Synthesis, Characterization, and Antitumor Activity of Poly(maleic anhydride-*co*-vinyl acetate-*co*-acrylic acid)

Hatice Kaplan Can,[1] A. Lale Doğan,[2] Zakir M. O. Rzaev,[3] Ayşegül Hasegeli Uner,[2] Ali Güner[1]

[1]*Department of Chemistry, Faculty of Science, Hacettepe University, Beytepe, 06532 Ankara, Turkey*
[2]*Department of Basic Oncology, Institute of Oncology, Hacettepe University, 06100 Ankara, Turkey*
[3]*Department of Chemical Engineering, Faculty of Engineering, Hacettepe University, Beytepe 06532 Ankara, Turkey*

Received 13 February 2004; accepted 15 May 2004
DOI 10.1002/app.21834
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: The ternary copolymerization of maleic anhydride (MA), vinyl acetate (VA), and acrylic acid (AA) [P(MA-*co*-VA-*co*-AA)], which is considered to be an acceptor–donor–acceptor system, was carried out in 1,4-dioxane with benzoyl peroxide as an initiator at 70°C under a nitrogen atmosphere. Constants of complex formation for the monomer systems in the study were determined by UV–visible (hydrogen-bonding complex) and ^{1}H-NMR (charge transfer complex) methods, respectively. The results show that polymerization of the P(MA-*co*-VA-*co*-AA) system proceeds by an alternating terpolymerization mechanism. It is shown that the synthesized copolymers have typical polyelectrolyte behavior, ability for reversible hydrolysis–anhydrization reactions, and semicrystalline structures. In these cases, including radical polymerization, and formation of semicrystalline structures, the hydrogen-bonding effect plays a significant role. The in vitro cytotoxicities of the synthesized terpolymer and alternating copolymer were evaluated using Raji cells (human Burkitt lymphoma cell line). The antitumor activities of prepared anion-active copolymers were studied using methyl–thiazol–tetrazolium colorimetric assay and 50% of the cytotoxic dose of each copolymer and terpolymer were calculated. Hydrolyzed P(MA-*co*-VA-*co*-AA) and P(MA-*alt*-AA) copolymers have sufficiently high antitumor activity, which depends on the amount of hydrogen-bonding carboxylic groups and their regular distribution in the side chain of functional macromolecules. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 100: 3425–3432, 2006

Key words: terpolymerization; vinyl acetate; maleic anhydride; acrylic acid; hydrogen bonding; thermal behavior; in vitro cytotoxicity; antitumor activity

INTRODUCTION

Synthetic polymers, water soluble or in the form of hydrogels, nanoparticles, dendrimers, or microspheres, are materials with which we are in daily contact or that are under development as materials for medical applications. At the end of the last century, synthetic polymers successfully replaced a number of natural materials, either because the latter were in short supply or because the physicochemical characteristics of synthetic polymers exceeded those of materials available from natural sources. Many functional polymers used as bioengineering polymer systems have found applications in tissue reconstruction and as surgical materials. Moreover, they have great potential to be used in tissue engineering as artificial organs and prostheses, in bone bonding and repairing, in the immobilization of cells and enzymes, as biosensors, and as different delivery systems for gene therapy and drug delivery.[1] In addition, the majority of these polymers show a multitude of biological activities in their own right (e.g., antitumor, antibiotic, antiviral, and antithrombotic activities, as well as inhibition of efflux pumps such as P-glycoprotein).[1–4] Despite their many clinical benefits, anticancer drugs often cause significant side effects because of their overall cytotoxicity and their lack of tissue specificity.[5] One approach to overcome this limitation is to make use of macromolecular derivatives.[6,7] In principle, these tissues can be addressed by conjugating the parent drugs to polymers, thereby enhancing both their tissue specificity and effective concentration at tumor sites. In fact, in contrast to low molecular weight anticancer drugs, polymer-based therapeutics have been found to accumulate more in tumor tissue because of an enhanced permeability and retention effect at these sites.[8] Naturally occurring polyanionic polymers are known to possess innate physiological properties. These natural macromolecules include polysaccharides, glycoproteins, and polynucleotides. The polyanionic functionality of these macromolecules is produced by sulfonate, phosphate, and carboxylate groups.[3]

Correspondence to: H. Kaplan Can (hkaplan@hacettepe.edu.tr or hkaplancan@yahoo.com).

Contract grant sponsor: Hacettepe University Research Foundation; contract grant number: 00 02 601 008.

