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Copolymers of N-(4-bromophenyl)-2-methacrylamide with 2-hydroxyethyl methacrylate

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Abstract. The radical-initiated copolymerization of N-(4-bromophenyl)-2-methacrylamide (BrPMAAm) with 2-hydroxyethylmethacrylate (HEMA) was carried out in 1,4-dioxane solution at 70°C using 2,2'-azobisisobutyronitrile (AIBN) as an initiator with different monomer-to-monomer ratios in the feed. The copolymers were characterized by FTIR, ^{1}H- and ^{13}C-NMR spectral studies. Gel permeation chromatography was employed for estimating the weight average ($\overline{M}_w$) and number average ($\overline{M}_n$) molecular weights and polydispersity index (PDI) of the copolymers. The copolymer composition was evaluated by nitrogen content (N for BrPMAAm-units) in polymers, which allowed the determination of reactivity ratios. Monomer reactivity ratios for BrPMAAm (M_1)-HEMA (M_2) pair were determined by the application of conventional linearization methods such as Fineman-Ross (F-R), Kelen-Tüdős (KT) and Extended Kelen-Tüdős (EKT) and a non-linear error invariable model method using a computer program RREVM. The characterizations were done thermogravimetric analysis (TGA). The antimicrobial effects of polymers were also tested on various bacteria, and yeast.

Keywords: *thermal properties, N-(4-bromophenyl)-2-methacrylamide, monomer reactivity ratios, antimicrobial effects*

1. Introduction

Several studies have been done in our laboratories on the synthesis of N-monosubstituted acrylamides [1–3] and their radical copolymerization with commercial monomers. These studies clearly show that the nature as well as position of the substituent had a large effect on monomer reactivity ratios, glass transition temperatures and antimicrobial properties. Copolymers with reactive or functional monomers are gaining importance steadily. The potentially wide range of applications for functionalized polymeric materials has made these materials important. 2-Hydroxyethyl methacrylate (HEMA) hydrogels are materials with a large number of biomedical applications, such as contact lenses, artificial implants, drug delivery systemes [4, 5], due to their biocompatibility, hydrophilicity, softness, high water content and permeability. Considerable work on polymers and copolymers of HEMA has been published in the recent years [6–8]. The understanding of copolymerization kinetics has gained great importance in recent decades. Because of this fact, the prediction of monomer reactivity ratios becomes a valuable quantitative aspect. Moreover, copolymerization is an important and useful way to develop new materials. Copolymerization modulates both the intramolecular and intermolecular forces exercised between like and unlike polymer segments. Therefore, properties such as the glass transition temperature, melting point, solubility, crystallinity, permeability, adhesion, elasticity, and chemical reactivity may be varied within wide limits [9]. Most existing procedures for calculating reactivity ratios can be classified as linear least-squares (LLS), and non-linear least-squares (NLLS) methods. It is accepted that LLS methods such as those proposed by Finemann and

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Ross [10], and by Kelen and Tüdős [11], can only be applied to experimental data at sufficiently low conversion, because the calculation is based on the differential copolymerization equation [12]. The extended Kelen-Tüdős method [13] involves a rather more complex calculation.

We report here the homopolymer and copolymers of BrPMAAm with HEMA using different feed ratios. The copolymer composition was determined by elemental analysis. The effect of BrPMAAm content on the thermal properties of resulting copolymers was investigated. Homopolymer and copolymers were also tested for their antimicrobial properties against microorganisms such as *Staphylococcus aureus COWAN I, Bacillus subtilis ATCC 6633, Escherichia coli ATTC 25922, Klebsiella pneumonia FMCS, Pseudomonas aeruginosa DSM 50071, and yeast, Candida tropcalis ATCC 13803, Candida globrata ATCC 66032, and Candida albicans CCM 314.*

2. Experimental

2.1. Materials

BrPMAAm monomer was prepared as reported [14]. 2-hydroxyethyl methacrylate (HEMA) was purchased from Merck which was purified by vacuum distillation at 68°C/7 mmHg. α,α'-azobisisobutyronitrile (AIBN) was recrystallized from chloroform-methanol. 1,4-dioxane, diethylether, (Merck), were analytical grade commercial products and used as received.

