

Synthesis, Characterization and Antimicrobial activity of Amidoximated Acrylic Copolymer

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Abstract: Copolymer membranes were fabricated into DMP and copolymer was treated with HA in aqueous solution at 60-80°C. In the amidoximation process, a part of nitrile groups of copolymer is converted into amidoxime groups. The characterization of virgin and amidoximated acrylic copolymer membranes were carried out by using various techniques as Fourier transform infrared, X-ray diffraction, thermogravimetric analysis, Scanning electron microscope etc. With the amidoximation process various significant changes in the copolymer properties such as functionality, crystallinity, thermal behavior, hydrophilicity, elemental composition and surface morphology were observed.

Keywords – amidoximation, copolymer, crystallinity, thermal behavior, surface morphology

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I. Introduction

Water plays an important role for the environment and for the co-evolution of the life on Earth [1]. Waste water discharges in fresh waters. Coastal seawaters are the main source of stool microorganisms, with pathogens [2,3]. The control of the growth of microbes, such as bacteria, fungi and yeast in nature is one of the fundamental concepts for the survival of higher species [4]. Membrane filtration is an efficient technique to eliminate particles, organic matter and microorganisms from drinking water and wastewaters. On Comparison with other methods, membrane method is superior method for improving the quality of water [5]. Polyacrylonitrile (PAN) and acrylonitrile based copolymers possess excellent membrane forming property, exhibit excellent chemical resistance to most of the solvents and good thermal and mechanical stability [6, 7]. PAN membranes has also used for membrane bioreactor and protein filtration [8, 9]. PAN hollow fiber membranes are already used in dialyzers that enable to focus molecule protein removals and high-flux dialysis therapy [10]. The copolymerization of acrylonitrile (AN) with incorporation of different appropriate acrylic and vinyl monomers during polymerization process some of them mainly includes methacrylic acid, acrylic acid (AA), itaconic acid, acrylamide and styrene is extensively reported in the literature [11-13]. Several natural and engineered nanomaterials have been reported such as chitosan, photo catalytic titanium dioxide, fullerene, gold and silver nanoparticles which are widely used as antimicrobial agent [14] in water disinfection related systems [15]. These nanoparticles also consist of electrical, visual means optical, biological properties, good electrical and thermal conductivity [16]. NS have been found to effectively inactivate and kill for a broad range of microbes [17] yet its antimicrobial mechanism has not been fully elucidated.

II. Materials And Methods

All the chemicals used such as Toluene, Polyvinyl alcohol, Silver nitrate, α Azobisisobutyronitrile, Acrylonitrile, Acrylic acid, DMF, NaOH, HCl etc. were of AnalaR grade and were obtained from Fluka or Sigma Aldrich. Silver nitrate (AgNO_3), fructose, Polyvinyl alcohol (PVA) with molecular weight of 115000 were also obtained from Sigma Aldrich and Fluka. All the chemicals were used normally without doing any purification. But all these solvents were dried and distilled by using standard method before using. Our methods for the synthesis of Polymerization, Molecular Weight Determination, Fabrication of Copolymer, Hydroxylamine treatment and Amidoximes content evaluations have been already published in our research paper [18].

III. Methods

Bulge Behaviour:

Bulge behaviour of copolymer membranes having AO content 3.5 meq/g was carried out with distilled water in water bath. 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.

Preparation of Nanosilver:

Wet chemical method was used for the preparation of Nanosilver. Silver nitrate was transformed into NS by reducing it with fructose in aqueous solution. 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 then kept this solution in refrigerator at 4°C. Reduction reaction mechanism due to fructose can be given as [20].



Immobilization of NS:

To immobilize NS into the copolymer film, a fix weight of AO 3.5 copolymer membrane was first swelled into distilled water at 90°C in fixed temperature water bath. The swollen films instantly transferred into NS solution carrying vessel and a moderate shaking was performed for few minutes. After it, films were 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 films once more vacuum dried at 95-100°C for 240 minutes for further use.

IV. Instrumentation

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. 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. TEM measurement of NS particles were carried out using a PHILIPS CM -12 system which was used to analyze the morphological characteristics such as size and shape of NS.

V. 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. AATCC 100-1998 method was followed for this study. The antimicrobial activity was performed against model bacteria *E. coli* and bacteria *S. aureus*.

