

Volume 7, Special Issue 7, June 2018

International Journal of Innovative Research in Science, Engineering and Technology

An ISO 3297: 2007 Certified Organization

One Day National Conference on "Recent Trends in Mechanical Engineering & Applied Sciences 2018" (RTME & AS-2018)"

17th May 2018

Organized by

Department of Mechanical Engineering and Applied Science, Sri Krishna Institute of Technology, Bangalore, India

Antimicrobial Studies Of Copoly(N-Vinyl Pyrrolidone – Dimethyl Amino Ethyl Methacrylate)

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ABSTRACT: (N-Vinylpyrrolidone - Dimethyl amino ethyl methacrylate) is a copolymer containing an antimicrobial monomer hence exhibits antimicrobial property. Quaternization of this copolymer will enhance the antimicrobial property. The copolymer (NVP-DMAEMA) was synthesized by free radical mechanism in presence of benzoyl peroxide as an initiator. The obtained copolymer was quaternized with ethyl bromide. Copolymer and quaternized saltwas characterized by FTIR and NMR. The antimicrobial property of these compounds was investigated by agar well diffusion method. Two gram +ve bacterial strains used were *Bacillus pumillus* – 1640 and *Staphylococcus aureus* – 3160, two gram –ve bacterial strains were *Escherichia coli* – 45 and *Enterobacter aerogenes* – 2822. The fungal strains used were *Fusarium oxysporum* – 2087, *Aspergillus niger* – 4325 and *Penicillium sp*. All compounds showed a better inhibition zones.

I. INTRODUCTION

Polymers are the molecules with high molecular weights. One of the important criteria for a molecule to be antimicrobial active is its functional group. There are a few functional groups which are highly antimicrobial active such as hydroxyl, carboxyl or amino groups. Polymers are covalently linked with such functional groups before polymerization. The polymers which are capable of killing the microorganisms are grouped as antimicrobial polymers. One of the ways of synthesizing antimicrobial polymer is by quaternizing the polymer to get quaternary ammonium and quaternary phosphonium salts. Cell walls of microbes are negatively charged. The positive charged molecules are easily adsorbed on them. Quaternary salts of ammonium and phosphonium contain positively charged groups. Adsorption of these is favored when compared to the other antimicrobial polymers.

II. LITERATURE SURVEY

N-Vinylpyrrolidone is a biocompatible monomer with low cytotoxicity. The two donor atoms oxygen and nitrogen present in it makes it hydrophilic. [1,2]. As it can form many copolymers with a variety of monomers [3,4], it has very much extensive range of applications in various fields [9-13].

Both homo and copolymers of N-Vinylpyrrolidone find their applications not only as biologically active compounds but also as sorbents, coagulants and flocculants [7,8]. Its copolymers are also used in cancer treatment [9], blood purification therapy [10] and as antiviral [11].

N, N-Dimethyl amino ethyl methacrylate is a water soluble, monofunctional acrylate monomer. Poly (N,N-Dimethylaminoethylmethacrylate) pDMAEMA can inhibit the growth of bacteria on different materials viz, glass, filter paper and plastic [12,13]. Its antibacterial activity may be increased by quaternization, because quaternization enhances the positive charge density and amphipathic character of its copolymer & polymer. [14,15].



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Copolymerization of NVP with N, N-Diethyl amino ethyl methacrylate results in an antimicrobial copolymer which can be quaternized with ethyl bromide to enhance the antimicrobial nature.

III. METHODOLOGY

Materials

N-Vinylpyrrolidone (Sigma-Aldrich), N, N-Dimethyl amino ethyl methacrylate (Kodak company, USA) were mixed with Fuller's earth separately and kept overnight to settle. Supernatant liquid was collected separately. Benzoyl peroxide, acted as initiator, Ethyl bromide (Chems Worth, Surat, India) which acts as quaternising agent and other solvents were used as received from S D Fine chemicals.

