Synthesis of An Amidoximated Acrylic Copolymer Membrane (AACM) Treated with Nano Silver Particles to Study the Antibacterial Efficiency of The Membrane

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Abstract

Polymers with cationic functional groups are proven to be an effective antimicrobial barrier. Particles, organic debris, and microbes can be well removed from drinking water using polymer membrane filtration. A variety of copolymers are utilized in the synthesis of such membranes. In this study, a copolymer membrane was synthesized first, and then Amidoximation was used to convert a portion of the copolymer's nitrile groups to amidoxime groups. The membrane was treated with HA at 60-80°C in an aqueous solution to obtain an Amidoximated film. After being treated with Nano Silver particles, the efficiency of the prefabricated and particularly produced copolymer film as an antibacterial water disinfectant was analyzed. Various techniques were used to analyse the virgin and Amidoximated Acrylic Copolymer Membranes (AACM), including X-ray diffraction (EDX), Fourier Transform Infrared (FTIR), Scanning Electron Microscope, Transmission Electron Microscope, and Thermogravimetric analysis. Many substantial changes in the copolymer characteristics, including functionality, crystallinity, thermal activity, hydrophilicity, elemental composition, surface morphology, and antibacterial activity, were identified during the Amidoximation phase. It was revealed that an Amidoximated copolymer membrane outperforms an untreated membrane in terms of antibacterial activity.

Keywords: Copolymer membrane, Amidoximation, Nano Silver Particles, Functionality of Fabricated Copolymer Membrane, Antibacterial Activity

1. Introduction

Water is considered life on this planet and is essential to the Earth's climate and the evolution of life [1]. Costal seawaters are the critical source of stool bacteria, including several diseases [2,3]. The management of microorganisms such as bacteria, fungus, and yeast in nature is one of the key elements for the survival of higher species [4]. Membrane filtration is an efficient way to remove particulates, organic materials, and microbes from drinking water. Polymers with cationic functional groups are proven to be an effective antimicrobial barrier. Particles, organic debris, and microbes can be well removed from drinking water using polymer membrane filtration. Copolymers are commonly used to synthesize such membranes. A variety of copolymers are utilized in the synthesis of such membranes. The membrane technique is superior to other approaches for improving water quality [5]. Development and advancement of new long-acting antimicrobial polymeric surfaces is always necessitated for destroying harmful germs and preventing infections in drinking water. Low molecular weight antimicrobial compounds have been proven ineffective in applications such as surface coatings, blends, and composites [6]. Over the last two decades, several
researchers have developed and extensively tested antimicrobial polymers against Gram-positive and Gram-negative bacteria, viruses, yeast, and fungus species [7]. Positively charged polymers attract negatively charged bacterial cell surfaces. The membrane-forming capabilities of polyacrylonitrile (PAN) and acrylonitrile-based copolymers are remarkable in this concern as they are chemically resistant to most solvents. They also have strong thermal and mechanical stability, making them more effective in membrane production [6, 7]. PAN membranes have also been employed as membrane bioreactors for protein filtration [8, 9]. PAN hollow fiber membranes are already used in dialysis machines that focuses on molecular protein removal and high-flux dialysis therapy. Surface area, zeta potential, pore diameter, surface roughness, and antibacterial agent inclusion are all elements that influence a membrane's antibacterial effectiveness [10, 21].

