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Microbial Screening of Copolymers of N-Vinylimidazole with Phenacyl Methacrylate: Synthesis and Monomer Reactivity Ratios

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N-vinylimidazole (VIM), and phenacyl methacrylate (PAMA) copolymerized with different feed ratios using 1,4-dioxane as a solvent and α,α'*-azobisisobutyronitrile (AIBN) as an initiator at 60°C. Structure and composition of copolymers for a wide range of monomer feed were determined by elemental analysis (content of N for VIM-units) and by Fourier transform infrared spectroscopy through recorded analytical absorption bands for VIM (*$670\,cm^{-1}$ *for C-N of imidazole ring) and PAMA (*$1730\,cm^{-1}$ *for C═O of ester group) units, respectively. Monomer reactivity ratios for VIM (*M_1*)-PAMA (*M_2*) pair were determined by the application of conventional linearization methods such as Fineman-Ross (F-R) and Kelen-Tüdös (KT) and a nonlinear error invariable model method using a computer program RREVM. The molecular weights (*$\overline{M}_w$ *and* $\overline{M}_n$*) and polydispersity indices of the polymers were determined using gel permeation chromatography (GPC). Thermal behaviors of copolymers with various compositions were investigated by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). Also, the apparent thermal decomposition activation energies (*ΔE_d*) were calculated by Ozawa method using the SETARAM Labsys TGA thermobalance. The antibacterial and antifungal effects of polymers were also tested on various bacteria, fungi and yeast.*

Keywords N-vinylimidazole, phenacyl methacrylate, monomer reactivity ratios, thermal behaviors, microbial screening

Introduction

Imidazole containing macromolecules have been suggested as carrying an active moiety of several electrolytic enzymes (1, 2). The imidazole ring is present in most proteins (i.e., histamine, histidine, etc.) and is partly responsible for their catalytic activity. Consequently, extensive studies of the catalytic behaviors of monomeric and polymeric (3) imidazole have been reported. Thus, Sebille and coworkers (4, 5) have developed a new coating technology for protein separations using poly(vinylimidazole) and/or poly-(vinylimidazole)-poly(N-vinylpyrrolidone) block copolymers. The roles of imidazole groups in polymers, such as metal-ion complexation, councounterion binding and dye binding, have been extensively studied (6, 7). Furthermore, the conformational behavior

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of poly(N-vinylimidazole) (PVIM) has been described in terms of the nature of the solvent, quaternizing group, ionic strength and counterion type (8, 9). The conditions for protein separations were optimized by varying the copolymer composition and the amount adsorbed on the non-porous silica supports. N-vinylimidazole-4-aminostyrene copolymer as a new tailor-made steric stabilizer for polyaniline colloids was synthesized by free-radical precipitation copolymerization of monomers in benzene at 70°C using 2,2′-azobisisobutyronitrile(AIBN) as an initiator (10). Silane (γ-methacryloxypropyltrimethoxysilane)-modified poly(N-vinylimidazole) copolymer coatings has been shown to have good corrosion protection and adhesion promotion capabilities for copper substrate in severe environments (11). It has been reported that the polymerization of 1-vinylimidazole at high monomer concentration is accompanied by the degradative addition of a growing radical to the 2-position of the imidazole ring of the monomer (12). The template radical polymerization of N-vinylimidazole (VIM) along poly-(methacrylic acid) in water at 50°C was studied by Grampel et al. (13).

Polymethacrylates with keto side chains have attracted increasing interest (14–16). Functionalized polymers have been employed as catalysts (17, 18), reagents (19–21) and in the immobilization of transition complexes (22, 23).

It is well known that physical properties and sequence length distribution are important characteristics of copolymers. The most fundamental quantity characterizing a copolymer is its compositions on a molar basis, which eventually is used for determination of the relevant monomer reactivity ratios. Spectroscopic methods, preferably ^{1}H-NMR, ^{13}C-NMR (24, 25), IR and Fourier transform IR (FTIR) (26) and UV (27) spectroscopy are probably the most widely used methods for analysis of copolymers, and determination of r_1 and r_2. In general, FTIR spectroscopy can provide not only qualitative but also very good quantitative analysis.

Thermogravimetric analysis (TGA) has been widely used to investigate the decomposition characteristics of many materials. Some methods have already been established to evaluate the kinetic parameters from thermogravimetric data (28, 29).

In a previous study (30), we described the synthesis and characterization of phenacyl methacrylate (PAMA) monomer and its homopolymer. In the present work, the results of radical copolymerization of VIM with phenacyl methacrylate (PAMA), determination monomer reactivity ratios using different method including FTIR spectroscopy and the effects of imidazole units in copolymer composition-thermal behavior relationships are presented and discussed. Homo- and copolymers have been characterized in their antimicrobial activity against microorganism such as bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*), fungi (*Aspergillus niger*, *Trichoderma lignorum*, *Trichoderma viridis*) and yeast (*Candiada albicans*, *Saccharomyces cerevisiae*, *Candiada utilis*).

Experimental

Materials

PAMA monomer was prepared as reported (30). N-vinylimidazole (VIM) monomer (Aldrich) was freed from the inhibitor by washing with 5% NaOH solution followed by it being distilled under vacuum before use: b.p. 78°C/13 mmHg. 1,4-Dioxane, chloroform, methanol, and ethanol (Merck), anhydrous magnesium sulphate (Aldrich) were used as received. α,α'-Azobisisobutyronitrile was recrystallized from chloroform-methanol. All other chemicals were analytical grade commercial products, and they were used without any further purification.

