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Research Article

Synthesis and Antibacterial Evaluation of N-Vinylcarbazole - Co-Phenoxy Methacrylate Copolymers Using Fourier-transform infrared-Attenuated Total Reflection

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ABSTRACT

The scope of the present study is focused to explore the antimicrobial properties of poly N-vinylcarbazole - co-phenoxyethyl methacrylate (NVC-Co-POEMA). Copolymers of NVC with POEMA at different feed compositions were prepared by free-radical solution polymerization at 60 ± 5°C using benzoyl peroxide as an initiator. The copolymers were characterized by Fourier-transform infrared (FTIR) and ^{1}H- nuclear magnetic resonance spectroscopy. Thermogravimetric analysis and differential scanning calorimetry of the copolymers showed high thermal stability and higher Tg values. The *in vitro* antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Salmonella paratyphi* was studied and compared. The antibacterial activity of the copolymer is higher than that of the homopolymer. This shows that copolymerization with phenoxy methacrylate moiety plays a very important role in the antibacterial activity. This has been confirmed with FTIR-attenuated total reflection spectra.

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INTRODUCTION

Contamination by microorganisms is of great concern in several areas such as medical devices, healthcare product, water purification systems, hospital and dental office equipment, food packaging, food storage, and household sanitation.[1,2] One possible way to avoid the microbial contamination is to develop materials possessing antimicrobial activities. Consequently, biocidal polymers have received much attention in recent years. Usage of antimicrobial polymers promise enhancing the efficacy of some existing antimicrobial agents and minimizing the environmental problems accompanying conventional antimicrobial agents by reducing the residual toxicity of the agents, increasing their efficiency and selectivity, and prolonging the lifetime of the antimicrobial. Over the past decade, the use of polymeric materials for the administration of pharmaceuticals and as biomedical devices has increased dramatically. For years, dentures, dental fillings, and denture bases have been made using methacrylic ester polymers.[3,4] These polymers can also be used to prevent tooth decay in natural teeth by serving as a barrier which can be coated over the surface of teeth. The dimensional behavior of these bone-cement composites has been reported.[5,6] Lutz[7] prepared polymers from meth(acrylate) moiety and poly(ethylene) glycol which can be used for building a wide variety of modern materials such as biosensors, artificial tissues, smart gels for chromatography, and drug carriers. Both hard and soft contact lenses have been made using polymethacrylates.[8-10] Hydrogels comprising poly(2-hydroxyethyl methacrylate) are used in soft contact lenses.[11,12] The main objective of the study was to synthesize and characterize acrylic copolymers having antimicrobial to evaluate their properties. Considering the above objective, N-vinylcarbazole (NVC) was copolymerized with phenoxyethyl methacrylate (POEMA). Fourier-transform infrared (FTIR) and ^{1}H-nuclear magnetic resonance (NMR) spectroscopy were used to characterize the copolymers. The copolymers showed high thermal stability and higher Tg. It was predicted by the thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) values. Homopolymers and copolymers have been characterized in their antimicrobial

activity against microorganisms. Their activities were tested with bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis,* and *Salmonella paratyphi*. Finally, the copolymers antibacterial activity is confirmed by FTIR-attenuated total reflection (ATR) using *S. aureus* bacteria as an example.

SYNTHESIS OF COPOLYMERS

The copolymers were synthesized from the appropriate amounts of different monomer using benzoyl peroxide as initiator. The reactants were dissolved in 25 mL of chlorobenzene to obtain a homogeneous solution in a standard polymer tube. Dry nitrogen gas was flushed into the reaction mixture. The reaction vessel was then immersed in a thermostatic water bath maintained at 60 ± 5°C. After desired period, the tubes are removed from the water bath and cooling was done under running tap water. The solution was then poured into ice-cold methanol to precipitate the copolymer. The copolymers were purified by repeated precipitation of chloroform from solution in chloroform and dried in vacuum oven at 45°C for 24 h. The schematic representation of the synthesis of copolymers is shown in Table 1.

The schematic representation of the synthesis of poly (NVC - co-POEMA) is shown in Figure 1.

