Electrospun Polyamide6–Chitosan Nanofibers Reinforced with Zinc Oxide Nanoparticles and Functionalized MultiWalled Carbon Nanotubes: Some Physical, Mechanical Properties and Bacterial Rejection

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Abstract: With the fast development of nanotechnology, the preparation of electrospun nanofibers received increasing interest. Because of their large specific area and superior functional abilities, electrospun nanofiber membranes play an essential role in various fields, particularly in wastewater treatment. The goal of this study is to develop bacterial rejection membranes of Polyamide6/Chitosan nanofiber membranes that have pores larger than some bacterial cell types present in wastewater. Polyamide6/ Chitosan nanofiber membranes were prepared by electrospinning technique. The fabricated filters were characterized using Field emission scanning electron microscopy (FeSEM), water contact angle assessment. The diameters of nanofibers in the range of (116.88 – 237.34) nm were achieved. The bacterial removal efficiency of Polyamide6/ Chitosan nanocomposites on coliform and streptococci were studied. The results showed that Polyamide6/ Chitosan/1.5% Zinc Oxide nanoparticles, and (20 – 25) min. of reaction time resulted in the best removal efficiency: 99 % for coliform and 99.99 % for *Streptococci*. The bacterial cells were entrapped and damaged after passing through the membrane, as seen by Fe-SEM images. As a result, these PA6/ Chitosan nanocomposites membranes appear promising for decontamination of water.

Keywords: Electrospinning, Wastewater Treatment, Bacterial Rejection, Electrospun Nanofiber Membranes

1. Introduction

Electrospun nanofiber membranes (ENMs) are the newly developed class of membranes that give rise to a novel way to the treatment of wastewater [1]. The main characteristics of this appearing technique such as lesser energy consumption, cheaper and lighter process in comparison to the present traditional techniques. High porosity and surface-volume ratio is also two of this technique's main benefits [2]. Electrospun nanofibers' structure offers great potential in terms of permeability, selectivity, and low fouling. [3].

Chitosan is a common biopolymer. It has several uses in microbial bio-sorption and the removal of metals from wastewater because it is the only naturally occurring cationic polymer [4]. Additionally, it's amine groups is correlated with high rate of antibacterial activity [5,6].

Carbon nanotubes (CNTs) have been widely exploited as adsorbents for microbial, inorganic and organic wastewater pollutants and act as scaffold in wastewater filtration systems [7,8].

ZnO NPs has diverse morphologies and has substantial antibacterial efficiency against a diverse range of bacterial species studied by a large group of researchers. ZnO is being recognized as an antibacterial agent for both microscale and nanoscale formulations [9].

Inclusion of nanofiller applied in combination with chitosan was also described as antibacterial substances, enhancing the total inhibitory zone around the membranes [10]

2. Materials and methods

2.1. Materials

Carboxyl Multiwalled carbon nanotubes and Zinc Oxide nanoparticles purchased from (VCN materials, Iran) and (Sky Spring nanomaterials, USA) respectively, polyamide6 obtained from (Changfeng chemical, China) and chitosan (medium molecular weight) supply from (Glentham / UK), formic acid purchased from (Alpha Chemika, India).

2.2 Preparation of electrospinning solutions

Polyamide6 (25gm) and (3gm) chitosan powder were dissolved in 100 ml formic acid at room temperature applying magnetic stirrer overnight, the ratio of polyamide6 / chitosan was (98.5 / 1.5 vol / vol) [11], then the ratio of (wt./ vol. 1.5 Multiwalled Carbon Nanotubes ,1.5 Zinc Oxide Nanoparticles) was added to the polymers' solution and stirred for 1 h. to form three solutions Polyamide6/Chitosan, Polyamide6/Chitosan/1.5% Zinc Oxide Nanoparticles and Polyamide6/ Chitosan / 1.5% MultiWalled Carbon Nanotubes. After that each solution was homogenized for 15 minutes with a homogenizer (300 VT Ultrasonic Homogenizer / USA) to prepare various nanofiber membranes.

