

Synthesis, Characterization, and Antimicrobial Activity of Acrylic Copolymers

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ABSTRACT: 2,4-Dichlorophenyl methacrylate (2,4-DMA) and vinyl acetate (VAc) were copolymerized with different feed ratios using dimethyl formamide (DMF) as a solvent and 2,2'-azobisisobutyronitrile (AIBN) as an initiator at 70°C. The copolymers were characterized by infrared (IR) spectroscopy. Copolymer compositions were determined by ultraviolet (UV) spectroscopy. The monomer reactivity ratios were evaluated by the Fineman–Ross method. Average molecular weight and polydispersity index were determined by gel permeation chromatography (GPC), and the

intrinsic viscosities of polymers were also discussed. Thermogravimetric analyses of polymers were carried out under a nitrogen atmosphere. The homo- and copolymers were tested for their antimicrobial activity against selected microorganisms. © 2003 Wiley Periodicals, Inc. *J Appl Polym Sci* 89: 895–900, 2003

Key words: copolymerization; gel permeation chromatography (GPC); thermal properties

INTRODUCTION

Contamination by microorganisms is of great concern in several areas, such as, medical devices, healthcare products, water purification system, hospital and dental office equipment, food packaging, food storage, etc. Consequently, biocidal polymers have received much attention in recent years.¹ One possible way to avoid microbial contamination is to develop materials with antimicrobial activities.²

Oh and co-workers^{3–5} synthesized 2,4,4'-trichloro-2'-acryloyloxy diphenyl ether and its homo- and copolymers with methyl methacrylic acid (MMA), styrene, and 2-hydroxy ethyl methacrylate and tested their bactericidal activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* microorganisms. The results showed excellent growth inhibition of these bacteria by the copolymers. Nonaka and co-workers⁶ prepared acryloyloxy trialkyl phosphonium chloride-based copolymers, which showed antibacterial activity against *Escherichia coli* and *S. aureus*.

The present investigation was carried out to modify acrylics by incorporation of acrylic monomer bases in 2,4-dichlorophenyl methacrylate (2,4-DMA) to make the resultant copolymers useful in pharmaceuticals, pesticides, paints, coatings, inks, adhesives, and cosmetics.⁷ This article discusses the synthesis and characterization of the monomer and homo- and copolymers using different feed ratios. The copolymer composition

was determined by ultraviolet (UV) spectroscopy, and molecular weight was measured by gel permeation chromatography (GPC). Thermal analyses of polymers are also included. The antimicrobial activity of homo- and copolymers against microorganisms such as bacteria (*Bacillus subtilis*, *E. coli*, *Staphylococcus citreus*), fungi (*Aspergillus niger*, *Sporotrichum pulverulentum*, *Trichoderma lignorum*), and yeast (*Candida utilis*, *Saccharomyces cerevisiae*, *Pichia stipitis*) was determined.

EXPERIMENTAL

All the chemicals used were of analytical grade. The solvents and monomers were purified by conventional methods.⁸

Synthesis of 2,4-dichlorophenyl methacrylate (2,4-DMA)

Methacryloyl chloride was prepared by reacting methacrylic acid with benzoyl chloride.⁹ The esterification was performed with methacryloyl chloride and 2,4-dichlorophenol (2,4-D). Absolute ethyl alcohol (400 mL) and NaOH (0.2 mol, 8.0 g) were added to a three-necked flask that was equipped with a stirrer, condenser, and thermometer. The flask was placed in a waterbath and the contents were stirred until all the NaOH dissolved. Next, 2,4-D (0.2 mol, 32.6 g) was added to the reaction mixture, which was then heated to 60°C for 30 min with stirring, cooled to room temperature, and then cooled to 0–5°C with ice. Freshly prepared methacryloyl chloride (0.21 mol, 20.5 mL) was added in a dropwise manner to the cooled reac-

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tion mixture and stirred for 90 min. The mixture was then poured into a crushed ice–water mixture where a cream-colored product was separated out. The product was filtered, washed thoroughly with cold water, dried at 35°C under vacuum, and recrystallized from petroleum ether. The formation of monomer was confirmed by infrared (IR) and proton nuclear magnetic resonance ($^1\text{H-NMR}$) techniques.

