



A Comparative Study on the Antimicrobial Property of Quaternised Copolymers N-vinylpyrrolidone - Dimethyl Amino Ethyl Methacrylate and N-vinylpyrrolidone - Vinyl Benzyl Chloride

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ABSTRACT

N-vinylpyrrolidone - Dimethyl amino ethyl methacrylate (NVP-DMAEMA) is a copolymer containing an antimicrobial monomer hence exhibits antimicrobial property. NVP-vinyl benzyl chloride (NVP-VBC) is another copolymer with chloride functional group which makes it antimicrobial. Quaternization of these will enhance the antimicrobial property. The copolymers NVP-DMAEMA and NVP-VBC are synthesized by free radical mechanism. The obtained copolymers are quaternized with suitable reagents. Copolymers and quaternized salts are characterized by Fourier transform infrared and nuclear magnetic resonance. The antimicrobial property of these compounds is investigated by agar well diffusion method. Two Gram-positive bacterial strains used are *Bacillus pumilus* – 1640 and *Staphylococcus aureus* – 3160, two Gram-negative bacterial strains are *Escherichia coli* – 45 and *Enterobacter aerogenes* – 2822. The fungal strains used are *Fusarium oxysporum* – 2087, *Aspergillus niger* – 4325, and *Penicillium sp.* All compounds are showing better inhibition zones.

Key words: N-vinylpyrrolidone - Dimethyl amino ethyl methacrylate, N-vinylpyrrolidone - Vinyl benzyl chloride, Fourier transform infrared, Nuclear magnetic resonance, Quaternization, Antimicrobial activity.

1. INTRODUCTION

Antimicrobial molecules are substances which are capable of killing microbes such as bacteria, fungi, and virus. Conventional antimicrobial molecules are chemicals with smaller molecular weight. One of the disadvantages of these is their residuals. Even though the molecules successfully deactivate the microbes, their residues are toxic to the environment.

Polymers are the molecules with high molecular weights. One of the important criteria for a molecule to be antimicrobial active is its functional group. If a polymer is prepared by introducing such functional group, it becomes antimicrobial active. 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.

N-vinylpyrrolidone (NVP) is a biocompatible monomer with low cytotoxicity. The two donor atoms oxygen and nitrogen present in it makes it hydrophilic [1-3]. As it can form many copolymers with a variety of monomers [4-8], it has very much extensive range of applications in various fields [9-13].

Both homo and copolymers of NVP find their applications not only as biologically active compounds but also as sorbents, coagulants, and flocculants [14-16]. Its copolymers are also used in cancer treatment [17], blood purification therapy [18] and as antiviral [19].

N,N-dimethyl amino ethyl methacrylate (DMAEMA) is a water soluble, monofunctional acrylate monomer. Poly (N,N-DMAEMA) (pDMAEMA) can inhibit the growth of bacteria on different materials, viz., glass, filter

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paper and plastic [20-23]. Its antibacterial activity may be increased by quaternization because quaternization enhances the positive charge density and amphiphilic character of its copolymer and polymer [24,25].

4-vinyl benzyl chloride (VBC) is dual functional monomer. It contains a benzyl group, chloride group, and a double bond. Its copolymers can also be quaternized and used as membrane in alkaline cells. The quaternized copolymer with 2-chloroethylvinylether exhibits antimicrobial property against a wide range of microbes [26].

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. Similarly, NVP can also be copolymerized with VBC and further quaternized with triethylamine and triphenylphosphine to get a group of antimicrobial compounds.

In the present work, we are comparing the antimicrobial activity of the all the compounds with suitable standard agents.

2. EXPERIMENTAL

2.1. Materials

NVP (Sigma-Aldrich), VBC, and N,N-DMAEMA (Kodak company, USA) were mixed with Fuller's earth separately and kept overnight to settle. The supernatant liquid was collected separately. 2,2-azo-bis-isobutyronitrile (AIBN) (Chems Worth, Surat, India), acted as initiator, triethylamine (SD Fine Chemicals, Mumbai, India), triphenylphosphine (Himedia, Mumbai, India), ethyl bromide (Chems Worth, Surat, India) which act as quaternizing agents and other solvents were used as received from S D Fine Chemicals.

