceutical forms "Tablets" charged with antibiotic agent "Cefalexin" and study its release in the intestinal medium. Both cellulose extracted will be tested against Gram positive, negative and fungal pathogen.

This work is organized into four chapters:

- Chapter I: This is devoted to a general presentation of the Alfa plant, including its botanical classification, geographical distribution, morphology, the chemical composition of its fibers and the various extraction methods used to isolate its cellulose.
- Chapter II: This chapter deals in detail with cellulose: its history, molecular and supramolecular structure, physico-chemical properties, crystallinity, and the various chemical modifications it can undergo (esterification, etherification, oxidation, etc.), in particular for the production of derivatives such as cellulose triacetate.
- Chapter III: describes the experimental part, which is classified into three stages, first part, and the extraction of cellulose from two different sources: plant alfa, and waste paper. The processes used (alkaline and acid treatments, drying, etc.) are detailed, as are the observations made on the yields, visual appearance and purity of the celluloses obtained, the second part describe the modification of cellulose extracted to prepare the cellulose triacetate CTA then the CTA used as polymeric matrix to prepare cefalexin loaded Tablets and study its release. The Cellulose extracted and CTA were characterized by FTIR and DRX. The third part is the biological study of cellulose against four pathogens.
- Chapter IV: describes the interpretation of the results obtained from the IR spectrums and XRD patterns, the cefalexin released percentages , and the inhibition zones of biological tests.

This work is part of an approach to valorizing local plant resources and recyclable waste, as part of a greener, more environmentally-friendly chemistry aimed at producing biodegradable materials with high added value.

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Chapter I

# The Alfa plant

# (*Stipa Tenacissima*)

## 1. General information on Alfa fiber:

Alfa is a typically Mediterranean perennial herb, growing in clumps about 1m to 1m20 high forming vast sheets. It does not disappear in winter **[1]**. It grows spontaneously and independently, particularly in arid and semi-arid environments, and marks the limits of the desert: where the Alfa stops, the desert begins **[2, 3]**.

This plant belongs to the Grass family, Stipae tribe, Stipa genus. This genus includes, in addition to Alfa (Stipa tenacissima L., the only species exploited), around 250 species, 7 of which are thought to be found in Algeria **[1] [4]**.

**Figure 01:** Illustrations of the Alfa plant in its raw state **[5, 6]**.

Alfa (from the Arabic ***Halfa***) or Sparta (*Esparto* grass in English) occupies a well-defined geographical area, originating in arid and semi-arid Mediterranean regions excluding desert areas: North Africa (Morocco, Algeria, Tunisia and Libya) and Southern Europe (Spain, Portugal and Italy) **[7, 8]**. The narrow location of this grass ensures that these countries have a monopoly on its exploitation and sale as a raw product. The distribution known to date is shown in (table.01) **[2] [9- 11]**.

**Table 01:** Estimated distribution of Alfa in 2012 **[2]**

| Country | Number of hectare |
|---|---|
| Algeria | 4.000.000 ha |
| Morocco | 3.136.000 ha |
| Tunisia | 600.000 ha |
| Libya | 350.000 ha |
| Spain | 300.000 ha |

The use of Alfa (*Stipa tenacissima*) dates back to ancient times. However, its widespread application as an industrial plant began in the late 19th century, when it was adopted by Scottish papermaking industries as a raw material, following a process developed by Routledge **[4].** Due to its low water requirements and the absence of a need for insecticides or harmful pesticides, Alfa holds both ecological and economic value. Furthermore, the plant contributes significantly to combating soil erosion and desertification **[12]**.

**2. Plant Morphology:**

The Alfa plant (*Stipa tenacissima*) features a cylindrical stem (tige) and typically grows in dense clumps, reaching up to one meter in height due to its slender, narrow leaves **[2]**. It is a robust perennial grass characterized by a thick central stem with leaves and branches. Multiple stems often emerge from the same root system and grow in a circular pattern when viewed from above. The roots (racines) can extend deeper than one meter into the soil, while the stems reach about one meter in height. Between the roots and stems lies the rhizome, a crucial structure for plant regeneration **[13, 14]**.

The root system is highly branched, with numerous nodes where secondary roots develop, giving the plant a strong anchorage in the soil an important adaptation for the arid regions it inhabits **[15]**. The stems and lower leaves are covered with fine hairs (velvety texture) and a waxy coating, which help trap airborne particles. This contributes to reducing wind erosion by preventing the spread of sand and dust, thereby mitigating a major factor in desertification **[12]**.

The wax coating also limits water evaporation, enabling Alfa to survive in hot, dry conditions by minimizing water loss **[16]**. It is highly resilient to extreme temperatures, withstanding night-time lows of –20°C and daytime summer highs of up to 40°C **[1][17]**.

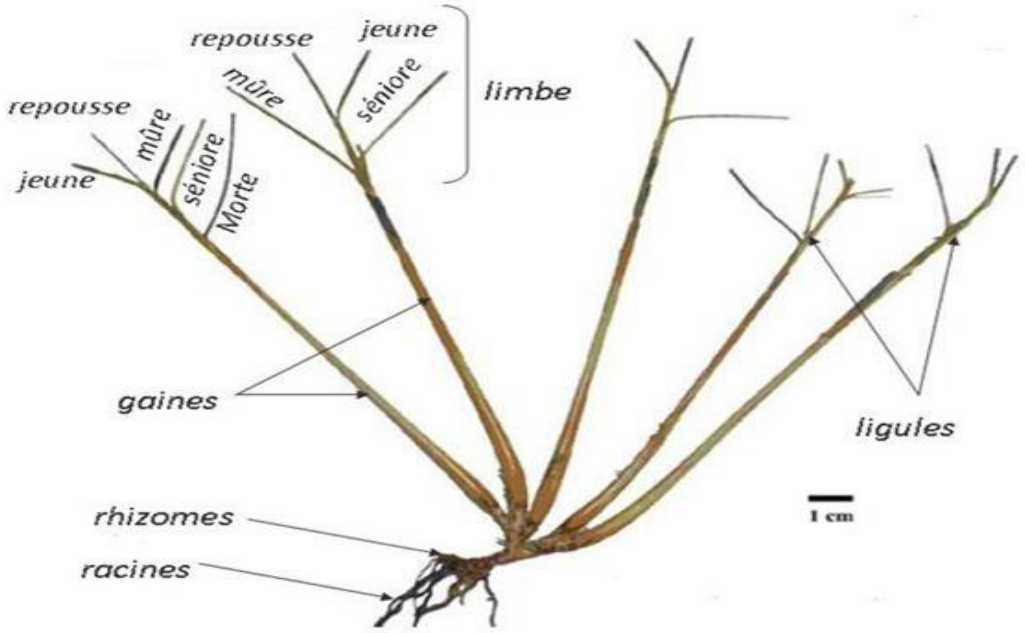


**Figure 2:** Esparto plant with indication of main parts **[2] [18,19,20]**.

## 3. Leaf fibres:

The main reason these fibres are typically longer than grain fibres is because the leaves are frequently very large. In addition to textile applications (such as textile fibres for textiles and tricots), they are the primary raw material used to make cables and cords **[21]**. The rough Alfa's cellulosic fibres are made of leaf fibres **[22]**.

## 4. Fibre structure and morphology:

Fiber structure is typically heterogeneous. The smallest structural units are cellulosic fibrils, which measure between 2 to 5 mm in length and 5 to 10 µm in diameter. These fibrils are tightly bound together by hemi-cellulose to form the basic fiber structure **[22]**. When observed under an optical microscope, the cross-section of the fibrils appears irregular in shape **[22]**. The overall fiber diameter is approximately 50 µm, as shown in Figure 12. The fibers are further grouped into bundles, known as *faisceaux*, through the binding action of lignin and pectins **[23]**. The lumen, a small central cavity that facilitates water transport in living plants, is narrow. A cross-section of the fiber bundles reveals a non-circular shape with an average diameter of about 200 µm.

Ultimately, the plant stem (tige) is formed by the aggregation of these fiber bundles **[22-24].**

## 5. Chemical composition of Alpha:

Chemical composition depends on the species, the age of the plant, climatic conditions, soil composition, and the extraction method used **[25]**.

**Table 02:** The chemical composition of Alfa **[23], [26-30].**

| Fibre | Cellulose (%) | Hemi-cellulose (%) | Lignin (%) | Pectin (%) | Wax |
|---|---|---|---|---|---|
| Alfa | 44 – 47 | 22 – 35 | 19 - 24 | 4 | 2 |

### 5.1. Cellulose:

Cellulose is the primary structural component of plant cell walls and one of the most abundant natural polymers on Earth. It is a polysaccharide made up of long chains of β-D-glucose units linked by β-1,4-glycosidic bonds, forming highly ordered, linear structures. These chains group together through hydrogen bonding to form microfibrils, which provide mechanical strength and rigidity to plant tissues **[31-34]**.

In fibrous plants such as Alfa (*Stipa tenacissima*)**,** cellulose plays a critical role in the composition of the cell walls of fiber cells. The cellulose content in Alfa can range from 40% to 50%**,** depending on factors like plant age, environmental conditions, and extraction methods. Alfa fibers are known for being rich in cellulose, which makes them suitable for industrial applications such as papermaking**,** composites, and biodegradable materials. The cellulose is embedded in a matrix of other components, such as hemi-cellulose**,** lignin**,** pectins, and wax, which contribute to the overall structure and function of the fibers.

Due to its high cellulose content**,** low water and chemical input requirements, and natural resistance to pests, Alfa is considered an eco-friendly and sustainable source of cellulose for both traditional and modern industrial uses. **[35-40]**

### 5.2. The Hemicellulose:

Hemicellulose is located in the cell walls of plant cells and has a structure that is somewhat similar to cellulose (figure 3) **[21]**. However, there are key differences between the two. Unlike cellulose, which has a crystalline structure and greater strength, hemicellulose is amorphous and therefore mechanically weaker **[12]**. Moreover, while cellulose is composed solely of β-glucose monomers, hemicellulose

consists of a variety of sugar monomers, such as xylose, mannose, galactose, rhamnose (a deoxy-hexose derived from mannose), and arabinose **[7] [1, 2]**.

Due to the structural similarities between hemicellulose and cellulose, hemicellulose is able to form hydrogen bonds with cellulose microfibrils. It also interacts with other cell wall components, contributing to the overall cohesion of the wall. Hemicellulose is likely to be involved in covalent bonding with pectins and extensins, further reinforcing the structural integrity of the plant cell wall **[7]**.

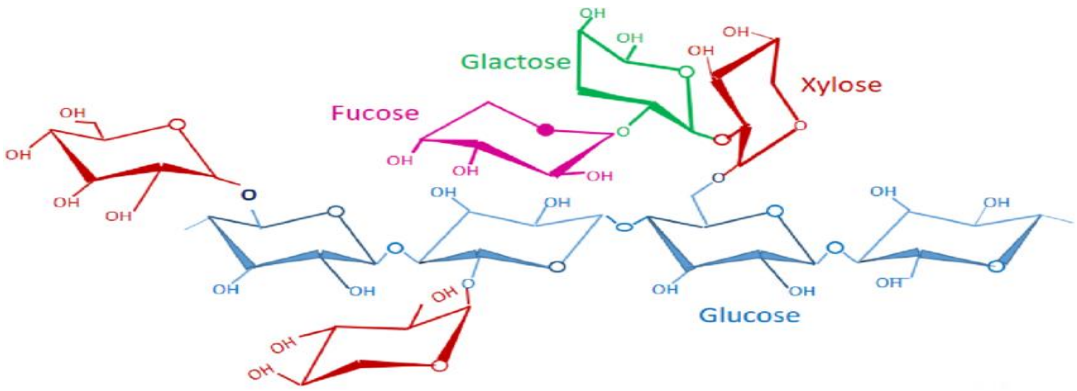


**Figure 03**: Chemical structures of hemi-cellulose

### 5.3 Lignin:

Although lignin is one of the major components of plant biomass—alongside cellulose and hemicellulose—it ranks second in abundance after cellulose. Its main functions include providing rigidity**,** water resistance**,** and strong protection against biological degradation, acting as a natural barrier **[41, 42][10].**

Lignin plays a crucial role in the structural integrity of Alfa and other plants. It is essential in the bonding of fibers, contributing to the formation of Alfa fibrils. Without lignin, Alfa fibers would not hold their structure. During the fiber extraction process, non-cellulosic components like lignin are broken down to isolate the usable fibers. This removal process, called lignolysis (or lignin degradation), is a key step in processing lingo-cellulosic materials.

Lignin does not have a uniform structure. Instead, it consists of a variety of molecules due to the presence of two alternating functional groups. Because of this complexity, the term “lignin” refers to a family of related compounds rather than a single molecule. Structural analysis shows that lignin contains aromatic (-Ar) and

aliphatic (-R) groups **[11]**, but its precise molecular architecture is still not fully understood. However, techniques such as UV-Vis spectroscopy provide insight into its general structure (see Figure 4). Lignin's strong covalent bonds give it excellent chemical and biological resistance **[12]**, which also makes it difficult to remove during the extraction of Alfa fibers.

Mechanically, lignin is relatively weak **[32][10]**. Unlike cellulose, which has a well-defined, repeating polymeric structure, lignin is amorphous and made up of three-dimensional polymers derived from three primary monolignols (phenylpropane units) **[43, 44]**:

- Coumaryl alcohol (H unit)
- Coniferyl alcohol (G unit)
- Sinapyl alcohol (S unit)

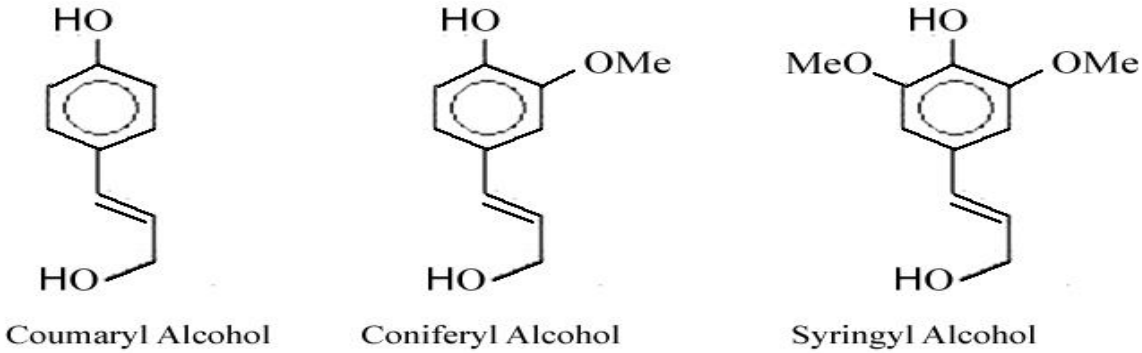


**Figure 4:** Representation of lignin components

**5.4. The pectine :**

Pectins are plant-based polysaccharides primarily found in stems and fruits, including the tiges of Alfa plants **[10].** They play a key role in binding fiber bundles (faisceaux) and have structural similarities to hemicelluloses. In fact, as fruits ripen, pectins often transform into hemicelluloses, showing how closely related their structures are **[16]**.

Like hemicellulose, pectins are made of glucose-based monomers, but they are chemically distinct because they contain carboxyl (-COOH) groups, which hemicelluloses lack. Structurally, pectins form linear chains similar to cellulose, and these can form strong hydrogen bonds due to their linearity and the presence of carboxyl groups **[32][3]**.

One of the main chemical properties of pectins is their gelling ability. In solution, they increase viscosity, and their gelling behavior depends on factors like concentration,

pH**,** degree of esterification (DE), and total solids. In alkaline conditions, carboxyl groups promote pectin chain bonding and gel formation. In contrast, high acidity and methylation also enhance gelation and pectin solubility. There are several types of pectins based on their solubility and chemical structure **[45, 46]**:

- **Protopectins** – water-insoluble forms
- **Pectinic acids** – partially or fully esterified polygalacturonic acids
- **Pectinates** – salts of pectinic acids
- **Pectic acids** – non-esterified polygalacturonic acids
- **Pectates** – salts of pectic acids

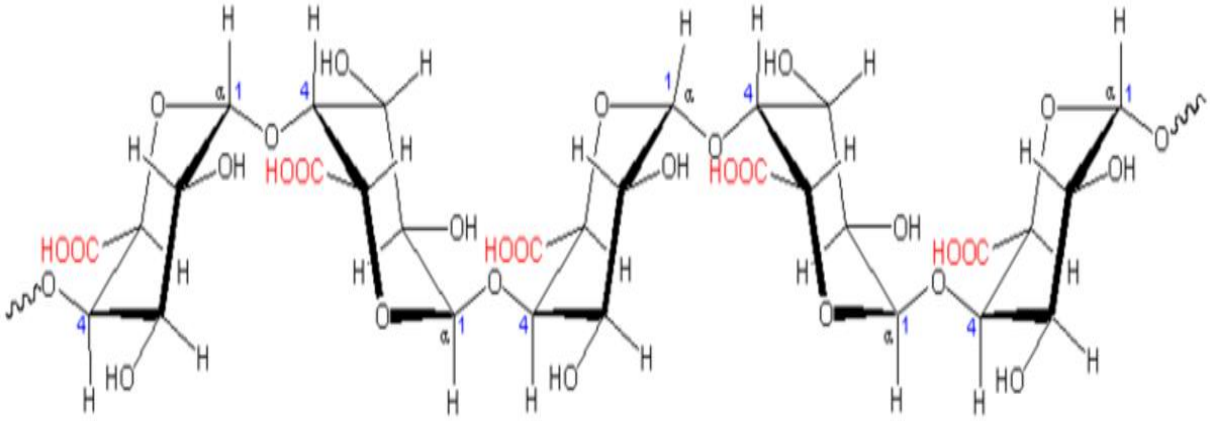


**Figure 5:** structural diagram of a pectin chain **[47, 48]**.

**5.5. the cires: [10]**

Waxes (cires) are lipid compounds found in a thin outer layer on the surface of plant stems (tiges) **[32]**. The structure and composition of this waxy layer can vary depending on the part of the plant and the stem itself. Waxes are completely hydrophobic and act as barriers to water and gas**,** significantly reducing plant transpiration **[31]**. In addition to limiting water loss, waxes serve several protective functions. They help shield the plant from harsh environmental conditions and insect attacks**.** The most common types of lipids present in plant waxes include:

- Hydrocarbons (C21–C35)
- Wax esters (C34–C62)
- Ketones (C23–C33)
- Alcohols (C22–C33)
- Fatty acids (C16–C32) **[32][20].**

The following table presents the chemical composition of plant fibres and their percentages:

**Table 3:** Percentage chemical composition of plant fiber constituents **[41] [49]**.

| Fibres | Cellulose | Hemicelluloses | Lignin | Pectin | Wax(Cires) |
|---|---|---|---|---|---|
| Cotton | 85-90 | 5,7 | 0,7-1,6 | 0-1 | 0,6 |
| Linen | 71 | 18,6- 20,6 | 2,2 | 2,3 | 1,7 |
| Hemp | 70-74 | 17,9-22,4 | 3,7-5,7 | 0,9 | 0,8 |
| Jute | 61,1-71,5 | 13,6-20,4 | 12-13 | 0,2 | 0,5 |
| Ramie | 68,6-76,2 | 13,1-16,7 | 0,6-0,7 | 1,9 | 0,3 |
| Sisal | 66-78 | 10-14 | 10-14 | 10 | 2 |
| Coco | 32-43 | 0,15-0,25 | 40,5 | 3 | 4 |
| **Alfa** | **45** | **24** | **24** | **5** | **2** |

## 6. Fiber Extraction Processes of Alfa

The complete process of extracting Alfa fibers consists of three main stages: **pretreatment, extraction,** and **post-treatment** [11]. Pretreatment includes mechanical brushing and soaking, while post-treatment involves drying and separating the fibers.