IR (cm^{-1}): 2925 ($\nu_{\text{C-H}}$), 1743 ($\nu_{\text{C=O}}$), 1642 ($\nu_{\text{C=C}}$), 1333 ($\nu_{\text{C-O}}$); 1232 and 1160 ($\nu_{\text{C-O-C}}$), 890 (—CH bending mode of vinyl group), 720 (rocking mode of vinyl group), 670 ($\nu_{\text{C-Cl}}$), 1590 and 1488 (bands due to phenyl ring). $^1\text{H-NMR}$ (ppm) (60 MHz): 2.080 (3H) (methyl protons); 6.416 (1H) and 5.811 (1H) (Non-equivalent methylene protons); 7.383–7.471 (1H; aromatic proton), 7.031–7.236 (2H; aromatic proton).

Copolymerization

Homo- and copolymerization were carried out in dimethyl formamide (DMF) using 2,2'-azobisisobutyronitrile (AIBN) as an initiator (see Fig. 1). Predetermined quantities of 2,4-DMA, vinyl acetate (VAc), DMF, and AIBN were mixed in a round-bottomed flask equipped with mechanical stirrer and reflux condenser. The reaction mixture was heated at 70°C with constant stirring. Sodium acetate was used as a buffer for homo- and copolymers of VAc monomer to prevent hydrolysis of VAc. The mixtures were then cooled to room temperature and slowly poured, with constant stirring, into a large excess of acetone/water (50:50) that was used as a nonsolvent. Solid polymers were purified by repeated precipitation with the acetone/water mixture from solution in DMF and finally dried under vacuum.

Characterization

IR spectra were recorded with a NICOLET-400D FTIR spectrophotometer on solid samples in the KBr pellets. Copolymer compositions and reactivity ratios were determined with a Shimadzu-160A-recording UV–visible (UV–Vis) spectrophotometer. Molecular weights of the polymers were determined by GPC with a Waters 600 E chromatograph equipped with a 410-RI detector and calibrated with polystyrene standards. A Schott–Gerate viscosity measurement system was

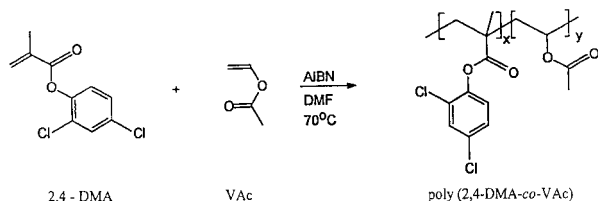


Figure 1 Synthesis scheme of poly(2,4-DMA-co-VAc).

