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Synthesis, Characterization and Bactericidal Properties of Poly(N-vinyl-2-pyrrolidone-co-Maleic anhydride-co-N-Isopropyl acrylamide)

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Summary: Radical-initiated terpolymerization of maleic anhydride (MA), N-vinyl-2-pyrrolidone (NVP) with N-isopyropyl acrylamide (NIPA) has been prepared as a way to obtain new water-soluble polymers. Structure, composition and thermal behaviour of synthesized terpolymers were determined by FTIR, UV-vis, ^{1}H NMR spectroscopy, elemental analysis (N content), differential scanning calorimetry, thermogravimetric and differential thermal analysis, gel permeation chromatography analysis (GPC), scanning electron microscopy (SEM) and X-ray diffraction analysis (XRD). The terpolymer composition-structure-property relationship indicates that the semicrystalline structure of terpolymers with different reaction times, degrees of crystallinity, and thermal behaviour depends on the content of carboxyl and amide-containing monomer linkage. The antimicrobial activities the terpolymers were evaluated against pathogen bacteria: *Staphylococcus aureus, Salmonella enteridis, Streptococcucs faecalis*, *Eschericia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa*. The antimicrobial activity was explored by the well-diffusion technique. All the studied polymers, containing biologically active moieties in the form of ionized cyclic amide and carboxylic acid groups, were more effective against Gram-positive than Gram-negative bacteria. The bactericidal effect of terpolymers against Gram negative bacteria increased with the increasing reaction times.

Keywords: bactericidal effect; maleic anhydride; N-vinyl-2-pyrrolidone; synthesis; water-soluble polymers

Introduction

Numerous water-soluble polymers, for the most part acrylic derivatives and vinyl type polymers have been investigated for use as macromolecular drug carriers,[1] protein hybrids[2] and advanced applications in biotechnology.[3] It has been pointed out that the copolymerization reactions provide an excellent way for the preparation of macromolecules with specific chemical structures and for the control of properties such as hydrophilic/hydrophobic balances, polarity and solubility.[4] Thus, the biochemical properties of the water-soluble copolymers such as interactions with proteins and adsorption of biological compounds will depend upon the monomers or comonomers of which they are made.[5] Poly(N-vinyl-2-pyrrolidone) (PNVP) is one of the most frequently investigated classes of materials for use in medicine and in other applications which interface with biological systems.[6] The principal reason for successful NVP copolymers applications is their excellent biocompatibility with living tissues and extremely low cytotoxicity.

NVP as an electron donor monomer shows an increase in its propagation rate constant in a polar solvent, due to solvatation of NVP monomer and growing macroradicals.[7] The radical copolymerization/crosslinking method was used for preparation of

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NVP containing biodegradable, thermoresponsive and biocompatible hydrogels for drug delivery.[8,9]

Polymers of N-isopropyl acrylamide (NIPA) exhibit pH and thermal sensivity and have been used in biologically active systems as cation active polymers soluble in water and physiological medium,[10–13] as well as carrier systems for DNA delivery, for affinity separation of genotoxins and as reversible bioconjugates. [14–16]

MA, which contains two adjacent carboxylic acid groups in anhydride form and reactive carbonyl groups that may be subjected to numerous reactions, was selected for polymerization with NVP and NIPA. Alternating copolymers of MA can be regarded as preactivated polymers due to the presence of anhydride moieties susceptible to the reaction with a primary amine of a biomolecule. [17]

In the last few years, synthetic water-soluble polymers have become of great interest in the biological field.[18–24] Some polymers have been investigated as biocides (bactericidal effect) because of advantages with respect to the monomeric analogues.[25,26] In previous work, poly(NVP-co-MA)/diethylethanolamine macrocomplexes were synthesized and tested for bactericidal activity against *Salmonella enteridis and Klebsiella pneumoniae* microorganisms. Excellent growth inhibition of these bacteria by the macrocomplexes was observed. [27]

A literature survey reveals that the synthesis, characterization and antimicrobial activity of poly(N-vinyl-2-pyrrolidone-co-maleic anhydride-co-N-isopropyl acrylamide) terpolymers [(Poly(NVP-co-MA-co-NIPA)] have not been reported. In this paper the synthesis and characterization of water-soluble terpolymers NVP with MA and NIPA are reported. A determination of monomer ratios using ^{1}H NMR and elemental analysis methods, evaluation of structural peculiarities and the effect of H-bonding and –NH···O = C- complexation on the monomer ratios have been carried out. Additionally, composition-structure-thermal behavior relationships for the synthesized terpolymers have been established.