The extraction stage involves several steps, primarily based on soda treatment. This soda treatment alone is used initially but does not yield satisfactory results. Therefore, a second step is added. This second step is the beginning of a bleaching process applied to the best samples from the soda treatment. However, the results are still not optimal because soda only removes lignin, and bleaching does not eliminate anything.

To eliminate pectins, bleaching is replaced with enzymatic treatment. Pectinase enzymes are effective in removing pectins. The pectinase stage does indeed improve the outcomes of the soda treatment. However, the fibers remain somewhat rigid, possibly due to the presence of residual lignin.

Since soda can remove lignin, an additional treatment was applied to the best samples already bleached. This new extraction cycle — "enzyme–soda" — ultimately produces the best fibers.

### 6.1. Pretreatments [50, 51]

### 6.1.1. Mechanical Brushing

Initially, Alfa stems were not mechanically brushed during extraction, and the entire material was used. However, the results were unsatisfactory due to the hardness and rigidity of the raw material. When the raw material is finer, chemical treatments in later stages can be more effective because more surface area is exposed. Mechanical brushing helps solve the issue of stem stiffness. This brushing process involves moving the stems longitudinally through comb-like tools. The bristles force the stems to pass through fine teeth, helping to split them and make them more uniform. Samples from brushed stems are generally finer and less rigid than those from unbrushed stems, which justifies including brushing as a pretreatment step.

### 6.1.2. Soaking (Trempage)

Soaking involves immersing the Alfa material in saltwater (brine). The purpose is to remove surface impurities like waxes, sand, and dust from the stems. By eliminating these substances, the stems become "open" and more receptive to subsequent treatments. Historically, Alfa producers soaked the stems in seawater, a technique still used today but improved in labs with controlled higher temperatures to speed up the process. The salt concentration varies across the world. To mimic Mediterranean seawater, concentrations of 27–38 g/L are used, mainly with sodium chloride (NaCl) and water.

In general, the duration must be long enough for the waxes to have time to dissolve in the saltwater. In the literature, the following conditions are often mentioned: 12 hours at 80°C or 24 hours at 60°C. To understand the difference between two options, mechanically brushed stems are dried in an oven for 24 hours at 60°C, then soaked under the specified conditions, washed with distilled water, and finally re-dried in an oven for 24 hours at 60°C. The stems are weighed before and after soaking, so the amount of dissolved material can easily be calculated. Drying before and after soaking is meant to evaporate the water from the stems so that the exact dry mass can be determined. This allows the mass difference to represent the actual amount of material eliminated during soaking.

## 6.2. Extraction

### 6.2.1. Soda Treatment

The function of soda is to delignify the alfa stems. The stems are hard, long, and thick. Lignin is the component that connects the cellulose fibrils together to form the stems. In the laboratory, soda is available in the form of pellets and must be dissolved to obtain an aqueous solution with the desired concentration.

Depending on the temperature, the soda solution is poured into a beaker. Beakers are used for temperatures up to 100°C under atmospheric pressure by placing them in a water bath filled with water and placed on a hot plate. Under these conditions, the use of beakers in water is limited to the boiling level of water.

The water bath is used to maintain a uniform temperature in the beaker and soda solution. For this reason, it is likely that the soda does not act regularly, resulting in a less homogeneous outcome. To achieve temperatures above 100°C, baby bottles are used. The maximum temperature for this method is 140°C. A reducing agent, which also acts as an antioxidant, may be added to protect the cellulose filaments against degradation (oxidation) by soda. The Na2O4S2 used as a reducing agent is only available in the form of a white powder and is soluble in water and aqueous soda solutions **[50, 51].**

#### 6.2.2. Replacement of Bleaching by Enzymatic Treatment

A single soda treatment, possibly followed by bleaching, is not enough. Either the results are too rigid due to lignin and residual pectins, or the results are too gelatinous due to high temperatures and pressure. Since pectins are similar to pectic fibers, the goal is to continue with less rigid treatments, meaning with soda concentrations below 0.25N or under 140°C. The goal is to obtain finer and more flexible fibers through the elimination of pectins. Enzymes are used in this phase and are generally applied to samples previously treated with soda at 150°C or 100°C (with 0.25N soda) to remove pectin residues from the fibers **[50, 51]**.

#### 6.2.3. Addition of an Extra Soda Treatment after Enzymes

In conclusion, an additional treatment is necessary to obtain finer and less rigid results. It is evident that preliminary research has not been completed, but the best samples obtained are selected and treated again. These samples are produced under the following conditions:

- Soda: 0.25N at 140°C for 1h to 2h with 0.5% to 1.5% reducing agent
- Enzymes: 5 ml/l at 38°C for 2h with a pH value of 7

### 6.3. Post-Treatments

#### 6.3.1. Drying

In general, the fibers are dried in an oven at a temperature of 50°C to 60°C for 12 hours or more until completely dry. This step is one of the most difficult to execute due to how the fibers are placed in the oven. After extraction, samples are placed in a beaker to be washed with distilled water. The fibers are not aligned in parallel, but rather placed freely. This position is maintained in the oven so that the fibers do not

stick together either during or after drying. Otherwise, it becomes difficult to separate them **[50, 51]**.

#### 6.3.2. Separation of Fibers after Drying

The separation of fibers is necessary because drying does not occur under optimal conditions and results in a mixture of fibers of varying qualities. This step is similar to a mechanical combing or carding of the fibers. As with wool fibers, the separation is done manually or with a brush to obtain the most flexible and precise results. This stage is important for fiber use. It is recommended to frequently handle and clean the fibers during this process **[50, 51]**.

## 7. Extraction techniques of Alfa fibers (Methods) :

Cellulose does not exist in pure form in plants; it is tightly bound with other components such as lignin, hemicellulose, and waxes. Therefore, extracting pure cellulose from plant biomass requires breaking down these complex structures. Several extraction methods mechanical, chemical, enzymatic, and solvent-based are used, either alone or in combination, to isolate cellulose with high purity and yield. Each method has its own advantages and limitations depending on the source material and intended application. [48] [19].

### 7.1. Mechanical extraction

Mechanical extraction is a physical method used to isolate plant fibers by breaking down plant structures mainly the stems or leaves without using chemical agents. It relies on crushing, beating, brushing, or grinding to separate cellulose-containing fibers from non-cellulosic materials like lignin, hemicellulose, and woody parts.

This technique is often a pre-treatment step before chemical or enzymatic extraction, as it opens the plant structure and increases the accessibility of cellulose.

**a- Teillage (Retting/Decortication)**

Mechanical extraction involves separating the woody core from plant stems using physical force, such as crushing and beating. This method is commonly used to extract fibers from plants like flax and hemp. Traditionally, the stems were handled manually using tools like the "tilleul" or "écang" lever-operated devices used to break the stems **[2][52- 54]**.

**b- Brushing with Metal Dents**

In this method, brushes equipped with metal teeth comb through the leaves along their longitudinal axis. This action reduces the diameter of the leaves and simultaneously removes some of the woody material. As a result, the fibers become softer and more pliable, although they may still contain non-cellulosic components. This process helps to open up the leaf structure, making it more accessible for subsequent chemical treatments.

**c- Grinding and Milling**

The processes are used to break down plant materials such as stems, leaves, or bark—into smaller, finer particles. This is done using machines like grinders, mills, or pulverizers, which apply physical force (such as pressure, friction, or impact) to reduce the size of the material. The main goal of this process is **to** increase the surface area of the plant material. When the material is finely ground, it becomes more exposed and easier to interact with in later stages, such as chemical, enzymatic, or solvent treatments**.** These treatments rely on contact with the cellulose, so a greater surface area improves efficiency and extraction yield.

**d- High-Pressure Homogenization**

- Plant material is forced through narrow channels at high pressure.
- Breaks cell walls and separates fibers.

**e- Steam Explosion (partially mechanical) [55, 56]**

- Uses high-pressure steam followed by sudden decompression.
- Physically disrupts the structure and helps fiber release.

**6.2. Chemical extraction:**

Several methods based on the chemical separation of cellulose from other non-cellulosic components are found in the literature. These methods help to avoid the disadvantages of mechanical extraction and, above all, offer significant savings in time and energy. In this section, we present the main chemical extraction methods for plant fibers **[57-61]**.

**a. Kraft Procédé :**

This alkaline treatment is designed to remove lignin, pectins, and hemicelluloses by using a solution of sodium hydroxide (NaOH) and sodium sulfide ($Na_2S$). Sodium sulfide acts as a reducing agent, helping to protect cellulose and prevent its oxidation. The cooking process (known as "cuisson") is typically carried

out at temperatures between 170°C and 175°C for a duration of two to four hours. During this process, sodium sulfide undergoes hydrolysis, producing sulfur, sodium hydrogen sulfide (NaHS), and hydrogen sulfide ($H_2S$). These sulfur-containing compounds interact with lignin, breaking it down into more soluble forms known as thiolignins. Sulfur and its byproducts thus contribute significantly to the delignification process. The term "white liquor" refers to the chemical mixture used in the treatment, while the term "black liquor" describes the residue containing dissolved cell wall components removed during the process **[58, 60]**.

**b. Bisulfate process:**

The bisulfite process allows for the separation of cellulose fibers from lignin using different forms of sulfur-based acids. The separation is achieved by exploiting pH-dependent reactions involving either sulfite ($SO_3^{2-}$) or bisulfite ($HSO_3^-$) ions. These reactions typically involve calcium, sodium, ammonium, or magnesium bisulfite reacting with free sulfur dioxide to break down lignin. Sulfur dioxide is produced by burning elemental sulfur in an excess of air, and it directly forms bisulfite upon contact with water. The process operates within a pH range of 1.5 to 5, at temperatures between 130°C and 160°C, and may last from 4 to 14 hours depending on the specific base used **[58, 62]**.

**c. Acidic process:**

The non-cellulosic components are eliminated by the action of an acid, preferably sulfuric acid, which converts lignine into soluble lignosulfonic acid, or chlorhydric acid, which, thanks to its chlorates, forms chlorolignines soluble in sodium hydroxide **[63]**.

**e. Soude process :**

This method uses sodium hydroxide (NaOH) to dissolve non-cellulosic substances such lignine, pectine, and hemicellulose as well as the other components that make up the reserve and outer layer of plant tige **[64]**. The treatment's temperature, pressure, concentration, and duration should be determined by the plant's kind, age, and lot in order to prevent the cellulosic fibers from degrading **[65, 66]**. It is

advised to monitor the solution's pH and adjust it to about 7. It is possible to add reducing agents to stop the cellulose from oxidizing.

### 6.3. Enzymatic extraction:

Biological extraction of Alfa (Stipa tenacissima) fibers is an environmentally friendly method that uses natural microbial or enzymatic processes to isolate fibers from the plant material. Unlike chemical methods that rely on harsh substances, biological extraction allows for the gentle degradation of non-cellulosic components such as pectins, hemicelluloses, and lignin, which bind the fibers together. Microorganisms or added enzymes like pectinases, cellulases, and hemicellulases break down these binding materials while preserving the integrity of the cellulose fibers **[58, 59]**. This technique offers significant advantages, including lower environmental impact, preservation of fiber strength, and reduced energy consumption. However, it also presents challenges such as longer processing times and variability in results due to differences in microbial activity and environmental conditions. Despite these limitations, biologically extracted Alfa fibers are well-suited for use in eco-friendly textiles, biocomposites, and paper manufacturing, aligning with sustainable development goals **[67, 68].**

## 7. Application domains:

Alfa has numerous and diverse applications, which can be grouped into two main categories based on the type of material used **[63][69- 70]**:

- **The stems (tiges) of Alfa:**

In traditional crafts, these stems are spun or braided to create various products such as ropes and woven goods, including mats, baskets, trays, tapes, and twine. It is estimated that households living in Alfa-growing regions use around 50 kg per year for such artisanal purposes (see Figure 26) [46].

- **Grazing:** The nappes alfatières make up a pastoral reserve area for both wild animals (gazelle) and livestock (boeufs, moutons, chameaux, etc.). Due to its low nutritional value and relative neglect by animals in favor of more

valuable pastoral resources, it makes up a vast stock that enables animal survival for years.

- **Fuel:** Alfa has a higher calorific value of 4666 Kcal/kg for brins aged 1 year and 5160 and 5163 Kcal/kg for brins aged 2 and 3 years, respectively **[69]**. This gives it a significant energy use in the form of combustible briquettes that can be used in place of or in place of firewood.

**Figure 6**: Examples of traditional crafts made from Alfa, including baskets, mats, espadrilles, and more.

➢ **The fibers of alfa:**

- **Papermaking paste:** Alfa paper, known for its high quality, emerged in the late 19th century, highlighting the economic importance of this plant. The paper industry is the largest consumer of Alfa. According to Figure 27 **[71]**, the national society of cellulose and paper of alfa (SNCPA – Tunisia) produces approximately 25,000 tons of paper and 12,000 tons of pulp per year. This paper is mainly used for premium applications, including writing paper, cigarette paper, filter paper, and capacitor paper for electronics.
- **Nonwovens:** Work has been done to replace the glass and carbon fibers, which has been expensive and affects the final product's return price. For example, in the orthopedic field, the non-tissés are used as a couch of reinforcement for emboitures **[72]**.
- **Analogously**: research has been done to create composites based on alfa fibers in a polypropylene, polyester, or PVC matrix. With the goal of creating biodegradable composites with good mechanical and acoustic properties and less environmental impact, natural fibers are being used more and more these

days. However, this kind of application has several implementation challenges, such as cohesiveness issues with the used matrix **[73, 74]**.

**Figure 7:** Illustrations of Alfa paste marketed by SNCPA

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Chapter II

# The Cellulose and its modification

## 1. History:

The first description of cellulose dates back to 1838, when Anselme Payen proposed that plant cells are almost all made of the same fibrous material that survives treatment with either ammoniac or acid. For the first time, the name "cellulose" was used in 1839 in a French Academy report on Payen's investigations **[1].** It then took a little over five hundred years to determine its basic chemical formula ($C_6H_{10}O_5$), which Weill Statter had discovered **[2].**

Numerous extraction techniques were developed throughout the 19th century in an effort to obtain the purest cellulose possible:

- 1853: the process of cuissoning salt at 170°C allowed for the production of a marron-based packaging paste with a 70–80% cellulose content;
- 1866: the Bisulfite acide process using calcium acid ($Ca(HSO_3)_2$) and sulfur dioxide ($SO_2$) at 140°C;
- 1879: the Kraft process based on salt and sulfur (Na2S) **[3].**
- In 1920, German chemist Hermann Staudinger (1881–1965) determined the polymer structure of cellulose. The compound was first chemically synthesized in 1992 by Kobayashi and Shoda without the use of biologically derived enzymes **[4]**.

## 2. Definition:

Cellulose is the most abundant organic substance in nature, accounting for over 50% of biomass, or about 90 billion tonnes, that are synthesized by terrestrial plants. It is also one of the main polysaccharides that make up plant paroi (polysaccharides pariétaux) **[5].** It is a structurally significant component of 95–99% cotton and 45–50% dry mass wood. Its annual production is approximately 1012 tons. **[5, 6].** The term "cellulose" indicates that a sugar "ose" is produced by cells [15]. The elemental mass composition of cellulose macromolecules is 49.4% oxygen, 44.4% carbon, and 6.2 percent hydrogen

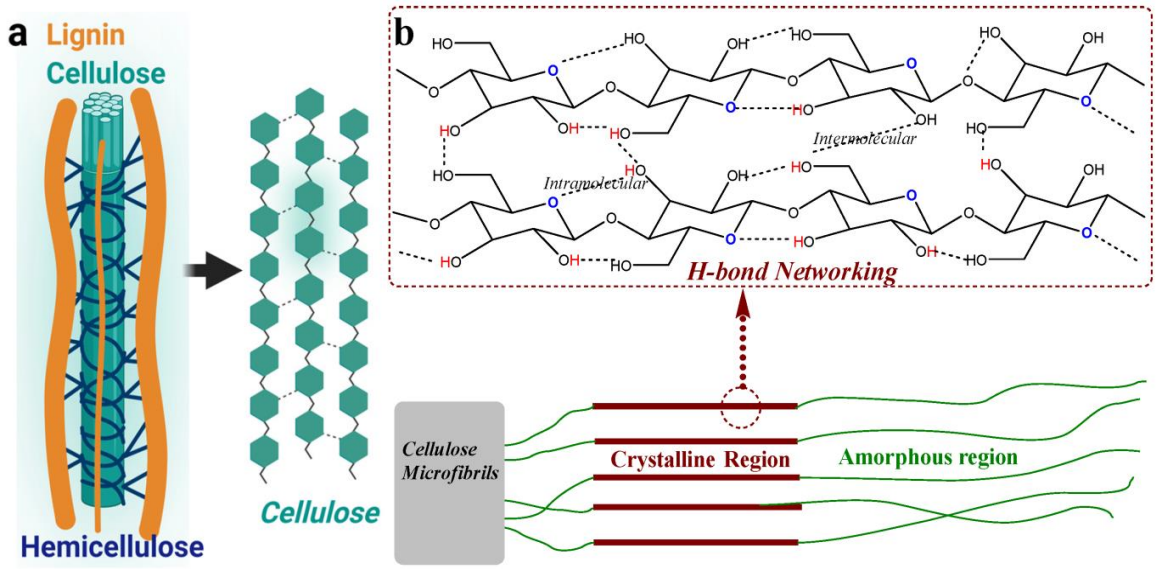



**Figure 1:** (a) Cellulose in natural form (coexisting with lignin and hemicellulose). (b) Network of intermolecular H-bonding within the cellulose structure

**Table 1:** Cellulose content of various plant species **[7]**

| **Plant species** | **Cellulose content (%)** |
|---|---|
| Cotton | 95-99 |
| Wood | 40-50 |
| Corn bran | 17-20 |
| Straw | 50-60 |

The cellular walls of plants are supported and protected by the cellulosic fibers. This polymer is also biosynthesised by a bacterium called Acetobacter xylinum via a different method **[8]**.

## 3. The cellulose biosynthesis :

With a variation in content of 30% to 50%, cellulose is the main component of cell walls, where it ensures the support of vegetative organisms **[9]**. Additionally, cellulose can be found in the skeleton of some marine animals or can be obtained from bacteria or algae **[10, 11]**. Its chemical structure is well understood, however its crystallnity and fibrous structure are less clear.

Its structure can be described at many scales:

**a) At the molecular scale:**

Cellulose is represented by a linear macromolecule of glucopyranose units linked by β-(1,4) bonds. Cellulose is synthesized at the level of the plant cell's plasma membrane by a cellulose synthesis enzyme complex (CESA) grouped by six to form subunits, themselves grouped by six to form rosettes of the order of 25 nm in size. Each rosette thus produces 36 cellulose chains **[12]**;

**b) Aggregation scale:**

Chemical composition, spatial conformation of cellulose chains, and Van der Walls's liaisons **[13]** all tend to make the chains more similar to one another. The presence of hydroxyl groups in C2, C3, and C6 enables hydrogen bonds to form both within and between molecules, forming ordered crystal formations called microfibrilles, which range in size from 5 to 20 nm.