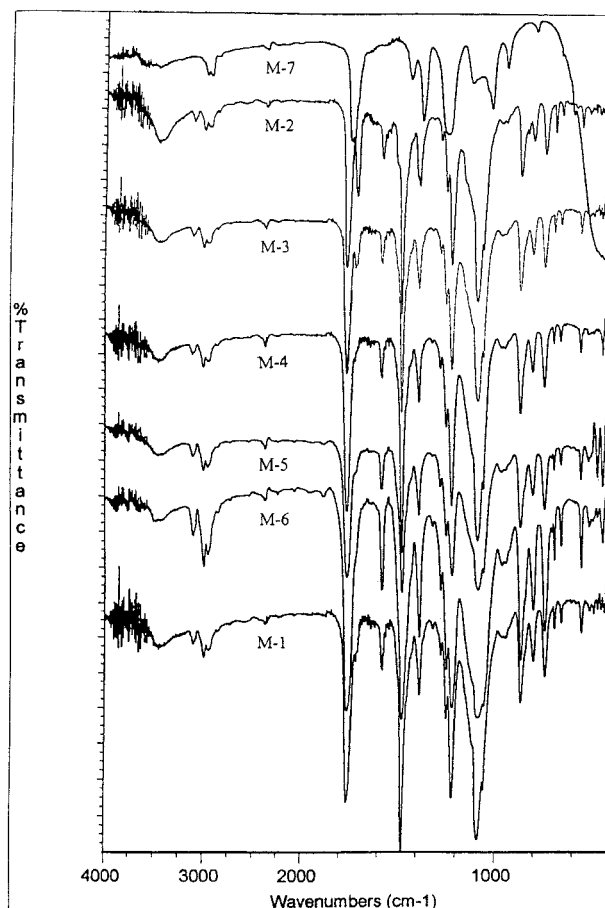


Figure 2 IR spectra of poly(2,4-DMA), poly(VAc), and their copolymers.

used to determine the viscosity of the polymer samples. Thermogravimetric analysis (TGA) was performed with a Du Pont 951 thermal analyzer at a heating rate of 10°C/pmin in static air atmosphere. Differential thermal analysis (DTA) was performed with DuPont-9900-differential thermal analyzer at a heating rate of 10°C/pmin in a nitrogen atmosphere.

Antimicrobial activity

The synthesized homo- and copolymers were tested against different microorganisms that are commonly employed for biodegradability tests. Bacterial strains (*B. subtilis*, *E. coli*, and *S. citreus*), fungi (*A. niger*, *S. pulverulentum*, and *T. lignorum*), and yeast (*C. utilis*, *S. cerevisiae*, and *P. stipitis*) were grown in nutrient broth (N-broth) and Subourand's dextrose broth medium.

Screening of acrylic copolymers for antibacterial activity:

A 5%, (v/v) inoculum of bacterial culture was used to inoculate 100 mL of a solution of N-broth (control) and test media (100 mL of a solution of N-broth + 50 mg

TABLE I
Copolymer Compositions and Reactivity Ratios of 2,4-DMA and VAc

Sample code no.	Monomer feed composition		Conversion (%)	Composition of 2,4-DMA in copolymer [m_1]	Reactivity ratio	
	2,4-DMA [M_1] (mol)	VAc [M_2] (mol)			r_1	r_2
M-1	1.00	—	—	—		
M-2	0.20	0.80	8.58	0.215		
M-3	0.40	0.60	8.20	0.428		
M-4	0.50	0.50	9.13	0.521	0.96	0.20
M-5	0.60	0.40	9.88	0.615		
M-6	0.80	0.20	8.56	0.830		
M-7	—	1.00	—	—		

of polymer sample). The resultant solution incubated on a rotary shaker (200 rpm) at room temperature, and 0.5-mL liquid samples were withdrawn at specified time intervals (24–48 h). After suitable dilution with (DNS) reagent and distilled water, the optical density was measured at 660 nm and calculated as optical density per milliliter (i.e., growth). This method is based on the principle that as the growth proceeds, the cell number increases, which leads to an increase in the optical density of the medium.

Screening of acrylic copolymers for antifungal activity

Fungal cultures show filamentous growth, so an optical method cannot be used to monitor its growth. Gravimetric analysis (GA) was used to determine the dry cell mass. A 10% (v/v) inoculum was added to the sterile control medium (without polymer) and test medium (100 mL control + 50 mg of polymer sample) in flasks that were then incubated at room temperature on a rotary shaker (200 rpm) for 48 hs. The contents of the flasks were filtered with cheese cloth, and the cell pellets were dried to a constant weight. The pH of and remaining sugar in the supernatant were checked. Sugar analysis was carried out with DNS reagent.¹⁰ A suitable amount of diluted supernatant was made to 1.0 mL and then 1.0 mL of DNS was added. This mixture was boiled for 10 min and then cooled. The final volume was made to 12.0 mL by

adding distilled water, and the optical density was recorded at 540 nm with a spectrophotometer.

