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Efficacy of Synthesized Amidoximated Acrylic Copolymer Membrane Treated with Nano Silver Particles for its Antibacterial Property

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Abstract:

Antimicrobial barriers are made of polymers of cationic functional groups.PolymerMembrane filtration is considered as highly effective method for removing particles, organic matter, and microorganisms from drinking water.These membranes are usually made from copolymers. In the present work, fabrication of a copolymer membrane wasdone first and a portion of the copolymer's nitrile groups is converted to amidoxime groups through the amidoximation process. After that the mebrane was treated with HA in an aqueous solution at 60-80°C to form an Amidoximated film. These fabricated and specially synthesized copolymer film after the treatment with Nano Silver particles were then studied for its efficacy as antibacterial water disinfectant. Several techniques such as Fourier Transform Infrared (FTIR), X-ray diffraction (EDX), Thermogravimetric analysis, Scanning Electron Microscope, and Transmission Electron Microscope were used to characterize the virgin and amidoximated acrylic copolymer membranes. Many significant changes in the copolymer properties, such as functionality, crystallinity, thermal activity, hydrophilicity, elemental composition, and surface morphology, were observed during the amidoximation phase along with their antibacterial activity. It was found that an amidoximated copolymer membrane is more efficient than the untreated membrane in context to its antibacterial activity.

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

I. Introduction:

Water is vital to the climate and the evolution of life on Earth [1].The primary source of stool microorganisms, including pathogens, is Costal seawaters [2,3]. One of the basic principles for the survival of higher organisms is the regulation of microbes such as bacteria, fungi, and yeast in nature [4]. Membrane filtration is a highly effective method for removing particles, organic matter, and microorganisms from drinking. In comparison to other approaches for improving water quality, the membrane approach is superior [5]. It is important to develop new long-acting antimicrobial polymeric surfaces that can destroy pathogenic microorganisms and prevent infections. In applications such as surface coatings, blends, and composites, low molecular weight antimicrobial agents are found to be ineffective [6].Antimicrobial polymers have been synthesized and tested against Gram-positive and Gram-negative bacteria, viruses, yeast, and fungiby several researchers in the past two decades extensively [7]. The negatively charged bacterial cell surface is attracted by

positively charged polymers.Polyacrylonitrile (PAN) and acrylonitrile-based copolymers have excellent membrane forming properties, and are chemically resistant to most solvents. Also, they have good thermal and mechanical stability that makes them more efficient for their usage in membrane formation [6, 7]. PAN membranes have also been used for protein filtration and as membrane bioreactors [8, 9]. Dialyzers that emphasize molecule protein removals and high-flux dialysis therapy already use PAN hollow fiber membranes. The antibacterial performance of a membrane is determined by a number of factors, including surface area, zeta potential, pore diameter, surface roughness, and antibacterial agent inclusion [10, 21]

On the other hand, in some previous researches it has been established that antibacterial activity is needed in nanofiber membranes for biomedical filtration devices to prevent bacterial contamination under moisture conditions while maintaining appropriate biocompatibility [11].The copolymerization of acrylonitrile (AN) with the inclusion of various suitable acrylic and vinyl monomers during the polymerization process is considerably documented in the literature [11-13]. These monomers mostly include methacrylic acid, acrylic acid (AA), itaconic acid, acrylamide, and styrene. Several natural and engineered nanomaterials have already been identified as antimicrobial agents by the researchers [14] in water disinfection systems, including chitosan, photo catalytic titanium dioxide, fullerene, gold, and silver nanoparticles [15]. The Nano-Silver particles consist of electrical properties, optical properties, biological properties, and thermal conductivity [16]. NS (Nano-Silver) have been found to effectively inactivate and kill a broad range of microbes [17-19].The fabricated AgNPs and functionalized PAN nanofibers (PAN-AgNPs) show substantial antibacterial efficacy against Escherichia coli and Staphylococcus aureus bacteria, along with long-term durability. In the current study a similar approach has been made to fabricate and synthesize a copolymer film after the treatment with Nano Silver particles to study their efficacy as water disinfectant.Nanofiber membranes, in particular, are a promising filtration platform that enables semi-permeable conduct for vapors and gases as well as high filtration efficiency for a wide range of applications, from indoor air filters to personal protective equipment.

