

Synthesis, Characterization, Thermal Stability and Antibacterial Activity of Coumarin Based Methacrylate Copolymers

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Abstract Monomer of 7-methacryloyloxy-4-methylcoumarin (MAOMC) was synthesized and characterized by FTIR, ¹H-NMR and ¹³C-NMR spectroscopy. Copolymers of MAOMC with butoxyethylmethacrylate (BOEMA) at different feed compositions were prepared by free radical solution polymerization at (70 ± 1) °C in ethylmethylketone (EMK) using benzyl peroxide (BPO) as an initiator. The copolymers were characterized by FTIR and ¹H-NMR spectroscopy. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) of the copolymers showed moderate thermal stability and higher *T_g* values. Gel permeation chromatography (GPC) was used to find out the molecular weights of the different copolymers. Antibacterial activities of the copolymers were also investigated against the selected pathogenic bacteria's. The antibacterial activity of the copolymer increases as the MAOMC content increases in the copolymer. This shows that coumarin moiety plays a very important role in the antibacterial activity.

Keywords: 7-Methacryloyloxy-4-methylcoumarin; Copolymerization; Thermogravimetric analysis; Antibacterial activity.

INTRODUCTION

In recent years many polymers with reactive functional groups are being synthesized, tested and used not only for their macromolecular properties but also for the properties of their functional groups. These functional groups provide an approach to a subsequent modification of the polymers for specific end applications^[1]. The use of antimicrobial polymers offers promise for enhancing the efficacy of some existing antimicrobial agents. These polymers minimize the environmental problems generated by conventional antimicrobial agents. One method of achieving the antimicrobial polymers is the preparation of monomers containing biocide moieties and then polymerization subsequently or copolymerization with another monomer^[2–5]. Kenawy and coworkers discussed on the requirements of antimicrobial polymers, factors affecting the antimicrobial activities, methods of synthesizing the antimicrobial polymers, major field of applications, future and perspectives in the field of antimicrobial polymers in their review article^[6].

Coumarin derivatives are important sources of heterocyclic compounds of pharmacological interest because of various physiological and biochemical properties. A review article dealing with the varied physiological activities of coumarin derivatives has been published, describing their anticoagulant, antibacterial, antihelminthic, hypothermal properties and vasodilatory action^[7]. Coumarin polymers have not received considerable attention in the literature. However, the reported coumarin polymers possess variety of functions and appear to be interesting. Although there are a huge number of reports on monomeric coumarin derivatives, there are only a few reports on coumarin polymers^[8, 9]. During the last twenty years, the study of the biological activities of

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coumarin derivatives has been the aim of many researchers^[10–13]. Furthermore it has been reported by different scientists that coumarin derivatives incorporating thiazole^[14], azetidinone^[15] and oxazole^[16] rings were also found to possess interesting antibacterial and antifungal activities.

Although many papers published on the synthesis of coumarin and its derivatives are available^[17–20], very little attention has been paid to the incorporation of coumarins into polymers^[21–23]. Patel and co-workers^[24] synthesized and characterized a new functional acrylic monomer, 7-acryloxy 4-methyl coumarin and copolymerized it with methyl acrylate and studied their thermal behavior and antimicrobial activity. Brahmabhatt and coworkers^[25] prepared poly(3-phenoxy-coumarin ethylene)s under Knoevenagel and Perkin condition and photometric assay method was used to determine their toxicity effect on various fungal and bacterial strains. These polymers showed good biological activity.

Due to various physiological and biochemical properties of coumarin, our interest is to synthesize and develop copolymers containing coumarin moieties. With this view, the synthesis of 7-methacryloyloxy-4-methylcoumarin (MAOMC) and its copolymerization with butoxyethylmethacrylate (BOEMA) by free radical solution polymerization is under taken. The prepared monomer and copolymers are characterized by FTIR, ¹H-NMR and ¹³C-NMR spectroscopy. The thermal properties of the copolymers are analyzed by TGA and DSC. Gel permeation chromatography is employed to determine the molecular weights of the synthesized copolymers. Antibacterial activity tests of the copolymers of different compositions are carried out against selected bacteria like *Bacillus cerus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Klebsiella planticola*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. In order to know the potency of the tested copolymers, the obtained results were compared with the standard drugs.