Microbial strains

Antimicrobial activity was screened by Agar well diffusion method against two gram +ve bacterial strains, *Bacillus pumillus – 1640* and *Staphylococcus aureus – 2822*, two gram –ve bacterial strains, *Escherichia coli – 45* and *Enterobacter aerogenes – 2822*. The fungal strains used were *Fusarium oxysporum – 2087* and *Aspergillus niger – 4325* collected from MTCC, Chandighar. *Penicillium sp.* Fungus was extracted from soil by the students of SIT, Tumkur, Karnataka.

Synthesis and quaternization of the copolymers

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N – Vinylpyrrolidone and N, N – Dimethyl amino ethyl methcrylate were taken in 1:1 molar in three necked round bottom flask. 0.5g benzoyl peroxide was added to the same flask which acts as initiator. Ethyl alcohol added acted as solvent. The mixture was heated at 65° C under nitrogen atmosphere for 6 hours. A thick viscous liquid formed was washed well with acetone and dried at 40° C in vacuum. This is named as D. The copolymer was dissolved in ethyl alcohol and required amount of ethyl bromide was added to it. This solution was stirred continuously for 6 hours in a water bath maintaining a temperature of 60° C. The reaction was stopped after a duration of 6 hrs and washed with 1,4 – dioxane solvent. It was precipitated with acetone and dried under vacuum at 40° C.

Copolymer characterization

Copolymers were characterised by Fourier – Transform Infrared Spectroscopy using Jasco FTIR at Sapala Organics Private Limited, Hyderabad, on KBr pellets in the range of 400 - 4000 cm⁻¹.

The Nuclear magnetic Resonance Spectrum of both proton and carbon (H^1 NMR and C^{13} NMR) for copolymers were recorded in Brucker AV – 300 spectrometer at Sapala Organics Private Limited, Hyderabad.

Antimicrobial activity test

Antimicrobial activity was screened by Agar well diffusion method [16]against four bacterial strains (two gram +ve and two gram –ve) and three fungal strains. Nutrient agar culture medium plates were prepared and swabbed using sterile L-shaped glass rod with 100µl of 24h mature broth culture of individual microbial strains. The wells were made by using sterile cork borer of 6mm diameter. The pure solvent DMSO is used as negative control. The compounds of different volumes were dispersed in the same solvent. Simultaneously the standard antibioticStreptomycin sulphate($5\mu g/50\mu l$) for bacteria and Fluconazole ($5\mu g/50\mu l$) for fungal as positive controls were tested against the pathogens.Varied volumes of compounds loaded into the wells of the Petri plates were used to assess the activity of the compounds. Then the plates were incubated at 37°C for 24-36 hours, the zone of inhibition was measured in millimeter of the every well and the values were noted. Triplicates were maintained in all volumes and the average values were calculated for the ultimate antimicrobial activity.



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Synthesis of Copolymer (NVP-DMAEMA) (D)



Quaternization of Copolymer (NVP-DMAEMA) with Ethyl bromide.



III. SIMULATION RESULTS

Characterization of copolymer:

Solubility Measurements:

The copolymer and quaternized salt were found to be soluble in polar solvents They were insoluble in non – polar solvents.

FTIR spectral studies of Copolymer D

The FTIR spectrum of the copolymer is as shown in the figure



FTIR spectrum of Copolymer (D)

Important peaks are to be considered are NVP $-C=O \ 1671 \text{ cm}^{-1}$, -C-N-C- stretching 1446 cm^{-1} , $C-N \ 1328 \text{ cm}^{-1}$ DMAEMA $-C=O \ 1729 \text{ cm}^{-1}$, $-CH_2-N-(CH_2) \ 1151 \text{ cm}^{-1}$.