Experimental Part

Materials

NVP monomer (Merck) was distilled before use. It had the following characteristics: NVP, bp 92.0-95.0 °C, $n_D^{20} = 1.512$, $d_4^{20} = 1.04$. MA monomer (Aldrich) was purified by recrystallization from anhydrous benzene and sublimation at reduced pressure. mp 52.8 °C. NIPA monomer (Aldrich) was purified before use by distillation at reduced pressure and recrystallization from diethyl ether solution: b.p. 91.5 °C, m.p. 61.6 °C. α,α′-Azobisisobutyronitrile (AIBN) (Acros) was recrystallized twice from methanol: mp 102.5 °C. 1,4-Dioxane (Merck), methanol (Fluka), diethyl ether (Merck), and benzene (Aldrich), dimethyl sulfoxide (DMSO) (Fluka), dimethyl formamide (DMF) (Fluka), chloroform ($CHCl_3$) (Merck) and CH_2Cl_2 (Merck) were used ass received.

Polymer Preparation

Terpolymerizations of NVP, MA with NIPA using different reaction times (6, 12, 24 and 36 h) were carried out in 1,4-dioxane at 65 °C with AIBN radical initiator at constant total concentration of monomers under the nitrogen atmosphere. Reaction conditions for systems: monomer ratios of NVP/MA/NIPA= 1:2:1. Monomers, 1,4-dioxane and AIBN were placed in a dry, three-necked, round-bottomed flask under a statik atmosphere of dry nitrogen. The flast was placed in a thermostated glycerin bath at 65 °C. The NVP-MA-NIPA terpolymers were isolated from the reacted mixture by precipitation with diethyl ether, then washed with several portions of benzene and dried at reduced pressure.

Terpolymer compositions were found by elemental analysis (N content for NIPA and NVP units) and ^{1}H NMR spectroscopy using integral areas of characteristic monomer absorptions.

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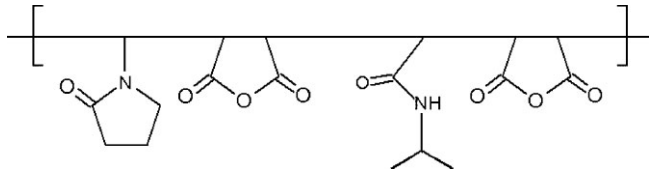

Figure 1.
Poly(NVP-co-MA-co-NIPA).

Terpolymers prepared from 1:2:1 NVP/MA/NIPA molar ratio of initial monomers has the following average characteristics:

Poly(NVP-co-MA-co-NIPA) terpolymer (reaction time: 24 h) (Figure 1):

Monomer unit ratio, m_1: 24.25 m_2: 46.50 m_3 : 29.25; Content of N (by elemental analysis); $[\eta]_{in}$ 0.77 dL/g in DMSO at $25 \pm 0.1\,^{\circ}C$; T_g 95 °C [by differential scanning calorimetry (DSC)] and T_m 368.37 °C [by DSC and differential thermal analyses (DTA)].

FTIR spectra (KBr pellet), cm^{-1}: 3100-3020 broad bands for NH secondary amide, 2984, 2910 and 2865 CH stretching in CH, CH_2 and CH_3 groups, 1883 antisym. C = O stretching, 1816 sym. C = O stretching of anhydride group, 1800-1765 H-bonded C = O stretching, 1730 C = O of free anhydride group, 1715, 1693 and 1665 broad triplet band for complexed anhydride C = O group, 1630 (s) C = O amide I band, 1530-1515 broad NH amide II band, 1454 CH_2 scissor vibration and CH_3 antisym. deformation, 1410 C-N stretching, 1380 and 1370 doublet band for CH_3 deformation in the isopropyl group, 1271 trans-amide III band, 1040-991 C-O, C-O-C stretching and CH_3 rocking, 1085 NH bending in -NH···O = C-, 890 C-C stretching of main chain, 747 NH deformation, 595-673 CH bending for anhydride unit.

^{1}H NMR spectra (in DMSO-d_6 at 27 °C), ppm: $2CH_2$ (pyrrolidone ring) 3.08-3.40, 3.65-3.95 CH (CH-N backbone) for NVP unit; 2H, CH 4.38-4.49 for MA unit; 2H, CH_2 1.89-1.94; 1H, CH 2.45-2.52; 1H, NH 6.89-7.06; 1H, CH 4.11-4.20; 6H, CH_3 1.12-1.21 for NIPA unit.

Polymer Characterization

FTIR spectra of the terpolymers (KBr pellets) were obtained using a Mattson-1000 Model spectrophotometer in the 4000-400 cm^{-1} range. ^{1}H NMR spectra were recorded using a Bruker Avance DPX400 (400 MHz) spectrometer with DMSO-d_6 as a solvent at 27 °C. DSC, DTA and thermogravimetric (TGA) analyses of terpolymers were performed using a Shimadzu DSC-60 calorimeter and Shimadzu TG-DTA 60/60H Thermal Analyzer, respectively, in a nitrogen atmosphere at a heating rate of 10 °C/min. The CHNS-932 Model LECO Elemental Analyzer was used for the determination of C, H and N contents in the terpolymers synthesized. Absorption spectra were obtained using a UNICAM UV2-100 UV-visible spectrophotometer equipped with 1 cm quartz cells and 10^{-5} $mol \cdot L^{-1}$ solutions in deionized water and DMSO in the range of 200-600 nm. GPC analyses were performed at 30 °C using THF as eluent at a flow rate of 1.0 mL/min. A refractive index detector was used as a detector. The instrument (Polymer Laboratories PL-GPC 220) was calibrated with a mixture of polystyrene standarts using GPC software for the determination of the number-average molecular weight (M_n), weight-average molecular weight (M_w) and polydispersity index (PDI) of the terpolymer sample. Crystallization behaviours of terpolymers were determined by D8-Advance-Bruker-AXS diffractometer employing CuKα ($\lambda = 1.54{,}184$ Å) radiation over the range $5^o \leq 2\theta \leq 50^\circ$. Crystallinity degrees of (X_c) of synthesized terpolymers were determined by area ratio method using the following equation:[28]