EXPERIMENTAL

Materials

Methacryloyl chloride was prepared from methacrylic acid and acyl chloride using the procedure of Stempel *et al.*^[26]. Butoxyethylmethacrylate (Sigma-Aldrich) was distilled under reduced pressure and collected in a dry clean amber colored bottle and kept in the refrigerator at 5 °C. Benzoyl peroxide (BPO) was recrystallized from chloroform-methanol (1:1). The AR grade EMK, hexane, chloroform and methanol were purified by the conventional methods. Triethylamine is dried using sodium metal. Bacterial medium Muller Hinton Agar was purchased from Himedia, Mumbai.

Measurements

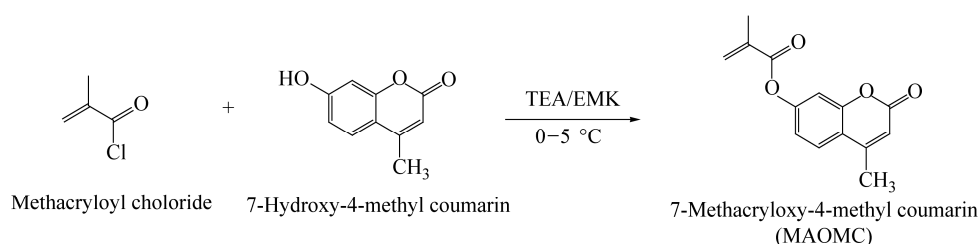
FTIR spectra of the monomer and the copolymers were recorded on a Perkin-Elmer FTIR spectrophotometer with KBr pellets. ¹H-NMR and ¹³C-NMR spectra were recorded in a BRUKER 500 MHz using CDCl₃ solvent. Thermogravimetric analysis (TGA) was performed with a TA Instruments Hi-Res TGA 2950 thermal analyzer at a heating rate of 10 K/min under nitrogen atmosphere. The glass transition temperature was determined with a Perkin-Elmer DSC d7 differential scanning calorimeter at a heating rate of 10 K/min in nitrogen. Molecular weights (M_w and M_n) of the polymers were determined by a Waters 501 gel permeation chromatography instrument equipped with a refractometer index detector. Tetrahydrofuran was used as an eluent at a flow rate of 1.0 mL/min and polystyrene standards were employed for calibration.

Synthesis of 7-Hydroxy-4-methylcoumarin

7-Hydroxy-4-methylcoumarin was prepared according to the procedure reported in the literature^[27]. A three necked flask fitted with a thermometer, a mechanical stirrer and a dropping funnel containing 250 mL of sulphuric acid (H₂SO₄) and resorcinol (0.23 mol) was maintained at temperature less than 10 °C. Then ethylacetoacetate (0.26 mol) was added drop wise with constant stirring. The stirring was continued for about 18 h and the contents of the flask were poured into the crushed ice. The crude product separated out was dissolved in 5% NaOH solution. To this solution H₂SO₄ (2 mol/L) was added until it becomes acidic, then it was filtered, dried and recrystallized from 95% ethanol. The product yield was 78% (mp: 186–188 °C).

Synthesis of 7-Methacryloyloxy-4-methylcoumarin (MAOMC) Monomer

The monomer, 7-methacryloyloxy-4-methylcoumarin (MAOMC), was synthesized as follows. A three-necked round-bottomed flask fitted with a thermometer, a stirrer, and a dropping funnel containing 7-hydroxy 4-methyl coumarin (5 g, 0.033 mol), triethylamine (6.9 mL, 0.049 mol) and ethylmethyleketone (300 mL) was cooled between -5°C to 0°C . Methacryloyl chloride (3.87 mL, 0.039 mol) in ethylmethyleketone (25 mL) was added dropwise in to the flask maintained between -5°C to 0°C . The reaction was allowed to proceed for 1 h at 0°C -5°C with constant stirring, then another 2 h at room temperature. The reaction mixture was washed with water followed by 1% NaOH solution to remove the traces of methacryloyl chloride. The organic layer was dried in anhydrous sodium sulphate. The product was purified by recrystallization from methanol, and found to be white crystals (Scheme 1). The product yield was 83% (mp: 102°C – 104°C). The formation of the monomer was confirmed by FTIR (Fig. 1a), ^1H -NMR (Fig. 2a) and ^{13}C -NMR spectra (Fig. 3).