**c) At the macro-scale:**

This is the representation of the organization of fundamental blocks. The microfibrilles join together to produce larger fibers (15–20 µm). Multiple couches of microfibrilles with a clearly defined orientation make up the fibers **[14].**

## 4. The structure of Cellulose [16]

### 4.1. Molecular Structure:

The first steps in structural characterization of cellulose date back to 1920, when Staudinger determined the polymer structure of cellulose **[17].** Then, studies by Irvine and Hirst [18] and Freudenberg and Braun **[19]** demonstrated that the cellulose's carbons C2, C3, and C6 carry hydroxyl groups. The discovery by Haworth et al. **[20]** that cellobiose is the fundamental component of cellulose has made it possible to comprehend that a homo-polymer composed of units of anhydroglucopyranose bound by β-glycosidic bonds is at work. It was then demonstrated by Chu and Jeffrey **[21]** that cyclic D-glucopyranose had a conformation resembling 4C1. Cellobiose is the unit of repetition. It is made up of two 180°-oriented glucose motifs, one centered on the glycosidic bonds C1-O-C4. Chemically, the two ends of the cellulose chains are not equivalent. An end is made up of a D-glucopyranose with an anomer carbon

bonded to a glycosidic bond and a secondary free alcohol function on the C4. The other extreme is a unit of D-glucopyranose with a free carbon atom; as a result, it has a cyclic function that is balanced with a minor aldehydic form. This "reducing" end has the ability to reduce Cu2+ ions in a solution of Fehling ions in Cu+. This gives cellulose a chemical polarity **[22]**.

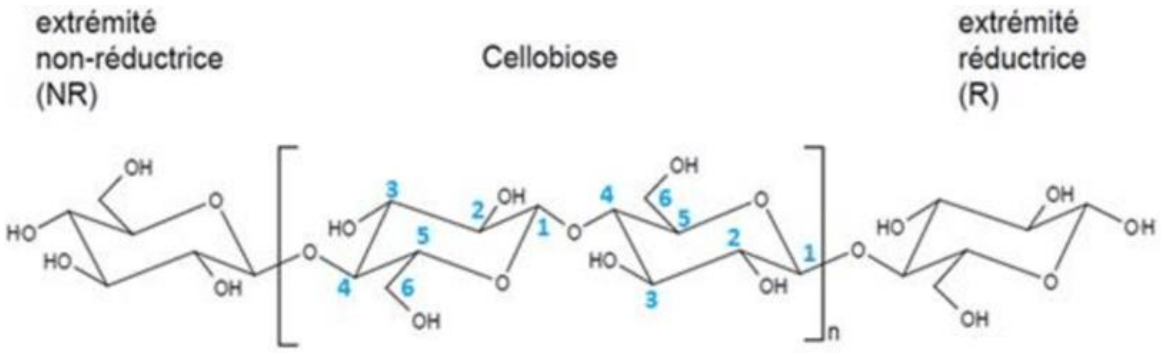



**Figure 2:** Chemical structure of cellulose **[23][24].**

Each unit of anhydroglucose contains three free hydroxyl groups: two secondary alcohol functions on carbons 2 and 3 and a primary alcohol function on carbon 6. In contrast to the axial position of the liaisons with the hydrogen atoms, these and other glycosidic-related liaisons are equatorial **[25]**.

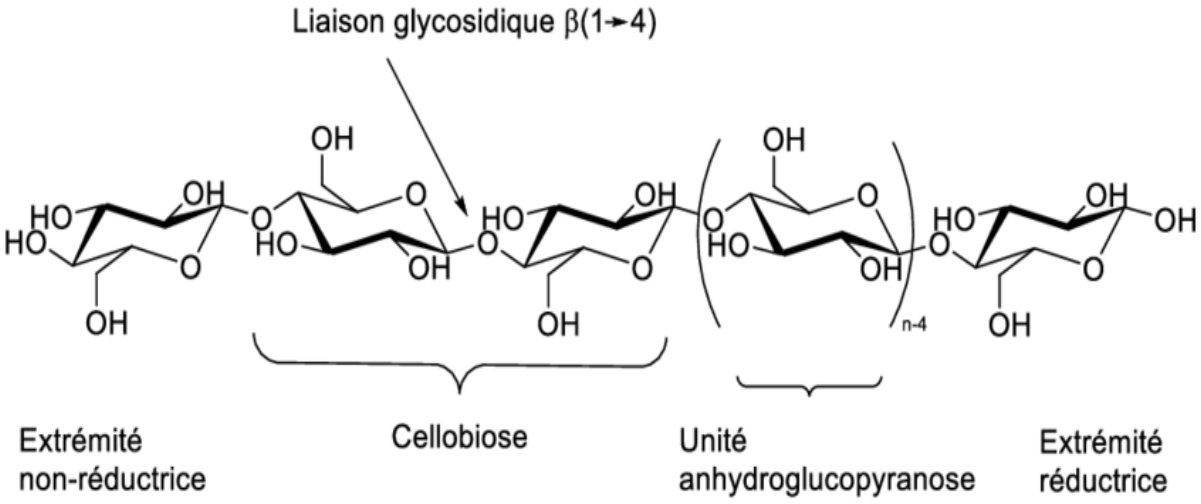



**Figure 3:**Structure moléculaire de la cellulose **[26]**

### 4.2. Supramolecular structure :

The three hydroxyl groups found in cellulose's chemical structure enable the creation of hydrogen bonds both inside and between molecules, resulting in a highly ordered structure that has been the focus of considerable research for several decades **[27, 28].** The structure of native cellulose was described by Gardner and Blackwell **[29]** as a monoclinic maille with two chains. This is because two allomorphes for native cellulose, known as type I, have been identified using a new technique called RMN 13C in its solid state. The allomorphe Iα has a triclinic maille and a chain by maille, while the allomorphe Iβ has a monoclinic maille and two chains by maille **[29, 30]**. The ratio of Iα and Iβ allomorphs varies depending on the species.Phase Iα is primarily found in cellulose produced by primitive organisms like bacteria or algae, whereas phase Iβ is primarily found in cellulose produced by higher plants (such as bois and cotton) and the envelope of a marine animal called a tunicier **[31, 32].** The allomorphe Iα can be converted into the thermodynamically more stable phase Iβ by hydrothermal treatment at 260 °C or by recuitting in organic solvents with different polarities **[33, 34].**

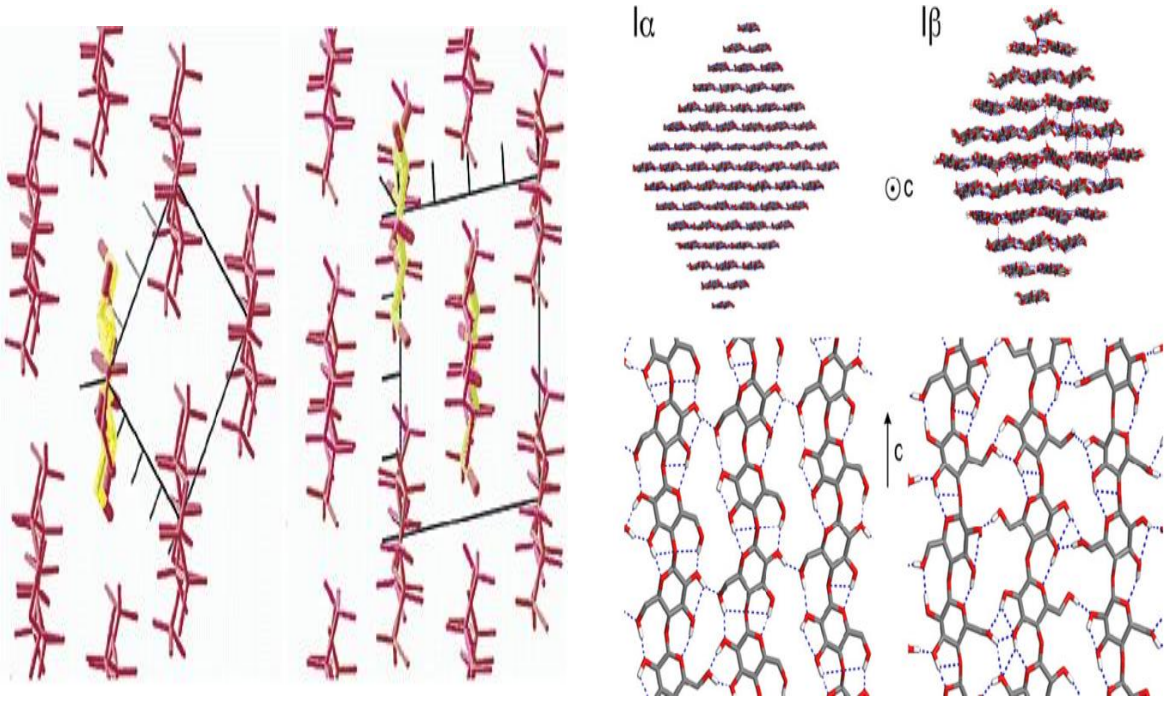



**Figure 4:** Représentations schématiques des mailles élémentaires des allomorphes Iα et $I_\beta$ de la cellulose I et du réseau de liaisons hydrogène intra et inter-chaînes **[35].**

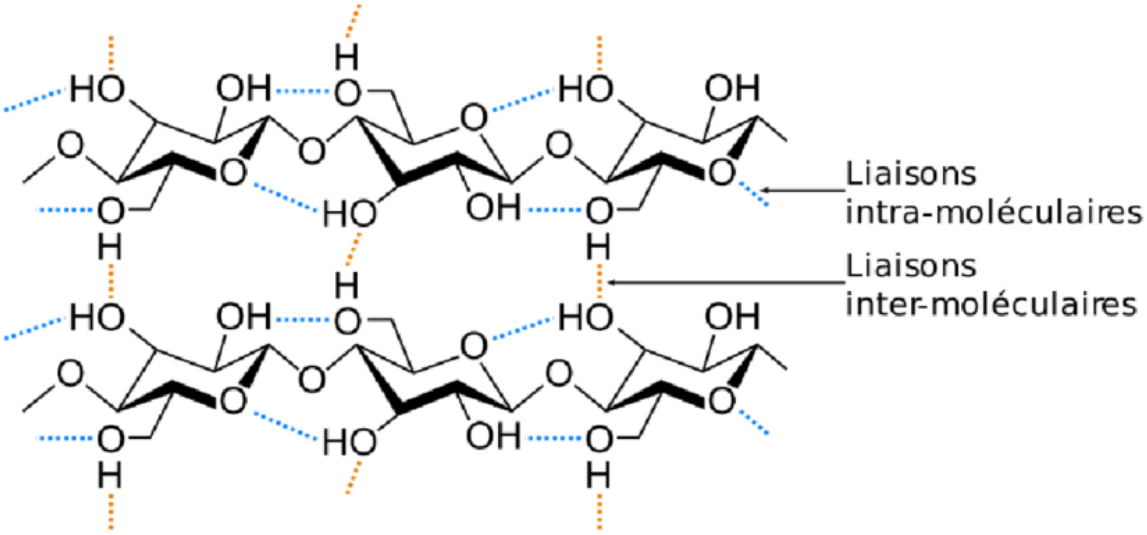
## 5. Crystallinity:

Cellulose is a semi-crystalline polymer. Its crystallinity rate ranges from 43 to 56% for wood and is roughly 60% for cotton. In crystallographic regions, molecules exhibit nearly perfect alignment, forming a stable network of hydrogen bonds

between and within molecules. This results in strong chain rigidity. Although there is little degree of organization in amorphous zones, chains are oriented randomly. **[36, 37, 38].**

Cellulose crystallization is made possible by the linear nature of its chain. The numerous hydroxyl groups (-OH) are responsible for the physicochemical behavior of cellulose. Thus, depending on their position in the glucose unit, two types ofhydrogen bonds can be established **[36, 37, 38]**:

- Intramolecular bonds, between two adjacent hydroxyl functions in the same cellulose chain, which stabilize the cellulose in its linear orientation and give it some rigidity; **[39,40]**
- Intermolecular bonds, between two adjacent hydroxyl functions of two chains, which reliently bind multiple macromolecules and maintain them in parallel **[41,42].**




- **Figure 5:** Intramolecular and intermolecular hydrogen bridges between two adjacent cellulose macromolecules **[43, 44]**.

Therefore, the coupling of several cellulose chains promotes the production of an ordered, partially crystallin state and enables the formation of a more complex structure known as microfibrilles. Cellulose microfibrilles are made up of partially

ordered crystallinous and amorphous zones, as opposed to completely disordered ones **[40]**.

There are various ways to measure the cellulose's crystallinity rate. The most popular ones are infrared spectroscopy (which reports characteristic band areas), X-ray diffraction (which compares the spectra of cellulose I and II), and RMN 13C spectroscopy **[43] [45]**. The degree of crystallinity is determined by the cellulose's origin and subsurface characteristics **[46].** The cellulose's crystallinity rate ranges from 40 to 50% for wood, 60% for cotton, and up to 70% for some marine algae **[47].** The existence of these intermolecular and intramolecular hydrogen bonds makes cellulose insoluble in water and most organic solvants [46] [48]

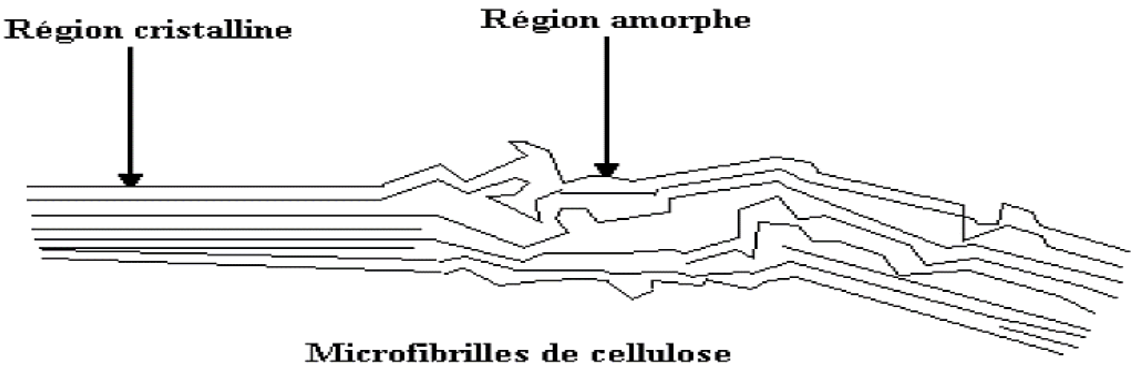



**Figure 6:**Schematic representation of the crystalline and amorphous zones of cellulose **[42]**.

## 6. The properties of cellulose:

- The molar mass of the cellulose chain varies widely (between $1.5.10^4$ and $2.5.10^6$ g.mol-1 ), depending on the ori;gin of the plant and the extraction treatment used;
- The molar mass of the (anhydroglucose) unit is 162.1 g/mol[49] ;
- The density of cellulose is typically between 1.50 and 1.55 g/cm3 ;
- Specific heat Cp: 1.32 to 1.78 J/g.K (at 273 K) **[50];**

- Elongation at break: 20 to 40% ;
- Coefficient of thermal expansion: $80.10^{-6}$ $K^{-1}$ ;
- Thermal conductivity: 0.06 W/m.K at 23°C;
- Cellulose fiber is flammable **[51]** ;
- Degradation temperature: 330-350°C **[52];**
- Glass transition temperature (Tg): 220-245°C amorphous, 243-433°C crystalline;
- Enthalpy of crystallization: 121.8 KJ/g **[53]** ;
- Cellulose is difficult to dissolve, being insoluble in water and most organic solvents.
- Solvents capable of dissolving cellulose include **[54,55]**:
  - Aqueous solvents: complexing agents such as cuprammonium hydroxide

cuprammonium hydroxide (cuam), cupriethylene diamine (cuen) or

cadmium ethylenediamine (cadoxen) **[56,57]** ;

  - Non-aqueous solvents: DMAC (dimethylacetamide), with amine compounds (cupriethylenediamine) or salts (lithium chloride...), lithium chloride...), N-methylmorpholine oxide (NMMO) ;
  - Certain ionic liquids such as 1-butyl-3-methylimidazolium (BminCl) or 1-ethyl-3-methylimidazolium (EminAc) **[58]**.

## 7. Chemical modifications of cellulose:

The two most frequent cellulose modifications are the esterification and etherification of the cellulose's hydroxyl groups. These chemical modification techniques are used to generate a variety of cellulosic derivatives that are hydrosoluble or soluble in organic solvents. However, there are other kinds of transformation, such as oxydation and deoxyhalogénation. It is feasible to perform on

cellulose all the necessary alterations to primary (C6) and secondary (C2, C3) alcohols, ether bonds (β(1-4)), and, at a lower level, aldehyde functions**[59, 60]**.

Based on the changes made to the polymère, these various reactions can be divided into two groups **[61]:**

- Modifications to the substance itself (oxydation of the glycol bond, oxydation of the primary alcohol function)
- Modifications to the hydroxyl groups

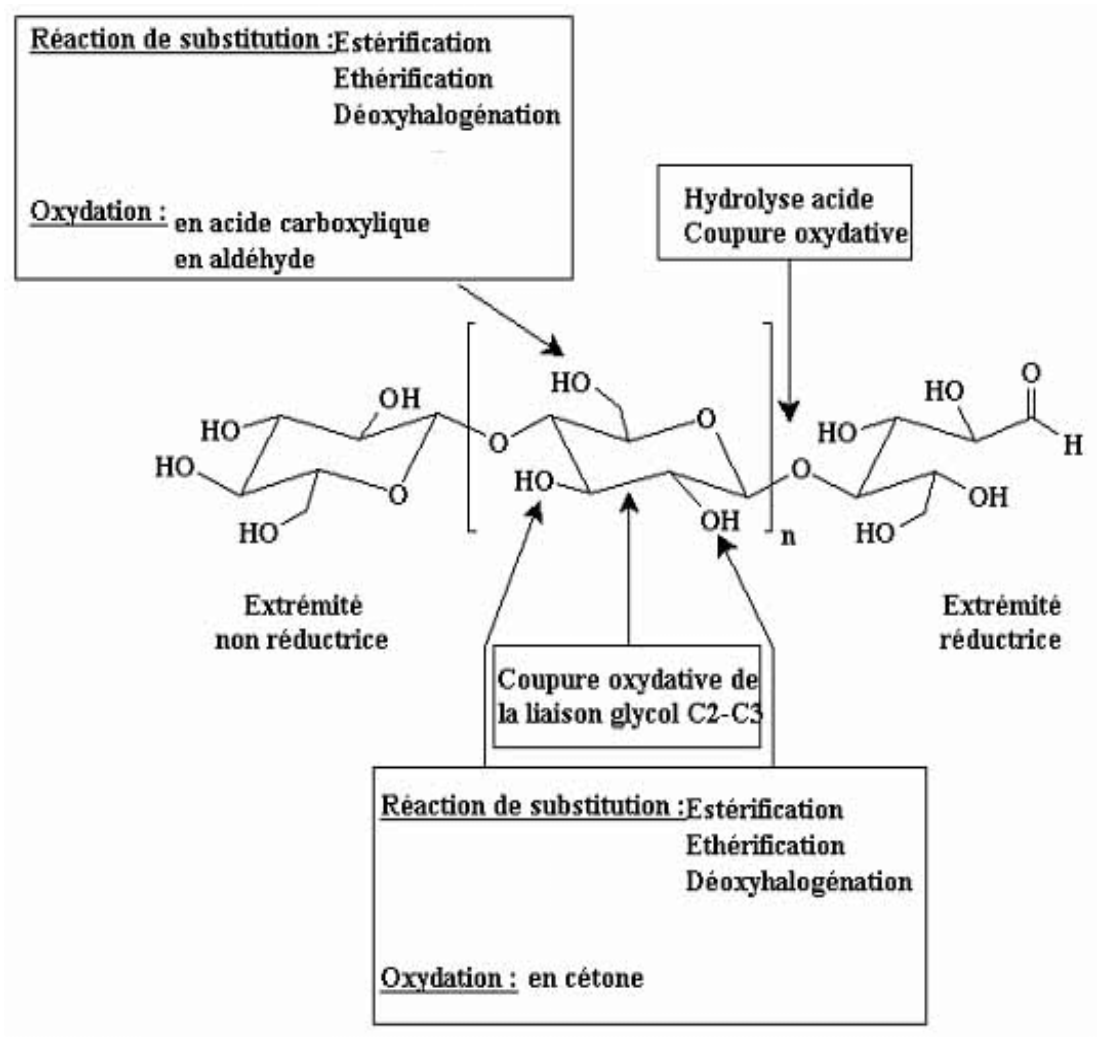



**Figure 7:** Most common chemical modifications related to polymer structure **[62]**.

### 7.1. Selective oxydation of cellulose:

With two contiguous secondary hydroxyles and a primary alcohol function, cellulose can be thought of as a polytril. An oxydant agent can convert the primary hydroxyle found on the carbon C6 of that cellulosic material into a carboxylic acid. Other forms of oxidation may occur at the secondary hydroxyle level. One of them enables the oxidation of alcohols to aldehydes and the breakdown of cellulose by breaking the C2-C3 bond. This process, known as periodic oxidation, is carried out in the presence of sodium periodate (NaIO4).This kind of oxidative coupling can also be accomplished by action on cellulose from nitrate of ammonium citrate (CAN), which will cause the disruption of the C2-C3 bond to form an aldehyde function and a radical function on a carbon containing a hydroxyle group **[64]**.