$$X_c = \frac{{}_o s^2 I_c(s) d}{{}_o s^2 I(s) d} \quad (1)$$

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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 derivation of the diffracted rays from the incident X-rays and λ is the wavelength); $I(s)$ and $I_c(s)$ are the intensities of coherent X-ray scatter from both crystalline and amorphous regions and from only crystalline region of polymer sample, respectively, and d is interplanar spacing.[28] The surface morphologies of the polymers were utilized using a Jeol model JSM-6060LV scanning electron microscope (SEM). Viscosities of the terpolymers were determined for solutions in DMSO at 25 °C at a concentration of 0.5 g/dL using a Ubbelohde viscometer. Intrinsic viscosities were obtained by extrapolation of relative viscosity plots to zero concentration.

Microbiological Studies

The antimicrobial activities of the synthesized terpolymers were determined by the well-diffusion method.[27,29] In this work, *Staphylococcus aureus, Salmonella enteridis, Streptococcucs faecalis* (Gram-positive) and *Eschericia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* (Gram-negative) were used to assess the antibacterial activities of synthesized terpolymers. The bacterial subcultures for *Staphylococcus aureus, Salmonella enteridis, Streptococcucs faecalis*, *Eschericia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were obtained from Gazi University, Faculty of Science and Arts, Department of Biology. *Staphylococcus aureus, Salmonella enteridis, Streptococcucs faecalis, Eschericia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* liquid cultures were prepared in brain heart infusion broth for the antimicrobial activity tests. All polymers were dissolved in deionized water at concentrations of 10 mg/ml. The solutions were filtered through a Millipore membrane filter (0.45 μm, Millipore, USA). Deionized water was found to have no antimicrobial activity against any of the organisms. Approximately 1 cm^3 of a 24 h broth culture containing 10^6 cfu/cm^3 of was placed in sterile Petri dishes. Moltent nutrient agar (15 cm^3) kept at 45 °C was then poured into the Petri dishes and allowed to solidify. Six millimeter diameter holes were then punched carefully using a sterile cork borer and completely filled with the test solutions. The plates were incubated for 24 h at 37 °C. After 24 h, the inhibition zone that appeared around the holes in each plate was measured.

Results and Discussion

Terpolymer Structure-Composition and Property Relationships

Poly(NVP-co-MA-co-NIPA) terpolymers were synthesized by radical-initiated polymerization in an anhydrous medium. From the structural peculiarities of the monomers of the ternary systems under study, it can be predicted that the formation of different types of intermolecular complexes, such as the hydrogen-bonded complex between MA, NVP (proton donor) and NIPA (proton acceptor) may occur. It is important to note that N-isopropyl acrylamide can also easily form proton-donor complexes through intermolecular H-bonding.[30]

The observed tendency toward to alternation in the polymerization of both monomer systems can be explained from the complexing tendencies of the monomers. Intraction between monomer/comonomer molecules and monomers/growing macroradicals, on the chain growth, are responsible for the formation of terpolymers with a primarly alternating structure as shown in the following Figure 2.[30,31]

Three characteristic peaks (3.08-3.40 ppm $2CH_2$ in pyrrolidone ring NVP unit, 4.38-4.49 ppm CH in maleic unit and 4.11-4.20 ppm CH in isopropyl group NIPA unit) can be identified in the ^{1}H NMR spectra of the terpolymers and used as analytical signals for quantitative determination of terpolymer composition. The signals around δ 6.89-7.06 ppm appeared on increasing the amount of the NIPA unit in the terpolymer. Its intensity increases with an increase in ratio of the NIPA unit to

