SYNTHESIS, CHARACTERIZATION AND ANTI MICROBIAL STUDIES OF COPOLY (N-VINYLPYRROLIDONE – MALEIC ANHYDRIDE) WITH N, N-DIETHYLAMINOETHANOL

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Abstract - A copolymer of N-vinylpyrrolidone (NVP) and Maleic anhydride (MA) is synthesized by free radical copolymerization with 2,2-Azo-bisisobutyronitrile(AIBN) as initiator at 650C in dioxane solution, under nitrogen atmosphere. The copolymer obtained is characterized by FTIR and NMR. The copolymer is grafted with N,N-Diethylaminoethanol (DEAE) in two different ratios. The antimicrobial property of these compounds is investigated by agar well diffusion method. Two gram +ve bacterial strains used are Bacillus pumillus – 1640 and Staphylococcus aureus – 3160, the gram –ve bacterial strain used is Escherichia coli – 45 . The fungal strains used are Fusarium oxysporum – 2087 and Aspergillusniger – 4325.

Keywords - Antimicrobial activity, Copolymerization, Maleic anhydride, N-vinylpyrrolidone,

I. INTRODUCTION

Copolymers with reactive functional groups are gaining importance not just because, they are macromolecules, but also for the behavior of these groups present in them [1]. The reactive functional groups present in the copolymer lead to the specific application and it modifies the properties of resultant from the parental polymer chain.[2]. This has emerged with a wide range of studies on the polymers as 'functional polymers'.

Maintaining hygienic is a challenging task in medicinal devices, health care products, hospitals, water purification systems, food storages and so on. The dangerous attacking agents are microbes which grow multiplying themselves in the minimum favorable conditions. One of the possible ways to evade microbial contamination is to introduce the agents possessing antimicrobial properties [3][4]. Antimicrobial polymers are those, which are capable of killing pathogenic micro-organisms. The advantage of these over conventional antimicrobials are their selectivity, increased efficiency and handling safety. [5] [6].

Kenawyet.al., has reported the antimicrobial activities of some linear copolymers with quaternary ammonium and phosphonium salts [4] [6]. Patel et al found that homo and copolymers of N-vinylpyrrolidone (NVP) and 2,4-dichlorophenyl methacrylate (2,4 DMA) were effective in inhibiting selective microorganisms [3]. AyhanTemiz et al. studied the antimicrobial behavior of Poly (N-vinylpyrrolidone - alt-Maleic anhydride) and it's Poly (ethyleneimine) macro complexes in aqueous solution and reported that Gram – positive bacteria affected by the complex but Gram – negative bacteria are not affected [7].

N-vinylpyrrolidone is a good biocompatible monomer due to its hydrophilic nature and low toxicity [8]. 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 [9]. Copolymer of 2-hydroxymethylmethacrylate (HEMA) and N-vinylpyrrolidone is used in pharmaceutical application, such as, cancer therapy [10]. Copolymer of NVP and n-butylmetharylate are used for blood purification therapies [11]. Fullerene (60) C complexed with Poly (N-vinylpyrrolidone) is used in antiviral activity [12].

Maleic anhydride is a bifunctional monomer with a double bond and anhydride which on hydrolysis forms maleic acid. The copolymers of Maleic anhydride are being used as antitumor agents [13], as drug carriers, as modifiers of proteins [14].

In the present work, a copolymer of NVP and MA is synthesized. It is characterized by FTIR, H1 NMR and C13 NMR. The synthesized copolymer is hydrolyzed. The product formed is made to react with N-diethylaminoethanol to form a complex in two different ratios. The complexes formed are tested for antibacterial activity.

II. EXPERIMENTAL

2.1 Materials used:

2.1.1. Chemicals:

N-vinyl-2-pyrrolidone (NVP) (Sigma-Aldrich) was purified before use by distillation under moderate vacuum. Maleic anhydride (MA) (Lobachem) was purified before use by recrystallisation from

anhydrous benzene solution and sublimation in vacuum. 2,2-Azobisisobutyronitrile (AIBN) was purified by successive crystallization from chloroform-methanol mixture. N,N-Diethylaminoethanol was purified by distillation. The solvents used as copolymerization medium, for precipitation and extraction were all of analytical grade.

2.1.2. Microbial strains

Antimicrobial activity was screened by Agar well diffusion method against two gram +ve bacterial strains, Bacillus pumillus – 1640 and Staphylococcus aureus – 2822, one gram –ve bacterial strain, Escherichia coli – 45. The fungal strains used were Fusarium oxysporum – 2087 and Aspergillus niger – 4325 collected from MTCC, Chandighar.