This compound is then greffed by acrylic nitrile, which, upon polymerization, results in the creation of a greffe copolymer: cellulose/polyacrylonitrile. It is also possible to convert the hydroxyle functions at positions 2 and 3 into cetones **[65,66]**.

### 7.2. Modifications to the hydroxyle groups of cellulose:

#### 7.2.a . Deoxy substitutions:

The two most common desoxy-substitution reactions are aminocellulose synthesis and desoxy-halogenations.

- **The process of desoxyhalogenation:**

To functionalize cellulose, the derived halogens may serve as the starting point for substitution reactions. This reaction preferentially occurs on carbon 6 and then on position 3. Disubstitution can be accomplished via action, either by a homogenous sulfuryl chloride phase or by a mixture of tribromoimidazole and triphenylphosphine on cellulose **[67][68]**.

- **L'amination:**

After protecting positions 2 and 3 and tosylating position 6, Tiller et coll. completed the cellulose's amination. In a basic environment, this position 6 is changed to a diamine to produce a cellulosic amine compound. In this case, the fixed chain is either an aromatic polyamine or a lengthy chain that is terminally positioned**[69]**. These

amino acids were synthesized to act as a support for the immobilization of enzym;es used as biological capteurs **[70]**.

### 7.2.b. Cellulose Ethers and Analogs

The halogénénures have the ability to etherify cellulose. In the majority of cases, the chains that are greffated are functionalized by carboxylic functions (carboxymethylcellulose (CMC)) or hydroxyl groups (hydroxypropyl cellulose (HPC), hydroxyéthylcellulose (HEC)). The carboxymethylation of cellulose is the reaction of the acid-non-chloroacétique with cellulose in the presence of a base. A long-chain amine (in a homogeneous phase) can then amidate the carboxylic function to change this CMC **[71]**. The resultant product is a polymer with hydrophobic properties **[72].** nFurthermore, partially ethylened celluloses (HPC, HEC, and CMC) are soluble in water, and their free hydroxyl groups can be substituted by reactions with long-chain hydroxyl compounds or alkyl ethers to produce hydrosoluble cellulosic polymers **[73]**.

### 7.2.c. Cellulose esters and their analogs

Cellulose esters are a class of cellulose derivatives formed by replacing the hydroxyl (-OH) groups on the glucose units of cellulose with ester groups (-O-COR). This modification improves the solubility, flexibility, and processing of cellulose, making it suitable for a wide range of applications. These cellulose esters can be produced, for instance, via phosphorylation or sulfatation reactions **[74]**. The resulting products are agents that may have anticoagulant properties or good filmogenic qualities, such as tosylcelluloses **[75]**. Last but not least is the potential for cellulose nitrate synthesis, which is used, among other things, as vernis to wood **[76]**.

The most common types of cellulose esters include:

- **Cellulose acetate** (from acetic acid)
- **Cellulose nitrate** (from nitric acid)
- **Cellulose propionate** and **cellulose butyrate** (from propionic and butyric acids, respectively)

These modifications alter the natural properties of cellulose, making the esters more **thermoplastic**, **hydrophobic**, and **soluble** in organic solvents, which pure cellulose is not. As a result, cellulose esters are widely used in various industries **[77, 78].**

## 7.3 CELLULOSE ACETATE:

### 7.3.1. Overview:

In 1865, Schützenberger was the first to synthesize cellulose acetate. By reacting the acetate anhydride with cotton cellulose heated in a closed tube at 180°C **[79, 80],** Produced on a large scale starting in the early 1920s, it continues to be the most significant of the cellulose's organic esters at the industrial level. In the most traditional acetylation process, native cellulose fibers are gradually converted to cellulose by a mixture of glacial acid and anhydride acid in the presence of a catalyst such as sulfuric or perchloric acid **[81, 82].**

Once the elaboration process was improved. This compound is used in many different fields, such as the production of fibers (for textiles), plastic materials (thermoplastiques for moulage), and films (filtering membranes). Its yearly production is approximately 106 t **[83-86].**

### 7.3.2. Chemical composition and explanation

The organic cellulose esters are called cellulose acetates. Their chemical structure is derived from that of cellulose, with hydroxyl groups that have been partially or completely replaced by acetate groups. The degree of substitution in acetyl (designated DS, which also denotes the mass in acetyl groups) is given significant weight in the cellulose esterification process. It consists of numbers 0 through 3. Its value is fundamental since the polymer's physical and chemical properties result in dependence **[87]**.

### 7.3.3. The process of cellulose esterification:

The most widely used method for producing cellulose acetate is esterification of cellulose using acid anhydrides. This fabrication breaks down into four separate phases **[88]**:

**a) A preliminary cellulose activation treatment** is required. The used cellulose is distinguished by a high DPv (usually between 1000 and 7000) and a strong concentration of cellulose known as alpha (at least 94%, very pure cellulose, traditionally seen in cotton linters). It is preferable to dry using glacial acid rather than

an over-drying method in order to have the most reactive cellulose possible. After that, the cellulose is crushed and kneaded using pure acetate **[89]**.

**b) The acetylation** is accomplished by adding the catalyzing acid (H2SO4) and the acetic anhydride. It then forms a cellulose triacetate. As the reaction progresses, the cellulosic fibers disintegrate more and more. Only in the presence of an excessively significant amount of an acetic anhydride can the complete dissolution occur. The end of the esterification is then indicated by her **[90].** Two phenomena are caused by this reaction: the hydroxyl groups in cellulose react, cellulose chains fission, and therefore the cellulose's DP decrease. It is important to pay close attention to the temperature that promotes the hydrolysis of cellulose because this reaction is very exothermetic **[91].**

Acetylation is a useful technique for reducing the number of hydroxyl groups in cellulose through the action of a mixture of glacial acid and anhydride acid in the presence of a catalyst such as sulfuric or perchloric acid, which increases hydrophobicity and decreases hydrogen bonds. The cellulose acetate was first synthesized by Schutzenberger in 1865, and it began to be produced on a large scale around the beginning of the 1920s **[92].**

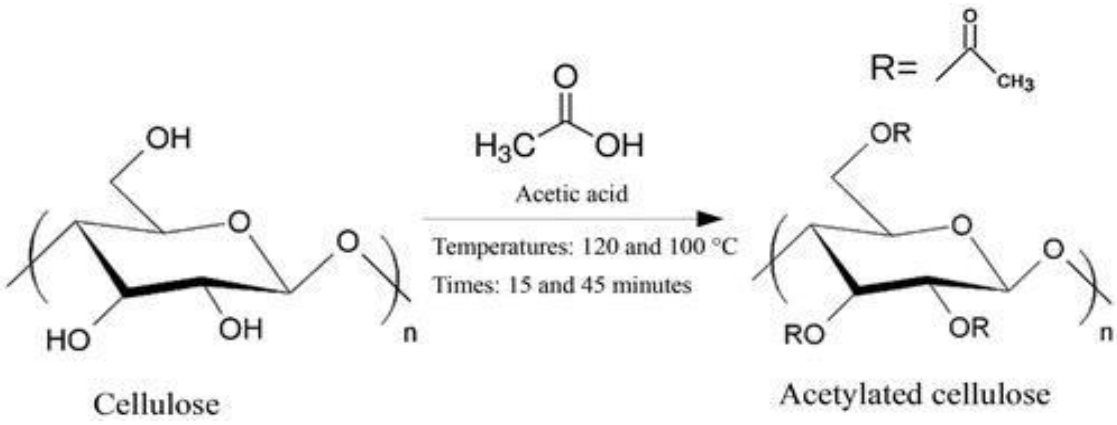



**Figure 8:** Cellulose acetylation reaction **[93]**

**c) The purpose of hydrolyzing** is to change the triacstate that is produced after acetylation into a derivative that is soluble in acetone. Water is added to the previous mixture (an acidic solution) to enable the hydrolysis of triacétate into diacétate. The

sulfuric acid is also a catalyst for this step. Primarily, it enables the DS of synthesized derivatives.

**d) Precipitation and purification** is the final step, After adjusting the acetate's DS, cellulosic acetate is precipitated by adding water to the solution and then letting the precipitate return to the water. This purification is essential for getting rid of chemical agent traces and recycling chemical acid. **[92]**

### 7.3.4. Cellulose triacetate CTA:

In 1948, cellulose triacetate (C12H16O8) gradually replaced nitrate in the production of supports. Its exceptional non-flammability qualities are what gave rise to its moniker as "socially responsible film." It is produced by esterifying cellulose in the presence of an acid; sulfuric acid catalyzes the reaction. Its industrial manufacturing uses significant amounts of organic solvents (methanol, n-butanol, and methylene chloride). In place of the previously mentioned esters, the addition of plasticizers (triphenylphosphate, butyl or ethylene palatal) strengthens its plastic qualities.

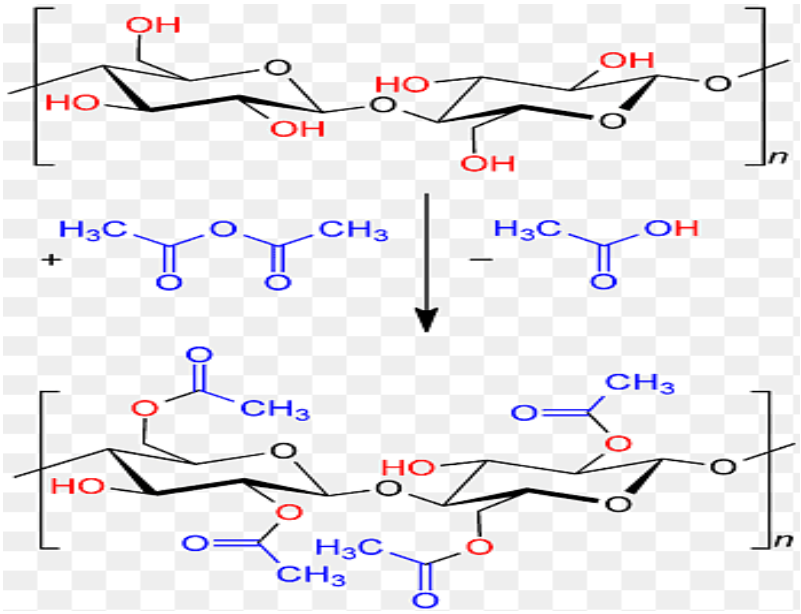



**Figure 9 :** Cellulose Triacetate synthesis

The first signs of the "vinegar syndrome" appeared towards the end of 1980, revealing the fragility of cellulose-based triacetate supports. This degradation process, which is exclusive to triacetate, involves two different hydrolysis reactions that result in the loss of a portion of the acetate groups and breaks in the main polymer chain. These two reactions are auto-catalyzed by the addition of metal particles that may be present in the containers as well as by the acidic vapours caused by the breakdown of the triacetate molecule **[94]**. The synthesis of cellulose triacetate from ethanolic acid is often slow and results in the creation of very few products. Acid is frequently replaced with its anhydride, also known as ethanolic anhydride.

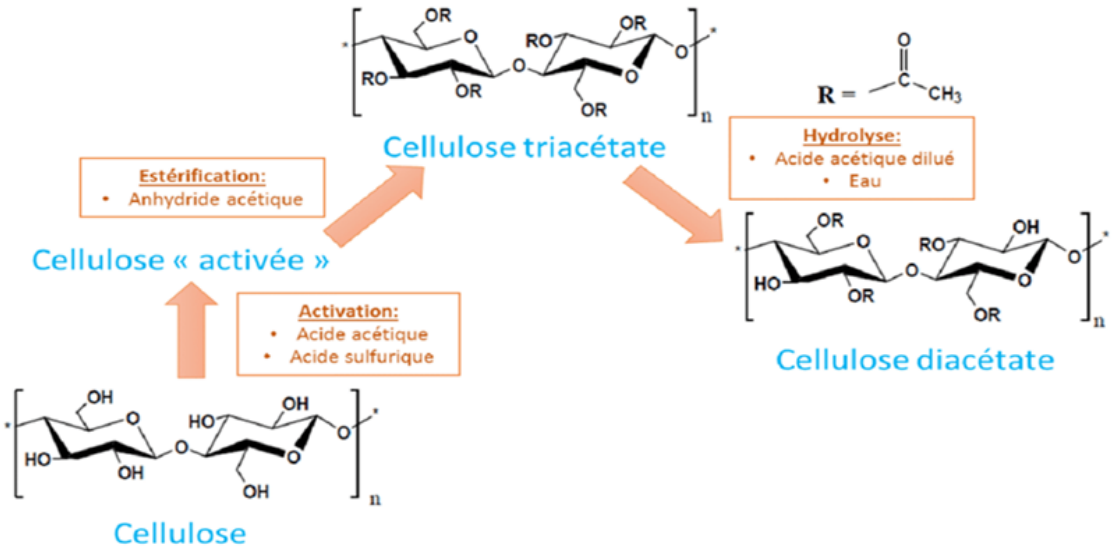



**Figure 10 :** Cellulose diacetate synthesis diagramI.4.

## 8. Applications:

Commercial use is the main production of cellulose paper, when Kraft is used to separate the cellulose from the lignine. Cellulose fiber is used in the textile industry. To make rayon, you can use natural fibers like cotton, lin, and others either directly or by transformation. the use of cellulose and cellulose charge powder as building blocks for medications, food thickening agents, emulsifiers, and stabilizers. The scientists use cellulose for liquid chromatography and filtration with a minced couche. Cellulose is used in electrical construction and as an insulating material. It is utilized in everyday household items including coffee filters, sponges, and collyre adhesives.Cellulose is used in electrical construction and as an insulating material. It's used in common household items like coffee filters, disinfectants, laxatives, and adhesives, as well as

films. Although plant cellulose is still a significant source of fuel, it can also be used to treat animal waste cellulose to create bio-butanol **[95]**.

• Cellulose triacétate is widely used in textiles, electrical isolants, optically transparent plastics, and photographic films.

• Cellulose diacetate can be used as a material for eyeglass frames, as a synthetic fiber for cigarette and gaming filter construction, as a strengthening material for optoelectronic devices, and in the delivery of osmotically administered medications. In applications involving nanocomposites, cellulose acetate is used as an additive to enhance the traction resistance and host polymer matrix module **[91]**.


**Figure 11:** Proposed different applications of alpha-cellulose in a conceptual biorefinery

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Chapter

# III

# Experimental Part

# I. Introduction

The extraction of natural polymers from different parts of plants has been studied for decades. With the development and growing interest in materials and biopolymers, in recent years, much research is being conducted on the identification of new sources of plant molecules, especially from plants with medicinal properties; because isolated biomaterials play an important role due to their effects, used also in the field of materials, and also in other industrial applications.

It is in this context that we are interested by extracting Cellulose from a plant source as Alfa plant "*Stipa Tenacissima*" and from waste of paper. After Extraction, the cellulose extracted was modified to obtain "Cellulose Triacetate CTA" using esterfication process. The cellulose and CTA were characterized by FTIR and DRX to confirm that the products have been properly synthesized. For using our polymer cellulose triacetate extracted as a polymeric matrice in pharmaceutical formulations, we introduced it to prepare two different discs charged with an antibiotic agent "Cefalexine" and study its release in physiological medium.

## II. Materials and Methods

### II.1. Plant and waste paper material

- ✓ Alfa plant (*stipa tenacissima*) (Figure1)was harvested and collected from the Wilaya of Ain Temouchent, in the EL Msaid- Amria commune region to be used as raw material for cellulose extraction.
- ✓ White waste papers were acquired from the different offices of a university.

**Figure1:** The plant Alfa (*stipa tenacissima*)

## II.2. Chemicals

**Table 1:** Chemicals, solvents and reagents used in this work

| Chemical products | Chemical formula | Purity (%) | M (g/mol) |
|---|---|---|---|
| Sodium hydroxide | NaOH | 97 | 40 |
| Ethanol | $C_2H_6O$ | 96 | 46.07 |
| Sulfuric acid | $H_2SO_4$ | 95-97 | 98.08 |
| Distilled water | $H_2O$ | 99.5 | 18 |
| methanol | $CH_4O$ | 99.5 | 32,04 |
| Toluene | $C_7H_8$ | 99.5 | 92,14 |
| Acetic acid | $C_2H_4O_2$ | 99.5 | 60,05 |
| Anhydride Acid | $C_4H_6O_3$ | 98 | 102 |
| Oxygen water | $H_2O_2$ | 85 | 34.02 |

## II.3. Laboratory Materials and equipments:

- Electric scale
- Magnetic stirrer
- The static oven
- Bath- Marie agitation.
- Ultraviolet UV spectroscopy

- X-ray diffractometry
- Infrared spectroscopy has transformed Furrier (FT-IR).
- Beakers.
- Watch glass.
- Spatula
- Funnel
- Erlenmeyer
- Magnetic bar.
- Filter paper.
- Graduated Cylinders
- Volumetric flasks.
- Support.
- Buchner.
- Droplets.
- Pestle and mortar.
- The balance of chemical.
- Crystal maker.
- Vacuum pump
- Thermometer
- Glass petri dishes
- Crystallizer
- Test tubes
- Refrigerant
- Balloon
- Hot plate
- Soxhlet

**Soxhlet Setup description**

The Soxhlet extractor is a glass device that allows for material extraction. It is mostly used in the preparation of pre-analysis samples, the identification of grasses in water, detergents, etc. An extractor, a refrigerator, and a balloon make up a Soxhlet outfit. This latter

has a tube system that allows the reservoir to be viewed. A cellulose cartouche that is placed in the reservoir to receive the compound to be extracted must be used to complete the system.

