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Bioengineering Functional Copolymers. IX. Synthesis and Characterization of Stimuli-Responsive Poly(*N*-isopropylacrylamide-*co*-*N*-vinyl-2-pyrrolidone) and Its Cu(II) Complexes

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Abstract

Radical copolymerization of *N*-isopropylacrylamide (NIPA) with *N*-vinyl-2-pyrrolidone (VP) were carried out with 2,2'-azobisisobutyronitrile (AIBN) as an intiator in *N,N'*-dimethylformamide solution at 65°C under nitrogen atmosphere. Cu(II)–copolymer macrocomplexes were prepared by incorporation of copolymers with copper sulfate in aqueous solution at 40°C. Structure and composition of the obtained copolymers, and the formation of coordinated Cu(II)-complexes between amide VP unit and Cu(II) ions were studied by FTIR spectroscopy, DSC and TGA-DTG methods, as well as by the electrical conductivity measurements. Compositon of the copolymers synthesized in a wide range of monomer feed ratios were determined by FTIR and ^{1}H (^{13}C) NMR–DEPT-135 spectroscopy. The monomer reactivity ratios were determined by Fineman-Ross, Kelen-Tüdös and non-linear regression methods. It was observed that studied monomer pair has some tendency to alternation in the chosen monomer feed ratios due to formation of intermolecular interaction through H-bonding and N→O=C coordination. The copolymers predominantly show amorphous structure while their Cu(II)–macrocomplexes exhibit crystalline phase. The conductive properties of the synthesized Cu(II)–poly(VP-*co*-NIPA) complexes was also discussed.The synthesized poly(NIPA-*co*-VP)s show temperature sensitiveness (T_S) and higher glass-transition temperature (T_g) and thermal stability, polyelectrolyte and stimuli-responsive (temperature- and pH-sensitive) behavior, and can be attributed to the class of bioengineering functional copolymers useful for application in various gene- and bioengineering processes, drug delivery systems, and biomacromolecule conjugations.

Key Words: Functional copolymers, stimuli-responsive, poly(NIPA), *N*-vinyl-2-pyrrolidone, Cu(II) complexes.

INTRODUCTION

The temperature and pH sensitive stimuli-responsive polymers as synthetic models for the reversible cold denaturation of proteins have great potential in application as biocatalysts [1,2], drug delivery systems [3-5], superabsorbents [6], human gene vectors [7], in separation and purification of metal ions [8,9] and biomolecules [10]. One of the important properties of *N*-subsitituted acrylamide polymers is that they exhibit a first-order phase transition (collapse) caused by a small change in external conditions such as temperature and pH of

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medium, solvent composition, electric fied, etc. Most results were obtained for polyacrylamide as a sensitive temperature and pH sensitive homo- and various amphiphilic copolymers of NIPA [4,11-15]. Temperature and pH sensitive poly(NIPA) as a important bioengineering polymer was a subject of many extensive investigations in the field of modern biotechnology, specially in the fields of cell and enzyme immobilization, controlled drug delivery and gene delivery, and protein dewatering process [16-18]. Enzymatic characteristics of these biosystems were changed significantly, depending on the hydrophilicity of the functional polymer-modifier and the degree of modification. Recently, synthesis and characterization of maleimide-terminated oligo(*N*-isopropyl acrylamide) [19] and amphiphilic *N*-isopropyl acrylamide-acrylic acid random and graft copolymers [20], which can be used as a temperature-sensitive polymer to a genetically-engineered protein, new water-soluble at low temperature and shear-responsive poly[(*N*-isopropylacrylamide)-*co*-(*N,N*-(dimethylaminopropyl methacrylamide)] and their alkyl bromide derivatives having a delicate balance between thermosensitive, hydrophobic and ionic groups [21-23], cationic stimuli-responsive acrylic acid-terminated poly(*N*-isopropyl acrylamide) potentially useful as carrier for gene delivery, conjugates of poly(*N*-isopropyl acrylamide) with amino acids as prodings [24-26].

Synthesis and characterization of copolymers of *N*-isopropyl acrylamide with maleic and citraconic anhydrides and their macrobranched (polyethylene oxide) and macrocomplexed (polyethylene imine) potentially useful as carrier for gene delivery and conjugates with biopolymers, have been reported [13-15].

Homo- and copolymers of *N*-vinyl-2-pyrrolidone (VP) are of considerable academic and industrial interest due to their unique properties allowing to use of these polymer systems in lithography as light sensitive thin coatings for printing plates, for preparation of separating membranes for ultrafiltration, biocompatible polymers with low toxicity and carriers of biologically active compounds, sorbents, coagulants and floculants [27,28]. VP (M_1) has been copolymerized with a considerable variety of functional comonomers (M_2), such as styrene ($r_1 \sim 0$ and $r_2 = 14.8$) [29], vinyl acetate [30], 2-vinyl- and 4-vinylpyridines ($r_1 = 0.3$ and $r_2 = 4.3$, and $r_1 = 0.5$ and $r_2 = 3.9$) [31], vinylcyclohexane ($r_1 = 0$ and $r_2 = 1.1$) [32], maleic anhydride [33,34], hydroxypentyl maleimide [35], and other monomers [36-38]. Homo- and copolymers of VP can form reversible association complexes with dyes of some reactive polymers [39]. Anufrieva et al. [40] studied complexing properties and conformational transitions of temperature sensitive poly(*N*-vinyl pyrrolidone-*co*-*N*-vinylcaprolactam), poly(VC-*co*-VP) macromolecules, depending on the comonomer unit content in the copolymer, in aqueous solutions by luminescence method. It was established that the complexing ability of copolymers with small molecules (phenol, sodium dodecyl sulphate, etc) even at a low content of VC units exceeds that of poly(VP). Recently, Eeckman et al. [41] have reported synthesis and characterization of thermosensitive copolymers of NIPA with hydrophylic comonomers, such as acrylamide, methacrylamide, *N*-methyl-*N*-vinylacetamide, *N*-vinylacetamide, 4-acryloyl morpholine and VP. Compositions of copolymers, including poly[NIPA-*co*-VP(2.1-18.3 mol %)]s with relatively lower content of VP units were determined by ^{1}H NMR spectroscopy.