2.3. Electrospinning technique

The prepared solutions were placed in 10 ml syringe fitted with a (22 gauge) stainless steel needle tip. The electrospinning process was performed at a voltage of 25 KV and a flow rate of 1 ml / h. The needle tip was 0.8 mm in diameter, and the distance was 15cm between electrodes [12]. The process parameters were identical for all solutions. The fibers were dried and kept until used.

2.4 Characterization of nanofibers

The nanofibers surface was sputtered with Ag to improve electrical conductivity and then examined using field emission scanning electron microscope (Fe-SEM) (model Inspect

F50, Spain). Average fibers diameter and average pore size were statistically analyzed using Fe-SEM images, image J and Origin 2021. Each image has a total of 100 data points. EDX was used to identify the membranes' elemental composition [13].

2.5 Contact angle

To assess hydrophobic/ hydrophilic properties of the membranes, the contact angle was obtained using (optical contact angle, China). A video camera was used to record the change of the droplet shape after 1 µl of DD was placed on the membrane surface. The measurements were taken at three different locations on the membrane surface, and the mean WCA was calculated [10].

2.6. Mechanical properties

The mechanical characteristics of the membranes were determined using a tensile mechanical tester (Tinus Oslen, H50 KT) which included a 5N load cell and the rate of extension was 0.5 mm / min. The experiment repeated three times to obtain the results average value [14].

2.7. Bacterial rejection

Bacterial rejection by prepared electrospinning membranes was also examined by immobilizing nanofiber membrane onto Millipore filter under vacuum filtration. A wastewater used was collected from Al-Azizya treatment plant/ Wasit province. Following that, waste water samples were filtered via the membranes. Then the bacterial densities include (coliform, F. coliform, streptococci and F. streptococci) were calculated using the diluting, spreading, incubating and counting of bacterial colonies numbers techniques described below [12].

For total coliform, Fecal coliform, *streptococci* and *Fecal streptococci* count, the most probable number method, described by [15], was used to dilute sample with normal saline. Using MacConkey broth as the cultured medium and for gas detection, the Durham tube was used (for both coliform and fecal coliform) and Azide Dextrose broth (for streptococci and fecal streptococci). the tubes were incubated for 24-48 hrs. at 37℃.

The results were calculated by using the equation:

Numbers of cells/ ml = Most probable number× reverse of mild dilution (1)

The antibacterial capabilities of the filters are expressed as a reduction percent (%) as follow:

Reduction percent (%) = (A-B)×100/A (2)

Where A and B = the numbers of viable bacterial cell before and after treatment, respectively. [16].

2.8. Statistical analysis

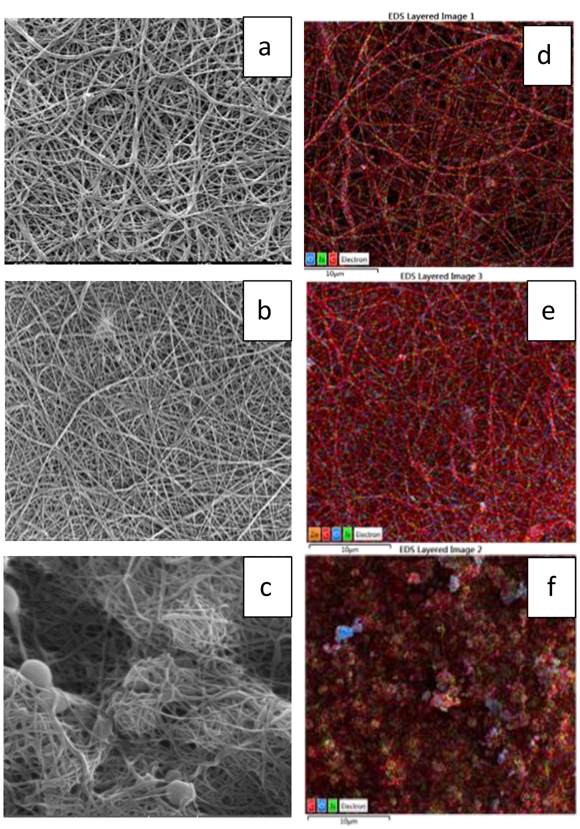
The data were examined utilizing Origin 2021 program. Data are provided as Mean ± SD. the Kruskal-Wallis test was applied to compare reduction efficiency. P< 0.05 was thought to be statistically significant.