Characterization Techniques

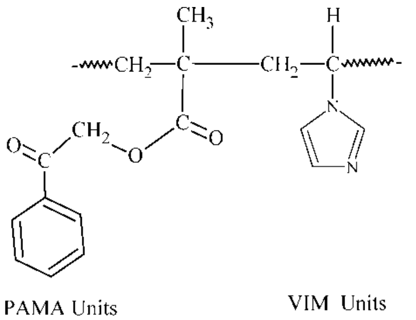
Infra-red spectra were obtained with a Jasco 460 Plus FTIR spectrometer using KBr pellets in the 4000–400 cm^{-1} range, where 10 scans were taken at 4 cm^{-1} resolution. For the composition analysis of copolymers, specifically contents of VIM and PAMA units, characteristic absorption bands of 670 cm^{-1} (for VIM unit), 1730 cm^{-1} (for PAMA unit) were used as analytical bands. The least changing absorption band of 1040 cm^{-1} was used as a standard band ($A = \log(I_o/I)$, $\Delta A^i = A^i/A1040$) to calculate the copolymer compositions. Molecular weight; ($\overline{M}_w$ and $\overline{M}_n$) of the polymer was determined using Waters 410 gel permeation chromatography equipped with a RI detector and calibrated with polystyrene standards. Thermal data were obtained by using a Setaram DSC-131 instrument at a heating rate of 20°C min^{-1} and a Labsys TGA thermobalance at a heating rate of 10°C min^{-1} in N_2 atmosphere. Elemental analyses were carried out by a LECO-932 microanalyzer.

Results and Discussion

Copolymerization

The monomer pair studied differs by nature of the conjugation between double bond and functional group VIM is an electron-donor monomer with π(C=C vinyl) ← p(N) ← π' (N=C imidazole ring)-conjugated system and PAMA is an electron-acceptor comonomer with π(C=C acrylic → π'(C=O ester)-conjugated system. These distinctive structural peculiarities allow to predict that the monomers may show sufficient activity in radical-initiated copolymerization. Copolymerizations of VIM with PAMA, having six different feed compositions were carried out in 1,4-Dioxane at 60 ± 0.1°C using AIBN (1%, based on the total weight of monomers) as an initiator. Appropriate amounts of VIM with PAMA, and 1,4-Dioxane was mixed in a polymerization tube, purged with N_2 for 20 min, and kept at 60 ± 0.1°C in a thermostat. The reaction time (≈1 h), was selected to give conversions less than 10% to satisfy the differential copolymerization equation (31). After the desired time the copolymers were separated by precipitation in ethanol and reprecipitated from CH_2Cl_2 solution. The polymers, purified by reprecipitation to avoid the formation of homopolymers. The polymers were finally dried over vacuum at 40°C to constant weight. The amounts of monomeric units in the copolymers were determined by elemental analysis (N content for VIM units) and FTIR spectroscopy using 670 cm^{-1} (C–N bond for VIM unit) and 1730 cm^{-1} (C=O bond for PAMA unit) as absorption bands for quantitative analysis (Scheme 1).

The constituent monomeric units of the copolymer are as follows:



Scheme 1. The constituent monomeric units of the copolymer.

FTIR spectra (KBR pellet), cm^{-1}: 3205 ($\upsilon_{C=N}$), 1643 ($\upsilon_{C=C}$), 815 and 670 (C–N) for VIM unit and 1730 and 1690 ($\upsilon_{C=O}$ ester and ketone), 1180–1020 (C–O and C–O–C) and 970 (δ_{C-O}) for PAMA unit; 2985 (υ_{aC-H} in CH_3), 2945 (υ_{aC-H} in CH_2), 2910 (υ_{sC-H} in CH_2 and CH_3), 1450 (δ_a CH_2), 1370 (δ_a CH_3), 1270 (δ_s CH_2) and 1235 (δ_s CH_3).

Copolymer Composition

Molar fractions (in mol%) of comonomer units (m_1 and m_2) in VIM (M_1)-PAMA(M_2) copolymers using FTIR analysis data are calculated according to the following equations (32):

$$m_1 = \frac{\Delta A^{670}/M_{VIM}}{\Delta A^{670}/M_{VIM} + \Delta A^{1730}/M_{PAMA}} 100 \tag{1}$$

$$m_2 = \frac{\Delta A^{1730}/M_{PAMA}}{\Delta A^{670}/M_{VIM} + \Delta A^{1730}/M_{PAMA}} 100 \tag{2}$$

where $m_1/m_2 = [\Delta A^{670}/M_{VIM}]/[\Delta A^{1730}/M_{PAMA}]$, $\Delta A = A^{i}/A^{1040}$ (standard band), M_{VIM} and M_{PAMA} are molecular weights (g/mol) of VIM and PAMA monomer units, respectively.

Results of FTIR analyses of VIM-PAMA copolymers for various initial monomer ratios as model systems synthesized are illustrated in Figure 1 and Table 1, respectively. Copolymer compositions calculated using elemental analysis data (content of N) were in very good agreement with those obtained from FTIR analysis (Table 1). On the basis of FTIR analysis data (Figure 1) the values of absorption bands for the comonomer units are calculated and then used for the determination of copolymer compositions according to Equations (1) and (2).