2.2. Microbial Strains

Antimicrobial activity was screened by Agar well diffusion method against two Gram-positive bacterial strains, *Bacillus pumilus* – 1640 and *Staphylococcus aureus* – 2822, two Gram-negative bacterial strains, *Escherichia coli* – 45 and *Enterobacter aerogenes* – 2822. The fungal strains used were *Fusarium oxysporum* – 2087 and *Aspergillus niger* – 4325 collected from Microbial Type Culture Collection, Chandigarh. *Penicillium* sp. fungus was extracted from soil by the students of SIT, Tumkur, Karnataka, India.

2.3. Synthesis and Quaternization of the Copolymers

NVP and N,N-DMAEMA were taken in 1:1 molar in three necked round bottom flask. 0.5 g of AIBN 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 h. 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 h in a water bath maintaining a temperature of 60°C. The reaction was stopped after a duration of 6 h and washed with 1,4-dioxane solvent. It was reprecipitated with acetone and dried under vacuum at 40°C (Figure 1).

The monomers NVP, VBC in equimolar concentration and solvent 1,4-dioxane, initiator AIBN were taken in round bottom three necked flask. The polymerization process was conducted at 70°C with continuous stirring for about 6 h in an oil bath by maintaining inert atmosphere with nitrogen gas. When the reaction mixture became thick viscous liquid, the polymerization process was stopped and the mixture was cooled to 30°C. With the mixture of diethyl ether/hexane the copolymer was washed well to remove residual monomers. It was reprecipitated again with the same mixture. The formed crystals were dried at 40°C in vacuum. The copolymer was named as V. The calculated amount of copolymer V was made to react with triethylamine and triphenylphosphine by dissolving in 1,4-dioxane in two parallel reactions. The reaction was carried out for 6 h at about 60-70°C in the presence of N₂ gas to maintain an inert atmosphere. The thick viscous liquid formed was cooled to room temperature and washed with ethyl alcohol to remove the unreacted reactants and dried in vacuum at 40°C (Figure 2).

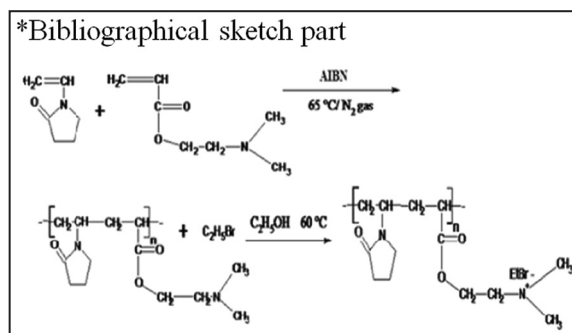


Figure 1: Scheme of preparation of copolymer D and quaternization.

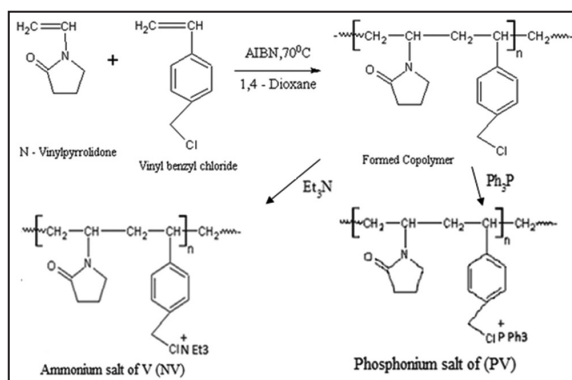


Figure 2: Scheme of preparation of copolymer V and quaternization.

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 (^1H NMR and ^{13}C NMR) for copolymers were recorded in Bruker AV – 300 spectrometer at Sapala Organics Private Limited, Hyderabad.

