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Article

Microwave-Assisted Extraction of Cellulose from Aloe Vera Plant Residue and Preparation of CNC/PVA Hydrogels

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Abstract: Cellulose nanocrystals are of major research interest because of their use in biodegradable bioplastics and food packaging, biomaterials, medical and pharmaceutical applications, cosmetics, electronics, and construction materials. This study introduces an approach combining microwave-assisted extraction (MAE) of cellulose from Aloe Vera plant residue and subsequent isolation of cellulose nanocrystals (CNCs) utilizing sulfuric acid hydrolysis. The MAE process, characterized by its rapid heating and penetrating ability, was optimized to maximize cellulose yield from raw biomass sources under various conditions (microwave power, solvent ratio, and time). Following extraction, the purified cellulose was subjected to sulfuric acid hydrolysis under controlled conditions to yield CNCs, with the aim of preserving the intrinsic crystalline structure while enhancing the material's physicochemical properties. The search for biocompatible and biodegradable materials suitable for biomedical applications has led to significant interest in hydrogels composed of natural polymers. Among these, hydrogels synthesized from cellulose and polyvinyl alcohol (PVA) have emerged as promising candidates due to their unique physical properties and compatibility with biological tissues. This study presents the formulation, characterization, and potential biomedical applications of a cellulose/PVA hydrogel. The hydrogel was synthesized to enable the formation of a semi-crystalline network that imparts the material with enhanced mechanical properties.

Keywords: cellulose; microwave-assisted extraction; cellulose nanocrystals; sulfuric acid hydrolysis; renewable biomass; polyvinyl alcohol (PVA); Borax; hydrogel

1. Introduction

In recent years, the studies of new non-traditional renewable sources for the production of biodegradable nanomaterials have decreased. Most of the natural resources are rich in cellulose, which have yet to be fully utilized to produce cellulose derivatives like cellulose microfibrils (CMF), cellulose nanofibrils (CNF), and cellulose nanocrystals (CNC). These valuable cellulose-rich materials represent promising avenues for innovation and development in the field of sustainable materials. These non-conventional sources present a significant alternative to traditional sources like wood and cotton [1]. Nanocellulose can be derived from various sources like plants, microorganisms, and aquatic animals such as tunicates, all of which are rich in cellulose. Banana leaves, corn cobs, cotton, ramie, rice husks, wood, sugarcane bagasse, sisal leaves, wheat straw, aloe vera leaves and coconut husks are all promising candidates for extracting nanocellulose from plant sources [3].

Nanocellulose is a biopolymer consisting of a crystalline fiber structure embedded in an amorphous matrix of lignin, pectin, and hemicellulose. It can be categorized primarily into two groups: cellulose nanocrystals (CNCs) and cellulose nanofibers (CNFs) [4]. The categorization of nanocellulose is determined by various factors including nanocellulose's inherent properties, dimensions, extraction techniques, and intended applications. The properties of these nanomaterials are significantly influenced by the source of cellulose and the methods employed during processing.

While the chemical properties of acid-hydrolyzed cellulose nanocrystals (CNCs) and mechanically extracted cellulose nanofibers (CNFs) may exhibit similarities, their physical attributes differ [5]. Several techniques, including mechanical and/or chemical procedures, have been investigated throughout the years to extract CNFs from lignocellulosic biomass [6]. CNCs, also known as cellulose substances, are categorized as sustainable bio-based nanomaterials generated from the degradation of the lignocellulosic biomass. Nanoscale dimensions with numerous intrinsic properties targeting various applications, ranging from packaging to biomedical, are obtained [7]. Additionally, inherent properties including low density, renewability, biodegradability, adjustable surface chemistry, non-toxicity, and increased thermal stability are significant. Each of these properties plays a crucial role in various fields, particularly in the context of material science, environmental sustainability and health. By processing these properties, materials can be engineered to meet specific performance requirements while minimizing negative impacts on human health and the environment [7].