On the other hand, polymeric metal complexes, moreover, are of great significance in different fields of chemistry, such as catalytic reactions, mining separations, biochemistry, medicine, and environmental chemistry [42]. Several functional hydrophilic polymers with chelating ligands used for complexation in the homogeneous phase have been investigated. These hydrophilic and noncrosslinked functional polymers that are able to complex metal ions have been termed polychelatogens which are characterized by two component: the polymer backbone which usually provides solubility and stability; and the functional groups which are necessary for metal complexation. Different approaches, introduction of metal-complexing groups into the polymers backbone, and varying the type of polymeric chain were investigated [43]. Metal macrocomplexes often show a specific catalytic activity based on the large ligand comparable to metal enzymes; they are considered to be analogues of synthetic enzymes. Complexation of polymeric ligand with metal ions and ligand substitution reaction of polymer-metal complexes are used to separate metal ions and/or small molecules [43]. Chelating of metal ions in solution is an important process in several areas, for example, design of ligands as therapeutic reagents for the treatment of metal intoxication [44,45], design of antibiotics that owe their antibiotic action to specific metal complexation [46], design of complexes to act as imaging agents [47] in the body, design of functional groups for chelating ion-exchange materials [48], and catalysts [49,50].

As evidenced from above mentioned short reviewed analysis, growing interest and much effort have been focused on the synthesis of various pH- and temperature sensitive copolymers of NIPA and evaluation of their suitability for the bioengineering applications. On the other hand, considerable progress has been demonstrated in the synthesis of bioengineering polymer systems on the base of VP homo- and copolymers, which in generally do not show stimuli-responsive behavior at physiological temperature. Thus, it can be proposed that copolymers of NIPA with VP can serve as ideal initial materials for developing new generation of bioengineering functional polymers with a wide range of useful properties for medical, pharmaceutical and gene engineering applications.

In the present article, results of synthesis and characterization of poly(NIPA-*co*-VP)s and their Cu(II) macrocomplexes with different compositions by the radical-initiated solution copolymerization of NIPA and VP in a wide range monomer ratios (Scheme 1), and the copolymer composition-stimuli responsive relationship are described and discussed. Special attention is spared on the effect of H-bonded interactions in the monomer feed and between monomers and growing macroradicals, the copolymer composition-property (thermal and stimuli-responsive behavior) relationship of synthesized copolymers, as well as the studies of the Cu(II)-macrocomplex composition–property (thermal behavior and conductivity) relationship, and to evaluation of the conductivity of synthesized Cu(II)–poly(VP-*co*-NIPA) complexes.

EXPERIMENTAL SECTION

Materials

NIPA monomer (Aldrich) was purified before use by distillation under vacuum and recrystallization from diethyl ether solution: b.p. 91.5°C/2 mm, m.p. 61.8°C (by DSC); ^{1}H NMR spectra ($CHCl_3$-d_1), ppm: 1H NH 7.75, 1H CH= multiplet 6.19-6.26, 2H CH_2= two doublets at 6.11-6.16 and 5.45-5.48, 1H CH (isopropyl group) 4.16 and 6H CH_3 (isopropyl group) 1.12. VP monomer (Aldrich) was purified by distillation in moderate vacuum: 2,2'-Azobisiso-butyronitrile (AIBN) (Fluka) was twice recrystallized from methanol: m.p. 102.5°C.

Copolymerization Procedure

Copolymerizations of NIPA with VP using various monomer feed ratios were carried out in *N,N'*-

dimethylformamide (DMF) at 65°C with AIBN radical initiator at constant total concentration of monomers under nitrogen atmosphere. Reaction conditions: $[M]_{total}$ = 1.66 mol/L, [AIBN] = 4.55.10^{-3} mol/L and monomer ratios of [NIPA]/[VPBA] = 0.25–4.0, conversion ≤ 10% (for the determination of monomer reactivity ratios). Appropriate quantities of monomers, DMF and AIBN were placed in a standard pyrex-glass tube, and the reaction mixture was cooled by liquid nitrogen and flushed with dried nitrogen gas for at least 2 min, then soldered and placed in a thermostated silicon oil bath at 65 ± 0.1°C. The NIPA-VP copolymer was isolated from reacted mixture by precipitation with diethyl ether. Copolymer was dissolved in 1,4-dioxane and precipited by diethyl ether, then washed with several portions of benzene and diethyl ether, and dried under vacuum at 40°C. The copolymer compositions were found by FTIR and ^{1}H NMR spectroscopy using analytical bands (3070 cm^{-1} for NIPA unit and 1495 cm^{-1} for VP unit) and integral area of chemical shifts of monomer functional groups, respectively, for quantitative analysis. Poly(NIPA-*co*-VP) prepared from equimolar ratio of initial monomers has the following average characterictics: The number-average molecular weight M_n 10,200 g/mol, the weight-average molecular weight M_w 13,600 g/mol and the dispersity index 1.33 (by GPC); the monomer unit ratio, m_1: m_2 = 48.7 : 51.3 (by ^{1}H NMR); $[h]_{in}$ 0.24 dL/g in 1,4-dioxane at 25 ± 0.1°C; temperature sensitiveness T_s 57.8°C, *DH* 10.0 J/g, glass-transition temperature T_g 147.9°C, D*H* 0.29 J/g (by DSC) and decomposition temperature T_d 375°C (by TGA).

FTIR spectra (KBr pellet), cm^{-1}: 4375 (w-m) and 4050 (w) combination of stretching and deformation of CH_2 and CH_3 groups, 3440 (vs, broad) complexed ternary amide of VP, 3300 (s, broad) NH stretching of *trans*-associated secondary amide, 3070 (m) overtone NH amide bending band, 2975-2810 (s-m), antisym. and sym. CH stretching in CH and CH_2 backbone, and CH_2 and CH_3 side-chain groups, 2125 (m, broad) H-bonded NH stretching, 1665 (vs, broad) C=O stretching band of pyrrolidone ring and C=O stretching amide I band, 1550 (s, broad) NH deformation-amide II band (only in associated *trans* form) coupled with C-N stretching, 1495 (m) CH_2 scissor vibration and CH_3 antisym. deformation, 1470 (s) H-bonded NH deformation in -NH…O=C-, 1422 (s) and 1440 (s) strong sharp bands due to CH_2 bending or C-N stretching, 1385 (s) and 1368 (s) two bands for CH_3 sym. deformation in isopropyl group, 1338 (w) and 1315 (m) CH_3 sym. deformation, 1290 (s) *trans*-amide III band 1230 (s) CH_2 wagging, 1175 (s) broad C–N stretching or CH_3 rocking, 1100 (m) CH_3 rocking, 1020 (m) NH bending in –NH…O=C–, 980 (m-w) CH_3 rocking, 935 (s-m) CH_2 wagging and twist or CH_3 rocking coupled with skeletal modes, 900 (m-w) CH out-of-plane deformation, 845 (s) CH_2 pyrrolidone ring, 730 (m) CH_2 rocking or NH wagging, 650 (m) N-C=O bending, 575 (w) C=O out-of-plane bending.