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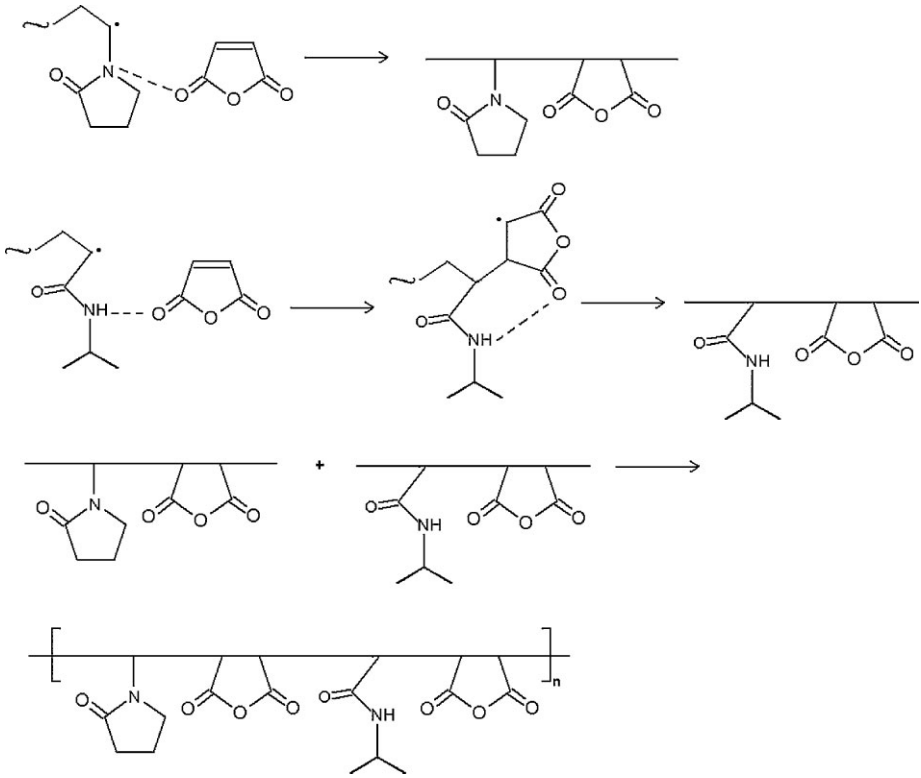

Figure 2.
Chain growth in the radical polymerization of NVP with MA and NIPA.

other units in the terpolymer. Terpolymer compositions calculated using elemental analysis data (content of N) were in reasonable agreement with those obtained from ^{1}H NMR analysis. Results of ^{1}H NMR and elemental analysis for the terpolymers are summarized in Table 1.

FTIR spectra of the terpolymers are characterized by the typical absorption bands for NIPA units (3100-3020 cm^{-1} broad bands for secondary NH amide or for H-bonded OH group of acid fragments in the partially hydrolyzed anhydride units, 2984-2865 cm^{-1} CH bands at the isopropyl group, 1630 cm^{-1} strong C = O amide band I, 1530-1515 cm^{-1} strong NH amide II band and 1271 cm^{-1} amide III band) and anhydride units (1883 and 1816 cm^{-1} symmetrical and antisymmetrical C = O bands, 1040-991 cm^{-1} C-O and C-O-C anhydride

Table 1.
^{1}H NMR and elemental analysis data for determining the composition of Poly(NVP-co-MA-co-NIPA) terpolymers.

Reaction time	Monomer feed			N	Copolymer composition					
h	mol%			%	mol%					
					^{1}H NMR analysis			Nitrogen analysis		
	NVP	MA	NIPA		m_1	m_2	m_3	m_1	m_2	m_3
6	25	50	25	7.09	27.70	44.90	27.40	27.32	45.12	27.56
12	25	50	25	7.85	25.45	46.30	28.25	26.00	45.97	28.03
24	25	50	25	8.30	23.90	47.00	29.10	24.25	46.50	29.25
36	25	50	25	8.52	21.50	48.60	29.90	22.05	48.20	29.75

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bands). Spectra of these terpolymers also contain characteristic bands for H-bonded C=O groups (1800-1765 cm^{-1} for anhydride C=O and 1715-1665 cm^{-1} broad band for amide C=O) and H-bonded secondary amide NH group (1085 cm^{-1} NH deformation of secondary H-bonded amide in –NH···O=C- complex). The intensity of C=O streching band increased relative to the amide I peak of NIPA, when the level of MA in the terpolymer was increased. It can be proposed that intra- and intermolecular H-bonded fragments are most probably formed between alternating NVP-MA-NIPA of macromolecules as follows: (Figure 3)

The UV-vis spectra of poly(NVP-co-MA-co-NIPA) was obtained using solutions in DMSO and deionized water. Figure 4(a), (b) obtains spectra for poly(NVP-co-MA-co-NIPA) in deionized water and DMSO, respectively. Their first absorptions (λ_{max}) appeared at 242 nm for poly(NVP-co-MA-co-NIPA) (reaction time: 6, 12, 24 and 36 h). The bands at 242 nm (reaction time: 6h, in deionized water) corresponded to $n \rightarrow \pi^*$ (C=O group in anhydride unit) transition.