Scheme 1 Synthesis of 7-methacryloyloxy-4-methylcoumarin (MAOMC) monomer

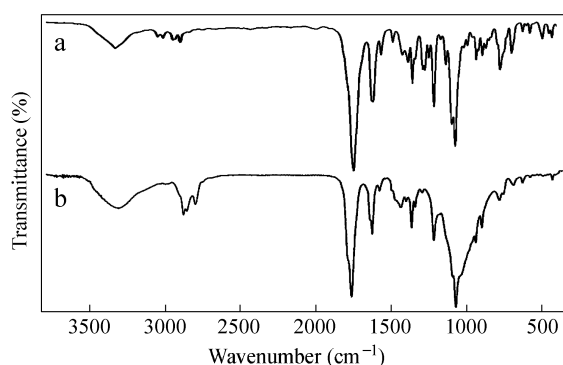


Fig. 1 IR spectra of (a) 7-methacryloyloxy-4-methylcoumarin and (b) copoly(MAOMC-co-BOEMA)

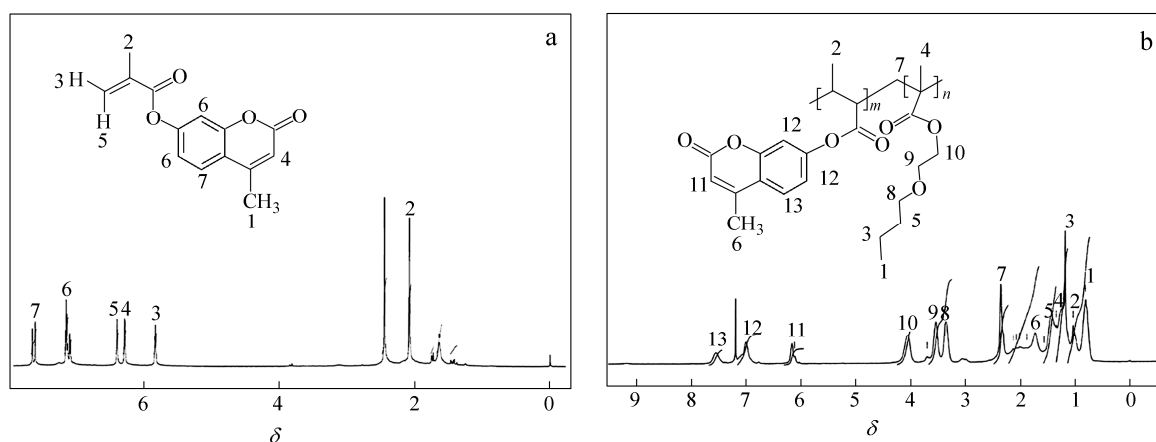


Fig. 2 ^1H -NMR spectra of (a) 7-methacryloyloxy-4-methylcoumarin and (b) copoly(MAOMC-co-BOEMA)

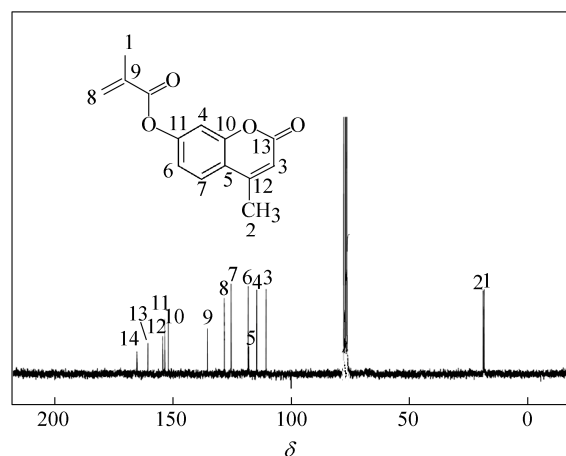
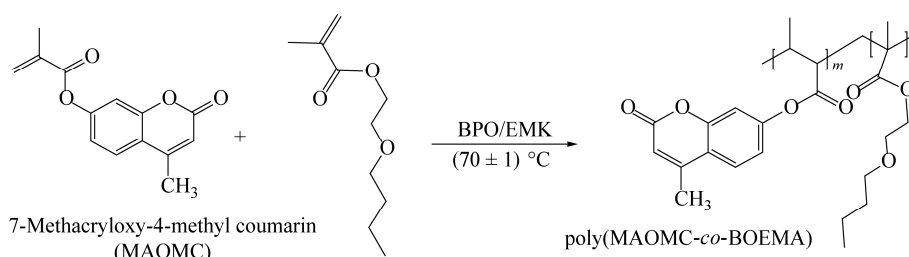


Fig. 3 ^{13}C -NMR spectrum of 7-methacryloyloxy-4-methylcoumarin (MAOMC)

Copolymerization of (MAOMC-co-BOEMA)

Copolymerization was carried out in EMK using BPO as an initiator. Predetermined quantities of MAOMC, BOEMA, EMK and BPO were placed in a polymerization tube and the mixture was flushed with oxygen free nitrogen gas for 30 min. The tube was tightly sealed and immersed in an oil bath at $(70 \pm 1)^\circ\text{C}$. After the required time, the reaction mixture was poured into excess of hexane. The precipitated polymer was filtered off and purified by repeated precipitation from chloroform and finally dried (Scheme 2).



