SYNTHESIS, CHARACTERISATION AND ANTIBACTERIAL ACTIVITY OF COPOLYMER (N-VINYLPYRROLIDONE – MALEIC ANHYDRIDE) WITH N- DIETHYLETHANOLAMINE

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Abstract

A copolymer of N-vinylpyrrolidone (NVP) with maleic anhydride (MA) is synthesized by radical copolymerization with 2,2-Azo-bisisobutyronitrile(AIBN) as initiator at 65^0C in dioxane solution, under nitrogen atmosphere. The copolymer obtained is characterized by FTIR and NMR. The copolymer is made to interact with N,N-Diethylaminoethanol (DEAE) in different ratios. The susceptibility of some Gram - negative bacteria like Klebsiella aerogenes NCIM-2098, Escherichia coli NCIM-5051, Pseudomonasaeruginosa NCIM2242, Pseudomonas desmolyticum NCIM-2028 and a Gram positive bacteria Staphylococcus aureus NCIM-5022, to the copolymer and its compound with N,N-Diethylaminoethanol of different compositions in microbiological medium is studied by Agar well diffusion method. The resulting polymeric antibacterial material is useful in a number of medicines and food industry.

Keywords: *N-vinylpyrrolidone, Maleic anhydride, Copolymerization, N, N-diethylaminoethanol, FTIR, NMR, Antibacterial activity.*

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1. INTRODUCTION

Copolymers with reactive or functional monomers are steadily gaining importance. Many copolymers with reactive functional groups are now being synthesized, tested and used not only for their macromolecular properties, but also for the properties of functional groups [1]. These functional groups provide an approach to a subsequent modification of the polymer for the specific end applications [2]. Nowadays, a strong demand prevails for "functional polymer" with very specific properties. Functional groups give a polymer structure a special character substantially different from the inherent properties of the basic polymer chain [3].

The antimicrobial property of the polymers plays an important role for many of its applications. Contamination by microorganisms is of great concern in several areas such as medical devices, health care products, water purification systems, hospital and dental equipments etc. One possible way to avoid microbial contamination is to develop the polymeric materials possessing antimicrobial properties [4-10]. Antimicrobial polymers are those, which are capable of killing pathogenic micro-organisms. These polymeric biocides can significantly reduce the loss of antimicrobial activity associated with volatization, photolytic decomposition, dissolution and permeation migration. Moreover, increased efficiency, selectivity and handling safety are additional benefits that are realized [11, 12].

Antimicrobial activity of some linear copolymers, containing quaternary ammonium and phosphonium salts has been reported by Kenawy et al. [12, 5, 10]. 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 [4]. It has been reported that polymers prepared using 2,4 - DMA showed strong inhibitory effect towards such tested microorganism as bacterial strains (S. Aurus, S. Citreus and E.Coli), molds and yeasts, while poly(VP) has been shown to have relatively lower antimicrobial activity. Gottenbos et al. reported that quaternary ammonium silane – coated silicone rubber showed antimicrobial properties against adhering bacteria, both Gram – positive and Gram – negative [13]. Ayhan Temiz 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 [14].

N-vinylpyrrolidone is a good biocompatible monomer due to its hydrophilic nature and low toxicity [15-17]. 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 [18-22]. Homo and copolymers of N-vinylpyrrolidone (VP) are of considerable academic and industrial interest due to their unique properties, allowing the use of these polymer systems in lithography as light sensitive thin coatings for printing plates, for the preparation of separating membranes for ultra filtration, biocompatible polymers with low toxicity and carriers of biologically active compounds, sorbents, coagulants and flocculants [23-27]. Copolymer of 2-hydroxymethylmethacrylate (HEMA) and N-vinylpyrrolidone is used in pharmaceutical application, such as, cancer therapy [28]. Copolymer of NVP and n-butylmetharylate are used for blood purification therapies [29]. Fullerene (60) C complexed with Poly (N-vinylpyrrolidone) is used in antiviral activity [30].