2.2. Copolymer Synthesis:

Both monomers N-vinylpyrrrolidone and Maleic anhydride were taken in equimolar in a three necked round bottom flask. They were dissolved in 1,4-dioxane which acts as solvent. The reaction was carried out in presence of AIBN as initiator at 650C under nitrogen atmosphere for about 48 hours. The copolymer formed was isolated from the reaction mixture and purified from the solvent by washing several times with diethylether. It was precipitated by treating with acetone. The copolymer was separated by centrifugation and dried at 400C under vacuum.

The copolymer (M) prepared was made to react with N, N-diethylaminoethanol at 400C in aqueous solution in different concentrations 1:1(M1), 1:2(M2). The copolymer (M), the copolymer complexes (M1, M2) were tested for antibacterial activity.

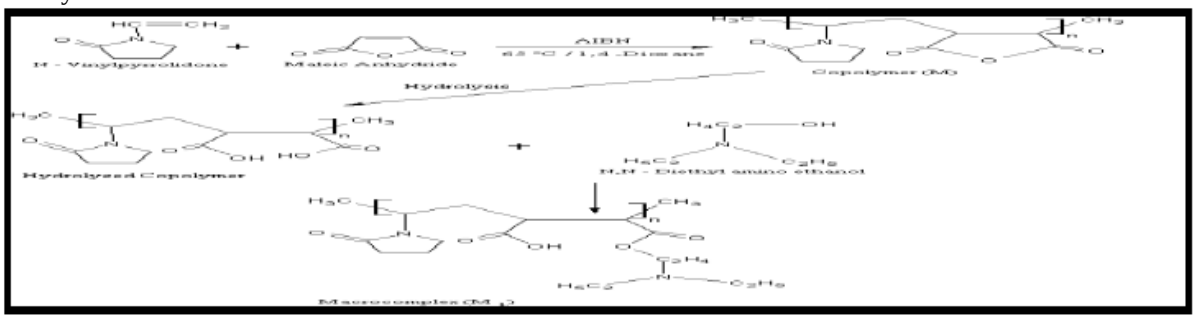
2.3. Copolymer characterization

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 – 4000cm-1.

The Nuclear magnetic Resonance Spectrum of both proton and carbon (H1 NMR and C13 NMR) for copolymers were recorded in Brucker AV – 300 spectrometer at Sapala Organics Private Limited, Hyderabad.

2.4. Antimicrobial activity test

Antimicrobial activity was screened by Agar well diffusion method [15]against three bacterial strains (two gram +ve and one gram –ve) and two 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(5µg/50µl) for bacteria and Fluconazole (5µg/50µl) 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.



Scheme - Preparation of Copolymer (M) and its compound M[1]

III. RESULTS AND DISCUSSION

3.1 Solubility Measurements

Solubility of the copolymer and its graft were examined in varieties of organic solvents ranging from polar to non polar. They were soluble in polar solvents like tetra hydrofuron, dimethyl sulfoxide, dimethylformamide, dimethylacetamide, isopropyl alcohol and methanol. Whereas, insoluble in non-polar ethyl acetate, chloroform, 1,4-dioxane, chloro benzene, toluene, n-hexane, n-heptane, cyclo hexane, carbon tetrachloride, diethyl ether and benzene.

3.2. Characterization of Copolymer

3.2.1. FTIR Spectral Studies of copolymer

Figure 1 shows FTIR spectrum of the copolymer.

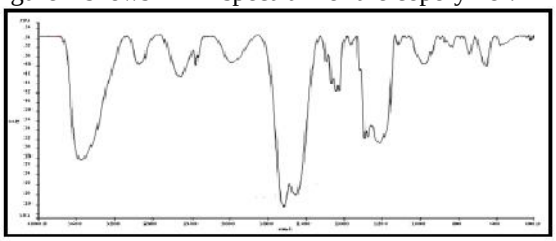


Fig 1: FTIR Spectrum of prepared NVP – MA Copolymer

Only important peaks are assigned.
NVP 1653.13cm-1 –C=O,1441.30cm-1 C-N-C, MA 1991.74cm-1anhydride ring, 984.25cm-1 cyclic anhydride, Range 649.99 and 739.61cm-1 C-(CH2)-C of parent chain

3.2.2. H^1_ NMR Spectral studies of copolymer M

H^1 NMR of the copolymer is as shown in the figure 2. Solvent – Methyl alcohol, Standard – TMS