Scheme 2 Synthesis of copolymer (MAOMC-co-BOEMA)

Solubility Studies

Solubility of the copolymer was tested in various polar and non-polar solvents. About 5–10 mg of the copolymer was added to about 2 mL of different solvents in a test tube and kept overnight. The solubility of the polymers was noted after 24 h.

Antibacterial Sensitivity Assay

The antibacterial activity of the copolymer (MAOMC-co-BOEMA) was tested by agar disc diffusion method. About 0.5 mL of the test bacterial sample was spread uniformly over the solidified Mueller Hinton agar in each Petri plate using sterile glass spreader. The discs with 5 mm diameter made-up of Whatmann No. 1 filter paper were taken, impregnated with the solution of the different copolymer compositions and were placed on the medium using sterile forceps. The plates incubated for 24 h at 37°C , were visually examined and the diameter values of the zone of inhibition were measured. By repeating the above procedure, triplicate results were recorded and tabulated.

RESULTS AND DISCUSSION

Solubility

The solubility of the newly prepared copolymers in various polar and non-polar solvents was tested at room temperature. The copolymers were easily soluble in various solvents such as chloroform, dichloromethane, dimethylformamide, dimethylsulfoxide, ethylmethylketone, toluene and acetone *etc.*

Spectroscopic Analysis of Monomer-7-methacryloyloxy-4-methylcoumarin (MAOMC)

FTIR spectrum of MAOMC is shown in Fig. 1(a): 3061 cm^{-1} ($-\text{CH}$ stretching vibration of the aromatic ring), 2982 cm^{-1} ($-\text{CH}_3$), 1726 cm^{-1} (broad, $\text{C}=\text{O}$ of methacrylate and of coumarin moiety), 1618 cm^{-1} ($\text{C}=\text{C}$), 1265 cm^{-1} (asymmetric $\text{C}-\text{O}-\text{C}$), 1141 cm^{-1} (symmetric $\text{C}-\text{O}-\text{C}$), 880 cm^{-1} ($-\text{CH}$ bending mode of vinyl group) and 749 cm^{-1} (rocking mode of vinyl group).

^1H -NMR of MAOMC is shown in Fig. 2(a) (δ , 500 MHz): 2.17 (3H, $-\text{CH}_3$ in methacrylate unit), 1.75 (3H, CH_3 in coumarin moiety), 6.3 and 5.8 (2H, non-equivalent methylene protons) and 7.08–7.65 (3H, aromatic protons).

^{13}C -NMR of MAOMC is shown in Fig. 3 (δ , 500 MHz): 160.5 ($\text{C}=\text{O}$ of ester group of coumarin), 165.1 ($\text{C}=\text{O}$ of ester of methacrylate), 135.3 ($\text{CH}_2=$), 114.5–155.2 (benzene ring carbons), 18.7 (CH_3- in coumarin moiety) and 18.3 (CH_3- in methacrylate).

Spectroscopic Analysis of Copolymer (7-Methacryloyloxy-4-methylcoumarin-co-butoxyethylmethacrylate)

The FTIR spectrum of the copolymer, poly(MAOMC-co-BOEMA) is shown in Fig. 1(b). The spectrum confirms the copolymer in all aspects. Poly(MAOMC-co-BOEMA) shows bands at 2957 cm^{-1} and 2933 cm^{-1} which may be attributed to the asymmetric and symmetric $\nu_{\text{C}-\text{H}}$ stretching vibration of methylene group, whereas the absorption at 1452 cm^{-1} is due to the bending vibration of $\nu_{-\text{CH}}$ group. The strong absorptions at 1388 cm^{-1} and 1368 cm^{-1} are related to the $\nu_{\text{C}-\text{H}}$ stretching vibrations of CH_3 and CH_2 groups. The broad band at 1732 cm^{-1} is assigned to $\nu_{\text{C}=\text{O}}$ stretching vibration of the ester group of the copolymer. A sharp band at 1260 cm^{-1} and a broad band at 1130 cm^{-1} are assigned to $\nu_{\text{C}-\text{O}-\text{C}}$ symmetric and asymmetric stretching vibration of the ester group.