The use of maleic anhydride (MA) copolymers in medicine or pharmacy was described as antitumor agents, [31-33] drug carriers, supporters for enzymes or protein modifiers [34-37]. Antitumor functional polymers were synthesized by reaction of poly (MA-co-VP) with hydroxyl and amino containing physiologically active compound [38-40]. By the intermolecular reactions of poly (MA-co-styrene) [43] and poly(MA-co-methyl methacrylate) [41] with 3,6 – diamino-10-methylacridiniumchloride (acriflavine as an antiseptic agent) in DMF and poly[(*N*-isopropylacrylamide-*co*-maleic anhydride)-*g*-poly(ethylene oxide)] using triethylamine as catalyst [44], new derivatives of these copolymers with antimicrobial properties were also synthesized.

In the present work, a copolymer of NVP and MA is synthesized. It is characterized by FTIR, H^1 NMR and C^{13} 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.

2. EXPERIMENTAL

2.1 Materials

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

Antibacterial activity was screened by Agar well diffusion method [45] against five bacterial Strains Gram-ve *Klebsiella aerogenes* NCIM-2098, *Escherichia coli* NCIM-5051, *Pseudomonas aeruginosa NCIM2242*, *Pseudomonas desmolyticum* NCIM-2028 and a Gram +ve bacteria *Staphylococcus aureus* NCIM-5022.

2.2 Copolymer Synthesis

The copolymer of N-vinylpyrrolidone with maleic anhydride was prepared by radical initiated copolymerization in 1,4-dioxane at 65^0C in the presence of AIBN as initiator in three necked round bottom flask under nitrogen atmosphere using equimolar monomer mixture. The reaction mixture was continuously stirred for 48 hours. Copolymer was isolated from the reaction mixture and purified from 1,4 - dioxane solution to diethylether (Figure -1). It was precipitated with acetone. The precipitate was washed well with acetone. The copolymer was then isolated by centrifugation and dried at 40^0C under vacuum to constant weight.

The copolymer (M) prepared was made to react with N, N-diethylaminoethanol at 40^0C 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.