Molecular Weights of the Polymers

The molecular weights of the polymers were determined by 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 ($\overline{M}_w/\overline{M}_n$) of polymer samples are presented in Table 2. The polydispersity index of the polymers ranges between 1.69 to 1.93. The theoretical values of ($\overline{M}_w/\overline{M}_n$) for polymers produced via radical recombination and disproportionation are 1.5 and 2.0, respectively (33).

Determination of Monomer Reactivity Ratios

The monomer reactivity ratios for the copolymerization of VIM with PAMA was determined from the monomer feed ratios and the copolymer composition. The Fineman-Ross (FR) (34), and Kelen-Tüdös (KT) (35) methods were used to determine the monomer reactivity ratios. Results from the analysis for F-R and K-T are presented in Table 3. According to the FR method the monomer reactivity ratios can be obtained by the equation:

$$G = Hr_1 - r_2 \tag{3}$$

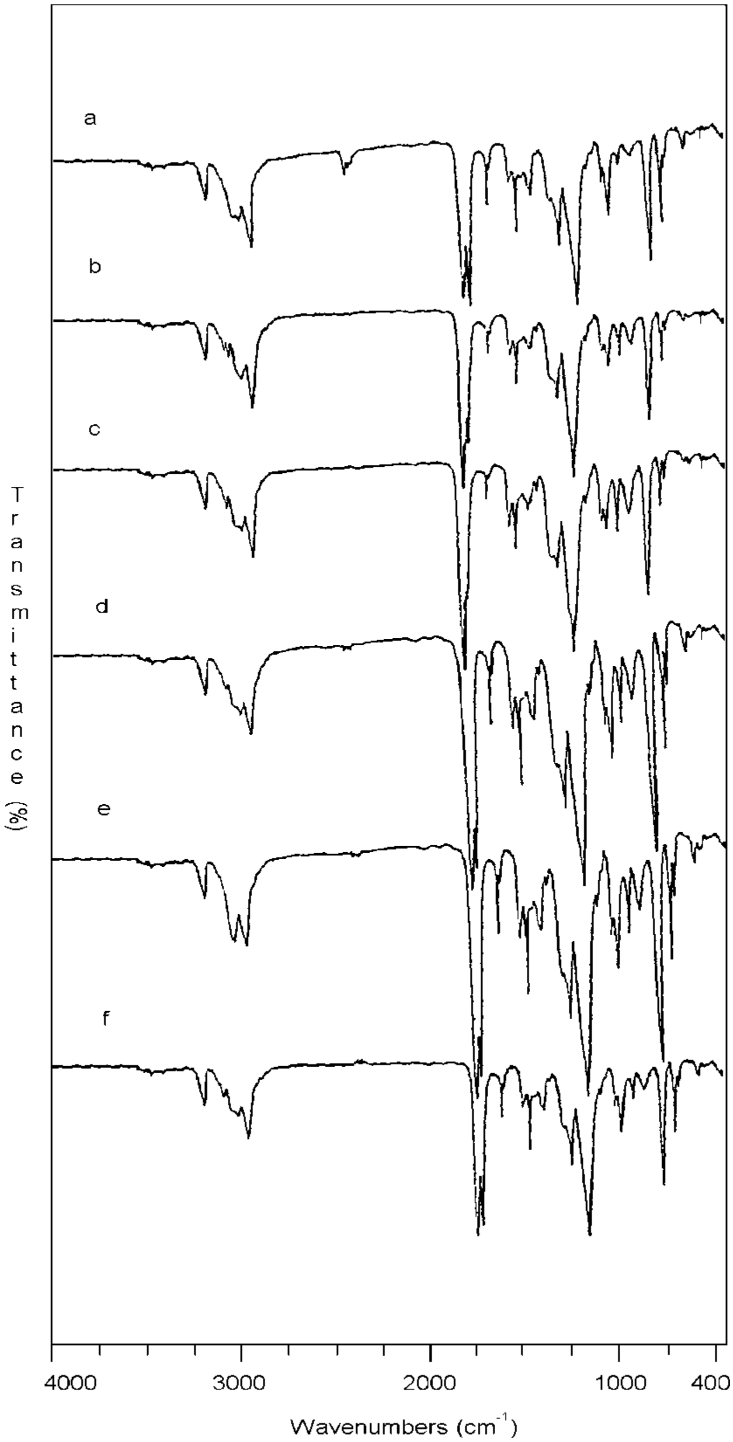


Figure 1. FTIR spectra of VIM-PAMA copolymers with different compositions (mol% of VIM-unit); (a) 0.057, (b) 0.139, (c) 0.218, (d) 0.309, (e) 0.394 and (f) 0.577.