CHARACTERIZATION OF COPOLYMER: POLY (NVC - CO-POEMA)

FTIR spectrum

The FTIR spectrum of poly (NVC - CO-POEMA) is shown in Figure 2. The band at 2979 cm^{-1} can be assigned to the aromatic C–H asymmetric stretching. The bands at 1454 and 1483 cm^{-1} may be attributed to the ring vibration of NVC moiety. A sharp band at 3444 cm^{-1} can be assigned to the phenoxy. The C–H in-plane deformation of aromatic ring has been observed at 1223 cm^{-1} and that of vinylidene group at 1335 cm^{-1}. The band at 3061 cm^{-1} can be assigned to the -CH_3 stretch vibration. CH_2 stretch vibration appears at 2948 cm^{-1}. A very strong band at 1727cm^{-1} belongs to the carbonyl group and −C−O−C− bands at 1244 and 1223 cm^{-1}. The bands at 1590 cm^{-1} and 1488 cm^{-1} are due to the phenyl group. The aromatic ring and the oxygen bond appears around 1727 cm^{-1}, while aromatic C-C appears around 1624 cm^{-1}. The C-O frequency can be attributed to the band at 1335 cm^{-1}. The -CH bending mode of vinyl group appears at 890 cm^{-1}, while the rocking mode at 670 cm^{-1}.[13,14]

^{1}H-NMR spectrum

^{1}H-NMR spectra of poly (NVC - co-POEMA) is shown in Figure 3. A hump at 0 ppm corresponds to TMS, an internal standard. The methyl protons occur at 2.06 ppm. The non-equivalent methylene protons at 5.59 ppm and equivalent methylene protons appeared at 1.81 ppm. The resonance signals between δ = 7.19-7.67 ppm are due to aromatic protons of NVC. The signals at δ = 1.55-2.16 ppm are due to the γ-methylene protons and methine protons of NVC unit. While the signals at δ = 0.88-2.16 ppm, and the multiplets between δ = 1.0-2.28 ppm are due to methyl and backbone protons of NVC and POEMA. The alkoxy protons appeared at 4.00 ppm.[15,16] Thus, the ^{1}H-NMR spectrum confirms the chemical structure of the copolymer.

Table 1: Synthesis of poly (NVC - co-POEMA)

Monomer 1:Monomer 2 ratio	NVC	POEMA	Yield (%)
0.20:0.80	0.2577	1.0180	70
0.50:0.50	0.3865	0.3819	86
0.80:0.20	0.3865	0.0954	75

NVC: N-vinylcarbazole, POEMA: Phenoxyethyl methacrylate

TGA of poly (NVC - co-POEMA)

The thermal decomposition of the copolymer was characterized by TG curves. The TGA curves [Figure 4] clearly indicate that all the copolymers undergo single-step decomposition process. The measured results were shown in Table 2. Different copolymer ratio exhibits excellent thermal stability with decomposition temperature ranges from 0 to 600°C.[15,16] The copolymers exhibited no significant weight loss on being heated to 600°C. The thermal stability of the copolymer increases as the NVC moiety is increased.

DSC of poly (NVC - co-POEMA)

The glass transition temperature of the polymer was studied by DSC in the temperature range 0°C-200°C under nitrogen atmosphere. The glass transition temperature (T_g) of poly (NVC - CO-POEMA) was found to be 98.19°C [Figure 5]. T_g increased by the copolymerization. This is due to the presence of POEMA and complex group which is polar in nature that increases the intermolecular force, interchain attraction, and cohesion which lead to decrease in free volume and increase in Tg.[17]

MICROBIAL SCREENING

The synthesized homopolymers and copolymers were tested against different microorganisms which are commonly employed for biodegradability tests.[1] Bacterial strains (*S. aureus*, *E. coli*, *B. subtilis*, and *Salmonella paratyphi*).[18-21]

Antibacterial activity

Screening of acrylic copolymers for antibacterial activity

The inoculums for the experiment were prepared in fresh nutrient broth from preserved slant culture. McFarland standards were used to standardize the inoculums by adjusting the turbidity of the culture. By the addition of sterile saline or broth, we can adjust the turbidity of the culture.[22,23] Cotton wool swabs on wooden applicator or plastics were prepared and sterilized by autoclaving or dry heat (only for wooden swabs) by packing the swabs in culture tubes, papers, tins, etc. By dipping in alcohol and burning off the alcohol, we have sterilized the forceps. The standardized inoculums were inoculated in the plates prepared earlier (aseptically) by dipping a sterile in the inoculums. The excess of inoculums was removed by passing, pressing, and rotating the swab firmly against the side of the culture tube above the level of the liquid. Streak the swabs all over the surface of the medium 3 times. The plates were then rotated through an angle of 60°