2.2. Copolymerization

Homo- and copolymerization reactions were carried out in 1,4-dioxane using 2,2'-azobisisobutyronitrile (AIBN) as an initiator. Predetermined quantities of BrPMAAm, 2-hydroxyethyl methacrylate (HEMA), 1,4-dioxane (4 mol·l^{-1}), and AIBN were mixed in a round-bottomed flask equipped with mechanical stirrer and reflux condenser. The initiator concentration was $12 \cdot 10^{-3}$ mol·l^{-1}. The solution was purged with nitrogen for about 10 min, and the reaction mixtures were purged again for several minutes prior to heating. The reaction mixture was heated to 70°C with constant stirring. The mixtures were then cooled to room temperature and slowly poured, with constant stirring, into a large excess of diethylether that was used as a nonsolvent. Solid polymers were purified by repeated precipitation with the diethylether from solution in 1,4-dioxane and finally dried under vacuum.

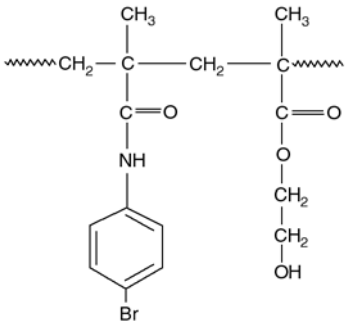
2.3. Characterization techniques

Infra-red spectra were measured on a Jasco 460 Plus FT-IR spectrometer. ^{1}H- and ^{13}C-NMR spectra of the polymers were recorded in DMSO-d_6 with tetramethylsilane as the internal standard using a Gemini Varian 200 MHz NMR spectrometer. Thermal data were obtained by using a Setaram Labsys TG-DSC/DTA thermobalance in N_2 atmosphere. Elemental analyses were carried out by a LECO CHNSO-932 auto microanalyser. Molecular weight; ($\overline{M}_w$ and $\overline{M}^n$) of the polymers were determined using Waters 410 gel permeation chromatography equipped with a differential refractive index detector and calibrated with polystyrene standards.

3. Results and discussion

3.1. Copolymer characterization

The copolymerizations of BrPMAAm with HEMA can be represented according to Figure 1.



poly(BrPMAAm-co-HEMA)

Figure 1. The structure monomeric units of the copolymer

3.1.1. FTIR spectrum

The FTIR spectra of the homo- and copolymer are shown in Figure 2. The FTIR spectra confirmed the structure of polymers in all aspects. In the FTIR spectra (a) of HEMA; the broad band at 3500 cm^{-1} is the most characteristic for hydroxyl group at HEMA units. A strong band at at 1740 cm^{-1} which is attributed to the ester carbonyl of HEMA units. The other characteristic bands confirmed the structure of HEMA polymers in all aspects. In the FTIR