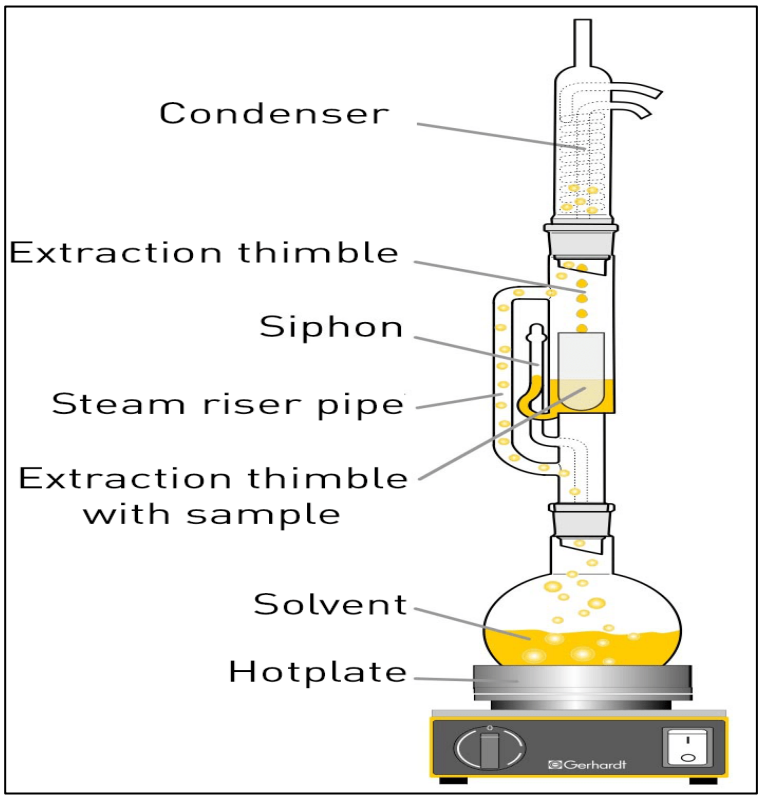



**Figure2:** Schematic representation of a classic Soxhlet extraction unit

## II.4. Methods of Cellulose Extractions

### II.4.1. Solid-liquid extraction by Soxhlet:

#### a) Experimental protocol

The plant material sample (*stipa tenacissima*) was first rinsed with distilled water and then cut into small pieces to make the extraction process easier. We took 23 grams of *stipa tenacissima* (Alfa) and subjected it to Soxhlet extraction (Figure 2) to remove waxes, resins, fats, low molecular weight carbohydrates, and other extractives using a solvent mixture of 400 ml toluene-ethanol (2:1 v/v) for 6 hours **[1].**

Next, the sample was placed in 400 ml of 1M NaOH at 25°C for 8 hours. The mixture was filtered, and the recovered solid corresponded to cellulose, while the filtrate contained hemicelluloses and lignins (Figure3).

**Figure 3 :** Solid-liquid extraction of Cellulose by Soxhlet

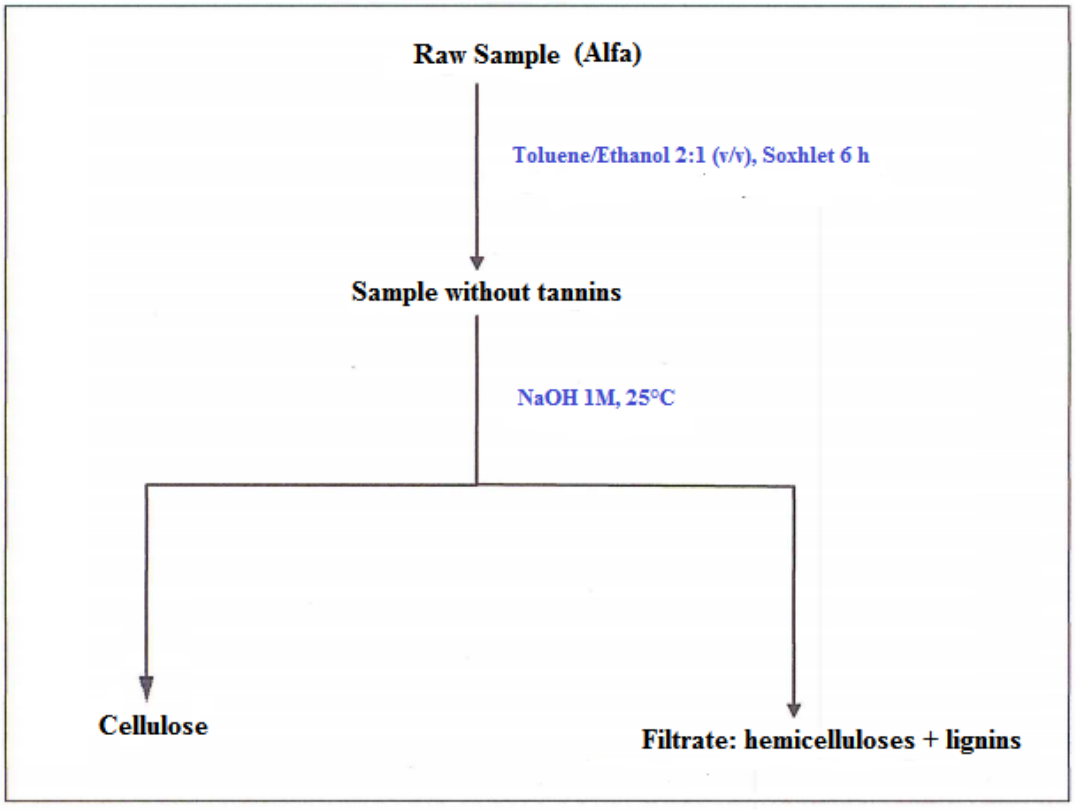



**Figure 4 :** cellulose extraction protocol in basic medium

**b) . Cellulose Bleaching Procedure**

In a 500 ml crystallizer containing a solution of 18% NaOH and hydrogen peroxide ($H_2O_2$) in a 2:1 volume ratio, the extracted cellulose was placed and kept at 40°C for one day. A color change from yellow to off-white was observed. The sample was then filtered and rinsed with distilled water until reaching a neutral pH (around 7). Next, the sample was treated with a sodium hypochlorite solution (commercial bleach, 12%). The bleaching process lasted one full day, resulting in a fully whitened product **[1].**

Finally, the solid was filtered, dried at 40°C for two days, and then analyzed using FTIR spectroscopy.

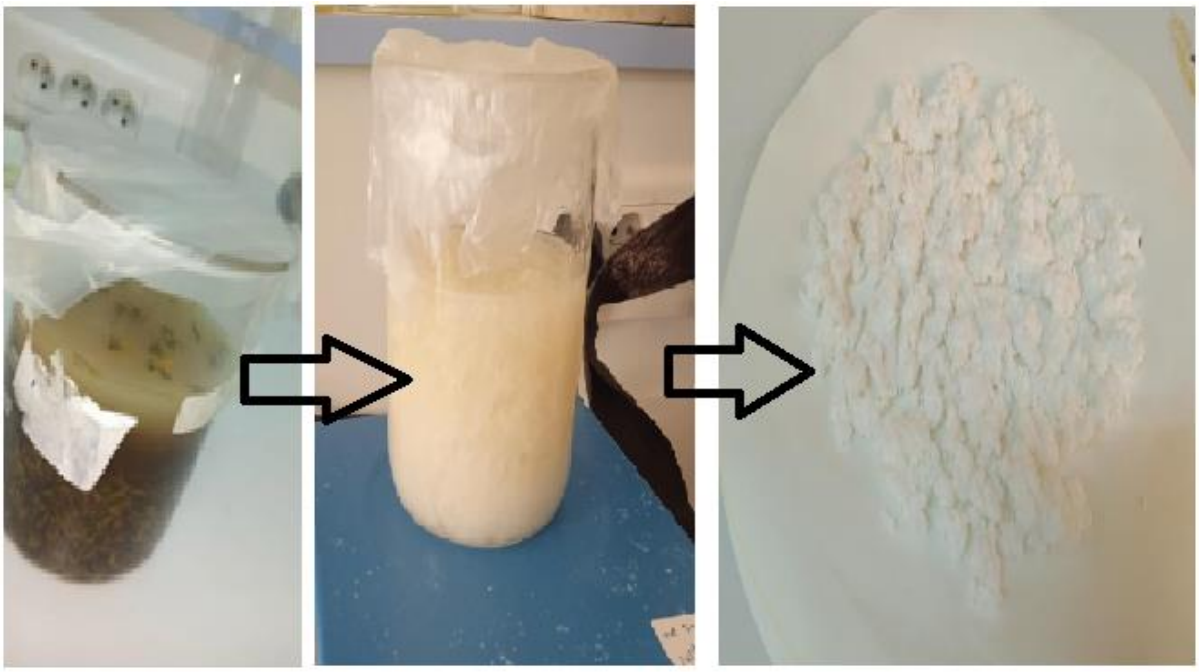


**Figure 5:** Cellulose extracted (P1) after bleaching procedure

**II.4.2. Cellulose extraction from waste paper:**

Cellulose, a natural polymer found in plant cell walls, is a valuable raw material for various industrial applications. Waste paper, which contains a high percentage of cellulose, offers an environmentally friendly and cost-effective source for its extraction. Recovering cellulose from waste paper not only reduces environmental pollution but also supports recycling and sustainable resource use. The extraction process typically involves removing inks, lignin, and other impurities to isolate purified cellulose for further use **[2]**.

**a) Experimental Procedure:**

The wastepaper was cut into pieces that are 1 square inch in size or smaller and then drenched in distilled water added with borax to achieve a pH of 8 for 12 hours at room temperature. The mixed waste paper was filtered and then mixed again with distilled water and borax to achieve a pH of 8 and a consistency of 1-2%. The mixture was fed to a blender for five minutes to produce fine pulp. The fine pulp was filtered and deinked and washed with detergent. This process was repeated until the filtrate was no longer gray. The pulp was rinsed with distilled water, filtered and pressed with a cheese cloth. This process was repeated until the filtrate has no more suds. The pulp was bleached to further remove smaller ink residues. Afterwards, the pulp was filtered and pressed several times using a cheese cloth**.**

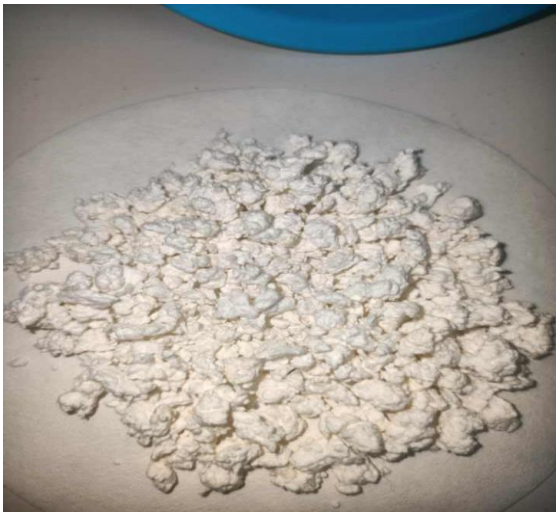


**Figure 6:** Cellulose extracted from waste of paper (P2)

**II.5. Cellulose Modifications:**

**II.5.1. Synthesis of Cellulose Triacetate (CTA)**

In a 250 ml flask equipped with a thermometer, 1.5 g of Cellulose extracted from both materials (Alfa and Waste Paper) are introduced, 12 ml of pure acetic acid and two drops of 95% sulfuric acid are added. The mixture is brought to reflux between 60-70 ° C for 30 min, the married bath is released and cooled with water, 12 ml of acetic anhydride are then added from the top of the refrigerant in small quantities, the mixture is heated again at 70°C until the cotton has completely disappeared (approximately 15 min).The medium is cooled and 5 ml of an aqueous solution of acetic acid at 20% by volume are added. The mixture is heated again between 60 and 70° C. (for 10 min).After complete cooling, the contents of the flask were poured into a 400 ml beaker, 100 ml of hot distilled water were added slowly while stirring,

the cellulose triacetate precipitated, filtered through a Büchner then the product was washed with the water. The washing operation is repeated several times until neutralization, the final product is dried in the oven at 60°C **[3, 4].**

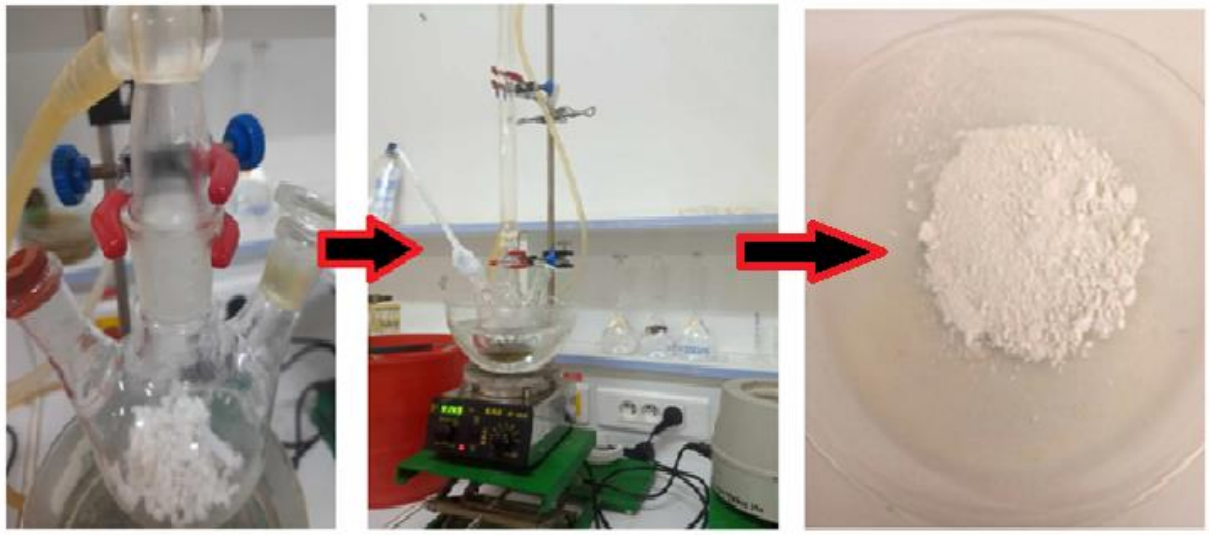


**Figure 7:** Reflux heating assembly used to CTA synthesis

- **Extraction yield**

The Yield percentage of cellulose extracted was calculated by the following equation:

$$Y\% = ( m_1 / m_0 ) \times 100$$

**Y%:** Percentage yield.

**$m_1$:** weight of extracted cellulose.

**$m_0$:** weight of the Alfa plant used.

- **Solubility test:**

A solubility test was carried out using various solvents: water, methanol, acetone, DMSO, sodium hydroxide (Na OH) ,solution acid (HCl) ,solution pH 1.2 buffer , solution at pH 7.4 , DMC, and chloroform. In each case, 0.1 g of cellulose extracted either from Alfa plant (P1) or

from paper waste (P2) was added to 5ml of solvent, and the solutions were stirred at room temperature

## II.6.Tablets formulation

The cellulose extracted from Alfa plant was used to prepare tablet formulations containing an antibiotic agent “Cefalexin”

### II.6.1. Preparing the tablets

Two tablets of different compositions containing cefalexin were formulated (Table 02). The Tablets were prepared manually, using a few milligrams of cefalexin dispersed in Cellulose Triacetate CTA. Absolute Ethanol is then sprayed into sufficient quantity of the two mixtures thoroughly crushed to obtain a final mass.

**Table 2:** The different formulations were presented in the next table

| **Tablets (quality)** | **Before drying m(g)** | **After drying(g)** | **Yield %** |
|---|---|---|---|
| **T1:** 0,2g (CTA) + 0,05g(Cefa)  | 0,35 | 0,23 | 90% |
| **T2:** 0,2g (CTA) + 0,1g(Cefa) | 0.38 | 0.27 | 92% |

## III. Characterization

The cellulose extracted from Alfa and waste paper and the prepared disc were characterized with different methods to confirm the good structures of our products. All the techniques are grouped in the figure 6.

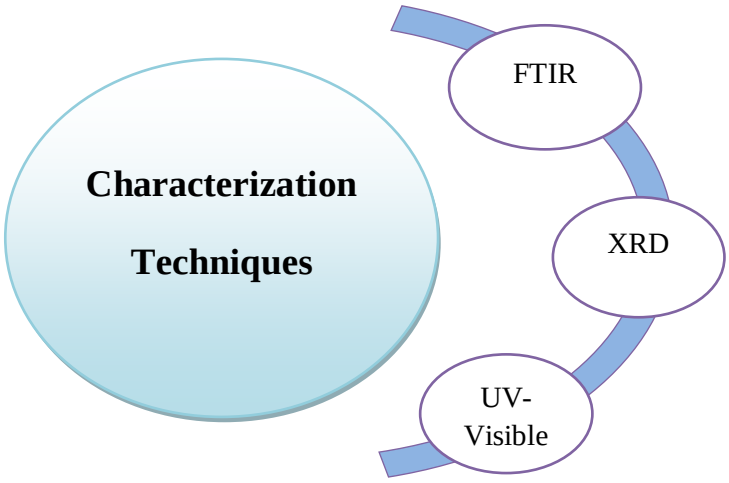



**Figure 8:** Characterization techniques used

### III.1. Principal of Infrared spectroscopy Fourier transform (FTIR)

Infrared (IR) spectroscopy is a widely used technique for identifying compounds and their functional groups (chemical bonds) in extract mixtures. Each type of bond absorbs infrared light at a specific wavenumber**,** producing a spectrum. By comparing this spectrum with a reference library, unknown compounds can be identified. This method was used to detect functional groups and observe any new bonds formed after extraction and modification **[5]**.

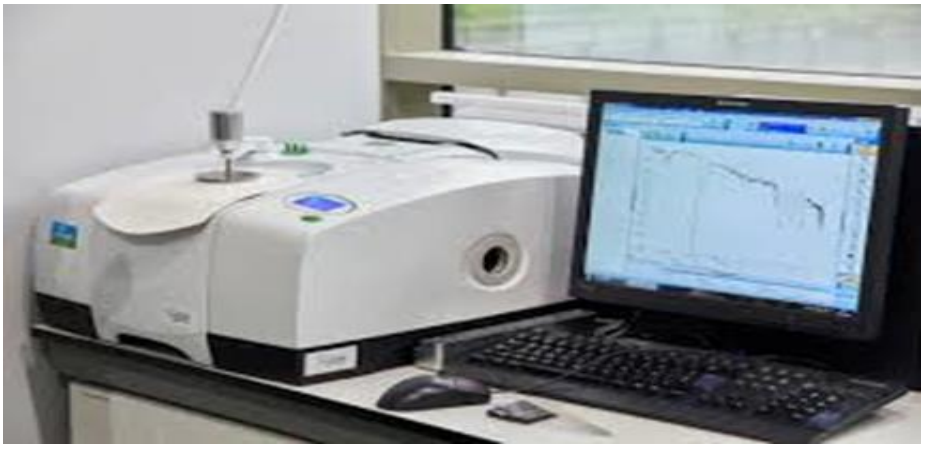


**Figure 9:** Infrared spectroscopy Fourier transforms (FT-IR).

### III.2. Principle of X-Ray Diffraction (XRD):

X-ray diffraction (XRD) is a technique used to identify the crystalline phases in a material and to determine the arrangement and spacing of atoms. It works by directing X-rays

onto a sample; as the rays interact with the atomic planes, they produce a diffraction pattern due to interference. This pattern, called a diffractogram, reflects the material's crystal structure and is recorded as a spectrum. XRD can be performed using a solid piece or a small amount of powder.