2.5. Antimicrobial Activity Test

Antimicrobial activity was screened by Agar well diffusion method [27] 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 dimethyl sulphoxide (DMSO) is used as negative control. The compounds of different volumes were dispersed in the same solvent. Simultaneously the standard antibiotic streptomycin sulfate (5 $\mu\text{g}/50$ μl) for bacteria and fluconazole (5 $\mu\text{g}/50$ 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 Copolymer

FTIR(KBr): 'D' 1671, 1446, 1729, 1151 cm^{-1} ; 'QD' 1670, 1440, 1289, 3434 cm^{-1} ; 'V' 1681, 1443, 1330, 3053, 1557 cm^{-1} ; 'NV' 1667, 1441, 1330, 3413 cm^{-1} ; N+ H 2682 cm^{-1} ; 'PV' 1667, 1441, 1328, 2961, P-Ph 1111 cm^{-1} , 1436 cm^{-1} .

^1H NMR of D: (DMSO) 1.3-2.4 (5H, Me), 7.2, 7.9 (2H, Me), 3.2(1H, Me) 5.6 (1H, Me) and 6.0 (1H, Me).

^{13}C NMR of D: (DMSO) 170 (C), 180 (C), 138 (C), 48 (CH₂), 24 (CH₂), 30 (CH₂), 125-130 (CH₂, 2CH₃).

^1H NMR of V: (Chloroform) 2.0 (2H, Me), 2.2 (2H, Me), 2.4 (2H, Me), 7.4 (1H, Me), 6.4 (1H, Me), 4.5 (2H, Me), 1.6-2.4 (CH₂, Me).

^{13}C NMR of V: (Chloroform) 17.5 (CH₂), 23.4 (CH₂), 30.1 (CH₂), 175.2 (C), 125.6 (CH), 144.7 (CH), 47.5 (CH₂).

Table 1: Antimicrobial activity of copolymers and quaternized salts against on pathogenic organisms.

Copolymer	Grafted compound	Volume (μl)	Bacterial strains				Fungal strains		
			Bc	Sa	Ec	Ea	Fu	An	Pe
Standard	Streptomycin sulfate	(5 $\mu\text{g}/50$ μl)	30	30	35	30			
Standard	Fluconazole	(5 $\mu\text{g}/50$ μl)					11	8	-
NVP-VBC	V	50	30	15	8	18	-	8	15
		100	30	20	-	22	11	8	20
		200	30	21	15	8	10	10	25
	NV	50	15	29	23	12	12	8	20
		100	25	15	15	18	14	9	12
		200	30	21	35	13	13	10	10
	PV	50	12	No clear zone	16	No clear zone	18	5	No clear zone
		100	25		22		19	12	
		200	25		29		19	10	
NVP-DMAEMA	D	50	15	20	20	-	11	5	8
		100	15	23	11	13	11	-	15
		200	15	20	21	10	20	-	10
	QD	50	35	20	28	-	-	8	-
		100	15	10	11	-	-	8	-
		200	25	12	22	-	-	10	-

==Inactive, NVP=N-vinylpyrrolidone, DMAEMA=Dimethyl amino ethyl methacrylate, VBC=Vinyl benzyl chloride, Bc=*Bacillus pumilus*, Sa=*Staphylococcus aureus*, Ec=*Escherichia coli*, Ea=*Enterobacter aerogenes*, Fu=*Fusarium oxysporum*, An=*Aspergillus niger*, Pe=*Penicillium* sp.

3.2. Antimicrobial Activity

The antimicrobial properties of the polymers and their compounds were evaluated 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* and *Penicillium* sp. by agar well diffusion method.

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

From Table 1, it is evident that the copolymer of NVP-DMAEMA and NVP-VBC and their quaternized salts are showing a better inhibition for all microbes selected for the study. A clear zone of inhibition is not observed for phosphonium salt of copolymer V, may be due to the diffusion of salt into the agar. Quaternized salt of D is also inactive toward bacterium *E. aerogenes* and fungi *F. oxysporum* and *Penicillium* sp.

4. CONCLUSION

The copolymers NVP-DMAEMA and NVP-VBC were synthesized by free radical polymerization. They were quaternized by suitable reagents. All were characterized by FTIR and NMR. The antimicrobial activity of all was determined by Agar well diffusion method. A validated zone of inhibition was observed with all compounds. Copolymer NVP-DMAEMA, NVP-VBC and quaternized salt of NVP-VBC were exhibiting a better antibacterial and antifungal property. Whereas, quaternized salt of NVP-DMAEMA was not that much antimicrobial active.

