



Utility of a New Non-Toxic Antibacterial Cellulose Membrane Prepared via Casting Technique

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Abstract

The present study describes the Facile preparation of antibacterial cellulose acetate (CA) membranes by using novel non-toxic antibacterial cyanoacetyl microcrystalline cellulose (NACAMC). Different concentrations of (NACAMC) were blended with CA to prepare three antibacterial cellulosic membranes namely: M1, M2 and M3 having hindrance activity against tested strains. The Fourier-transformed infrared spectroscopy (FT-IR) was used to investigate the changes in chemical and physical membrane characteristics. The obtained membranes (M1, M2, and M3) were visually evaluated by scanning electron microscopy (SEM). M2 and M3 are characterized by a pore-free upper surface and a porous bottom surface of the membranes in comparison with the CA blank membrane. The water uptake ratio was measured to be 175%, 426% and 707% for M1, M2 and M3 respectively. Also, the average pore size distribution for prepared membranes was measured. The Mechanical properties of M1 and M2 were measured which indicates that the tensile strength and elongation are higher in M2 than M1. Antibacterial activity for membranes and (NACAMC) against 5 pathogenic strains were evaluated. The prepared membranes could be developed for several uses as in wound dressing applications, food packaging, and water treatment antifouling membranes.

Keywords: Cellulosic membranes; Non-toxic antibacterial cellulose; Biodegradability; antifouling; Rice straw; Water treatment.

1. Introduction

Rice straw is one of the cereal straws produced in large quantities worldwide every year [1]. Mainly, it contains polysaccharides like cellulose and hemicelluloses [2]. However, in developing countries, such straw is under-utilized and is regarded as agricultural waste. Instead of open burning this causes pollution problems to the environment. Nowadays, attention has been given to rice straw as cheap and available feedstock for the production of valuable chemicals and materials [3].

Recently, we patented a solar pulping Mini-mill for pulping rice straw [4]. Using the obtained pulp in different preparations [5]. Also, lignin/silica derived from rice straw was utilized as an antioxidant and reinforcement in rubber composites [6,7].

The transformations of cellulose by acetylating

reaction producing cellulose acetate that provides a bio-based raw material [8, 9], which has wide industrial applications in coatings, cigarette filters, filtration membranes, and pharmaceutical products [10–12].

Cellulose acetate (CA) membranes have been widely used in food packaging as well as in reverse osmosis systems. Membranes prepared using Cellulose acetate have good characteristics such as high potential flux and good desalting. Due to this advantage, CA membranes have been used in the research technology and commercial membranes and also have applications in Microfiltration (MF), Ultrafiltration (UF), Nano-filtration (NF), and gas separation [13,14].

One of the most popular methods for the preparation of membranes is the blending of natural polymers

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with synthetic ones [15, 16]. Also, the preparation of membranes composed of only blended natural polymers, such as natural rubber and chitosan, [17] chitin and cellulose, [18], or N, O-carboxymethyl chitosan, and cellulose has been reported [19]. To date, cellulose filter membranes with different pore sizes can be used commercially to remove various sizes of substances. They can remove bacteria, viruses, antibiotics, pesticides, synthetic dyes, oil and grease, heavy metals, and even dissolved salts [7, 16]. Within this context, the cellulose acetate membrane loaded with silver nanoparticles was used as an antibacterial membrane where cellulose acetate plays the role of a stabilizer to control the dispersion and growth of the Ag particles [20], in this context, Antibacterial electrospun cellulose acetate/ silver-sulfadiazine nanofibers composites were prepared and used for wound dressing applications [21].

In our previous studies we aimed to synthesize new biologically active compounds [22-25], Non-toxic antibacterial cyanoacetyl microcrystalline cellulose (NACAMC) was synthesized utilizing rice straw pulp [26-28]. In the present work, this novel green biologically active compound (NACAMC) was blended with cellulose acetate in an attempt to prepare antibacterial membranes and studying their chemical and physical properties..

2. Materials and experimental techniques

2.1. Materials:

The following Chemicals were used: cellulose acetate with an acetyl content of 39.8 (Acros Organic company, New Jersey, USA and N,N-dimethyl formamide (DMF), were analytical grade-products purchased from Sigma-Aldrich, Merck. The novel non-toxic antibacterial cyanoacetyl microcrystalline cellulose (NACAMC) was prepared in our lab.