On the other hand, it has been demonstrated in several prior studies that antibacterial activity is considerably required in nanofiber membranes for biomedical filtration devices in order to minimize bacterial contamination under moist conditions while retaining biocompatibility [11]. The copolymerization of acrylonitrile (AN) with various acrylic and vinyl monomers during the polymerization process has been significantly described in the literature [11-13]. Methacrylic acid, acrylic acid (AA), itaconic acid, acrylamide, and styrene are the most common monomers used for such interventions. Researchers [14] have already discovered several natural and artificial nanomaterials as antimicrobial agents in water disinfection systems, including chitosan, photo catalytic titanium dioxide, fullerene, gold, silver nanoparticles [15]. Due to their electrical characteristics, optical properties, biological qualities, and thermal conductivity Nano-Silver (AgNPs) particles are considered as a best alternative for the purpose [16]. NS (Nano-Silver) particles can effectively destroy and inactivate a wide variety of microorganisms as described in the previous researches [17-19]. The produced AgNPs and functionalized PAN nanofibers (PAN-AgNPs) have significant antibacterial activity and long-term durability against Escherichia coli and Staphylococcus aureus bacteria. A similar approach was used in the current research to fabricate and synthesize a copolymer film after the effective treatment with Nano Silver particles to determine its effectiveness as a water disinfectant. Nanofiber membranes, in particular, are a promising filtration platform for vapors and gases, as well as excellent filtering efficiency, in a variety of applications ranging from interior air filters to personal protective equipment. Also, the major objectives of the present work were to perform the Amidoximation of acrylic copolymer membranes, then to characterize the virgin and the Amidoximated acrylic copolymer membranes by using various techniques to study the efficiency of the synthesized membrane in this process for its antimicrobial properties.

2. Materials and Methods

Toluene, Polyvinyl alcohol, Silver nitrate, Azobisisobutyronitrile, Acrylonitrile, Acrylonitrile, Acrylic acid, DMF (Dimethylformamide), NaOH (Sodium Hydroxide), HCl (Hydrochloric Acid), and other compounds were utilised in this study.

All of the chemicals were AnalR reagent grade, and they were purchased from Fluka and Sigma Aldrich. Silver nitrate (AgNO3), fructose, and polyvinyl alcohol were also given by Sigma Aldrich and Fluka (PVA).

All of the compounds were utilised in their natural state, without being purified. Before being used further, all of these solvents were dried and distilled using a conventional process [20]. Polymerization synthesis, Molecular Weight Determination, Copolymer Fabrication, Hydroxylamine treatment, and Amidoximes content analyses have all been published in one of our prior works [13].
2.1 Bulge Behaviour Determination

The slow diffusion of solvents into polymer chains, results in a swollen polymeric membrane. Bulge behaviour of copolymer membranes having Amidoxime (AO) content 3.5 meq/g was carried out with distilled water in water bath as shown in Table 1. After bulging, the excess water was removed by filter paper and the puffy sample was again weighted. The degree of bulging was calculated as [19]:

\[
\text{Bulge} \, (\%) = \frac{W_s - W_0}{W_0} \times 100
\]

Where, \( W_0 \) and \( W_s \) are the weight of the dry and swollen membrane, respectively.

| Abbreviations used | Chemical Details |
|--------------------|------------------|
| AO 3.5             | Acrylic copolymer membrane contains 3.5 meq/g amidoxime content. |
| NS320              | Nano Silver (NS) synthesized by 20-minute reduction time. |
| NS20               | NS synthesized by 40-minute reduction time. |
| NS60               | NS synthesized by 60-minute reduction time. |
| AO0.5+NS320        | AO 0.5, membrane contains NS formed by 20-minute reduction time. |
| AO0.5+NS60         | AO 0.5, membrane contains NS formed by 40-minute reduction time. |

2.2 Preparation of Nano Silver Particle (AgNPs)

Wet chemical method was used for the preparation of Nano silver (NS) particles. Silver nitrate was first converted to NS in an aqueous solution by reducing it with fructose. The solution of silver ions was prepared by dissolving 30 mg silver nitrate in 100 ml of distilled water under constant stirring condition at 25-30°C of temperature. Then fructose and polyvinyl alcohol were added in it as a stabilizing agent. Colourless solution changed into light yellow colour indicated the formation of NS particles. This solution was then kept in refrigerator at 4°C. Reduction reaction mechanism due to fructose can be given as [20].

\[ 2\text{Ag}^+ + \text{H}_2\text{O} + \text{CH}_2\text{OH}-(\text{CHO})_4-(\text{CHO}) \rightarrow 2\text{Ag}^0 + 2\text{H}^+ + \text{CH}_2\text{OH}-(\text{CHO})_4-\text{COOH} \]