Synthetic polycarboxylic acid polymers were found to produce a broad spectrum of immunological effects. They induce interferon; modify the reticuloendothedial system; and display immunoadvujant, antiviral, and antitumor activity.[9] The synthetic carboxylic acid polymers were first investigated as poly(acrylic acid), poly(methacrylic acid), poly(ethylene-*co*-maleic anhydride), and oxidized polysaccharides.[10] Among these polymers, the hydrolyzed form of divinyl ether maleic anhydride copolymer (DIVEMA, also called pyran copolymer), which contains carboxylic acid groups, exhibits high antitumor activity together with toxic side effects.[11]

The aim of the study was to obtain new biologically active polymers using anhydride-containing monomers. Polymers containing anhydride groups are expected to show considerably high biological activities because the anionic character of the polymers formed after their hydrolysis is similar to that of DIVEMA. In this study, poly(MA-*co*-vinyl acetate-*co*-acrylic acid) [P(MA-*co*-VA-*co*-AA)] and P(MA-*co*-AA) were prepared by the ternary and binary copolymerization of the corresponding monomers. The structural properties of these polymers were identified by Fourier transform IR (FTIR), differential scanning calorimetry (DSC), and thermogravimetric analyses (TGA). Monomer reactivity ratios and the equilibrium of complex formation for the monomer systems in the study were determined by UV–visible (UV–vis, H complex) and ^{1}H-NMR ([charge transfer complex (CTC)] methods, respectively. The in vitro cytotoxicities of P(MA-*co*-VA-*co*-AA) and P(MA-*co*-AA) were evaluated using Raji cells (human Burkitt lymphoma cell line). The antitumor activities of prepared anion-active copolymers were studied by 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay and the 50% of cytotoxic doses (LD_{50}) were calculated.

EXPERIMENTAL

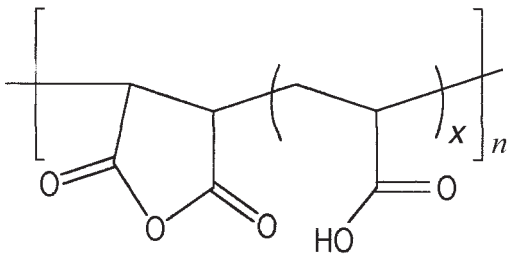
Materials

VA and AA (Fluka) were distilled before use. They had the following average characteristics: VA, bp 72.5°C, d_4^{20} 0.9315, n_D^{20} 1.3950; AA, bp 141.6°C, mp 13.5°C, d_4^{20} 1.0510, n_D^{20} 1.4215. MA (Fluka) was purified before use by recrystallization from anhydrous benzene and by sublimation in vacuo (mp 52.8°C). Benzoyl peroxide (BP, Fluka) was purified by recrystallizing twice from chloroform solution by methanol and drying under a vacuum (mp 106°C). Other reagents including organic solvents were purified by ordinary methods.

Copolymerization

Copolymerization of MA with AA and terpolymerization of MA, VA, and AA were carried out in similar conditions in 1,4-dioxane in the presence of BP (0.1%) as an initiator at 70°C under a nitrogen atmosphere. The P(MA-*alt*-AA) and P(MA-*co*-VA-*co*-AA) were isolated from the reaction mixture by reprecipitation with anhydrous methanol and *n*-hexane, respectively. Copolymers were purified by reprecipitating twice from dioxane solution with *n*-hexane and by washing with several portions of hexane, benzene, and diethyl ether. Then, they were dried *in vacuo* at 50°C to a constant weight with almost quantitative yields.

The terpolymer was purified by several reprecipitations from anhydrous acetone solution with hexane and by washing with hexane and benzene. It was dried in vacuo at 60°C to a constant weight with quantitative yields. The copolymer and terpolymer synthesized by the use of 1 : 1 and 1 : 2 : 1 molar ratios of initial monomers, respectively, had the following characteristics[12]:



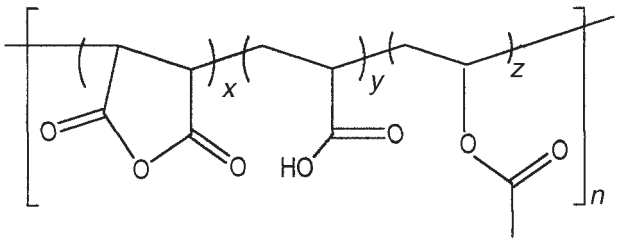
where x = 1.12 (AA unit = 52.83), yield = 75%, glass-transition temperature (T_g) = 111°C, melting temperature (T_m) = 153°C (DSC analysis), crystallinity = 26% (X-ray diffraction [XRD] analysis), intrinsic viscosity ([η_{in}]) in 1,4-dioxane at 25°C = 1.25 dL g^{-1}, acid number (AN) = 878 mg KOH/g, monomer unit ratio in copolymer (m_1/m_2) = 1 : 1.12.