Innovative microwave-assisted extraction (MAE) techniques have been developed, offering advantages that surpass traditional methods. These include enhanced reproducibility and minimal sample manipulation, as well as reduced solvent volume, exposure time, and energy consumption during the extraction process [8]. Microwave-assisted extraction (MAE) processes were recently developed, demonstrating advantages that overcome possible issues of the traditional approaches. The aim of the present study is the development of a green technique for the extraction of CNCs from Aloe Vera leaves by MAE by minimizing the cost food-related applications and overcoming the major drawbacks of traditional extraction procedures [9]. The MAE set up and parameters are provided in section 2.2, while cellulose, CNCs samples and CNC/PVA hydrogel are thoroughly analyzed using Fourier-Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and Dynamic Mechanical Analysis (DMA) [10].

Hydrogels are polymeric materials that have a 3D structure and the ability to absorb and reversibly release large amounts of water and biological fluids [11]. The hydrogels can also respond to various condition changes such as pH, temperature, ions as well as magnetic or electric fields [12]. According to the origin of the polymers, hydrogels can be divided into natural or synthetic. Those based on natural polymers derive from hydrophilic polymers, such as cellulose, gelatine, chitosan, hyaluronic acid, as well as some of their derivatives. In addition to natural polymers, there are also hydrogels based on synthetic polymers, which typically include polymers such as polyethylene glycol (PEG), polyacrylic acid (PAA), polyacrylamide (PAAm), polyvinyl alcohol (PVA), as well as their copolymers [13].

PVA, a synthetic polymer, is both safe and eco-friendly, designed to degrade naturally. Its versatility has led to its adoption across diverse industries such as medicine, packaging, food production, and papermaking [14]. Moreover, there's potential for PVA in biomedical uses like tissue engineering, cell culture, and crafting implants for vascular systems. It has been combined with a range of fibers and micro-sized cellulose-based materials, such as cellulose nanocrystals (CNCs), to improve its properties. Given that CNCs are hydrophilic, PVA-CNC hydrogels present are an environmentally friendly option for biomaterials development. These hydrogels can be customized to be lightweight and biodegradable [15]. PVA and CNC interact via secondary interactions, which enhances the mechanical properties of the hydrogel [16].

Cellulose-based hydrogels present characteristics that make them suitable for use in biomedical applications. Cellulose is non-toxic, which is essential for any material intended for use in biomedical applications. Cellulose-based hydrogels typically exhibit high water absorption capacity due to the hydrophilic nature of cellulose [17], which enables them to retain significant amount of water, making them suitable for applications such as wound dressings, where moisture management is crucial for healing promotion. They can also be engineered to have a wide range of mechanical properties, porosity, and degradation rates, depending on the application. Overall, the properties such as biodegradability, biocompatibility, non-toxicity, hydrophilicity, and structural versatility makes them possible candidates for numerous biomedical applications, offering potential benefits in terms of safety, efficacy, and performance [18]. When incorporated into hydrogels, cellulose acts as a reinforcing material, enhancing the mechanical strength of the hydrogel matrix. The presence of

cellulose fibers or nanoparticles within the hydrogel network helps to distribute mechanical stresses more effectively, resulting in improved elasticity and structural integrity. Furthermore, cellulose's ability to form hydrogen bonds with water molecules contributes to the hydrogel's stability and swelling behavior. The hydrophilic nature of cellulose facilitates water absorption, which is beneficial for applications such as tissue engineering and drug delivery [19].

By incorporating therapeutic agents, such as growth factors or anti-inflammatory drugs, into the hydrogel matrix, controlled release can be achieved, aiding in tissue regeneration, and reducing inflammation in the affected area. Moreover, they can be combined with other biomaterials, such as ceramics or polymers, to create composite materials with enhanced properties suitable for specific bone repair applications. These composite materials can offer a synergistic approach to address complex bone defects or injuries [20].

