

Nanocellulose – Turmeric Composite for Wound Dressing

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Abstract

Nanocellulose have undergone rapid development in recent years as promising biomedical materials because of their excellent physical and biological properties, in particular their biocompatibility, biodegradability and low cytotoxicity. Turmeric is used very often over wounds in India. Use of turmeric for wounds heals the cut, prevents infection and also reduces the pain. Curcumin, the therapeutic component of turmeric shows anti-inflammatory, anti-bacterial and anti-viral properties. Use of turmeric for wounds fastens the healing process and also reduces the risk of formation of scar. In this report, nanocellulose was prepared by bleaching and acid hydrolysis of rice husks. Turmeric powder is obtained by mechanical grinding of dried turmeric roots. Later nanocellulose and turmeric composite was prepared. The antibacterial activity of prepared composite was examined against *E. coli* and *Staphylococcus aureus*.

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1. Introduction

We are familiar with cellulose, which is the most abundant natural, biodegradable source which exists in plant cell wall, has been extensively used in the manufacture of paper, fibres, textiles, etc. When this cellulose is broken down to small fibres in nanoscale, it gains some excellent mechanical, thermal and biological properties, which is termed as 'nanocellulose'. Regarding wound dressings, nanocellulose possess an important characteristics which is their ability to absorb exudate during the dressing process, and its removal from a wound surface after recovery [1]. Nanocellulose have been synthesized from different sources such as oats husks [2]. Nanocellulose fibers have been prepared using several mechanical processes [3-5].

Turmeric (*Curcuma longa*) has been used in India for thousands of years as a spice and medicinal herb. Powdered turmeric contains curcumin which constitutes 3.14% (on average) [6]. Curcumin, the active agent in turmeric, has antifungal, antimicrobial, anti-inflammatory and antibacterial properties, which aids in reducing bacterial infection and better healing of wounds. Using turmeric for wounds heals the cut, prevents infection, fastens the healing process and also reduces the risk of scar formation.

For increased biocompatibility the use of curcumin with other polymeric material to form composites have received attention. Various researchers had prepared different materials

like Suwantong et al.,[7-8] prepared ultrafineacetate fibre mats containing curcumin, Chen et al.,[9] prepared PLA/curcumincomposite membrane by electrospinning, Gopinath et al.,[10] incorporatedcurcumin into collagen films to support wound healing.

Nanocellulose - turmeric composite can be prepared by using nanocellulose indifferent proportions with turmeric. Different methods of synthesis can be preferred based on the application of composites. They have potential application in wound dressing, food-packaging and other bio-medical applications because of potent anti-oxidant, anti-bacterial properties of turmeric caused by presence of compound named as curcumin in it.

Wound healing is a complex biological event. Proper wound care is important to restore the skin barrier functionality. So, wound dressings developed from natural and synthetic materials have been used depending on their properties such as elasticity, pH maintenance in wound environment, compatibility with topical therapeutic agents, optimum oxygen permeability, non-toxic, non-antigenic properties and ability to avoid bacterial infection. Different dressing material like NFC (Nano-fibrillar cellulose) polymer, CMC (Carboxymethyl cellulose) can be used in skin graft treatment, natural polymers like polysaccharides (Alginate, Chitin, Chitosan), proteins, proteoglycans can also be used because of their biocompatibility and biodegradability. Polymers like PLA, PVP, PVA, PEG exhibit in-vivo and in-vitro wound healing properties. The selection of dressing material plays an important role in recovery and aesthetic appearance of regenerated tissues.

In this report, nanocellulose was obtained from rice husks using bleaching and acid hydrolysis at a mild temperature (45 °C) followed by ultrasonication [11]. Rice husks were used as the starting material because it contains cellulose about 40-50%, lignin 25-30%, ash 15-20% and moisture 8-15% [12]. A lot of work has been done only on the compound present in the turmeric known as curcumin. To the best of our knowledge this is the first work done on the preparation of nanocellulose - turmeric composite. So, in this report we are preparing this composite for the application of wound dressing. Prepared samples were characterized by the SEM, EDAX and XRD. Antibacterial studies were done for the prepared composite.