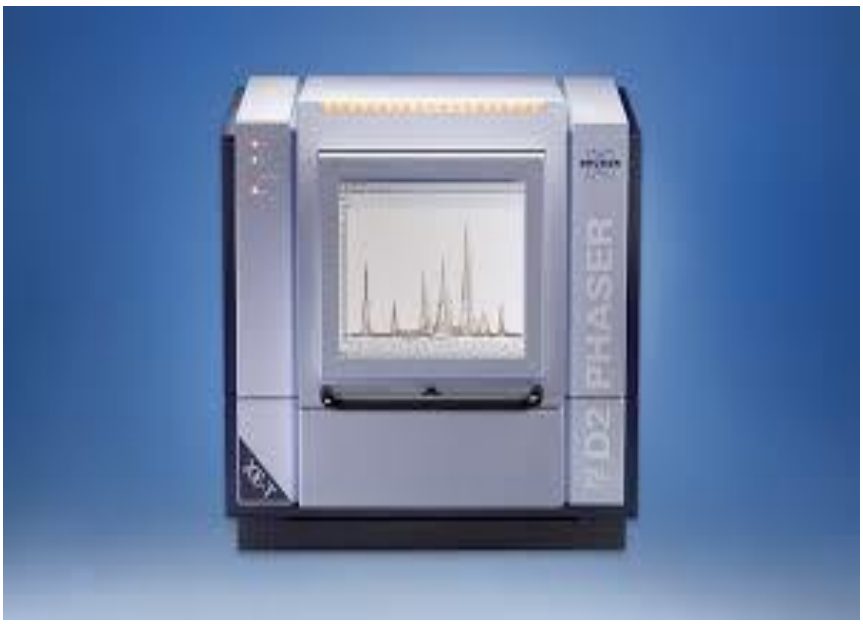



**Figure 10:** X-Ray Diffraction.

## IV. Study of the kinetics of the release of active principle in pH=7.7

The release of the active ingredient was monitored using a UV-Vis spectrometer, previously calibrated at the wavelength $\lambda_{max}$ of the active ingredient used (cefalexin) in the medium considered.

In the case where the drug is dispersed alone in the coating matrix, its release by diffusion through this matrix depends on three essential factors:

- The speed of "penetration" of the liquid into the dosage form through the polymer-matrix structure.
- The speed of "dissolution" of the active ingredient in the trapped liquid.
- The "diffusion" of the active ingredient through the polymer matrix.

The objective of this kinetic study is to compare the "prolonged and controlled" effect on the release of this active principle through polymeric matrice based on CTA which modified from Cellulose Extracted (Alfa).

## IV.1 Factors influencing material transfers

- **Medium stirring speed** (rotation speed fixed at 750 r.p.m for all experiments).

- **The temperature of the medium** All our experiments were carried out at constant temperature 37°C (human body temperature),
- **The nature of the medium, pH and volume**
- **"Non-sink" method:** where the chosen volume (100 ml) is used for the entire experiment. The concentration of the active ingredient increases during the experiment
- **“Sink” method:** the volume is constantly renewed by virgin liquid, the volume used is therefore greater

The first method (non-sink) is much easier to perform, and it is this method that we used in all our experiments

## IV.2. Composition of the study environment

Based on the results obtained from previous work [6] about tablets formulations based on cefalexine and different polymeric matrices, we found that good release of CF from tablets was greater in medium pH= 7.7 compared with acidic medium. According to the results , we have done the kinetics of CF in pH=7.7 medium only.

For our various kinetic studies, we chose to reconstitute environment physiological pH = 7.7, these environments correspond to the longest stay times most important during the digestive tract, it is prepared in accordance with standards described by the American Pharmacopoeia U.S Patent XX:

- **Intestinal environment of pH = 7.7:**

  HCl: 0.1N (20ml), Borax (sodium tetra borate 10 hydrate): 0.025N (500ml), Distilled water: (1 liter)

## IV.3. Measurement conditions

The kinetics corresponding to the different forms were carried out under the same operating conditions:

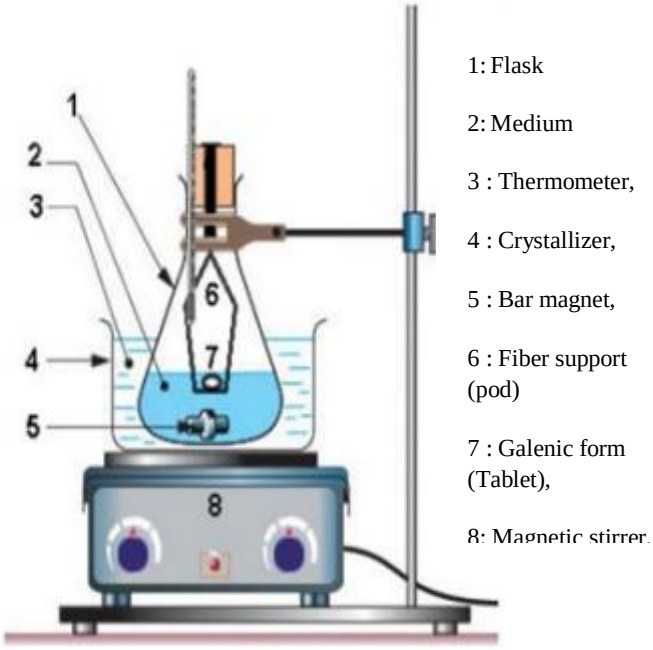
- Disk preparation: is done in the same way as explained.
- The support of the discs: it is made of fiber glass, a little high compared to the magnetic bar in order to avoid the shocks which may occur there and also allows good agitation and circulation of the liquid around the galenic form.
- Temperature (37°C), Stirring (500 r.p.m)
- Initial volume in the bottle (100 mL) and volume of the test portions (1 mL), in order to ensure better reproducibility of the results and to be able to compare them.
- Maximum wavelength: measurements are made using a UV device.

## IV.4. Kinetics of CF release from discs

### IV.4 .1 Operating Mode and Experimental Device

In a 500mL capacity bottle, the dosage form was placed in 100mL of the study environment (pH=7.7). The medium was maintained at 37°C and stirred at a rotation speed of 500 r.p.m using a magnetic stirrer. At each time “t” the disk is removed from the bottle, rolled

on Joseph paper to remove the film of liquid which had formed, then weighed, and at the same time a volume Vp = 1mL of the liquid medium is taken. The volume taken diluted by a dilution volume Vd =10ml of the same physiological medium. The density optical (OD) is then determined by UV for each sample **[3, 6-7].**




1: Flask

2: Medium

3 : Thermometer,

4 : Crystallizer,

5 : Bar magnet,

6 : Fiber support (pod)

7 : Galenic form (Tablet),

8: Magnetic stirrer.

**Figure 11**: Experimental device for releasing tablet

### IV.4 .2. The rate of PA released

The rate of PA released is therefore calculated in relation to the real mass of active agent according to the following relationship:

$$\%PA = (mt/mi).100$$

$$mt = D.O \,.Vd \,.\, MM \,/\, \varepsilon.Vf$$

**mt:** The mass of active ingredient at time "t"

**mi:** Initial mass of the active ingredient**.**

**Vd:** The volume of the dilution flask in ml

**Vf:** The volume of the release liquid contained in the bottle in ml

**MM:** The molar mass of the principle of the active ingredient (g/mol)

## V. Biological study of Extracted Cellulose

To demonstrate microbial activity of cellulose extracted from Alfa (P1) and from waste of paper (P2), three bacterial strains and one fungal strain were tested against the prepared cellulose.

- **Bacterial strains**

The bacterial strains used are referenced and coded as follows:

- Gram-negative bacteria:
  - Escherichia coli ATCC25922.
  - Pseudomonas ATCC 27853
- Gram-positive bacteria: Staphylococcus aureus ATCC25923.
- "Yeast" fungal strains: Candida albicans ATCC10231

- **Mueller-Hinton agar**

Mueller-Hinton agar is the only solid culture medium for the study of sensitivity that has been validated by the NCCLS. It is recommended to always use agar Mueller Hinton for agar diffusion tests, depending on the guidelines international standards and the NCCLS. Since the way Mueller-Hinton agar is prepared may affect the results of the disk diffusion procedure, it is very important to refer to Section C below for instructions on preparation and quality control of this environment **[8, 9]**

### V.1. Agar diffusion method (well method):

This is the basic technique used to study a substance's ability to exert an antimicrobial effect. It is also known as the agar dilution method for determining active extracts. Mueller-Hinton agar was prepared, autoclaved for 20 minutes at 130C°, and then flowed into Petri dishes.

After the plates are dried, a hole is made in the center of the agar using the upper part of a Pasteur pipette. The resulting cavities are then filled with a small amount of cellulose extracted from Alfa (P1) and cellulose extracted from paper waste (P2). The plates were incubated at 30°C for 24 hours, to then compare the diameters in order to test the inhibitory activity **[10]**. The inhibitory action is manifested by the formation of a halo around the wells. The results are read by measuring the diameters of the inhibition zones.

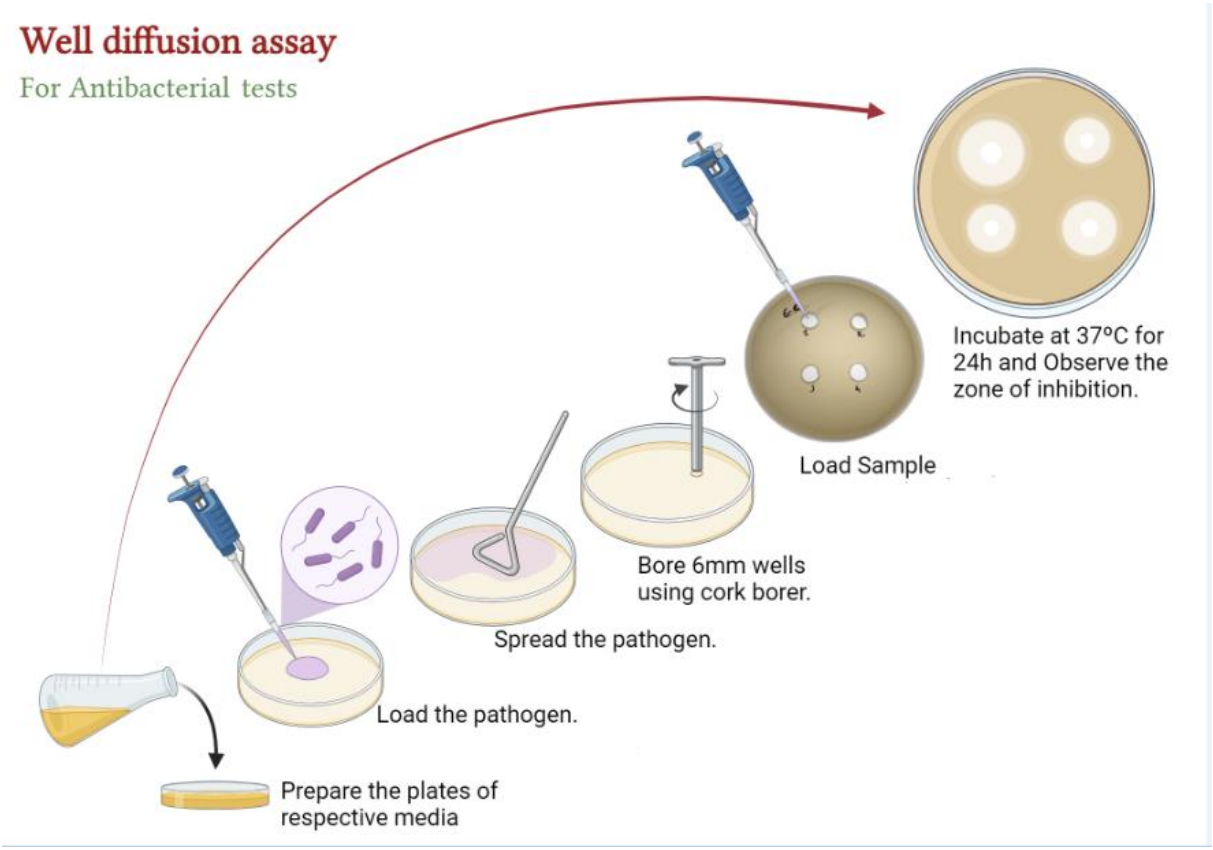



**Figure 12:** Schematic representation of the Well diffusion method

**a- Preparation of the suspension:**

A bacterial suspension with a density equivalent to the standard of 0.5 Mac Farland (0.1-0.08) which corresponds to $10^8$ colony forming units per milliliter (CFU/ml) this at a wavelength of 620 nm.

**b- Seeding:**

Seeding is carried out by sterile swabs on Petri dishes containing MH agar. A swab is dipped in the standardized bacterial suspension then rubbed over the entire agar surface, from top to bottom in tight streaks. The operation is repeated three times, rotating the box 60° each time. The boxes thus inoculated were left for 15 minutes.

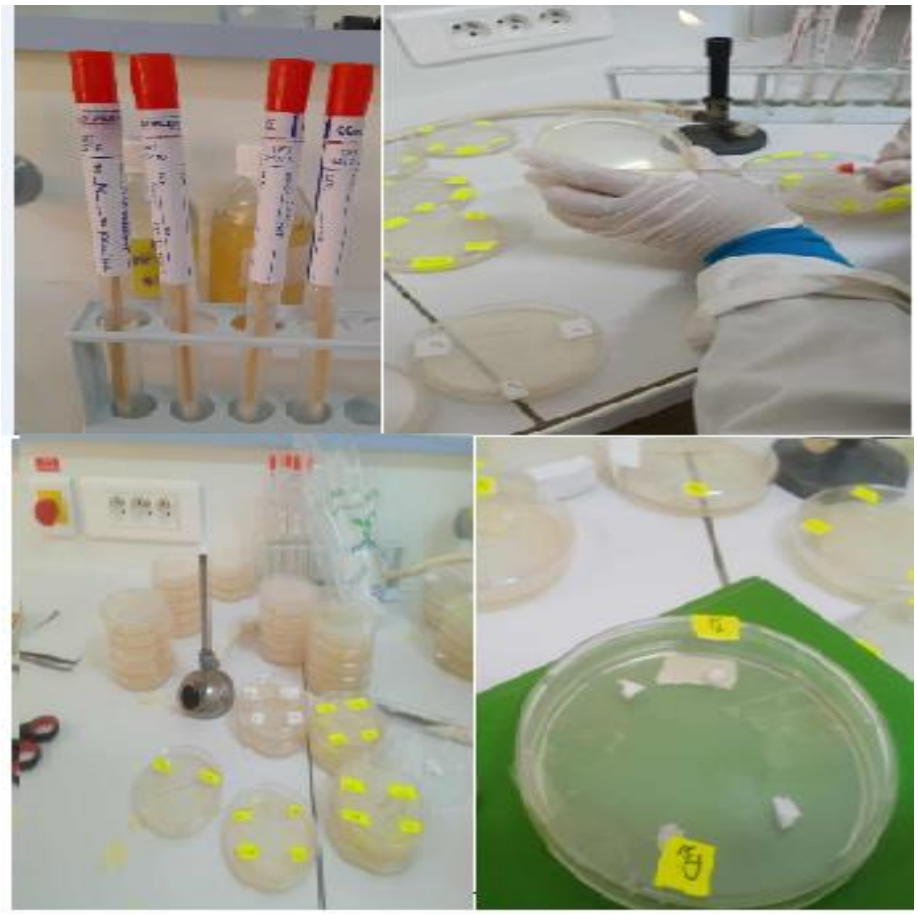


**Figure 13:** Preparation of the suspension and Seeding of culture medium

Chapter

# IV Results and Discussion

## 1. Determination of Yield %:

Before characterizations and application of the prepared polymers P1, P2 and CTA, we calculate the Yield % with the equation cited in Chapter 3. The percentages are classified on Table 1.

**Table 1:** Calculation of Yields % results

| Polymers | P1 | P2 | CTA |
|---|---|---|---|
| Yield % | 50 % | 90 % | 98 % |

## 2. Solubility tests:

A solubility test was carried out using various solvents: water, methanol, acetone, DMSO, sodium hydroxide (Na OH) ,solution acid (HCl) ,solution at pH 1,2 buffer solution at pH 7,4 , DMC, and chloroform. Both types of cellulose (from Alfa P1 and from waste of paper P2) were found to be insoluble in all the tested solvents. However, after heating despite its limited solubility, is a remarkable biopolymer due to its structural stability, rigidity, and wide range of potential applications.

**Table 2:** Results of solubility test of P1 and P2 at room temperature

| **Solvents** | **Water** | **Methanol** | **Acetone** | **DMSO** | **NaOH** | **HCl** | **Buffer pH 1.2** | **Buffer pH 7.7** | **DMC** | **Chlorofom** |
|---|---|---|---|---|---|---|---|---|---|---|
| **P1** | - | - | - | - | - | - | - | - | - | - |
| **P2** | - | - | - | - | - | - | - | - | - | - |

## 3. Characterizations

The extracted Cellulose from Alfa and from waste of paper and the cellulose triacetate were analyzed by infrared spectroscopy and X-Ray Diffraction ; we were able to identify some characteristic bands of the prepared polymers.

### 3.1. Fourier transforms infrared spectroscopy (FTIR):

The functional groups of samples were detected by infrared spectrometry at Fourier transform FTIR-600 (Agilent Technologies Cary Spectrophotometer), in the range of 4000-500 cm-1 wavelengths.

### a) Cellulose from Alfa (P1):

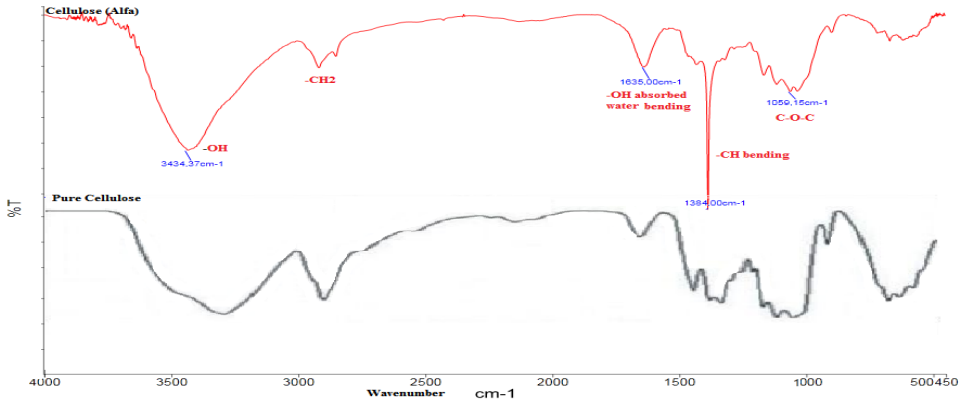



**Figure 1:** IR spectrum of Cellulose extracted from Alfa (P1)

The IR spectrum presented in the (Figure 1) indicates the presence of major characteristic absorption bands:

- Strong absorption at 3434.37 $cm^{-1}$ related to the stretching of –OH group
- Peak around 2950 $cm^{-1}$ assigned to the stretch vibration and bending vibration of C-H bond of methylene group
- Peak at 1636 $cm^{-1}$ corresponds to the bending of the absorbed water (-OH)
- Peak at 1384 $cm^{-1}$ correspond to the –CH bending and band at 1059 $cm^{-1}$ for C-O-C bonds

Those peaks were compared with the commercial cellulose [11] which confirm the good correlation and good extracted of polymer.