FTIR spectra (film, cm^{-1}); νOH 3060 (broad, in COOH), νCH_3 2950 (as) and 2880 (s), νCH_2 2930 (as) and 2870 (s), νCOOH 2545 (broad), νC═O 1836 (as) and 1766 (s, C═O in anhydride unit), νC═O 1730 (C═O in ester group), νC═O 1585 (as, in COO), δCH_2 1478 and 1443 (doublet), δCH_3 1385 and 1357 (doublet), δC—O 1240–1170 (ester and carboxyl), νC—O═C 1035 (in anhydride unit), δOH 943 (out of plane OH bending), δCH 886 and 871 (doublet), δCH_3 842 (rock), δCH_2 720 (rock), δCH 645 (in main chain from anhydride unit), δO═C═O 560 (s, bend of COOH) and 645 (in main chain from anhydride unit), δO═C═O 578 (s, bend of ester group).

In the FTIR spectra of hydrolyzed copolymers, the characteristic bands for anhydride units disappeared, and new bands in the field of 1970, 1585, and 1630 cm^{-1} relating to COOH groups appeared, as well as an increase in intensity of 3060 and 2545 cm^{-1} broad bands.

TABLE I
Test Concentrations Used in MTT Assay

Polymer sample	Test concentration (μg/mL)
Poly[maleic anhydride-*alt*-acrylic acid]	1, 5, 10, 50, 100
Poly[maleic anhydride-*co*-acrylic acid-*co*-(vinyl acetate)]	10, 50, 100, 200, 400



where x, y, and z are monomer unit fractions: x = 50.02 mol %, y = 20.97 mol %, z = 30.34 mol %, yield = 95%, T_g = 186.5°C, T_m = 201°C (DSC analysis), crystallinity = 12% (XRD analysis), $[\eta_{in}]$ in dioxane at 25°C = 1.36 dL g^{-1}, AN = 710 mg KOH/g, monomer unit ratio in copolymer ($m_1/m_2/m_3$) = 1 : 2 : 1.

FTIR spectra (film, cm^{-1}): νOH 3060 (broad, in COOH), νCH_3 2950 (as) and 2880 (s), νCH_2 2930 (as) and 2870 (s), νCOOH 2545 (broad), νC═O 1836 (as) and 1766 (s, C═O in anhydride unit), νC═O 1730 (C═O in ester group), νC═O 1585 (as, in COO), δCH_2 1478 and 1443 (doublet), δCH_3 1385 and 1357 (doublet), δC—O 1240–1170 (ester and carboxyl), νC—O═C 1035 (in anhydride units), δOH 943 (out of plane OH bending), δCH 886 and 871 (doublet), δCH_3 842 (rock), δCH_2 720 (rock), δCH 645 (in main chain from anhydride unit), δO═C═O 560 (s, bend of COOH).

Antitumor activity of polymers

Cell culture

Raji cells (human Burkitt lymphoma cell line) were maintained in RPMI 1640 culture medium supplemented with 10% heat-inactivated fetal bovine serum, 2 m*M* L-glutamine, 100 IU/mL penicillin, and 100 g/mL streptomycin at 37°C in a humidified incubator containing 5% CO_2.

Preparation of copolymers for cytotoxicity assay

P(MA-*alt*-AA) (100 mg/mL) and P(MA-*co*-AA-*co*-VA) (200 mg/mL) stock solutions were prepared in distilled water and filtered (0.45 μm, Millipore) for sterilization. Stock solutions were kept at room temperature. Copolymers were diluted with culture medium just before use. The test concentrations are given in Table I.

In vitro cytotoxicity assay

The in vitro cytotoxicities of the copolymers were determined by the MTT assay method.[13] Briefly, 2 × 10^4 cells/well were seeded in 96-well plates and 50 μL of sample solutions were added into each well. After 72-h incubation, 25 μL of MTT solution (1 mg/mL final concentration) was added to each well and the plates were incubated for a further 4 h. The formazan produced was solubilized by adding 80 μL of lysing buffer consisting of 23% SDS dissolved in a solution of 45% DMF (pH 4.7). After an overnight incubation at 37°C, the optical densities at a 540-nm wavelength were measured using a microplate reader (Spectramax Plus, Molecular Devices). Cells incubated in culture medium alone served as a control for cell viability (untreated wells). All assays were performed in quadruplicate and mean ± standard deviation values were used to estimate the inhibition rate. The LD_{50} was defined as the concentrations of samples that reduced the absorbance of the treated cells by 50%.