Screening of acrylic copolymers on yeast

A 5% (v/v) inoculum of yeast culture was added to the sterile control medium and test medium (100 mL control + 50 mg of polymer sample), and the same procedure as described for determination of antibacterial activity was followed.

RESULTS

The copolymerization of 2,4-DMA with VAc in DMF solution was studied in a wide composition range, with mole fractions of 2,4-DMA ranging from 0.2 to 0.8 in the feed. The reaction time was selected to give a conversion of <10 wt % to satisfy the differential copolymerization equation.

The IR spectra of all the resins show the C—H stretching frequency of the aromatic moiety at ~ 3000 cm^{-1} . The C—H stretching vibrations of the aliphatic group appear in the range 3000 – 2900 cm^{-1} . The bending modes of the CH_3 group are attributed to absorption at ~ 1470 and ~ 1390 cm^{-1} (δ CH_3) respectively. The bending of the CH_2 group also appeared at ~ 1440 cm^{-1} . The strong absorption at 1740 cm^{-1} may be mainly due to the C=O stretching mode, whereas a large broad band at 1230 cm^{-1} may be due to the

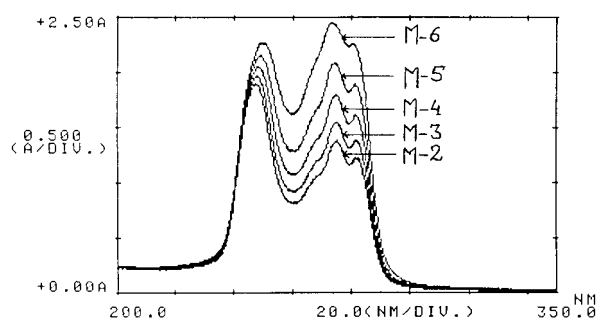


Figure 3 UV spectra of copolymers of 2,4-DMA and VAc.

TABLE II
Average Molecular Weight and Intrinsic Viscosity Values for Poly(2,4-DMA), Poly(VAc), and their Copolymers

Sample code no.	\bar{M}_n	\bar{M}_w	Polydispersity index	Intrinsic viscosity [η] ($\text{dL}\cdot\text{g}^{-1}$)
M-1	7974	19800	2.48	0.028
M-2	7892	19980	2.53	0.025
M-3	7420	18523	2.50	0.023
M-4	7085	16350	2.31	0.022
M-5	6815	15435	2.26	0.020
M-6	6788	15083	2.21	0.017
M-7	7910	19972	2.52	0.026

TABLE III
TGA Data for Poly(2,4-DMA), Poly(VAc), and their Copolymers

Sample code no.	% Weight loss at various temperatures (°C)				Decomposition temperature range (°C)	T_{\max} (°C) ^a	IPDT (°C) ^b	Activation energy (E_A) (KJ·mol ⁻¹) ^c
	250	350	450	550				
M-1	1.7	35.7	89.9	—	330–495	354	358	138
M-2	4.5	72.0	91.7	—	210–435	328	330	132
M-3	3.4	59.9	99.8	—	205–450	338	345	125
M-4	5.2	68.0	89.9	99.0	225–525	332	338	129
M-5	3.8	67.1	90.8	—	220–521	335	340	125
M-6	4.4	62.2	93.3	—	217–510	338	341	119
M-7	4.5	69.0	82.5	99.8	210–525	327	328	136

^a Temperature for maximum rate of decomposition.

^b Integral procedural decomposition temperature.

^c Determined by Broido's method.

C—O—C stretching mode characteristic of an acetate group. The prominent sharp band at 670 cm⁻¹ is attributed to C—Cl.¹¹ The IR spectra lend support to the structure of the resins prepared (Figure 2).