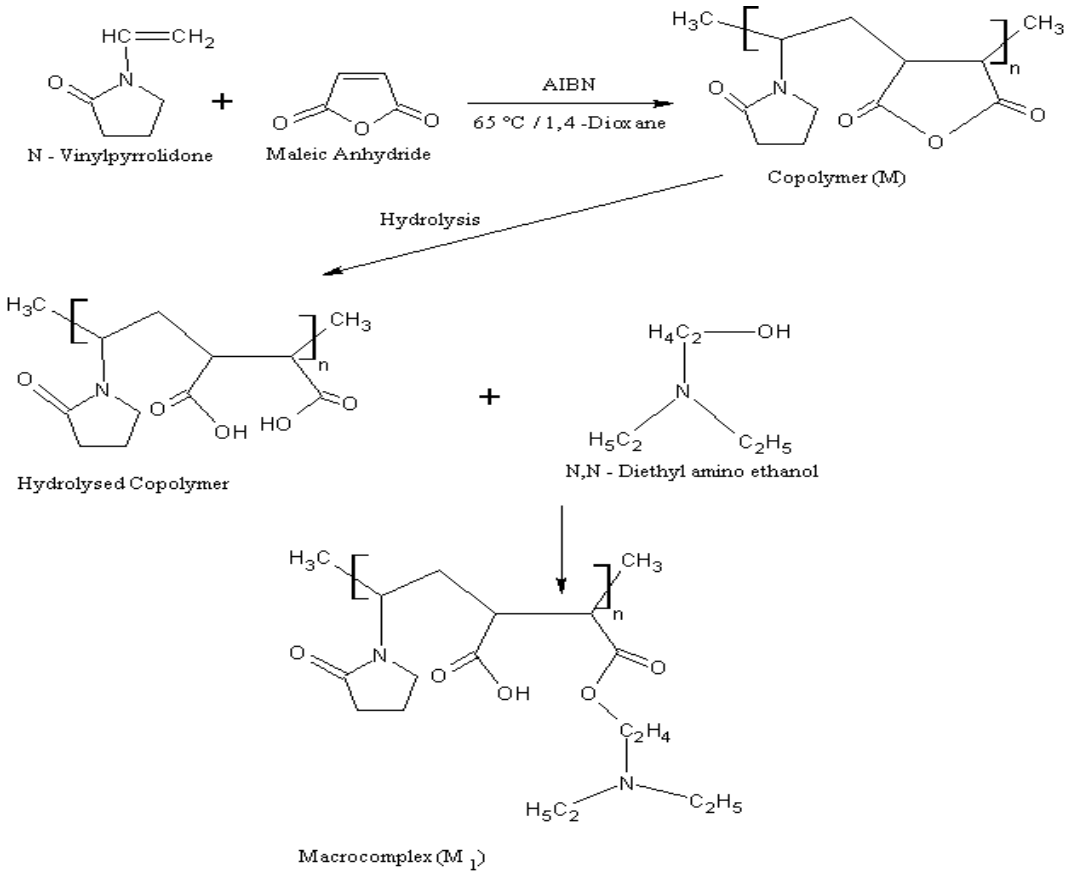

Fig 1: Reaction scheme of N-Vinylpyrrolidone and Maleic anhydride, hydrolyzed copolymer and poly(NVP-Co-MA) / DEEA macro complex

2.3 Copolymer Characterization

Copolymers were characterized by FTIR spectra using Shimadzu – 1800S spectrometer on KBr pellets in the range of 400-4000 cm^{-1}. The H^1-NMR and C^{13}-NMR (Brucker AMX-400, at Indian Institute of Science, Bangalore) spectra of copolymer were recorded in methanol as solvent and tetramethylsilane (TMS) as an internal standard.

2.4 Antimicrobial Activity Test

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 bacterial strains. The wells were made by using sterile cork borer of 6mm in each petri-plate. Varied concentrations of given compound (1000µg/well) were used to assess the activity of the compounds. The compound dispersed in sterile water acts as a negative control and the standard antibiotics Ciprofloxacin (5µg/50µl) (Hi Media, Mumbai, India) acts as positive control were tested against the bacterial pathogens. Then the plates were incubated at 37 °C for 24 – 36 hours. The zone inhibition was measured in millimeter for the every well and also the values were noted. Triplicates were maintained in every concentration and the average values were calculated for the ultimate antibacterial activity.

3. RESULTS AND DISCUSSION

3.1 Characterization of Copolymer

3.1.1 Solubility Studies

Solubility of the Copolymer was tested in various polar and non polar organic solvents. It was soluble in solvents like tetrahydrofuron, dimethylsulfoxide, dimethylformamide, dimethylacetamide, isopropylalcohol and methanol. Whereas, it was insoluble in ethylacetate, chloroform, 1,4-dioxane, chlorobenzene, toluene, n-hexane, n-heptane, cyclohexane, carbontetrachloride, diethyl ether and benzene.

3.1.2 Fourier Transform Infrared (FTIR) Spectral Studies

The structure of copolymer was confirmed by FTIR spectra as shown in Figure -2.