Statistical analysis

The LD_{50} values were estimated using nonlinear regression analysis [graphPAD Version 2.0 ISI(R) Software].

Measurements

The H-complex formation in the MA—AA monomer system was studied by the UV method using a Hitachi 100-50 model UV–vis double-beam spectrophotometer and 1,4-dioxane solutions of monomer mixtures with various monomer ratios at 20 ± 0.1°C.

The equilibrium constant of MA···AA CTC formation was determined by the ^{1}H-NMR method using a Bruker DPX300 NMR spectrometer with 300-MHz frequency, CH_3═CO═CH_3-*d*, and deuterated *p*-dioxane as the solvent, and tetramethylsilane as the internal standard at 25 ± 0.1°C.

The FTIR spectra of copolymer films or thin coatings on KBr pellets were recorded with Nicolet 510 FTIR spectrometer in the 4000–400 cm^{-1} range, and 30 scans were taken at 4 cm^{-1} resolution. For the composition analysis of the terpolymers, specifically the contents of VA and AA units, characteristic absorption bands of 1360 (VA unit), 1630 (MA–maleic acid units), and 1580 cm^{-1} (AA unit) 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 ANs of the anhydride-containing copolymers and terpolymers were determined by a known nonaqueous titration method.

The intrinsic viscosities of the synthesized copolymers and terpolymers were determined in 1,4-dioxane

at 25 ± 0.1°C in a concentration range of 0.1–1.0 dL g^{-1} using an Ubbelohde viscometer.

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

The powder diffraction patterns of synthesized samples were recorded using a Philips manual spectrogoniometer employing Cu Kα (λ = 1.54184 Å) radiation over the range $5° \leq 2\theta \leq 50°$.

The degrees of crystallinity of the new products were determined by the area ratio method:

$$X_c = \int_0^\infty s^2 I_c(s) ds \Big/ \int_0^\infty s^2 I(s) ds \qquad (1)$$

where s is the magnitude of the reciprocal-lattice vector, which is given by $s = (2 \sin \theta)/\lambda$, θ is one-half the angle of deviation of the diffracted rays from the incident X rays, λ is the wavelength, $I(s)$ is the intensity of coherent X-ray scatter from a specimen (both crystalline and amorphous), and $I_c(s)$ is the intensity of coherent X-ray scatter from the crystalline region. In this method, the areas of amorphous and crystalline parts of the patterns were calculated.

RESULTS AND DISCUSSION

Identification of anhydride-containing polymers

From the structural peculiarities of the monomers of the ternary systems under study, it may be predicted that the formation of the two types of intermolecular complexes, such as the CTC between the double bonds of MA (electron acceptor) and VA (electron donor) and the hydrogen-bonded complex between MA (═C═O, proton acceptor) and AA (═COOH, proton donor) proceeds as follows:

$$\mathrm{MA + VA} \xleftrightarrow{K_c} \mathrm{[MA\cdots VA]}$$

(I)

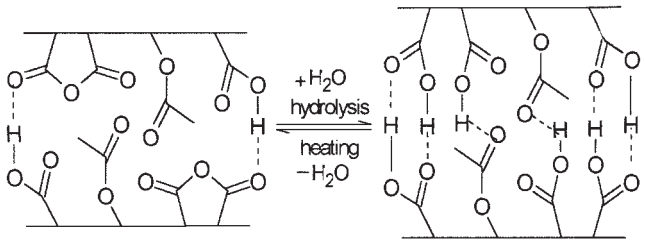
$$\mathrm{MA + AA} \xleftrightarrow{K_H} \mathrm{[MA \quad AA]} \xleftrightarrow{K_c} \mathrm{[MA\cdots AA]}$$

(II) **(III)**

where (**I**) is the CTC; (**II**) is the only hydrogen-bonded complex forming through >C═O· · ·HOOC— bonds; and (**III**) is the CTC forming because of charge transfer from the AA double bond to the MA double bond, which is caused by the effect of hydrogen bonding between functional groups of comonomers.