An additional feature is that "smart" cellulose hydrogels can be created that are capable of being used as drug carriers, where proteins and peptides will be protected from the host environment but will be capable of allowing the acceleration as well as the retardation of their release, as the polymer chains will expand or contract [21]. Smart" cellulose hydrogels can be engineered to function as advanced drug carriers with controlled release capabilities. These hydrogels possess the ability to respond to various stimuli, such as pH, temperature, or specific biochemical signals, allowing for precise modulation of drug release kinetics. The expansion and contraction of polymer chains within the hydrogel matrix plays a crucial role in achieving controlled release profiles [22]. This results in the ability to remotely control drug delivery from a control switch, thus presenting a significant advantage in many biomedical applications. Finally, cellulose nanocrystals, which are isolated through acid hydrolysis of native cellulose, can be used to reinforce the polymeric hydrogel due to its mechanical properties [23].

2. Results and Discussion

2.1. FT-IR Spectrum

In Figure 1a the spectrum of aloe vera samples show bands of ether R-OR group at 1017 cm^{-1} and secondary alcohol R-OH group at 1238 cm^{-1} . An ether ROOR group is seen at 1230 cm^{-1} and an aromatic group is seen at 1421 cm^{-1} . A band of nitro NO_2 group is also seen at 1600 cm^{-1} . These bands show the probable chromophoric groups likely to be present in the gel of aloe vera. The strong absorption band at 1600 cm^{-1} is due to $\text{C}=\text{C}$ stretching which indicates the presence of vinyl ether and aloin compound [25].

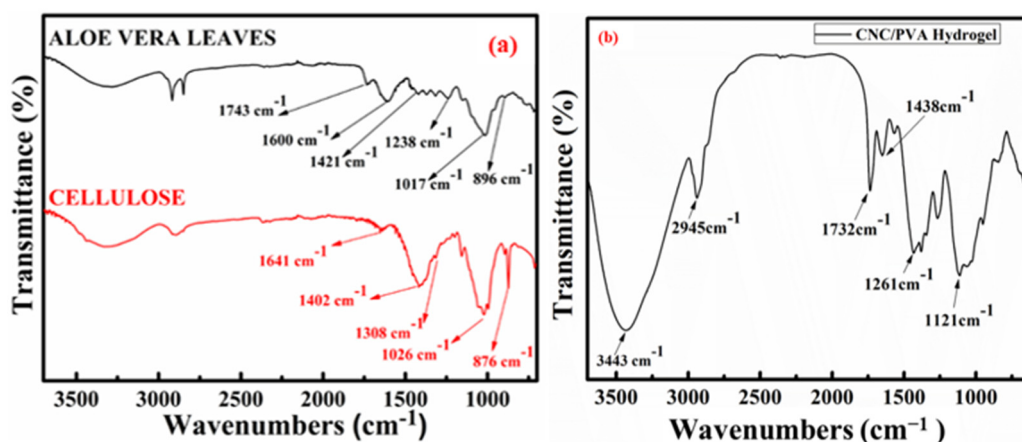


Figure 1. FTIR spectra of a) pure aloe vera leaves and extracted cellulose and b) CNC/PVA Hydrogel.

Also in Figure 1a the observed peaks in the wave number range of $3660\text{--}2900\text{ cm}^{-1}$ are characteristic for stretching vibration of O-H and C-H bonds in polysaccharides. The broad peak at 3331 cm^{-1} is characteristic for stretching vibration of the hydroxyl group in polysaccharides. This

peak also includes inter- and intra-molecular hydrogen bond vibrations in cellulose. Typical bands assigned to cellulose were observed in the region of 1630–900 cm^{-1} . The peaks located at 1641 cm^{-1} correspond to vibration of water molecules absorbed in cellulose. The absorption bands at 1402, 1308, 1026 cm^{-1} and 876 cm^{-1} belong to stretching and bending vibrations of $-\text{CH}_2$ and $-\text{CH}$, $-\text{OH}$ and $\text{C}-\text{O}$ bonds in cellulose [26]. Figure 1b shows the FTIR spectra of PVA/CNC hydrogel. A band around 3443 cm^{-1} is observed in all spectra and is assigned to the free O-H stretching vibration of the OH groups. O-H stretching vibration from the intramolecular hydrogen bonds within the PVA and intermolecular hydrogen bonding between hydroxyl groups of PVA and the CNC are responsible for this band. The addition of CNC causes a shift toward higher wavenumber. The peak for C-H stretching vibrations from alkyl groups is present at 2945 cm^{-1} in all formulations. The peak around 1732 cm^{-1} is attributed to the C-O stretching from the residual acetate groups in the PVA matrix. However, the intensity of that peak was reduced with the addition of CNC. The band at 1261 cm^{-1} is attributed to the C-O stretching vibration. The reduction in peak intensity is due to the formation of hydrogen bonds between PVA and CNC. The peak at 1438 cm^{-1} ascribed to C-H deformation vibration. The peak at 1121 cm^{-1} could be attributed to the C-H bending vibration of CH_2 group [27].