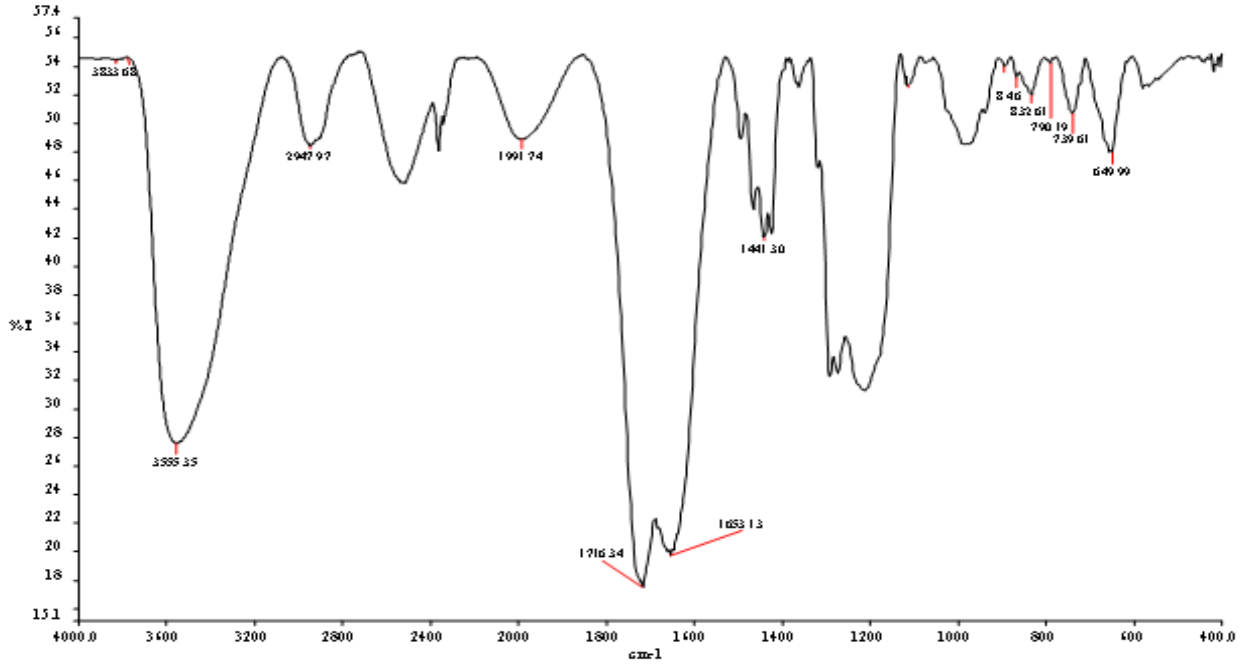

Fig 2: FTIR Spectra of prepared NVP – MA Copolymer

The structure of the copolymer is confirmed by FTIR spectra as shown in Fig-2. Only important peaks are assigned.

NVP 1653.13cm^{-1} –C=O, 1441.30cm^{-1} C-N-C,

MA 1991.74cm^{-1}anhydride ring, 984.25cm^{-1} cyclic anhydride,

Range 649.99 and 739.61cm^{-1} C-(CH2)-C of parent chain

3.1.3 Nuclear Magnetic Resonance Spectroscopy (H^1_ NMR):

The structure of copolymer is also confirmed by NMR spectral studies.

H^{1-} NMR spectrum of copolymer is as shown in Fig –3.

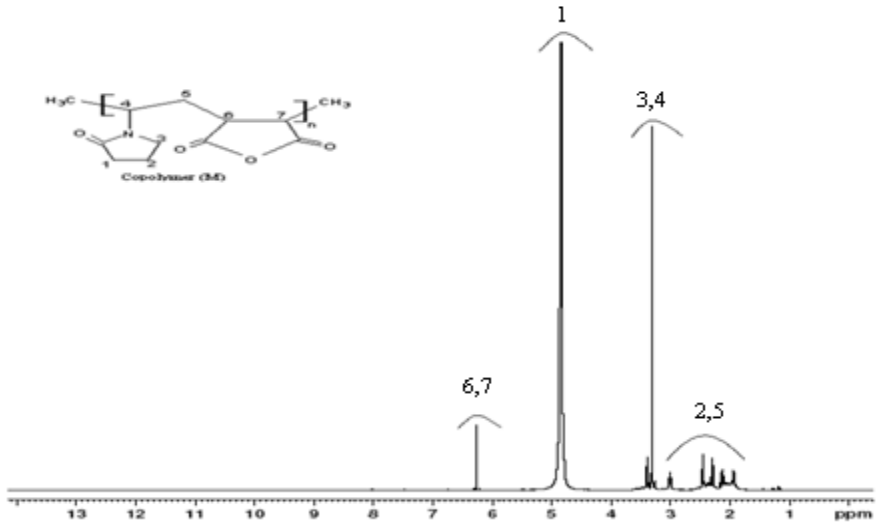

Fig 3: H^1 NMR Spectrum of Copolymer

Ring methyl protons in NVP resonate at 4.9 δ, 2.3 δ and 3.2 δ. CH_2 main chain back bone resonates at 3.5-2.5 δ. Protons of maleic unhydride resonate at 6.2 δ.

3.1.4 Nuclear Magnetic Resonance Spectroscopy (C^{13}_ NMR):

The structure of copolymer was also confirmed by C^{13}– NMR spectrum as shown in Fig –4.