2.2. Membrane preparation:

The CA/(NACAMC) membrane has been prepared by wet phase inversion process as shown in Figure 1. The polymer solution has been prepared by dissolving CA and (NACAMC) in DMF. The composition of CA/(NACAMC) membranes are shown in Table 1. The homogeneous polymer solution was casted on a glass plate substrate using a film applicator. Then, the casted membrane was immersed in a coagulation bath (pure water) at $20 \pm 2^\circ\text{C}$. As the final stage, the prepared membranes were dried at $80-90^\circ\text{C}$ for 10 minutes.

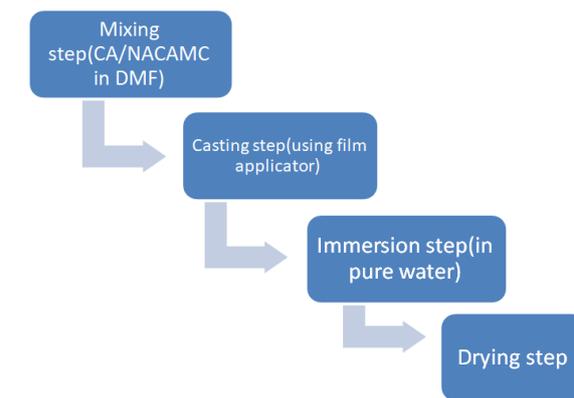


Figure 1: Block flow diagram for CA/(NACAMC) membrane preparation by phase inversion process.

Table 1

Composition of CA/(NACAMC) membranes.

Membrane	CA%	(NACAMC)%	DMF%
M _{blank}	15	0	85
M ₁	19.3	3.8	76.9
M ₂	11.9	4.76%	83.33
M ₃	8.33	8.33	83.33

2.3 Membrane characterization:

2.3.1 Membrane Morphology:

Scanning electron microscopy (SEM) was used to examine the morphology of CA/(NACAMC) casted membranes; however, samples of membranes were coated with gold to provide electrical conductivity. After that, these membranes were studied by Scanning Electron Microscopy of Model JEOL-JXA-840A and were applied at different kV with magnification 10x up to 400,000x.

2.3.2 Water Uptake and Membrane Stability

Water uptake was measured at room temperature for the prepared membranes. The prepared membranes were soaked in water at room temperature for 24 h to determine the water uptake ratios to detect their ability to be used in desalination membranes.

$$\text{Water Uptake ratios } \% = 100 * \frac{(m_1 - m_0)}{m_0} \% \quad (1)$$

m_0 and m_1 are the weights of dry and wet membranes (g) respectively, To minimize the experimental errors, the membrane uptake of each sample was measured several times and the results were reported as average use equation (1).

The membrane stability with pressure and water flow rate was tested using membrane testing set-up as shown in Fig.2 the water tank was filled with pure water; the water is pumped at different pressures through the prepared membrane. Then, Water tank was filled with a synthetic concentration of NaCl solution (5000 ppm), the saline solution is pumped at different pressures (0:15 bar) through the prepared membranes (M_2). The assessments of membrane performance as a function of several operating variables has determined using performance testing unit. The prepared membrane has tested to check their mechanical strength under different operating pressure. The performance tests have been carried out at ambient temperature under an operating pressure up to the required pressure. The permeate water flux (F) of the tested solution is determined from the following equation:

$$F = (m/a \cdot t) \quad \text{kg/m}^2 \cdot \text{t} \quad (2) \quad [16]$$

Where; m: permeate mass in kg, a: membrane active area in m^2 , t: time in hour

The rejection (R%) percentage can be calculated as follow:

$$R\% = \frac{(C_f - C_p) \times 100}{C_f} \quad (3) \quad [16]$$

Where;

C_f : the concentration of the feed solution, C_p : the concentration of the permeate

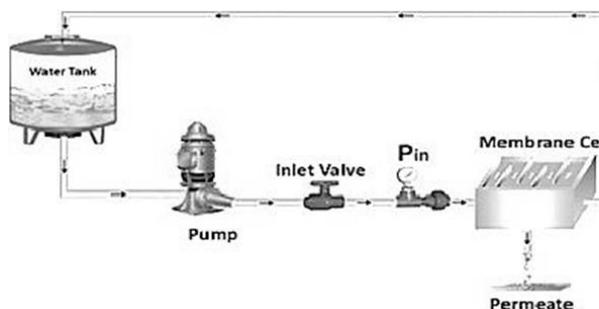


Figure 2: The schematic of membrane testing set-up.