2. Research Method

Chemicals

Sodium Hydroxide (NaOH) 5% (w/v), Acetic Acid, Hydrogen Peroxide, 63.7% (w/v) Sulphuric Acid, Chloroform, Glycerine, Turmeric Roots, Distilled Water, Unpurified Rice Husks.

This work is divided into two parts:

- (1) Separation of Cellulose nanofibre from rice husks
- (2) Preparation of Nanocellulose - Turmeric composite.

1. Separation of Cellulose nanofibre from rice hull

1.1 Chemical Composition

Rice husks content represents 20% dry weight of harvested rice. It contains 36-40g/100g cellulose, 12-19g/100g hemi-cellulose, ash represents 12g/100g i.e. silica (80-90g/100g). Two types of cellulose can be isolated from the cellulose nanocrystals and nanofibrils.

1.2 Bleaching of the rice husks

The bleaching of the rice husks was performed in two steps. The first step was an alkaline pre-treatment [13]. Rice husks (20 g) were immersed in 200 ml of sodium hydroxide (NaOH) 5% (w/v) at 90°C for 60 min at constant vigorous stirring. The material was washed with distilled water until it reached a neutral pH and was dried at 40°C for 24 h. In the second step, approximately 20 g of the husk (pre-treated) was dispersed in 250 ml of a

peracetic acid solution (50% acetic acid, 38% hydrogen peroxide and 12% distilled water) at 60°C and stirred for 24 h [2]. The fibres were filtered, continuously washed with distilled water and centrifuged at 3000 rpm for 10 min to maintain the pH value between 6-7 and dried at 35°C for 14 h in an air-circulating oven. The bleached rice hull was stored for further use.

1.3 Preparation of the cellulose nanofibres from rice husks

Weight approximately 10 g of bleached rice husks and dispersed in 100 ml of 65.67% (w/v) sulphuric acid and distilled water (for dilution) at 45°C and vigorously stirred for 1 h. Cold distilled water (200 ml) was added to stop the reaction. The sulphuric acid was partially removed from the resulting suspension through centrifugation at 5,000 rpm for 30 min. The non-reactive sulphate groups were removed using centrifugation followed by filtration with distilled water until the pH value was between 6-7. Repeat this procedure for several times until the pH is maintained. The neutral suspension was ultrasonicated (Ultrasonic Processor – Fisher Scientific) for 15 min, 4-5 drops of chloroform were added and then the suspension was stored in a refrigerator. A 50 ml aliquot was dried at 35°C for 14 h in an air-circulating oven for X-ray Diffraction.

2. Preparation of Nanocellulose - Turmeric composite

A fine turmeric powder was obtained by mechanical grinding of dried turmeric roots was mixed with the extracted nanocellulose in the following proportions by weight (1:1, 1:2, 1:3) for the preparation of composite. Prepared composite was bonded to the mulmul cotton fabric by dipping the fabric in the composite solution, until a thick coating was obtained and dried at room temperature. Figure 1. shows the prepared nanocellulose-turmeric composite dressing material. Prepared composite was studied for anti-bacterial activity using gram positive and negative bacteria.