Colony count method:

A suspension of *E. coli* or *S. aureus* was prepared from fresh colonies in Muller Hinton Broth. All the three samples (0.05 g) were put separately into contact with 5 ml bacterial suspension in MHB having 10^6 CFU/ml. All the samples were shaken and incubated at 35-37°C for 24 h. After 24 h, the suspensions were shaken again and successive dilute solutions were prepared and surviving bacteria were counted by the spread plate method. 200 μl of the inoculum were uniformly spread on nutrient agar plate. The plates were incubated at 35-37°C and the colonies were counted after 24 h. All the experiments were carried out under sterile conditions. Antimicrobial efficiency was expressed according to AATCC 100 and calculated as:

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

- 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,
- R (%) is the percent reduction ratio which indicated antimicrobial efficiency.

VI. Results And Discussion

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

Table 1: Composition of samples

AO _{3.5}	Acrylic copolymer membrane contains 3.5 meq/g amidoxime content.
NS _{R20}	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.

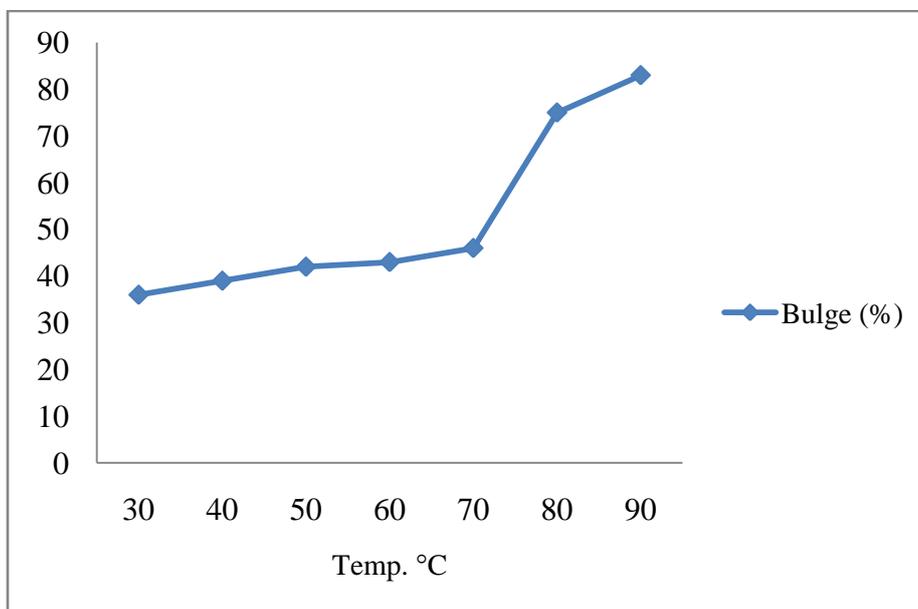


Fig. 1: Bulge behaviour of membrane AO 3.5 at different temperatures

Table 2: Bulge behaviour of the membrane AO 3.5 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”.

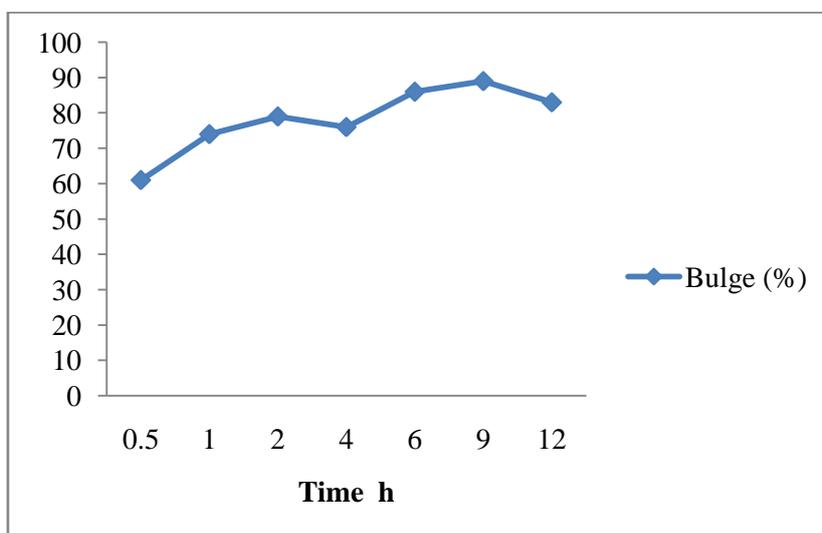


Fig. 2: Bulge behaviour of membrane AO 3.5 at 90°C with respect to time

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

Table 3: Equilibrium swelling behaviour 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 Ag^+ ions in to NS (Ag^0) particles, colourless silver nitrate solution turned in to light yellow solution and the colour 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 was obtained at 383, 395 and 404 nm for 20 min, 40 min and 60 min reduction time, respectively.