^{1}H NMR spectra (in $CHCl_3$-d_1 at 27 °C), ppm: (5 and 6) 6H, CH_3 (in NIPA isopropyl group) 1.13, (1) 2H, CH_2 (backbone in NIPA unit), (2) 1H, CH (backbone in NIPA unit) and 2H, CH_2 (backbone in VP unit) 1.25-2.12, (10) 2H, CH_2 (VP ring) 2.35, (11) 2H, CH_2 (VP ring) 3.21, (9) 2H, CH_2 (VP ring) 3.41, (4 and 8) 1H, CH (in NIPA side-chain and VP backbone) 3.62-4.15, (3) 1H NH (in NIPA amide group) 6.09-7.28 (broad signal).

In the similar conditions, poly(NIPA) and poly(VP) homopolymers were synthesized and characterized. Poly(NIPA): $[\eta]_{in}$ 0.012 dL/g in dioxane at 25 ± 0.1°C, T_s 45.7°C and T_g143.5°C (by DSC). ^{1}H NMR spectra (in $CHCl_3$-d_1), ppm: 1H NH broad peak with centred at 6.44, 1H CH (isopropyl group) 4.24, 1H CH backbone 2.32, 2H CH_2 backbone 1.86-2.07 and 6H CH_3 (isopropyl group) 1.38. Poly(VP): $[h]_{in}$ 0.061 dL/g in dioxane at 25 ± 0.1°C, T_s 62.9°C and

T_g 172.7 (by DSC). ^{1}H NMR spectra (in $CHCl_3$-d_1), ppm: 1H CH backbone 3.95-4.11, 2H CH_2 (N-CH_2 ring) doublet centred at 3.45, 2H CH_2 (CO-CH_2 ring) doublet at 2.43-2.59, 2H CH_2 (C-CH_2-C ring) doublet centred at 2.27 and 2H CH_2 backbone around 1.64-1.94.

Preparation of Complexes

The synthesis of Cu(II)–poly(VP-*co*-NIPA) macrocomplexes includes the following procedure: In a typical example, copolymer was resolved in 0.05 M $CuCl_2.H_2O$ solution then stirred vigorously for 45 min at 25°C. The solid polymeric Cu(II)-complex was precipitated instantaneously and filtered to obtain. The excessive copper ions in the complex were washed by deionized water. Then the solid powder was dried under vacuum at 50°C for 48 h. For all the synthesis, molar ratio of copolymer (VP-unit):$CuCl_2$ = 1:1 was used. Synthesized complexes were characterized FTIR spectroscopy and DSC and TGA analyses, as well as by electrical conductivity measurement.

Copolymer Characterization

The molecular weights (M_n and M_w) and the dispersity index (M_w/M_n) of the copolymers were determined by Gel-Permeation Chromatography using a Shimpack 804 column with THF as a mobile phase-eluent at flow rate of 1.0 mL/min at 35°C. Standard polystyrene was used for molecular weight calibration. The intrinsic viscosities of the copolymers with different compositions were determined in dioxane at 25 ± 0.1°C in the concentration range of 0.1-1.0 g/dL using an Ubbelohde viscometer. Fourier transform infrared (FTIR) spectra of the copolymers (KBr pellet) were recorded with FTIR Nicolet 510 spectrometer in the 4000-400 cm^{-1} range, where 30 scans were taken at 4 cm^{-1} resolution. ^{1}H{^{13}C} NMR (DEPT-135) spectra were recorded on a JEOL 6X-400 (400 MHz) spectrometer with $CHCl_3$-d_1 as the solvent at 27°C. The compositions of the copolymers synthesized using various monomer feed ratios were determined by known FTIR [51] and NMR methods [13] and were achieved by comparing the absorbance values of NH (amide) and CH_2 (VP ring) bands and the integrals of the CH_3 and NH regions in FTIR and ^{1}H NMR spectra of NIPA and VP units, respectively.

Thermal Behavior

Differential scanning calorimetry (DSC) and differential (DTG) and gravimetrical (TGA) thermal analyses of copolymers were performed on a DuPont TA 2000 calorimeter and Setaram Labsys TG-DTA 12 Thermal Analyzer, respectively, under nitrogen atmosphere at a heating rate of 10°C/min. Before these analyses, all the polymer samples (10 mg) were thermotreated at 110°C during 30 min to remove their thermal history, followed cooling to 20°C.

Coil-Globule Transition Temperature

The lower critical solution temperature (LCST) measurements were performed in a UV spectrophotometer (UV 1602, Shimadzi, Japan) equipped with a heating system and temperature control unit. The temperature of the polymer solutions (1.0 wt.%) at pH 4.0 (an acetic acid/acetate buffer) or 7.4 (a phosphate buffer) was increased at a rate of 1.0°C/min starting from room temperature, and the absorbance of the solutions was periodically recorded at a wavelength of 500 nm. The LCST value as a coil-glouble transition temperature of studied polymer in aqueous solution was calculated from the absorbance-temperature curve [24].

RESULTS AND DISCUSSION

The Monomer Reactivity Ratios

Taking into consideration the monomer structural peculiarities, especially the character of double bond conjugation, NIPA is an electron acceptor monomer with p(C=C)→ p'(C=O) conjugation, while VP is an electron donor monomer with *p*(*N*)→p(C=C) conjugation, and therefore, they have different values of polarity and specific activity of their radicals: e_1 = -0.71 and Q_1 = 0.26 for NIPA monomer [13] (e_1 = -0.26 and Q_1 = 0.41 for *N,N*-dimethylacrylamide as a model monomer) [52] and e_2 = -1.62 and Q_2 = 0.088 for VP comonomer [52]. Macromolecules of both NIPA and VP homopolymers have amphiphilic character containing highly polar secondary (NIPA) and ternary cyclic (VP) amides, which confirm their polar and hydrophilic attracting properties, while backbone methylene and methine groups in both monomers, and side chain isopropyl fragment in NIPA unit provide the hydrophobic properties. Both polymers possess higher tendency to form complexes with various negatively charged natural and synthetic polymers, and consequently these polymers find wide applications in bioengineering and medicine [28,53]. On the other hand, the formation of H-bonded complex is most possible between amide and pyrrolidone ring carbonyl (-NH…O=C-) groups and also the complex between highly polar ternary amide of VP and NIPA carbonyl group (>N→O=C-) if these monomers are employed as a copolymerized monomer pair. In this case, the monomers must have sufficient activity in free-radical copolymerization thanks to the interaction between functional groups of the comonomers or macroradicals.