Table 2: TGA analysis data of poly (NVC - co-POEMA)

Poly (NVC - co-POEMA) composition	IDT	Decomposition temperature range (°C)	Temperature (°C) of weight loss (%) of the copolymers				
			10	30	50	70	90
20:80	267	0-600	289	357	397	404	416
50:50	270	0-600	290	367	401	425	469
80:20	275	0-600	302	370	412	432	485

NVC: N-vinylcarbazole, POEMA: Phenoxyethyl methacrylate, TGA: Thermogravimetric analysis

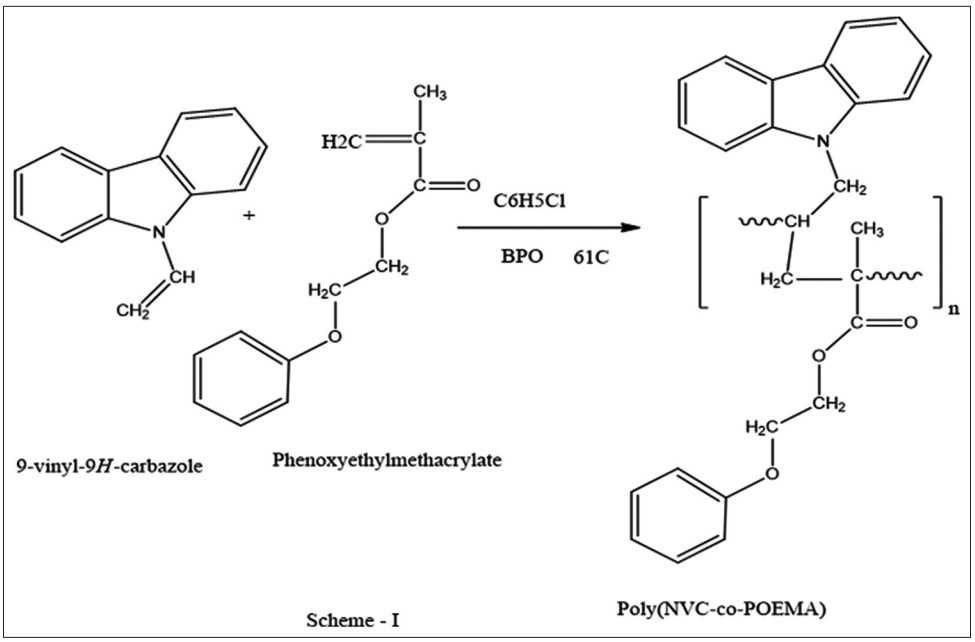

Figure 1: Synthesis of poly (N-vinylcarbazole - co-phenoxyethyl methacrylate)

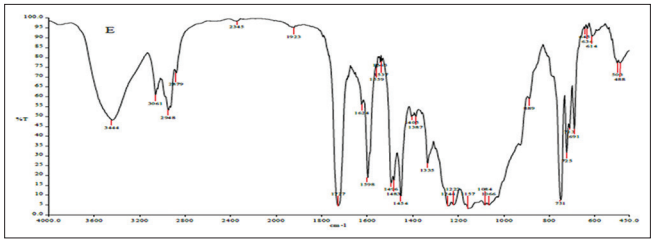

Figure 2: Fourier-transform infrared spectrum of poly (N-vinylcarbazole - co-phenoxyethyl methacrylate)

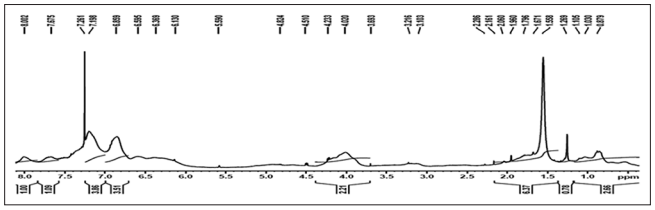

Figure 3: ^{1}H nuclear magnetic resonance spectrum of poly (N-vinylcarbazole - co-phenoxyethyl methacrylate)

after each application. At last, pass the swab round the edge of the agar surface. The drying of inoculums was done at room temperature by the lid closed.