FTIR spectral studies of QD

The FTIR spectrum of the copolymer is as shown in the figure



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FTIR spectrum of QD

Important peaks to be considered are

NVP -C=O 1670cm⁻¹, -C-N-C stretching 1440cm⁻¹, DMAEMA OC-OH stretching 1289cm⁻¹, Quaternary ammonium salt (N+) 3434 cm⁻¹,

H¹NMR spectral studies of the Copolymer D

TheH1- NMR of the copolymer is as shown in figure.Solvent - Dimethyl sulfoxide, Standard - TMS



H¹ NMR spectrum of the copolymer (D)

All the protons of ring methylene and around amine resonate in the region 1.3 to 2.4. Protons present closer to carbonyl carbon of amine resonate at 7.2, 7.9. Methylene groups present on NVP and DMAEMA resonate at 3.2, 5.6 and 6.0.

C¹³ NMR spectral studies of Copolymer D

TheC¹³-NMR of the copolymer is as given in the figure.Solvent - Dimethyl sulfoxide, Standard–TMS



C¹³ NMR spectrum of Copolymer (D)

Carbon present on the carbonyl group of NVP and ester in DMAEMA resonate at 170 and 180 respectively. Carbon present closer to ester group in DMAEMA resonates at 138. Methylene groups present in the ring of NVP resonate at 48, 24, 30. Methylene groups present around the amine group resonate at 125 to 130.

Antimicrobial Screening Test



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The antimicrobial activity of copolymer D and its quaternized salts was examined against four bacterial strains *viz.*,gram+ve bacteria *Staphylococcusaureus* and *Bacilluspumillus*, gram –ve bacteria like *Escherichiacoli* and *Enterobacteraerogenes* and three fugal strains *Fusariumoxysporum*, *Aspergillusniger* and *Penicilliumsp*. The standard antibacterial used was Streptomycin sulphate. The standard antifungal used was Fluconazole. The inhibition zone in mm for the copolymer and its graft was given in the table

	Grafted Compound	Volume (µl)	Bacterial Strains				Fungal Strains		
Copolymer			Bc	Sa	Ec	Ea	Fu	An	Pe
Standard	Streptomycin sulphate	(5μg/ 50μl)	30	30	35	30			
Standard	Fluconazole	(5μg/ 50μl)					11	8	-
NVP - DMAEMA	D	50	15	20	20	-	11	5	8
		100	15	23	11	13	11	-	15
		200	15	20	21	10	20	-	10
		50	35	20	28	-	-	8	-
	QD	100	15	10	11	-	-	8	-
		200	25	12	22	-	-	10	-

Table: Antimicrobial activity of D & QD against on Pathogenic Organisms



Bc: Bacillus pumillus, Sa:Staphylococcus aureus, Ec:Escherichia coli, Ea:Enterobacteraerogenes, Fu:Fusariumoxysporum, An:Aspergillusniger, Pe:Penicilliumsp, '-' inactive



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Photographic representation of antifungal test
a. Fusarium oxysporum for D,
b. Aspergillius niger for D, c. Aspergillius niger for QD,
d. Penicillium sp for D, d. Penicillium sp for QD,

Representative photographs showing Antimicrobial activity in Agar well diffusion method for D and QD

IV. CONCLUSION

The copolymer (NVP –DMAEMA) was synthesized. Its structure was confirmed by FTIR, H^{1} –NMR and C^{13} –NMR. The antimicrobial activity of copolymer was determined by Agar well diffusion method. It was demonstrated that the copolymer has a positive effect in controlling the growth of the gram +ve, gram –ve bacterial and fungal strains. The zone inhibition for gram +ve bacteria is larger when compared to other bacteria. It deactivates *Fusarium oxysporum* and *Penicilliumsp* better than standard Fluconazole. This copolymer can be used as substitute for standard Streptomycin and Fluconazole.



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The copolymer (NVP – DMAEMA) was quaternized with ethyl bromide. Its structure was confirmed by FTIR. The antimicrobial activity of quaternized copolymer was determined by Agar well diffusion method. It was demonstrated that the copolymer has a positive effect in controlling the growth of the gram +ve bacteria and *E.coli*. The zone inhibition for gram +ve bacteria is larger when compared to other bacteria. It acts as anti fungal in the presence of fungal strain *Aspergillus niger*. It acts as a good anti bacterial agent and anti fungal to a small extent.

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