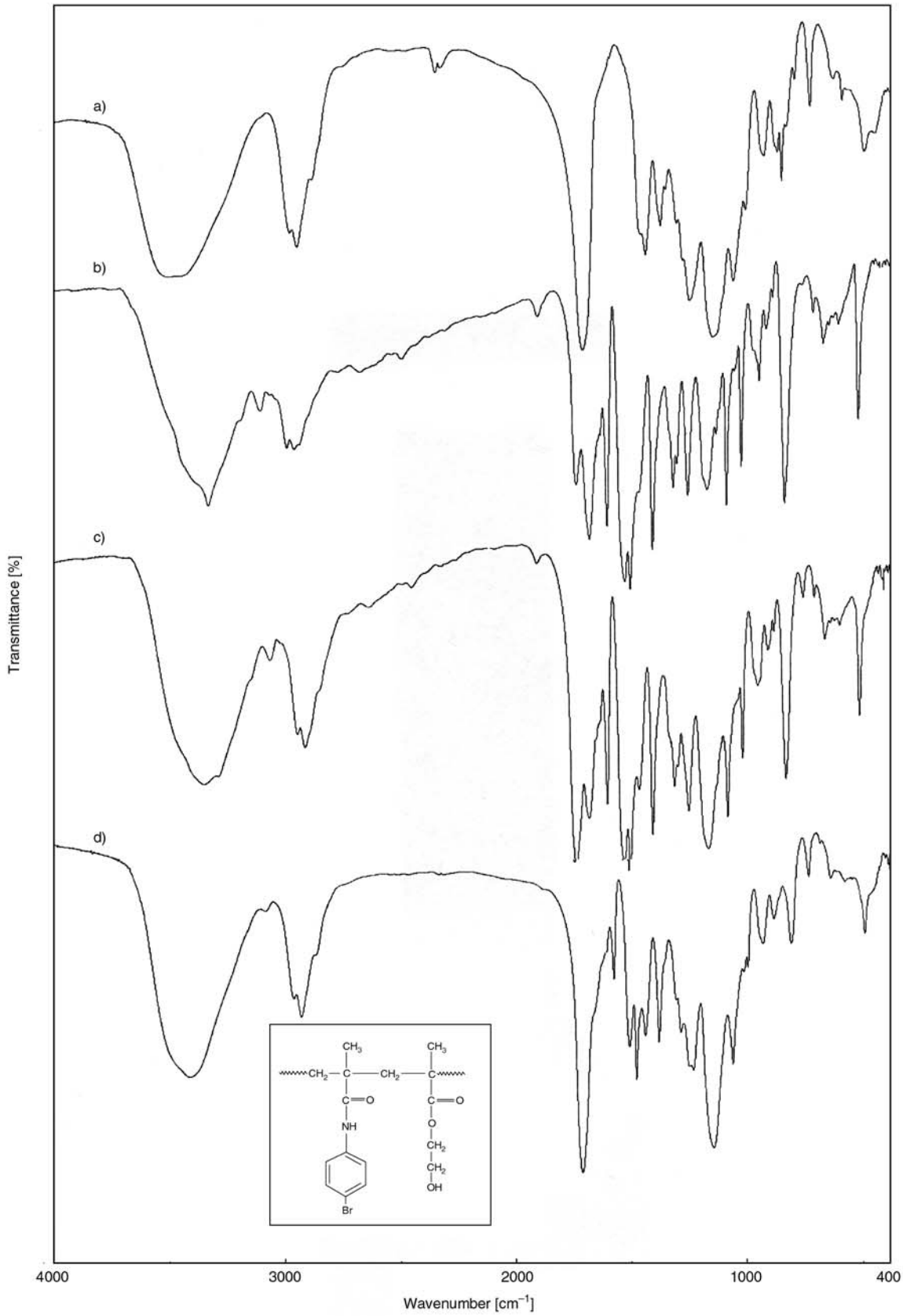


Figure 2. FTIR spectrum of a) poly(HEMA), b) poly(BrPMAAm-co-HEMA) (0.64:0.36), c) poly(BrPMAAm-co-HEMA) (0.28:0.72), and d) poly(BrPMAAm)

spectra of (b, c and d) the band at 3320 cm^{-1} (–NH in the BrPMAAm unit) is the most characteristic for the polymer. The peak at 3050 cm^{-1} corresponds to the C–H stretching of the aromatic system. The symmetrical and asymmetrical stretching bands due to the methyl and methylene groups are observed at 2985, 2940 and 2865 cm^{-1}. The peak at 1740 cm^{-1} is attributed to the ester carbonyl stretching of HEMA units. The absorption at 1700 cm^{-1} could be assigned for a complex stretching vibrations of C=O and C–N, while the strong absorption at 1300 cm^{-1} could be attributed predominantly to C–O stretching. The broad band at 1440 cm^{-1} could be due to the C–N scissoring vibration of the –N–C=O group. The ring breathing vibrations of the aromatic nuclei are observed at 1600, 1505 and 1470 cm^{-1}. The asymmetrical and symmetrical bending vibrations of methyl groups are seen at 1455 and 1380 cm^{-1}. The C–H and C=C out of plane bending vibrations of the aromatic nuclei are observed at 790 and 565 cm^{-1}, respectively.

3.1.2. ^{1}H-NMR spectrum

The ^{1}H-NMR spectra of the copolymer poly(BrPMMAm-co-HEMA) (0.61:0.39) and its assignments are shown in Figure 3a. The chemical shift assignments for the copolymers were based on the chemical shifts observed for the respective homopolymers. The resonance signals at 9.88 ppm correspond to the NH protons of the BrPMAAm unit. The aromatic protons show signals between 7.62 and 7.20 ppm. The spectrum shows two signals at 4.81 and 4.02 ppm, which are due to $–CH_2CH_2–OH$ group at HEMA units. The backbone methylene groups show signals at 1.54–2.01 ppm. The signals obtained at 1.22 and 1.10 ppm are due to the α-methyl protons of both the monomer units.

3.1.3. ^{13}C-NMR spectrum

The proton decoupled ^{13}C-NMR spectrum of the copolymer poly(BrPMMAm-co-HEMA) (0.61:0.39) and its assignments are shown in Figure 3b. The amide carbonyl of BrPMAAm appeared at 166.1 ppm while the ester carbonyl of HEMA appeared at 168.6 ppm. The aromatic carbons of BrPMAAm unit in copolymer appeared at 142.2, 136.3, 127.4 and 124.0 ppm, respectively. The hydroxyethyl carbon atoms of HEMA unit appeared at 64.4, 66.4 ppm, respectively. The signals due to the backbone methylene carbon atoms are observed at 58.2 and 56.0 ppm, while those of the tertiary carbons is observed at 45.1 and 43.3 ppm. The α-methyl carbon atoms of both monomeric units give a series of resonance signal at 18.2 ppm.

