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# Micro and nanocellulose in electrospinning processes



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## **Riassunto in italiano**

La cellulosa è il più abbondante biopolimero sulla terra, può essere ottenuta da diverse piante (tuberi, canna da zucchero, cotone) e da biomasse (alghe e batteri o animali marini, come i tunicati). Negli ultimi anni è cresciuto molto l'interesse di utilizzare questa materia prima perché è rinnovabile, sostenibile e biodegradabile. Queste sono caratteristiche che al giorno d'oggi sono messe in prima posizione, soprattutto perché si cerca di ridurre l'utilizzo di materiali derivanti dal petrolio. La ricerca e la scienza si stanno muovendo sempre di più verso materie prime estratte da risorse rinnovabili come la micro e nanocellulosa, che rientra in questa categoria. Normalmente essa viene impiegata come materiale di rinforzo (come filler in matrice polimerica) grazie anche a delle caratteristiche aggiuntive come il basso costo, la buona reperibilità e la non tossicità. Inoltre, non viene usata solo come filler ma anche, nell'industria cartaria, farmaceutica, nell'imballaggio, per la realizzazione di filtri e anche nel campo biomedico nell'ingegneria dei tessuti. Lo scopo principale di questa tesi è approfondire il campo della micro e nanocellulosa utilizzata come rinforzo in fibre polimeriche e membrane fibrose ottenute tramite elettrofilatura (electrospinning), una tecnica versatile che permette di filare nanofibre polimeriche a partire da una soluzione tramite l'applicazione di un forte campo elettrico. Infatti, la micro e nanocellulosa è stata introdotta in diversi sistemi polimerici (come ad esempio in alcool polivinilico, e ossido di polietilene) prima dell'electrospinning, per migliorare le caratteristiche meccaniche delle fibre e delle membrane fibrose ottenute.

Dopo aver descritto la cellulosa (Capitolo 1) e il metodo di electrospinning (Capitolo 2), questa tesi riporta lo stato dell'arte sull'uso della micro e nano-cellulosa nel processo di electrospinning (Capitolo 3). Infine, nel Capitolo 4, viene presentata una semplice proposta di ricerca sperimentale sull'argomento. In particolare, viene proposta la formazione di membrane fibrose bio-nano-composite formate da una matrice chitosanica e da cristalli di cellulosa estratti dalla bagassa delle canne da zucchero come filler. Queste membrane sono completamente biodegradabili, presentano buone caratteristiche in termini di proprietà meccaniche e potrebbero trovare applicazione nel campo dell'imballaggio alimentare.

La cellulosa ha una struttura gerarchica e complessa. Le molecole di cellulosa sono allineate tra loro formando delle microfibrille che sono composte da una parte cristallina e una parte amorfa, a loro volta messe insieme a formare le macrofibrille o anche definite come aggregati di fibrille. Queste ultime sono tenute insieme da una matrice composta da lignina e emicellulosa. Questa particolare struttura che appare molto compatta garantisce una buona resistenza; per questo motivo sono necessari diversi processi (meccanici e chimici) per poter ottenere la micro e nanocellulosa. La cellulosa è un polimero lineare, la sua unità strutturale è formata da due anelli di anidroglucosio  $(C_6H_{10}O_5)_n$ ; dove 'n' può essere uguale a 10000-15000 e dipende dalla fonte di cellulosa. I monomeri della cellulosa sono legati tra loro tramite legami glucosidici (legami  $\beta$ -1-4), i quali si formano grazie all'eliminazione di una molecola d'acqua. Ogni monomero ha tre gruppi -OH che sono responsabili della formazione di legami idrogeno. I legami che si vengono a formare inter-catena sono molto forti e sono quelli che garantiscono stabilità e che rendono la cellulosa insolubile in alcuni solventi.

Per quanto riguarda la struttura di una fibra di cellulosa, essa è divisa in diverse parti: la lamella mediana, la parete primaria e quella secondaria. La parete primaria è quella più sottile ed è composta principalmente da microfibrille di cellulosa, la parte rimanente è emicellulosa. La parete secondaria è quella più spessa e può essere suddivisa a sua volta in parte esterna S1, mediana S2 e interna S3. La sezione S2 è quella predominante e le microfibre si posso disporre parallelamente le une con le altre o in modo perpendicolare. L'orientazione delle fibre è definita secondo un angolo, detto 'microfibrillar angle', che influenza le proprietà meccaniche del material, come la rigidezza. L'angolo delle fibre poste parallelamente è minore rispetto a quello delle fibre poste perpendicolarmente. Al centro della fibre si trova il lumen, un canale dove passa la linfa.

La lignina è un polimero idrocarburico con costituenti alifatici e aromatici; è costituita da diversi acidi fenilpropilici e alcoli; nel complesso ha una struttura molto ramificata. Questa complessa struttura e l'elevato grado di polimerizzazione hanno lo scopo di conferire stabilità e rigidità alle fibre di cellulosa. La lignina funge infatti da matrice per bloccare le fibre di cellulosa tra di loro, rendendole stabili e resistenti alle pressioni esterne. Invece l'emicellulosa è una miscela di polisaccaridi, in particolare zuccheri con 5 atomi di carbonio (xilosio, arabinosio) e 6 atomi di carbonio (galattosio, ramnosio). L'emicellulosa, data la somiglianza con la cellulosa, forma con essa legami idrogeno e in combinazione con questa funge da materiale strutturale.

L'estrazione delle microfibrille di cellulosa (con l'eliminazione di lignina ed emicellulosa) può essere eseguita tramite tre diversi metodi: meccanici, chimici e enzimatici. L'estrazione delle microfibrille può essere complessa e, a seconda della fonte da cui si desidera estrarre la cellulosa, si possono selezionare diversi trattamenti. Per quanto riguarda il legno e le piante, devono essere effettuati pretrattamenti per eliminare la matrice formata da lignina ed emicellulosa. Per la cellulosa estratta dai tunicati, invece, è necessario rimuovere il mantello e la matrice proteica che circonda l'animale. Per quanto riguarda le alghe, devono essere eseguite fasi di purificazione per rimuovere la matrice della parete algale. Infine, per la cellulosa batterica, la crescita deve essere controllata durante la coltura e successivamente devono essere eliminati i batteri e i mezzi di coltura tramite purificazione. I metodi meccanici richiedono molta energia perché normalmente utilizzano un alto livello di pressione ed energia cinetical metodi chimici utilizzano invece diversi agenti volti a sciogliere la matrice lignina-emicellulosa che si trova attorno alle microfibre di cellulosa. I principali metodi meccanici sono la macinatura, la crio-frantumazione, l'omogeneizzazione con raffinatori, e i trattamenti ad ultrasuoni ad alta intensità. Per quanto riguarda i trattamenti chimici, che generalmente affiancano quelli meccanici per ridurne l'energia legata all'estrazione, quelli più utilizzati sono la delignificazione, l'esplosione di vapore, l'ossidazione mediante TEMPO e la carbometilazione.

Dopo l'estrazione delle microfibrille, tramite un'idrolisi acida è possibile ottenere i nanocristalli di cellulosa, la parte maggiormente utilizzata come filler. L'idrolizzazione viene fatta con acidi perché

la parte cristallina ha una buona resistenza e rimane intatta, mentre la parte amorfa viene attaccata, solubilizzata e successivamente allontanata tramite una filtrazione.

La tecnica dell'elettrofilatura è diventata sempre più interessante per la produzione di fibre micro e nanometriche partendo da diverse soluzioni polimeriche. Questa tecnologia è stata ampiamente studiata nell'ultimo decennio, non solo per la sua grande versatilità nella produzione di membrane ma anche principalmente per la sua capacità di formare fibre che vanno da pochi nanometri a pochi micron, che risultano quindi difficilmente ottenibili con altre tecniche. L'elettrofilatura può essere utilizzata con un gran numero di polimeri, sia sintetici (poliammidi, poliolefine, poliesteri, aramidici e poliacrilato) che naturali (polisaccaridi, polipeptidi e proteine).

Le nanofibre preparate mediante elettrofilatura mostrano un enorme potenziale di applicazione in diverse aree: oltre al loro utilizzo come rinforzo per lo sviluppo di materiali compositi, esse trovano impiego per sistemi di filtrazione, per indumenti protettivi, in ambito biomedico per realizzare protesi di tessuti molli, scaffold per l'ingegneria tissutale e sistemi di drug delivery ed in ambito energetico per realizzare sensori, attuatori ed elettrodi.

L'impianto di elettrofilatura è composto da una pompa a siringa, un generatore ad alta tensione e un collettore. I filamenti polimerici si formano grazie a due elettrodi carichi di polarità opposta, posti uno a contatto con la soluzione e l'altro con il collettore. La soluzione viene espulsa dalla filiera metallica tramite un piccolo foro, il campo elettrico ad alta tensione viene applicato al flusso di fluido polimerico (soluzione) e le cariche libere vengono indotte nel fluido all'interno della siringa. Questi ioni si muovono in risposta al campo elettrico applicato verso l'elettrodo di polarità opposta e, di conseguenza, le forze di trazione vengono trasferite al fluido polimerico. Dopo un periodo di induzione, la carica interna raggiunge la condizione critica e un getto di fluido erompe sulla punta dell'ago, determinando la formazione del cono di Taylor. A causa dell'interazione tra il getto, il campo elettrico esterno e la repulsione delle cariche all'interno, il getto subisce una flessione, allungandosi più sottilmente. Durante il volo del fluido verso il collettore, il solvente evapora lasciando le fibre solide che si raccolgono sul collettore.

Le parti che compongono l'apparecchiatura sono semplici, ma possono esserci delle difficoltà nell'impostazione dei parametri sperimentali. Il processo di elettrofilatura è infatti influenzato da diversi parametri, che possono avere un grande effetto sulle fibre che si ottengono. La morfologia delle nanofibre dipende dal processo, dal sistema e dai parametri ambientali. Poiché lo scopo dell'elettrofilatura è creare membrane costituite da fibre con un diametro nanometrico, è necessario limitare i difetti dei filamenti polimerici che possono formarsi durante il processo, definiti come 'beads' e giunzioni. Per questo motivo è necessario controllare accuratamente tutti i parametri.

Il parametri di sistema sono costituiti dalla viscosità, dalla concentrazione della soluzione, dalla conducibilità e dalla tensione superficiale della soluzione in esame. La viscosità è un parametro che, verificato nel processo di elettrofilatura, determina le caratteristiche morfologiche delle fibre. Inoltre bisogna considerare che la viscosità è proporzionale alla concentrazione del polimero. Il solvente gioca un ruolo fondamentale perché diminuisce la viscosità della soluzione. In generale, se la viscosità è alta, si ottengono fibre uniformi con meno imperfezioni, ma all'aumentare della

viscosità crescono i diametri delle fibre. Ovviamente la concentrazione della soluzione è un altro parametro che determina delle caratteristiche importanti delle fibre elettrofilate. Viene definita una concentrazione minima al di sotto della quale il processo diventa "electrospray" e una concentrazione massima oltre la quale la soluzione è eccessivamente viscosa per garantire un processo continuo. Il diametro delle fibrea aumenta con l'aumentare della concentrazione del polimero secondo una relazione di legge di potenza. Il parametro della tensione superficiale è una funzione del solvente utilizzato nella soluzione ma è indipendente, a differenza della viscosità, dalla concentrazione del polimero. Diversi solventi generano caratteristiche differenti; quando si ha una maggiore densità di carica sulla superficie della soluzione (ad esempio aggiungendo una piccola percentuale di sale), si ha come risultato una diminuzione dei difetti e diametri più piccoli delle fibre. Infine, la conducibilità della soluzione dipende dal polimero, dal solvente e dalla presenza di sali. All'aumentare della conducibilità, il diametro delle fibre diminuisce. Se la conducibilità è eccessivamente bassa, non si possono formare fibre continue ma si otterranno difetti. Tuttavia le soluzioni eccessivamente conduttive sono altamente instabili sotto campi elettrici intensi e la regione di instabilità è molto marcata, portando a fibre con diametri molto irregolari. L'aggiunta di sale è spesso importante per aumentare la conduttività della soluzione e per produrre fibre più uniformi. È stato anche studiato che l'aggiunta di alcol al solvente può aumentare la conducibilità. La cosa più importante è che questi additivi non rimangano nelle fibre una volta raccolte e quindi non abbiano conseguenze sul materiale elettrofilato.

I parametri di processo invece sono la tensione di filatura, la distanza di lavoro tra la punta dell'ago e il collettore, la portata di soluzione introdotta e le disposizioni dei componenti dell'apparecchiatura (ad esempio set up orizzontale o verticale). La portata può essere controllata con una pompa e si è riscontrato che una portata inferiore implica diametri di fibre inferiori. Una portata troppo alta non va bene perché le fibre non possono asciugarsi completamente prima di raggiungere il collettore. Per quanto riguarda la tensione applicata, esiste una quantità minima al di sopra della quale si possono formare le fibre. Se la tensione aumenta al di sopra della soglia minima, le fibre mostrano inizialmente diametri progressivamente più piccoli, a causa delle crescenti forze repulsive all'interno del getto che ne favoriscono l'assottigliamento, ma se la tensione è troppo alta il volume della soluzione nella punta dell'ago diminuisce, non si ha più la formazione del cono di Taylor e di conseguenza si ha come risultato delle fibre con difetti ('beads'). La differenza di potenziale ottimale applicata dipende comunque dalle proprietà della soluzione di filatura: peso molecolare del polimero e viscosità. È importante controllare la distanza tra la filiera e il collettore, perché se questa non è sufficiente c'è un'evaporazione incompleta del solvente. Il design del collettore è studiato per favorire l'allineamento delle fibre poiché la traiettoria del getto di polimero è soggetta ad instabilità a causa della tensione applicata e dei parametri utilizzati. Il collettore, oltre alla sua più semplice forma piana, ad esempio può essere anche un cilindro rotante. Le fibre vengono raccolte sulla superficie del cilindro in modo circonferenziale e si allineano.