The results obtained from the UV-vis of spectra of terpolymers at different wavelengths near maximum absorption in DMSO and deionized water are presented. As evidenced from the comparative analysis of these spectra, there is almost no change in the values of λ_{max} as a function of the type of solvent or polymerization time for the NVP-MA-NIPA terpolymers. Going from deionized water to DMSO (increasing the donor number from 18.0 to 29.8), the λ_{max} value increases 12 nm. As seen from the UV-vis spectra, the λ_{max} value shifts (bathocromic effect) with increasing NVP, MA and NIPA levels jards in the copolymer, and with increasing polymer chain length.

The synthesized copolymers display different thermal properties, including melting and glass-transition behaviors, depending on the content of hydrogen bonding fragments.[29] The TGA curves for poly(NVP-co-MA-co-NIPA) indicate that there are two-stages of decomposition. The first weight loss for the copolymers occurs around 146-220 °C and is the result of the loss of free water and solvent. The second weight loss is around 401-468 °C. The second-stage of decomposition is due to the rupture of the polymer chain. Poly(NVP-co-MA-co-NIPA) (reaction time: 24 and 36 h) has a higher decomposition temperature than do the other copolymers (reaction time: 6 and 12 h).

Figure 5 contains the DSC thermograms for both terpolymers prepared at different reaction times. These results indicate that the intensity and position of the higher temperature endo-peaks, which are associated with the melting point T_m, significantly depend on the monomer (NVP, MA and NIPA) ratios in the terpolymers, and especially on the degree of their alternation. It is known that the high melting points of terpolymers are associated with

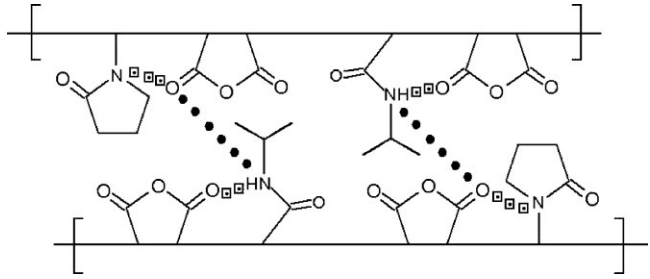

Figure 3.
Intra- and intermolecular interactions in Poly(NVP-co-MA-co-NIPA): • = intermolecular interaction, ⊡ = intramolecular interaction.

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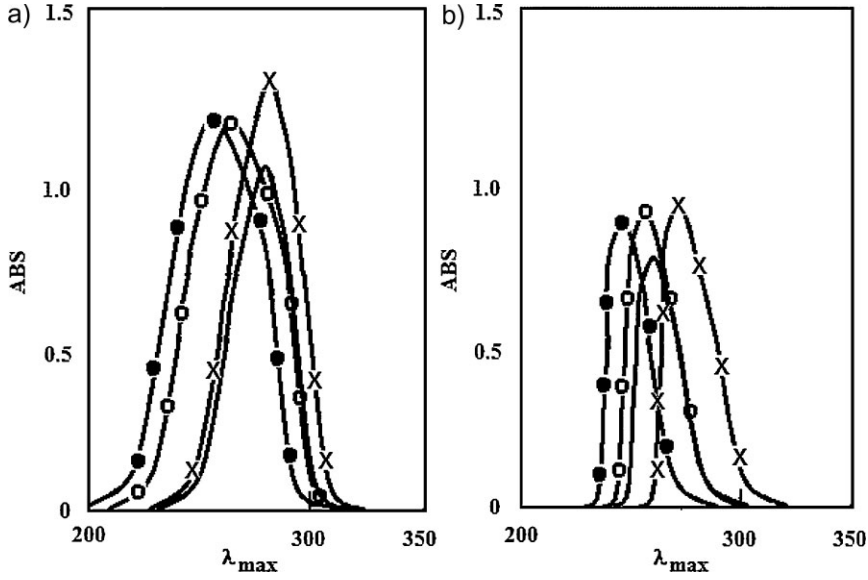

Figure 4.
UV-vis spectra of terpolymers (a) DMSO (10^{-5} M); (b) in deionized water (10^{-5} M); preparation time • = 6h, ◘ = 12 h, - = 24 h, X = 36 h.