Copolymer compositions and reactivity ratios

The synthesis reaction of copolymers is shown in Figure 1. The average composition of each copolymer sample was determined from the corresponding UV spectrum. Assignment of the absorption in the UV spectrum allows for the accurate evaluation of the content of each kind of monomeric unit incorporated into the copolymer chains. From monomer feed ratios and copolymer compositions, the reactivity ratios of 2,4-DMA and VAc were determined by the Fineman–Ross (*F-R*) method.¹² The copolymer compositions and reactivity ratios are presented in Table I. The UV spectra of copolymers of 2,4-DMA and VAc with different feed ratios are shown in Figure 3.

Molecular weights and viscosity measurements

The number and weight-average molecular weights of poly(2,4-DMA), poly(VAc), and five samples of copolymer were obtained by GPC. The polydispersity index of homo- and copolymers varies in the range 2.21–2.53. The intrinsic viscosities (η) were obtained by extrapolating $\eta_{sp} \cdot c^{-1}$ to zero concentration. The molecular weights and viscosities are presented in Table II.

Thermal analysis

Thermogravimetric analysis (TGA)

TGA data for homo- and copolymers, presented in Table III, clearly indicate that all polymers undergo single-step decomposition. Activation energy (E_A) and integral procedural decomposition temperature (IPDT) were determined by the method of Broido and

Doyle.^{13,14} Thermal studies show that the polymers undergo decomposition in the range 210–525°C.

Differential thermal analysis (DTA)

DTA results for homo- and copolymers are presented in Table IV. The activation energy for thermal degradation and reaction order were determined by Reich's method.¹⁵ The activation energy for thermal degradation of polymers ranges from 115 to 140 KJ·mol⁻¹.

Antimicrobial activity

The antimicrobial activity of poly(2,4-DMA), poly(VAc), and poly(2,4-DMA-*co*-VAc) on bacteria, fungi, and yeast are shown in Figures 4, 5, and 6, respectively. In the case of antibacterial activity, poly(2,4-DMA) shows the lowest growth (20%) and the other copolymers exhibit 20–38% growth. In the case of antifungal and anti-yeast activities, poly(2,4-DMA) shows 18% growth, and the other copolymers show 18–38% growth.

TABLE IV
DTA Data for Poly(2,4-DMA), Poly(VAc), and their copolymers

Sample code no.	T_1 (°C) ^a	T_2 (°C) ^b	T_p (°C) ^c	Activation energy (E_A) (KJ·mol ⁻¹) ^d	Reaction order
M-1	338	494	402	140	1
M-2	320	496	405	132	1
M-3	321	470	400	130	1
M-4	315	503	427	128	1
M-5	312	520	421	115	1
M-6	310	524	417	121	1
M-7	330	485	385	130	1

^a Starting temperature of DTA trace.

^b Ending temperature of DTA trace.

^c Peak maxima temperature of DTA trace.

^d Activation energy by Reich's method.

