Synthesis and Antitumor Activity of Poly(3,4-dihydro-2H-pyran-*co*-maleic anhydride-*co*-vinyl acetate)

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ABSTRACT: The radical-initiated terpolymerization of 3,4-dihydro-2H-pyran (DHP), maleic anhydride (MA), and vinyl acetate (VA), which were used as a donor–acceptor–donor system, was carried out in methyl ethyl ketone in the presence of 2,2′-azobisisobutyronitrile as an initiator at 65°C in a nitrogen atmosphere. The synthesis and characterization of binary and ternary copolymers, some kinetic parameters of terpolymerization, the terpolymer-composition/thermal-behavior relationship, and the antitumor activity of the synthesized polymers were examined. The polymerization of the DHP–MA–VA monomer system predominantly proceeded by the alternating terpolymerization mechanism. The *in vitro* cytotoxicities of poly(3,4-dihydro-2H-pyran-*alt*-maleic anhydride) [poly(DHP-*alt*-MA)] and poly(3,4-dihydro-2H-pyran-*co*-maleic anhydride-*co*-vinyl acetate) [poly(DHP-*co*-MA-*co*-VA)] were evaluated with Raji cells (human Burkitt lymphoma cell line). The antitumor activity of the prepared anion-active poly(DHP-*alt*-MA) and poly(DHP-*co*-MA-*co*-VA) polymers were studied with methyl–thiazol–tetrazolium testing, and the 50% cytotoxic dose was calculated. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 96: 2352–2359, 2005

Key words: hydrophilic polymers; copolymerization; water-soluble polymers; antitumor activity FT-IR

INTRODUCTION

Water-soluble anhydride-containing copolymers, used as polyanions, and their functional derivatives have high biological and physiological activity and, in particular, antimicrobial and antitumor properties.[1,2] Polyanions are also known for their potential to stimulate the immune system and invoke activity against tumors, viruses, and bacteria.[2–5] Still another mode of their action has been reported to induce interferon.[6] Copolymers of dihydropyran and its derivatives with acrylic acid, which contain tetrahydropyran rings and free carboxylic groups on the polymer backbone, and an alternating cyclocopolymer of divinyl ether (acyclic analogue of dihydropyran) with maleic anhydride (MA) have exhibited antitumor activities *in vitro*. [1–7]

3,4-Dihydro-2H-pyran (DHP) and its derivatives easily undergo complex-radical alternating copolymerization with MA.[3–10] The low molecular weight of the synthesized copolymers [number-average molecular weight (M_n) = 1000–7500] can be explained by degradative chain transfer to the pyran monomer in the alternating copolymerization.[9,10] Poly(3,4-dihydro-2H-pyran-*alt*-maleic anhydride) [poly(DHP-*alt*-MA)] and its derivatives, which consist of different substituents (e.g., acetoxy, alkoxy, methoxycarbonyl, formyl, and tosyloxymethyl groups) in the 2-position of the tetrahydropyran ring of the copolymer backbone, have higher antitumor activity against selective tumor cells *in vitro* and *in vivo*.[10] Hydroscopic and water-soluble copolymers of dihydopyran and its 2-carboxylate derivative with acrylic acid and acrylamide, synthesized by radical copolymerization, also have antitumor activity (against Lewis Lond carcinomas, carcinomas, malignant tumors, and melanomas).[6] An alternating copolymer of DHP and fumaronitrile has been synthesized by radical-initiated copolymerization in methyl ethyl ketone (MEK) at 70°C under a nitrogen atmosphere.[11] A pyran-containing alternating copolymer (M_n = 22,000, softening point = 183–190°C) has also been synthesized by the cationic copolymerization of 2,3-dihydropyran and 2-thiophenealdehyde in the presence of BF_3OEt_2 at −78°C in dichloroethane.[12] IR and NMR spectroscopy for this copolymer suggest that the copolymerization proceeds simply through the vinylene (dihydropyran) and aldehyde groups. Thus, it can be assumed that the synthesis of new functional alternating copolymers of dihydropyran with a high-density carboxylic acid functionality along the macromolecular chain is important in the fields of polymer science and macromolecular engineering.

In this study, the results of the radical-initiated terpolymerization of DHP, MA, and vinyl acetate (VA), which were used as a donor–acceptor–donor system, some kinetic parameters, the terpolymer-composition/thermal-behavior relationship, and the antitumor activity of

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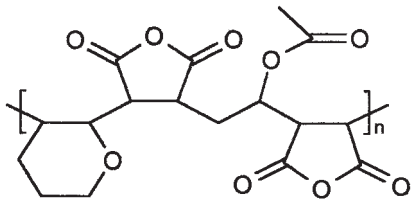
the synthesized terpolymer with an alternating structure were examined. The *in vitro* cytotoxicities of poly(maleic anhydride-*co*-vinyl acetate-*co*-3,4-dihydro-2H-pyran) and poly(maleic anhydride-*co*-3,4-dihydro-2H-pyran) were evaluated with Raji cells (human Burkitt lymphoma cell line). The antitumor activities of the prepared anion-active copolymers were studied with methyl–thiazol–tetrazolium (MTT) testing, and the 50% cytotoxic dose (LD_{50}) was calculated for the copolymers.