3.1.4. Molecular weights of the polymers

The molecular weights of the polymers were determined by gel permation chromatography (GPC) with polystyrene and tetrahydrofuran as the standard and solvent, respectively. The weight average ($\overline{M}_w$) and number average ($\overline{M}_n$) molecular weights and the polydispersity indexes (PDI); ($\overline{M}_w/\overline{M}_n$) of polymer samples are presented in Table 1. The

Table 1. Summary of composition and molecular weights of the Poly(BrPMAAm-co-HEMA) systema

Sample	$M_{BrPMAAm}$[b]	M_{HEMA}[c]	N [%][d]	Conv. [%][e]	$m_{BrPMAAm}$[f]	m_{HEMA}[g]	$\overline{M}_w \cdot 10^{-4}$	$\overline{M}_n \cdot 10^{-4}$	PDI[h]
Copoly-1	0.10	0.90	0.541	9.6	0.05	0.95	3.22	1.73	1.86
Copoly-2	0.20	0.80	1.439	8.2	0.14	0.86	2.88	1.65	1.75
Copoly-3	0.30	0.70	2.264	10.8	0.24	0.76	2.44	1.28	1.90
Copoly-4	0.40	0.60	2.553	7.3	0.28	0.72	2.70	1.63	1.66
Copoly-5	0.50	0.50	3.257	11.2	0.39	0.61	3.10	1.80	1.72
Copoly-6	0.60	0.40	3.598	7.3	0.45	0.55	3.08	1.73	1.78
Copoly-7	0.70	0.30	4.103	10.5	0.54	0.46	2.92	1.50	1.94
Copoly-8	0.75	0.25	4.437	7.6	0.61	0.39	2.56	1.50	1.70
Copoly-9	0.80	0.20	4.561	9.4	0.64	0.36	3.44	1.83	1.88
Copoly-10	0.90	0.10	4.974	8.7	0.74	0.26	2.78	1.65	1.68
Poly(BrPMAAm)	1.0	–	–	88.4	1.0	–	2.80	1.52	1.84
Poly(HEMA)	–	1.0	–	88.6		1.0	3.04	1.69	1.80

[a]Polymerization conditions: 1,4-dioxane solution (4 mol·l^{-1}); temperature:70±1°C; initiator:AIBN (0.1%, based on total molar of monomers) ($12 \cdot 10^{-3}$ mol·l^{-1}). [b]The molar fraction of BrPMAAm at feed. [c]The molar fraction of HEMA at feed. [d]Determined by elementeal analysis. [e]Determined by gravimetrically. [f]The molar fraction of BrPMAAm in copolymer, obtained from elementel analysis. [g]The molar fraction of HEMA in copolymer, obtained from elementel analysis. [h]Poly dispersity index.