II. Materials and Methods:

Toluene, Polyvinyl alcohol, Silver nitrate, Azobisisobutyronitrile, Acrylonitrile, Acrylic acid, DMF (Dimerthylformamide), NaOH (Sodium Hydroxide), HCl (Hydrochloric Acid), and other chemicals were used in the present research work. All the chemicals were of AnalaR (Reagent Grade) grade and were obtained from Fluka and Sigma Aldrich. Sigma Aldrich and Fluka also provided the silver nitrate (AgNO3), fructose, and polyvinyl alcohol (PVA). All of the chemicals were used as it is, without further purification. However, all of these solvents were desiccated and distilled by using standard method before using [20].The methods for the synthesis ofPolymerization, Molecular Weight Determination, Fabricationof Copolymer, Hydroxylaminetreatment and Amidoximescontentevaluations have been already published in our one of the previous studies [13].

Bulge Behavior Determination:

Swelling is caused by the slow diffusion of solvents into polymer chains, resulting in a swollen polymeric membrane. Bulge behavior 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 of 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.

Table 1: Composition of samples

$AO_{3.5}$	Acrylic copolymer membrane contains 3.5 meq/gamidoxime content.
NS_{R20}	Nano Silver (NS) synthesized by 20-minute reduction time.
NS_{R40}	NS synthesized by 40-minute reduction time.
NS_{R60}	NS synthesized by 60-minute reduction time.
$AO_{3.5}$-NS_{R20}	$AO_{3.5}$ membrane contains NS formed by 20-minute reduction time.
$AO_{3.5}$-NS_{R40}	$AO_{3.5}$ membrane contains NS formed by 40-minute reduction time.
$AO_{3.5}$-NS_{R60}	$AO_{3.5}$ membrane contains NS formed by 60-minute reduction time.

Preparation of Nano silver:

Wet chemical method was used for the preparation of Nano silver (NS) particles. Silver nitrate was 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 at 25-30°C. Then fructose and polyvinyl alcohol were added in it as a stabilizing agent. Colorless solution changed into light yellow color indicates the formation of NS particles,and thenthis solution was kept in refrigerator at 4°C. Reduction reaction mechanism due to fructose can be given as [20, 25].

$$2Ag^+ + H_2O + CH_2OH\text{-}(CHOH)_4\text{-}(\cancel{CHO}) \quad 2Ag^o + 2H^+ + CH_2OH\text{-}(CHOH)_4\text{-}COOH$$

Immobilization of Nano Silver:

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. Then the swollen films wereinstantly transferred into NS solution carrying vessel and with a moderate shaking for few minutes. After it, films were dried at 95-100°C for 120 minutes in vacuum ovenand washed in distilled water to remove excess NS particles and other chemical impurities. The formed films were once again vacuumed dried at 95-100°C for 240 minutes for further use.

Instrumentation Used:

Synthesis of NS was confirmed by UV-visible Spectroscopy of solutions. UV 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 360scanning electron microscope for the study of surface as well as fracture of NS immobilized copolymer. The morphological characteristics of NS particles

were analyzed using a PHILIPS CM -12 method, which was used to analyze the morphological characteristics of NS particles such as size and shape.

Antimicrobial Studies:

All the samples were sterilized by γ-rays, so that preexisting microbes can be eliminated. Viable cell count method was acquired to examine antimicrobial nature of samples. The American Association of Textile Chemists and Colorists (AATCC) 100method was followed for this study. 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 bacteria S. aureus.

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 three samples (0.05 g) was inoculated with a 5 ml bacterial suspension in MHB containing 10^6 CFU/ml.Both of the samples were shaken and incubated for 24 hours at 35-37°C. The suspensions were shaken again after 24 hours, and successive dilute solutions were prepared, with the remaining bacteria counted using the spread plate process. 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 the tests were carried out in a sterile setting. 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 the inoculated test specimen after 24 h incubation with untreated sample,B is the number of bacteria according to "A" conditions with NS modified samples, and R (%) is the percent reduction ratio which indicated antimicrobial efficiency.

III. Results and Discussion:

Bulge behavior of membranes was carried out at different temperaturesas shown in Table-2to study the hydrophilicity in aqueous system (Fig. 1). From figure it is clear that swelling increases slowly up to 60°C and it increases rapidly at beyond 60^0 and up to 70°C and after passes 70°C the swelling enhanced quickly up to about 80°C and after it almostit is at the level off. This behavior indicates that there is a sudden change in copolymer structure at about 70^0-80°C.