### b) Cellulose from Waste of paper (P2):

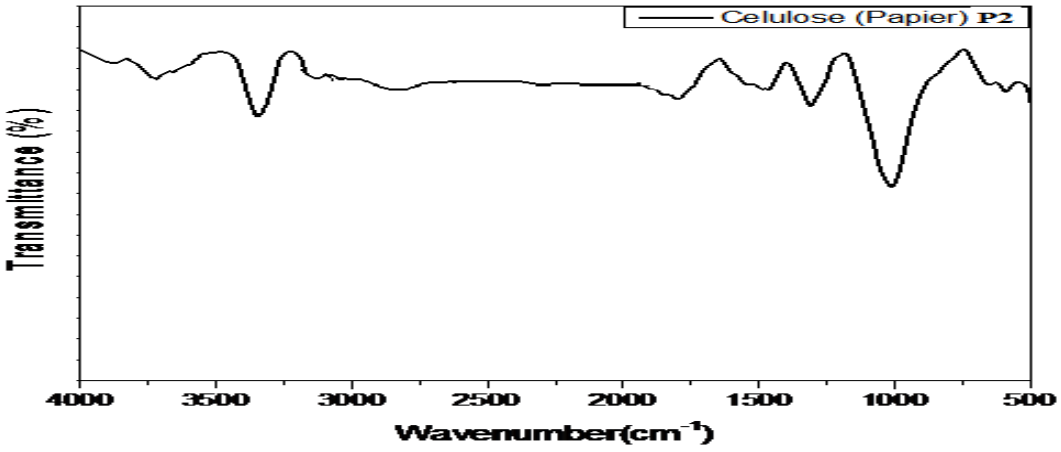



**Figure 2:** IR spectrum of Cellulose extracted from paper (P2)

The IR spectrum presented in the (Figure 02) indicates the presence of major characteristic absorption bands of cellulose compared with the spectrum of P1 and pure cellulose, we observed the peaks :

- At around 3474.55 $cm^{-1}$ related to the stretching of –OH group
- Small band around 2900 $cm^{-1}$ assigned to the stretch vibration and bending vibration of C-H bond of methylene group
- Peak at 1670 $cm^{-1}$ corresponds to the bending of the absorbed water (-OH)
- Peak at 1319.074 $cm^{-1}$ correspond to the –CH bending and band at 1040.417 $cm^{-1}$ for C-O-C bonds

This peaks confirmed that the cellulose extracted from waste of paper was good synthesis.

**c) Cellulose Triacetate prepared from P1**

The presented figure is the spectrum of CTA prepared from cellulose extracted from Alfa (P1). The spectrum was compared with the previous research [**3, 6]**

The CTA spectrum confirms the achievement of product. We observed the characteristic peaks : at 3548.39 $cm^{-1}$ for –OH vibration , 2955.36 $cm^{-1}$ for –C-H aliphatic, 1785 $cm^{-1}$ for C=O ester carbonyl , and specially the peak at 1391 $cm^{-1}$ indicate the acetyl group –C-$CH_3$ which means the acetylation process in this study was successful.

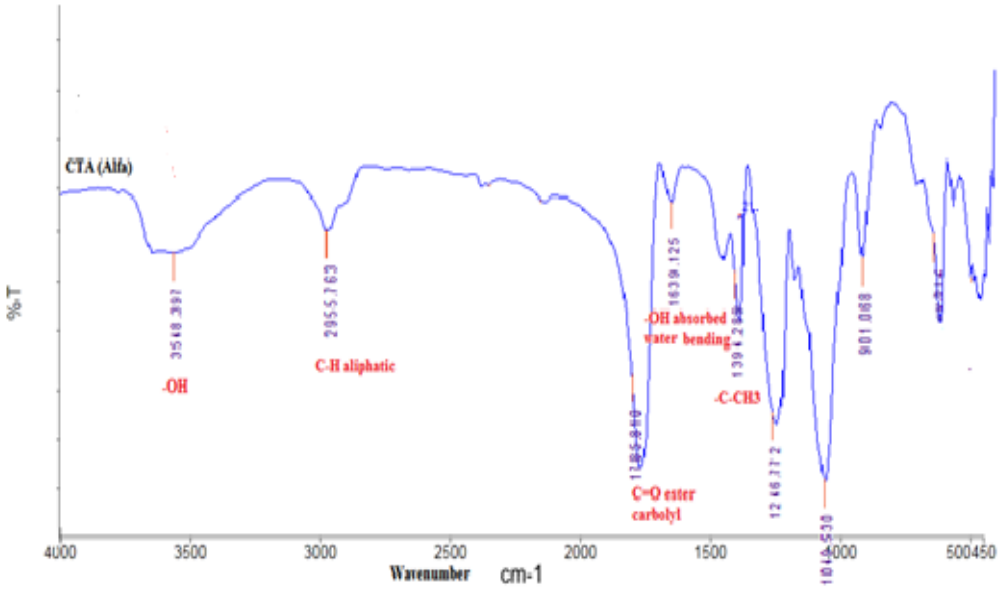



**Figure 3:** IR spectrum of CTA

**d) IR of Antibiotic agent (Cefalexin), CTA and Tablet 1 prepared:**

The following figure 4 represents the sum of specters of Cefalexin, Cellulose Triacetate CTA and the tablet prepared based on these products:

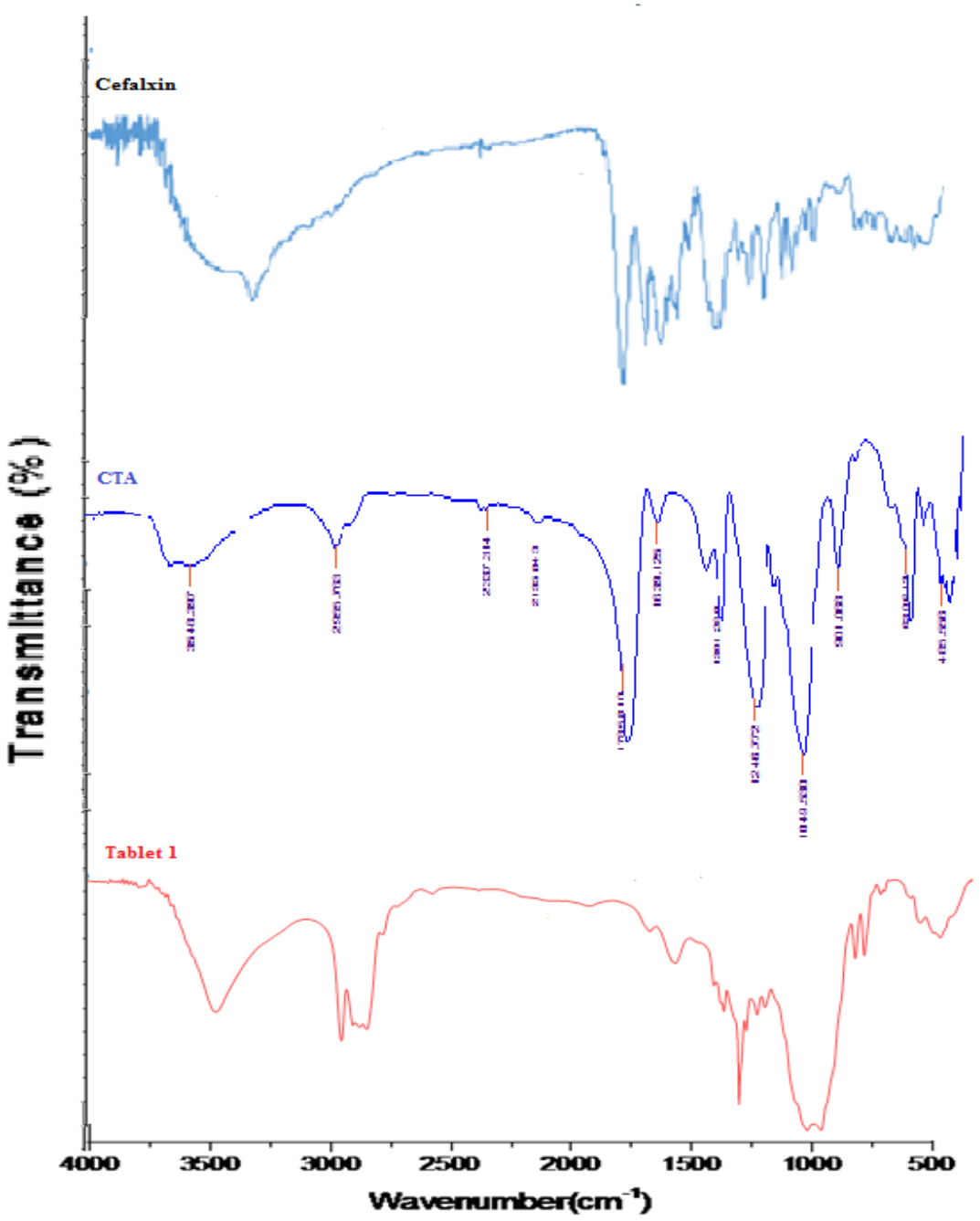



**Figure 4:** IR spectrum of Cefalexin, CTA and Tablet 1

The active substance “Cefalaxin” was characterized by FTIR. the IR spectrum presented in the (Figure 4) indicates the presence of major characteristic absorption bands at 3315.05 cm-1 (N-H), bands 1857.12 cm-1 may be due to the presence of C=O acid. A band at 1735.63 characterizing the C=O ketone bond, and another band at 1690.25 cm-1 indicating the presence of the carbonyl group C=O of the amide function, 3072.06 cm-1 CH (aromatic) and around 1645.79 cm-1 -C=C- (aromatic).

The IR spectrum of Tablet 1 based on the polymer (CTA) and CF allowed us to observe the main characteristic bands relating to the different groups present. We note the presence of the characteristic bands of the O-H alcohol group located at 3477.90$cm^{-1}$, as well as that the characteristic bands N-H 2975.53$cm^{-1}$, 2870.61 $cm^{-1}$ corresponding to aliphatic C-H, and around 1761.34 cm-1 C=O, 1667.94$cm^{-1}$, 1599.59 $cm^{-1}$, 1491.87 C=C aromatic vibrations

The results show that the tablet has good correlation between Cefalexin and CTA and the CF was dispersed on the polymer matrix CTA. The main absorption bands of CF which appear clearly in the disk spectrum

### 3.2. X-Ray Diffraction (XRD):

#### a) Cellulose from Alfa (P1) and Cellulose from paper (P2):

The following patterns represent the XRD pattern of Cellulose extracted from Alfa and paper (Figure 5 and 6)

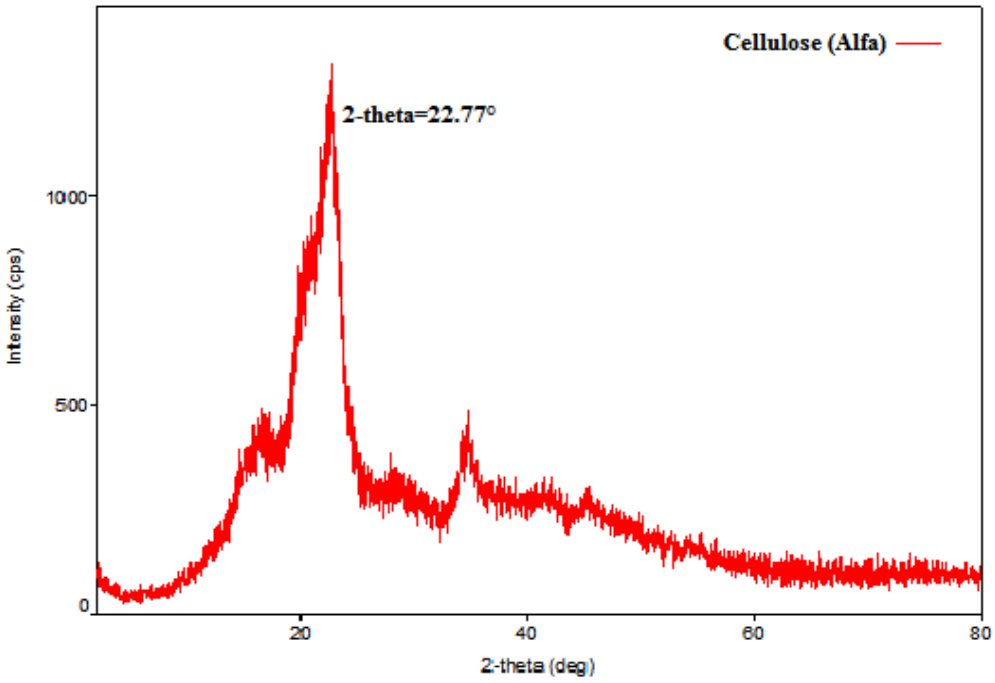



**Figure 5:** XRD pattern of Cellulose from Alfa (P1)

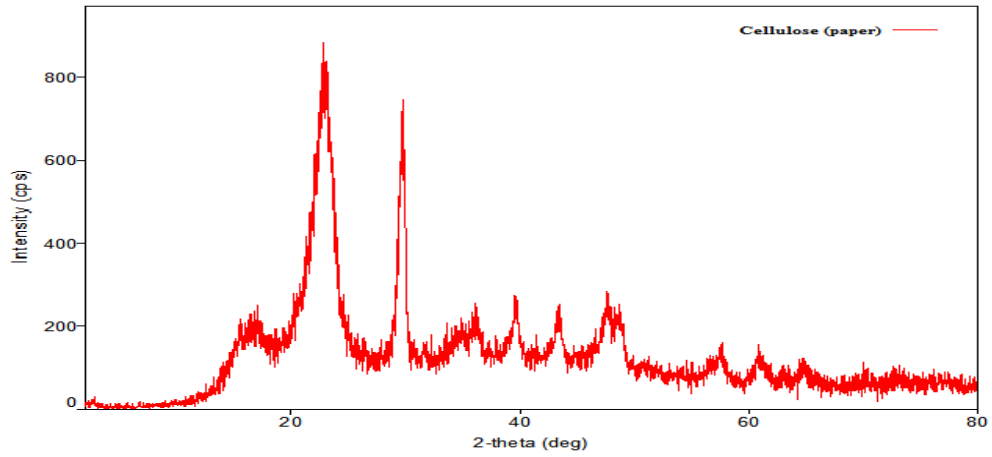



**Figure 6:** XRD pattern of Cellulose from Paper (P2)

Figure 5, presents the XRD of Cellulose extracted from Alfa Plant , The diffractogram clearly shows the presence of an intense peak at 2Θ=22.77° of crystallinity and shows also a broadband presents amorphous properties therefore the Cellulose polymer is semicrystalline polymer**[12].** The diffractogram of Cellulose extracted from paper (Figure 6) clearly shows the presence of characteristic crystallinity peaks which appear in the form of a more intense peak as 2Θ=16.03°, 23.15°, 29.85°, 36.18°, 39.72° and 48.60° **[13, 14].**

**b) Cellulose Triacetate CTA from Alfa plant:**

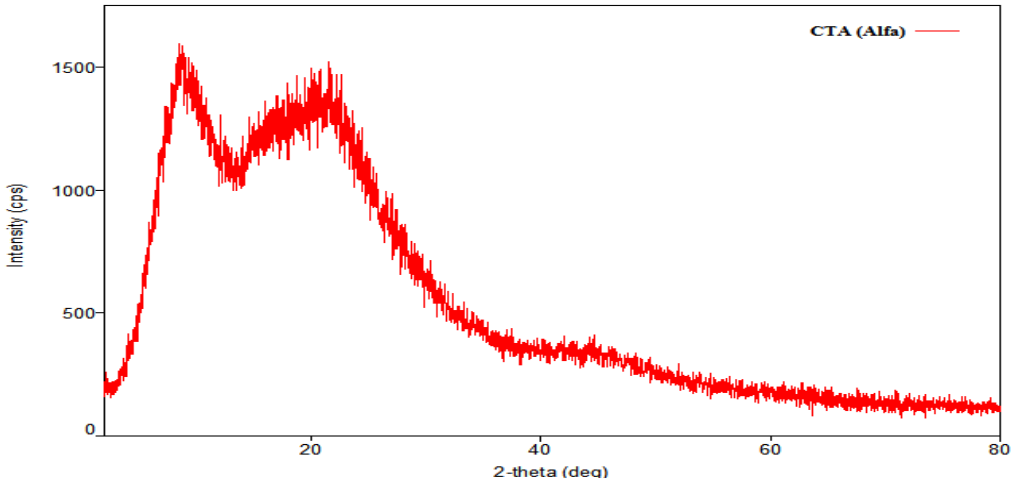



**Figure 7:** XRD pattern of Cellulose Triacetate CTA

The XRD pattern of CTA modified from cellulose extracted from Alfa plant shows tow amorphous broad band. This indicates the amorphous structure of CTA.

## 4. Study of the kinetics of the release of active principle in pH=7.7

The PA release kinetic curves illustrate the quantity of PA released as a function of time in the environment pH 7.7.

The release of the active ingredient was monitored using a UV-Vis spectrometer, previously calibrated at the wavelength $\lambda_{max}$= 297nm of the active ingredient used (cefalexin) in the medium considered (table 1). For our various kinetic studies, we chose to reconstitute environment physiological pH = 7.7, these environments correspond to the longest stay times most important during the digestive tract. The basic medium was the good medium for the cefalexin release on previous studies [**6**]. The release of CF from the two discs prepared was doing on the medium pH=7,7.