Table 1
FTIR analysis data for determining the composition of VIM-PAMA copolymers synthesized from various initial monomer mixtures

						Copolymer composition in mole fraction			
	Feed composition in mode fraction					By FTIR analysis		By nitrogen analysis	
Sample code no	VIM (M_1)	PAMA (M_2)	$^a\Delta A^{670}$ (VIM unit)	$^a\Delta A^{1730}$ (PAMA unit)	Elementel (%) N	m_1	m_2	m_1	m_2
M-1	—	1.00	—	—	—	—	1.00	—	1.00
M-2	0.10	0.90	0.084	3.037	0.80	0.057	0.943	0.056	0.944
M-3	0.20	0.80	0.280	3.772	2.02	0.139	0.861	0.136	0.864
M-4	0.35	0.65	0.360	2.810	3.48	0.218	0.782	0.223	0.777
M-5	0.50	0.50	0.402	1.950	5.24	0.309	0.691	0.316	0.684
M-6	0.65	0.35	0.420	1.400	7.20	0.394	0.605	0.408	0.592
M-7	0.80	0.20	0.428	0.680	11.60	0.577	0.423	0.581	0.419
M-8	1.00	—	—	—	—	1.00	—	1.00	—

*A*nalytical absorption bands: 670 cm^{-1} (υ_{C-N} in imidazole ring) and 1730 cm^{-1} ($\upsilon_{C=O}$ in PAMA unit).
$^a\Delta A^{670}$ (or ΔA^{1730}) $= A^{670}$ (or A^{1730})$/A^{1040}$; where A^{1040} is an absorption of least changing b and.

Table 2

Differantiel scanning calorimetry, molecular weight data of polymers

Sample code no	$\overline{M}_w \times 10^{-4}$	$\overline{M}_n \times 10^{-4}$	$\overline{M}_w/\overline{M}_n$	Tg (°C)	ΔH_o^d (J/g)	ΔCp (J/g·K)
M-1	2.91	1.66	1.75	105	1.8726 (Endo)	−0.0955
M-2	2.86	1.54	1.86	108	2.8995 (Endo)	−0.1690
M-3	3.20	1.84	1.74	115	3.6449 (Endo)	−0.2327
M-4	1.98	1.06	1.87	123	4.8078 (Endo)	−0.2820
M-5	2.54	1.50	1.69	135	3.1224 (Endo)	−0.1892
M-6	3.12	1.80	1.73	146	2.3144 (Endo)	−0.1210
M-7	2.26	1.30	1.74	153	2.2218 (Endo)	−0.1022
M-8	2.36	1.22	1.93	172	4.2730 (Endo)	−0.2610

where the reactivity ratios, r_1 and r_2 correspond to the VIM with PAMA monomers respectively. The parameters G and H are defined as follows:

$$G = F(f - 1)/f \quad \text{and } H = F^2/f \tag{4}$$

with

$$F = M_1/M_2 \quad \text{and } f = m_1/m_2 \tag{5}$$

M_1 and M_2 are the monomer molar compositions in feed and m_1 and m_2 the copolymer molar compositions.

Alternatively the reactivity ratios can be obtained using the KT method, which is based on the equation:

$$\eta = (r_1 + r_2/\alpha)\xi - r_2/\alpha \tag{6}$$

where η and ξ are functions of the parameters G and H:

$$\eta = G/(\alpha + H) \quad \text{and} \quad \xi = H/(\alpha + H) \tag{7}$$

and α a constant which is equal to $(H_{max} \cdot H_{min})^{1/2}$, H_{max}, H_{min} being the maximum and the minimum H values, respectively from the series of measurements. From the linear plot of η as a function of ξ the values of η for $\xi = 0$ and $\eta = 1$ is used to calculate the reactivity ratios according to the equations:

$$\xi = 0 \Rightarrow \eta = -r_2/\alpha \quad \text{and} \quad \xi = 1 \Rightarrow \eta = r_1 \tag{8}$$

The graphical plots concerning the methods previously reported are given in Figures 2 and 3.

Several non-linear methods have been attempted to determine monomer reactivity ratios (36–38). 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 (38). The r_1 and r_2 values from methods such as F-R, K-T and RREVM are presented in Table 4. The 95% joint confidence region for the determined r_1 and r_2 values using RREVM is shown in Figure 4.

Table 3
F-R and K-T parameters for copoly(VIM/PAMA) system

$F = M_1/M_2$	$f = m_1/m_2$		$G = F(f-1)/f$		$H = F^2/f$		$\eta = G/(\alpha + H)$		$\xi = H/(\alpha + H)$	
0.111	0.060^a	0.059^b	-1.739^a	-1.770^b	0.205^a	0.209^b	-0.991^a	-1.006^b	0.117^a	0.119^b
0.250	0.161^a	0.157^b	-1.303^a	-1.342^b	0.388^a	0.398^b	-0.672^a	-0.689^b	0.200^a	0.204_b
0.538	0.278^a	0.287^b	-1.397^a	-1.336^b	1.041^a	1.008^b	-0.539^a	-0.522^b	0.402^a	0.394^b
1.000	0.447^a	0.462^b	-1.237^a	-1.164^b	2.237^a	2.164^b	-0.327^a	-0.313^b	0.591^a	0.583^b
1.857	0.651^a	0.689^b	-0.996^a	-0.838^b	5.297^a	5.005^b	-0.145^a	-0.128^b	0.774^a	0.764^b
4.000	1.364^a	1.387^b	1.067^a	1.116^b	11.730^a	11.536^b	0.080^a	0.085^b	0.883^a	0.882^b

Reaction conditions: Solvent:1,4-dioxane; Conversion $<10\%$; α (arbitrary constant) = $(H_{max} \cdot H_{min})^{1/2}$: 55 (from FTIR analysis) and 1.55 (from elemental analysis).

[a] By FTIR analysis.

[b] By Nitrogen analysis.