The equilibrium constant (K_H) of the hydrogen-bonded complex at various λ_{max} values are determined using UV–vis spectroscopy data. The corresponding equations from the Benesi–Hildebrand and Ketelaar results obtained can be given in the following form: a K_H value of 469 L mol^{-1} for the Benesi–Hildebrand at 270 nm and a K_H value of 447 L mol^{-1} for Ketelaar at 270 nm were found. As seen from these values, the use of both methods allows us to obtain the close results for the MA—AA system.[14] Using the appreciable displacement of chemical shifts observed from the ^{1}H-NMR and Hanna–Aushbaugh equation allows us to determine the K_c value for the MA· · ·AA CTC from a plot of $1/\Delta_{exp}$ versus 1/AA. The K_c values were 0.056 ± 0.003 (0.051 by least squares analysis) and 0.16 ± 0.01 L mol^{-1} (0.17 by least squares analysis) at 25 ± 0.1°C in deuterated acetone and dioxane, respectively.[14]

From the known model of the H bond, an electrostatic interaction should also be a foreseen possibility and consequence of charge transfer interactions in hydrogen-bonded complexes. Formation of CTC in the MA—AA monomer system can be explained between the electron acceptor MA double bond and the electron donor AA double bond (only in the mixtures with MA after realizing a hydrogen bonding). Using these peculiarities of the system, anhydride-containing copolymers and terpolymers are synthesized. In the FTIR spectra of hydrolyzed co- and terpolymers, characteristic bands for anhydride units disappeared and new bands at 1970, 1585, and 1630 cm^{-1} relating to —COOH groups appeared, as well as increased intensity of the 3060 and 2545 cm^{-1} broad bands.[12] These terpolymers are easily transformed to the anhydride forms after thermotreatment at 120–130°C during 15 min. This observed reversible hydrolysis–heating process is presented in Scheme 1.



Scheme 1 The reversible hydrolysis–hydrogen bonding–heating process in anhydride-containing co(ter)polymers.

Practically all water-soluble polymers exhibit typical polyelectrolyte behavior, that is, a decrease in the reduced viscosity with an increase in the polyelectrolyte concentration.[15] These anhydride-containing copolymers significantly differ from other water-soluble polymer systems because their solution process is accompanied by spontaneous hydrolysis of anhydride linkages with the formation of strong hydrogen-bonded carboxylic fragments in the side chains of macromolecules (Scheme 1).

The possibility of the formation of specific molecular side-chain fragments through H bonding in most alternating copolymers and terpolymers containing free carboxyl groups can allow us to use these polymers for the fabrication of stable mesophases of side-chain polymers, compatible polymeric systems, stable hydro- and sol–gels, stable organized molecular films with nonlinear optical properties, liquid crystalline polymers with flexible backbones, biomolecular complexes, and other specific polymer materials. One of the important intra- and intermolecular structural characteristics of polyfunctional polymers is the structural regularity and bond flexibility of their macromolecules. When the polar polymers possess H-bonding capabilities, the most energetically favored crystal structures will tend to capitalize on these features.[16]

The synthesized terpolymers display different thermal properties, including melting and glass-transition behaviors, depending on the content of hydrogen-bonding fragments.[17] The results of the DSC and TGA studies of the terpolymers are summarized in Table II. The MA—VA—AA terpolymers with different compositions have characteristic glass-transition endotherms (T_g) in area of 146.0, 153.5, 186.5, and 197.5°C, as well as different decomposition behaviors. An appreciable increase in the T_g values with decreasing AN values from 725 to 703 mg KOH/g is observed. Similarly, the T_m changes and the intensity of the endotherm is decreased by a shift of the T_m to a higher temperature region. This seems to be related to the crystalline phase that is formed through intermolecular H bonding between the free —COOH groups containing functional macromolecules. The average value of the T_g/T_m ratio (Table II) is approximately 0.91, which agrees with the known value of this relationship for polar polymers proposed by Boyer.[18]

Scheme 1 illustrates the molecular organization through intermolecular hydrogen bonding for P(MA-*co*-VA-*co*-AA) macromolecules, especially for its hydrolyzed derivative, which suffered reversible anhydridization by heating at 110°C in the solid state.

DSC curves of MA—VA—AA and MA—VA—methyl acrylate (as a model comonomer) terpolymers that were recorded beginning at −100°C show that the T_m exotherm is essentially shifted to a relatively low temperature region (from 186.5 to 125°C for the MA—VA—AA terpolymer). This fact can be explained by the change of the crystallization mechanism and by the formation of complexed macromolecules with different physical structural fragments with a lower temperature condition. The TGA thermogram of the hydrolyzed terpolymer differs from the same curve of the initial terpolymer and it has the multistep character of the decomposition process. A first step of decomposition is related to the dehydration reaction and it transfers acid units to anhydride forms in the side chains of the macromolecules.