^1H -NMR spectrum of poly(MAOMC-co-BOEMA) is shown in Fig. 2(b). (δ , 500 MHz). The signals at $\delta=7.0$ –7.54 are due to aromatic protons and the signal at $\delta=7.54$ is for non equivalent proton of aromatic ring. The signals of two different methylene protons appeared at $\delta=1.03$ and $\delta=1.25$. The peaks appeared at $\delta=3.69$ and 4.01 is due to two different $-\text{CH}_2$ units, between oxygen atoms of BOEMA unit. The $-\text{CH}_3$ proton of MAOMC and BOEMA shows signal at $\delta=1.73$ and 0.80 respectively. The formation of copolymer was evident from the disappearance of signals at 6.3 (2H, $\text{CH}_2=$) and appearance of broad signals at 2.3, due to CH_2 backbone chain (2H, $-\text{CH}_2$).

Copolymer Composition

The composition of the monomeric units in the copolymer was determined using ^1H -NMR spectra, by the distribution of protons in the two units from the distinct and well separated resonance peaks. This method is a well established one. Thus the mole fraction of MAOMC in the copolymer was determined from the ratio of the integrated values of the intensities of aromatic protons of MAOMC with the rest of the aliphatic protons of MAOMC and BOEMA units (Table 1).

Table 1. Composition data of the free radical copolymerization of MAOMC with BOEMA in EMK solution

No.	Feed composition M_1	Conversion (%)	Copolymer composition m_1
1.	0.20	24	0.26
2.	0.50	41	0.64
3.	0.80	62	0.91

Thermal Degradation of Copolymers

The thermal behavior of copolymers was characterized using TG traces. The TGA curves (Fig. 4) clearly indicates that all the copolymers undergo single step degradation pattern which suggests their purity. The

measured results are shown in Table 2. Copolymers of different composition exhibit excellent thermal stability with decomposition temperatures varying from 90 °C to 420 °C. Thermal stability of the copolymer increases as the coumarin moiety increases^[28].

Table 2. TGA data of poly(MAOMC-*co*-BOEMA)

No.	poly(MAOMC- <i>co</i> -BOEMA)	Decomposition temperature range (°C)	Temperature (°C) at weight loss (%)				
			10%	30%	50%	70%	90%
1	20:80	99–390	223	289	324	353	378
2	50:50	120–395	135	299	332	365	385
3	80:20	95–415	274	325	355	389	412

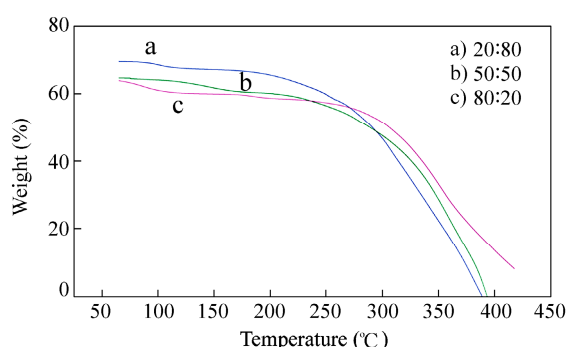


Fig. 4 TGA curves of poly(MAOMC-*co*-BOEMA)

Glass Transition Temperature

The glass transition temperature of copolymers of different ratios 20:80, 50:50 and 80:20 are 106 °C, 113 °C and 138 °C respectively (Fig. 5). The increase in the T_g value of the copolymers is due to the increase in the chain rigidity.

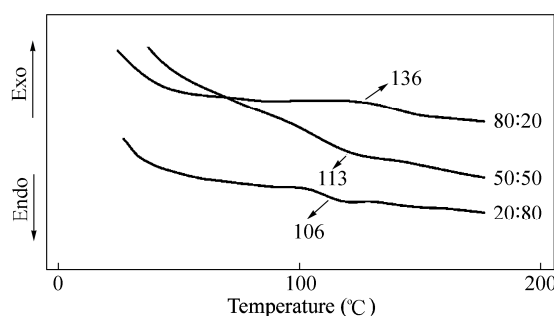


Fig. 5 DSC curves of poly(MAOMC-*co*-BOEMA)

Molecular Weights of the Copolymers

The weight-average (M_w) and number average (M_n) molecular weights and polydispersity indices of different copolymers of poly(MAOMC-*co*-BOEMA) determined by GPC are presented in Table 3. The values of polydispersity index are very close to 2.0. The theoretical values of M_w/M_n for polymers produced *via* radical combination and disproportionation were 1.5 and 2.0 respectively^[29]. This suggests that poly(MAOMC-*co*-NVP) was produced mainly *via* termination by disproportionation. Further the values of M_w/M_n of these polymers indicate a strong tendency for chain termination by disproportionation when the mole fraction of MAOMC is higher than that of the BOEMA in the feed.

