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Indian
Journal of Advances in
Chemical Science

Indian Journal of Advances in Chemical Science S1 (2016) 52-55

Antimicrobial Copolymers of N-vinylpyrrolidone

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Received 16th April 2016; Revised 25th April 2016; Accepted 02nd May 2016

ABSTRACT

Antimicrobial polymers can be synthesized in many methods. They can be further modified to alter their action toward microbes. N-vinylpyrrolidone (NVP) is biocompatible monomer which on copolymerization can form an antimicrobial copolymer. Acrylic acid (AA) is an unsaturated carboxylic acid which under goes copolymerization. The carboxylic acid functional group introduces an antimicrobial property to the copolymer formed. Maleic anhydride (MA) is another monomer that can be used to make the copolymer as antimicrobial. On hydrolysis, it becomes dicarboxylic acid. These two copolymers differ by one carboxylic acid group. These copolymers are further esterified with N,N-diethylaminoethanol (DEAE). This introduces amino group into the copolymer and the antimicrobial property will be enhanced. The copolymers of (NVP-AA) and (NVP-MA) are synthesized by free radical copolymerization. Further they are esterified with DEAE. The resulting copolymers and macro-complexes are characterized by Fourier-transform infrared and nuclear magnetic resonance spectral studies. The antimicrobial properties of these are studied by Agar well diffusion method. The studies are made on the Gram-positive bacteria Staphylococcus aureus - 3160 and Bacillus pumilus - 1640, Gram-negative bacteria Escherichia coli - 45 and Enterobacter aerogenes - 2822, and two fungal strains Fusarium oxysporum - 2087 and Aspergillus niger - 4325. The standard antibacterial used was streptomycin sulfate. The standard antifungal used was fluconazole. The inhibition zones formed are of considerable diameter. These copolymers and macro-complexes may be used as a substituent for the standards used.

Key words: *N-vinylpyrrolidone, Acrylic acid, Maleic anhydride, Diethylaminoethanol, Fourier-transform infrared, Nuclear magnetic resonance, Antimicrobial studies.*

1. INTRODUCTION

Antimicrobial polymers are those, which are capable of killing pathogenic micro-organisms. Polymeric antimicrobial agents may enhance the efficacy of some existing antimicrobial agents and minimize the environmental problems accompanying the residual toxicity of the agents in addition to prolonging their lifetime [1]. Synthetic polymers with functional groups, which are antimicrobial active, are widely used to prevent the growth of micro-organisms on the surface of materials such as antifouling paints, antibiotics, in soil sterilization, and in water treatment. Increased efficiency, selectivity, and handling safety are additional benefits that are realized [2,3].

N-vinylpyrrolidone (NVP) is a good biocompatible monomer due to its hydrophilic nature and low toxicity [4,5,6]. The amide group of NVP has a high affinity for several small and large molecules that are known as good hydrogen-bond acceptors and has been copolymerized with a variety of monomers [7-9].

Acrylic acid (AA) is a carboxylic acid containing unsaturated bonds. The presence of vinyl and carboxylic group has made still more active. The unsaturated bonds are responsible for the addition reactions and also the polymerization. Esterification, amidation, and self-crosslinking properties of AA monomer help to produce crosslinked or grafted polymer molecules with special properties.

Maleic anhydride (MA) is an organic, colorless, or white solid with an acrid odor. On hydrolysis it produces maleic acid, when made to react with alcohols, half-ester is generated.

Copolymerization of NVP with AA and MA results in antimicrobial active copolymers, as these two possess functional groups which are antimicrobial active. In

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this work, we have esterified these with N, N-Diethyl amino ethanol and antimicrobial activity is studied by Agar well diffusion method.

2. EXPERIMENTAL

2.1. Materials

NVP (Sigma-Aldrich), AA (SD Fine Chemicals) are mixed with Fuller's Earth and kept overnight to settle. Supernatant liquid (washed monomer) is collected separately. Hydrogen peroxide (SpectroChem) 30% was used as received. MA (Lobachem) was purified before use by recrystallization from anhydrous benzene solution and sublimation in vacuum. 2,2-azobisisobutyronitrile (AIBN) was purified by successive crystallization from chloroform-methanol mixture. N,N-diethylaminoethanol (DEAE) was purified by distillation. Other solvents are of AR grade with 99% purity. They are used as received from SD Fine Chemicals, Mumbai, India.