Scheme 1

EXPERIMENTAL

Materials

DHP was supplied by Fluka (Buchs, Switzerland) and was purified before use by distillation (bp = 86.5°C, density (d_4^{20}) = 0.9280, refractive index (n_D^{20}) = 1.4410). ^{1}H-NMR ($CHCl_3$-d_1, ppm): 6.40 (1H, doublet, —OC**H**═), 4.71 (1H, multiplet, ═C**H**—CH_2—), 3.90 (2H, triplet, —OC**H**$_2$—), 1.51–2.29 ppm (4H, multiplet, —C**H**$_2$—C**H**$_2$—). Fourier transform infrared (FTIR; cm^{-1}): 3083 (CH═), 2959–2877 (CH_2), 1659 (C═C), 1473 (CH_2), 1267–1081 (C—O), 895 (CH═), 730 (CH═, cis form).

MA (Aldrich) was purified before use by recrystallization from anhydrous benzene and by sublimation *in vacuo* (mp = 52.8°C). VA (Fluka) was purified by distillation (bp = 72.5°C, d_4^{20} = 0.9315, n_D^{20} = 1.3950). 2,2′-Azobisisobutyronitrile (AIBN; Fluka), used as an initiator, was twice recrystallized from a chloroform solution by methanol. Other reagents, including organic solvents, were purified by ordinary methods.

Synthesis of the copolymers

Copolymerization was carried out with AIBN ([AIBN] = 2.16×10^{-2} mol/L) as an initiator at 65°C in degassed tubes in MEK at different total monomer concentrations (1.5, 2.84, or 5.12 mol/L) under a nitrogen atmosphere. After the reaction occurred for a given time, the contents of the tubes were poured into a large amount of *n*-hexane to precipitate the copolymer, and the obtained powderlike product was separated by ultracentrifugation. It was then purified by multiple washings in *n*-hexane and diethyl ether and was redeposited by filtration. Ternary and binary copolymers synthesized with this method under similar conditions were dried at 40°C to a constant weight and were characterized by a chemical method (the titration of anhydride units), FTIR spectroscopy, and thermal analysis [differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA)].

Under similar conditions, DHP–MA and MA–VA binary copolymers were prepared with an equimolar ratio of the comonomers. These binary copolymers had the following characteristics.

Poly(DHP-*alt*-MA)

Acid number: 605 mg of KOH/g. Intrinsic viscosity ($[\eta]_{in}$): 0.067 dL/g in MEK at 25 ± 0.1°C. Glass-transition temperature (T_g): 169.6°C (by DSC).

Poly(maleic anhydride-*alt*-vinyl acetate)

Acid number: 617 mg of KOH/g. $[\eta]_{in}$: 0.11 dL/g in MEK at 25 ± 0.1°C. T_g: 134°C (by DSC).

Poly(3,4-dihydro-2H-pyran-*co*-maleic anhydride-*co*-vinyl acetate) [poly(DHP-*co*-MA-*co*-VA)]

This was an alternating terpolymer (Scheme 1) synthesized with a 1:2:1 monomer ratio.

Acid number: 314 mg of KOH/g. $[\eta]_{in}$: 0.08 dL/g in MEK at 25 ± 0.1°C. T_g: 156.7°C (by DSC). FTIR (KBr pellet, cm^{-1}): 3500 (OH stretching in COOH) and 3200 (hydrogen bonding; for the partially hydrolyzed anhydride unit, 2980 (v_{as} CH in CH_3), 2950 (v_{as} CH in CH_2), 2875 (v_{as} CH in CH_3), 2650 (intramolecular hydrogen bonding), 1860 (v_{as} C═O) and 1780 (v_s C═O; for the anhydride unit), 1743 (C═O; for the ester unit), 1634 (C═O; for the shifted conjugated C═O stretching band), 1450 (scissor vibrations of CH_2), 1430 (δ_{as} CH_3 asymmetric deformation), 1360 (δ_{as} CH_3 of acetate), 1220–1180 (stretching of anhydride C—O—C), 1210–1200 (δ CH_3), 1255–1240 (δ CH_3 of acetate), 1130 (broad stretching of C—O in dihydropyran), 920 (CH_3 rocking in acetate), 620–595 (δ CH in CH—CH anhydride units). (For the FTIR spectrum in film, see Fig. 1.)

Measurements

Kinetic studies of the terpolymerization were performed dilatometrically in the steady state (conversion ≤ 10%). The values of the initial copolymerization rate (R_p) were calculated as follows:

$$R_p = (\Delta V \times 10^3)/(V\delta_M \Delta t M) \quad (1) \qquad (1)$$

where $\Delta V = \pi r^2 \Delta h$ (where r = 0.4 cm is the radius of the capillary in the dilatometer and Δh is the change in the level in the dilatometer at time Δt) is the volume of the reaction mass at time Δt (s), $\delta_M = (\rho_m)^{-1} - (\rho_p)^{-1}$ (where ρ_m and ρ_p are the densities of the monomer mixture and copolymer at a given reaction temperature) is the con-