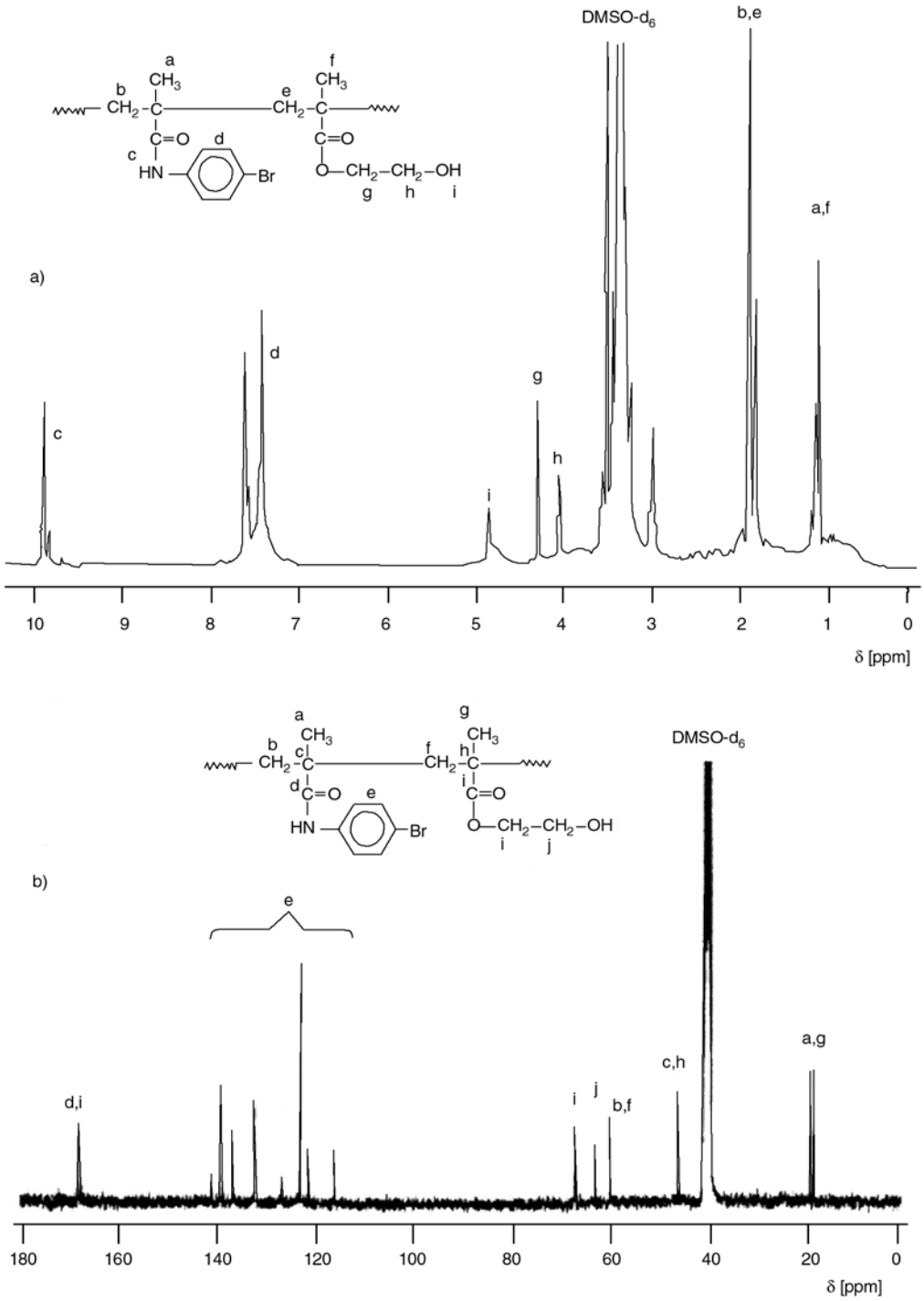


Figure 3. a) ^{1}H-NMR spectra and b) ^{13}C-NMR spectra of copoly(BrPMAAm-co-HEMA); m_1:m_2: (0.61:0.39)

polydispersity index of the polymers ranges between 1.66 and 1.94. The theoretical values of PDI for polymers produced via radical recombination and disproportionation are 1.5 and 2.0, respectively [15]. This suggests that polymers were produced mainly via termination of growing chain by disproportionation.

3.2. Copolymer composition and monomer reactivity ratios

The monomer reactivity ratios for the copolymerization of BrPMAAm with HEMA were determined from the monomer feed ratios and the copolymer composition. The classical approach for acquiring copolymer data was to isolate the copolymers from each of 10 feed compositions at early

conversions and analyze the copolymer compositions by elemental analyses. The analytical data for copolymerization of BrPMAAm with HEMA as an example are illustrated in Table 1. The plot of the mole fractions of BrPMAAm (M_1) in the feed *vs.* that in the copolymer (m_1) is shown in Figure 4. It clearly indicates that the composition of BrPMAAm in the copolymer is always lower than that in the feed. The Fineman-Ross (FR) [10], and Kelen-Tüdős (KT) [11] and Extended Kelen-Tüdős (EKT) [13] methods were used to determine the monomer reactivity ratios. The graphical plots concerning the methods previously reported are given in Figures 5, 6 and 7; whereas the reactivity ratios are summarized in Table 2. The monomer reactivity ratios determined by conventional linearization methods are only approximate and are usually employed as good starting values for non-linear parameter estimation schemes. In the determination of the monomer reactivity studies, the curves of the F-R, K-T and ext.K-T methods are quite different from straight lines, because, these methods are conventional linearization methods.