**Table 3:** Represents the values of λ max and Ɛmax of Cefalexin in the different media

| **Active substance** | **medium pH** | **λ max (nm)** | **Ɛmax (L.mol-1 .cm-1 )** |
|---|---|---|---|
| **Cefalexin** | 7.7 | 297 | 102.7 |

The graph present the release kinetic curves of two different tablets based on CTA and Cefalexin:

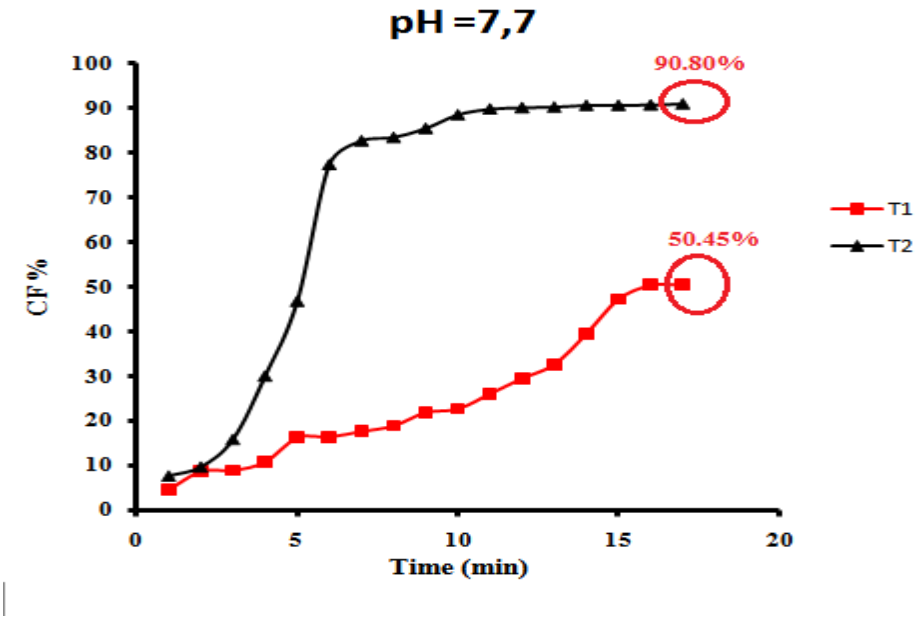



**Figure 8**: % active ingredient cefalexin released as a function of time at pH=7.7 of T1 and T2 (T =37°C, 500 r.p.m)

For all the formulation loaded with CF, the percentage of active ingredient released is greater in the medium at pH=7,7. Classic curve-shaped release profile was observed in the two cases: At early times, the release rate was high and then gradually increased during the observation period (the slope of the curve decreased steadily with time).

This type of release profile is consistent with the hypothesis that the diffusion of the active ingredient through the polymer plays a major role in the release kinetics. Upon contact of the formulation with the medium, the medium enters the system and dissolves the CF. since CF is moderately soluble in water and the initial charge of CF is relatively low, we could expect CF to dissolve quickly. Once dissolved, the CF diffuses through the coating polymer towards the dissolution medium.

The release of active ingredient Cefalexin (Figure 8) from Tablet1 (0.2gCTA/0.05gCF) was 50.45% and Tablet 2 (0.2gCTA/0.1gCF) was 90.80% in the basic medium pH 7.7. The basique medium more influenced the morphology of a crystalline polymer by transferring it into an amorphous structure therefore quick release. Both release percentage from T1 and T2 presents that the amount of CF loaded on the tablet was released after 120min.

The amorphous morphology of CTA which allows the absorption of liquid in the formulation. The release was around 50% in pH7.7 for T1 and for T2 was around 90%. It can be explained that the increase comes from the absorption of the liquid from the medium and as a result Cefalexin finds it easy to dissolve and diffuses through the polymer wall.

According to the results, The CF release study confirmed that the Cellulose Triacetate CTA prepared from Cellulose extracted from Alfa plant was a good matrix to prepare pharmaceuticals forms.

## 5. Biological study of Extracted Cellulose

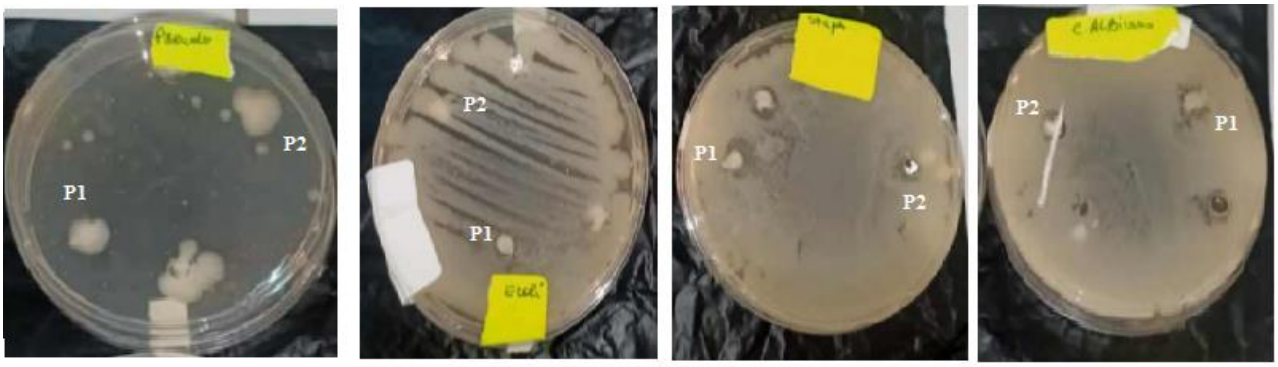
For the antibacterial study, we tested the antibacterial effectiveness of the Cellulose extracted from Alfa plant (P1) and from wastepaper (P2) on the bacterial strains, then the microorganisms were grown and inoculated, which are the positive , negative bacterial and fungal. The scale for estimating antimicrobial activity is given by **[19]** classified the diameter of the zones of inhibition (D) of microbial growth as follows:

- Resistant (-): D< 8 mm
- Sensitive (+): 9mm <D <14mm
- Very sensitive (++): 15mm <D< 19 mm
- Extremely sensitive (+++): D >20 mm

After incubation at 24h at 37°c, we see an increase in the diameters of inhibition zones. The cellulose showed some degree of antibacterial activity as can be seen in

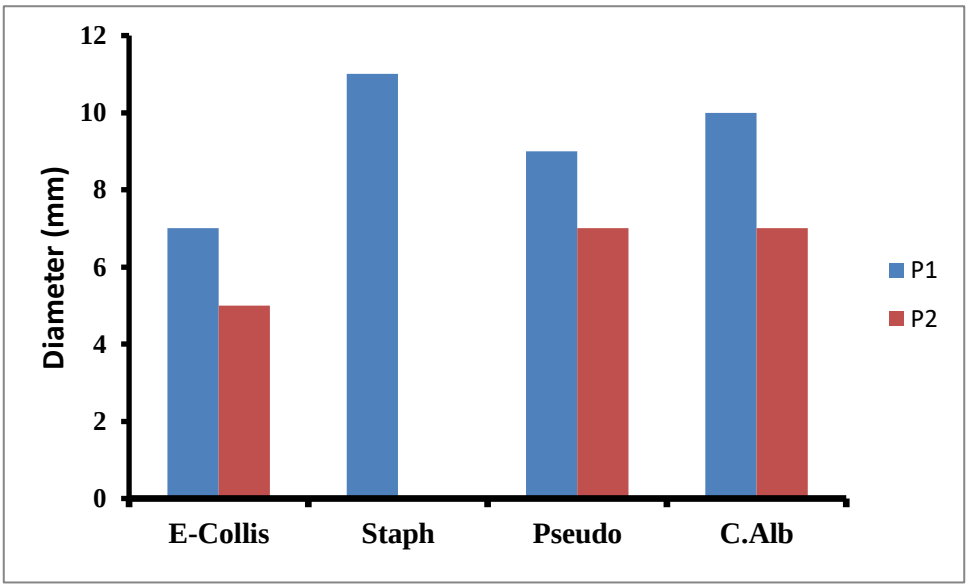
the Petri dishes used in the diffusion test (Figure 9). The average size of the inhibition zone measured for all strains is shown in Table 2.

**Figure 9:** Antibacterial activity of cellulose extracted against various strains bacterial pathogens.




The results of antibacterial activity were collected in the following Histogram. We observe that the polymer has a good inhibition zones with all strains tasted with different diameters:

- For Cellulose extracted from Alfa plant (P1) , the highest inhibition zone was observed with staph strain with diameter 11 mm.
- For the cellulose extracted from wastepaper (P2), the highest inhibition zones was observed with Pseudomonas and candida albican with 7mm. P2 shows negative results with Staph strain




**Histogram01:** Diameter Zone of inhibition produced by extracted Cellulose against various bacteria.

The inhibition zones were observed with the extracted cellulose, it suggests:

- Either residual antibacterial compounds remained adsorbed on or embedded in the cellulose.
- Or the cellulose structure itself facilitates bacterial stress, possibly due to surface charge, porosity, or interactions with bacterial membranes.

The antibacterial inhibition zones observed around the cellulose extracted from *Stipa tenacissima* via the Soxhlet method are probably attributed to the presence of residual bioactive substances that were not completely eliminated during the extraction. These substances, such as phenolic compounds or waxes, are recognized for their antimicrobial activity. Furthermore, the physical properties of the extracted cellulose like its surface texture, porosity, and potential chemical interactions may also play a role in inhibiting bacterial growth by inducing stress or capturing essential nutrients.

The antibacterial inhibition zones observed around cellulose extracted from waste paper may be attributed to residual chemical additives or processing agents used in the original paper manufacturing process. Waste paper often contains **inks, dyes, bleaching agents, fillers**, or **coatings**, some of which can possess antimicrobial properties, especially if not entirely removed during the extraction process.

In addition, the structure of the regenerated or extracted cellulose itself such as **high surface area, porosity**, or **hydrophilic functional groups** can create an environment that is **unfavorable for bacterial adhesion and proliferation**. This may lead to localized stress or nutrient deprivation for the microbes, thereby inhibiting their growth.

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Conclusion

# General Conclusion

# General Conclusion

The extraction of natural polymers from plant sources has long been of scientific interest, especially with the growing demand for sustainable and bioactive materials. In recent years, research has increasingly focused on identifying plant-based biopolymers, particularly from species with medicinal potential, due to their broad applicability in pharmaceuticals and various industries.

Within this context, our study explored cellulose extraction from both the Alfa plant (*Stipa tenacissima*) and waste paper. The extracted cellulose was chemically modified through esterification to produce cellulose triacetate (CTA). Structural characterization using FTIR and XRD confirmed the successful synthesis. To evaluate its pharmaceutical potential, CTA was used as a polymer matrix to formulate discs loaded with the antibiotic cefalexin, and its drug release behavior was assessed in physiological conditions.

Functional groups of samples were detected by infrared spectrometry at Fourier transform FTIR-600 (Agilent Technologies Cary Spectrophotometer), in the range of 4000-500 cm-1 wavelengths. The IR spectrums of both cellulose extracted from Alfa plant P1 and from wastepaper P2 indicates the presence of major characteristic absorption bands of cellulose compared with the spectrum of pure cellulose. The CTA spectrum confirms the achievement of product. We observed the presence of characteristic peaks : at for –OH vibration ,–C-H aliphatic, C=O ester carbonyl , and specially the peak at 1391 $cm^{-1}$ indicate the acetyl group –C-$CH_3$ which means the acetylation process in this study was successful.

The IR spectrum of Tablet 1 based on the polymer (CTA) and CF allowed us to observe the main characteristic bands relating to the different groups present. The results show that the tablet has good correlation between Cefalexin and CTA and the CF was dispersed on the polymer matrix CTA. The main absorption bands of CF which appear clearly in the disk spectrum

The diffractogram XRD of Cellulose extracted from Alfa Plant clearly shows the presence of an intense peak at $2\Theta=22.77°$ of crystallinity and shows also a broadband presents amorphous properties therefore the Cellulose polymer is semi crystalline polymer**.** The diffractogram of Cellulose extracted from wastepaper shows

the presence of characteristic crystallinity peaks which appear in the form of a more intense peak as 2Θ=16.03°, 23.15°, 29.85°, 36.18°, 39.72° and 48.60°. The XRD pattern of CTA modified from cellulose extracted from Alfa plant shows tow amorphous broad band. This indicates the amorphous structure of CTA.

The CF release kinetic curves illustrate the quantity of cefalexin released as a function of time in the environment pH 7.7. In a basic medium (pH 7.7), the release of cefalexin from two tablets containing cellulose triacetate (CTA) was significantly different depending on the drug load. Tablet 1 (0.2g CTA/0.05g CF) released about 50.45% of the active ingredient, while Tablet 2 (0.2g CTA/0.1g CF) achieved a higher release of 90.80% after 120 minutes. The basic environment appears to alter the crystalline structure of CTA into a more amorphous form, enhancing liquid absorption and promoting drug diffusion. The higher cefalexin concentration in T2, combined with the amorphous nature of CTA, facilitated faster and more efficient release of the antibiotic through the polymer matrix.

For the antibacterial study, we tested the antibacterial effectiveness of the Cellulose extracted from Alfa plant (P1) and from wastepaper (P2) on the bacterial strains. We observe that the polymer has a good inhibition zones with all strains tasted with different diameters, for Cellulose extracted from Alfa plant (P1) , the highest inhibition zone was observed with staph strain with diameter 11 mm. we obtained also for the cellulose extracted from wastepaper (P2), the highest inhibition zones was observed with Pseudomonas and candida albican with 7mm. P2 shows negative results with Staph strain. the structure of the regenerated or extracted cellulose itself such as high surface area, porosity, or hydrophilic functional groups can create an environment that is unfavorable for bacterial adhesion and proliferation. This may lead to localized stress or nutrient deprivation for the microbes, thereby inhibiting their growth.

Cellulose extracted from the Alfa plant and wastepaper offers a sustainable, eco-friendly solution for wastewater treatment. Its natural abundance, biodegradability, and strong adsorption capacity make it effective for removing dyes and pollutants, supporting green chemistry and environmental protection, this interested application will be in our perspective in future works.

**ملخص:** في هذا العمل، نهتم باستخلاص السليلوز من مصدر نباتي، مثل نبات الحلفاء *Stipa Tenacissima*، ومن نفايات الورق. بعد الاستخلاص، تم تعديل السليلوز المستخلص للحصول على " ثلاثي أسيتات السليلوز "CTA عن طريق الأسترة. تم توصيف كل من السليلوز و CTAباستخدام تقنية FTIR و .DRXلاستخدام بوليمر CTAالمحضر كمصفوفة بوليمرية في التركيبات الصيدلانية، قمنا بإدخاله في تحضير قرصين مختلفين محملين بمضاد حيوي، "سيفالكسين"، ودرسنا إطلاقه في وسط فيزيولوجي. تم اختبار P1 و P2من الناحية الميكروبية ضد أربع سلالات بكتيرية. أظهرت النتائج أن أطياف الأشعة تحت الحمراء للسليلوز المستخلص من نبات الحلفاء (P1)ومن نفايات الورق (P2) تشير إلى وجود نطاقات امتصاص مميزة رئيسية للسليلوز مقارنة بطيف السليلوز النقي. كما أظهرت نتائج FTIR توافقًا جيدًا بين السيفالكسين و CTAفي القرص، وأن CF كان موزعًا على مصفوفة CTA البوليمرية. تظهر النطاقات الرئيسية لامتصاص CF بوضوح في طيف القرص.يُظهر حيود الأشعة السينية (DRX) لعينة P1 بوضوح أن البوليمر شبه متبلور. بينما يكشف ملف DRX لـ CTA المعدّل من P1 عن بنية غير متبلورة (أمورفية). أظهرت نتائج إطلاق CF من القرصين المحتويين على ثلاثي أسيتات السليلوز (CTA) اختلافًا كبيرًا حسب كمية الدواء المحمّل. وأوضح الاختبار الميكروبي أن البوليمر يُظهر مناطق تثبيط جيدة، حيث ظهرت جميع السلالات بأقطار مختلفة. بالنسبة للسليلوز المستخلص من نبات الحلفاء(P1) ، تم تسجيل أكبر منطقة تثبيط بقطر 11 مم ضد سلالة المكورات العنقودية. كما تم تسجيل أعلى مناطق تثبيط للسليلوز المستخلص من نفايات الورق (P2) ضد بكتيريا *Pseudomonas* وفطر *Candida albicans*بقطر 7 مم.

**الكلمات المفتاحية:** السليلوز، نبات ستيبا تيناسيما، ثلاثي أسيتات السليلوز، سيفاليكسين، حركية الإطلاق، النشاط الميكروبي

**Résumé :** dans ce travail que nous nous intéressons à l'extraction de cellulose à partir d'une source végétale, comme la plante Alfa « Stipa Tenacissima », et de déchets de papier. Après extraction, la cellulose extraite a été modifiée pour obtenir du « triacétate de cellulose CTA » par estérification. La cellulose et le CTA ont été caractérisés par FTIR et DRX. Pour utiliser notre polymère CTA préparé comme matrice polymère dans des formulations pharmaceutiques, nous l'avons introduit dans la préparation de deux disques différents chargés d'un agent antibiotique, la « céfalexine », et avons étudié sa libération en milieu physiologique. P1 et P2 ont été testés microbiens avec quatre agents pathogènes. Les résultats ont montré que les spectres IR de la cellulose extraite de la plante Alfa P1 et des déchets de papier P2 indiquent la présence de bandes d'absorption caractéristiques majeures de la cellulose par rapport au spectre de la cellulose pure. Les résultats IR montrent une bonne corrélation entre la céfalexine et le CTA dans le comprimé et que le CF était dispersé sur la matrice polymère CTA. Les principales bandes d'absorption du CF apparaissent clairement dans le spectre du disque. Le diffractogramme DRX de P1 montre clairement que le polymère est semi-cristallin. Le profil DRX de CTA modifié à partir de P1 révèle la structure amorphe. Les résultats de libération de CF à partir de deux comprimés contenant du triacétate de cellulose (CTA) étaient significativement différents selon la charge en médicament. Le test microbien a montré que le polymère présente de bonnes zones d'inhibition, toutes les souches ayant des diamètres différents. Pour la cellulose extraite de la plante Alfa (P1), la zone d'inhibition la plus élevée a été observée avec une souche de staphylocoque de 11 mm de diamètre. Nous avons également obtenu pour la cellulose extraite de vieux papiers (P2), les zones d'inhibition les plus élevées ont été observées avec Pseudomonas et Candida albican de 7 mm.

**Mots clés :** Cellulose, plante Stipa Tenacissima, triacétate de cellulose, céfalexine, cinétique de libération, activité microbienne

**Abstract:** In this work we are interested by extracting Cellulose from a plant source as Alfa plant "*Stipa Tenacissima*" and from waste of paper. After Extraction, the cellulose extracted was modified to obtain "Cellulose Triacetate CTA" using esterfication process. The cellulose and CTA were characterized by FTIR and DRX. For using our polymer CTA prepared as a polymeric matrice in pharmaceutical formulations, we introduced it to prepare two different discs charged with an antibiotic agent "Cefalexin" and study its release in physiological medium. P1 and P2 were tested microbial with 4 pathogens. The results showed The IR spectrums of both cellulose extracted from Alfa plant P1 and from wastepaper P2 indicates the presence of major characteristic absorption bands of cellulose compared with the spectrum of pure cellulose. The IR results show that the tablet has good correlation between Cefalexin and CTA and the CF was dispersed on the polymer matrix CTA. The main absorption bands of CF which appear clearly in the disk spectrum. The diffractogram XRD of P1 clearly shows that the polymer is semi crystalline polymer. The XRD pattern of CTA modified from P1 shows the amorphous structure. The results of release of CF from two tablets containing cellulose triacetate (CTA) was significantly different depending on the drug load. From microbial test, We observe that the polymer has a good inhibition zones with all strains tasted with different diameters, for Cellulose extracted from Alfa plant (P1) , the highest inhibition zone was observed with staph strain with diameter 11 mm. we obtained also for the cellulose extracted from wastepaper (P2), the highest inhibition zones was observed with Pseudomonas and candida albican with 7mm.

**Key words:** Cellulose**,** *Stipa Tenacissima* Plant, Cellulose Triacetate, Cefalexin, Kinetics of release, microbial activity