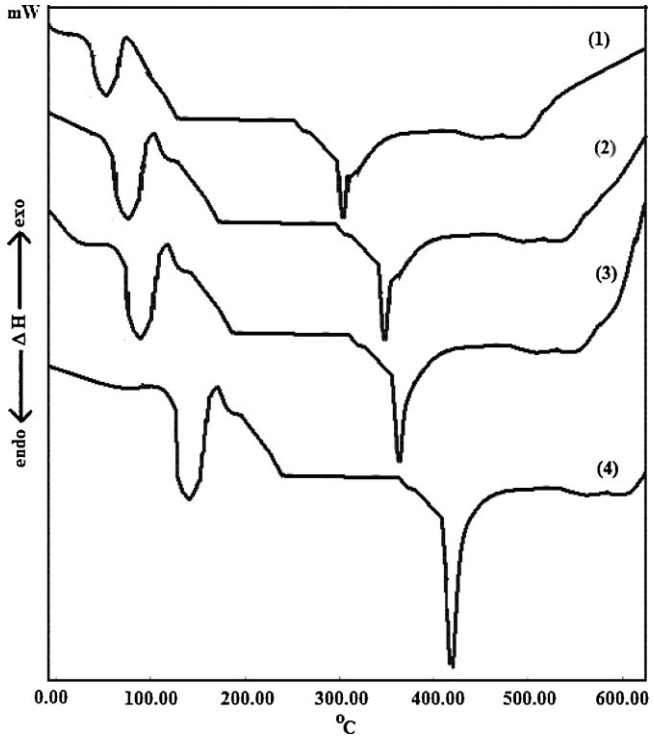

Figure 5.
DSC thermograms of terpolymers formed at different reaction times: (1) 6h, (2) 12 h (3) 24 h, (4) 36 h.

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Table 2.
Thermal behavior of a NVP: MA: NIPA terpolymer.

Reaction time	DSC and DTA analysis					TGA analysis		
(h)	T_g °C	ΔH mJ	T_m °C	ΔH mJ	T_d °C	T_i °C	T_m °C	T_f °C
6	75.60	300.90	300.10	202.00	290.77	110.00	135.80	162.30
12	81.25	230.90	340.50	340.95	320.80	132.80	165.00	180.13
24	94.62	738.27	365.75	647.59	368.37	145.67	192.59	220.37
36	120.15	430.09	410.20	343.77	409.10	160.15	220.12	247.80

many factors including intra- and intermolecular structural regularity and rigidity of macromolecules.[29]

The lower temperature endo-effects in the DSC curves, associated with the glass transition temperature (T_g), change insignificantly with increasing NVP and NIPA levels in the terpolymers. This result indicates that the mechanism of glass-transition is similar in these terpolymers.[32]

The values of T_g, T_m and ΔH for the terpolymers are presented in Table 2. It is shown that an increase in reaction time and an increase of NVP and NIPA content in the terpolymers increases the value of T_m from 300 to 410 °C. The observed relationship among the Tm values for the four terpolymers is related to the effects of carbonyl group of the MA unit and the amide group of the NIPA unit which give rise to both intra and intermolecular H-bonded fragments in the terpolymers. The higher values of T_m (reaction time: 36 h) are observed for the terpolymers with composition 1:2:1. NVP: MA: NIPA Therefore, a rigid H-bonded structure provides terpolymers with high T_m. This seems to be related to the crystalline phase that is formed through intermolecular hydrogen bonding between free –COOH groups.[32]

Figure 6 contains DTA curves for the terpolymer produced at different reaction times. The first broad endo-effects around 75.60-120.15 °C on the DTA curves might be related to a ''physical aging'' of H-bonded terpolymers which is accompanied by the breaking of inter- and intramolecular -NH···O = C- bonds of the macromolecules. It is necessary to note that the character and position of these peaks, as well as T_g from the DSC curves, are unaltered for the terpolymer. The same values were obtained from DTA and DSC. The lower temperature endotherm on the curves are related to T_m as on the DSC curves. The flowing (T_f) and decomposition (T_d) temperature regions for the terpolymers are around 162-248 and 291-409 °C.

The results of DSC and DTA studies for the copolymer produced at different reaction times indicate the formation of a semicrystalline structure which might arise from H-bonded intermolecular alternating fragments of macromolecules.[28]

The results of DSC and XRD studies of the terpolymer composition-thermal behavior relationship and crystallinity for the synthesized terpolymers can also as an additional confirmation of the formation of the intermolecular and/or intramolecular H-bonded macromolecular structure in the systems. Figure 7 shows the X-ray diffraction (XRD) patterns of poly(NVP-co-MA-co-NIPA) produced at different reaction times (24 h and 36 h). Poly(NVP-co-MA-co-NIPA) (36 h) powders exhibit ''sharp peak'' at 2θ angles around 24°. This peak is a characteristic of terpolymer, and it is sharper in poly(NVP-co-MA-co-NIPA) (24 h). It indicates terpolymers crystallization to a certain extent. Semicrystalline structure is also confirmed by X-ray diffraction analysis of terpolymers. As evidenced from XRD patterns, illustrated Figure 7, terpolymers consist of amorphous phase and crystallinity (X_c= 32-39%), the degree of which depends on the content of MA and NIPA in terpolymer. The values of crystalline and amorphous phase area and crystallinity (%) for terpolymers are presented in Table 3.