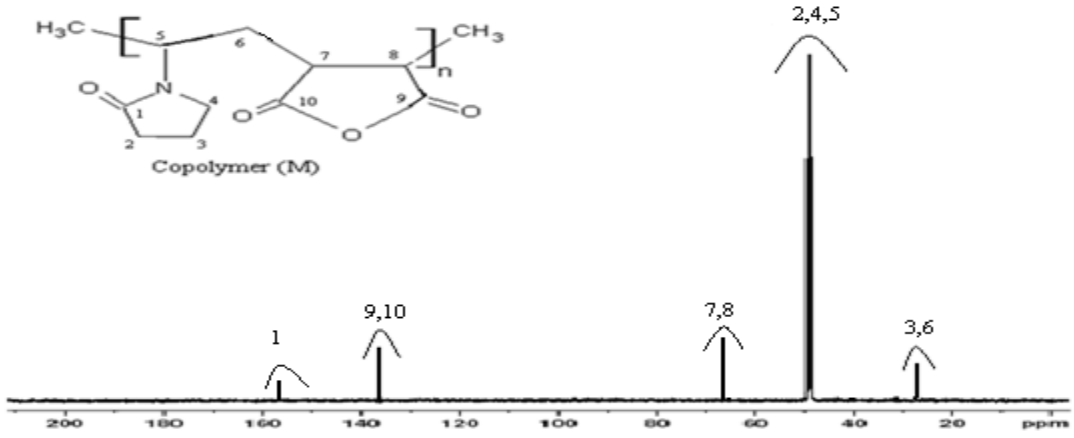

Figure 4: C^{13} NMR Spectrum of Copolymer

Ring methylene carbon of NVP resonates at 49.6 δ, 43.59 δ and 23.8 δ.
C=O of NVP respnates at 157 δ,
CH_2 Main chain back bone resonates at 48.2 and 21.2 δ.
Carbon atoms of maleic unhydride ring resonate at 136.13 δ

3.2 Anti bacterial Properties of Copolymer and its Compounds:

The antibacterial properties of compounds were evaluated against four Gram-ve *K. aerogenes, E. coli, P. aeruginosa,* and *P. desmolyticum* and one Gram +ve *S.aureus* bacterial strains using agar well diffusion method. In agar well diffusion method the compounds showed significant antibacterial activity on all the four Gram-ve bacterial strains. *K. aerogenes, E. coli, P.aeruginosa P. desmolyticum* and then another bacteria Gram +ve *S.aureus* is inactive. The zone of inhibition with the concentration 1000 µg per well is as shown in Fig.5. The data of zone of inhibition is depicted in Table 1.

Table-1: antibacterial activity of (M, M1, M2) on Pathogenic bacterial Strain

Sl.no	Treatment	*Klebsiella aerogenes(B1)* (Mean±SE)	*Escherichia coli (B2)* (Mean±SE)	*Pseudomonas aeruginosa(B3)* (Mean±SE)	*Staphyloccus aureus (B4)* (Mean±SE)	*Pseudomonas desmolyticum(B5)* (Mean±SE)
I	Standard (5µg/50µL)	12.67±0.33**	13.67±0.33**	13.67±0.33**	13.67±0.33**	14.67±0.33**
II	M (100µg/100µL)	1.33±0.33**	1.33±0.33**	2.67±0.33**	inactive	2.67±0.33**
III	M1 (1000µg/100µL)	2.67±0.33**	2.00±0.00	3.67±0.33**	inactive	3.00±0.00
IV	M2 (1000µg/100µL)	1.67±0.33**	2.67±0.33**	5.67±0.33**	inactive	1.00±0.00

Values are the mean ± SE of clear zone in mm. Symbols represent statistical significance,

*$P < 0.05$, **$P < 0.01$ as compared with the control group.

From this table, it is evident that the Copolymer (NVP – MA) is showing a better inhibition for the bacteria *Pseudomonas aeruginosa(B3)* and *Pseudomonas desmolyticum(B5).* The macro complex M1 and M2 are showing a better inhibition towards *Pseudomonas aeruginosa(B3).* Among all the compounds M2 is showing a very good antibacterial property.