Le condizioni ambientali che interessano il processo di electrospinning sono generalmente una temperatura atmosferica e un'umidità non elevata, perché se c'è un aumento di quest'ultima si creano dei pori sulla superficie delle fibre formate.

Per aumentare le proprietà meccaniche delle membrane create tramite il processo di elettrofilatura, può essere utilizzata come filler la nanocellulosa. La nanocellulosa, che comprende sia nanofibre di cellulosa (CNF) che nanocristalli di cellulosa (CNC), ha ottime caratteristiche: ha un alto effetto rinforzante a basso dosaggio, non è tossica, è rinnovabile e generalmente biocompatibile. Per queste caratteristiche è un buon materiale come riempitivo in una matrice per la produzione di materiali nanocompositi.

Diversi polimeri sono stati usati come matrice con un riempitivo di nanocellulosa, inclusi polimeri naturali, come alginato, acetato di cellulosa, e polimeri sintetici, come polietilenglicole (PEO), polistirene (PS), poli (ɛ-caprolattone) (PCL), acido polilattico (PLA), polivinil acetato (PVA), acido poliacrilico (PAA), etilene-vinil alcol, poli (metil metacrilato). Nel complesso, indipendentemente dal polimero utilizzato come matrice, la nanocellulosa introdotta migliora le caratteristiche meccaniche ed i diametri ottenuti dopo la realizzazione dei compositi con elettrofilatura sono sempre nell'ordine di grandezza dei nanometri.

È importante considerare come le sospensioni di cellulosa possano essere miscelate con la soluzione polimerica e utilizzate per la produzione di compositi fibrosi mediante elettrofilatura. Il solvente utilizzato per la produzione del composito è infatti fondamentale e il processo può essere suddiviso in elettrofilatura in mezzi acquosi ed elettrofilatura in mezzi non acquosi (solventi organici).

La soluzione in un mezzo acquoso implica la solubilità della matrice polimerica in acqua e una buona disperdibilità della nano-cellulosa nella miscela. I polimeri idrosolubili presi in considerazione e analizzati sono PVA, PEO e PAA.

Nel caso di elettrofilatura in mezzo non acquoso, l'imporante che il solvente sia compatibile con il polimero e produca una miscela stabile con la nanocellulosa, considerando che quest'ultima è un materiale idrofilo. I polimeri presi in considerazione e analizzati sono stati PLA e PCL.

L'introduzione di nanocellulosa porta alla crescita della conduttività della soluzione da elettrofilare e di conseguenza una diminuzione del diametro delle fibre. Bisogna anche considerare che la nanocellulosa aumenta la viscosità della soluzione e quindi i parametri di processo devono essere modificati. In generale, tutti i sistemi presentati in letteratura mostrano un aumento delle proprietà meccaniche (modulo di Young e resistenza a trazione) e termiche (aumento della temperatura di transizione vetrosa) con l'aumento della concentrazione del filler. Tuttavia, se la quantità di riempitivo nella matrice è troppo elevata, il composito ottenuto potrebbe avere caratteristiche fragili (ridotto allungamento a rottura), e il modulo elastico potrebbe diminuire. Per questo motivo è importante scegliere la corretta concentrazione di nanocellulosa, che normalmente non è superiore al 5 % in peso.

Nella parte finale della tesi, facendo riferimento alla ricerca bibliografica fatta e alla conoscenze acquisite grazie alla ricerca scientifica, sono state tratte le conclusioni proponendo un lavoro di ricerca che mira ad ottenere un bio-nano composito sotto forma di membrana fibrosa ottenuto tramite elettrofilatura, formato da chitosano come matrice e nanocristalli di cellulosa come riempitivo,. L'applicazione di interesse è l'imballaggio alimentare. E' stato scelto come applicazione il packaging alimentare perché negli ultimi anni è diventato un campo di grande interesse, molte ricerche si sono concentrate sulla ricerca di nuovi materiali in sostituzione di quelli derivanti dai combustibili fossili. Lo scopo è promuovere la "green economy", trovare alternative adeguate, competitive con le plastiche più utilizzate (polietilene e polipropilene), riducendo i rifiuti di difficile smaltimento.

Il materiale proposto come matrice è il chitosano che, come dimostrato in letteratura, è possibile elettrofilare creando una membrana fibrosa. Una caratterista aggiuntiva per proporre questo materiale nel food packaging è costituita dalle sue proprietà antimicrobiche. Infatti, grazie alla sua struttura e in particolare al gruppo amminico che interagisce con le membrane cellulari microbiche, le quali causano le fuoriuscita di proteine e altri costituenti intracellulari di microrganismi, il chitosano interagisce con le membrane batteriche, inibendo l'iterazione diretta tra i costituenti secreti e l'alimento aumentando così la shelf-life.

I cristalli di cellulosa saranno estratti dalla bagassa di canna da zucchero. La canna da zucchero è stata selezionata perché è largamente disponibile, è uno scarto da biomassa e può essere convertita in diversi prodotti.

Gli obbiettivi proposti da questa ricerca sono:

- Ottenimento dei nanocristalli di cellulosa da utilizzare come riempitivo;
- Preparazione della sospensione da utilizzare nel processo di elettrofilatura;
- Ottimizzazione del processo di elettrofilatura;
- Caratterizzazione delle membrane elettrofilate bio-nanocomposite.

In conclusione, le membrane fibrose bio-nanocomposte proposte dovrebbero presentare proprietà antimicrobiche grazie al chitosano, e dovrebbero avere proprietà meccaniche migliorate grazie ai CNC. Sono quindi adatte come materiali da imballaggio per alimenti, anche considerando l'interesse mondiale alla riduzione della plastica e alla riduzione dell'inquinamento ambientale.

## Introduction

Cellulose is the most abundant biopolymer on earth. It can be obtained from a lot of plant resources and derived biomass: wood, plants (such as cotton, ramie, sisal, potato tubers, and sugar cane), sea animals (such as tunicates), algae, and bacteria. The interest in this material is given by the fact that it is renewable, recycleble, and biodegradable; these three characteristics are of great interest in recent years to replace materials deriving from fossil fuels. Therefore, cellulose can be defined as a sustainable and 'green' polymer (fig.1). [1]



Figure 1: Concept of "sustainable" bio-based product. [1]

The structure of cellulose is hierarchical: cellulose molecules align to form microfibrils, which have both crystalline and non-crystalline regions that merge together, and the cellulose microfibrils themselves are aligned and bound together into macrofibrils (or fibril aggregates) by a matrix of hemicellulose and either pectin or lignin. Micro and nano-cellulose (i.e., microfibrillated cellulose, cellulose nanofibers, cellulose nanocrystals) can be extracted from any cellulose source material by chemical or mechanical processes. It can be then used as reinforcing material thanks to its mechanical properties as well as its low cost, non toxicity and sustainability.

Micro and nano-cellulose can be introduced as a filler also in polymer fibers and fibrous membranes to enhance their properties. An interesting and versatile method to form polymer fibrous structures is the electrospinning. This technology uses electrical forces to produce fibers with nano- or microscale size from polymer solutions. During electrospinning, different parameters could be controlled: the viscosity of the solution, its concentration and surface tension, the voltage applied, the operating temperature and humidity, the geometry of the collector. These parameters influence the morphology of the fibers and their diameters. The obtained fibrous membranes find

application in different fields, such as filtration, packaging, tissue engineering, drug delivery. Interestingly a large set of polymers, both natural and synthetic, can be processed by electrospinning.

After describing cellulose (Chapter 1) and the electrospinning method (Chapter 2), this thesis reports the state-of-the-art on the use of micro and nano-cellulose in the electrospinning process (Chapter 3). Cellulose fillers have been introduced in different polymer systems (such as polyvinyl alcohol, polyethylene oxide and polycaprolactone) before electrospinning to improve the mechanical features of the obtained fibers and fibrous membranes.

Moreover, in Chapter 4, a simple experimental work plan on the topic is proposed. In particular, the formation of bio-nano-composite fibrous membranes made of a chitosan matrix and cellulose crystals extracted from cane sugar bagasse as filler is presented. The proposed membranes are completely biodegradable, show good characteristics in terms of mechanical properties and could find application in the food packaging field.

# Chapter 1 – Cellulose

Cellulose is a natural polymer, very abundant in nature, biodegradable, economical, and environmental-friendly. It can be easily procured from different sources and its annual production is estimated to be over  $7.5 \times 10^{10}$  tons. [2]

Its interesting chemical structure, the easy availability, and its chemical-physical characteristics have created great interest in many applications, such as food industry, paper production, pharmaceutical, and biomaterials. [3] [4]

In nature, cellulose does not exist as a single molecule but rather in the form of fibers resulting from the hierarchical organization of several cellulosic chains that are the result of the process of biosynthesis of cellulose.

In fig.2 it is shown the hierarchical structure of cellulose: cellulose molecules align to form microfibrils (elementary fibrils), which are composed by crystalline and non-crystalline part; the cellulose microfibrils themselves are bound together into macrofibrils (fibril aggregates) by a matrix of hemicellulose and either pectin or lignin. [3] Cellulose provides strength and stability to the plant cell walls, this particular structure gives cellulose good resistance, and cellulose fibers are responsible for numerous properties such as compactness and structural organization.



Figure 2: Schematics of cellulosic fiber structure. [4]

Cellulose can be obtained from different types of woods, plants, animals (tunicates), bacteria, and algae. Wood is one of the most abundant sources for extracting cellulose. In general, at first, the wood is purified from lignin, hemicellulose and impurities. Typical materials obtained are bleached pulp and dissolving pulp. Like wood, plants are an attractive source of cellulose mainly because they are abundant and extensively used in the textile industry, which deals with the harvesting, maceration/pulping, and processing of the product. Many plants have been studied for cellulose extraction such as cotton, ramie, sisal, potato tubers, soybean stock, or cane sugar. [3] Tunicates belong to the family of sea animals and are the only ones capable of producing cellulose microfibrils. They have a coat consisting of cellulose microfibrils embedded in a protein matrix. It is this thick leathery coat in their mature stage that is used as a source of cellulose microfibrils. The best-known species is call 'Ascidiacea'. [3]

Algae is another species that can produce cellulose microfibrils within the cell wall. There are differences between the cellulose microfibrils produced by different algae, due to the differences in the biosynthesis process. Some species used are: 'Valonia', 'Caldophora', or 'Micrasterias denticulata'. [3]

Bacteria can produce cellulose under a special culturing condition: bacteria secrete cellulose microfibrils, in particular a gel composed of 97% of water on the surface of a liquid medium. The advantage of bacterial-derived cellulose is that it is possible to regulate the culture conditions and thus control the formation of microfibrils and their crystallization. [3]

#### **1.1 Cellulose structure**

Cellulose is the main polysaccharide in the plant cell wall. Its molecule is a linear, unbranched polymer and is insoluble in water.[3] The cellulose chain is made of two anhydroglucose rings  $(C_6H_{10}O_5)_n$ , where n may be from 10000 to 15000, depending upon the source of cellulose. The repeating unit of cellulose (i.e, the two rings, the dimer) is named 'cellobiose' (fig. 3). [5]



Figure 3: structure of cellulose (cellobiose). [3]

In cellobiose structure there are two  $\beta$ -D glucopyranose units linked together by  $\beta$ -1-4 linkages, named glucosidic bonds, which are formed deleting a molecule of water. Each monomer has three hydroxyl groups that give an important characteristic to cellulose: [3] they are fundamental to form hydrogen bonds. These intermolecular linkages and van der Waals forces promote parallel stacking of multiple cellulose chains forming elementary fibrils: for this reason, cellulose is a stable polymer and has a high axial stiffness.

The chain of cellulose, thanks to its bonds, is ordered in a crystalline part and has an amorphous region (fig. 4). Normally the crystalline part is extracted, resulting in cellulose nanocrystals. [3] Different extraction methods are described in Chapter 1.2.



Figure 4: crystalline and amorphous regions in a cellulose fibril. [3]

The interchain hydrogen bonds in the crystalline regions are strong, thanks to this configuration the resulting fibers have good strength and insolubility in most solvents. [5]

As described above, single cellulose microfibrils or elementary fibrils hold together forming aggregates, macrofibrils, thanks to a matrix of lignin and hemicellulose. Fig. 5 show the different layers present in a plant cell wall: the median lamella, also known as intercellular substance, the primary wall and the secondary wall.