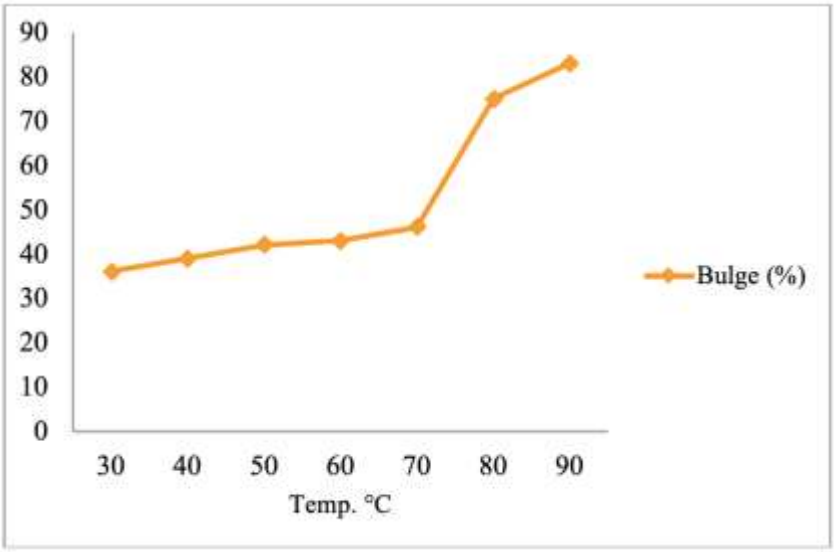

Figure 1: Bulge behavior of membrane AO 3.5 meq/g at different temperatures

Table 2: Bulge behavior of the membrane AO 3.5meq/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".

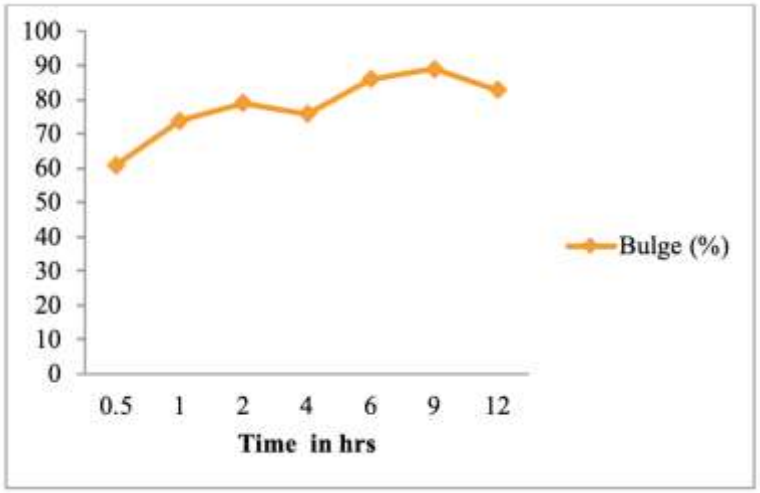

Figure 2: Bulge behavior of membrane AO 3.5 meq/g at 90°C with respect to time

The bulge linearly increases up to 2 hrs. and then tends to almost saturate (Table 3). The results indicate that almost maximum bulge is achieved only in 2 hrs. of time.

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

UV- Visible spectroscopy:

Results show that on transformation of (silver) Ag^{+} ions in to NS (Ag°) particles, colorless silver nitrate solution turned in to light yellow solution and the color shifted from light yellow to light green with the passes of time, which confirms the formation of NS particles in solution, the UV-vis absorption spectra of solutions with different reduction time were recorded in the range of 300-600 nm confirmed it quantitatively and represented in Figure 3. The plasmon peak for NS with λ_{max} values were obtained at 383, 395 and 404 nm for 20 min, 40 min and 60 min reduction time, respectively.

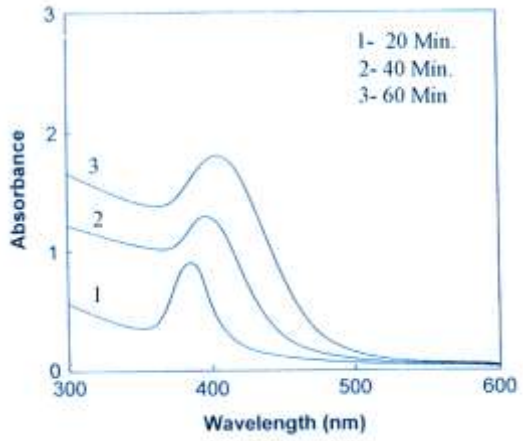

Figure 3: UV-vis absorption spectra of NS solutions.

On 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.