2.3 Immobilization of Nano Silver Particles

To immobilize NS into the copolymer film, a fix weight of AO 3.5 meq/g copolymer membrane was first swelled into distilled water at 90°C in fixed temperature water bath. The swollen films were then instantly transferred into NS solution carrying vessel along with moderate shaking for few minutes. The manufactured films were then dried at 95-100°C for 120 minutes in vacuum oven and washed in distilled water to remove excess NS particles and other chemical impurities. The synthesized films were then once again vacuumed dried at 95-100°C for 240 minutes for further use.
2.4 Instrumentation Used

Synthesis of NS was confirmed by UV-visible Spectroscopy. UV-visible spectroscopy was carried out by Perkin Elmer Lambda E Z 201 spectrophotometer which was used for the determination of Plasmon peak of NS. Energy Dispersive X-Ray (EDX) studies of samples were carried out using STEREOSCAN 360 scanning electron microscope for the study of surface as well as fracture of NS immobilized copolymer. The morphological characteristics of NS particles were analysed using a PHILIPS CM -12 method, which was used to analyse the morphological characteristics of NS particles such as size and shape.

2.5 Antimicrobial Studies

All the samples were sterilized by γ-rays, so that pre-existing microbes can be eliminated. Viable cell count method was acquired to examine antimicrobial nature of samples. The American Association of Textile Chemists and Colourists (AATCC) 100 method was followed for this purpose. As this method is considered to be known to evaluate the antibacterial activity over the textiles. The antimicrobial activity was performed against model bacteria E. coli and S. aureus bacteria [23].

2.6 Method of counting colonies

Fresh colonies of E. coli or S. aureus were used to create a suspension in Muller Hinton Broth (MHB). Each of the samples (0.05 g) were inoculated with a 5 ml bacterial suspension in MHB containing 10^6 CFU/ml. Both of the samples were well shaken and incubated for 24 hours at 35-37°C. After 24 hours, the suspensions were shaken again, and dilute solutions were made, with the remaining bacteria enumerated using the spread plate method. 200 µl of the inoculum were uniformly spread on nutrient agar plate. The plates were incubated at 35-37°C for 24 hours, and the colonies were counted again. All of these tests were carried out in a sterile setting to avoid any contamination. Antimicrobial efficiency was expressed according to AATCC 100 and calculated as:

\[ R(\%) = \frac{A-B}{A} \times 100 \]

Where, A is the number of bacteria recovered from an inoculated test specimen after 24 hours of incubation with an untreated sample, B is the number of bacteria recovered with NS modified samples under "A" conditions, and R (percent) is the percent reduction ratio that indicates antimicrobial efficiency.

3. Results and Discussion

Bulge behaviour of membranes was carried out at different temperatures as shown in Table-2 to study the hydrophilicity in aqueous system (Fig. 1). From figure 1 it is clear that swelling of the membrane increases slowly up to 60°C and it increases rapidly beyond 60° and up to 70°C and once it passes 70°C the swelling enhanced quickly up to about 80°C and after it almost it is at the level off. This behaviour indicates that there is a sudden change in copolymer structure at about 70-80°C.
Figure 1: Bulge behaviour of membrane AO 3.5 meq/g at different temperatures

Table 2: Bulge behaviour of the membrane AO 3.5 meq/g at different temperatures

| Temperature (°C) | Bulge (%) |
|------------------|-----------|
| 30               | 36        |
| 40               | 39        |
| 50               | 42        |
| 60               | 43        |
| 70               | 46        |
| 80               | 75        |
| 90               | 83        |

Bulge measurement was carried out at 90°C temperature with respect to time and is given in “Fig. 2”.

Figure 2: Bulge behaviour of membrane AO 3.5 meq/g at 90°C with respect to time
The bulge linearly increases up to 2 hours and then tends to almost saturate (Table 3). The results indicate that almost maximum bulge is achieved only in 2 hours.

Table 3: Equilibrium swelling behavior of membrane AO 3.5 at 90°C with respect to time

| Time (min.) | Swelling (%) |
|------------|--------------|
| 30         | 59           |
| 60         | 72           |
| 120        | 77           |
| 240        | 75           |
| 360        | 87           |
| 540        | 90           |
| 720        | 81           |

3.1 UV-Visible spectroscopy

Results show the transformation of (silver) Ag⁺ ions in to NS (Ag) particles. A colourless silver nitrate solution turned in to light yellow solution and the colour shifted from light yellow to light green with the passage of time, which confirms the formation of NS particles in solution. The UV-visible absorption spectra of solutions with different reduction time were recorded in the range of 300-600 nm which confirmed it quantitatively and represented in Figure 3. The plasmon peak for NS with $\lambda_{\text{max}}$ values were obtained at 383, 395 and 404 nm for 20 min, 40 min and 60 min reduction time, respectively.