The 'median lamella' is mainly composed of peptide substances and lignin. The primary wall is the thinnest part and is mainly made up of cellulose fibers; the remaining part is hemicellulose. The secondary wall, the thicker one, can be divided into three parts: external S1, median S2, internal S3. Layer S2, the one detailed in the inset of fig. 5, is the thickest and the microfibers can be arranged parallel or perpendicular to the fiber itself. Microfibers placed in parallel have a smaller angle than those placed in the other direction. The angle between the fiber axis and the microfibrils is called the microfibrillar angle: this is important for the mechanical characteristics of cellulose, such as stiffness, and therefore also of the final product. [2] [6] [6] At the center of the structure is the lumen, a channel through which the lymph passes.



Figure 5: scheme of cellulose's fiber. [6]

The morphological structure of cellulosic fibers includes other constituents, as mentioned above, in addition to the cellulose itself: lignin and hemicellulose. The proportions between these constituents vary according to the different parts of the trunk and the type of plants.

Lignin (fig. 6) has a complex structure: it is an hydrocarbon polymer with aliphatic and aromatic constituents, it is made up of different phenylpropyl acids and alcohols and overall has a very branched structure. This complex structure and the high degree of polymerization have the purpose of conferring stability and stiffness to the cellulose fibers. It acts as a matrix to block the cellulose microfibrils between them, making them stable and resistant to external pressure [7]



Figure 6: Structure of lignin. [6]

Hemicellulose is a mixture of polysaccharides, especially sugars with 5 carbon atoms (xylose, arabinose) and 6 carbon atoms (galactose rhamnose), has several final branches. Hemicellulose,

given the similarity with cellulose, forms hydrogen bonds with it and thus acts as a structural material. [8] However, hemicellulose differs from cellulose:

- It contains different sugar whereas cellulose is done by  $1,4-\beta$ -D glucopyranose units.
- It has a pendant side group, for this, does not have a crystalline nature.
- The degree of polymerization is less than for native cellulose; it is around 50-300 while cellulose has a degree 10-100 times greater.

#### 1.1.1 Polymorphism of cellulose

There are four different polymorphs of cellulose: cellulose I, II, III, and IV, as shown in fig. 7.

Cellulose I is the polymorph found in native cellulose that can be converted to cellulose II thanks to re-crystallization or other processes called mercerization with aqueous sodium hydroxide.

Native cellulose can have two sub-allomorphs forms:  $I_{\alpha}$  and  $I_{\beta}$ . Cellulose  $I_{\alpha}$  can be found in lower plants, primitive organism as algae and bacteria, while the  $I_{\beta}$  in higher plants. These two forms differ for their hydrogen bonds, as shown in fig. 8. [2]  $I_{\alpha}$  is formed of a triclinic unit cell with one cellulose chain in the cell, while  $I_{\beta}$  is composed of a monoclinic cell with two cellulose chains. The  $I_{\alpha}$  form is more metastable and can be converted in  $I_{\beta}$ , which is more thermodynamically more stable, by high-temperature annealing. [2]

Cellulose II has a different configuration from cellulose I because it is constituted by an antiparallel organization instead of a parallel one Also, Cellulose II is the most stable crystalline configuration. [9]

Cellulose III can be formed by treatment of cellulose I and II with ammonia. It has been showed that it can be divided in  $III_I$  and  $III_{II}$ ; where the pedic indicates the type of cellulose it derives from. [9]

Lastly, cellulose IV is formed heating cellulose  $III_I$  and  $III_{II}$  up to 260 <sup>0</sup>C in glycerol. [9]



Figure 7: polymorphs of cellulose and the main steps to obtain them. [9]



Figure 8: the two alternative hydrogen-bond networks in cellulose  $I_{\alpha}$  (top) and in cellulose  $I_{\beta}$  (bottom).[2]

#### **1.2 Cellulose extraction**

The extraction of microfibrils of cellulose can be done by three different methods: mechanically, chemically, and enzymatically.

Some considerations must be made: mechanical methods need a lot of energy because normally they use a high level of pressure and kinetic energy. The product obtained has the same composition as the original feed. Whereas, chemical methods use several agents aimed at dissolving the lignin-

hemicellulose matrix that is around cellulose fibers. Therefore, the energy consumption is lower but more harmful chemicals are employed.

A combination of the two methods may normally be used to obtain a higher yield of cellulose with lower energy and using less chemical agents.

The extraction of microfibrils can be complex and the treatments can vary depending on the source from which cellulose is extracted. As far as wood and plants are concerned, pre-treatments must be performed to eliminate the matrix formed by lignin and hemicellulose (mainly by mechanical and chemical processes). For the cellulose extracted from the tunics, on the other hand, the mantle surrounding the animal as well as the protein matrix must be removed. For algae instead purification steps must be performed to remove the algal wall matrix. Finally, for bacterial cellulose, growth must be controlled during culture and then the elimination of bacteria and any media has to be performed. [3]

### 1.2.1 Mechanical pulping

The main mechanical methods are grinding, homogenization with refiners, cryo crushing, and highintensity ultrasonic treatments.,

• Grinding and cryo crushing

Cellulose passes between a static grindstone and a rotating one (1500 rpm), the purpose is to break hydrogen bonds by shear forces and to individualize the nanofibers from the pulp (fig. 9). Cellulose fibers pass-through disks several times, gradually reaching the nanometric size; at some point there is no change in the morphology of the fibers, and defibrillation can be considered complete. A drawback is that this method can sometimes reduce the length of the fibers, which is an important feature. [10]

In order to reduce the energy employed to extract microfibrils, liquid nitrogen can be applied for freezing the fibers (cryo crushing). During this process the formation of ice crystals occurs, which exert pressure on the cell wall causing them to rupture and the release of microfibrils. [10] Normally this method is used for extracting microfibrils from agricultural crops. After this process, a grinder is used for the complete extraction. For example, Chakraborty et al. [11] immersed at first the fibers in liquid nitrogen but subsequently used a high-impact grinder with a cast iron mortar and pestle. This step was performed to impart sufficient energy to break the microfibrils of the fiber walls and form individual fibrils of very low diameter [11]



Figure 9: Schematic representation of a grinder. [9]

• Homogenization and microfluidization

The homogenization consists in pumping cellulose at high pressure through a spring high pressure loaded valve that opens and closes in rapid succession, as shown in fig. 10.

Before this step, normally it is better to do a refining process because it gradually detaches the external cell wall and exposes the layer S2 (the one with more fibers), facilitating the next treatment and reducing energy consumption. The refining process consists in forcing the fibers in solution through rotor and stator disks, having a surface with roughness. The fibers are exposed to repeated cyclic stress.[1]



Figure 10: Schematic representation of homogenizer. [9]

An alternative to the homogenizer is the microfluidizer (fig. 11). The wood pulp passes through a z-shaped chamber (with channel dimensions that are usually 200–400 micrometers) under great pressure. The applied shear rate is very high and therefore leads to the formation of very thin fibers. This equipment has been used a lot recently because it allows to obtain fibers with more uniform sizes. [9]



Figure 11: Schematic representation of microfluidizer. [9]

• High-intensity ultrasonic treatments

The use of the ultrasonic technique for the isolation of cellulose nanofibers is an emerging method. During this process, ultrasound energy is transferred to the cellulose chains through a process called cavitation, a phenomenon consisting of the formation of vapor zones within a fluid which then implode producing a characteristic noise. The energy provided by cavitation is about 10–100 kJ/mol, which falls within the hydrogen bond energy scale. [12] Thus, the ultrasonic impact can gradually disintegrate the micron-sized cellulose fibers into nanofibers. Cheng et al. [13] used this technique to separate fibrils. Initially they immersed the material in distilled water for more than 24 h (concentration: 2% by mass) and then treated it for 30 min with a power of 80% using high-intensity ultrasound to isolate the fibrils. The ultrasounds produce a very strong mechanical oscillating power so that the cellulose fibrils can be isolated from the cellulose fibers by the action of the hydrodynamic forces of the ultrasounds; then centrifugation is performed to completely separate the fibrils from the treated material. [13]

### 1.2.2 Chemical pulping

Chemical pulping normally accompanies mechanical pulping, to reduce the overall energy used to extract the cellulose. Chemical pulping can also be classified as a pre-treatment: given the complex structure of the cellulose, it helps the extraction work.

Delignification is a process, normally done before the mechanical pulping, used in wood and plants for degrading lignin. The lignin molecules are brought in solution and then removed with a wash. This process is called 'Kraft' pulping: the chemicals used are sodium hydroxide, NaOH, and sodium sulfide,  $Na_2S$ , with –OH and –HS as active anions. The latter is the main dignifying agent, while the hydroxides keep the lignin fragments in solution. [1] Then the product obtained from delignification can be bleached by chemicals as  $H_2O_2$ ,  $ClO_2$  and  $O_3$ . The purpose of this step is to obtain a bleached product with reduced impurities. [1] The steam explosion was invented by Mason in 1927. [1] This process can be defined as a pretreatment: it is used to remove the lignocellulosic material from cellulose fibers. The lignocellulosic material is placed in a cylinder with other waste and subsequently pressurized with saturated steam at pressure up to 1000 psi. The saturated steam creates high internal pressure; when it is released defibrillation occurs due to the sudden decompression. During the process, products as sugar and phenolic compounds are produced, thanks to the hemicellulose hydrolysis and depolymerization of lignin.[1]

The effect of this process can be summarized in:

- Cleavage of some accessible glycosidic links.
- Cleavage of lignin–carbohydrate complex bonds.
- Minor chemical modification of lignin and carbohydrates.

Another pre-treatment that helps the extraction of fibers is TEMPO-mediated oxidation (2,2,6,6 tetramethyl-1-piperidinyloxy), which aims at modifying the surface of the native cellulose, into which they can be introduced carboxylate and aldehyde functional groups. [14] The principle of this pre-treatment consists in the oxidation of cellulose fibers by adding NaClO to aqueous cellulose suspensions in the presence of catalytic quantities of TEMPO and NaBr at pH 10-11 at room temperature. The hydroxyl groups of the cellulose are then converted to carboxylate groups via the aldehyde groups and only NaClO and NaOH are consumed. As a result, the nanofibrils within the fibers separate better from each other due to the repulsive forces between the carboxylates, which break the hydrogen bonds. Also, compared to the energy consumption of repeated cycles of a high-pressure homogenizer (700–1400 MJ/kg), TEMPO-mediated oxidation pre-treatment dramatically decreases consumption to values below 7 MJ/kg. [9]

Carboxymethylation is another chemical pre-treatment. The carboxymethylation reaction takes place, allowing the introduction of ionizable groups into the cellulose structure, facilitating its delamination. Then a mechanical process allows the production of microfibrils by applying high shear forces on the cellulose fibers, but reducing the energy consumption during fibrillation. A decrease in energy was noted, from 5.5 MWh/t to 2.2 MWh/t after pre-treatment. [9]

#### **1.2.3 Enzymatic method**

In the previous paragraphs efficient chemical pre-tretements have been shown, but the used chemicals can release toxic products into the environment. Therefore, in the last few years there has been a growing interest in replacing these processes with other alternatives such as the enzymatic method. These processes use enzymes like Xylanases to attack the hemicellulose and the interface between lignin and cellulose. Moreover, they increase the extent of fine material in comparison with chemical treatments as acid hydrolysis. [9]

## **1.3 Cellulose nanocrystals**

As described in Chapter 1.1, the cellulose fibers are composed of a para-crystalline or disordered region and a crystalline one. Cellulose nanocrystals (fig.12) are also defined as microcrystals, whiskers, microcrystallites, or nanofibers. [2]

The main process for the isolation of the cellulose nanocrystals is acid hydrolysis. In fact, the crystalline part of cellulose has good acid resistance and remains intact. [2] The hydrolyzation process consists in dispersing the cellulose microfibers in water and treating the suspension with sulphuric acid (65 wt%) and controlled temperature, agitation and time. The suspensionis heated at 60 °C for 20 min to achieve complete hydrolysis, then it is diluted with water and washed with successive centrifugation, followed by dialysis to remove any free acid molecules. [15] The collected suspension is sonified in a small volume to isolate crystals. Finally, as the last step, it is passed through a filter to remove any particles. [15]



Figure 12: dried dispersion of cellulose nanocrystals. [2]

Once the amorphous phase is removed, the nanocrystals are rigid and rod-like, have a reduced size distribution and are shorter than the fibrils. The advantages of cellulose nanocrystals are:

- High modulus of elasticity, flexibility, transparency and crystallinity, low thermal coefficient of expansion; moreover they contains hydroxyl groups. For example, cellulose nanocrystals have an elastic modulus (E) of about 150 GPa, a value that can compete with other materials, such as Klevar (130 GPa). [14]These characteristics allow the formation of solid films or liquid suspensions. [9] Moreover cellulose nanocrystals can be used as a filler for producing bio-composite materials.
- Low production cost and abundant source. [1] [16] [15]
- Renewability and environmentally friendly. Natural fiber composites are regarded as a substitute for traditional materials, the biopolymer is a great idea because can be competing with plastic materials. Also, it has been demonstrated that polymers made of natural fibers can fix carbon in nature and reduce  $CO_2$  emissions into the atmosphere.[16]

The fields of application of nanocellulose, which includes cellulose nanofibers (CNFs) and cellulose nanocrystals (CNCs), are: reinforcement as a filler, paper industry, electronic products, packaging materials, biological fabrics or functional scaffold materials, drug carriers. In particular, it is used as a filler for the production of composite materials thanks to its mechanical properties. Moreover, the large specific surface allows a low concentration of filler to obtain a good strengthening effect; Nanocellulose, has a