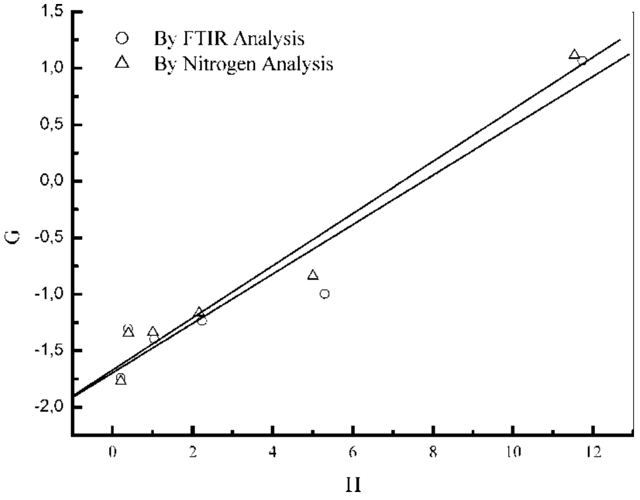


Figure 2. FR plots for determining monomer reactivity ratios in copolymerization of VIM (M_1) and PAMA (M_2) data of FTIR and elemental analysis.

For VIM and PAMA systems, the r_2 values are higher than the r_1 values. The higher r_2 value of PAMA confirms the higher reactivity of PAMA. The reactivity ratio values (r_1 and r_2) of copoly(VIM-PAMA) are less than one. The product $r_1 \cdot r_2$ indicates that the system copolymerizes randomly in the polymer chain, although there is a possible tendency for alternation.

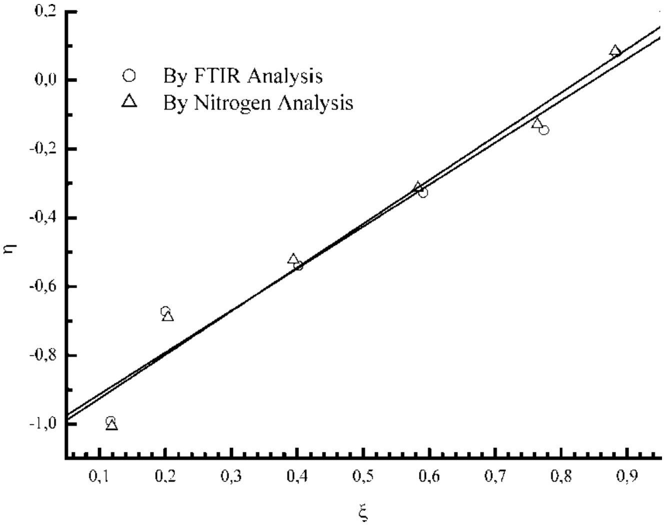


Figure 3. KT plots for determining monomer reactivity ratios in copolymerization of VIM (M_1) and PAMA (M_2) data of FTIR and elemental analysis.

Table 4

Copolymerization parameters for the free radical copolymerization of VIM with PAMA

Method	r_1[a]	r_2[a]	r_1r_2
Fineman-Ross	0.2187[b]; 0.2308[c]	1.6958[b]; 1.6706[c]	0.3709[b]; 0.3856[c]
Kelen-Tüdös	0.1843[b]; 0.2178[c]	1.6052[b]; 1.6317[c]	0.2958[b]; 0.3554[c]
RREVM	0.1885[b]; 0.2150[c]	1.6063[b]; 1.6223[c]	0.3028[b]; 0.3488[c]

[a] r_1 and r_2 are the monomer reactivity ratios of VIM and PAMA, respectively.
[b] Calculated by FTIR analysis.
[c] Calculated by nitrogen analysis.

Differential Scanning Calorimetry Experiments

The glass transition (Tg) temperatures were determined by a Setaram 131 DSC. Samples of about 5–8 mg held in sealed aluminum crucibles and the heating rate of 20°C/min under a dynamic nitrogen flow ($5\,L \cdot h^{-1}$) were used for the measurements. From DSC measurements, Tg was taken as the midpoint of the transition region. All the copolymers show a single Tg, showing the absence of a mixture of homopolymers or the formation of a block copolymer. Poly(VIM) is a thermally more stable polymer. Its Tg value is at 172°C. The Tg of the copolymers increases with an increase in VIM content in the copolymers. The results clearly indicate that Tg values of copolymers depend on the composition of comonomers and increase with increasing VIM content in the polymer chain. It can be seen that the observed Tg increases with increasing VIM and presents a striking positive deviation with respect to linearity, which can be associated with a lower free volume, mobility and flexibility than a mixture of VIM and PAMA units.