According to Figure 1, the water soluble P(MA-*alt*-AA) and P(MA-*co*-VA-*co*-AA) display typical polyelectrolyte behavior, that is, a decrease in the reduced viscosity with an increase in the polyelectrolyte concentration; the degree of ionization increases and the ions that are produced form an ionic environment with a size that is higher than the diameter of the polymer coil. The repulsion among the ions increases the rigidity of the chain, expanding the polymeric coil with a consequent

TABLE II
Hydrogen-Bonding Effect on Glass-Transition Temperature (T_g), Melting Temperature (T_m), and Destruction (T_d and ΔH_d) Behaviors of VA–MA–AA Terpolymer with Different Compositions

Terpolymer composition (mol %)			AN	T_g	T_m		T_d	ΔH_d
m_1	m_2	m_2	(mg KOH/g)	(°C)	(°C)	T_g/T_m	(°C)	(J/g)
30.3	50.0	19.7	729.3	146.0	161.3	0.905	198	405
32.5	50.2	17.3	725.4	153.4	182.5	0.842	207	412
33.9	45.1	21.0	711.2	186.5	201.0	0.928	218	439
35.1	45.5	19.4	703.4	197.5	206.3	0.957	219	456

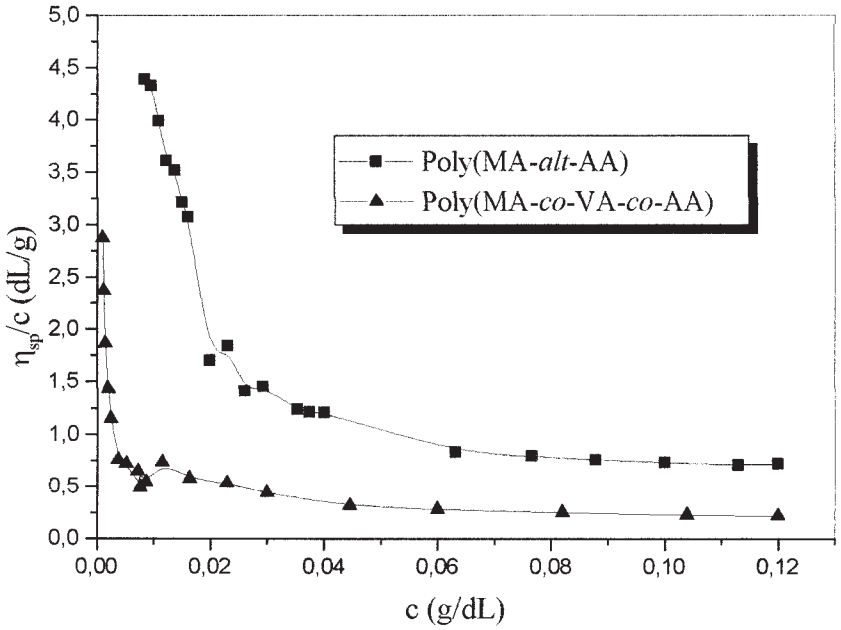


Figure 1 The polyanionic behavior of P(MA-*alt*-AA) and P(MA-*co*-VA-*co*-AA) in water at 25°C.

increase of the viscosity. The charge density and solution configuration of these polymers were varied over a wide range by the proper choice of substituents on the backbone of the molecule and substitutions on the carboxyl groups.[10] In the AA and MA copolymer, functional groups are attached to immediately adjacent carbon atoms of the carbon backbone atoms separated by a methylene unit. These properties of the polyanionic polymers are different from the other types of polymers by means of the ionic strength as well as charge density.

In vitro cytotoxicity of anhydride-containing polymers

Anionic polymers such as polyphosphates and polycarboxylates of either natural or synthetic origin have