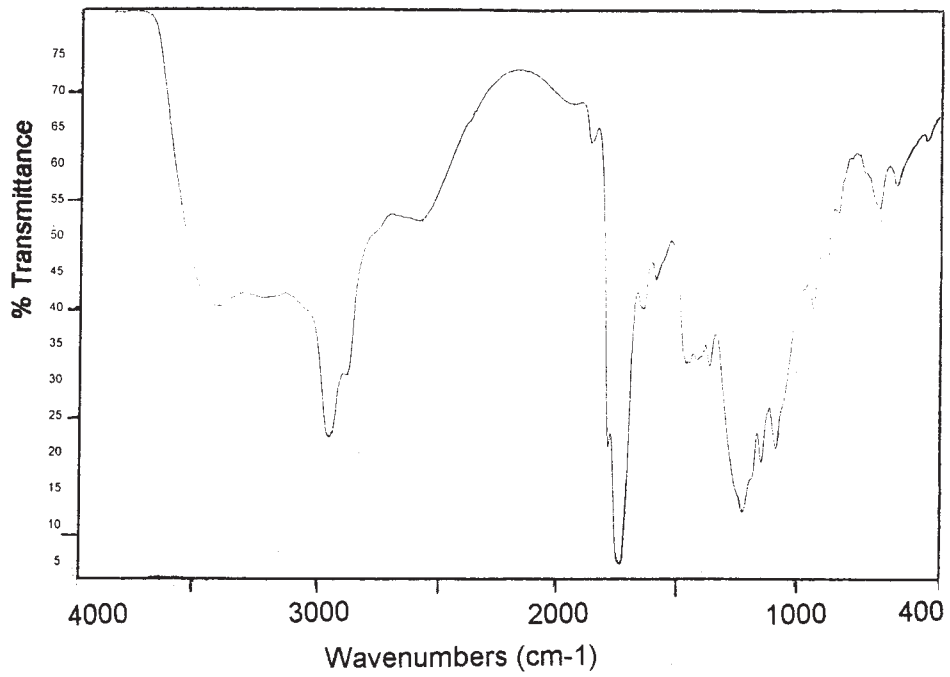


Figure 1 FTIR spectrum of partially hydrolyzed poly(DHP-*co*-MA-*co*-VA).

stant of contraction in the copolymerization, M is the total molecular mass of the monomers (g/mol), and V is the volume of the initial monomer mixture (mL). For the ternary monomer system studied, δ_m was 0.247.

FTIR spectra of the copolymer films and thin coatings on KBr pellets were recorded with a Nicolet FTIR 510 spectrometer (Madison, WI) in the 4000–400-cm^{-1} range; 30 scans were taken at a 4-cm^{-1} resolution. For the composition analysis of the terpolymers, specifically the concentrations of the DHP and VA units, characteristic absorption bands at 1130 (for the DHP units) and 1360 cm^{-1} (for the VA units) were used as analytical bands. The least changing absorption band of 1130 cm^{-1} was used as a standard band [$A = \log(I_0/I)$, $\Delta A^i = A^i/A^{1130}$] to calculate the terpolymer compositions.

The copolymers synthesized were characterized by FTIR spectroscopy for the determination of VA and DHP unit contents. The absorption value ratios between characteristic analytical bands of 1360 cm^{-1} (for VA unit), 1130 cm^{-1} (for DHP) and 1630 cm^{-1} (for MA-maleic acid units) the least changing absorbtion band of 1130 cm^{-1} as a standard band ($A = \log(I_0/I)$, $\Delta A^i = A^i/A^{1130}$) were used to calculate the polymer compositions.

Molar fractions (in mol %) of comonomer units (m_1, m_2, and m_3) in $VA(M_1)$—$MA(M_2)$—DHP (M_3) terpolymers using FTIR analysis data are calculated according to the following equations:

$$m_1 = \frac{\Delta A^{1360}/M_1}{\Delta A^{1360}/M_1 + \Delta A^{1630}/M_2 + \Delta A^{1130}/M_3} \cdot 100$$

$$m_2 = \frac{\Delta A^{1630}/M_2}{\Delta A^{1360}/M_1 + \Delta A^{1630}/M_2 + \Delta A^{1130}/M_3} \cdot 100$$

$$m_3 = \frac{\Delta A^{1130}/M_3}{\Delta A^{1360}/M_1 + \Delta A^{1630}/M_2 + \Delta A^{1130}/M_3} \cdot 100$$

where $m_1/m_2 = [\Delta A^{1360}/M_1]/[\Delta A^{1630}/M_2]$, $m_1/m_3 = [\Delta A^{1360}/M_1]/[\Delta A^{1130}/M_3]$, $m_2/m_3 = [\Delta A^{1630}/M_2]/[\Delta A^{1130}/M_3]$, $\Delta A = A^i/A^{1130}$ (standard band), M_1, M_2, and M_3 are molecular weights of *VA*, *MA*, and DHP monomer units, respectively.

The acid numbers of the anhydride-containing copolymers and terpolymers were determined by a known nonaqueous titration method.[13]

The $[\eta]_{in}$ values of the synthesized copolymers and terpolymers were determined in MEK at 25 ± 0.1°C in the concentration range of 0.1–1.0 g/dL with an Ubbelohde viscometer.