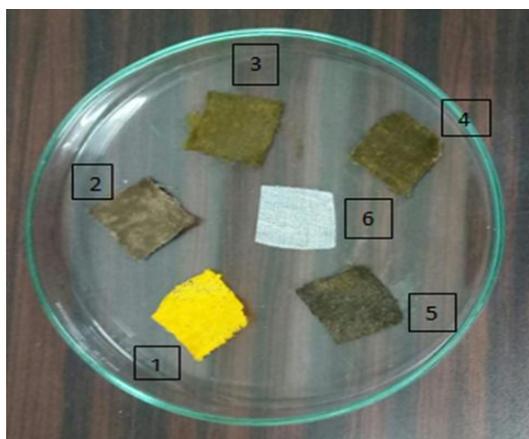


Figure 1: (1) Turmeric coated muslin cloth, (2) Nanocellulose coated muslin cloth, (3) 1:1 ratio nanocellulose-turmeric composite coated muslin cloth, (4) 2:1 ratio nanocellulose-turmeric composite coated muslin cloth, (5) 3:1 ratio nanocellulose-turmeric composite coated muslin cloth, (6) Non coated muslin cloth

3. Results and Analysis

X-ray diffraction:

The XRD spectra of the nanocellulose is shown in figure 2. Nanocellulose showed a characteristic peak at $2\theta = 23.19^\circ$. The crystallinity index of nanocellulose was analysed using X-Ray diffraction. The relative crystallinity index (CI) of nanocellulose was calculated using the Segal et al., [14], which is, $CI (\%) = \frac{(I_{002} - I_{am})}{I_{002}} \times 100$, in which I_{002} is the maximum intensity of the 002 peak at approximately $2\theta = 20-22^\circ$ and I_{am} is the intensity corresponding to the peak at $2\theta = 18^\circ$. The crystallinity index of the nanocellulose was 61.69%. This is in agreement with the XRD graph observed in the literature [11].

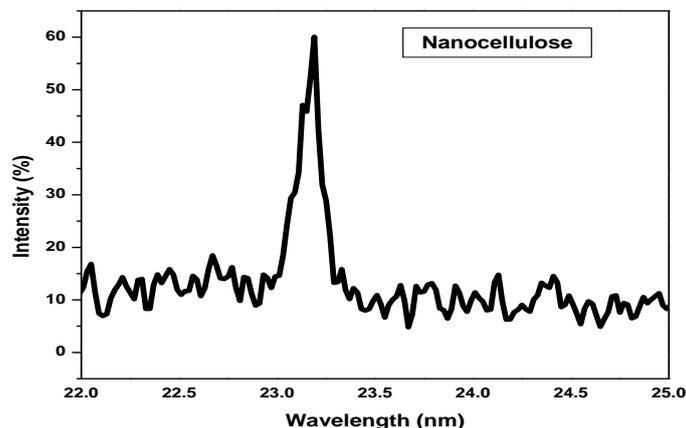


Figure 2. X-ray diffractogram of nanocellulose.

Scanning electron microscope (SEM) analyses:

The morphology of nanocellulose and nanocellulose-turmeric composite was studied using SEM. Figure 3 (a and b) shows the structure of nanocellulose. Thenanocellulose is present in the form of particles along with some rod shapedfibres. Figure 4 (a and b) shows the surface of nanocellulose-turmeric composite. The surface of the nanocellulose-turmeric composite showed flaky structure. It was observed that nanocellulose was coated with turmeric particles.