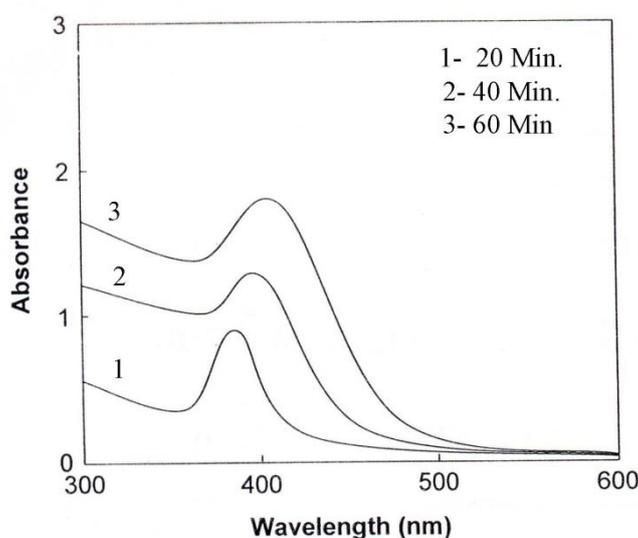


Fig. 3: UV-vis absorption spectra of NS solutions.

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.



Fig. 4: TEM images of NS_{R60}

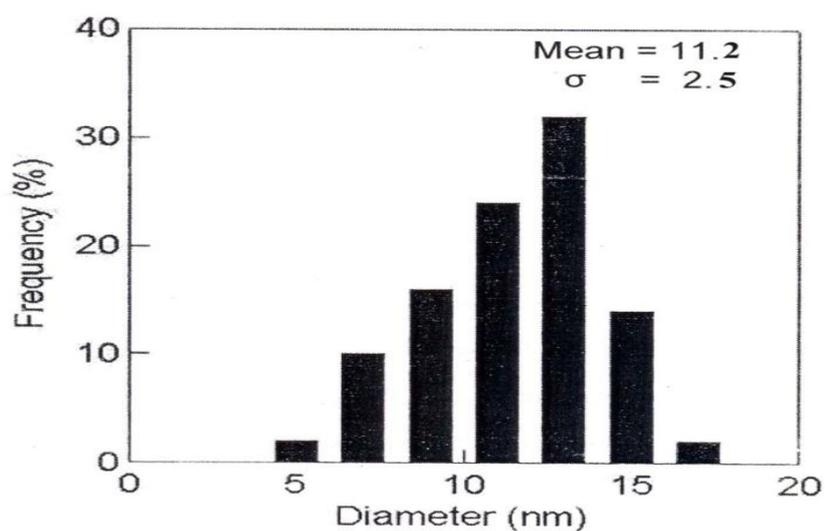


Fig. 5: Particle size distribution histogram of NS_{R60} evaluated from TEM images

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.

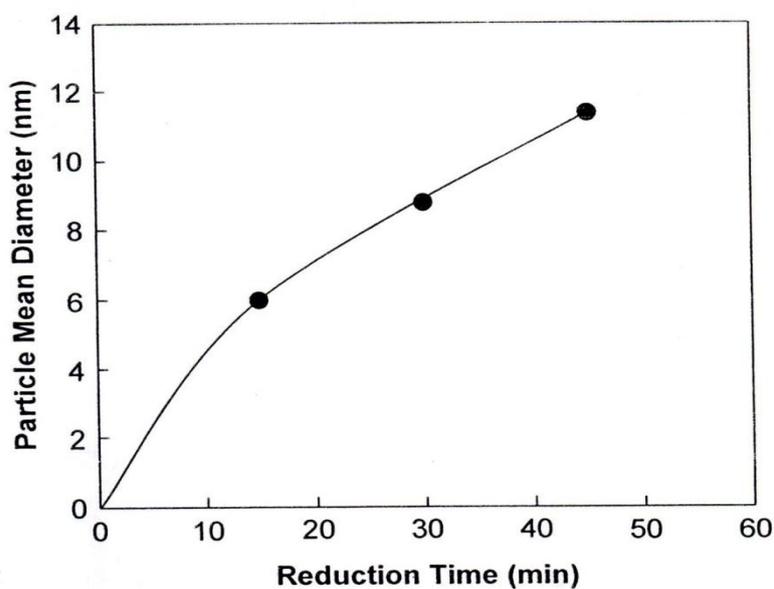


Fig. 6: NS particle mean diameter (nm) with different reduction time

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 a assessment of the number of viable colony 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 $\text{AO}_{3.5}$ membrane.