DSC and TGA for the copolymers and terpolymers were carried out with a DuPont V4.1C 2000 and a DuPont TA 2000 (Boston, MA) in a nitrogen atmosphere at a heating rate of 5°C/min.

Cell culture

Raji cells (human Burkitt lymphoma cell line) were maintained in an RPMI 1640 culture medium (Sigma Chemical Co., St. Louis, MO) supplemented with 10% heat-inactivated fetal bovine serum (Biochrom KG, Berlin, Germany), 2 m*M* L-glutamine, 100 IU/mL penicillin (Sigma-Aldrich Co., Ltd., Irvine, Ayrshire, UK), and 100 μg/mL streptomycin at 37°C in a humidified incubator containing 5% CO_2.

TABLE 1
Test Concentrations Used in the MTT Assay

Copolymer	Test concentration (μg/mL)
DHP–MA	75–150–300–750–1500
DHP–MA–VA	60–120–240–480–600

Preparation of the copolymers for the cytotoxicity assay

Poly(DHP-*alt*-MA) (30 mg/mL) and poly(DHP-*co*-MA-*co*-VA) (600 mg/mL) stock solutions were prepared in distilled water and filtered (Millipore Ltd., Hertfordshire, UK; 0.45 μm) for sterilization. The stock solutions were kept at room temperature. The copolymers were diluted with a culture medium just before use. The test concentrations are given in Table I.

In vitro cytotoxicity assay

The *in vitro* cytotoxicities of poly(DHP-*alt*-MA) and poly(DHP-*co*-MA-*co*-VA) were determined by the MTT assay method.[14] Briefly, 2×10^4 cells per well were seeded in 96-well plates, and 50-μL sample solutions were added to each well. After 72 h of incubation, a 25-μL MTT solution (1 mg/mL final concentration) was added to each well, and the plates were incubated for 4 h more. The produced formazan was solubilized by the addition of 80 μL of a lysing buffer of 23% sodium dodecyl sulfate (SDS) (Sigma Chemical Co., St. Louis, MO) dissolved in a solution of 45% dimethylformamide (pH 4.7). After overnight incubation at 37°C, the optical density (OD) values at a wavelength of 540 nm were measured with a microplate reader (Spectramax Plus, Molecular Devices, Sunnyvale, CA). The cells incubated in the culture medium alone served as controls for cell viability (untreated wells). All assays were performed in quadruplicate, and mean values (plus or minus the standard deviation) were used to estimate the inhibition rate. LD_{50} was defined as the concentration of a sample that reduced the absorbance of the treated cells by 50%.

Statistical analysis

The LD_{50} values were estimated with nonlinear regression analysis [Graphpad, version 2.0, ISI(R) Software, Inc., San Diego, CA].

RESULTS AND DISCUSSION

Copolymerization

The studied ternary monomer system differs by the nature of the conjugation between the double bond and functional group, which contains two donor–acceptor monomer pairs, such as DHP (weak donor)–MA (strong acceptor), with a known equilibrium constant of charge-transfer-complex (CTC) formation (K_c = 0.11 L/mol), and MA–VA (donor), with K_c = 0.06 L/mol.[15] On the other hand, the two donor monomers are a noncopolymerized pair under the chosen conditions for the terpolymerization. The homopolymerization of DHP and MA can also be ignored under these conditions. However, the binary copolymerization of DHP–MA[9,15] and MA–VA[3,19] monomer pairs separately proceeds by a complex-radical alternating copolymerization mechanism. These distinctive structural peculiarities allow the prediction of the following chain-growth reactions, in which the monomers may show sufficient activity for radical-initiated terpolymerization. The chain growth via free and complexed monomers can be expressed as follows:

$$\sim\mathrm{DHP}^{\cdot} + \mathrm{MA} \xrightarrow{k_{12}} \sim\mathrm{MA}^{\cdot} \quad (2)$$

$$\sim\mathrm{MA}^{\cdot} + \mathrm{DHP} \xrightarrow{k_{21}} \sim\mathrm{DHP}^{\cdot} \quad (3)$$

$$\sim\mathrm{MA}^{\cdot} + \mathrm{VA} \xrightarrow{k_{23}} \sim\mathrm{VA}^{\cdot} \quad (4)$$

$$\sim\mathrm{VA}^{\cdot} + \mathrm{MA} \xrightarrow{k_{32}} \sim\mathrm{MA}^{\cdot} \quad (5)$$

$$\sim\mathrm{DHP}^{\cdot} + \mathrm{MA}\ldots\mathrm{DHP} \xrightarrow{k_{1c1}} \sim\mathrm{DHP} - \mathrm{MA}^{\cdot}\ldots\mathrm{DHP} \rightarrow \sim\mathrm{DHP}^{\cdot} \quad (6)$$

$$\sim\mathrm{DHP}^{\cdot} + \mathrm{MA}\ldots\mathrm{VA} \xrightarrow{k_{1c}}{}_2 \sim\mathrm{DHP} - \mathrm{MA}^{\cdot}\ldots\mathrm{VA} \rightarrow \sim\mathrm{VA}^{\cdot} \quad (7)$$