2.2. XRD Analysis

X-ray diffraction (XRD) analysis of aloe vera leaves typically reveals diffraction peaks corresponding to the crystalline structure of the materials present in the leaves. Aloe vera leaves are composed of various compounds, including cellulose, hemicellulose, lignin, and other polysaccharides.

The XRD pattern may show peaks associated with the crystalline components, such as cellulose, Figure 2a, which usually appear around 2θ values of 14.8°, 16.5°, and 23.6°. These peaks can provide information about the crystallinity and structural properties of the cellulose and other crystalline components in the aloe vera leaves, Figure 2a [24]. X-ray diffraction (XRD) analysis of cellulose typically shows diffraction peaks indicative of its crystalline structure. The XRD pattern usually displays sharp peaks at specific 2θ values, typically around 18.8°, 22°, and 29.7°. The intensity and position of these peaks can provide information about the crystallinity, crystal size, and orientation of the cellulose molecules within the sample [25]. The PVA/CNC hydrogel exhibits a diffraction peak at $2\theta=21^\circ$, with decreased density and a shoulder peak at $2\theta=28.2^\circ$, with increased density, which suggests the physical interaction of PVA and CNC, Figure 2b. This indicates that the incorporation of CNCs and PVA does not affect the crystalline structure of PVA matrix. This means that the PVA were well dispersed in the CNCs suspension to form the PVA/CNC hydrogel [26].

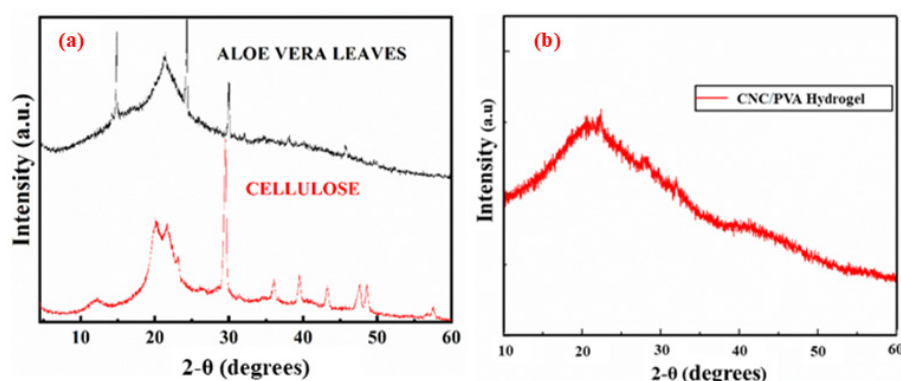


Figure 2. XRD diffractograms of a) pure aloe vera leaves and extracted cellulose and b) CNC/PVA hydrogel.

2.3. Scanning Electron Microscopy (SEM)

Figure 3a shows the amorphous form and surface morphology that were demonstrated in the SEM. The micrograph shows the presence of surface holes that correspond to the pores present in the material. These images revealed a porous and irregular surface (different heterogeneous surface structures) with deep pores such as granules, nanofibers, nanotubes, nanospheres, microspheres, and flakes and aggregation of irregularly shaped grains, well interconnected that appear brighter, and flakes like a flat surface revealed.

Bleaching with H_2O_2 after the hydrothermal method helps to eliminate the rest of lignin and further disintegration leading to the development of cellulose micro fibrils. This is clearly observed from Figure 3b. The bleached pulp exhibits smoother and uniform fibril surface, confirming the removal of non-cellulosic components. Morphological examination of the CNCs is essential because the source of cellulose and hydrolysis technique has profound influence on the dimension and properties of nanocellulose. SEM image, Figure 3c, of CNCs shows highly agglomerated and rough surface. It can be clearly observed that there is considerable reduction in size after acid hydrolysis due to the successful removal of amorphous phase.