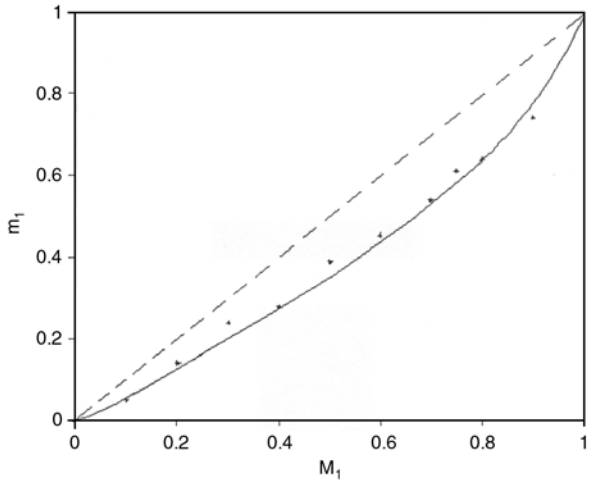


Figure 4. Copolymer composition diagram for poly(BrPMAAm-co-HEMA) system. (M_1: Feed composition in mole fraction for BrPMAAm; m_1: Copolymer composition in mole fraction for BrPMAAm).

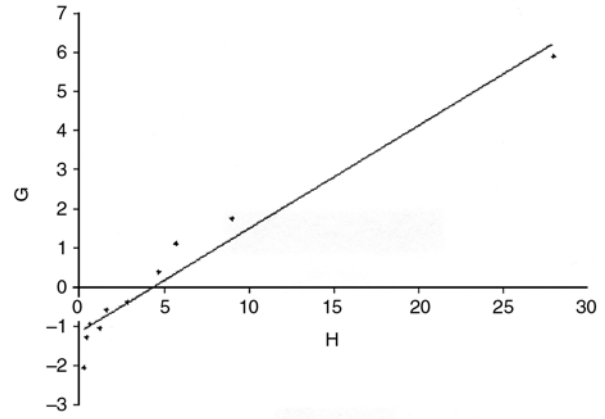


Figure 5. FR plots for determining monomer reactivity ratios in copolymerization of BrPMAAm (M_1) and HEMA (M_2) data of elemental analysis

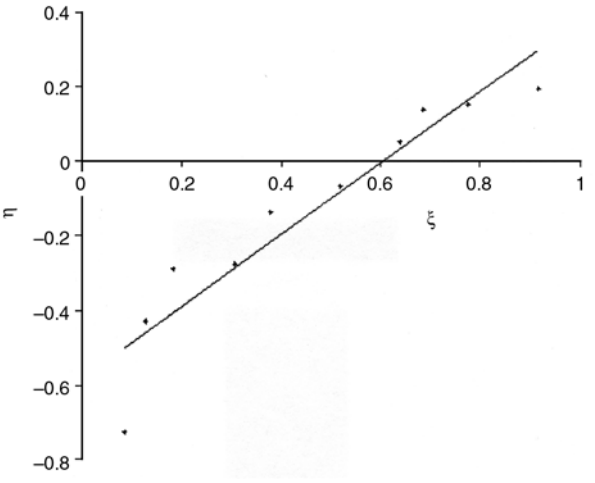


Figure 6. KT plots for determining monomer reactivity ratios in copolymerization of BrPMAAm (M_1) and HEMA (M_2) data of elemental analysis

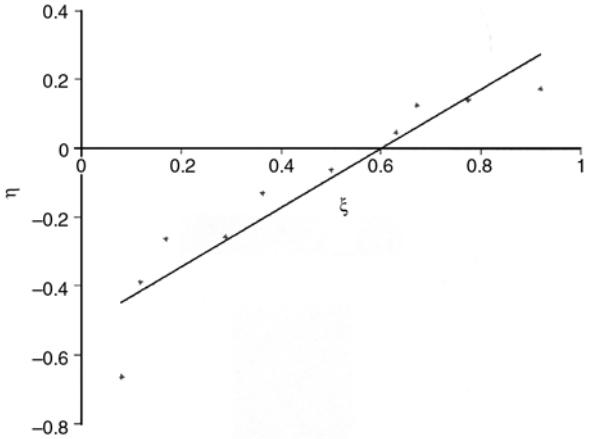


Figure 7. Ext.KT plots for determining monomer reactivity ratios in copolymerization of BrPMAAm (M_1) and HEMA (M_2) data of elemental analysis

Table 2. Comparison of the monomer reactivity ratios of BrPMAAm with HEMA by various methods

System	Methods	r_1	r_2	$r_1 \cdot r_2$	$1/r_1$	$1/r_2$
Copoly(BrPMAAm-HEMA)	F-R	0.2668	1.1374	0.3034	3.7481	0.8792
	K-T	0.3790	1.5178	0.5752	2.6385	0.6588
	Ext.K-T	0.3488	1.5275	0.5328	2.8669	0.6547
	RREVM	0.4587	1.6100	0.7385	2.1800	0.6211