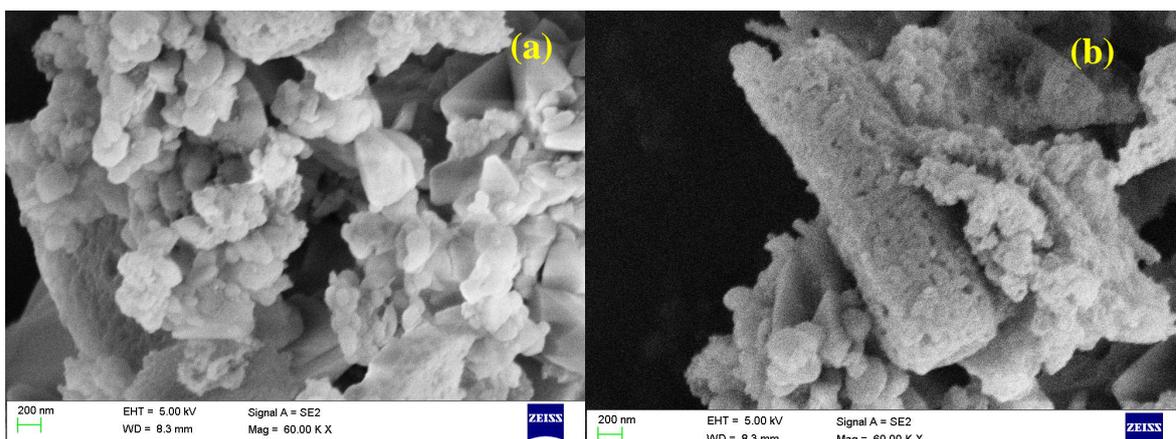


Fig.3 (a, b): SEM images of prepared Nanocellulose

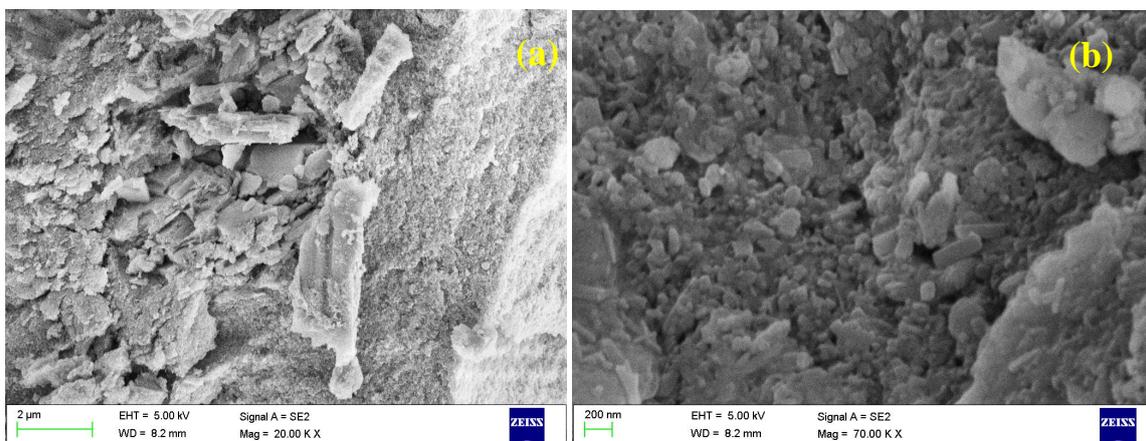


Fig.4 (a, b): SEM images of Nanocellulose- Turmeric composite with different

Energy dispersive spectroscopy (EDAX):

Figure 5(a) shows the elemental analysis of nanocellulose and figure 5(b) shows the elemental analysis of the nanocellulose coated with turmeric. These images clearly show an increase in the atomic percentage of oxygen in the sample containing cellulose coated with turmeric. There was 5 atomic percent increase in oxygen levels. Nanocellulose and turmeric both has oxygen present in it, which leads to the increase in oxygen level in the composite. Nanocellulose and turmeric shows the presence of all the elements in it.