3. Results and discussion

3.1. Characterization of polymer nanocomposite fibers

The structure of the prepared electrospun fibers was homogenous, randomly arranged with lacking of beads in polyamide6/Chitosan and Polyamide6/ Chitosan /1.5% Zinc Oxide Nanoparticles membranes, **Figure 1. (a and c)**, while less beaded fibers were shown in **Figure 1. (b)** for Polyamide6 / Chitosan / 1.5 % Multi-Walled Carbon Nanotubes membrane.

The nanofibers contain C, N, O, Zn as a result to usage of (ZnO, MWCNTs) according to EDX analysis **(Figure1. d, e, f)**.



The average fiber diameters and the average pore size for the prepared membranes are illustrated in **Figure1**. **(g)**. Added of ZnO nanoparticles had little effects on the average diameter of Polyamide6/ Chitosan fibers. Because of the increased conductivity of their spinning precursor solution, the fibers containing MWCNTs have the lowest diameter (116.86) nm [17].

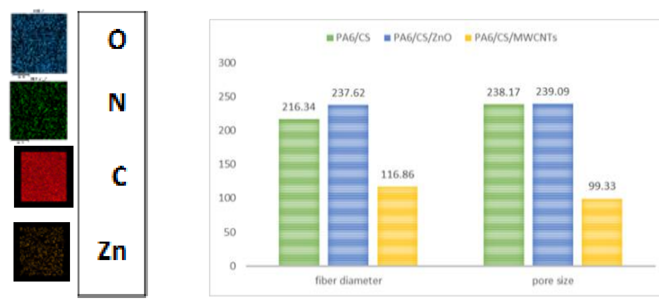


Figure1. (a, b and c) SEM images, (d, e and f), EDX, (g) Average fiber diameter and average pore size of Polyamide6 / Chitosan, Polyamide6 / Chitosan / 1.5 % Zinc Oxide Nanoparticles and Polyamide6 / Chitosan / 1.5 % MultiWalled Carbon Nanotubes Membranes.

3.2 Contact angle

Figure 2. display the contact angle value of Polyamide6 / Chitosan, Polyamide6 / Chitosan / 1.5 % Zinc Oxide Nanoparticles and Polyamide6 / Chitosan / 1.5 % MultiWalled Carbon Nanotubes membranes. The results show that the contact angle decrease with adding ZnO and MWCNTs respectively, which means it is more hydrophilic over blend nanofibers hydrophilicity, this is may be due to the hydrophilic behavior of ZnO NPs and raise

the surface roughness [18] or because of tunnel structure and MWCNTs modification [12, 19].

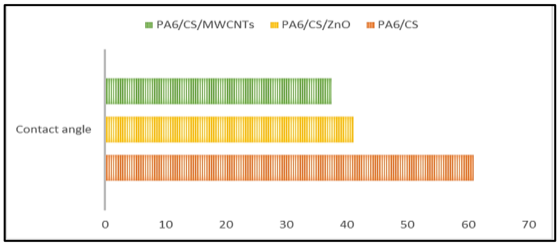


Figure2. Contact angle of Polyamide6 / Chitosan, polyamide6 / Chitosan / 1.5 % Zinc Oxide Nanoparticles and (Polyamide6 / Chitosan / 1.5 % MultiWalled Carbon Nanotubes Membranes.

3.3 Mechanical properties

The result of mechanical properties for Polyamide6 / Chitosan membrane of different compositions (PA6 / Chitosan, Polyamide6/ Chitosan / 1.5% ZnO and Polyamide 6/ Chitosan / 1.5 % MWCNTs) were examined to show the influence of different additives on the membrane mechanical behavior, results are displayed in **Figure 3.**

The results show slightly enhance in mechanical properties when added ZnO to the (Polyamide6/ Chitosan) membrane. This behavior is consistent with previous research, which indicates that a low quantity of ZnO and a sufficient dispersion of NPs enhanced the mechanical properties of nanocomposite [20].