Transmission Electron Microscopy:

On the observation for all the TEM images nanoparticles appear to be undispersed and spherical. The histograms for particle size distribution were obtained by counting 50 NS particles from TEM images for each sample in Fig. 4. The data were analyzed and are

presented in Fig.5. The histogram shows the particle size ranges from 2.7-13.0, 2.4-14.7 and 5.4 -16.1 nm 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_{R20}, NS_{R40} and NS_{R60}, respectively.

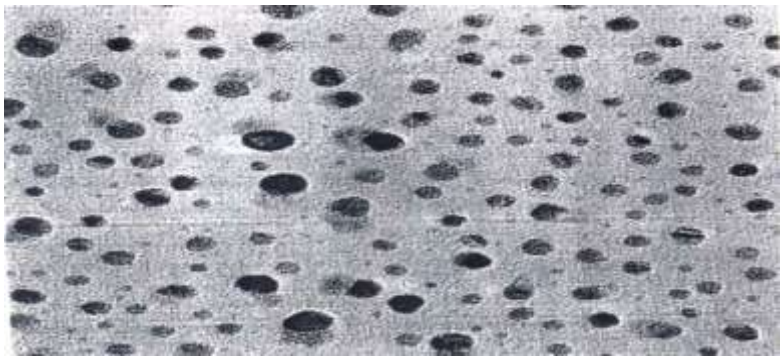

Figure4: TEM images of NS_{R60}

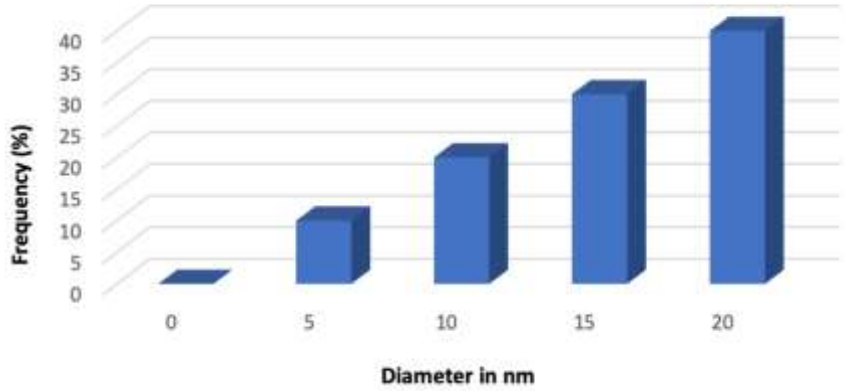

Figure 5: Particle size distribution histogram of NS_{R60} evaluated from TEM images

The variation of particle mean diameter with reduction time is presented in Fig.6. It is interesting to see that with the increase in reduction time the mean particle diameter increases.

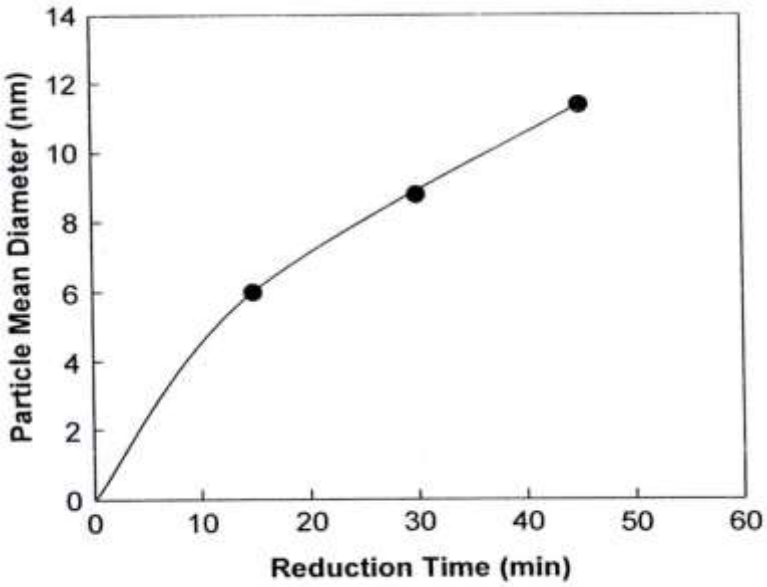

Figure 6: NS particle mean diameter (nm) with different reduction time

TEM and histogram shows that as the reduction time increases the NS particle intensity decreases whereas particle mean diameter increases. Different studies suggested that nucleation leads the increase in the number of scattering centers or number of particles for a given system therefore it provides an increase in the scattered intensity. It is consistent with the mechanism of the reduction of Ag+ ions and the association of Ag° atoms to produce metallic Ag particles [21-23]. NS particle becomes more stable with the increased reduction time as the particles become more homogenous in size allocation.