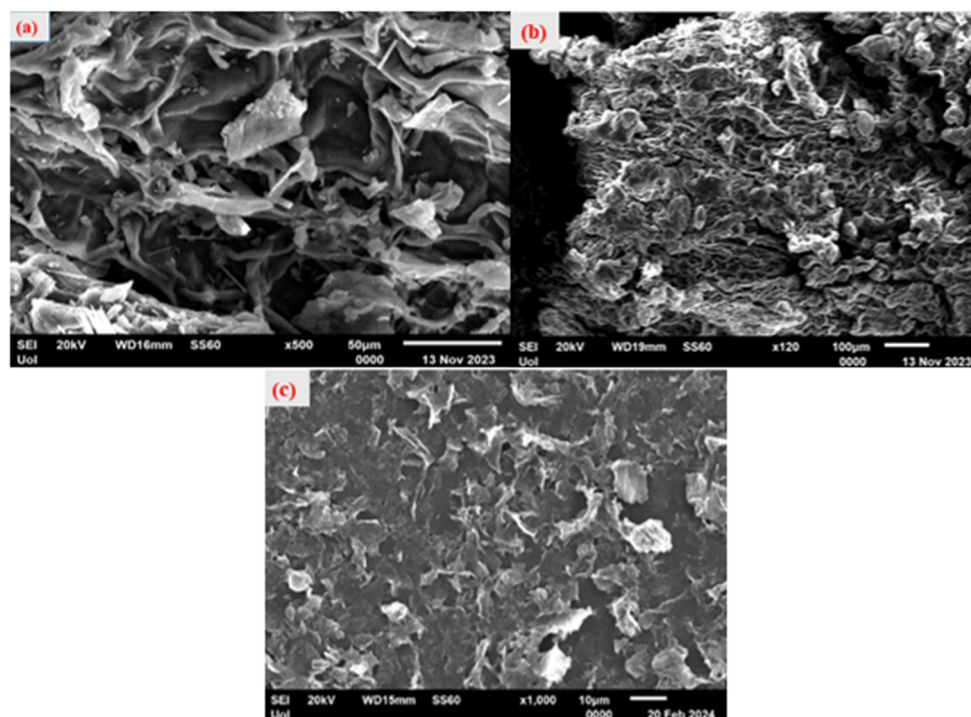


Figure 3. SEM images of a) pure aloe vera leaves, b) extracted cellulose and c) isolated CNCs.

To study the interior structure of the hydrogel, it was freeze-dried to prevent shrinking and examined using SEM imaging. Figure 4 shows that the hydrogel produced an open, interconnected, continuous, macroporous structure. Figure 4a shows tiny extensions reaching outwards from the matrix cell walls. It is possible that these projections are CNC whiskers. There were no such projections visible in Figure 4b, instead, lengthy filaments extending across many cells may be detected on occasion [27].

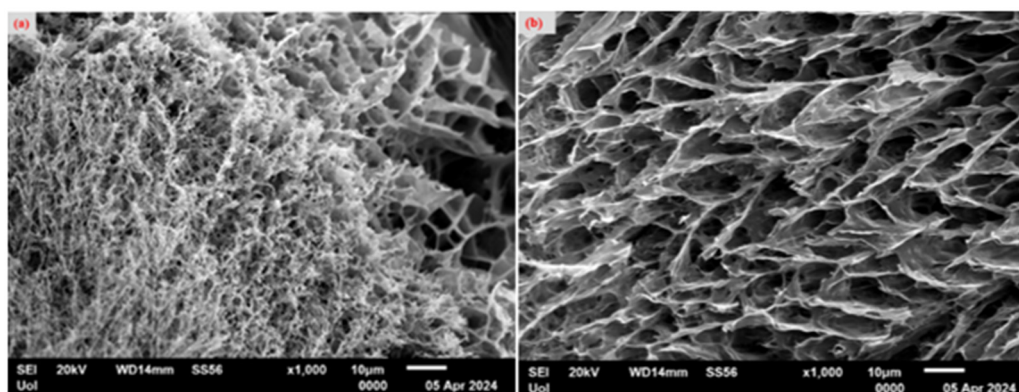


Figure 4. SEM images for CNC/PVA hydrogel.