$$\sim\mathrm{MA}^{\cdot} + \mathrm{DHP}\ldots\mathrm{MA} \xrightarrow{k_{2c1}} \sim\mathrm{MA} - \mathrm{DHP}^{\cdot}\ldots\mathrm{MA} \rightarrow \sim\mathrm{MA}^{\cdot} \quad (8)$$

$$\sim\mathrm{MA}^{\cdot} + \mathrm{VA}\ldots\mathrm{MA} \xrightarrow{k_{2c2}} \sim\mathrm{MA} - \mathrm{VA}^{\cdot}\ldots\mathrm{MA} \rightarrow \sim\mathrm{MA}^{\cdot} \quad (9)$$

$$\sim\mathrm{VA}^{\cdot} + \mathrm{MA}\ldots\mathrm{DHP} \xrightarrow{k_{3c1}} \sim\mathrm{VA} - \mathrm{MA}^{\cdot}\ldots\mathrm{DHP} \rightarrow \sim\mathrm{DHP}^{\cdot} \quad (10)$$

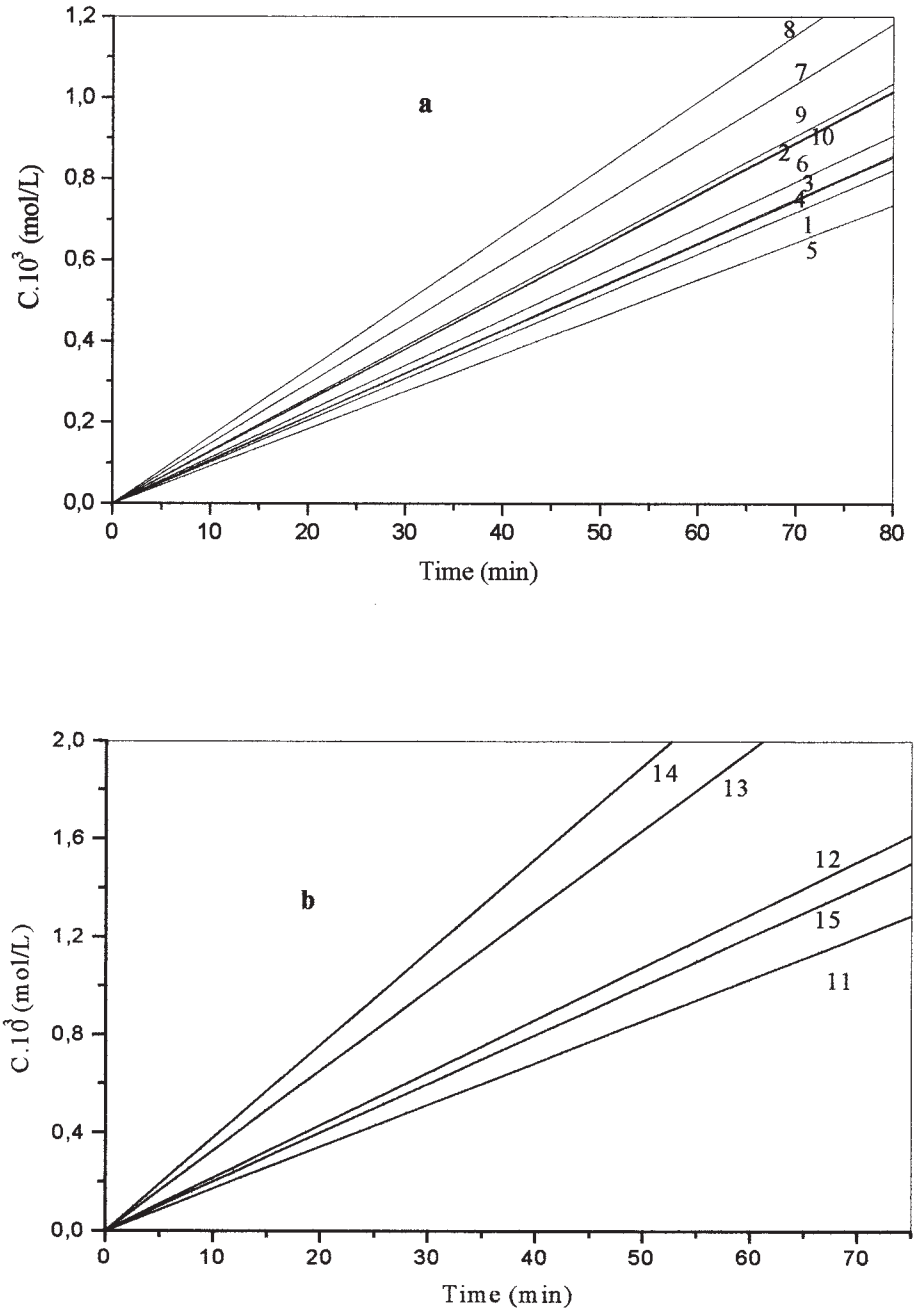


Figure 2 Conversion (C; mol/L) versus the reaction time (min) for the radical terpolymerization of DHP, MA, and VA in MEK at 65°C with various monomer ratios and total monomer concentrations. The monomer ratios were (1,6,11) DHP/VA = 35:35, (2,7,12) DHP/VA = 20:30, (3,8,13) DHP/VA = 25:25, (4,9,14) DHP/VA = 30:20, and (5,10,14) DHP/VA = 35:15. The total monomer concentrations were (1–5) 1.5, (6–10), 2.84, and (11–15) 5.12 mol/L. The MA concentration was constant (50 mol %).

$$\sim VA^{\cdot} + MA...VA_{c2} \xrightarrow{k_{3c2}} \sim VA - MA^{\cdot}...VA \rightarrow \sim VA^{\cdot} \quad (11)$$

As shown in these equations, because of the possible self-organization and selectivity of the reacted comonomers, the number of chain-growth reactions in the studied radical terpolymerization is limited to 10 reactions of the 19 theoretical reactions.