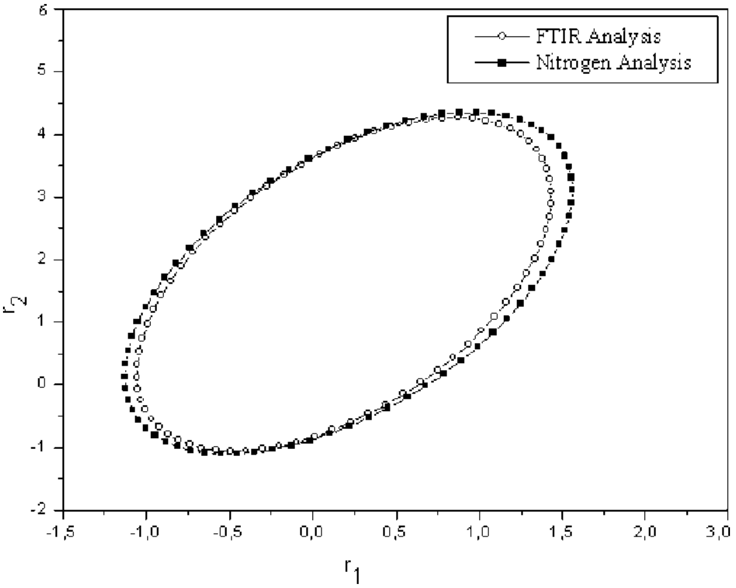


Figure 4. Reactivity ratio estimates with 95% confidence interval generated by EVM.

The DSC thermograms of polymers indicated endothermic degradation. Representative DSC thermograms of polymers are given in Figure 5. Data analysis was carried out with the Setaram software package. The enthalpy changes (ΔH_o^d) and heat capacity (ΔCp) during thermal degradation obtained from the DSC thermograms of polymers are given in Table 2.

Decomposition Kinetics

The thermal stabilities of the polymers were investigated by thermogravimetric analysis (TGA) in a nitrogen stream at a heating rate of $10°\mathrm{C} \cdot \mathrm{min}^{-1}$. In Figure 6, the TGA thermograms of polymers are shown. It is clear that three degradation stages for poly(VIM) and two degradation stages for poly(PAMA) are observed. The thermal degradation of poly-n-alkyl methacrylates typically produces the monomer as a result of depolymerization. The formation of cyclic anhydride type structures by intramolecular cyclization is another main process in degradation of these polymers. The latter produces some low molecular weight products, depending on the chemical structures of the side chain of poly-methacrylic esters.

For the study on the kinetics of thermal degradation of polymers, we can select the isothermal thermogravimetry (ITG) or the thermogravimetry (TG) at various heating rates (39). ITG is superior to obtain an accurate activation energy for thermal degradation, although it is time-consuming. In the case of thermal degradation of polymers, in which depolymerization is competing with cyclization or crosslinking due to the side groups, the TG at various heating rates is much more convenient than ITG for the investigation of thermal degradation kinetics. Therefore, in the present work, TG curves at various heating rates were obtained and the activation energies (ΔE_d) for thermal degradation of polymers were calculated by Ozawa's plot, which is a widely used method. Degradations were performed in the scanning mode, from 35 up to 500°C, under nitrogen flow ($20\,\mathrm{mL} \cdot \mathrm{min}^{-1}$), at various heating rates (β: 4.0, 7.0, 12.5, 15.0, and

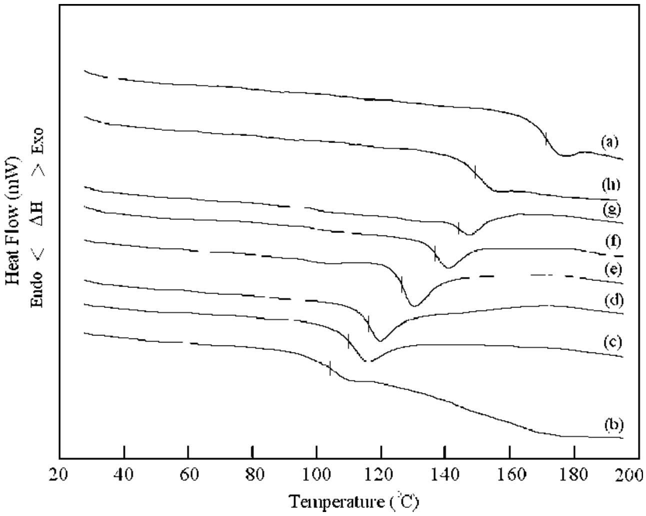


Figure 5. DSC thermograms of: (a) poly(VIM), (b) poly(PAMA) and (c)–(h) VIM-PAMA copolymers (mol% of VIM Units as in Figure 1).

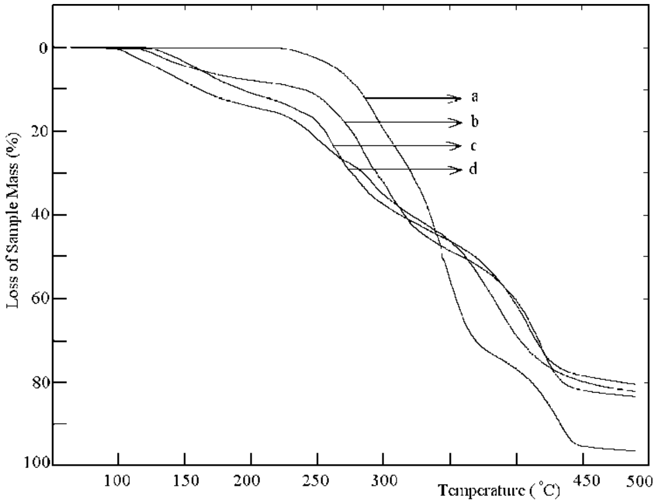


Figure 6. TGA curves of (a) poly(PAMA), (d) poly(VIM), (b) and (c) VIM-PAMA copolymers with different compositions (mol% of VIM-unit); (b) 0.309, and (c) 0.577.