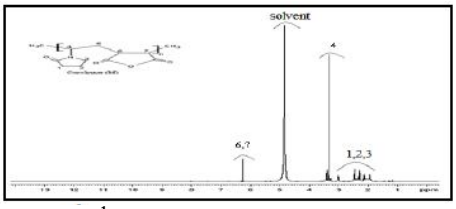


Fig 2. H^1 NMR Spectrum of Copolymer M

Ring methyl protons in NVP resonate at 1.8 δ, 2.2 δ and 2.8 δ. CH2 main chain back bone resonates at 3.5-2.5 δ. Protons of maleic unhydride resonate at 6.2 δ.

3.3.3. C13_ NMR Spectral studies of copolymer M

C13 NMR of the copolymer is as shown in the fig 3. Solvent – Methyl alcohol, Standard – TMS

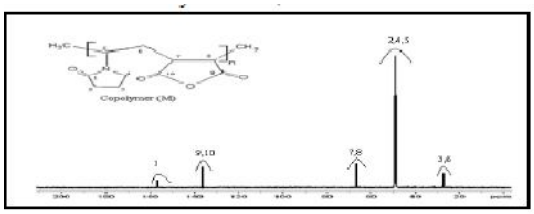


Fig 3. C^{13} NMR Spectrum of Copolymer M

Ring methylene carbon of NVP resonates at 49.6 δ, 43.59 δ and 23.8δ.

C=O of NVP resonates at 157 δ, CH2 Main chain back bone resonates at 48.2 and 21.2 δ.

Carbon atoms of maleic unhydride ring resonate at 136.13 δ

3.3.4. FTIR Spectral Studies of grafted compounds

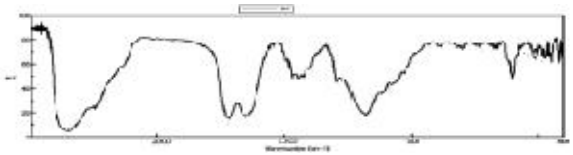


Fig 4. FTIR Spectrum of grafted copolymer in 1:1 ratio (M_1)

Only important peaks are assigned.
NVP –C=O 1650cm-1, C-N-C 1442cm-1, MA -OH not esterified 3417cm-1, Tertiary amine stretching 1179cm-1,

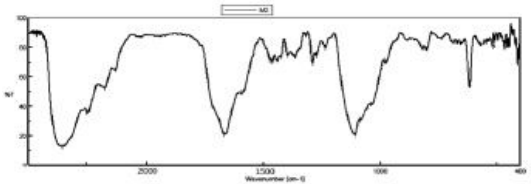


Fig 5. FTIR Spectrum of grafted copolymer in 1:2 ratio (M2)

Only important peaks are assigned.
NVP -C=O 1662cm-1, C-N-C stretching 1443cm-1, C-N 1326cm-1,
Tertiary amine stretching 3416cm-1, Parental chain 616cm-1.

IV. ANTIMICROBIAL SCREENING TEST

The antimicrobial activity of copolymer and its complex was examined against three bacterial strains viz., gram +ve bacteria Bacillus pumillus and Staphylococcus aureus, garm –ve bacterium Escherichia coliand two fungal strains Fusarium oxysporum and Aspergillus niger. The standard antibacterial used was Streptomycin sulphate. The standard antifungal used was Fluconazole. The inhibition zone in mm for the copolymer and its graft is given in the table 1.

Table 1.Antimicrobial activity of (M, M1 & M2) on Pathogenic Organisms

Test Sample	Volumes of the sample (µl)	Bacterial Strains			Fungal Strains	
		Bc	Sa	Ec	Fu	An
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

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

CONCLUSION

The Copolymer (NVP –MA) was synthesized. Its structure was confirmed by FTIR, H1_ NMR and C13_ NMR. It was made to react with DEAE after hydrolysis. Its graft was characterised by FTIR. The antimicrobial activity of copolymer and its macro complex was determined by Agar well diffusion method. It was demonstrated that the copolymer and its macro complex have a positive effect in controlling the growth of the gram –ve and gram +ve bacteria. The copolymer M is active in the presence of Fusarium oxysporum, but not with Aspergillus niger. Similarly the complex M2 is active in the presence of Aspergillus niger, but not with Fusarium oxysporum. Both fungal strains are susceptible in the presence of complex M2. This may be due to the nature of surface of the microbe and the combination of the functional groups within the single molecule of these macromolecules.

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