Figure 3: UV-visible absorption spectra of NS solutions.

During study it was found that with the progresses in reduction time, the absorption bands for NS broadened and shifted continuously towards larger wavelength which indicates the formation of larger NS particles.
3.2 Energy Dispersive X-ray Spectroscopy (EDX):

Fig. 4a and 4b shows the Energy Dispersive X-ray Spectroscopic (EDX) Results, which explain the qualitative elemental composition of virgin and Amidoximated samples.

![Figure 4a: EDX of Acrylic Copolymer Virgin And Membrane](image)

![Figure 4b: EDX Of Acrylic Copolymer A.O Content 3.3 Meq/G](image)

3.3 Transmission Electron Microscopy

During thorough observation for all the TEM images, NS particles appeared to be undispersed and spherical. The histograms for particle size distribution were obtained by counting 50 NS particles from TEM images for each sample as shown in Fig. 5. The analysis is represented in Fig. 6. The histogram shows the particle size ranges from 2.7-13.0, 2.4-14.7 and 5.4-16.1 nm respectively with mean diameter of 6.1, 8.7 and 11.2 nm with standard deviation of 1.8, 2.6 and 2.5 nm for NS,NS, and NS, respectively.
The variation in particle mean diameter with reduction time is presented in Fig. 7. It is interesting to see that with the increase in reduction time the mean particle diameter increases.
TEM and histogram show that as the reduction time increases, the NS particle intensity decreases, whereas particle mean diameter increases. Different studies suggested that nucleation leads to an increase in the number of scattering centers or the number of particles for a given system; therefore, it provides an increase in the scattered intensity. It is consistent with the mechanism of reducing Ag+ ions and the association of Ag° atoms to produce metallic Ag particles [21-22]. NS particle becomes more stable with the increased reduction time as the particles become more homogenous in size allocation.

3.4 Antimicrobial activity:

The antimicrobial activity of virgin as well as various NS containing samples were examined against model bacteria S. aureus (gram +ve) and bacteria E. coli (gram-ve) by an assessment of the number of viable colonies after being in contact with different samples for a period of 24 hours and are shown in “Fig 8, 9”. It can be seen that an average number of viable S. aureus and E. coli colonies decreased by 95% in both cases as compared to virgin AO ◄ membrane.

Figure 8: Antimicrobial activity of membranes against S. aureus (a) Control and (b) AO ◄ - NS ◄

Figure 9: Antimicrobial activity of membranes against E. coli (a) Control and (b) AO ◄ - NS ◄)
4. Conclusion

- Copolymer membranes having AO (Amidoxime) content with immobilized with NS particles were found to be very effective in the removal of microorganism impurities from drinking water.
- The EDX studies showed that the presence of NS in the membrane surface as well as in the inner side of polymer matrix increases the efficiency of the membrane further.
- TEM measurement of NS particles was carried out to analyse the morphological characteristics such as size and shape of NS and were successfully studied.
- As the antimicrobial activities of virgin as well as various NS containing samples were examined against gram positive bacteria S. aureus and gram-negative bacteria E. coli by the estimation of the number of viable colonies that shows the reduction of these bacterial colonies by the application of Amidoximated copolymer membrane.
- TEM measurement of NS particles was carried out to analyse the morphological characteristics such as size and shape of NS and were successfully studied.
- The reaction factors, such as temperature, time, and HA concentration, control the amidoxime content.
- The FTIR analysis revealed that the intensity of virgin and amidoximated acrylic copolymer declines with increasing AO concentration, confirming that as amidoximation occurs, the nitrile content of the copolymer reduces on a regular basis due to the conversion of nitrile groups to amidoxime groups. Also, in comparison to pure PAN, the presence of AA decreases the degradation temperature of virgin copolymer.

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