The tensile test revealed that incorporating Multiwalled Carbon nanotubes in (Polyamide6 / Chitosan) membrane produced nanocomposite with significant improved mechanical properties. This is because MWCNTs have superior mechanical characteristics. Furthermore, functionalization of MWCNTs improved its dispersion in PA6 / Cs matrix and that agree with [12].

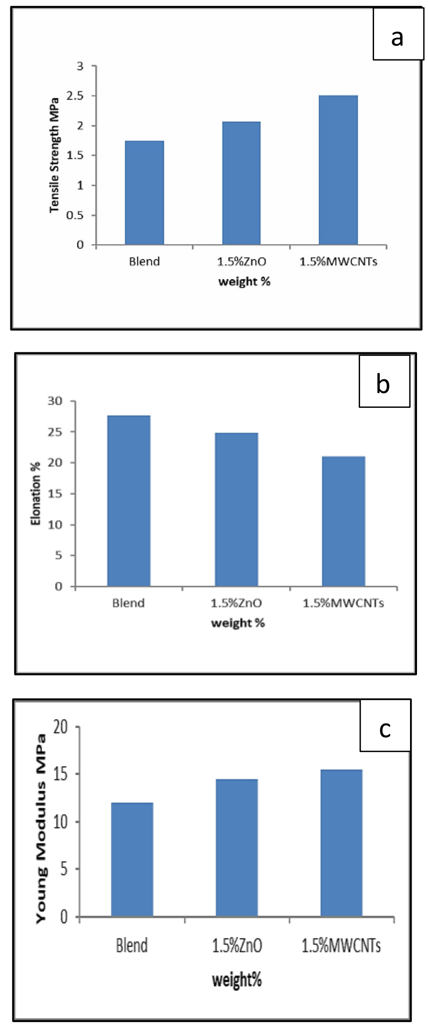


Figure 3. (a, b and c) Polyamide6/Chitosan, Polyamide6/Chitosan/1.5% Zinc Oxide Nanoparticles and Polyamide6/Chitosan /1.5%MultiWalled Carbon Nanotubes Membranes.

3.4 Bacterial rejection

The antibacterial activity of all prepared electrospun Polyamide6 / Chitosan, Polyamide6

/ Chitosan / 1.5 % Zinc Oxide Nanoparticles and Polyamide6 / Chitosan / 1.5 % MultiWalled Carbon Nanotubes nanofiber membranes against Coliform, Fecal coliform, Streptococci and Fecal streptococci were recorded in **Table1.**

In **Figure 4.** the results show that the highest reduction percent was above 99 % for tested bacteria (both gram-positive and gram-negative bacteria) after filtration of polluted water through Polyamide6 / Chitosan / 1.5% ZnO membrane filter, while the lowest reduction percent was 10% when using Polyamide6/Chitosan membrane as filter for gram negative bacteria and 25% for gram-positive bacteria.

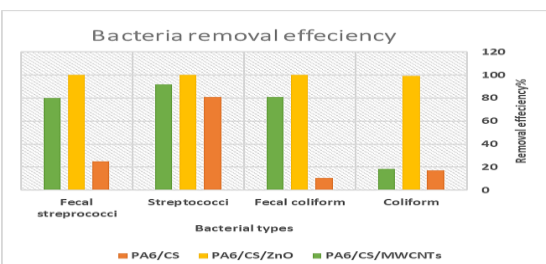


Figure 4. Bacteria removal efficiency by Polyamide 6/ Chitosan, Polyamide 6/ Chitosan/ 1.5% Zinc Oxide Nanoparticles and Polyamide6 / Chitosan / 1.5 % MultiWalled Carbon Nanotubes Membranes.