Table 1 compares the mechanical properties of composites with CNFs and CNCs as reinforcement. Both the reinforcements improve the characteristics of the polymer, but the difference is that CNFs have greater toughness, instead the addition of CNCs can increase the crystallinity making the nanocomposite more brittle. Finally, a general decrease in properties at high nanocellulose concentration (10%) can be noticed, and it is presumed to be caused by nanocellulose agglomerations. [17]

filler	content (wt %)	Young's modulus (MPa)	yield strength (MPa)	stress-at-failure (MPa)	strain-at-failure (%)	toughness (kJ/m <sup>3</sup> )
	0	760 ± 109	$14.2 \pm 0.9$	$12.8 \pm 0.4$	86 ± 14	$1161 \pm 20$
CNCs	1	820 ± 195	$15.9 \pm 0.1$	16.9 ± 2.1	495 ± 43	$6157 \pm 573$
	4	895 ± 141	$16.0 \pm 0.8$	$16.2 \pm 1.3$	504 ± 34	$6371 \pm 618$
	7	937 ± 150	$17.6 \pm 0.7$	$18.4 \pm 2.0$	526 ± 40	7083 ± 686
	10	758 ± 326	$15.3 \pm 0.2$	$13.8 \pm 1.7$	416 ± 43	4750 ± 705
CNFs	1	896 ± 99	$17.7 \pm 0.9$	$23.0 \pm 1.0$	491 ± 21	8267 ± 277
	4	994 ± 222	$20.8 \pm 0.7$	20.9 ± 1.7	$281 \pm 56$	5898 ± 539
	7	$1727 \pm 102$	$27.3 \pm 0.9$	26.9 ± 1.7	340 ± 62	9662 ± 112
	10	1235 ± 99	$14.4 \pm 0.5$	16.4 ± 6.7	89 ± 55	$1648 \pm 300$

*Table 1: Mechanical Properties of composites with CNFs and CNCs used as a filler and PEO as a matrix* [17]

Moreover, the introduction of nanocellulose as filler, in addition to increase the mechanical properties of the composite, creates a large specific area that allows to obtain a dense network,

which makes the system very hard for various molecules to pass through (oxygen and water vapor barrier properties). This is a property that the food packaging industry looks for. CNFs generally have better features for this application than CNCs, which show a higher oxygen permeability. This phenomenon is explained by the structural organization that is formed within the composite. CNFs create more entanglements, making it more difficult for small particles to spread. [18] [19] Table 2 reports some values of oxygen permeability of CNFs, as well as of different polymer films used in the food packaging industry.

Material	Oxygen permeability (cc μm/m <sup>2</sup> day kPa)	Conditions
NFC	0,6	65% RH
		23°C
NFC (carboxymethylated)	0,0006	0% RH
		23°C
NFC (carboxymethylated)	0.85	50% RH
		23°C
Cellophane	0.41	0% RH
		25-80°C
Polyethylene (PE)	50-200	50% RH
		23°C
Polyvinylidene chloride (PVdC)	0.1-3	50% RH
		23°C
Polyvinyl alcohol (PVOH)	0.20	0% RH
		23°C
Ethylene vinyl alcohol (EVOH)	0.01-0.1	0% RH
		23°C

Table 2: oxygen permeability of CNFs (here indicated as NFC) compared to the most used materials in food packaging. [19]

In summary, CNFs, used as a filler, are competitive with other petroleum-based materials, even though their water barrier properties are not the same. In fact, nanocellulose is a hydrophilic material and if there is an increase of humidity, fibrils of nanocellulose can be ruined because of the high swelling, thus losing their barrier properties. [19]

# **Chapter 2 – Electrospinning**

The technique of electrospinning has become an increasingly interesting technique for producing micro-to nano-scale fibers from a lot of materials. This has been extensively studied in the last decade, not only due to its great versatility in the production of fibers but also mainly due to its ability to form fibers ranging from few nanometers to few microns, result difficult to obtain through other techniques.[20] Electrospinning has created a lot of interest in different applications: drug delivery, tissue engineering, nanocomposite, filtration, cosmetics, protective clothing. [21]

As described below in Chapter 2.2, by changing the system and process parameters, it is possible to control the morphology of the electrospun fibers. In fig. 13, examples of different membranes that can be obtained with the electrospinning technique are shown: (a) straight and randomly oriented fibers; (b) bead-in-string and randomly oriented fibers; (c) straight, core-shell structure, and randomly oriented; (d) bead-in-string, core-shell structure, and randomly oriented; (e) straight and patterned. [22]



Figure 13 : differents types of fibrous membranes formed by electrospinning [22]

The electrospinning method works thanks to the electrostatic principle, in which polymeric solutions produced from different solvents and polymers are processed. The purpose is to form continuous fibers from an electrically charged jet of polymer solutions.

The electrospinning setup consists of a syringe pump, a high voltage pump, and a collector (fig.14). [23] Polymer filaments are formed thanks to two charged electrodes with opposite polarity, placed one in contact with the solution and the other with the collector. The solution is ejected out of the metal spinneret with a small hole, the solution evaporates leaving the solid fibers which are collected on the collector. It is important the distance between the spinneret and the collector because if it is not enough there is incomplete solvent evaporation. The potential difference depends on the properties of the spinning solution: polymer molecular weight and viscosity.[24]



Figure 14: typical electrospinning setup [23]

#### 2.1 Theory of electrospinning

The term '*electrospinning*' is very recent, the first patent on this has been published by Formhals in 1934, originally the term 'electrostatic spinning' was used. From 1934 to 1944, Formhals described an experimental setup for the production of polymer filaments using electrostatic force. He used a cellulose acetate solution and subjected it to an electric field.

From 1952 to 1971 several researchers studied how to produce nanometric size fibers. For example, Vonnegut and Neubauer [25] have studied how to produce streams of electrified uniform droplets of about 0.1 mm in diameter. They used a glass tube with capillary dimensions in diameter. The tube was filled with water or other liquid and a high voltage (5-10 kV) was introduced into the liquid. Another person that studied this process was Simons [26] who, in 1966, patented an

apparatus for the production of non-woven fabrics using electrical spinning. He used two electrodes, the positive one into the polymer solution and the negative one connected to a belt where the non-woven fabrics were collected. The most important observation that he did was that low viscous solutions generated short and thin fibers while more viscous solutions generated longer and more continuous fibers. [24]

In the 1960s, Taylor initiated studies on the jet forming process. He studied the shape of the polymer droplet produced at the tip of the needle when an electric field is applied. This conical shape of the jet was later named as the Taylor cone. [27]

Researcher Baumgarten in 1971, with the production of acrylic fibers with diameters in the range of 0.05-1.1 microns, described the electrospinning process which is used today. He used a steel capillary tube with constant size where the feed rate was controlled by a pump, a high voltage was connected to the tube, whereas the fibers were collected on a metal screen (collector).[24]

During electrospinning a high voltage electric field is applied to a polymer fluid flow (solution) through a thin nozzle and free charges are induced in the polymer fluid. These charged ions move in response to the applied electric field towards the electrode of the opposite polarity, and consequently, traction forces are transferred to the polymer fluid. After an induction period and when the internal charge reaches critical condition, a jet of fluid will erupt from the droplet on the tip of the needle, resulting in the formation of the Tylor cone. Because of the interaction between the jet and the external electric field and the repulsion of charges inside, the jet undergoes a bending or 'whipping' instability, stretching more thinly. [27]

Rutledge and co-workers [28] have studied these instabilities; they modeled the behavior of the jet in three different instabilities: Rayleigh instability and two 'conducting' modes. [20] The Rayleigh instability (fig. 15) is dominated by surface tension and is removed at high electric fields; whereas, the conducting modes are independent of surface tension and are dominated by electric forces. [20]



*Figure 15: photograph illustrating the instability region of the solution jet* [27]

#### 2.2 Parameters of electrospinning

As shown in fig. 14, the parts that make up the electrospinning equipment are simple, but there can be some difficulties in setting the experimental parameters. The electrospinning process is influenced by different parameters, which can influence the result. The morphology of nanofibres depends on the process, system, and environmental parameters:

- System parameters: viscosity, polymer concentration, conductivity, and surface tension of the solution;
- Environmental parameters: temperature, humidity;
- Process parameters: flow rate, electric field, collector composition, and geometry, vary disposition of equipment's components.

As the purpose of electrospinning is to create fibers with a nanometric diameter and also limit the defects in the form of beads and junction (fig. 16), [20] [29] all these parameters have to be accurately controlled.



Figure 16: (a) A random polymer fiber mesh produced by electrospinning a 9% PCL solution. (b) Electrospun mesh obtained using a 5% PCL solution; at low polymer concentrations defects in the form of beads and junctions are observed [20]

#### 2.2.1 System parameters

The solution viscosity is the most important characteristic to determine the fiber size and morphology during the spinning process. However, it has to be considered that the polymer concentration is proportional to the viscosity.

For instance, Koski et al. found that there are some concentration and viscosity required to electrospin polymer fibers made of poly(vinyl alcohol) (PVA). [20] PVA is a water-soluble synthetic polymer with the formula  $[CH_2CH(OH)]_n$ , which is possible to spin as long as  $[\eta] \cdot c > 5$ , where  $[\eta]$  is the intrinsic viscosity and c is the concentration. Whereas, for polyethylene glycol or polyethylene oxide (PEO), which has the formula H-(O-CH<sub>2</sub>-CH<sub>2</sub>)<sub>n</sub>-OH, fibers are obtained as long as  $[\eta] \cdot c > 10$ . [20]

The intrinsic viscosity  $[\eta]$  can be explained as the initial slope of the plot between specific viscosity and concentration. [23]The specific viscosity can be determined with the Huggins equation, used to determine the solution viscosity:

$$\eta_{sp}(\mathbf{c}) = [\eta] \cdot \mathbf{c} + k_H \cdot ([\eta] \cdot \mathbf{c})^2$$

where  $\eta_{sp}(c)$  is the specific viscosity, c is the concentration,  $k_H$  is the Huggins coefficient and the dimensionless product  $[\eta] \cdot c$  is considered as the Berry's number (B<sub>e</sub>), an index for controlling the diameter of electrospun nanofibers. [30]

To make the electrospinning process to take place, the first step is to introduce a solution to the device. The solution can be diluted or concentrated; if the solution is concentrated, there can be an interpenetration of the polymer chains, which also depends on the viscosity of the solution under examination. The product  $[\eta] \cdot c$ , named Berry's number, measures the overlap of the chains in solution. [31] So the solutions can be classified based on the Berry's number. In case of a dilute solution, without overlapping or interactions between chains, Be < 1. As the concentration increases, the iteration between the chains increases and a critical crossover concentration can be reached (c\*), the chains begin to overlap but the solution is homogeneous. At higher concentrations (c>c\*), the number of chain entanglements will be proportional to c. The value of c will depend on the average molecule weight, flexibility of the polymer chain (or the chemical structure of the polymer), the temperature and the nature of the solvent. [31] In conclusion, it is important to consider three different solution regimes for the polymer solution (fig. 17) with a different Berry's number. These regimes depend on the critical chain overlap concentration (c\*), which is the crossover concentration between the dilute and the semi-dilute concentration and the entanglement concentration (c<sub>e</sub>).



Figure 17: physical representation of the three solution regimes: (a) dilute, (b) semi-dilute unentangled, (c) semi-dilute entangled. [23]

In their article Gupta et al. [23] determined the critical chain overlap concentration for polymethylmethacrylate (PMMA): when the solution concentration was lower than c\* there was insufficient chain overlap to form polymer fibers, in the semi-dilute region ( $c^{*} < c < c_{e}$ ) could be formed fibers and beads occasionally and at last with a concentration  $c > c_{e}$  there were bead-free fibers (fig. 18). [20]



Figure 18: SEM photographs of electrospun nanofibers from different polymer concentration solution.[24]

There are other approaches to describe the intrinsic viscosity  $[\eta]$ . It can be calculated with the Fox-Flory relationship, where  $[\eta]$  is directly proportional to the root-mean-squared end-to-end distance

 $< R^2 > 0.5$  and inversely proportional to N monomers:  $[\eta] \sim \frac{< R^2 > 3/2}{N}$ . [23]

However, the intrinsic viscosity can be related to the Mark-Houwink-Sakurada equation, where  $[\eta]$  this time is directly proportional to the molecular weight M, and to constants K and a that depend on the polymer:  $[\eta] \sim K \cdot M^a$ .[23]

In conclusion, the viscosity is a parameter that determines the morphology characteristics of the fibers. Recalling the definition of viscosity as the energy dissipated by fluid in motion under shear stress applied, for a polymer the energy dissipation can be related to the friction between the molecules, which depends on the repeat unit of polymer and strength of intermolecular forces. In this case, the solvent plays a fundamental role because it decreases the viscosity when compared with a dry system. Consequently, it is possible to introduce a higher concentration into the solution to be electrospun. [32] If the viscosity is high, which depends also on polymer concentration, uniform fibers with fewer imperfections are obtained, but if the concentration is too high the fibers show larger diameters.