2.2. Microbial Strains

The antimicrobial activity of copolymers and their grafts were examined by Agar well diffusion method against four bacterial strains, i.e., Gram-positive bacteria *Staphylococcus aureus* 3160 and *Bacillus pumilus* 1640, Gram-negative bacteria *Escherichia coli* 45 and *Enterobacter aerogenes* 2822, and two fungal strains *Fusarium oxysporum* 2087 and *Aspergillus niger* 4325. The standard antibacterial used was streptomycin sulfate. The standard antifungal used was fluconazole.

2.3. Synthesis of Copolymer

The monomers NVP and AA were taken in 1:1 ratio and polymerization was carried out in tetrahydrofuran as solvent at 45°C maintaining an inert atmosphere by passing nitrogen gas and as hydrogen peroxide as an initiator. The process was carried for 3 h. The high viscous reaction mixture was poured into beaker and washed well with diethyl ether/hexane mixture. Dried at 40°C to evaporate the solvent.

The copolymer (B) prepared was made to react with DEAE to form an ester (EB) through acid group of AA in dimethyl sulfoxide solvent (Figure 1).

In a parallel reaction, the copolymer NVP with MA was prepared by taking monomers in 1:1 ratio by free radical addition polymerization. The monomers, 1,4-dioxane as solvent azobisisobutyronitrile as initiator were added to a round-bottomed flask with three necks. The reaction mixture was heated to about 50-60°C with a continuous stirring for about 48 h. The reaction mixture was allowed to attain laboratory temperature. Precipitation of the copolymer was done by acetone. This copolymer was then washed well with acetone and dried at 40°C.

This copolymer (M) prepared was made to react with DEAE at 40°C in aqueous solution in different concentrations 1:1 (M_1) and 1:2 (M_2) (Figure 2).

2.4. Copolymer Characterization

Copolymers were characterized by Fourier-transform infrared (FTIR) 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 (NMR) 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.

2.5. Antimicrobial Activity Test

Antimicrobial activity was screened by Agar well diffusion method [10] against four bacterial strains (two Gram-positive and two Gram-negative) and three fungal strains. Nutrient agar culture medium plates were prepared and swabbed using sterile L-shaped glass rod with 100 µl of 24 h mature broth culture of individual microbial strains. The wells were made using sterile cork borer of 6 mm diameter. The pure solvent DMSO is used as negative control. The compounds of different volumes were dispersed in the same solvent. Simultaneously the standard antibiotic streptomycin sulfate (5 µg/50 µl) for bacteria and fluconazole (5 µg/50 µl) 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 h, 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.

3. RESULTS AND DISCUSSION

3.1. Characterization of Copolymers and Grafts

FTIR (KBr): B 1644 cm^{-1}, 1444 cm^{-1}, 1330 cm^{1}, 1731 cm^{-1}, 3439 cm^{-1}, 650.9 cm^{-1}

EB: 1661 cm^{-1}, 1443 cm^{-1}, 1725 cm^{1}, 1290 cm^{-1}, 3428 cm^{-1}, 618.9 cm^{-1}

M: 1653 cm^{-1}, 1441 cm^{-1}, 1991 cm^{-1}, 984 cm^{-1}

M_1: 1650 cm^{-1}, 1442 cm^{-1}, 3417 cm^{-1}, 1179 cm^{-1}

M_2: O 1662 cm^{-1}, 1443 cm^{-1}, 1326 cm^{-1}, 3416 cm^{-1}

H^1 NMR: B (400 MHz, MeOD): 3.3 (4H,1.8, Me), 2.5 (H,17.2, Me) 4.2 (H,0.93, Me) 1.8 (2H, 3.5, Me), 2.8 (2H, 2.6, Me), 2.2 (2H, 3.5, Me), 11.2 (1H, s, COOH)

M: (400 MHz, MeOD): 1.8 (2H, Me), 2.2 (2H, Me), 2.8 (2H, Me), 3.5-2.5 (2H, Me), 6.2 (H, CH)

C^{13}– NMR: B (400 MHz, MeOD): 177 (C), 181 (C), 42 (CH), 30 (CH_2), 35 (CH), 68 (CH_2), 25 (CH_2), 58 (CH_2).

M_1: (400 MHz, MeOD): 49.6 (CH_2), 43.59 (CH_2), 23.8 (CH_2), 157 (C), 48.2 (CH_2), 21.2 (CH_2), 136.13 (CH).