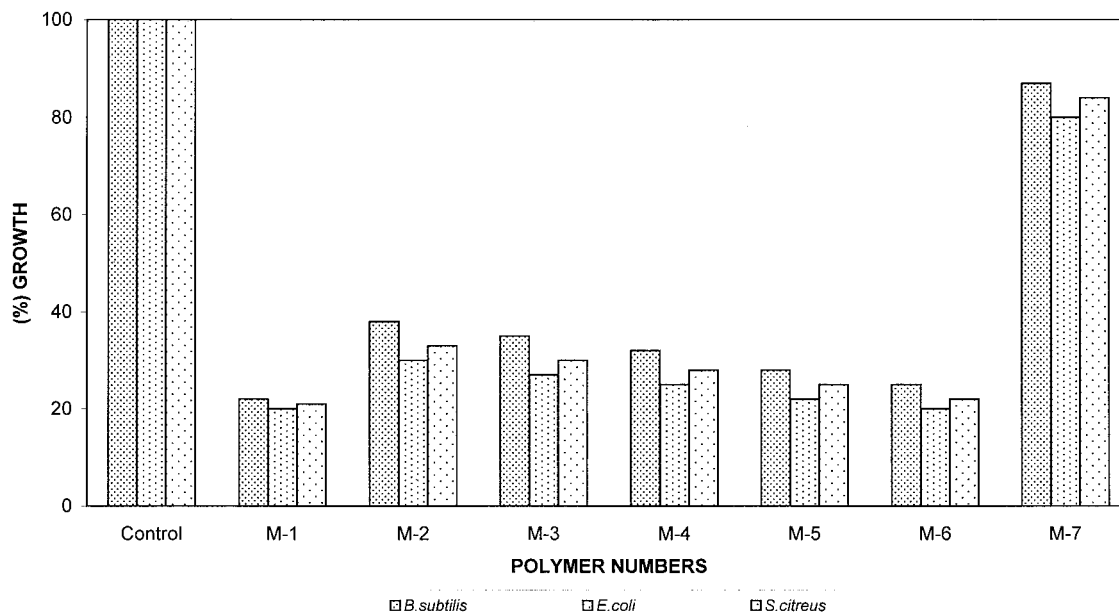


Figure 4 Effect of homo- and copolymers on the percent growth of bacteria.

DISCUSSION

The reactivity ratio values for 2,4-DMA (r_1) and VAc (r_2) from the $F-R$ plot are 0.96 and 0.20, respectively. When the r_1 and r_2 values are <1 , the system gives rise to azeotropic polymerization at a particular composition of the monomers, which is calculated with the equation,

$$N_1 = \frac{(1 - r_2)}{(2 - r_1 - r_2)} = 0.952,$$

where N_1 is the mole fraction of monomer 2,4-DMA in the feed. When the mole fraction of the monomer

2,4-DMA in the feed is 0.952, the copolymer formed will have the same composition as that of the feed. When the mole fraction of the feed is <0.952 with respect to 2,4-DMA, the copolymer is relatively richer in this monomer unit than in the feed. When the mole fraction of the monomer 2,4-DMA in the feed is >0.952 , the copolymer is relatively richer in the VAc unit than in the feed.

The GPC results show that the number average molecular weight (\bar{M}_n) ranges from 6700 to 7900, and weight average molecular (\bar{M}_w) weight varies from 15,000 to 19,800. These results also indicate that with