The concentration of the solution, which is correlated to viscosity, is an important characteristic because can determine the diameter and morphology of fibers. There is a minimum concentration below which the process becomes "electrospray" and a maximum concentration beyond which the solution is excessively viscous to ensure a continuous process. The fiber diameter increases with increasing polymer concentration according to a power-law relationship.[24] [20]

The surface tension parameter is a function of the solvent used in the solution but is independent, unlike viscosity, of the polymer concentration. Different solvents generate different characteristics to the solution; Zong et al. showed that adding a little percentage of salt (1 wt%), a higher charge density is brought on the surface of the solution and at the end higher elongation forces are imposed to the jet under an electrical field, resulting in smaller bead defects and thinner fiber diameters. [24]

The conductivity (the degree to which a specified material conducts electricity, calculated as the ratio of the current density in the material to the electric field which causes the flow of current) depends on the polymer, the solvent, and the presence of salt in the solution. As conductivity increases, the diameter of the fibers decreases. If the conductivity is excessively low, no continuous fibers can be formed but these have defects. However excessively conductive solutions are highly unstable under intense electric fields and the region of instability is very marked, leading to fibers with very uneven diameters. The addition of salt is often important to increase the solution conductivity for producing more uniform fibers with fewer beads. It has also been studied that the addition of alcohol to the solvent may increase conductivity. The most important thing is that these products do not remain in the fibers once they are collected and therefore do not have consequences on the electrospun material.

For example, Zong et al. found that PVA fiber diameters were decreased from  $214 \pm 19$  nm to  $159 \pm 21$  nm when NaCl concentration was increased from 0.05 to 0.2% (spinning conditions: solvent = water, voltage = 5 kV, distance to collector = 10 cm, flow rate = 0.2 mL/h). They found that salts with a small ionic radius produce smaller fibers due to the mobility of ions: with small ions, there is greater mobility and increased elongation forces exert on the fiber jet. [20]

#### 2.2.2 Environmental conditions

Generally, the atmospheric temperature is used for doing the electrospinning, but Mit-Uppatham et al. [33], studied the spinning of polyamide-6 fibers at a temperature ranging from 25 to 60  $^{0}C$ . They found that if there is an increase in temperature, there is a decrease in the diameter of the fiber, maybe because the increasing temperature means a decrease in viscosity.[33]

The humidity can change the morphology of fibers. Casper et al. [34], showed that if the humidity is increased, there is an appearance of circular pores on the surface of the fibers; the claim was made after they spun a polystyrene solution.[34]

According to the final morphology of fibers and to their diameters, the parameters could change; for example, if the purpose is to produce fibers with large diameters, the electrospinning process can be performed under vacuum.

#### 2.2.3 Process parameters

Electrospinning process parameters are spinning voltage, working distance, feed rate and arrangments of equipment's components.

The positioning of the various components of the apparatus has been studied; for instance, some researchers decided to place vertically the nozzle (fig. 19), leaving the polymer fluid drops with the help of gravity and the collector under the needle. Whereas, other researches preferred to keep the capillary tube, where there is the solution, to a certain angle for controlling the flow. [27] Others placed the capillary to a horizontal position (fig. 20) and thanks to an infusion pump collecting to the syringe controlled the polymer solution flow.



Figure 19: a vertical disposition of the electrospinning process [27]



Figure 20: a horizontal disposition of the electrospinning proces. [24]

The flow rate can be controlled with a pump, and it was found that a lower flow rate means smaller diameters of fibers. A too high flow rate is not good because fibers cannot completely dry before reaching the collector.

There is a minimum voltage above which fibers can form. If the voltage increases above the minimum threshold, the fibers initially show progressively smaller diameters, because of the

growing repulsive forces inside the jet that favor the thinning, but if the voltage is too high, the drop volume in the needle tip decreases, the Taylor cone cannot be formed and consequently beads are present in the mat. [20]

The distance between the tip and the collector and the design of the latter are variables that can be changed and depend on the final product required. There is a minimum distance required to allow the fibers sufficient time to dry before reaching the collector; if this distance is too far or too close, beads are created. This variable can also modify the fiber morphology. For instance, for a silk-like polymer with fibronectin functionality if the distance is small the result is flat fibers, but if the distance is greater rounder fibers are obtained.[20] [35]

The design of the collector has been studied to help the fiber alignment because the polymer jet trajectory is subject to instability due to the voltage applied and the parameters used.

The collector, besides its simplest plane shape, can also be a rotating cylinder (fig. 21). The fibers are taken up on the surface of the cylinder in a circumferential manner and they line up. If the surface speed of the cylinder is lower than the alignment speed, the fibers are collected randomly, but if the speed is too fast this can break the fiber jet. The ideal speed must be in the middle of the two boundary conditions. [24]



Figure 21: a schematic cylindric collector for electrospun fibers [24]

A collector with an auxiliary electrode can also be used (fig. 22). The fibers are always circumferentially directed on the collector but with the help of an auxiliary electric field. For example, fibers can be collected by using a Teflon tube of 4 mm in diameter, rotating at a speed of 1165 rpm above the charge grid. The polymer in solution was given a positive charge (+12 kV) and the auxiliary electrode, the aluminum grid, had a negative charge (-8 kV). Another collecting

system is a tube collector positioned between two charge plates for collecting electrospun fibers that have been orientated circumferentially to the longitudinal axis of the tubular structure (fig. 23). [24]



Figure 22: Aligning electrospun fibers with an auxiliary electrical field. (a) The aluminum grid is the auxiliary field to help the collection of fibers. (b)The tube collector between the two charge plates.[24]


*Figure 23: nanofiber deposition: (a) without and (b) with an auxiliary electrical field.*[24]

Finally, a thin wheel with a sharp edge can be used as a collector: the spun fibers are wrapped on a round thin disk, with a pointed edge where an electric field is concentrated (fig. 24). This is very similar to a bobbin, the only thing is that it is necessary to use a grounding because the fiber collected on the disk exerts a repulsive force on the fiber collected previously, as the nanofibers retain sufficient residual charges to repel each other. The presence of this grounding can influence the morphology of fiber disposition. [24]



Figure 24: Typical set up of a thin wheel with a sharp edge. [13]

In summary, it is difficult to isolate the effect of the different electrospinning parameters because they are all interrelated, as shown in fig. 25. For example, changing the solution concentration or viscosity affects other solution properties, such as conductivity and surface tension. Parameters have to be changed according to the target final product and according to the selected application.

Parameters		Fiber morphology		
Solution parameters	Concentration and Viscosity	High: increase in fiber diameter <sup>[57]</sup>		
	Molecular weight	Low: bead generation <sup>[58]</sup>		
	Solvent vola- tility property	Fast: uniform diameter of the ultrafine fibers		
	Conductivity	High: straight fibers; decrease in fiber diameter <sup>[47]</sup>		
		Low: bead generation		
	Surface tension	No conclusive link with fiber morphology <sup>[26]</sup>		
Processing parameters	Electric field strength	Strong: decrease in fiber diameter <sup>[47]</sup>		
	Flow rate	Fast: bead gen- eration; increase in fiber diameter <sup>[63]</sup>		
		Slow: decrease in fiber diameter <sup>[65]</sup>		
	Tip to col- lector distance	Bead generation with diameters that are too large or too small <sup>[47]</sup>		
Ambient parameters	Humidity	Increased humidity results in an increased number of pores on the fiber <sup>[58]</sup>		
	Temperature	Increased in temperature results in decreased the fiber diameter <sup>[47]</sup>		

*Figure 25: the relationship among the various electrospinning parameters*[36]

## 2.3 Applications

Electrospinning can be used with a large number of polymers:

- Synthetic polymers: polyamides, polyolefins, polyesters, aramid fibers and, polyacrylate.
- Natural polymers: polysaccharide, polypeptide, and proteins.

Nanofibers prepared by electrospinning show tremendous potential for application in several areas, from filtration to environmental and energy fields, chemical and biological sensing, and wound dressing.

In the following, the main application areas of electrospun mats are described.

### 2.3.1 Reinforcement for composite materials

The fibers produced by electrospinning could be used as reinforcement in composite materials. The composites with these nanofibers are produced for a variety of purposes, and the electrospun fibers are used especially to:

- Increase the mechanical properties (stiffness and tensile strength);
- Increase the laminate resilience (when a layer of nanofibers, which do not alter thickness and weight, is used).

For example, nylon 4,6 has been used as a reinforcement in an epoxy resin. Nylon 4,6 is an aliphatic polyamide formed by the polycondensation of 1,4-diaminobutane (putrescine) and adipic acid, and is characterized by high heat resistance. The electrospinning solution was done with a solution of nylon 4,6 (10 wt.-%) in formic acid (95%). After the process, the fibers obtained were incorporated into an epoxy resin forming films with a thickness of ca. 100 mm. The goal was to obtain a good dispersion between fibers and epoxy resin and a homogeneous fiber distribution leading to good mechanical characteristics. Moreover the composite was transparent: light is transmitted over the complete range of wavelengths in the visible spectrum ( $400\pm700$  nm). [37]

## 2.3.2 Filtration

Filtration is another application for electrospinning. Fibrous materials have the advantage of being efficient, thanks to the high surface area, and having low air resistance. Therefore, they find applications such as air filters: air compressed is passed through the filter that can capture fine oily particles (0.3 microns). Also, polymeric nanofibers can be electrically charged to modify the ability of electrostatic attraction of particle without increasing pressure drop, that is a limitation for filtration, and at the same time improve the efficiency. [36]

Cao et al. [38] have studied the relationship between the fiber diameter and the capacity of air filtration membranes, using electrospun fibers of polyacrylonitrile (PAN). Also polylactic acid (PLA) has been used to manufacture membranes for air filtration. [36]

Not only these polymers can be spun for producing filtration membranes but also PVA, polyethylene terephthalate (PET), polyurethane (PU), polyvinyl chloride (PVC), chitosan, cellulose acetate and others.

Moreover, two different polymers can be used together to increase the efficiency of the filtration membrane. For example, Wang et al. developed two-tier composite filtration membranes, incorporating together with an optimal blend ratio PVC and PU. Pure PVC has low strength and elongation behavior. With the addition of the PU, the membrane showed a linear elastic behavior much higher until reaching the yield point, this characteristic can be explained with the coexistence of bonding and nonbonding cross structure in the polymer blend membranes. [36]

#### 2.3.3 Protective clothing application

Electrospinning can be used to produce water and air vapor-permeable nanofiber membranes, reactive to nerve gases and other chemicals, to be employed for protective clothing applications. These structures have high porosity, but small pores that also resist the penetration of aerosol. A polymer used for producing these clothing is polybenzimidazole (PBI). It was dissolved in N, N-dimethylacetamide (DMAc) with a little lithium chloride (LiC1), only 4 wt% by weight was used to increase solution shelf life from days to several months. PBI polymer solution was prepared under nitrogen gas at a bath temperature above the boiling point of DMAc (185°C) for 4 hours with a refluxing condenser. After the preparation of the solution, it was electrospun The resulting jets were attracted toward a rotating cylinder. The typical potential difference between the spinneret and the grounded aluminum collector, which were separated by 6 centimeters, was between 10 and 25 kV. The diameter of the fibers produced was calculated as 300 nanometers. [39] [40]

#### 2.3.4 Biomedical applications

Electrospun mats can be used for medical prosthesis, in particular as soft tissue prosthesis (vascular, breast, blood vessels) or between the prosthesis and the tissue, in order to reduce the difference in properties between the two materials.

Moreover, scaffolds for tissue engineering mimicking the structure of the ECM (native extracellular matrix) can be produced. The ECM is a three-dimensional network of extracellular macromolecules, such as collagen, enzymes, and glycoproteins, that provides structural and biochemical support to surrounding cells. The principal functions of ECM are cell adhesion, cell-to-cell communication, and differentiation.

Tissue engineering uses scaffolds to provide support for cells and to regenerate new extracellular matrix which has been destroyed by disease, injury, or congenital defects. These scaffolds can be formed with a synthetic or natural polymer. The class of synthetic polymers includes those polymers that are biocompatible, bioabsorbable and being synthetic have reproducible properties, which can be adapted to a specific application. Examples of commonly used synthetic polymers for fibrous scaffolds are poly(L-lactic acid-co- $\varepsilon$ -caprolactone), PLCL, which formed scaffolds for attacking human umbilical vein endothelial cell, and poly lactic-co-glycolic acid, PLGA, which formed fiber systems with a good porosity and was demonstrated that mouse fibroblasts seeded on them and spread according to fiber orientation. [20]

Natural polymers have the characteristic of being abundantly present in nature, bioactive and biocompatible because they are derivatives of the components of the extracellular matrix. Some materials are not part of the extracellular matrix, but are however present in nature with similar composition and properties; they are used for making scaffolds, as they are well tolerated by the body. Examples of natural polymers used for tissue engineering scaffolds are:

- Collagen is widely used in tissue engineering for its biocompatibility, is a component of ECM, the solvent used for the electrospinning process is hexafluoro-2-propanol (HFP). The collagen network produced was demonstrated to be suitable for the proliferation of aortic smooth muscle cells;[20]
- Silk fibers that derive from silkworm and are biocompatible can be used as sutures on soft tissue;[20]
- The plasma serum protein fibrinogen is part of the wound healing. This protein has been spun and solubilized in a medium of 1,1,1,3,3,3-hexafluoro-2-propanol. [41] The scaffold produced with these protein fibers can be used as a biomedical gauze and as a material for neocartilage formation.[20]

Delivery of drugs to patients in the most physiologically acceptable manner has always been an important concern in medicine. The rate of drug release increases with the growing surface area of the drug and carrier material. The smaller dimension of the drug and the coating material required to encapsulate the drug, the better the drug can be absorbed. [24] The drug and the carrier materials can be mixed for electrospinning nanofibers, the drug can bound to the carrier in several ways:

- The drug is attacked on the surface of the carrier which is in the form of nanofibers;
- The drug and the carrier are in nanofiber form;
- The blend of drug and carrier are integrated into the fibers;
- The carrier material is electrospun in a tubular way and the drug is encapsulated.