2.4. Dynamic Mechanical Analysis (DMA)

Frequency sweeps were performed on the CNC/PVA hydrogel. Storage modulus and loss modulus obtained from dynamic frequency sweep measurements can give further information about the microstructure of CNC/PVA hydrogel, as shown in Figure 5a. It is shown that for a fixed temperature (35 °C), the storage modulus rises as the frequency increased. The increase in storage modulus as function of frequency indicates that the hydrogel retains a strong network structure while becoming more elastic and producing stable structures. Increasing the crosslinking density enhances the network's mechanical strength, leading to a higher storage modulus. Additionally, a high crosslinking density restricts chain mobility, resulting in reduced energy dissipation and thus a lower loss modulus. The restriction of the movement of polymer chains and the reduction of their ability to dissipate energy result the decrease of tan delta, as it is shown in Figure 5b [28].

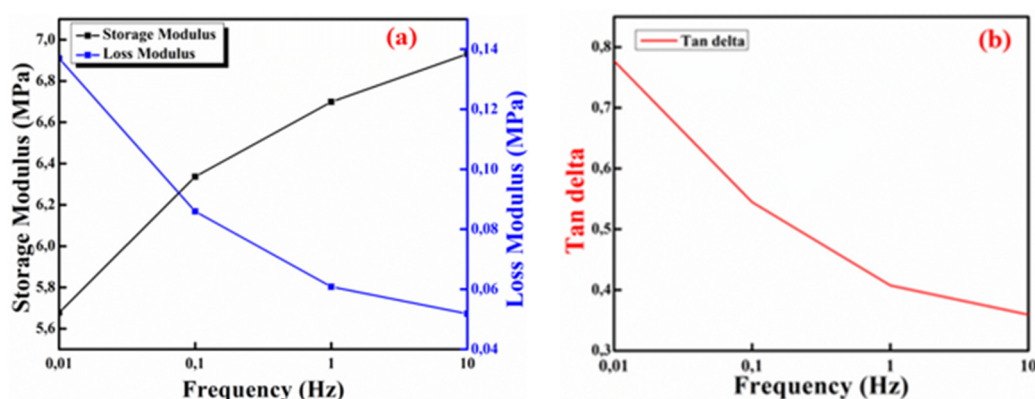


Figure 5. Dynamic Mechanical Analysis of CNC/PVA hydrogel.

3. Materials and Methods

3.1. Materials

Sigma-Aldrich (St. Louis, MO, USA) was the supplier of Sodium Hydroxide, Hydrogen Peroxide, Polyvinyl alcohol, and Borax. Aloe-Vera (av) waste leaves were supplied by the Greek Industrial Company, Hellenic Aloe, Ethnikis Antistaseos 21, Heraklion, Crete.

3.2. Cellulose Extraction

AV waste leaves were cut into small pieces and dried in an oven at 60 °C for 4 hours under vacuum. Then the dried AV leaves were blended in a blender until powder was collected. The collected powder (2g) and 50ml distilled water were immersed in a 100ml Teflon vessel. The vessel was placed in the microwave at 200 °C for 15 min. 25 bar pressure was reached at 1080 Watt. After

the microwave treatment, the sample was filtered with filter paper. Then a beaker containing 50 ml of 12% NaOH solution was heated at 80 °C for 2 hours. This procedure was repeated three times, the mixture was filtered, and the solid sample was dried at 60 °C for 4 h. The obtained sample was bleached with 30% H₂O₂ for 4 h at 60 °C. Then the bleached product was washed multiple times with distilled water and placed in an oven to dry, Figure 6.