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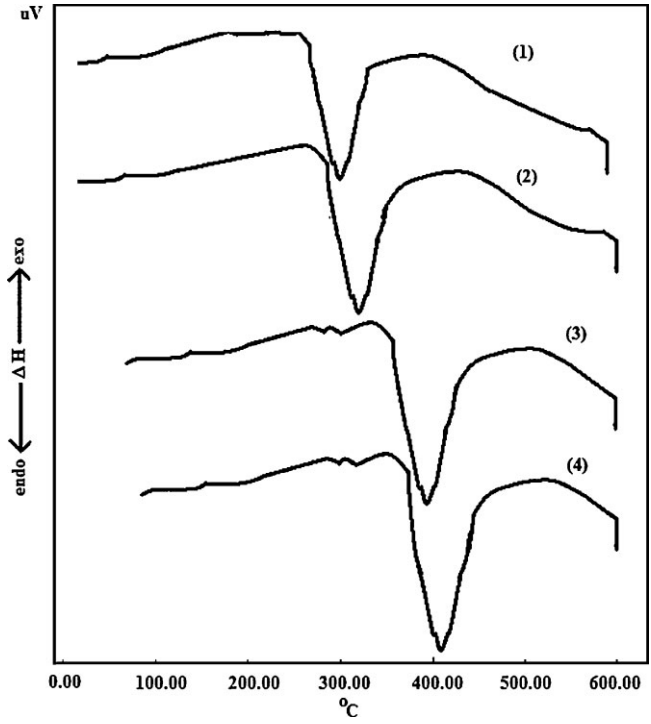

Figure 6.
DTA curves for poly(NVP-co-MA-co-NIPA) produced at different reaction times: (1) 6h, (2) 12 h (3) 24 h, (4) 36 h.

Poly(NVP-co-MA-co-NIPA) terpolymers have different morphologies (Figure 8). SEM images of terpolymers produced at different reaction times indicate the formation of intermolecular interaction alternating fragments of macromolecules (especially 36 h). This agrees well with thermal and XRD analysis. Terpolymer (reaction time: 36 h) has regular structure. Other terpolymers (reaction time: 6, 12 and 24 h) have porous structure.

The number-average molecular weight (M_n), weight-average molecular weight (M_w) and polydispersity index (PDI) values of terpolymers are presented in Table 4. Poly(NVP-co-MA-co-NIPA) terpolymers prepared at different reaction time show

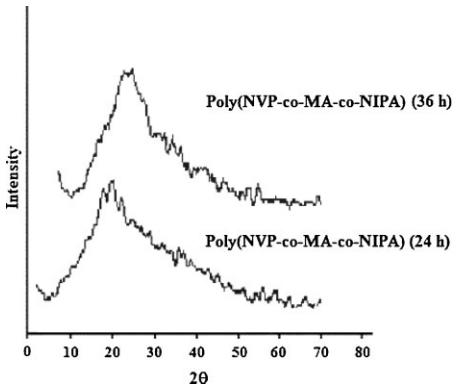

Figure 7.
X-ray diffraction patterns of poly(NVP-co-MA-co-NIPA) (24 h and 36 h).

Table 3.
Crystallinity of Poly(NVP-co-MA-co-NIPA) terpolymers.

Reaction time	Crystallinity (by XRD)		
h	Area		
	Cryst.	Amorph.	X_c (%)
6	30.1	65.3	32.2
12	32.7	58.9	33.4
24	37.6	63.4	37.5
36	39.7	59.2	39.2

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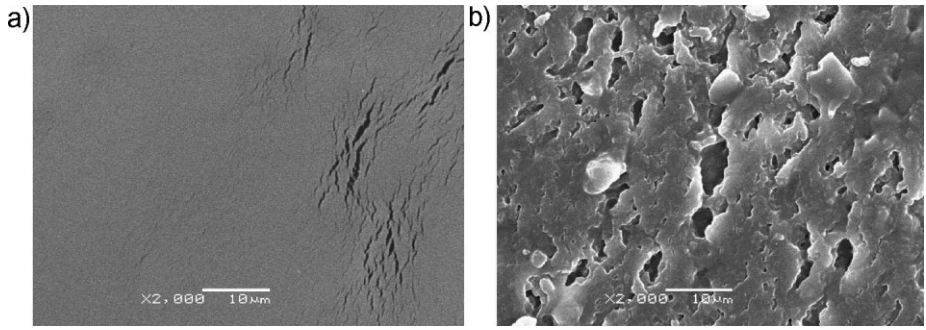

Figure 8.
SEM images of poly(NVP-co-MA-co-NIPA) produced at different reaction times: a) 36 h and b) 48 h.

Table 4.
Molecular weight averages of Poly(NVP-co-MA-co-NIPA) terpolymers.

Reaction time h	Molecular weight averages (by GPC)		
h	M_n	M_w (g/mol)	PDI
6	32,280	50,960	1.58
12	38,130	55,250	1.45
24	42,560	60,320	1.42
36	48,220	65,410	1.36

a continuous increase in M_n and M_w values with increasing NVP, MA and NIPA content in terpolymer structure.

Intrinsic viscosity of the terpolymers produced at different reaction times were determined in DMSO at $25 \pm 0.1\,^{\circ}C$ in the concentration range 0.1-1.0 g/dL using a Ubbelohde viscometer. [η]: 0.62 (reaction time: 6 h), 0.65 (12 h), 0.77 (24 h), 0.84 (36 h) dL/g.