To determine more reliable values of monomer reactivity ratios, a non-linear error-in-variables model (EVM) method is used utilizing the computer program, RREVM [16]. Various statistical treatments of the feed and copolymer compositions can be used to determine monomer reactivity ratios. The nonlinear methodology used selected values of r_1 and r_2, where the sum of the squares of the differences between the observed and the computed polymer compositions was minimized. With this criterion for the nonlinear least-squares method of analysis, the values for the monomer reactivity ratios were unique for a given set of data. The program produces monomer reactivity ratios for the monomers in the system with a 95% joint confidence limit determination. The joint confidence limit is a quantitative estimation of the validity of the results of the experiments and the calculations performed. This method of data analysis consists of obtaining initial estimates of the monomer reactivity ratios for the system and experimental data of comonomer charge amounts and comonomer amounts that have been incorporated into the copolymer, both in molar fractions. Tidwell and Mortimer [17] produced a nonlinear least-squares method that allowed rigorous applications of statistical analysis for reactivity ratios r_1 and r_2. This method is a modification or extension of the curve-fitting model and allows the calculations to be quantitatively analyzed. Extensive calculations are needed, but a computer program by Polic *et al.* [16] permits rapid data analysis of the nonlinear calculations. The 95% joint confidence regions for the determined r_1 and r_2 values using RREVM are shown in Figure 8. The r_1 and r_2 values from methods such as F-R, K-T, EKT and RREVM are presented in Table 2.

The microstructure of a polymeric material plays an important role in the behavior of the material toward a variety of biological systems and could be especially important in copolymerizations with monomers of different reactivities [18]. This implies that the type of copolymer prepared (i. e., random, alternating, or block) may affect the response elicited by the material in a biological environment. Monomer reactivity ratios provide a tool for estimating the average compositions of copolymers and the relative placement of reactive or functional monomers along the polymer chain [18]. The reactivity ratio values are also valuable

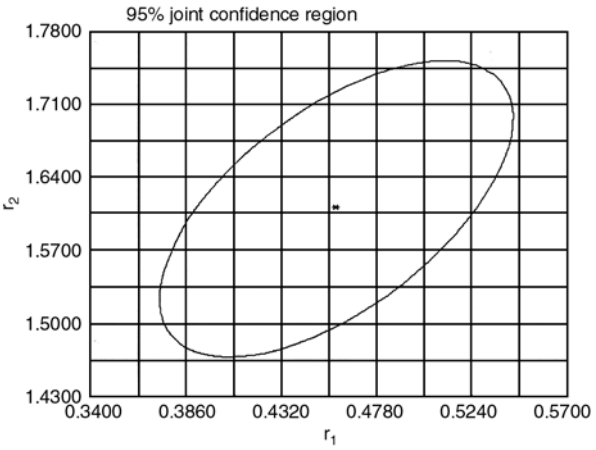


Figure 8. 95% joint confidence region of r_1 and r_2 values by RREVM for BrPMAAm-HEMA copolymer system

because the final composition of a copolymer is not simply dependent on the amounts of the two monomers present; this is especially true for monomers displaying substantial differences in the copolymerization rates. The final composition of a copolymer also depends on the method of monomer introduction, that is, whether the monomers are added all at once or incrementally over the course of the copolymerization. Both the composition and placement of monomers are dependent on the relative reactivity of each monomer in the system toward the growing polymer radicals, and vice versa.

The product of r_1 is less than 1 and r_2 is greater than 1, which indicates that the system leads to random distribution of monomer units with a longer sequence of HEMA units in the copolymer chain. Generally, neutral olefin molecules and those olefin molecules containing moderately electron-donating or electron-withdrawing groups favor free radical polymerization. HEMA consists of an electron-withdrawing ester group and an electron-donating methyl group and attached to an olefin molecule, while BrPMAAm consists of electron withdrawing phenyl amide group and an electron-donating methyl group attached to an olefin molecule. But the net charge on the HEMA molecule is less when compared to BrPMAAm, and therefore, the reactivity of HEMA is more than that of BrPMAAm. Moreover, the relative reactivity of the comonomers has to be decided not only in terms of the electronic effects, but also of the steric effects and the overall polarity of the molecule.