2.3.3 FT-IR spectral analysis (FT-IR):

FT-IR spectra of the prepared compounds were obtained with a JASCO FTIR-4100 E FT-IR spectrometer (Japan) operating in absorption mode, in the wavenumber range of $4,000\text{--}400\text{ cm}^{-1}$ by the prepared compounds mixing with KBr (potassium bromide) discs and using a model ATR PRO450-S single reflection measuring attachment.

FTIR spectrum has been extensively used in our research since it presents a relatively easy method of obtaining direct information on chemical changes that occur during the preparation of membranes obtained by mixing acetyl cellulose with different concentrations of (NACAMC) using the casting technique.

2.3.4 Mechanical properties

Mechanical properties of prepared membranes M_1 and M_2 were determined using a mechanical testing system (INSTRON-5500R, USA) [29].

2.3.5 Determination of the Surface Area and Pore Size of membranes

BET surface area, mean pore diameter, and total pore volume were determined using the Brunauer-Emmett-Teller (BET) method by the BET apparatus of a model (ChemBET-3000, Quanta-chrome, USA). Samples of a known weight of the membranes were cut into long strips and placed in a glass column of the apparatus, dried, and degassed by heating at 80°C for 3 hrs. The average area was determined using the BET single point.

2.3.6 Antibacterial activity of (NACAMC):

Petri dishes including freshly prepared sterilized nutrient agar medium for bacteria or potato dextrose agar for yeast (cold to 40°C) were prepared. Freshly prepared cell culture from *Candida albicans* ATCC 10231, *Bacillus cereus* ATCC 33018, *Escherichia coli* ATCC8739, *Pseudomonas aeruginosa* ATCC 9027, and *salmonella sp* were inoculated ($10\ \mu\text{m}$) in each Petri dishes and shake vigorously. The sterilized paper discs (6 mm) with the required doses of the tested samples were put on the Petri dishes surface. They were incubated at 30°C for 24 h then; the inhibition zone diameter was measured. The ability to inhibit the growth of Gram-negative bacteria and yeast was observed using an overlay method [30].

3. Results and discussion

3.1 Membrane Morphology

In this work, CA/(NACAMC) membranes were successfully prepared via casting method with a suitable solvent and water as a non-solvent. The morphology of the prepared M_{blank} , M_1 , M_2 , and M_3 membranes was studied by SEM as shown in Fig. 3, (a-d). It is obvious from the cross-sections that all

the membranes had asymmetric structure. On the other hand, the prepared membranes exhibit different structures function in different percentages of CA/(NACAMC). Mblank has a top layer (Fig. 3a) supported by a spongy-like structure at the intermediate underneath of bottom layers. M1 and M2 have a top layer (Fig. 3b,c) supported by a finger-like structure underneath, followed by a big macrovoids structure at the intermediate underneath of the bottom layer. M3 has a top layer (Fig. 3d) supported by a spongy-like structure underneath, followed by a big macrovoids structure at the intermediate underneath of the bottom layer. And it can be observed that the bottom layers of these membranes are porous and the top layers are less porous. High precipitation rates (small gelation times) lead to form asymmetric membranes with a “finger” like structure and low precipitation rates (long gelation times) lead to form asymmetric membranes with a “spongy” structure.

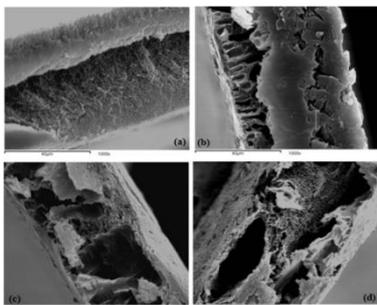


Figure 3: SEM views of the prepared membranes (a) Mblank (b) M1, (C) M2, and (d) M3.