5. REFERENCES

1. S. Vijaysekaran, T. V. Chirila, Y. Hong, S. G. Tahija, P. D. Dalton, I. J. Constable, I. L. McAllister, (1996) Poly(1-vinyl-2-pyrrolidinone) hydrogels as vitreous substitutes: Histopathological evaluation in the animal eye, *Journal of Biomaterials Science*, **7**: 685-696.
2. H. Ranucci, G. Spagnoli, L. Sartore, F. Bignottie, P. Ferruti, (1995) Synthesis and molecular weight characterization of end-functionalized N-vinyl-2-pyrrolidone oligomers, *Macromolecular Chemistry and Physics*, **196**: 763-774.
3. H. Y. Lee, S. A. Yu, K. H. Jeong, Y. Kim, (2007) Poly(vinyl pyrrolidone) conjugated lipid system for the hydrophobic drug delivery, *Macromolecular Research*, **15**: 547-552.
4. B. S. S. Reddy, R. Arshady, M. George, (1985) Copolymerization of N-vinyl-2-pyrrolidone with 2, 4, 5 - tri-chlorophenyl acrylate and with 2-hydroxyethyl methacrylate: Reactivity ratios and molecular weights, *European Polymer Journal*, **21**: 511-515.
5. S. Morariu, C. Hulubei, (2006) Radical copolymerization of functional N-substituted maleimides with N-vinyl-2-pyrrolidone, *High Performance Polymers*, **18**: 185-198.
6. G. Bauduin, B. Boutevin, M. Belbachir, R. Meghabar, (1995) Determination of reactivity ratios in radical copolymerization: A comparison of methods for a methacrylate/N-vinylpyrrolidone system, *Macromolecules*, **28**: 1750-1753.
7. S. Soundarajan, B. S. R. Reddy, (1991) Glycidyl methacrylate and N-vinyl-2-pyrrolidone copolymers: Synthesis, characterization, and reactivity ratios, *Journal of Applied Polymer Science*, **43**: 251-258.
8. S. Vijay Kumar, T. E. Musturappa, S. Prasannakumar, K. M. Mahadevan, B. S. Sheregara, (2007) N-vinyl pyrrolidone and ethoxyethyl methacrylate copolymer: Synthesis, characterization and reactivity ratios, *Journal of Macromolecular Science, Part A*, **44**: 1161-1169.
9. D. Radic, L. Gargallo, (1997) Synthesis, reactivity ratios, and solution behavior of vinylpyrrolidone - Co-monoalkylitaconate and vinylpyrrolidone-co-dialkyl itaconate copolymers, *Macromolecules*, **30**: 817-825.
10. D. Queroz, R. R. Vargas, O. Z. Higa, R. R. Ribeiro, M. Vitolo, (2002) Lactam-amide graft copolymers as novel support for enzyme immobilization, *Journal of Applied Polymer Science*, **84**: 767-777.
11. S. K. Bajpai, J. Sonkusley, (2002) Hydrogels for oral drug delivery of peptides: Synthesis and characterization, *Journal of Applied Polymer Science*, **83**: 1717-1729.
12. T. Beitz, J. Kotz, G. Wolf, E. Lleinpeter, S. E. Fribery, (2001) Poly(N-vinyl-2-pyrrolidone) and 1-Octyl-2-pyrrolidinone modified ionic micro emulsions, *Journal of Colloid and Interface Science*, **240**: 581-589.
13. M. Basri, A. Harun, M. B. Ahmad, C. A. N. Razak, A. B. Salleh, (2001) Immobilization of lipase on poly(N-vinyl-2-pyrrolidone-co-styrene) hydrogel, *Journal of Applied Polymer Science*, **82**: 1404-1409.
14. H. F. Mark, N. M. Bikales, C. G. Overberger, G. Menges, (1986) *Encyclopedia of Polymer Science and Engineering*, New York: Wiley Interscience.
15. N. Gatica, L. Gargallo, D. Radić, (1998) 2-Vinylpyridine-co-N-vinyl-2-pyrrolidone and 4-vinylpyridine-co-N-vinyl-2-pyrrolidone copolymers: Synthesis and reactivity ratios, *Polymer International*, **45**: 285-290.
16. F. Abbaszadeh, O. Moradi, M. Norouzi, O. Sabzevari, (2014) Improvements single-wall carbon nanotubes (SWCNTs) based on functionalizing with monomers 2-hydroxyethylmethacrylate (HEMA) and N-vinylpyrrolidone (NVP) for pharmaceutical applications as cancer therapy, *Journal of Industrial and Engineering Chemistry*, **20(5)**: 2895-2900.
17. M. Tjink, J. Janssen, M. Timmer, J. Austen,