Poly(NIPA-*co*-VP)s are synthesized by radical copolymerization method in the chosen conditions which are considered in experimental part. Composition of prepared copolymers using a wide range of monomer feed is not appropriate for determination by elemental (nitrogen analysis) because both monomer units contain *N* atoms. For this system, spectroscopic techniques are most convenient for the composition analysis of copolymers. In this study, Fourier transforms infrared (FTIR) and ^{1}H (^{13}C -DEPT-135) NMR analysis methods for evaluation of some structural peculiarities of synthesized copolymers and determination of copolymer compositions have been used.

Comparative analysis of FTIR spectra of homopolymers and copolymers indicates that the band at 1665 cm^{-1} (vs, broad) assigns to stretching of the H-bonded C=O in the VP unit. This band occurs at a lower frequency than in VP homopolymer (1695 cm^{-1}) indicating that it could not be attributed to carbonyl group in poly(VP) but to H-bonded carbonyl groups (–C=O…HN–), as secondary NH amide of NIPA units are involved. The band at 2125 cm^{-1} (m, broad) also associates with H-bonded NH stretching in NIPA units. The bands at 3440 (vs, broad) and 3300 cm^{-1} (s, broad), attributed to the NH stretching in NIPA units, show also changes. The broad band at 3300 cm^{-1} decreases while the one at 3440 cm^{-1} increases. The band at 3300 cm^{-1} could be assigned to H-bonded NH, mainly to the C=O of VP unit. The band at 3440 cm^{-1} (higher energy) is assigned to uncomplexed NH band.

The formation of above mentioned type of complexes is also confirmed by ^{1}H and ^{13}C NMR (DEPT-135) studies of copolymer structure, having different compositions. Results of comparative spectral analysis of homo- and copolymers, which are presented in Table 1, indicated that the chemical shifts of protons from NH (amide), CH (isopropyl) and CH_3 (isopropyl) groups of NIPA unit and from CH (backbone in CH-N), three CH_2 groups

Table 1. Effect of N→O=C coordination on the ^{1}H NMR chemical shifts the main groups in poly(NIPA-*co*-VP)s.

Copolymer composition (mol %)		NIPA unit ($\Delta\delta$, ppm = $\delta^f - \delta^c$) ^{a}			VP unit ($\Delta\delta$, ppm = $\delta^f - \delta^c$) ^{a}	
m_1	m_2	$\Delta\delta$ (NH)	$\Delta\delta$ (^{4}CH)	$\Delta\delta$ (^{8}CH)	$\Delta\delta$ (^{9}CH$_2$)	$\Delta\delta$ (^{11}CH$_2$)
100 ^{b}	0.0	6.44	4.24	-	-	-
69.9	30.1	-0.60	0.56	0.01	0.26	0.18
48.8	51.2	-0.59	0.56	0.02	0.26	0.25
25.3	74.7	-0.58	0.54	0.07	0.28	0.26
0.0	100 ^{b}	-	-	3.95	3.45	2.59

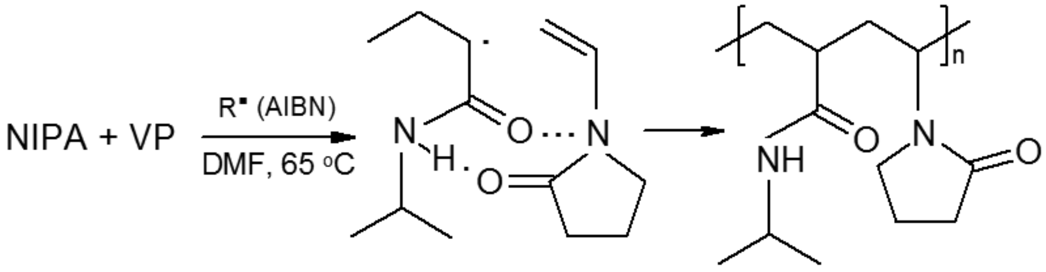
^{a} δ^f and δ^c are the chemical shifts of protons from monomer units in poly(NIPA), poly(VP) and poly(NIPA-*co*-VP); (-) negative value of $\Delta\delta$ means a displacement of chemical shift to weaker field.

^{b} The values of δ^f for homopolymers of NIPA and VP, respectively.

(pyrrolidone ring) of VP unit, which showed higher sensitivity to -NH…O=C- complexing, significantly change when they are introduced into copolymer chain. Full displacement of NH proton from 6.44 (for homopolymer NIPA) to 7.03 ppm (for copolymer) is observed for the alternating and random (content of VP units 74.7 mol%) copolymers. However in the case of random copolymer enriching with NIPA units, proton of NH appears in the form of two broad signals which can be attributed to the H-bonded (7.03 ppm) and uncomplexed (6.42 ppm) NH group, respectively.

As can be seen from these data, the most essential changes of chemical shifts [$\Delta\delta = \delta^f$(homopolymer) - δ^c (copolymer)] are observed for the amide NH ($\Delta\delta_{NH}$ = -0.59 ppm) and isopropyl groups ($\Delta\delta_{CH}$ = 0.56 and $\Delta\delta_{CH3}$ = 0.28 ppm) of NIPA linkage and methylene groups of VP ring in the copolymers with $m_1 >> m_2$, $m_1 \cong m_2$ and $m_1 << m_2$, respectively, providing maximum probability for the inter- and/or intramolecular interaction in copolymer macromolecules. It is a very important fact that this strong H-bonding effect takes place in the alternating copolymer (Table 1). Naturally, it can be proposed that observed H-bonding will be also affected by the conjugation of acrylic (acceptor) and vinyl (donor) groups in the studied monomer system. Taking into consideration of this observed effect, general scheme of donor-acceptor type of radical copolymerization can be illustrated as follows.