3.2. Water uptake and membrane stability

Water uptake of the membranes at room temperature determined by the amount of water taken up by membranes immersed in water. The ratio of water contents was determined for three membranes as, 175 % for M1 (the membrane can absorb more than 1.7 times of its weight) 426% is the water uptake of M2 (the membrane can absorb more than 4 times of its weight), and 707% for M3 (the membrane can absorb more than 7 times of his weight). The results reveal that increases in the presence of (NACAMC) in the membrane seem to increase the hydrophilic properties of the membrane which subsequently, lead to the increase in its water uptake and its swelling as shown in Fig. 4 these results could be developed to be used in membranes for water purification via pressure driven membrane processes or electro dialysis techniques. Fig. 4 showed that the swelling percentage % reached a steady-state after 1 day for membranes M1 and M2.

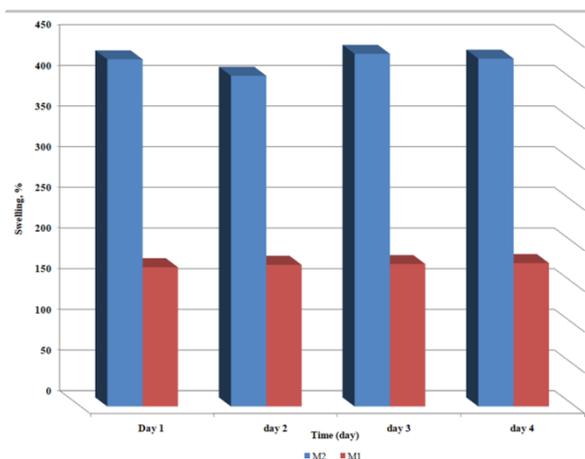


Figure 4: Effect of time on membrane swelling %.

From the result obtained in water uptake; thus this type of membranes could be applied in water treatment. Therefore, these membranes have been tested with membrane testing set-up apparatus. The membrane stability with operating pressure and water flow rate with membrane testing set-up apparatus showed that the membrane are stable at the working pressure without cracking or broken. The membrane flux was in average 125 kg/ m² t, and salt rejection was 45 to 37% for M2 membrane at operating pressure 5 bar and synthetic concentration of NaCl solution 5000 ppm. That results show that the prepared membranes could be developed to be used for water treatment purposes.

3.3. FT-IR spectral analysis:

The IR spectra of (NACAMC) Fig. 5 showed, the strong absorption OH broadband at ν 3435cm⁻¹, the presence of this band is attributed to the fact that chemical modification is not always sufficient enough to acetylate all the cellulose hydroxyl groups. The absorption at ν 2925 cm⁻¹ attributed to CH and CH₂ stretching. Cyano group Absorption was detected at ν 2220 cm⁻¹ and the carbonyl absorption detected at ν 1739cm⁻¹ as broadband attributed to the acetyl and the cyanoacetyl CO groups.

Spectroscopes of CA/(NACAMC) casted membranes showed a decrease in intensity of OH broadband at ν 3465 cm⁻¹ and the presence of the cyano group.

CA/(NACAMC) casted membranes containing investigated compounds revealed a shift in the wavelengths of the characteristic bands as well as a change in their intensity. It seems that this shift is attributed to the chemical binding of the investigated compound to the membrane.

3.4. Flexibility of membranes and Their Mechanical properties

A comparative study on the membranes with the naked eye revealed that the membrane M2 was flexible and foldable as shown in Fig. 6. It seems that the incorporated (NACAMC) enhances these properties and when the concentration of (NACAMC) increases the membrane becomes fragile and not flexible as in M3.

The Mechanical properties of prepared membrane M1 and M2 were determined (see Table 2). The tensile strength and elongation are higher in M2 than M1, which indicates that M2 has good mechanical properties. Further concentration of (NAMCC) caused the membrane (M3) to be fragile.

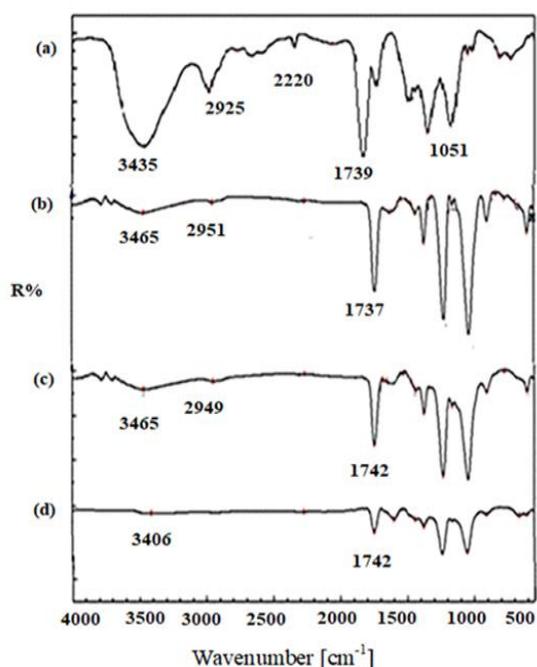


Figure 5: FT-IR (a) (NACAMC), (b) M 1,(c) M2 and (d) M3.