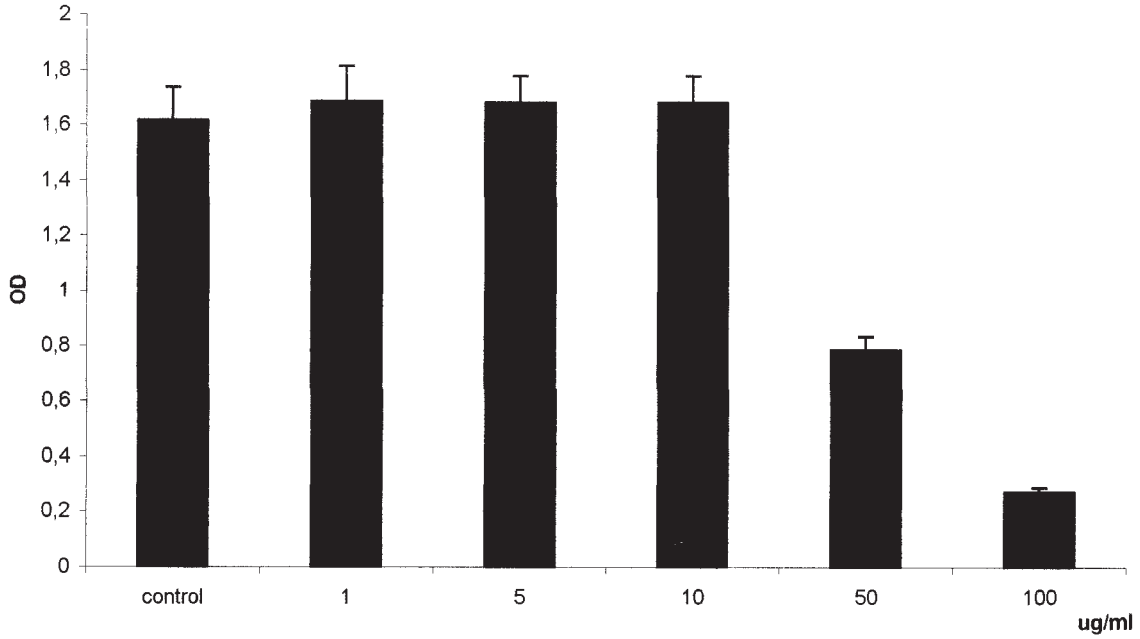


Figure 2 The cytotoxicity of P(MA-*alt*-AA) on Raji cells. The optical density (OD) values represent the mean ± standard deviation of four wells.

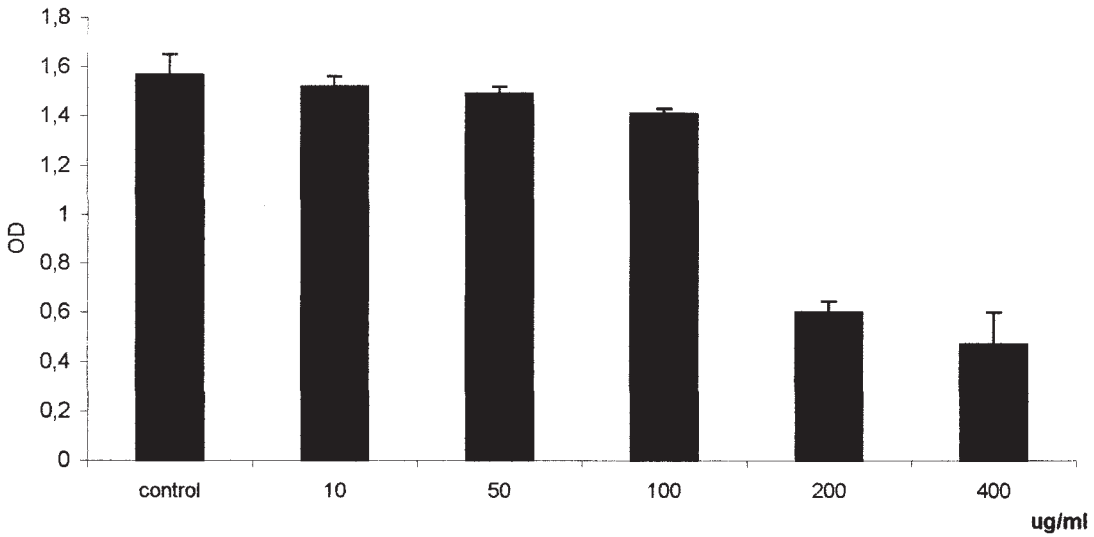


Figure 3 The cytotoxicity of P(MA-*co*-VA-*co*-AA) on Raji cells. The optical density (OD) values represent the mean ± standard deviation of four wells.

been shown to be potential inhibitors of transplanted tumors.[19] The dose–response curve of P(MA-*alt*-AA) on Raji cells is presented in Figure 2. The doses of copolymer up to 10 μg/mL resulted in nontoxic effects in tumor cells. The LD_{50} value was calculated as 44.596 μg/mL for P(MA-*alt*-AA). The lower the LD_{50} values are, the more efficiently the polymer kills the cancer cells.

The dose–response curve of P(MA-*co*-VA-*co*-AA) on Raji cells is presented in Figure 3. There was not cytotoxic effect at the 100 g/mL dose of the studied terpolymer. The LD_{50} value was calculated as 145.38 μg/mL for this terpolymer. The dose-dependent evaluation of anhydride-containing polymers revealed that P(MA-*alt*-AA) is more cytotoxic than the terpolymer. The cytotoxicity of the synthesized copolymer was superior to those of the control and P(MA-*co*-VA-*co*-AA) increased with increasing concentration (Table I). The P(MA-*co*-VA-*co*-AA) terpolymer has low polyelectrolyte behavior in comparison to the P(MA-*alt*-AA) alternating copolymer[20] (Fig. 1). This polyanionic character of the terpolymer depicts the low cytotoxicity behavior. VA fragments in the terpolymer give immobility to the polymer chains and low polyanionic character.