Copolymerizations were carried out to low conversions (≤ 10 %) in order to determine monomer reactivity ratios in the steady-state kinetics by using known terminal model of the Fineman-Ross (FR) [54] and Kelen-Tüdös (KT) [55] equations:



Scheme 1. Hydrogen bonding and -N…C=O coordination effects in radical copolymerization.

$$F(f-1)/f = (F^2/f)\, r_1 - r_2 \qquad (1)$$

$$\eta = (r_1 + r_2/\alpha)\, \xi - r_2/\alpha \qquad (2)$$

where $\eta = [F(f-1)/f] / (F^2/f + \alpha)$; $\xi = (F^2/f)/(F^2/f + a)$; F = [NIPA] / [VP] and $f = m_1/m_2$; α (arbitrary constant) = $\sqrt{}\ (F^2/f)_{min} \cdot (F^2/f)_{max}$.

For comparison, non-linear regression (NLR) procedure using a microcomputer program [56] has also been applied to recalculate copolymerization constants.

The synthesized poly(NIPA-*co*-VP)s were characterized by FTIR and ^{1}H NMR spectroscopy for the determination of NIPA and VP unit contents. The absorption value ratios between characteristic analytical bands of 3070 cm^{-1} (for NH band in NIPA unit), 1495 cm^{-1} (for CH_2 ring band in VP unit) and the least changing absorption band of 1370 cm^{-1} as a standard band ($A = \log (I_o/I)$, $\Delta A^i = A^i/A^{1370}$) were used to calculate the copolymer compositions. Molar fractions (in mol %) of comonomer units (m_1 and m_2) in copolymers using FTIR analysis data are calculated according to the following equations:

$$m_1 / m_2 = (\Delta Am_1^{3070} / \Delta Am_2^{1495}) \times (M_2/M_1) \qquad (3)$$

where M_1 and M_2 are molecular weights of NIPA and VP monomer units, respectively.

Molar fraction ratios of the comonomer units (m_1/m_2) in NIPA-VP copolymers using ^{1}H NMR analysis data were calculated according to the following equations:

$$m_1/m_2 = f = n_2\, Am_1(CH_3) / n_1 Am_2(^{10}CH_2 \text{ ring}) \qquad (4)$$

where Am_1 and Am_2 are the normalized areas per H from the corresponding functional groups of the monomer unit regions in ^{1}H NMR spectra; n_1 and n_2 are the integers of proton(s) in the functional group of the monomers.

On the base of FTIR analysis data the values of absorption bands for the comonomer units are calculated which are used for the determination of copolymer compositions according to equation (3). The results obtained are presented in Table 1. Results of ^{1}H (^{13}C) NMR (DEP-135) analyses of copolymers are summarized in Table 2 and 3. As evidenced from these data, change of NIPA and VP concentrations in monomer feed from 40 mol % to 60 mol % leads to the formation of copolymers with almost similar molar ratios of m_1/m_2 monomer units. The composition of copolymer is dependent on the monomer feed ratio and can be varied from random (for the high concentration of NIPA or VP) to alternating (for nearly equimolar concentration of monomers). Studied monomers have a tendency for the alternating copolymerization, especially at the given monomer feed compositions close to (1:1) (40:60 – 60:40).

Table 2. Copolymer composition–chemical shift relationship from ^{13}C NMR (DEPT 135) analysis of poly(NIPA-*co*-VP)s with different compositions.

Monomer unit ratio m_1/m_2	Chemical shifts of carbon atoms (δ, ppm)			
	NIPA unit		VP unit	
	$^{5,6}CH_3$	^4CH	^8CH	$^9CH_2$
69.9/30.1	23.0	42.7	48.6	36.0
48.8/51.2	22.8	45.1	48.4	42.5
25.3/74.7	18.7	46.8	46.7	45.1
Dd =	4.3	– 4.1	1.9	– 9.1

Table 3. FTIR and ^{1}H NMR analysis of copolymers synthesized from various monomer mixture.

monomer feed (mol %)		$\Delta A_1{}^a$ (3070 cm^{-1})	$\Delta A_2{}^a$ (1495 cm^{-1})	copolymer composition (mol %)	
		integral area			
[NIPA]	[VP]	$Am_1(CH_3)$	Am_2 (CH_2)	m_1	m_2
					(by FTIR)
80	20	0.575	0.253	69.06	30.94
60	40	0.459	0.324	58.18	41.82
50	50	0.500	0.470	48.90	51.10
40	60	0.406	0.592	40.65	59.35
20	80	0.342	0.945	26.20	73.80
					(by ^{1}H NMR)
80	20	0.555	0.056	69.90	30.10
60	40	0.421	0.097	59.18	40.82
50	50	0.742	0.260	48.74	51.26
40	60	0.598	0.753	44.25	55.75
20	80	0.188	0.273	25.30	74.70

[a] The values of $\Delta A_1 = 0.176$ and $\Delta A_2 = 0.273$ for poly(NIPA) and poly(VP), respectively; 1370 cm^{-1} is used as a less changed standard band.

The competitive H-bonding and coordinating interactions between monomer units in the copolymers with different compositions were also studied by means of ^{1}H{^{13}C} NMR. The analysis of ^{1}H and ^{13}C NMR spectra indicates that the chemical shifts of the protons or carbon atoms of the methine and methylene groups at nearly NH (NIPA) and cyclic amide (VP) fragments significantly change as compared with those in poly(NIPA) and poly(VP) macromolecules, respectively. Calculated values of $\Delta\delta$ ($\Delta\delta = \delta^f - \delta^c$, where δ^f and δ^c are chemical shifts of the methine and methylene protons or carbon atoms in homopolymers and complexed linkages of copolymers, respectively) are presented in Table 2 and 3. These observed changes may serve as an additional reasonable confirmation for the presence of complexed fragments in the structure of copolymer macromolecules.

Monomer reactivity ratios (r_1 and r_2) were evaluated using experimental data, presented in Table 4 and 5, from FR plots of (F^2/f) vs. $F(f-1)/f$ or KT plots of ξ vs. η and NLR analysis. As evidenced from these values, which are summarized in Table 3, copolymerization constants determined by FR or KT method and calculated by NLR method have similar values indicating good agreement between both FTIR and ^{1}H NMR analysis techniques. Obtained r_1 and r_2 values for NIPA–VP system are also close to those for methyl methacrylate (weak acceptor)–styrene (strong donor) pair ($r_1 = 0.46$ and $r_2 = 0.52$) a known as a better-copolymerization pair [57]. In general, these results suggest that the chain growth reactions proceed predominantly by the addition of NIPA monomer to ~VP$^{\cdot}$ macroradical through intermediate coordinating via NH→O=C and N→O=C bonding between functional groups of comonomers and their growing macroradicals.