3.2. Antimicrobial Activity

The antimicrobial properties of the polymers and their compounds were evaluated by Agar well diffusion method against two Gram-positive bacterial strains, *B. pumilus* and *S. aureus*, two Gram-negative bacterial strains, *E. coli* and *E. aerogenes*. The fungal strains used were *F. oxysporum* and *A. niger*.

The inhibition zone in mm for the copolymers and its quaternized salts are given in Table 1.

From this Table 1, it is evident that the copolymers of NVP-AA, NVP-MA, and their copolymers are showing a better inhibition for all microbes selected for the study. All are active toward Gram-positive bacteria and Gram-negative *E. coli*, But inactive toward another Gram-negative *E. aerogenes*. Only grafted compounds are active toward fungal strains but not the copolymers. The zones observed.

4. CONCLUSION

The copolymers (NVP-AA), (NVP-MA) were synthesized by free radical polymerization. They were grafted by N,N-diethyl amino ethanol and were characterized by FTIR and NMR. The antimicrobial activity was determined by Agar well diffusion method.

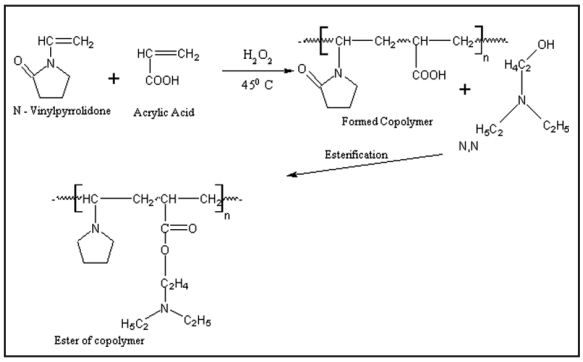

Figure 1: Scheme of synthesis of copolymer (N-vinylpyrrolidone-acrylic acid) (B) and grafting with N,N-diethyl amino ethanol (EB).

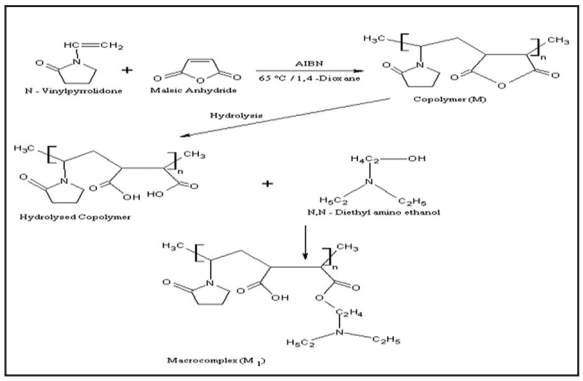

Figure 2: Scheme of preparation of copolymer (M) and its compound M_1.

Table 1: Inhibition zones of compounds

Copolymer	Grafted compound	Volume (µl)	Bacterial strains				Fungal strains	
			Bc	Sa	Ec	Ea	Fu	An
Standard	Streptomycin sulfate	(5 µg/50 µl)	30	30	35	30		
	Fluconazole	(5 µg/50 µl)					11	8
NVP-AA	B	50	20	11	10	15	-	-
		100	25	12	15	-	-	-
		200	25	19	20	-	-	-
	EB	50	12	12	11	-	21	8
		100	20	15	12	-	-	10
		200	20	14	17	-	15	12
NVP-MA	M	50	20	12	28	-	13	-
		100	25	15	11	-	12	-
		200	25	14	22	-	18	-
	M1	50	12	10	-	-	-	-
		100	20	16	8	-	-	-
		200	20	17	-	-	-	-
	M2	50	35	11	10	-	-	8
		100	15	12	18	-	-	8
		200	25	19	12	-	-	10

-=Inactive, Bc=*Bacillus pumilus*, Sa=*Staphylococcus aureus*, Ec=*Escherichia coli*, Ea=*Enterobacter aerogenes*, Fu=*F. oxysporum*, An: *Aspergillus niger*, NVP=N-vinylpyrrolidone, AA=Acrylic acid, MA=Maleic anhydride

A significant zone of inhibition was observed with all compounds. All the copolymers and grafted compounds are active toward Gram-positive bacteria and are selective to the Gram-negative and fungal microbes.

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***Bibliographical Sketch**

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