Most water-borne bacteria have a diameter bigger than 0.2 µm, as an example the size of E. coli is 0.5 – 2.0 µm and streptococci size is 0.5 – 1.2 µm. Since this membrane average pore size is less than the size of both bacterial types. Because of their large size, the bacterial cells entrapped in the electrospinning nanocomposite membrane, **Figure (5. a and b)**. In general, electrospinning nano-membrane eliminate all types of used bacteria including Escherichia coli, fecal coliform, etc... This result agrees with [21].

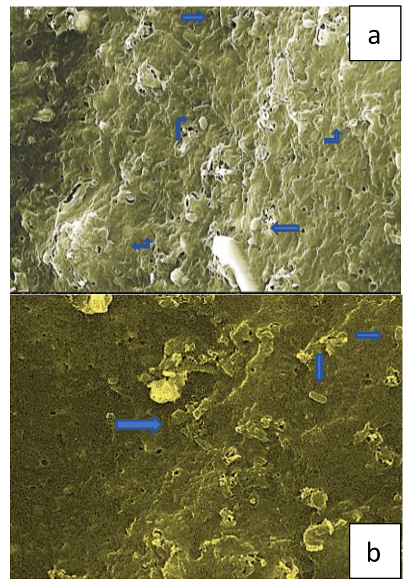


Figure 5.(a, b) Bacteria entrapped within nanofiber membrane.

Also, the results show that the three filters antibacterial activity was better for gram positive bacteria in comparison with gram negative bacteria. This maybe explain by the differences in the cell membrane components of these bacteria and this agree with [22] results and did not agree with [8].

However, complete microbe removal is not attainable since NPs may not be available to specific bacterial cell. In addition, the environment of work where the permeate was collected could have been contaminated with bacteria, which is normally inevitable [23].

Conclusion

Electrospinning technique was used to prepare Polyamide6 / Chitosan / MultiWalled Carbon Nanotubes and Polyamide6/ Chitosan / ZnO nanofiber membranes. The ions are effectively combined with the electrospun fibers, as seen by Fe-SEM and EDX results. Polyamide6 /Chitosan/ ZnO nanocomposites filter outperformed Polyamide6 / Chitosan and Polyamide6/ Chitosan / Multiwalled Carbon Nanotubes filters in terms of bactericidal activity. The rejection percentage for all types of used bacteria obtained with the Polyamide6 / Chitosan / 1.5 % (ZnO) membrane was above 99 %, which was higher than the rejection percentage obtained with Polyamide6 / Chitosan and Polyamide6/ Chitosan / 1.5% MultiWalled Carbon Nanotubes membranes respectively.

Hydrophilicity and mechanical strength of the Polyamide6/ Chitosan blend membrane increased when adding nanoparticles, and this can reduce antifouling impact, making the mat a viable candidate for water filtration and improving the membranes' efficiency at low pressure.

Because of their Nano sized pores, these nanofiber membranes significantly removed bacteria such as fecal streptococci and fecal coliform.

Table 1. Removal of (Coliform, Fecal coliform, Streptococci and Fecal streptococci) bacteria by Polyamide6 / Chitosan, Polyamide6/ Chitosan / 1.5 % Zinc Oxide Nanoparticles and Polyamide6/ Chitosan / 1.5 % MultiWalled Carbon Nanotubes Membranes.

Parameter	Polluted water	Polyamide6/ Chitosan	Polyamide6/ Chitosan/ 1.5% ZnO	Polyamide6/ Chitosan /1.5%MWCNTs
Fecal streptococci cell×10^3/ 100ml	20 ± 1 a	15 ± 1 a	0.0013 ± 0.0011 b	4 ± 1 ab
Streptococci cell 10^3/ 100 ml	1100 ± 414.1 a	210 ± 23.07 b	0.002 ± 0 b	93 ± 4.35 b
Total Coliform cell×10^3/ 100ml	1100 ± 163.7 a	912 ± 17.09 a	11 ± 1 b	895 ± 41.76 a
Fecal coliform cell×10^3/ 100ml	1100 ± 128,8 a	982 ± 100,47 a	0,002 ± 0,0017 b	210 ± 45,9 b

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