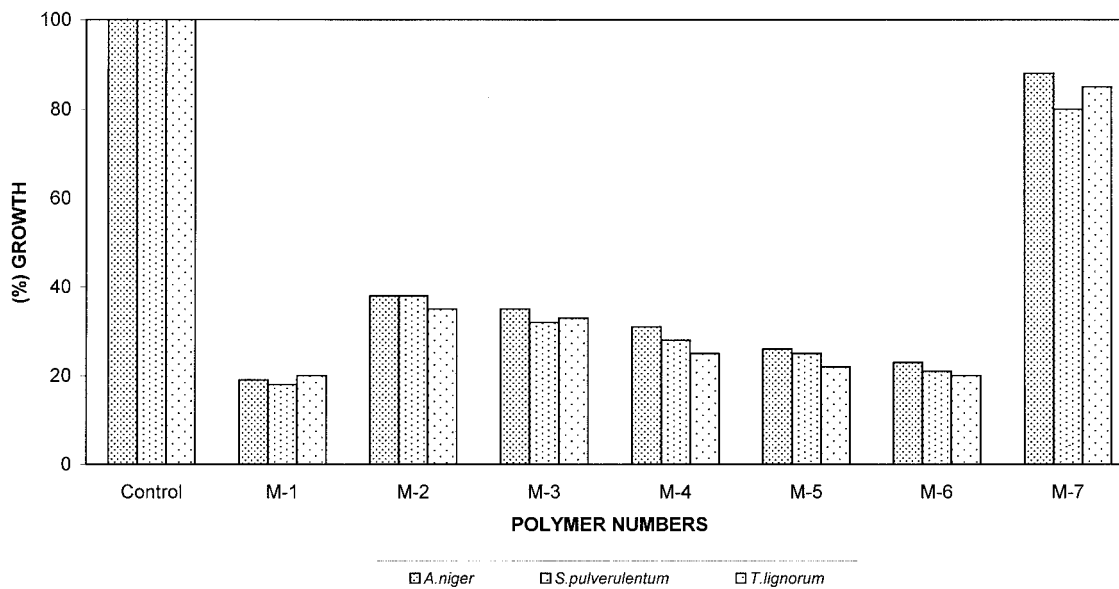


Figure 5 Effect of homo- and copolymers on the percent growth of fungi.

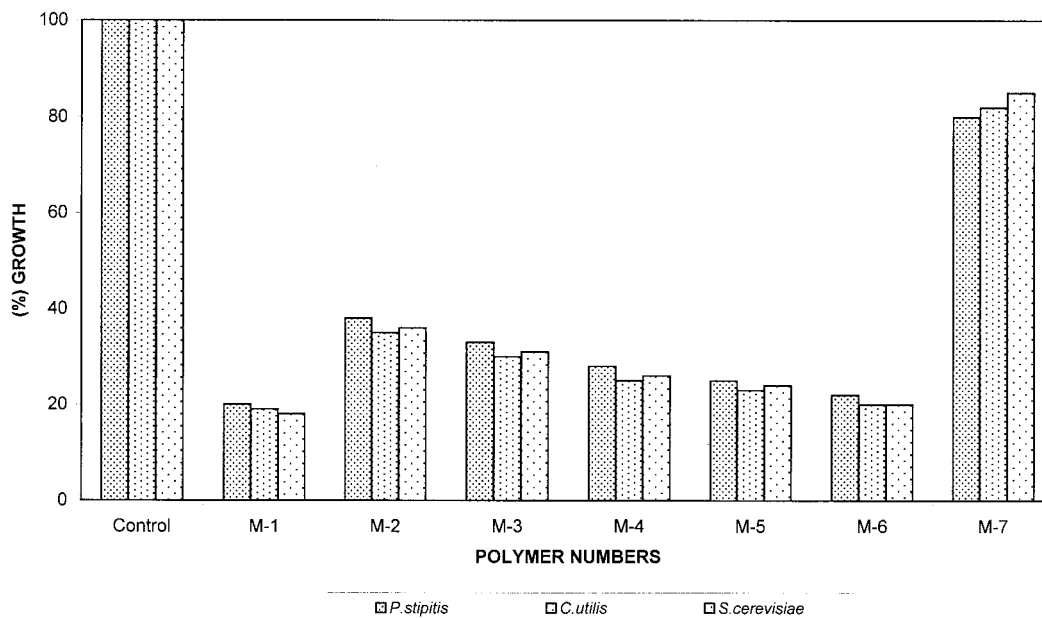


Figure 6 Effect of homo- and copolymers on the percent growth of yeast.

an increase in the 2,4-DMA content in the copolymers, the average molecular weights and viscosity decrease to a considerable extent, whereas the polydispersity index changes randomly.

Examination of the TGA data suggests that the homo- and copolymers follow a single-step degradation and possess moderate thermal stability. Also, incorporation of 2,4-DMA in certain polymers resulted in a slight increase in their thermal stability with respect to their individual homopolymers.

All copolymer systems impart almost similar antimicrobial activity against bacteria, fungi, and yeast. Polymers prepared with 2,4-DMA show strong growth inhibitory effects, towards the microorganisms tested. As the 2,4-DMA content increases in copolymers the growth of microorganisms is inhibited accordingly.

CONCLUSION

Copolymers of 2,4-DMA with VAc can significantly inhibit the growth of microorganisms within 48 h. It is

hoped that such copolymers will find use as antimicrobial agents.

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