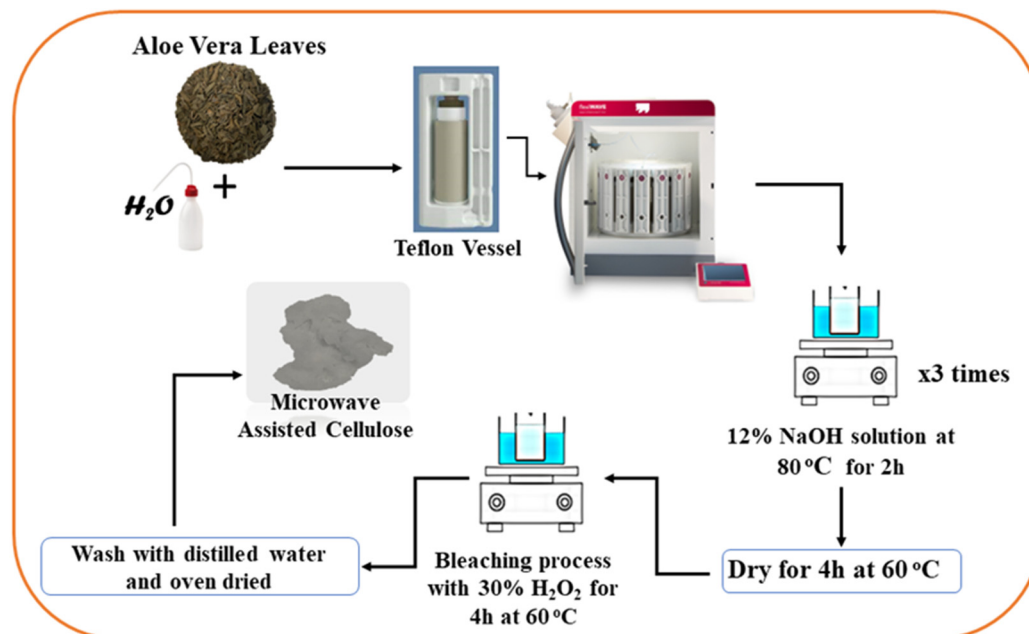


Figure 6. Schematic illustration of cellulose extraction.

3.3. Cellulose Nanocrystals Isolation

CNCs were extracted by subjecting the extracted CMF to sulfuric acid hydrolysis. For that, CMF was added to a preheated sulfuric acid solution (64 wt%) at 50 °C under mechanical stirring. Then, the mixture was diluted with cold distilled water to stop the reaction. The obtained mixture was centrifuged at 12,000 rpm for 15 min for several. Then, the obtained mixture was dialyzed against distilled water until it reached a neutral pH. Afterwards, the obtained CNC aqueous suspension was homogenized using a probe-type ultrasonic homogenizer for 5 min, Figure 7 [29].