The results of a kinetic study of the terpolymerization are presented in Figure 2. As indicated by these data, a change in the initial monomer ratio and a dilution of the monomer mixture can have a significant influence on the terpolymerization rate in the steady state. These effects are well illustrated by plots of the initial rate of terpolymerization (R_p) versus the monomer feed with different total monomer concentrations (Fig. 3). The curves and the shift of the maximum R_p values with the dilution of the monomer mixture indicate that an alternating copolymerization has

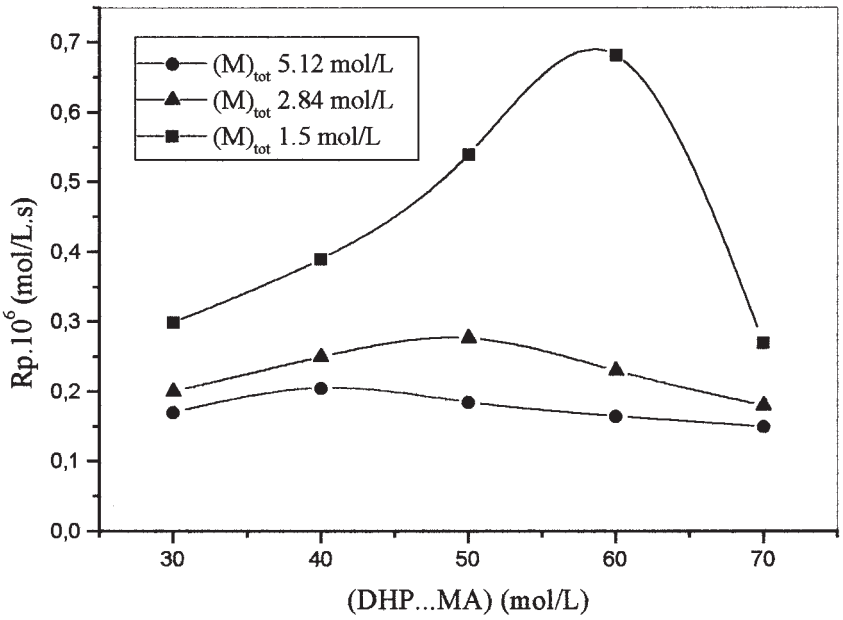
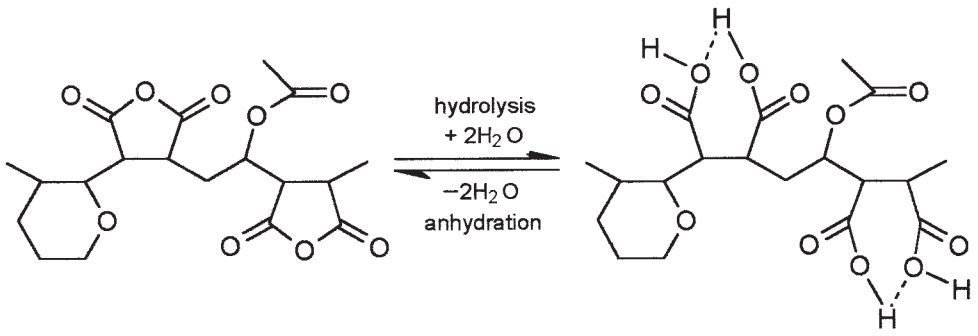


Figure 3 R_p versus the DHP, MA, and VA concentrations at total monomer concentrations of (■) 1.5, (▲) 2.84, and (●) 5.12 mol/L (for the reaction conditions, see Fig. 2).

been realized with the studied DHP–MA–VA ternary system, which proceeds by a mixed mechanism, that is, by the participation of both free and complexed monomers in the chain-growth reactions. A significant narrowing of the broad peak at the higher concentration of the monomers (total monomer concentration = 5.12 mol/L) may have resulted from the relatively high concentration of CTCs in the monomer mixture and from the increased probability of their participation in chain propagation.