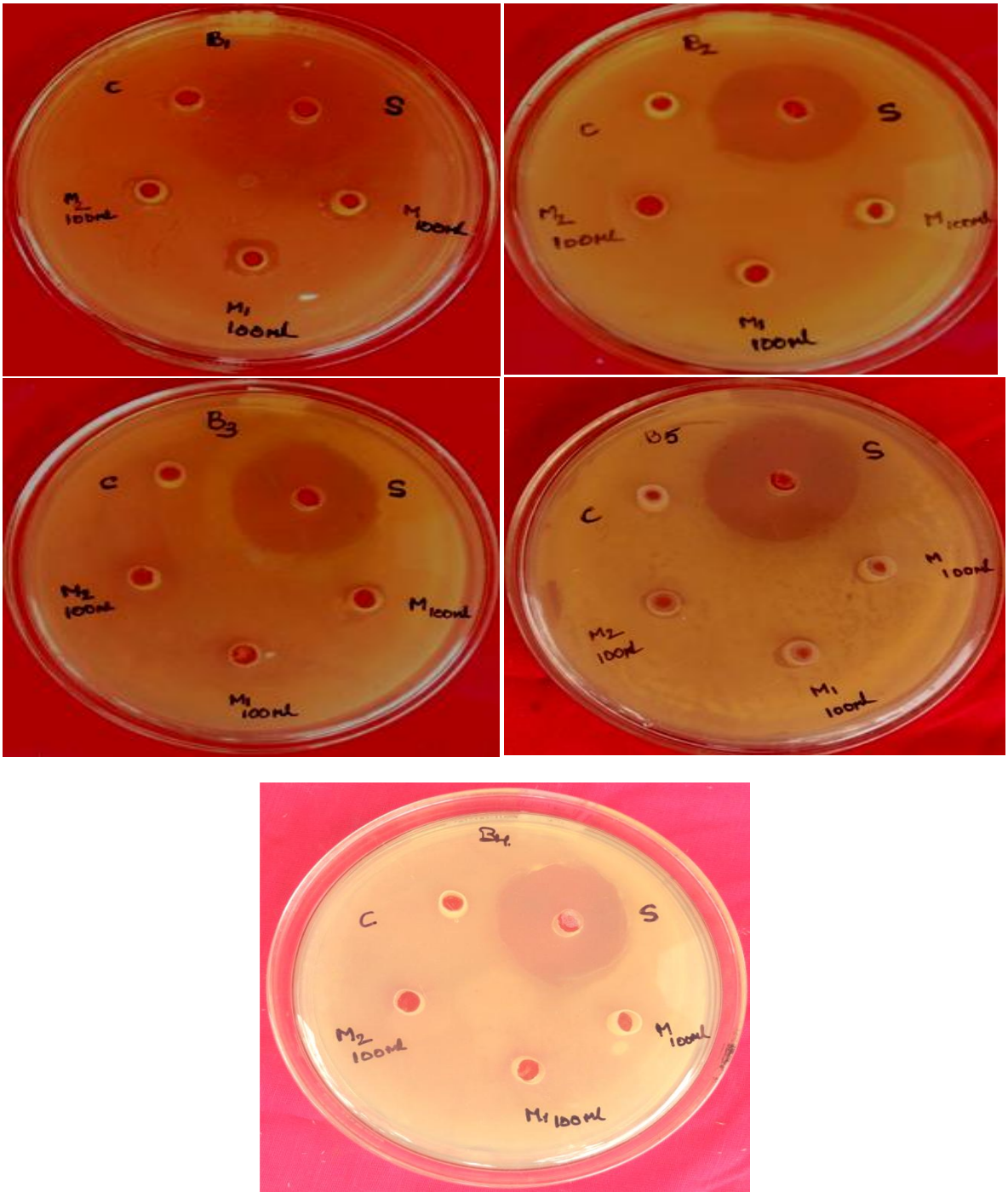

Fig 5: Representative photograph showing Antibacterial activity in Agar well diffusion method

4. CONCLUSIONS

The Copolymer (NVP –MA) was synthesized. Its structure was confirmed by FTIR, H^{1-} NMR and C^{13-} NMR. It was made to react with DEEA after hydrolysis. The anti bacterial 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 bacteria. Gram +ve Bacteria were found to be active in the presence of these compounds. This fact can be explained by the different responsive behavior of surface layer structures of these two bacteria. The activity of M2 is more when compared to M and M1. This may be due to the combination of the functional groups within the single molecule. This new polymeric biocide system can be recommended for the biomedical and food industry application.

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