PLA was electrospun for creating a membrane in which antibiotic drug Meloxin was loaded. [24]

In another example, nanofibers of PLGA and poly(ethylene glycol)-g-chitosan were used for loading the anti-inflammatory drug ibuprofen.[42]

## 2.3.5 Electrical applications

The electrospun fibers can be used as conductive nanofibers: sensors, actuators, porous electrodes for high battery (due to the fact that the rate of the electrochemical reaction is proportional to the surface area of the electrode), corrosion protection membranes.

Carbon nanofibers (CNF) are widely used in the electrochemical field, thanks to their conductivity and structural stability they can be used as energy storage devices. In energy storage devices, such as rechargeable batteries and supercapacitors, CNF can be used as materials for electrodes and substrates for supporting active metals (oxides). To form these nanofibers, electrospinning is used due to the low production cost and the final characteristics obtained of the product. [43] Zussman et al. [44] presented the realization of carbon nanofibers formed by the electrospinning of polyacrylonitrile and subsequently carbonized. The solvent used in the process was N, N-dimethylformamide (DMF) to obtain an 8% by weight solution. The polymer solution was electrospun from a 5 ml syringe with a needle with an internal diameter of 0.1 mm. The extruded solution was spun onto the cutting edge of a grounded collector disk. PAN fibers as spun were harvested on aluminum oxide (alumina) substrates which were attached to the edge of the pick-up wheel. For carbonization, the substrates were placed in a tubular oven and stabilized in the air for 30 min at 250 ° C, then carbonized for 1 hour in nitrogen at 750 ° C and finally heated to 1100 ° C in nitrogen for another hour. [44]

## **Chapter 3 - Use of micro e nano cellulose in the process of electrospinning**

Since the 1990's the interest in new materials and especially in composite materials with advanced mechanical properties has increased. Nowadays there is a particular focus on renewable and biodegradable materials, which can be used as a reinforcement or as a single material for different applications. [45][3]

In this chapter, the attention is focused on the cellulose, particularly on the employment of micro e nano cellulose, which has been described in Chapter 1, in the process of electrospinning.

Nanocellulose, which includes both cellulose nanofibers (CNFs) and cellulose nanocrystals (CNCs), has very good features: it has a high reinforcing effect at a low dosage, it is not toxic, renewable, and generally biocompatible. For these characteristics, it is a good material as a filler in a matrix for the production of nanocomposite materials for biomedical applications and filtration. [46]

It is important to explain how suspensions of micro and nanocellulose can be mixed with the polymer solution and used for producing fibrous composites by electrospinning. [14] [3] As shown in fig. 26, many polymers can be reinforced with nanocellulose fillers. In general, the introduced quantity of nanocellulose does not exceed 22 wt% and the diameters obtained after the realization of the composites by electrospinning is always in the nanometers range. From this figure, it is possible to deduce the growing interest in using nanocellulose as a filler and in this chapter, the impact of the nanocellulose after incorporation into the matrix through electrospinning has been analyzed.

The polymers that have been used as a matrix with a nanocellulose filler include natural polymers, such as alginate, cellulose acetate, and synthetic polymers, such as PEO, polystyrene (PS), poly( $\varepsilon$ -caprolactone), PLA, PVA, polyacrylic acid (PAA), ethylene-vinyl alcohol, PMMA. [47]

Nanocellulose	Matrices	Loading levels	Fiber diameters
CNC	Poly(ethylene oxide)	0,2, 0,4 wt%	300 ± 40 nm
		10, 20 wt%	154 ± 70 nm
		5-20 wt%	183 ± 22 nm
		1-10 wt%	300-400 nm
	Poly(e-caprolactone)	2.5-7.5 wt%	160 ± 40 nm
		1-5 wt%	166,4 ± 7,5 nm
	Polystyrene	6, 9 wt%	600-5400 nm
		1-7 wt%	2300 nm
	Poly(acrylic acid)	5-20 wt%	141 ± 60 nm
	Polyacrylamide	10, 20 wt%	221 ± 72 nm
	Poly(vinyl alcohol)	5-15 wt%	235 ± 110 nm
		5-30 wt%	$(47 \pm 7) \times 10^3 \text{ nm}$
	Ethylene-vinyl alcohol copolymer	1-8 wt%	285 ± 100 nm
	Cellulose	5-20 wt%	216 ± 5 nm
	Regenerated cellulose	0.42,	300-800 nm
		3.28 wt%	
	Cellulose acetate	5 wt%	-
	Alginate	2-50 wt%	-
	Chitosan/Poly(ethylene oxide)	50 wt%	301-1240 nm
	Lignin/poly(vinyl alcohol)	5-15 wt%	73 ± 5 nm,
			114 ± 4 nm
	Hordein/zein protein	1-5 wt%	860 ± 250 nm
	Poly(methyl methacrylate)	5-41 wt%	182-459 nm
	Poly(lactic acid)	1-10 wt%	405-642 nm
		1, 10 wt%	335 ± 144 nm,
			306 ± 90 nm
		1-7 wt%	300-600 nm
		1-10 wt%	600-900 nm
		1 wt%	50-500 µm
	Maleic anhydride grafted poly(lactic acid)	1-5 wt%	292 ± 66 nm
	Polyhydroxybutyrate	15 wt%	100-500 nm
		5-22 wt%	1–2,5 µm
CNF	Poly(ethylene oxide)	3,3-21 wt%	150-500 nm
	Poly(methyl methacrylate)	1-20 wt%	100-1800 nm
	Poly(vinyl alcohol) /waterborne	5 wt%	400-700 nm
	polyurethane		
	Poly(e-caprolactone)	0.2-0.5 wt%	470 nm
	Polystyrene	5-35 wt%	70-130 nm

*Figure 26: Variois polymers enhanced with nanocellulose: percentage by weight of inserted cellulose and diameter of fibers obtained* [14]

Overall, regardless of the polymer used as a matrix, the nanocellulose introduced improves the mechanical features. The solvent used for producing the composite by electrospinning is fundamental and the process can be divided into electrospinning in aqueous media and electrospinning in non-aqueous media of organic solvents. [14] The solution in an aqueous medium implies polymer matrix solubility in water and a good dispersibility of nanocellulose in the mixture; examples of water-soluble polymers are PEO, PVA, or alginate. Instead, an organic solvent can be used. It should be compatible with the polymer and has to produce a stable mixture with nanocellulose, considering that the latter is a hydrophilic material. Examples of such systems include PS in tetrahydrofuran,  $poly(\epsilon$ -caprolactone) in DMF, PAA in ethanol, poly(methyl)

methacrylate) in DMF, poly-hydroxybutyrate in chloroform/DMF, and PLA in chloroform/DMF. [14]

### 3.1 Process in aqueous medium

Nanocellulose fillers (CNCs or CNFs) are simpler to introduce in a hydrophilic matrix because it is more compatible thanks to the structure of cellulose. Moreover, in this case, water can be used as a solvent for electrospinning.

PVA is a water-soluble, semicrystalline, biodegradable, and biocompatible polymer; for these features, it is used for a lot of applications: tissue scaffolding, filtration materials, membranes, protective clothing, drug release. [48] [14] [47] An important feature of neat PVA is the fact of having low mechanical strength and integrity. Many studies have thus shown that the insertion of CNCs has increased these mechanical characteristics. [46] [49]

In the work of Peresin et al. [48], two PVA solutions were prepared to explain the effect of CNC addition. Two PVA, with the same molecular weight buT different residual acetyl content (the acetyl groups originated from the precursor vinyl acetate which has been hydrolyzed to produce PVA), were used. Based on the degree of hydrolysis, 98 % and 88 % respectively, PVA-98 and PVA-88 solutions were subjected to electrospinning. [48] [50] The solutions were prepared and the CNCs were added to obtain a final content of nanocrystals of 0, 5, 10 and 15 % (w/w), keeping the total concentration of PVA at 7%. The suspensions obtained have been kept under mechanical agitation at 80 °C for 120 min, and then they were cooled until room temperature. [48] [46] An horizontal equipment of electrospinning was used, the collector had 30 cm of diameter, covered by aluminum foil, the distance between the needle and the collector was 15 cm. [49] After the electrospinning, it has been observed that the more the concentration of CNCs in solution and the more the diameter of the fibers decreased. The average diameters of the fibers obtained were around 235 nm for PVA-98 and 275 nm for PVA-88. For the solution with the PVA-98 was observed that the diameter could reach also 188 nm with the addition of 15% (w/w) of CNCs. [49] This decrease in diameter by the addition of CNCs was explained as the nanocrystals increased the electrostatic charge density in the solution, increasing the elongation of the jet and decreasing the dimensions of the fibers.

The same result was obtained by Sanders et al. who proved the theory described above: they observed smaller diameters (233nm) with a solution composed of 80% of PVA and 20% of CNCs with respect to the unfilled PVA system. [46] [51]



*Figure 27: Scanning electron micrograph of electrospun fibers: (a) PVA-98, (b) PVA-88, with the addition of cellulose nanocrystal with the concentration of 0, 5, 10, 15 wt%* [49]

As fig. 27 demonstrates, with the increase in the amount of filler in the PVA solution there is a decrease in the diameter of the fibers obtained through electrospinning, but moreover, it must be considered that the addition of CNCs causes an increase in viscosity due to the fiber network interaction. This result can reduce the pumping capacity in the syringe that makes up the electrospinning apparatus, hindering the production of the fibers. One way to overcome this problem is to use high pressure to reduce the viscosity of the solution, thus making it ready for electrospinning. A second solution is to increase the diameter of the spinning needle. [46]

At last, according to fig. 28, it has been shown how the addition of CNCs can improve the Young's Modulus. The anisotropic alignment of CNCs in the PVA can give good mechanical properties to the composite, but a high concentration, more than 30 wt%, of microcrystalline cellulose can increase the fragility of the material by decreasing the Young's Modulus.[14]



Figure 28: Stress-strain curve of PVA-CNC systems [14]

**PEO**, like PVA, is a biodegradable, biocompatible, hydrophilic, and flexible polymer that forms hydrogen bonding with cellulose. PEO and CNF composite have a lot of applications: polyelectrolytes in batteries, biocompatible scaffolds, drug delivery systems, and packaging. [14] [19]

In the work of Fortunato et al. [52], the solution used in the electrospinning was prepared with pure water, PEO and CNFs. The PEO was dissolved in water and shook for 24h, then was introduced to the CNF aqueous suspension and for another 24 h has been mixed. Solutions with different concentration of nanocellulose (0, 0.14, 0.29, 0.56, and 1.12 wt%) and a constant polymer concentration were studied. The electrospinning used was composed of a syringe with a stainless steel needle with an internal diameter of 0.8 mm. The positive electric field, 10 kV, was applied to the needle tip and the negative one to the counter-electrode. The jet was target toward the negative electrode obtaining continuous and homogeneous fibers. [52] Two types of collectors were chosen, the first was an aluminum plate and the second one was a rotating drum with a radius of 10 cm and a width of 1 cm. he tip-to-counter-electrode distance was set to 20 cm. [52] After the addition of NFCs, an increase in conductivity was observed by a factor of two compared to pure PEO and also an increase in viscosity; this rise is due to the NFCs network forming structure within the dispersions. It has to be taken into account that conductivity and viscosity affect the morphology and size of the fiber diameter. The diameter of the fibers collected on the static collector varied between 400-500 nm (fig. 29), while the diameter of the fibers collected on the rotating drum resulted to be smaller, about 150 nm.[52]



*Figure 29: a SEM image showing fibers of pure PEO and PEO/CNFs. (a) 0 wt% of CNF, (b) 11.7 wt% of CNFs, a single fiber, (c) 11.7 wt% of CNFs, a fiber patch.*[52]

CNFs concentration not only affected the fibers diameter but also increased their mechanical properties, as can be seen in the stress/strain graph of fig. 30. CNFs improved Young's Modulus and tensile strength, while the only property deceased was the strain at break. Linear growth is shown between Young's Modulus and the concentration of CNFs: the value of the Young's Modulus increased of 170 MPa compared to pure PEO. [48][52]



Figure 30: stress-strain curve of PEO/CNFs with a various concentration of filler[47]

In another study, Park et al. [53] showed the incorporation of bacterial cellulose whiskers into PEO, again through the electrospinning process, demonstrating an increase in mechanical properties. Cellulose whiskers were obtained by acid hydrolysis of bacterial cellulose microfibrils. [4] The bacterium used for the extraction was Acetobacter xylinum. The bacterial fibrils of the cellulose were first obtained by acid hydrolysis in sulfuric acid at 65% by weight at 40 ° C for 16 hours under continuous stirring. After hydrolysis, the reaction was stopped by immersing the flask in an ice-water bath. Subsequently, the cellulose whiskers, those used as fillers, were obtained by thorough washing by centrifugation and dialysis. The final solution, ready for electrospinning, was composed of PEO and bacterial cellulose at 5% by weight. [53] The addition of cellulose crystals to PEO increased the viscosity and surface tension of the system making possible the electrospinning, the distance between the tip and the collector was 20 cm and the potential difference was gradually increased from 12 to 24 kV/cm. [53] The study was done by Park et al.; the authors showed that the fibers without whiskers were very smooth, while those with cellulose crystals were rougher and some crystals protruded: this phenomenon is caused by their aggregation during the process of electrospinning. But despite this, an increase in mechanical properties was shown: after the addition of the microcrystals (0.4 wt%) into the electrospun PEO fibers, their tensile modulus, tensile strength, and elongation were increased by 193.9%, 72.3%, and 233.3%, respectively. [53] In conclusion, the composite of CNFs and PEO can be a great new material, characterized by biodegradability and not toxicity. [54][55]

Also **PAA** was used as a matrix for the production of composite fibers by electrospinning. The nanofibers of PAA/CNCs showed diameters ranging from 80 to 500 nm. For the dispersion of CNCs (obtained by cotton cellulose) in the matrix of PAA, ester linkages between hydroxyls on the CNCs surface and carboxylic acids along the polymer chain were formed. This ester linkage is the cause of the water-insolubility of the composite and consequently of a mechanical and thermal enhancement. [48] To prepare PAA/CNCs solutions, CNC powder was first dispersed in ethanol by sonication for 5 min, followed by addition of PAA powder into the CNC suspension. Then electrospinning was used to produce fibers, its set-up consisted of a horizontally positioned plastic syringe with a flat-end metal needle operated by a syringe pump and a high voltage of 30 kV applied to the solution. [56] It has been shown that better the CNCs were dispersed in the suspension with the polymer and smoother and more uniform in size were the fibers. Fig. 31 shows that if the CNCs are isolated, the rods appear to align with the fiber axis, while if they are not dissolved very well there are some spherical agglomerates of CNCs.