In each Petri dish, sample discs such as NVC:POEMA (20:80), NVC:POEMA (50:50), NVC:POEMA (80:20), and HOMO (100 μg) discs (discs are soaked overnight in sample solution) and STD ciprofloxacin 10 μg, were placed with the help of sterile forceps. Diffusion was done by placing the Petri dishes in the refrigerator at 4°C at room temperature for 1 h and incubated at 37°C for 24 h. The zone of inhibition produced by different samples was observed. Measure it using a scale and record the average of two diameters of each zone of inhibition [Figure 6 and Table 3].[24-30]

FTIR-ATR SPECTRA OF THE POLY (NVC - CO-POEMA) WITH BACTERIA

The synthesized copolymer along with the biofouling bacteria (*S. aureus*) was investigated under ATR-FTIR. The infrared spectrum of poly (NVC - co-POEMA) is illustrated in Figure 7. The sample exhibited absorption bands due to hydroxyl stretch at 3402 cm^{-1}. A previous study shows that the hydroxyl group decreases when carbonate content increases. A weak band near 3005 cm^{-1} is also observed, resulting from C-H double bond stretching modes of unsaturated fatty acid chains. The spectrum shows the intense peak of 1014 cm^{-1} was associated with PO_4 antisymmetric stretching mode. The 951 cm^{-1} peak

corresponds to the PO_4 derived bands. The peaks between 1436 cm^{-1} and 1406 cm^{-1} are related to carbonated apatite. On the other hand, in the FTIR spectrum, the peaks at 2918 cm^{-1} are associated with asymmetric and symmetric vibrations of the CH_2 group. The peaks at 1315 and 1406 cm^{-1} correspond to the CH_2 band vibrations.[31] The ester COO vibrations occur at 1014 and 1250 cm^{-1}. The -O–C vibrations occur at the peak of 1014 and 951 cm^{-1}. Peak absorption shows a very small decrease at 1014, 951 to 703 cm^{-1}. The region between 1800 and 1500 cm^{-1} is dominated by the conformation-sensitive amide I and amide II bands, which are the most intensive bands in the spectra of nearly all bacterial samples and a band near 1715 cm^{-1} is assigned to a CHO stretching vibration, is routinely observed in the spectra of hydrated microbial cells and tissue material and is known as a sensitive probe of base pairing in nucleic acids. Similarly, the C=O vibration of ester at 1721 cm^{-1}, with the CH_2 vibration appearing at 732 cm^{-1} was characteristic of the bacterial samples. The incorporation of the antibacterial copolymer shows the absence of these peaks indicating the decline in the growth of the biofouling bacteria.[32] Furthermore, the effect of hydrophobicity on antibacterial activity was monitored using ATR-FTIR. The hydrophobic groups (e.g., methyl and methylene) show peaks at 2918, 1436, and 1406 cm^{-1}. Stronger intensity of these peaks indicates the hydrophobic nature of the copolymer, which in turn is an important property for antibiofouling activity. Thus, the infrared spectrum of copolymer with biofouling bacteria showed expected results with characteristic peaks.

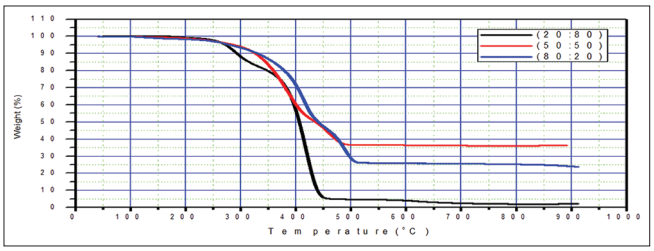

Figure 4: Thermogravimetric analysis spectrum of poly (N-vinylcarbazole - co-phenoxyethyl methacrylate)