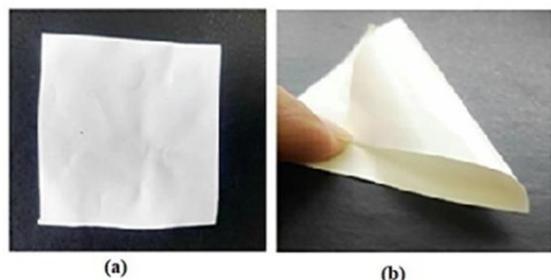


Figure 6: The stability of the membrane (a, b) M2.

Table 2

The Mechanical properties of prepared membranes M1 and M2.

Membrane Type	Elongation%	Tensile strength (N)
M1	5	5.7
M2	6.5	8.8

3.5. Surface area and pore size analysis

Surface area, as well as mean pore diameter and total pore volume, could be determined using the BET analysis (see Table 3). It could be noticed that with the increase of the NAMCC concentration, a decrease in the specific surface area, the pore volume and pore diameter of membranes occurred, probably due to the partial occupation of pores volume by NAMCC [31]. Also, the average pore size of the membranes, which ranged from 2–50 nm is categorized into mesoporous membranes [32].

Table 3

The average pore size distribution for prepared membranes.

Membrane Type	BET area m ² /g	Total pore volume (cm ³ /g)	Sample weight (g)	Mean pore diameter (nm)
M ₁	16.1	0.02237	0.1636	5.5567
M ₂	15.27	0.0204	0.1429	5.3707
M ₃	12.61	0.01627	0.1656	5.1617

3.6 Antibacterial activity

Antibacterial activity for membranes and cellulose acetate against 5 pathogenic strains namely: *Candida albicans* ATCC 10231 as yeast and four bacteria including *Bacillus cereus* ATCC 33018, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, and *salmonella* were evaluated. The results in Table 4 show that (NACAMC) has antimicrobial activities against all the tested pathogens with a degree of variation. *Bacillus cereus* recorded the highest inhibition zone (15mm) while *Pseudomonas aeruginosa* recorded the lowest inhibition zone (11mm). All tested strains could not grow on membranes M1 and M2 even M3 revealed an inhibition zone. Also when the tested samples were incubated for 3 weeks, all tested strains could not grow on the membranes. On the other hand, all tested strains grew on cellulose acetate [33].

This result indicated that the antimicrobial activity of (NACAMC) could be extended to the membranes, making membranes safe to be used in many industrial aspects and overcome the problem of reducing the

activities of the preservatives which happened when use cellulose acetate only in membranes [34].

Table 4.

The Inhibition zone (mean diameter of the disc in mm) as a criterion of the antibacterial activities of NAMCC and membranes.

	Tested strains			
	(NAMCC)	M ₁	M ₂	M ₃
<i>B. cereus</i> ATCC 33018	15	^a -ve	-ve	5
<i>Escherichia coli</i> ATCC 8739	12	-ve	-ve	-ve
<i>Pseudomonas aeruginosa</i> ATCC 9027.	11	-ve	-ve	-ve
<i>Salmonella sp.</i>	11	-ve	-ve	-ve
<i>Candida albicans</i> ATCC 10231.	13	-ve	-ve	-ve

^a-ve: means no microbial growth on the membrane.

4. Conclusion

This study presents the blending of novel nontoxic semi-natural antibacterial biodegradable cyanoacetyl microcrystalline cellulose (NACAMC) with cellulose acetate affording antibacterial membranes. Based on obtained results membrane M₂ could be used in wound dressing and hygiene food packaging materials. In this context, it's worth mentioning that the antibacterial function of these membranes does not affect after keeping it for one year in a dry atmosphere or water. The water uptake and permeability of these films indicate that this membrane could be developed for water treatment systems.

Conflicts of interest

“There are no conflicts to declare”.

Acknowledgment

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