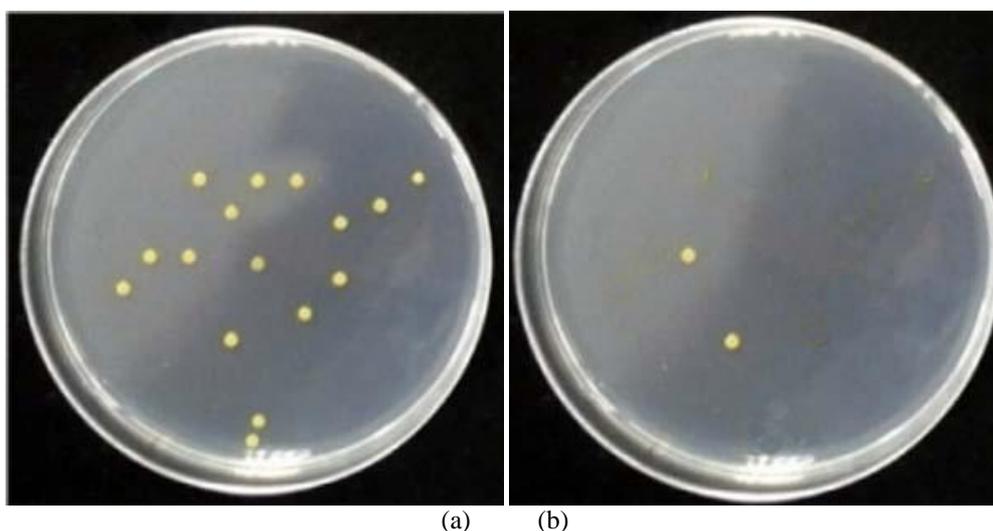


Figure 7: Antimicrobial activity of membranes against *S. aureus* (a) Control and (b) $\text{AO}_{3.5} - \text{NS}_{\text{R60}}$

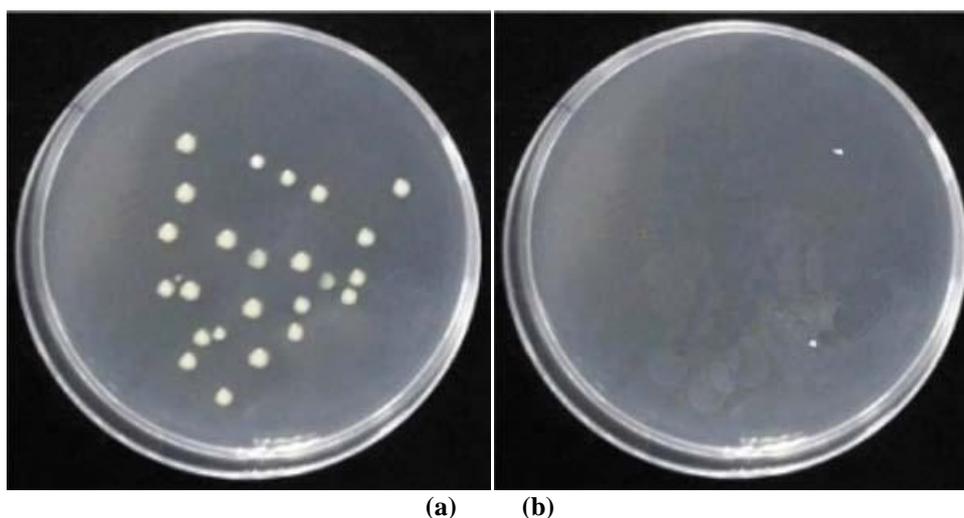


Figure 8: Antimicrobial activity of membranes against *E.coli* (a) Control and (b) $\text{AO}_{3.5} - \text{NS}_{\text{R60}}$

VII. Conclusion

Copolymer membranes having AO 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 negative bacteria *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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