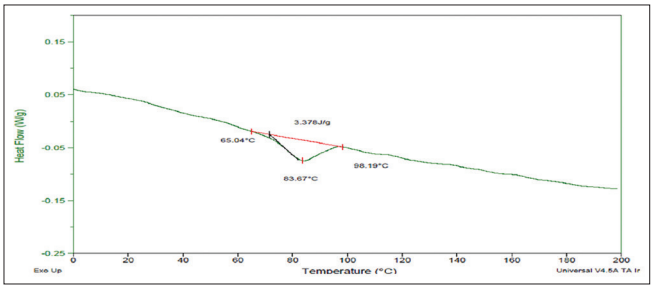

Figure 5: Differential scanning calorimetry of poly (N-vinylcarbazole - co-phenoxyethyl methacrylate)

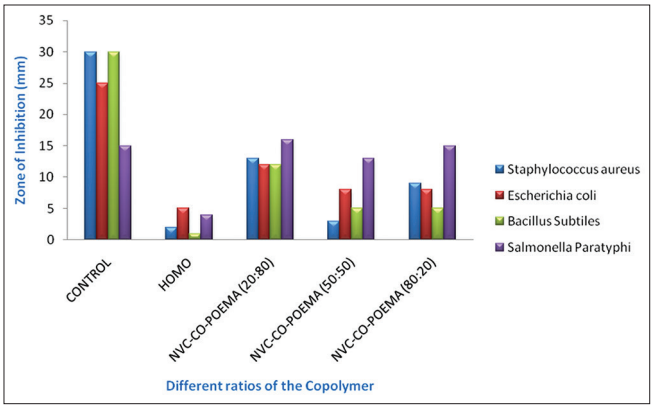

Figure 6: Antibacterial activity of poly (N-vinylcarbazole - co-phenoxyethyl methacrylate)

CONCLUSION

The microbial screening of the homopolymer and copolymers shows that the copolymer was most effective in inhibiting microorganism growth. As the percentage of NVC in the copolymers increases, the effectiveness of the copolymers to inhibit the growth of the microorganisms mostly decreases except in few cases. As expected, poly (NVC - co-POEMA) is effective in inhibiting the growth of microorganisms. Although the nitrogen present in the polymers appears to be the most important component to impart antimicrobial properties, it is to be remembered that the conformation of the polymers acquired under experimental conditions is a factor for their antigrowth activity. This might account for the reversal in the trend of activity of the copolymers in a few cases. Summarizing, the antimicrobial activity of the polymers has been attributed to the presence of nitrogen-containing aromatic heterocyclic ring in the polymers and their conformational changes took place during the copolymerization. The antibacterial activity has been confirmed by FTIR-ATR.

Table 3: Antimicrobial activity of poly (NVC - co-POEMA) against bacterial pathogens

Organisms	Zone of inhibition (mm)				
	STD ciprofloxacin (10 μg/disc)	Samples (100 μg/disc)			
		HOMO	NVC: POEMA (20:80)	NVC: POEMA (50:50)	NVC: POEMA (80:20)
S. aureus	30	02	13	03	09
E. coli	25	05	12	08	08
B. subtilis	30	01	12	05	05
S. paratyphi	15	04	16	13	15

NVC: N-vinylcarbazole, POEMA: Phenoxyethyl methacrylate, *S. aureus: Staphylococcus aureus, E. coli: Escherichia coli, B. subtilis: Bacillus subtilis, S. paratyphi: Salmonella paratyphi*

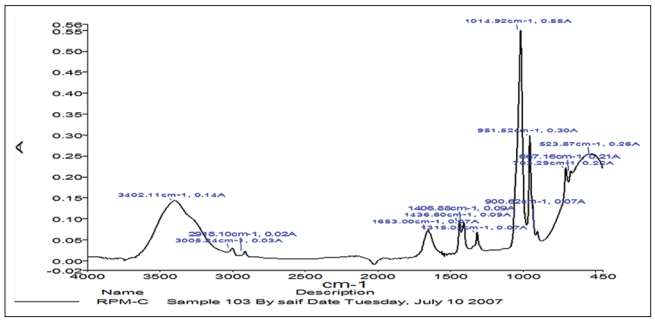

Figure 7: Fourier-transform infrared-attenuated total reflection spectra of poly (N-vinylcarbazole - co-phenoxyethyl methacrylate) with bacteria

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