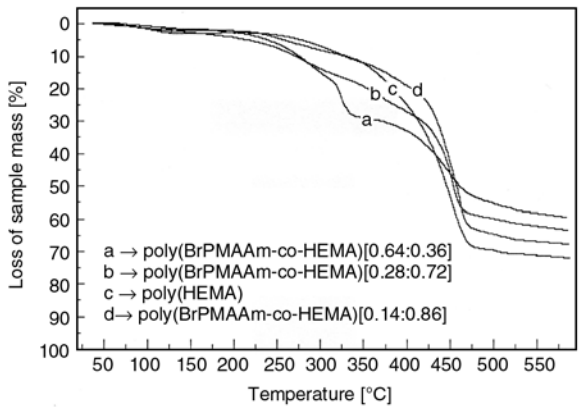


Figure 9. TGA curves of the investigated homo-and copolymers

3.3. Thermal properties

As evidenced from Figure 9, TGA curves have characteristic three-step decomposition regions. The first weight loss region appears around 50–150°C associated with dehydration of partially degradated of amide groups; secondary weight loss occurring around 150–300°C can be related to possible decarboxylation and/or other reactions of side-chain units. At last weight loss around 315–450°C indicates the main-chain degradation reactions and breakdown of the polymer backbone[18]. Copolymers show a high thermal stability which increases with increasing HEMA content in copolymers.

3.4. Antimicrobial screening

The biological activities of polymers were tested against different microorganisms using DMSO as the solvent. The sample concentrations was 100 μg. In this study, *Staphylococcus aureus COWAN I*, *Bacillus subtilis ATCC 6633, Escherichia coli ATTC 25922, Klebsiella pneumonia FMCS, Pseudomonas aeruginosa DSM 50071 have been used as bacteria, Candida tropcalis ATCC 13803, Candida globrata ATCC 66032, and Candida albicans CCM 314* as yeast.

The antibiotic sensitivities of the polymers were tested by using the antibiotic disk assay as described [19]. Muller-Hinton Agar 1.0% (w/v) beef extract, 2.0% (w/v) bactopeptone, 1.0% (w/v) glucose, 2.0% (w/v) agar was purchased from Difco. 1.5 ml of each prepared different cell culture was transferred into 20 ml of Muller-Hinton Agar (MHA) and mixed gently. The mixture was inoculated into the plate. The plates were rotated firmly and allowed to dry at room temperature for 10 minutes. Prepared antibiotic disks (100 μg) were placed on the surface of the agar medium [20]. The plates were kept at 5°C for 30 minutes then incubated at 35°C for 2 days. If a toxic compound leached out from the disc the microbial growth was inhibited around the sample. The width of this area expressed the antibacterial or antifungal activities by diffusion. The zones of inhibition of the microorganism growth of the standard samples, investigated polymers were measured with a millimeter ruler at the end of incubation period. The data reported in Table 3 are the average data of three experiments. The results were standardized against Kanamycin and Amphicillin under the same conditions. The results show that the investigated polymers have good biological activity comparable with control drugs such as Kanamycin (KAN) and Amphicillin (AMP). The first six copolymers showed good antimicrobial activities. Inhibition zone was significantly increased with HEMA content. In the case of bacteria and yeast, all copolymers allowed least growth (40–50%) were exhibited, because 2-hydroxyethyl methacrylate (HEMA) exhibits bio-

Table 3. Antimicrobial activity of compounds

	E.coli	Kleb.	Pseudo	Staph	Bacill	C.glo	C.tro	C.albi
Copoly-1	9	11	11	11	10	15	17	15
Copoly-2	8	10	11	11	9	10	11	10
Copoly-3	8	10	10	11	8	8	10	10
Copoly-4	8	8	8	10	8	8	9	8
Copoly-5	8	8	–	–	8	8	9	8
Copoly-6	8	–	–	–	8	8	8	8
Copoly-7	–	–	–	–	8	–	8	–
Copoly-8	–	–	–	–	–	–	–	–
Copoly-9	–	–	–	–	–	–	–	–
Copoly-10	–	–	–	–	–	–	–	–
KAN	20	19	21	21	22	22	22	21
AMP	19	19	23	22	21	21	22	23

compatibility, hydrophilicity, softness, high water content and permeability.

4. Conclusions

Poly(BrPMAAm) and the copolymers of BrPMAAm with HEMA were synthesized by free radical polymerization in 1,4-dioxane at 70°C. Characterization of copolymers were performed by FT-IR, ^{1}H-NMR and ^{13}C-NMR techniques. The r_1 value is less than 1, and r_2 is greater than 1. This indicates that the system forms a random copolymer with a longer sequence of HEMA units in the copolymer chain. The antimicrobial activity on the homo- and copolymers of BrPMAAm with HEMA was investigated. As the percentage of HEMA in the copolymers increases, the effectiveness of the copolymers to inhibit the growth of the microorganisms increases. It is to be remembered that the conformation the polymers acquired under experimental conditions is a factor for their antigrowth activity.

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