Table 3. GPC data for different Copolymers

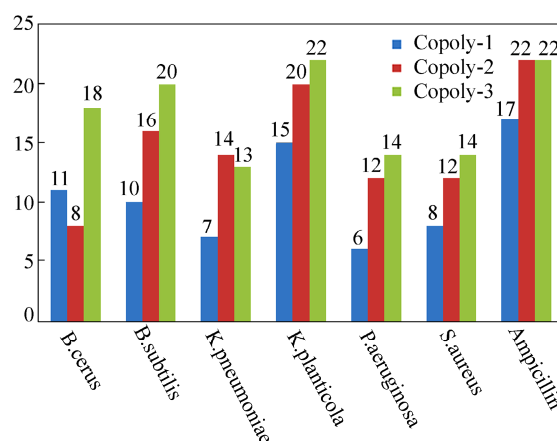
No.	Feed composition in copolymer		Molecular weights		
	MAOMC M ₁	BOEMA M ₂	$M_w \times 10^{-4}$	$M_n \times 10^{-4}$	M_w/M_n
1	0.20	0.80	2.75	1.81	1.52
2	0.50	0.50	3.36	2.07	1.62
3	0.80	0.20	3.15	1.92	1.64

Antibacterial Activity

Table 4 shows the antibacterial activity of copolymers on pathogenic and opprtuneic bacteria. The inhibition zone was observed against *P. aeruginosa*, *S. aureus*, *K. pneumoniae*, *B. subtilis*, *B. cerus* and *K. planticola*. In the quantitative assay 50 μ L of various copolymer solution were added in three respective wells. Ampicillin is used as reference standard for comparing the results. The zone of inhibition is increased when copolymer concentration is increased. Among the different copolymer ratios, the ratio 80:20 is giving good results when compared to others (Fig. 6). The benzene ring of the coumarin moiety will have electron rich as well as electron deficient centres. As the number of coumarin moiety increases these centres also increase. The micro organism depending on its nature will attack the electron rich or electron deficient centres. Thus it is concluded that as the percentage of MAOMC in the copolymer increases, the effectiveness of the copolymers to inhibit the growth of microorganisms increases^[30]. All the copolymer ratios show maximum inhibitory action against the pathogenic bacteria *K. planticola*, *P. aeruginosa* and *opportune bacteria B. subtilis*. The different copolymer ratios were moderately active against the disease causing bacteria *S. aureus*. The pneumonial fever causing bacteria *K. pneumoniae* having the minimum level of zone of inhibition. These results suggest that the copolymer ratio 80:20 which is vigorously involved in the antibacterial activity will be used for the antimicrobial based applications.

Table 4. Zone of inhibition of copolymers against pathogenic bacteria

No.	Bacterial strains	Zone of inhibition (mm)		
		Copoly-1 20:80	Copoly-2 50:50	Copoly-3 80:20
1.	<i>Bacillus cerus</i>	11	8	18
2.	<i>Bacillus subtilis</i>	10	16	20
3.	<i>Klebsiella pneumoniae</i>	7	14	13
4.	<i>Klebsiella planticola</i>	15	20	22
5.	<i>Pseudomonas aeruginosa</i>	6	12	20
6.	<i>Staphylococcus aureus</i>	8	12	14
7.	Ampicillin	17	22	22

**Fig. 6** Zone of inhibition of different copolymers against pathogenic bacteria

CONCLUSIONS

An attempt has been made to synthesised copolymers 7-methacryloyloxy-4-methylcoumarin-*co*-butoxyethylmethacrylate of different feed compositions. The copolymers were structurally characterized by FTIR, ¹H-NMR. Thermal stability of the copolymers was studied using TGA and DSC, the results show that 80:20 ratio is thermally more stable than the other, also the T_g value increases with the increase of MAOMC content in the copolymer. The antibacterial activity studies against different bacterial stains were reported. The good zone of inhibition was observed for the copolymer of ratio 80:20, which exhibited remarkable antibacterial activities against disease causing bacterial isolates such as *B. cerus*, *B. subtilis*, *K. pneumoniae*, *K. planticola*, *P. aeruginosa* and *S. aureus*.

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