Table 4. Fineman-Ross (FR) and Kelen-Tüdös (KT) parameters for determination of the monomer reactivity ratios for NIPA–VP pair.

FR-parameters		KT-parameters		
F^2/f	$F(f\text{-}1)/f$	$F^2/f + \alpha^a$	ξ	η
(by FTIR)				
7.17	2.21	8.29	0.864	0.266
1.62	0.42	2.74	0.590	0.154
0.96	0.04	2.08	0.460	0.021
0.65	-0.31	1.77	0.366	-0.173
0.18	-0.45	1.30	0.135	-0.349
(^{1}H NMR)				
6.90	2.28	8.01	0.861	0.285
1.55	0.47	2.66	0.583	0.177
1.05	-0.05	2.16	0.486	-0.023
0.57	-0.18	1.68	0.339	-0.107
0.18	-0.48	1.29	0.139	-0.372

^{b} α = 1.123 (by FTIR) and 1.110 (by ^{1}H NMR).

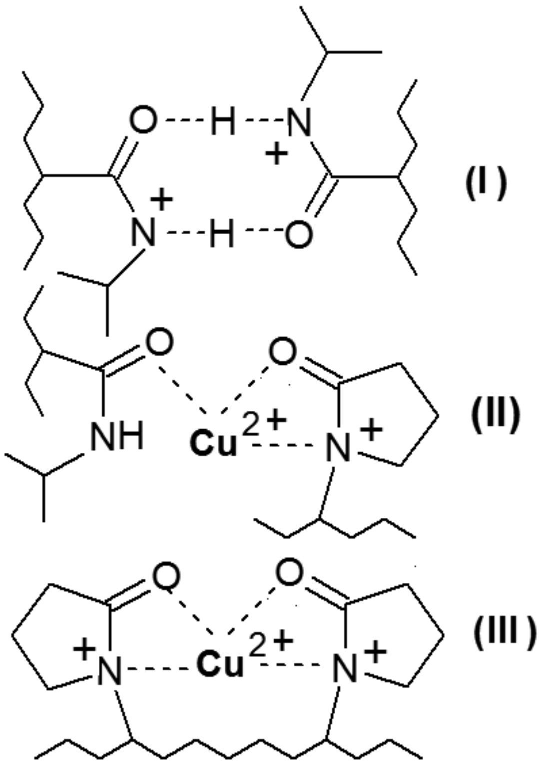
Table 5. The monomer reactivity ratios (r_1 and r_2) for NIPA (M_1) and VP (M_2) pair.

Method of calculation	r_1	r_2	(R^2)
FR-Equation	0.37	0.40	0.989
KT-Equation (FTIR)	0.44	0.50	0.968
Non Linear Regression	0.38	0.40	0.979
KT-Equation (^{1}H NMR)	0.47	0.50	0.970

Structure of Cu(II)-macrocomplexes

The structure of synthesized macrocomplexes was confirmed by FTIR spectroscopy by the comparative analysis of spectra of the homopolymers, copolymer and Cu(II)-copolymer complexes. The results from FTIR spectra of poly(VP) and poly(NIPA) and their Cu(II)-complexes show that the most essential changes are observed in the region of carbonyl groups which are directly take placed in complex formation with Cu(II) ions. The observed considerable decreasing in intensity of C=O bands in the spectra of Cu(II)-polymer complexes as comparison with their Cu-free analogues indicate the formation of physically crosslinked and intermolecular complexed ligands with rigid structure. This fact is also confirmed by the observed insolubility of Cu(II)-polymer complexes in the organic solvent and water.

Similar changes were observed in the spectrum of Cu(II)-poly(VP-*co*-NIPA)s. It is fact that when macromolecules transfer from a linear structure to a crosslinking form with formation of metal-ligand complexes the intensities of the main stretching (preferably) and bending bands are significantly decreased which are accompanied with decreasing of motion of macromolecular chain, and the backbone and neighboring side-chain bonds joining around cupper ions. These results allow us proposed the following probable structures in the form of Cu(II)-polymer ligands through Cu-monomer units complexing, preferably via VP linkage (structures II and III), as well as through H-bonding in the case of NIPA units (structure I), which are represented in Scheme 2.

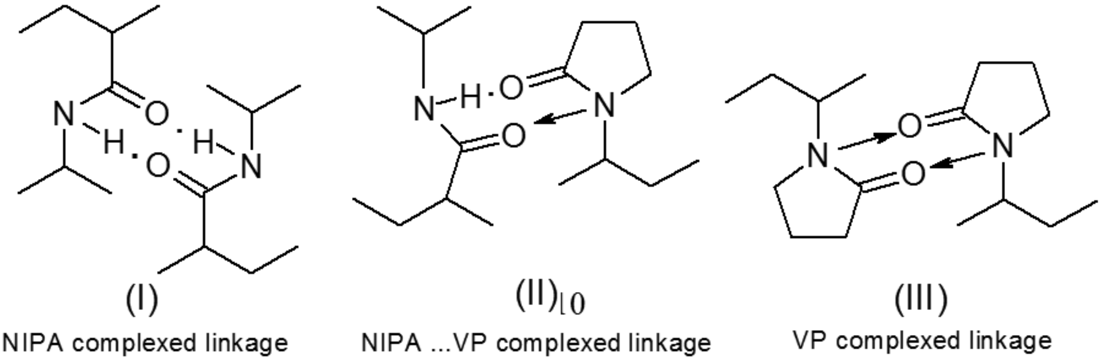


Scheme 2. Schematic representation of poly(VP-co-NIPA)/Cu-macrocomplex structures: (I): Hydrogen bonding complex between NIPA/NIPA linkages, (II): Coordinated Cu-complex between NIPA/VP linkages, (III): Coordinated Cu-complex between VP/VP linkages.