20.0°C · min^{-1}). In Figure 7, the TGA thermograms of poly(VIM-co-PAMA): (0.309 : 0.691) are shown. Samples of 5–8 mg held in alumina open crucibles, were used and their weights were measured as a function of temperature and stored in the list of data of the appropriate built-in program of the processor. The TGA curves were immediately printed at the end of each experiment and the weights of the sample were then transferred to a PC at various temperatures.

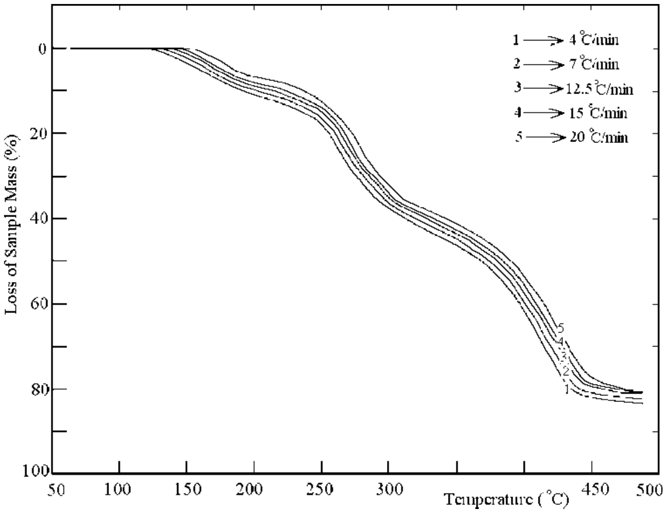


Figure 7. The thermal degradation curves of poly(VIM-co-PAMA): (0.309 : 0.691) at different heating rates.

According to the method of Ozawa (40), the apparent thermal decomposition activation energy, E_d, can be determined from the TGA thermograms under various heating rates, such as in Figure 7, and the following equation:

$$E_d = -\frac{R}{b}\left[\frac{d\log\beta}{d(1/T))}\right] \tag{9}$$

where R is the gas constant; b, a constant (0.4567); and β, the heating rate (°C/min). According to Equation (1), the activation energy of degradation can be determined from the slope of the linear relationship between log β and $1/T$, as shown in Figure 8; the ΔE_d values for polymers are given in Table 5. ΔE_d calculated from the Ozawa method is superior to other methods for complex degradation, since it does not use the reaction order in the calculation of the decomposition activation energy (41). Therefore, ΔE_d calculated from the Ozawa method was superior to the former methods for complex degradation.

Antibacterial and Antifungal Effects

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*, *Bacillus subtilis*, and *Escherichia coli* have been used as bacteria, *Aspergillus nigar*, *Trichoderma lignorum*, and *Trichoderma viridis* as fungi, with *Candiada albicans*, *Saccharomyces cerevisiae*, and *Candiada utilis* as yeast.

The antibiotic sensitivity of the polymers were tested by using the antibiotic disk assay as described (42). 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

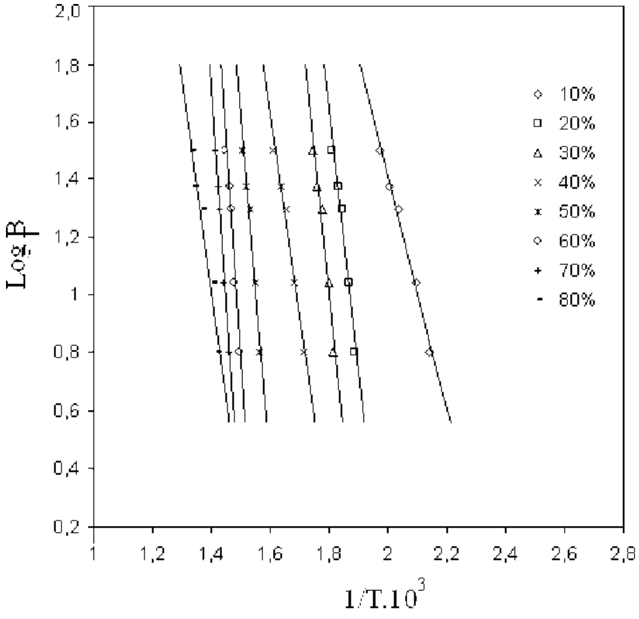


Figure 8. Ozawa's plots of logarithm of heating rate (β) vs. reciprocal temperature (1/T) at different conversions for a poly(VIM-co-PAMA): (0.309 : 0.691).

Table 5
The apparent activation energies of polymers under thermal degradation in N_2

Sample code no	Activation energy ΔE_d (kJ/mol) conversion (%) 10	20	30	40	50	60	70	80	Average
M-1	76.4	82.1	85.2	78.0	79.3	84.4	80.1	84.2	81.2
M-2	81.4	82.0	75.2	86.0	89.8	90.4	92.1	84.2	85.1
M-3	84.4	78.5	80.0	81.2	86.3	90.8	96.1	82.0	84.9
M-4	78.6	80.8	88.3	95.2	96.2	100.8	101.2	86.5	91.0
M-5	82.2	84.0	78.8	87.4	88.0	102.2	96.1	90.5	88.6
M-6	78.2	85.5	90.6	90.8	104.0	105.2	94.4	86.0	91.8
M-7	80.2	84.0	85.6	94.3	92.8	96.6	87.8	81.1	87.8
M-8	88.2	90.0	79.4	85.6	90.8	98.6	100.4	83.4	89.6