CONCLUSIONS

The ternary copolymerization of MA, VA, and AA, which is considered an acceptor_–donor–acceptor system, was carried out in 1,4-dioxane in the presence of BP as an initiator in a nitrogen atmosphere at 70°C. The constants of complex formation for the monomer systems in the study were determined by UV–vis (K_H, hydrogen-bonded complex) and ^{1}H-NMR (CTC, K_c) methods. Studies of the structural peculiarities (FTIR analysis) and structure–thermal behavior (T_g and T_m values) relationship of the synthesized polymers indicated that these polymer systems show typical polyelectrolyte behavior, having a semicrystalline structure because of the H-bonding effect and suffering reversible hydrolysis–anhydrization reactions. These anhydride-containing copolymers differ significantly from other water-soluble polymer systems because their solution process is accompanied by spontaneous hydrolysis of anhydride linkages with the formation of strong hydrogen-bonded carboxylic fragments in the side chains of the macromolecules (Scheme 1).

The in vitro cytotoxicities of P(MA-*co*-VA-*co*-AA) and P(MA-*alt*-AA) were evaluated using Raji cells. The LD_{50} values were calculated as 44.596 μg/mL for P(MA-*alt*-AA) and 145.38 μg/mL for P(MA-*co*-AA-*co*-VA). These results demonstrate that P(MA-*alt*-AA) has a more effective cytotoxic effect on tumor cells than P(MA-*co*-AA-*co*-VA).

This study, which was carried out in the Department of Chemistry and Oncology Institute of Hacettepe University, was financially supported by the Hacettepe University Research Foundation. The authors thank Serap Kavlak for viscosity measurements.

References

1. Hunter, A. C.; Moghimi, A. M. Drug Discov Today 2003, 7, 998.
2. Ringsdorf, H. J Polym Sci Polym Symp 1975, 51,135.
3. Ottenbrite, R. M. J Macromol Sci Chem 1985, A22, 819.
4. Putnam, D.; Kopecek, J. Adv Polym Sci 1995, 3, 355.
5. Soyez, H.; Seymour, L. W.; Schacht, E. J Controlled Release 1999, 57, 187.
6. Kim, G. C.; Jeong, J. G.; Lee, N. J.; Ha, C. S.; Cho, W. J. J Appl Polym Sci 1997, 64, 2605.

7. Choi, W. M.; Lee, N. J.; Ha, C. S.; Cho, W. J. Polym Int 1997, 43, 167.
8. Lee, N. J.; Ju, S. S.; Cho, W. J.; Kim, S. H.; Kang, K. T.; Brady, T.; Theodorakis, E. A. Eur Polym J 2003, 39, 367.
9. Ottenbrite, R. M. The Antitumor and Antiviral Effects of Polycarboxylic Acid Polymers in Biological Activities of Polymers, ACS Symposium Series 186; American Chemical Society: Washington DC, 1982.
10. Regelson, W.; Kuhar, S.; Tunis, M.; Fields, J. E.; Johnson, J. J.; Glusenkamp, E. W. Nature 1960, 186, 778.
11. Butler, G. J Polym Sci 1960, 48, 279.
12. Kaplan Can, H.; Rzaev, Z. M. O.; Güner, A. J Appl Polym Sci 2003, 90, 4009.
13. Hansen, M. B.; Nielsen, S. E.; Berg, K. J Immunol Methods 1989, 119, 203.
14. Kaplan Can, H.; Rzaev, Z. M. O.; Güner, A. J Mol Liq 2003, 111, 77.
15. Kavlak, S.; Kaplan Can, H.; Güner, A.; Rzaev, Z. M. O. J Appl Polym Sci 2003, 90, 1708.
16. Sperling, L. H. Introduction to Physical Polymer Science; Wiley: New York, 1992; p 209.
17. Rzaev, Z. M. O. Prog Polym Sci 2000, 25, 163.
18. Boyer R. F. Encyclopedia of Polymer Science and Technology; Bikales, N. M., Ed.; Interscience: New York, 1977; Suppl. Vol. 2, p 745.
19. Morahan, P. S.; Munson, J. A.; Baird, L. G.; Kaplan, M. A.; Regelson, W. Can Res 1974, 34, 506.
20. Ulbrich, K.; Etrych, T.; Chytil, P.; Jelnkova, M.; Rhova, B. J Controlled Release 2003, 87, 33.