Copolymer Composition-Thermal Behavior Relationship

Both NIPA and VP units in copolymers have amphiphilic character containing hydrophilic polar amide groups and hydrophobic methylene, methine and isopropyl groups in backbone and side chain, respectively. VP unit, containing Lewis basic carbonyl group, can be form H-bonding with proton-donating functional groups (Lewis acids) of other polymers. It is known that amphiphilic NIPA and VP units in their homo- and copolymers, which contain highly polar amide groups, confer polar-attracting and hydrophylic behavior, as well as complexing ability with metal ions and other active species [13-15,24,58,59]. These polar amide linkages have also a higher tendency to form intermolecular complexed linkages of $m_1 \ldots m_1$ (I), $m_1 \ldots m_2$ (II) and $m_2 \ldots m_2$ (III) between monomer units in the form of diads as in the following scheme (Scheme 3).

Thermal properties of copolymers were performed by DSC-TGA-DTG analysis. Obtained results are summarized in Table 6. DSC traces of copolymers show two endo-effects with very strong peaks around 82-95^{o}C and weak peaks at 140-150^{o}C. Observation of the absence of a visible loss in weights on the TGA and corresponding exo-peaks on the DTG curves in the first transition broad endo-peak region of DSC curves indicate that these endo-peaks are not related with elimination of water molecules, including complexed H_2O from copolymers. Therefore, these peaks associate with phase transition process, which proceed before glass-transitions, and can be related to “aging” H-bonding and coordinated macromolecular complexes. This specific transition in solid state as a thermal sensitiveness (T_S) is equal to temperature sensitivity (LCST) of copolymer in solution. Similar transition was previously observed in copolymers of NIPA with maleic anhydride [13,14]. Recently,



Scheme 3. Structure of intermolecular complexed linkages.

Table 6. Thermal behavior of poly(NIPA), poly(VP), poly(NIPA-*co*-VP)s with different compositions[a].

VP unit in	DSC			DTG		TGA		
copolymer	T_s	DH	T_g	DH[b]	T_d	loss weight (%) at (^{o}C)		
(mol %)	(^{o}C)	(J/g)	(^{o}C)	(J/g)	(^{o}C)	200	300	400
Poly(NIPA)	45.7	5.9	143.5	0.03	350	7.3	15.5	37.6
30.1	44.7	11.3	156.9	0.07	361	1.6	2.1	27.0
51.3	57.8	10.0	147.9	0.29	375	1.0	1.5	29.0
74.7	50.1	21.8	159.8	0.82	387	0.9	1.7	52.0
Poly(VP)	62.9	29.3	172.7	0.10	394	0.0	1.2	25.0

[a] All the homo- and copolymers were synthesized in similar conditions.
[b] Specific enthalpy values.

Eeckman et al. [41] have found thermal behavior of the thermosensitive copolymers of NIPA with various hydrophilic comonomers, including VP comonomer, in aqueous solution (phase separation or demixing temperature, T_d) [60] and solid state (single glass transition temperature, T_g) using DSC with cooling system and usual DSC analysis, respectively. Among these certain comonomers were found to be capable of rising T_d to a value slightly higher than the physiological temperature (37^{o}C) and to have adequate phase transition behavior [41].

It can be assumed that complexing force (compactivity of macromolecules) for the homopolymers (I) and (III), and for copolymer (II) increase from (I) to (III) which is confirmed by the obtained values of thermal behavior (T_s and T_g) by DSC analysis (Table 6). Relatively high value of T_s observed for an alternating copolymer (Table 6) can serve as a reasonable agreement for our above mentioned propose. As evidenced form TGA-DTG curves copolymers show one-step decomposition and sufficiently higher thermal stability, temperature decomposition of which is expected to be around 385-400^{o}C.

Thermal behavior of synthesized homo- and copolymers and their Cu(II)-macrocomplexes have been studied by thermal analysis methods such TGA-DTG and DSC techniques at a heating rate of 10^{o}C/min under nitrogen atmosphere. Obtained results are summarized in Table 7. From the characteristic TGA-DTG curves and fragments of DSC scans of the Cu(II)-poly(VP-*co*-NIPA), Cu-free homo- and copolymers exhibit typical glass-transition behavior, which are characteristic for amorphous physical state of polymers. By contrast, Cu(II)-macrocomplexes only exhibit melt-transition properties indicating the crystalline structure of these complexes. Distribution of monomer units (random or alternating) and monomer unit ratio in copolymers essentially influenced on the thermal behavior of Cu(II)-macrocomplexes: in the case of Cu(II) complexes of the random copolymers (Cu(II)-copolymer-I and Cu(II)-copolymer-III) is observed relatively low values of melting temperature (223 and 226^{o}C, respectively) while Cu(II) complex of alternating copolymer (Cu(II)-copolymer-II) is melt at 235.3^{o}C. This observed phenomenon can be explained by high content of VP–NIPA diad linkages in copolymer, which are responsible for the formation of more compact macromolecular structure through complexation of Cu(II) ions dominantly with VP linkages and H-bonding (preferably via NIPA secondary amide groups) as shown in Scheme 2.

Table 7. Thermal behaviour of homo- and copolymers of VP and NIPA and their Cu(II)-macrocomplexes.

(Co)polymers and their macrocomplexes	VP-unit content in polymer system (mol. %)	T_g[a] (°C)	T_m[a] (°C)	T_d[a] (°C)	TGA analysis Loss weight (%) at (°C)		
					200	300	400
Poly(VP)	100	172.7	–	394	0.0	1.2	25.0
Cu(II)-poly(VP)	63.6		228.2	260	0.0	14.5	14.9
Poly(NIPA)	0.00	143.5	–	350	7.3	15.5	37.6
Cu(II)-poly(NIPA)	0.00		236.9	275	0.0	13.0	16.5
Poly(VP-*co*-MA)-I (80:20)	74.7	159.8	–	387	0.9	1.7	52.0
Cu(II)-poly(VP-*co*-NIPA)-I	52.2		226.0	257	1.3	16.3	17.2
Poly(VP-*co*-MA)-II (50:50)	51.3	147.9	–	375	1.0	1.5	29.0
Cu(II)-poly(VP-*co*-NIPA)-II	39.4		235.3	266	0.0	10.0	10.2
Poly(VP-*co*-MA)-III (20:80)	30.1	156.9	–	361	1.6	2.1	27.0
Cu(II)-poly(VP-*co*-NIPA)-III	26.1		223.0	265	0.0	8.5	9.6

[a] Obtained values from DSC analysis.
[b] The maximum values of decomposition temperature in DTG curves.