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 h and are shown in “Fig 7, 8”. It can be seen that on an average number of viable S. aureus and E.coli colonies decreased by 95% for both cases as compared to virgin AO $_{3.5}$ membrane.

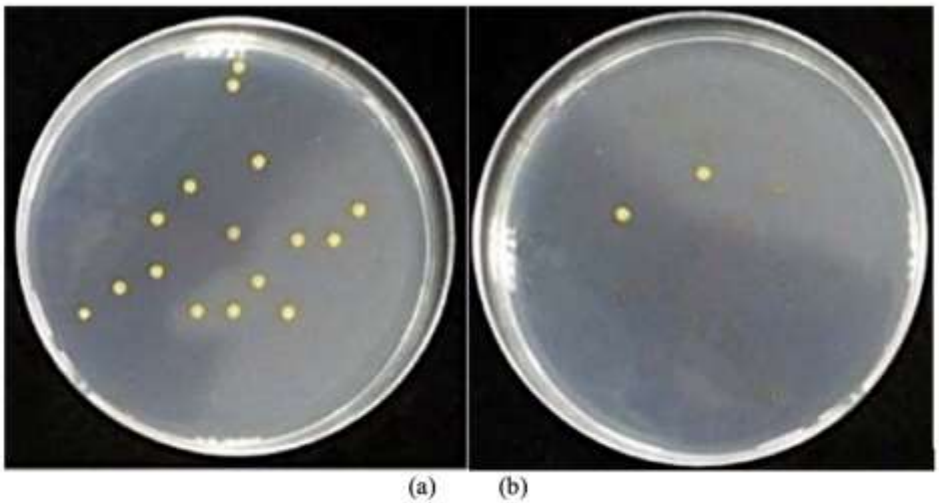

Figure 7: Antimicrobial activity of membranes against S. aureus (a)Control and (b) $AO_{3.5}$ - NS_{R60}

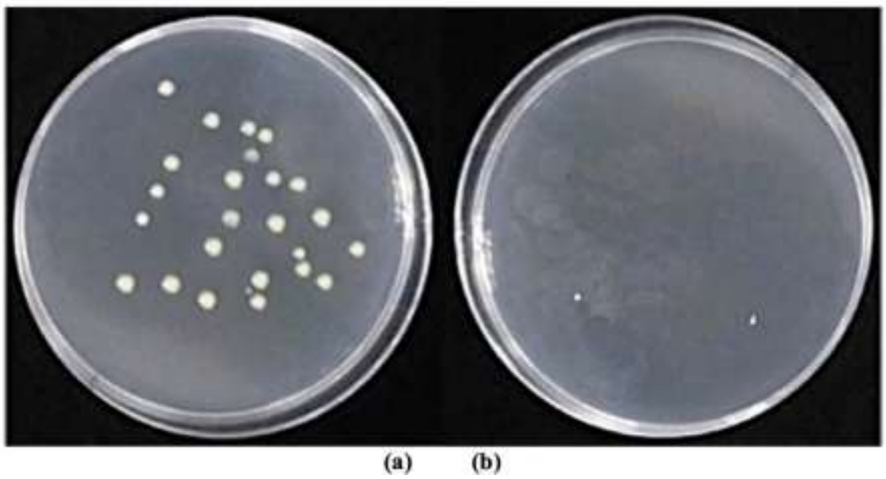

Figure 8: Antimicrobial activity of membranes against E. coli (a) Control and (b) $AO_{3.5}$ - NS_{R60})

IV. Conclusion:

Copolymer membranes having AO (Amidoxime) content were immobilized with NS particles. Synthesis of NS was confirmed by UV-visible Spectroscopy of solutions. EDX studies showed the presence of NS in the membrane surface as well as in the inner side of polymer matrix. TEM measurement of NS particles was carried out to analyze the morphological characteristics such as size and shape of NS. The antimicrobial activities of virgin as well as various NS containing samples were examined against gram positive bacteria S. aureus and gram negativebacteria E. coli by the estimation of the number of viable colony.This indicated that the NS penetrates within the swollen AO membranes and stays back. It can be seen that on an average number of viable S. aureus and E.coli colonies decreased by 95% for both cases as compared to virgin AO 3.5 membrane.

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