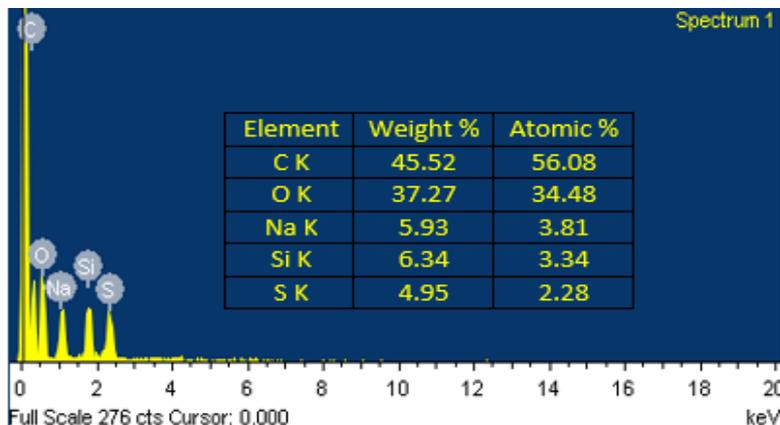


Figure 5 (a): EDAX of nanocellulose

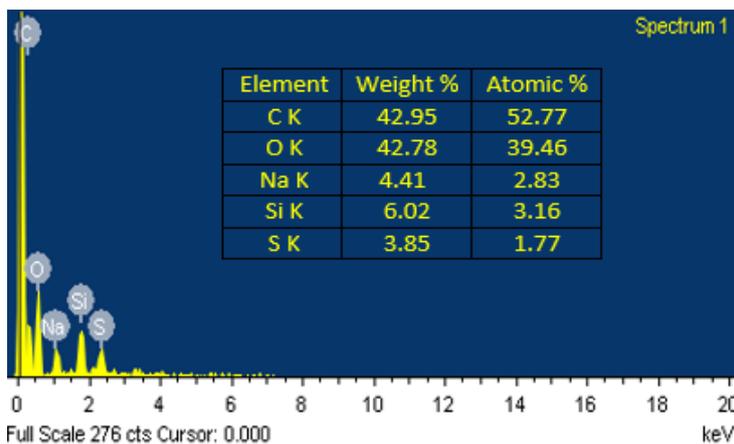


Figure 5 (b): EDAX of nanocellulose-turmeric composite

Antibacterial study of nanocellulose-turmeric composite band:

The antibacterial activity of nanocellulose-turmeric composite was checked against *Escherichia coli* (gram negative bacteria) and *Staphylococcus aureus* (gram positive bacteria). To investigate the antibacterial activity, the disk diffusion method was used. The disks with 1cm size were coated with nanocellulose-turmeric composites having different ratios i.e. 1:1, 2:1, 3:1 respectively and with only turmeric and nanocellulose. The bacterial suspensions of *E. coli* and *Staphylococcus aureus* were spread on the nutrient agar plates. The prepared disks were placed in direct contact with the agar medium. These plates were incubated at 37 °C for 24 hours. After incubation, the clear inhibition zone was observed around the disks coated with turmeric and nanocellulose-turmeric composite. The diameter of inhibition zone along with the diameter of coated disks was measured. The turmeric and nanocellulose-turmeric composite with 1:1 ratio showed large clear inhibition zone, while the composites with 2:1 and 3:1 ratio showed smaller clear inhibition zone, while only nanocellulose showed very small inhibition zone. This indicates that turmeric contributed to the antimicrobial activity of nanocellulose-turmeric composite. The antimicrobial activity goes on decreasing with the decreasing concentration of the turmeric. As nanocellulose itself has no antibacterial activity [1], hence decreasing the turmeric ratio decreases the antibacterial effect. The diameters of the inhibition zone of turmeric, nanocellulose and composite with varying concentration of nanocellulose are present in table 1.

Samples	Nanocellulose	Turmeric	1:1	1:2	1:3
Inhibition zone on <i>E. coli</i> (mm)	1.1	1.3	1.3	1.2	1.1
Inhibition zone on <i>S. aureus</i> (mm)	1.0	1.3	1.3	1.2	1.1

Table.1: Diameters of anti-bacterial inhibition zone of samples

4. Conclusion

The nanocellulose-turmeric composite was prepared from rice husk and dried turmeric roots. The characterization proves the formation of nanocellulose turmeric composite. From the antibacterial studies we can conclude that nanocellulose-turmeric composite has better wound healing and wound dressing properties at the ratio of 1:1 than the 2:1 and 3:1. This composite of nanocellulose along with the turmeric at 1:1 ratio provides protection against infections and its antibacterial studies perfectly serves the purpose of wound healing and dressing. Results showed that it is the facile method to develop a functional wound dressing material.

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