Figure 31: (a) a good alignment of CNCs in PAA, (b) a sphere of CNCs. [56]

Young's modulus and tensile strength were significantly improved with increasing CNCs loads in the nanocomposite fibers, the same result that was demonstrated with the other nanocomposites. Most importantly, the large increase in Young's modulus and maximum stress doubled and quadrupled respectively, due to heat-induced esterification between the CNCs surface hydroxyls and PAA carboxyls, making the nanocomposite fibers insoluble in water. [56] [48]. Moreover, linear PAA chains can easily slide by one another during the tensile deformation, showing low Young's modulus and tensile strength but high elongation. Instead, PAA/CNCs nanocomposites have a higher Young's Modulus and tensile strength thanks to the strong hydrogen bond created 'inter' chain. In table 3, the increment of these mechanical properties with increasing CNCs, considering a constant concentration of 4 wt% of PAA, is shown. [56]

PAA/CNC	Young's modulus (GPa)	Max. stress (MPa)	Elongation to break (%)
4/0	0.056 (1.8%)	0.29 (3.4%)	$136 \pm 7$
4/5	0.188 (2.1%)	0.68 (4.4%)	$126 \pm 6$
4/10	0.224 (0.4%)	0.84 (4.8%)	$67 \pm 4$
4/15	1.192 (1.4%)	3.26 (4.9%)	$60 \pm 3$
4/20	1.983 (2.4%)	4.51 (5.1%)	$36 \pm 2$
4/20 <sup>a</sup>	4.331 (0.9%)	16.70 (5.0%)	$17 \pm 1$

Table 3: Mechanical proprieties of PAA/CNCs, crosslinked by heating at 140 °C for 60 min [56]

#### 3.2 Process in non-aqueous medium

Before electrospinning, the polymer can be dissolved in organic solvents creating a matrice to prepare composite nanofibers with micro and nanocellulose. [47]

**PLA** is a biodegradable and biocompatible polymer, is formed by lactic acid, that is produced by the fermentation of renewable agricultural sources rich in carbohydrates, such as corn. [57] The major applications of this polymer are focused on the biomedical field as tissue engineering or also packaging: PLA has noted as bioplastic a good substitute to plastic generated from fossil-fuels [58] As pure PLA fibers normally have weak mechanical properties and low thermal stability, which limits their application in many fields, CNCs can be introduced to improve the mechanical properties. [59]

For example, Shi et al. [59] showed how through electrospinning it is possible to create biocomposites with CNCs as a reinforcement. For the process, at first, has been done a suspension of PLA and CNCs in a solvent of chloroform/DMF (4/1 v/v). To help mix the solution, before carrying out electrospinning, the mixture was stirred and heated for 30 minutes at 65 ° C. The final concentration of PLA in the mixture was 15% by weight. The compositions of the CNCs were 0, 1, 2, 5 and 10% by weight based on the weight of the PLA. The suspension was then loaded into a 1 ml plastic syringe with a stainless steel needle (internal diameter = 0.584 mm), applying a voltage of 15 kV. The flow rate of the suspension was calculated to be 1.5 ml h-1 by a pump and a collector made of grounded aluminum foil under the capillary tube was used as the collector. [59]

Like many of the composites previously analyzed, also in this case the result of the study showed that with the addition of filler the diameter of the electrospun fibers decreased. As shown in fig 32, the mean diameters of pure PLA fibers as spun and PLA nanocomposite fibers with 1% by weight of the CNCs load level were about 1.5 and 1 nanometers, respectively. Fiber diameters further decreased to 642 and 405 nm at 5 and 10% by weight of CNCs load levels, respectively. Fiber diameter distribution was more uniform in PLA / 5 wt% CNCs composite fibers than PLA / 10 wt% CNCs composite fibers. This was probably due to the fact that the higher concentration, in PLA / 10% CNCs by weight composite fibers, obstructed the needle head and thus caused discontinuous fibers obtained during the process.



*Figure 32: (a) pure PLA, (b) PLA/1 wt% CNC, (c) PLA/2 wt% CNC, (d) PLA/5 wt% CNC, (e) PLA/10 wt% CNC; and (g) the relationship between CNC content and the diameter distribution for PLA/CNC fibers.*[59]

Moreover, according to table 4, some thermal characteristics are changed with the addition of CNCs. The glass transition temperature decreased with increasing CNC contents, up to the addition of 5 wt%. The increase that is noted at 10 wt% is caused by the aggregations of the microcrystals causing not a good dispersion within the matrix and therefore molecular movements within the PLA chain. As far as the cold crystallization temperature is concerned, it is noted that the lowest data is obtained with the addition of 5 wt% of filler, and then there is an increase. This is always related to the explanation described above.[59]

Table 4 also shows that the crystallinity Xp of PLA increases with increasing CNC content by up to 5% by weight, indicating that a low amount of CNCs could act as nucleating agents to enhance the crystallization of the molecular chains of PLA. [59]

SAMPLE	<i>T<sub>g</sub></i> °C	<i>T<sub>c c</sub></i> ℃	X <sub>p</sub> %
PLA	60,4	123,3	0
PLA/1 wt% CNC	60,4	123,9	1,0
PLA/2 wt% CNC	60,2	122,9	2,6
PLA/5 wt% CNC	62,5	110,9	7,1
PLA/10 wt% CNC	60,5	113,8	3,3

Table 4: Thermal proprieties of PLA/CNCs fiber composites [59]

Regarding the mechanical properties, as noted with the previous composites, the addition of the filler is shown to increase the mechanical properties. In this case, looking at fig. 33, it can be seen that compared to pure PLA there is a large increase: the curve with the best characteristics is the one with the addition of 5 wt%. There is a relative increase in the elastic modulus as the filler concentration is increased but also a decrease in elongation at break due to the increase in crystallinity. [59]



Figure 33: Stress-Strain curve of PLA/CNCs [59]

In another work, Martínez-Sanz et al. [60] used the electrospinning process to improve the dispersibility of the CNC in the PLA film and not to obtain a composite. Bacterial CNCs were used (Gluconacetobacter xylinus, which is a polysaccharide-rich culture medium that can produce an almost pure bacterial cellulose layer). The electrospun fibers were generated from PLA and CNCs with hexafluoropropylene (HFP) as solvent. The solutions contained a total solid content of 6% by weight and the CNCs concentration was 15% by weight, relative to the weight of the PLA. The cellulose nanowhiskers, in the form of a partially hydrated precipitate, were dispersed in the solvent by applying intense homogenization for 2 min and sonication for 30 s and were then stirred with PLA at 40 ° C. The electrospinning apparatus was composed of a stainless steel needle with a diameter of 0.9 mm. The applied voltage was about 0-30 kV and the polymer solution was fed at a rate of 0.66 mL/h. The binder was a rectangular plate covered with aluminum foil. Finally, the distance between the needle and the plate was 10 cm and room temperature was used to perform the process. [60] Fig. 34 shows the dispersion of the CNCs after the electrospinning process and the subsequent formation of continuous film.



Figure 34: SEM micrographs show a good dispersion of CNC in PLA film [60]

**PCL** is biodegradable and biocompatible, for his characteristics can be used to create scaffolds with mechanical and biological properties that are similar to extracellular matrices (ECM). A problem to overcome is its hydrophobicity that could hinder the adhesion of cells, mobilization, and limit the mechanical strength, which is a feature fundamental for structural integrity, all characteristics required for the materials used in tissue engineering. For this reason, hydrophilic fillers such as microcrystals of cellulose have been used to improve cellular adhesion. [48] Before using the process of electrospinning for preparing nanofibers, to overcome the incompatibility between the polymer matrix and the filler, the grafting technique was used. CNC was grafted with PCL chains (fig. 35) by ring-opening polymerization (ROP). This technique consists in placing CNC in anhydrous toluene and improving dispersion by maintaining the suspension in a sonication bath for 5 min. Then a distillation for deleting residual water was performed, and PCL was added. The polymerization proceeded for 24 h under magnetic stirring at 120 °C, and a few drops of aqueous HCl (1M) were added to stop the reaction. The resulting PCL-

grafted CNC was purified by extraction with toluene for 24 h and then with dichloromethane for 24 h. [61] [62]



Figure 35: procedure of ROP for producing PCL-grafted CNC [62]

The electrospinning solutions were prepared with PCL in a solvent mixture of CHCl<sub>3</sub>:DMF (4:1) using a magnetic stirrer for 24 h at room temperature. The apparatus of electrospinning had a vertical standard configuration with two concentric needles and was connected to a high voltage power (15 kV). The polymer solution flew through the inner needle, and the solvent flew through the outer one. The collector was a grounded static plate covered with aluminum foil. The distance between the tip to the collector was 15 cm. [62] [63]

As before, it was demonstrated (fig. 36) that more CNCs are added, smaller are the diameters of the fibers obtained. The diameter of the nanofibers produced decreased from 640 nm for the neat PCL to 180 nm for the 5 wt% PCL-g-CNC. This reduction can be explained with the addition of the filler leading to an increase in the conductivity of the electrospinning solution. [64]



(a)

(b)



*Figure 36: SEM images of electrospun mats of (a) neat PCL, and PCL with (b) 1 wt % (c) 3 wt %, and (d) 5 wt % PCL-grafted CNC.*[61]

Sample	D (nm)	<i>T</i> <sub>m</sub> (°C)	$\Delta H_m$ (Jg <sup>-1</sup> )	$T_c$ (°C)	X <sub>c</sub> (%)
PCL	636.9±62.1	$60.0 \pm 2.1$	$80.9 \pm 0.1$	$41.3 \pm 1.4$	$58.0 \pm 0.1$
PCL + 1wt % PCL-g-CNC	$182.1 \pm 11.9$	$62.8 \pm 1.8$	$81.7 \pm 0.1$	$41.4 \pm 1.8$	$59.2 \pm 0.1$
PCL + 3wt % PCL-g- CNC	$164.7 \pm 8.9$	66.2±2.3	82.5±0.2	$44.3 \pm 1.4$	$61.0 \pm 0.2$
PCL + 5wt % PCL-g- CNC	$166.4 \pm 7.5$	$67.8 \pm 1.5$	84.4±0.2	$46.7 \pm 2.0$	63.2±0.2

*Table 5: Average fibers diameter (D), thermal properties and degree of crystallinity (Xc) of neat PCL and PCL-g-CNC* [61]

As shown in table 5, by increasing the CNCs content, there is not only a decrease in diameter but also an increase in melting temperature, degree of crystallinity, and temperature of crystallization. [64] Gravimetric analysis (TGA) showed that there is a decrease in the thermal stability of PCL with the addition of 1 wt %, 3 wt %, and 5 wt % of PCL-grafted CNC (fig. 37). The degradation temperature of neat PCL is higher than that of filled PCL because PCL-grafted-CNC has a shorter chain that the PCL matrix. [61]



*Figure 37: TGA graph of neat PCL and PCL with 1 wt %, 3 wt %, and 5 wt % PCL-grafted CNCs* [61]

Regarding the mechanical properties, the behavior appeared to be similar to other composites: there is an increase of Young's modulus and tension of break with the addition of filler. Moreover, there is a decrease in the elongation at the break because CNCs increase the brittleness of nano-composite. The behavior showed in fig. 38 indicates a good dispersion and interface between the PCL-grafted CNCs and the PCL matrix.[61]



Figure 38: Elastic modulus (E), tensile at break ( $\sigma_b$ ), and elongation at break ( $\mathcal{E}_b$ ) of the electrospun PCL/PCL-grafted-CNCs fibers [61]

In conclusion, thanks to its biodegradability and easy availability, nanocellulose can be a great filler to be used in polymeric fibers obtained by electrospinning. The fibrous nanocomposites presented can be used for different applications described before, such as tissue engineering scaffolds or filtration membranes. In general, the addition of CNCs or CNFs improves the mechanical proprieties (i.e. elastic modulus, load to failure) of the polymers. However, a good dispersion of the filler is the base for producing a nanocomposite with acceptable properties. Therefore, the main challenge that needs to be overcome is the agglomeration of CNCs or CNFs in an a-polar matrix, which could be done with chemical modifications.