Hydrolysis and thermal behavior

The synthesized binary and ternary copolymers can be easily dissolved in water. This solution process is accompanied by the full hydrolysis of the anhydride units and the formation of strong hydrogen-bonding fragments.[20] Maleic acid copolymers easily form intramolecular hydrogen bonds with different values of the dissociation constants for two carboxylic groups ($pK_1 > pK_2$).[3] The same opening of the anhydride ring also partially proceeds in the presence of atmospheric H_2O. The hydrolyzed copolymer again accepts an initial form by the anhydration of dicarboxyl units through a thermal treatment of its thin coating or film at 90–110°C for 20 min. Anhydration via dehydration does not excite crosslinking (intermolecular anhydration) and proceeds selectively because the copolymer has only intramolecular character; this is confirmed by its solubility and the identical structure for the initial terpolymers and copolymers. This reversible reaction of hydrolysis and anhydration can be presented as follows:



In the FTIR spectra of this copolymer, the characteristic bands for the anhydride units (1860 and 1780 cm^{-1}) disappear, 1945 and 1630-cm^{-1} bands related to carboxylic groups appear, and the intensity of the 3500-cm^{-1} band, related to acid units, significantly increases. DSC scans of the synthesized binary copolymers and terpolymers are shown in Figure 4. In the DSC curves of the copolymers, endothermic peaks for the MA–VA and

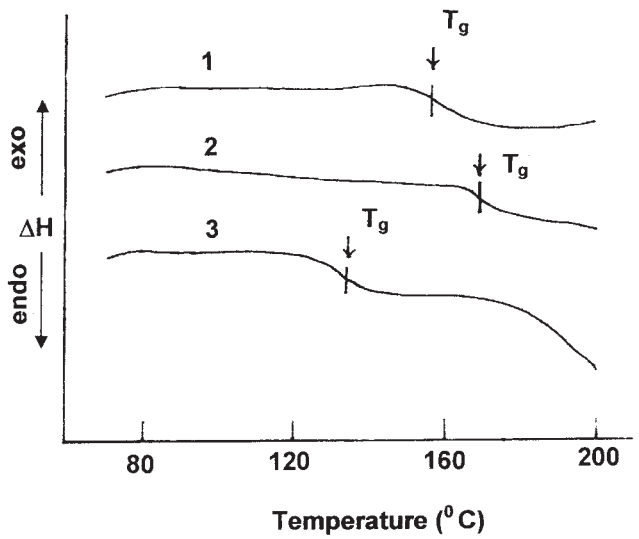


Figure 4 DSC thermograms of (1) the DHP–MA–VA terpolymer, (2) the DHP–MA copolymer, and (3) the MA–VA copolymer synthesized at a heating rate of 10°C/min under a nitrogen atmosphere.

DHP–MA binary copolymers and the DHP–MA–VA terpolymer appear in the fields of 134 [enthalpy (ΔH) = 0.0051 J/g], 169.6 (ΔH = 0.0014 J/g), and 156.9°C (ΔH = 0.038 J/g), respectively. These peaks are associated with T_g's of the copolymers with corresponding values of ΔH.[21] These copolymers were synthesized under similar conditions with 1:1 and 1:2:1 molar ratios of the monomers for the binary and ternary systems, respectively. The obtained T_g values indicate that the contribution of the monomer units and their fractions to the glass-transition process has a regular character; that is, the position of the T_g peak for poly(DHP-*co*-MA-*co*-VA) moves to a high temperature with respect to poly(maleic anhydride-*co*-vinyl acetate) and moves to a low temperature with respect to poly(DHP-*alt*-MA). The lower values of ΔH for the binary copolymers can be attributed to weaker interactions among the hard segments. However, a relatively high value of ΔH for the terpolymer can be explained by the presence of alternating –DHP–MA–VA–MA– segments in the macromolecular chain.

Antitumor activity

Anionic polymers such as polyphosphates and polycarboxylates of natural or synthetic origin have been shown to be potential inhibitors of transplanted tumors.[22–26] The *in vitro* cytotoxicities of the copolymers were determined by the MTT assay method.[14] The dose–response curve of poly(DHP-*alt*-MA) on Raji cells is presented in Figure 5. A dose up to 300 μg/mL of the DHP–MA copolymer was not cytotoxic in tumor cells. The LD_{50} value was calculated to be 444.676 μg/mL for this copolymer.

The dose–response curve of the terpolymer on Raji cells is presented in Figure 6. There was no cytotoxic effect at the concentrations tested. Poly(DHP-*alt*-MA) has cytotoxic potential in comparison with the poly(DHP-*co*-MA-*co*-VA) terpolymer. The cytotoxicity of the synthesized copolymer is superior to that of the terpolymer. This means that the synthesized polyanionic polymers have improved cytotoxicity against a normal cell line. Previously, we observed a similar effect for binary and ternary copolymers of an MA/VA/acrylic acid monomer system.[26,27] It was shown that the VA linkages in the terpolymer imparted immobility to the polymer chains and low polyanionic character.[27] The hydrolyzed copolymer has sufficiently high antitumor activity,