- Y. Aldenhoff, J. Kooman, L. Koole, J. Damoiseaux, R. van Oerle, Y. Henskens, D. Stamatialis, (2013) Development of novel membranes for blood purification therapies based on copolymers of N-vinylpyrrolidone and n-butylmethacrylate, *Journal of Material Chemistry B*, **1**: 6066-6077.
18. O. I. Kiselev, K. N. Kozeletskaya, M. Elu, L. V. Vinogradova, E. E. Kever, S. I. Klenin, V. N. Zgonnik, M. A. Dumpis, L. B. Piotrovskii, (1998) Antiviral activity of fullerene (60)C complexed with poly(N-vinyl pyrrolidone). *Doklady Akademii Nauk*, **361(4)**: 547-549.
 19. A. Kusumo, L. Bombalski, Q. Lin, K. Matyjaszewski, J. W. Schneider, R. D. Titlon, (2007) High capacity, charge-selective protein uptake by polyelectrolyte brushes, *Langmuir*, **23**: 4448-4454.
 20. S. B. Lee, R. R. Koepsel, S. W. Morley, K. Matyjaszewski, Y. Sun, A. J. Russell, (2004) Permanent, nonleaching antibacterial surfaces. 1. Synthesis by atom transfer radical polymerization, *Biomacromolecules*, **5**: 877-882.
 21. D. Roy, J. S. Knapp, J. T. Guthrie, S. Perrier (2008) Antibacterial cellulose fibre via RAFT surface graft polymerization, *Biomacromolecules*, **9**: 91-99.
 22. J. Huang, H. Murata, R. R. Koepsel, A. J. Russell, K. Matyjaszewski, (2007) Antibacterial polypropylene via surface-initiated atom transfer radical polymerization, *Biomolecules*, **8**: 1369-1399.
 23. L. A. B. Rawlinson, S. M. Ryan, G. Mantovani, J. A. Syrett, D. M. Haddleton, D. J. Brayden, (2010) Antibacterial effect of poly(2-(dimethylamino ethyl)methacrylate) against selected gram-positive and gram-negative bacteria, *Biomacromolecules*, **11(2)**: 443-453.
 24. H. Dong, J. Huang, R. R. Koepsel, P. Ye, A. J. Russell, K. Matyjaszewski, (2011) Recyclable antibacterial magnetic nanoparticles grafted with quaternised poly(2-(dimethylamino)ethyl methacrylate) brushes, *Biomacromolecules*, **12**: 1305-1311.
 25. Y. Luo, J. Guo, C. Wang, D. Chu, (2010) Quaternized poly(methyl methacrylate-co-butyl acrylate-co-vinylbenzyl chloride) membrane for alkaline fuel cells, *Journal of Power Sources*, **195(12)**: 3765-3771.
 26. E. R. Kenawy, F. I. Abdel-Hay, A. A. El-Magd, Y. Mahmoud, (2006) Biologically active polymer: VII. Synthesis and antimicrobial activity of some copolymers with quaternary ammonium and phosphonium groups, *Reactive and Functional Polymers*, **66(4)**: 419-429.
 27. C. Perez, M. Paul, P. Bazerque, (1990) An antibiotic assay by the agar well diffusion method, *Actabiologiae et Medicine Experiments*, **15**: 113-115.

***Bibliographical Sketch**

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