# Chapter 4 – Research Proposal: Preparation of bio-nano composite fibers made of chitosan and cellulose nanocrystals by electrospinning

In this chapter, I would like to draw my conclusions, proposing a research work that aims to obtain a bio-nano composite in the form of fibrous membrane, formed by chitosan as matrix and cellulose nanocrystals as filler, obtained via electrospinning. The application of interest is food packaging.

## 4.1 Motivation and state of the art

In recent years, food packaging has become a field of great interest, a lot of researches have focused on finding new materials to replace those deriving from fossil fuels. The purpose is to promote the 'green economy', find suitable alternatives, competitive with the most used plastics (polyethylene and polypropylene), and reduce waste that is difficult to dispose of.

It has been shown that packaging is a complex operation, many companies are involved in marketing food or drink in a suitable container for its conservation and distribution. The packaging is central in the production phase, this importance is given by the fact that the product must arrive intact to the consumer and also guarantee certain shelf life.[6]

There are three different categories of packaging: [65]

- Primary packaging, which is in contact with the food and is taken home by consumers;
- Secondary packaging, which covers the larger packaging, such as boxes, used to carry quantities of primary packaged goods;
- Tertiary packaging, which relates to transport.

In this work, the attention will be on primary packaging and the aim of the research is the development of a biopolymer suitable for food contact that complies with EU regulations. In particular, two EU directives have to be taken into account: "Plastic Materials and Articles Intended to Come into Contact with Food-stuffs" (90/128/EEC) with later amendments, and "Packaging and Packaging Waste Directive" (94/62/EEC). [66] These two EU legislations can be resumed as follows. [66] The 90/128/EEC lists the allowed monomers, additives, etc. to be used in the production of packaging materials for foods, sets standards for the production and use of packaging

materials and the labeling of these materials, and introduces specific migration requirements for numerous compounds. The 94/62/EEC includes rules for the production of packaging materials, directives for waste management, use of recycling, composting, and energy recovery by incineration.

A biopolymer can be defined as a polymer that has been extracted directly or indirectly from biomass. The prefix "bio" means that these materials are easy to dispose of (compostable) and have a low environmental impact.

To promote the green economy, the aim is to use a biodegradable polymer, that is, one that can degrade and become  $CO_2$ , water and mineral salts. This is not to be confused with the term compostable, which arrives at the same result but in this case the degradation of the material is not controlled by any parameter and occurs naturally, while the term biodegradable means that the degradation is controlled by parameters such as environmental factors and the composition of the subsoil. [67] [65] [68]

Biopolymers, which fall into the category of biodegradable materials, can be divided into different categories depending on the raw materials and the manufacturing process used for producing them: natural polymers, such as plant carbohydrates like starch, cellulose, chitosan, alginate, agar, and animal or plant origin proteins like soy protein, wheat gluten, gelatin, collagen; synthetic biodegradable polymers, such as PLA, poly(glycolic acid) (PGA), poly( $\epsilon$ -caprolactone) (PCL); biopolymers produced by microbial fermentation, such as microbial polyesters, like poly(hydroxy alkanoates) (PHAs) and poly( $\beta$ -hydroxybutyrate) (PHB). [69]

In this work, the biopolymer chitosan will be used as matrix for the fibrous composite membranes. Chitosan (fig. 39) is formed by the N-deacetylation of chitin and is a natural linear polysaccharide consisting of 1,4-linked 2-amino-deoxy- $\beta$ -d-glucan. Chitosan is the second most abundant natural polysaccharide after cellulose, is non-toxic, biodegradable, bio-functional, biocompatible and also it has been shown that it has strong antimicrobial and antifungal activities, a good feature for packaging. [70] [71]



Figure 39: The monomer of chitosan[70]

Chitosan solutions will be processed by electrospinning technique to obtain non-woven membranes formed by polymer nanofibers. As described in Chapter 2, this method allows to fabricate thin fibers with a micro-or nano-scale diameter through the application of a high voltage to a polymer solution. Chitosan has been used in electrospinning, but due to its high crystallinity and its molecular structure, it is difficult to solubilize in aqueous and organic media. A study by Salas et al. [42] reports as the optimal conditions for the electrospinning of chitosan the use as solvent of highly concentrated acid solutions, such as acetic, hydrochloric, formic and lactic acid. It was found [72] that only solutions with 80%, 85% and 90% of acetic acid produced uniform fibers, with optimal conditions of 3% by weight of chitosan on a solution of acetic acid at 90% (fig. 40). [72] Moreover, electrospun chitosan membranes have good proprieties for food packaging application, such as their anti-bacterial proprieties, a good skill for improving the shelf-life of foods. [73]



*Figure 40: SEM picture shows membrane produced via electrospinning with 3 m% of chitosan with 90 m% of acid acetic.* [72]

Nanocellulose will be used as filler, always respecting the idea of using a biodegradable and 'green' material. Also, cellulose is characterized by low cost due to the large availability of the raw material, as it is considered the most abundant resource.[55] Cellulose and nanocellulose are described in Chapter 1.

In this project, cellulose crystals will be extracted from sugar cane bagasse. Sugar cane was selected because it is largely available and can be converted into different products. Indeed it can be used to obtain sugar, but it can also undergo other transformations such as, for example, the obtaining of molasses-based on liquid sucrose or the production of energy given by the combustion of the

stems of the cane left after crushing. Lately, interest in this new raw material has increased, mainly thanks to its use for the production of bio-ethanol, a new resource to be used as a biofuel, and other energy applications to try to limit the use of fossil fuel resources as much as possible. All plant waste is also used as manure and fertilizer. Even sugar cane leaves, burned away from the cane stem before harvesting, are transformed with the addition of bagasse into 'second generation' ethanol.[74] For these reasons, sugar cane bagasse, being a biomass waste, can be the ideal raw material from which to extract cellulose and subsequently the cellulose crystals used as reinforcement.

The objective of using CNCs as a filler is to obtain an increase in the mechanical properties of the material thanks to their very high resistance to bending and rigidity. In Chapter 3 it has been shown that the addition of cellulose nanocrystals as a filler for creating a composite via electrospinning was able to improve mechanical characteristics, like Young's modulus and tensile strength. However, if the amount of the filler in the matrix is too high, the composite obtained could have features similar to brittle materials (reduced elongation at break), and the elastic modulus may drop. For this reason it is important to choose the correct filler's concentration. Normally it is not higher than 5 wt%.

In conclusion, the bio-nanocomposite fibrous membranes should present antimicrobial and antifungal properties thanks to chitosan, and should have enhanced mechanical properties thanks to the CNCs. They are thus proposed as food packaging materials.

## 4.2 Research objectives

In order to achieve the overall project goal described before, there are four main research objectives to be accomplished:

- Objective 1: Obtaining the cellulose nanocrystals to be used as filler;
- Objective 2: Preparation of the suspension to be used in electrospinning process;
- Objective 3: Optimization of the electrospinning process;
- Objective 4: Characterization of the bio-nanocomposite electrospun membranes.

## **Objective 1**

Cellulose fibers and subsequently CNCs will be obtained by sugar cane bagasse. The procedure described by Slavutsky et al., [75] which is based on the alkaline hydrolysis of the raw material, could be followed. Sugarcane bagasse is hydrolyzed with NaOH. Next, the fibers are filtered to remove the excess of NaOH and washed with distilled water. The bleaching process consists in the introduction of the material into a flask containing NaClO<sub>2</sub> and its shaking during 24 h at room

temperature. After that, the fibers are filtered and washed with distilled water until neutral pH is reached. CNCs are extracted from cellulose fibers, dispersing them in a solution of  $H_2SO_4$  into a flask under mechanical stirring. The excess of  $H_2SO_4$  is removed from the resulting suspension by centrifugation. Finally, the suspension is dialyzed against distilled water using a cellulose membrane until the pH reaches 6–7. [75]

The obtained CNCs are characterized by microscopy and light scattering in order to identify their dimensions.

## **Objective 2**

The suspension to be used for the electrospinning can be prepared by adding CNCs in a solution of chitosan and diluted acetic acid. Based on the literature, a 2% (wt/wt) acetic acid solution can be initially tested. [76] [77] The percentage of CNCs used as a filler could vary during the various laboratory tests: the amount should be optimized so that the membrane obtained have good mechanical properties, there is a good dispersion inside the matrix, and that the suspension used for electrospinning has the ideal viscosity for the process to take place. Remembering that the viscosity is proportional to the concentration of the solution and of the filler, there is a minimum and a maximum concentration for assuring the process. If the concentration is too high, the electrospinning process is not successful, due to excessive viscosity that could cause defects in the obtained fibers (beads or jet discontinuity). Based on the literature, a concentration of CNCs from 2 to 10 wt% (with respect to the polymer) can be used. The viscosity of the suspensions with different amount of CNCs will be assessed before electrospinning.

## **Objective 3**

Different electrospinning parameters can be varied to optimize the process. The voltage used for the process can range from 15 to 40 kV; the chosen collector is an aluminum foil and the distance used between the tip and the collector, for the evaporation of the solvent, is expected to be around 15 cm. [77] These are initial parameters that can be set based on previous studies, but they could be modified to optimize the electrospinning process and for changing the features of the membrane.

After electrospinning, the morphology of the obtained fibrous membranes will be analyzed by electron microscopy, and the results will be correlated to the process parameters used and to the composition of the suspension. In fact, it is known from the literature that the addition of CNCs generally decreases the diameter of the electrospun fibers.

## **Objective 4**

The bio-nanocomposite electrospun membranes will be fully characterized. The thermal proprieties will be studied by differential scanning calorimetry and thermogravimetric analysis. The mechanical properties will be assessed to select the best concentration of CNCs to be introduced into the chitosan matrix.

Moreover, in view of the application of the materials for food packaging, other tests, concerning the regulations mentioned in the introduction to this chapter (90/128/EEC, 94/62/EEC), should be performed. First of all, evaluate if the membrane obtained with a certain amount of filler is suitable as food packaging and if it respects the biodegradability conditions. Moreover, the antimicrobial features of the membranes could be assessed. Chitosan could have excellent characteristics for packaging because it has antibacterial characteristics, thanks to its structure, an excellent skill to increase the shelf life of foods. One of the reasons for the antimicrobial character of chitosan is its amino group that interacts with microbial cell membranes, which leads to the leakage of proteins and other intracellular constituents of microorganisms. And therefore, interacting with microbial membranes, chitosan can stop the passage, consequently if used in food packaging it can inhibit the iteration between the constituents secreted by the bacteria and the food. [78]

If all the considerations made are correct and proven by the results obtained, the final purpose is to propose the developed materials as a sustainable solution for food packaging, considering the worldwide interest in the reduction of plastic and consequent environmental pollution.

## 4.2.1 Research risks

The risks that could compromise or slow down the research and the results are:

- Poor dispersion of the cellulose crystals inside the matrix, because no surface treatment has been planned to increase the compatibility. Poor dispersion can lead to problems in the electrospinning process and strongly decrease the mechanical performances of the composite membranes. In the literature, it has been presented a grafting strategy to increase the compatibility between CNCs and chitosan. The CNCs can be functionalized using the methyl adipoyl chloride (MAC) reagent and then the functionalized CNCs can be reacted with the amino groups of the chitosan. [69]
- The membrane obtained do not have adequate characteristics to be used as food packaging. In this case, another biopolymer that can be used as a matrix, able to produce nanofibers through electrospinning, can be used.

## Conclusions

This thesis work focused on the study and description of cellulose and nanocellulose in particular. The latter can be obtained through mechanical and chemical treatments to break the cellulose strucrure and to extract the nanometric crystalline part.

Subsequently, the electrospinning process was analyzed; it is a process that allows to form polymer membranes made of fibers of nanometric diameter from polmer solutions. The electrospinning has the advantage of being a simple process, but the parameters that control the process are many and all interrelated (viscosity, concentration of the solution, applied voltage, size of the syringe used in the device, and also the shape of the collector that receives the final product).

The research was deepened, focusing on the nanocellulose used as a filler in a polymer matrix in the form of fibrous membranes obrated by electrospinning. In general, the literature results show that the addition of CNCs or CNFs as fillers leads to a decrease in the diameter of the and also to an increase in the mechanical and thermal properties of the obtained product. Research is very active in this field, especially for the numerous applications such nanocomposite membranes can have: filtration, tissue engineering (scaffolds), protective clothing, etc.

Taking into account the literature results and thanks to the multiplicity of research material available, this thesis work was concluded by formulating a research proposal. The idea is to create a nanocomposite fibrous membrane with biodegradable characteristics and potential application in food packaging. The material chosen is a composite of chitosan and CNCs used as a filler, thanks to their reinforcing properties. The bio-nanocomposite is produced by electrospinning, a process that allows the fine control of the obtained fiber morphology. This product could be proposed to the food industry to increase attention towards the 'green economy'.

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