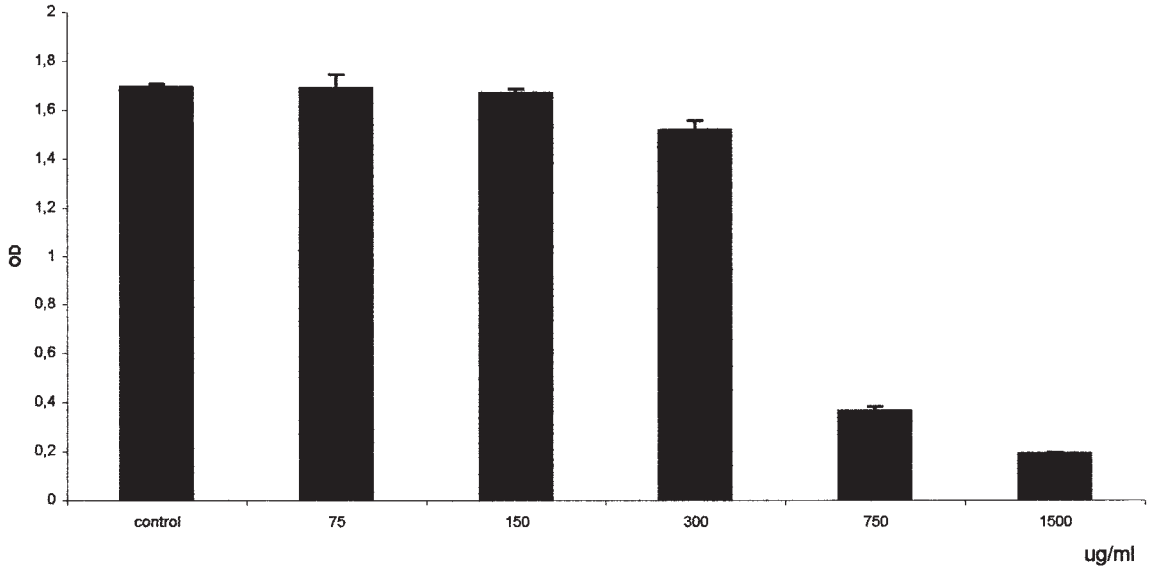


Figure 5 Cytotoxicity of poly(DHP-*alt*-MA) on Raji cells. The OD values are mean values (plus or minus the standard deviation) of four wells.

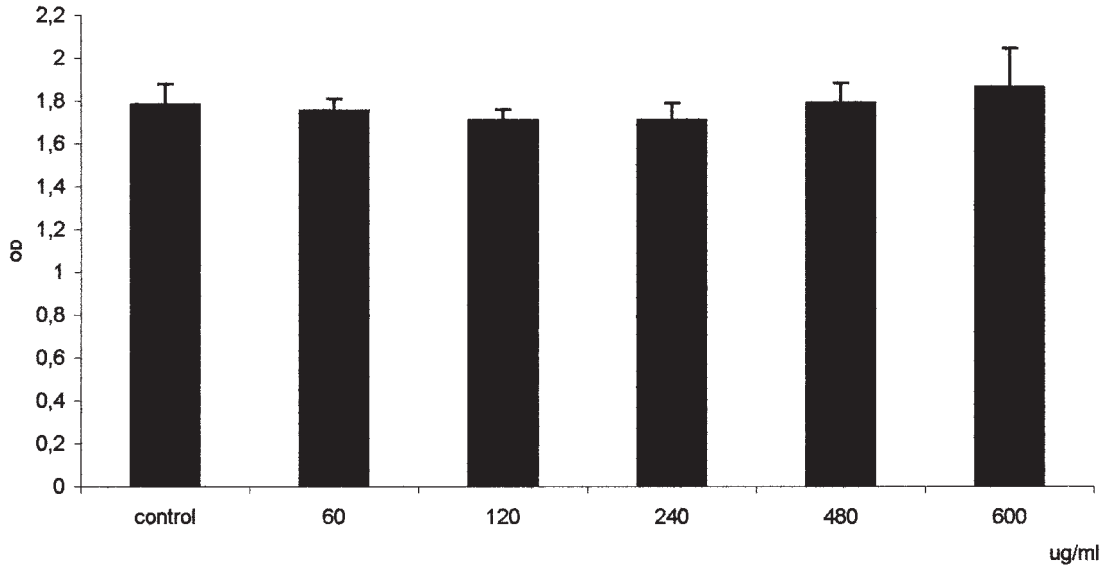


Figure 6 Cytotoxicity of poly(DHP-*co*-MA-*co*-VA) on Raji cells. The OD values are mean values (plus or minus the standard deviation) of four wells.

which depends on the amount of hydrogen-bonding carboxylic groups and their regular distribution in the side chains of functional macromolecules.

CONCLUSIONS

The synthesis, characterization, and antitumor activity of pyran–anhydride-containing binary and ternary copolymers were examined. Some kinetic peculiarities of the complex-radical terpolymerization and the terpolymer-composition/thermal-behavior relationships of the synthesized polymers with an alternating structure were also studied. The *in vitro* cytotoxicities of the synthesized poly(DHP-*alt*-MA) and poly(DHP-*co*-MA-*co*-VA) polymers were evaluated with Raji cells (human Burkitt lymphoma cell line). The antitumor activity of the prepared anion-active poly(DHP-*alt*-MA) and poly(DHP-*co*-MA-*co*-VA) polymers were studied by the MTT testing method with calorimetric assay measurements. Doses up to 300 μg/mL of DHP–MA were not cytotoxic in tumor cells. LD_{50} was calculated to be 444.676 μg/mL for DHP–MA. There was no cytotoxic effect with poly(DHP-*co*-MA-*co*-VA) at the concentrations tested.

This study was carried out in accordance with the "New Functional Polymers in Oncology" program of the Chemistry and Chemical Engineering Departments and Oncology Institute of Hacettepe University.

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