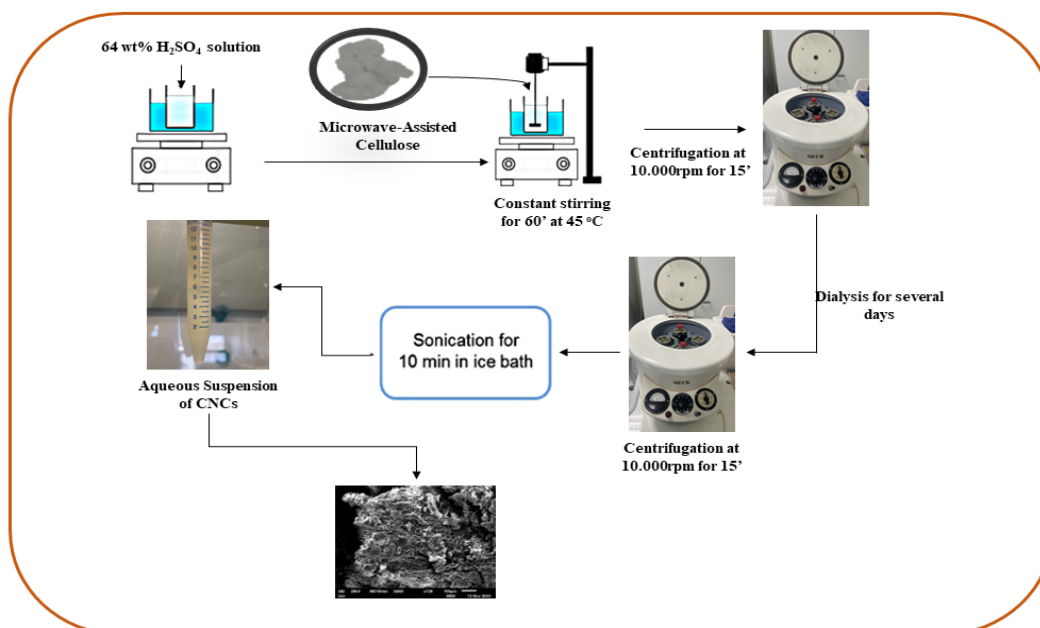
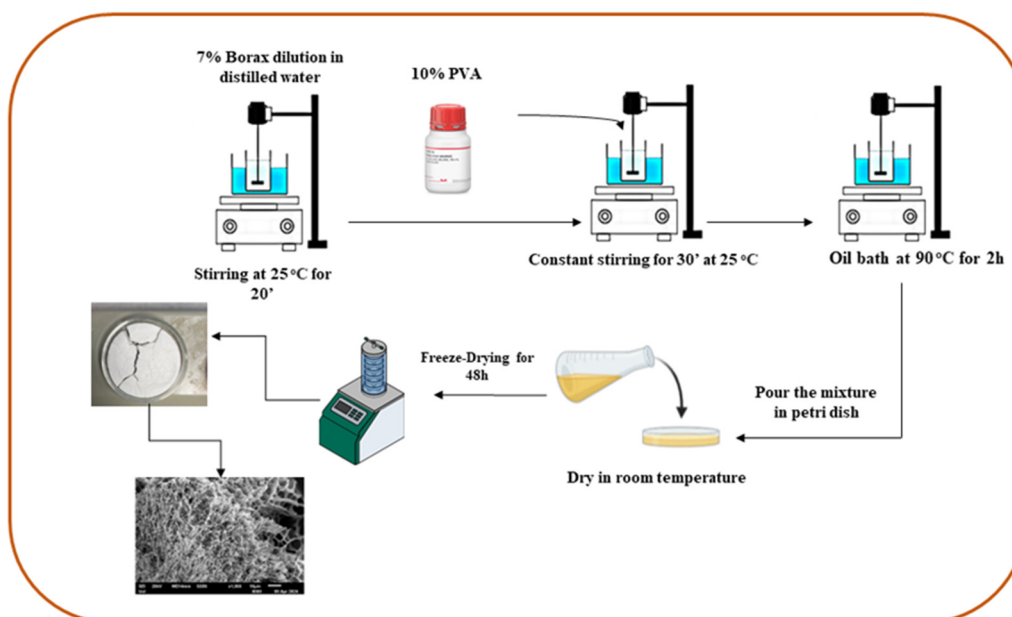


Figure 7. Schematic illustration of cellulose nanocrystals isolation.

3.4. PVA/CNC Hydrogel Preparation

For the preparation of the PVA-CNC hydrogels, in a beaker containing CNC suspension, 7% Borax was diluted under continuous stirring at 25 °C for 20 minutes. Then 10% of PVA was added to the same beaker stirring for another 30 min. After the fully dilution of PVA, the beaker was placed in oil bath at 90 °C for 2 hours under continuous stirring. The mixture was poured in a petri dish and was let to dry at room temperature. The sample was stored at -20 °C and then it was lyophilized to receive the porous hydrogel, Figure 8.

**Figure 8.** Schematic illustration of CNCs/PVA hydrogel preparation.

4. Conclusions

The prepared cellulose nanocrystal-based hydrogels were synthesized through a solution casting-evaporation method and characterized through all the analytical measurements. Cellulose was extracted from aloe vera leaves residue. Cellulose nanocrystals were then isolated using sulfuric acid. For the hydrogel preparation PVA was used because of its hydrogel forming properties and Borax also was added as cross-linker. Thus, we are willing to do more characterizations to prove that the prepared materials can be used as sustainable packaging materials or/and as drug delivery systems.

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