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 min. Prepared antibiotic disks (100 μg) were placed on the surface of the agar medium. The plates were kept at 5°C for 30 min 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 microorganisms growth of the standard samples, investigated polymers were measured with a millimeter ruler at the end of incubation period. The data reported in Figures 9, 10, and 11 are the average data of three experiments. The results show that the investigated polymers have good biological activity comparable with control drugs such as Kanamycin and Amphicillin. In the case of bacteria, poly(VIM) allowed least

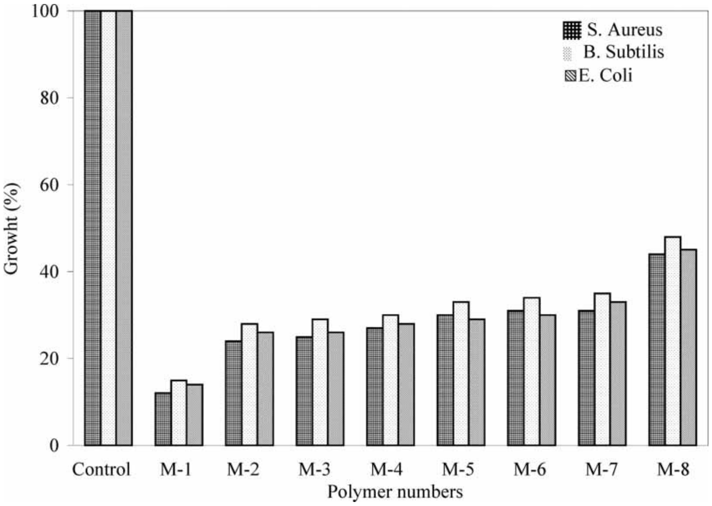


Figure 9. Effect of homo- and copolymers on percentage growth of bacteria.

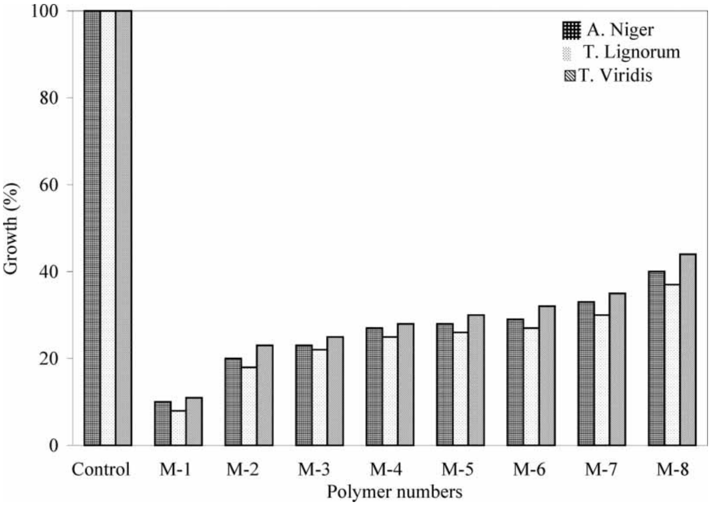


Figure 10. Effect of homo- and copolymers on percentage growth of fungi.

growth (50%) and with the copolymers, growths of 20–35 % were exhibited. Fungi and yeast in the presence of poly(VIM) showed 45% growth, while 20–35% growth was observed with copolymers. However, the polymers exhibit inhibition zone was significantly increased with VIM content.

Conclusions

Copolymers of PAMA with VIM have been prepared by free radical polymerization in 1,4-dioxane at 60°C. The reactivity ratios of the copolymers were estimated using linear and non-linear graphical methods. It was observed that the glass transition

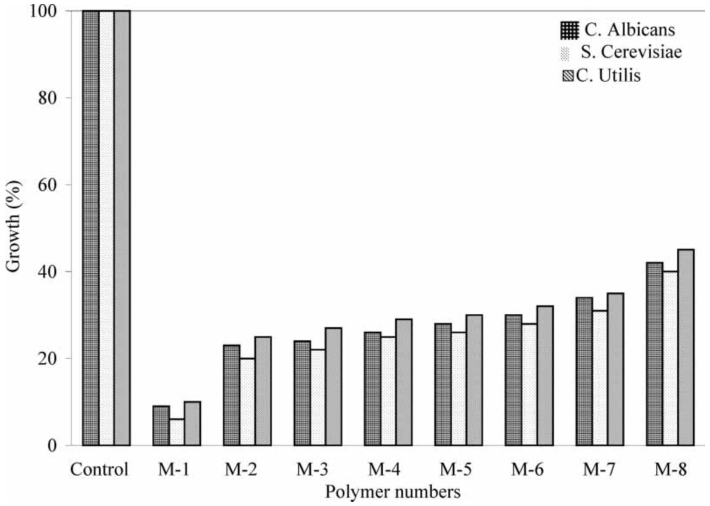


Figure 11. Effect of homo- and copolymers on percentage growth of yeast.

temperature of copolymers increased with increasing of VIM content in copolymers and thermal stability of copolymers increased up to 40% decomposition with increasing of VIM content in copolymers. The polymers have good biological activity comparable with control drugs such as Kanamycin and Amphicillin. As the percentage of VIM in the copolymers increases, the effectiveness of the copolymers to inhibit the growth of the microorganisms increases.

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