It is reasonable to presume that the viscosity of polymer solution would be dependent on concentration and the average molecular size of the sample and hence the molecular weight.

The NVP/MA/NIPA terpolymer prepared at different reaction time show a continuous decrease in intrinsic viscosity with decreasing NVP, MA and NIPA content in terpolymer structure. Viscosity and GPC results suggested that the NVP/MA/NIPA comonomer composition has a great effect on the resultant terpolymer molecular weight.

Bactericidal Effects of Polymer

The antimicrobial effect of the on different pathogenic bacteria terpolymers on these microorganisms under *in vitro* conditions has been studied. The antibacterial activities of terpolymers were determined against six bacteria (*Staphylococcus aureus, Salmonella enteridis, Streptococcucs faecalis, Eschericia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). The data for the antimicrobial tests are summarized in Table 5.

All polymers had different antibacterial activity *in vitro* against the tested bacterial strains. Terpolymer (reaction time: 36 h) showed more antimicrobial activity than

Table 5.
Invitro antimicrobial activity of polymers against Staphylococcus aureus, Salmonella enteridis, Streptococcucs faecalis, Eschericia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa (Diameter of zone, mm).

Polymer (Reaction time, h)	Gram-positive bacteria			Gram-negative bacteria		
	S. aureus	*S. enteridis*	*S. faecalis*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*
6	21	23	25	18	17	9
12	23	24	27	19	18	11
24	24	25	28	20	18	12
36	26	27	30	22	19	14

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other terpolymers (reaction time: 6, 12, and 24 h) against these strains. The highest inhibition of growth occurred for *Staphylococcus aureus*. The terpolymers generally affected *Staphylococcus aureus*, whilst the terpolymers were less affective against *Pseudomonas aeruginosa*. All polymers showed low antimicrobial activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Microorganisms used in the present study are responsible for many hospital infections. For example, *Staphylococcus aureus* can cause pneumonia, meningitis, endocarditis, toxic shock syndrome (TSS) and septicaemia.

All polymers studied, containing biologically active moieties in the form of carboxylic acid, anhydride, and amide groups and acid/amide complexed fragments, were more effective against Gram-positive bacteria than against Gram-negative bacteria.[34] This observation may be explained by different surface layer structural architectures of the biomacromolecules of the test bacteria. If the compounds can form covalent complexes or interact electrostatically with the cell wall, it is very probable that the compounds will show biocidal activity.[35]

The terpolymers containing pendant groups derived from hydrolysis with strong hydrogen bonding tendicies easily form self-assembled structure with polymer chains through COO-. +NH noncovalent interaction between free carboxylic groups of MA units and NIPA groups (Figure 2). As the ratio of COOH/N increases (i.e., the number of COOH groups increase) in the terpolymers, antimicrobial activity of the terpolymer toward Gram-positive bacteria increases.[33]

The bacteria size varied between 500 and 3000 nm. This value is 20-60 times higher than the size of terpolymers, which were in the size range of peptidoglycan network holes. These terpolymers can interact with the cell membrane, producing changes that would finally cause the death of the bacteria. However, another probable mechanism is the interaction with teichoic acid at the cell wall. This antity which contains phosphate bridges (negative charges) that could interact electrostatically with the polycations. This would permit the polymer to stay at the bacterial cell, blocking the ion exchange channels, inhibiting growth, and producing cell death. Another possibility is the interaction with peptidoglycan, producing degradation and finally cell death.[35]

Conclusion

The synthesis of poly(NVP-coMA-co-NIPA) by radical-initiated polymerization in anhydrous medium has been accomplished. This polymer, of varying molecular weight, is soluble in deionized water and various organic solvents. The terpolymers were characterized using UV-vis, FTIR, ^{1}H NMR, elemental (N content) analysis, thermal analysis (DSC, DTA and TGA), GPC, SEM and XRD. Using ^{1}H NMR spectroscopy and elemental analysis data reflecting copolymer composition, the values of m_1, m_2 and m_3 for monomer pairs were found. Terpolymers produced at longer reaction time display increased thermal stability. Lower these generated over those shorter reaction times. The results of DSC, DTA, TGA, XRD and SEM studies of the terpolymer provide evidence for the formation of an intermolecular H-bonded structure. Viscosity and GPC results suggested that the NVP/MA/NIPA comonomer composition has a great effect on the resultant terpolymer molecular weight. The new water-soluble terpolymer displays antimicrobial activity against three Gram-positive and three Gram-negative bacterial pathogens. With an increasing number of COOH and N units in the polymer, the antimicrobial activity was found to increase, possibly due to an increase in interaction between the cationic pendant moieties with the cell wall. It was observed that Gram-positive bacteria is more susceptible to inhibition by the terpolymer than is Gram-negative. This may be explained by the different responsive behavior of the surface layer structures

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of these two types of bacteria. This new water-soluble terpolymer may find use in medicine as drug component, in food industry or in the hospital as a disinfectant.

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