Polyelectrolyte and Stimuli-Responsive Behavior

A tendency of copolymers observed for the formation of macromolecular complexed linkages allows one to propose that these copolymers must as well show some polyelectrolyte behavior. This is confirmed by the observed dilution effect, i. e., increase in viscosity values with the dilution of dioxane solution of copolymer (Table 8). This phenomenon can be explained by specific behavior of complexed macromolecules and their conformational changes resulting in the expansion of polymer coil in the diluted solution. This fact relates the polyelectrolyte behavior of the studied copolymers and their temperature- and pH-sensitivities. Copolymers synthesized from various monomer feed ratios (NIPA:VP = 80:20, 50:50 and 20:80) show relatively narrow molecular weight distributions (Table 8) at high content of NIPA unit in copolymer.

The copolymers showed a pH-sensitivity depending on the VP content in copolymers. Since VP has a

Table 8. Effect of Copolymer Composition on the LCST Behavior, Viscosity and M_n Values.

copolymer compositon (mol %)		LSCT (°C) at pH		[h]$_{in}$ (dL/g) in dioxane at 25 °C	M_n (g/mol)
m_1	m_2	4.0	7.4		
Poly(NIPA)		32.2	27.6	0.012	3,200[a]
69.9	30.1	38.5	29.8	0.283	11,700
48.7	51.3	45.8	34.1	0.245	10,200
25.3	74.7	>59.6	>44.6	0.256	10,500
Poly(VP)		not observed		0.061	5,400[a]

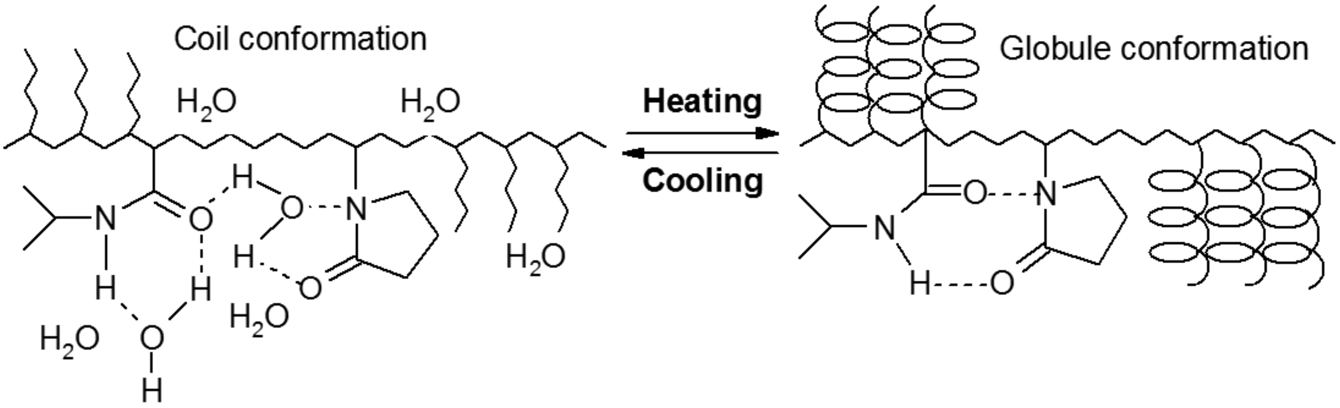
[a] Calculated values of viscosity-average molecular weights (M_v) using known Mark-Houwink parameters: $\alpha = 0.65$ and $K = 2.3 \times 10^{-4}$ for poly(NIPA) in methanol and $\alpha = 0.64$ and $K = 3.0 \times 10^{-4}$ for poly(VP) in methanol.

basic character, it caused an increase in LCST temperature at pH 4.0 more than at pH 7.4 (Table 8). All observed transitions were thermally reversible which was obtained by observing the changes in the absorbency values with the temperature of the polymer solutions measured with an UV-spectrophotometer at 500 nm as described above. As a general tendency, the absorbency increased with increasing temperature since the transparent polymer solution became turbid. Note that all transitions were thermally reversible so that the turbid copolymer solutions at the temperatures higher than LCST again turned into the transparent form when the temperature was decreased below LCST. Here, each experiment was repeated with the solution adjusted to two different pHs (pH: 4.0 and 7.4). When the VP ratio was increased, LCST temperature of the copolymer increased to higher temperatures from 32°C (LCST value of NIPA homopolymer) since VP has a hydrophilic character. However, Poly(VP) has an LCST which at a very high temperature (~147°C) so that incorporation of VP units to copolymer caused this increase in aggregation temperature. Poly(NIPA-*co*-VP) also showed a pH-sensitivity again depending on the VP ratio in the copolymer structure. Since VP has a basic character, it caused an increase in LCST temperature at pH: 4.0 more than at pH: 7.4. In acidic conditions basic VP units prefer to interact with solution rather than separation from solution causing higher LCST temperature. Similarly, VP units are not wanting to be in soluble form at basic pH as much as in acidic medium and cause an easy coil-globule transition of macromolecules (Scheme 4).

It can proposed that in this conformational change of macromolecules, complexed linkages are also place a significant role. Previously, we have observed a similar LCST change behavior for poly(NIPA)/poly(ethylene imine) system [24]. Obtained values of LCST at different pH of aqueous solutions allow to attribute these copolymers to the class of temperature and pH sensitive bioengineering amphiphilic polymers.

Conductivity

The measured values of conductivities of coordinated Cu(II)-macrocomplexes of copolymers of VP and NIPA at room temperature are summarized in Table 9. The value of conductivity [s, in (W.cm)$^{-1}$] were calculated from plots of current (nA) vs voltage (mV) for the poly(VP-*co*-NIPA) and Cu(II)-poly(VP-co-NIPA)s. The obtained values indicated that the copolymers showed higher trend to formation of conductive Cu(II)-macrocomplexes. Poly(NIPA-*co*-VP) with even higher content of VP unit is not showed conductive behaviour, while the



Scheme 4. Schematic representation of coil-globule transition in aqueous solutions of poly(NIPA-*co*-VP).

Table 9. Electrical conductivity (s) of Cu(II)-poly(VP-*co*-NIPA)s with different compositions.

Macrocomplexes	Content of VP-unit, (mol %)	Conductivity, s $(W.cm)^{-1}$
Poly(VP-*co*-NIPA)	74.7	Not observed
Cu(II)-poly(VP-*co*-NIPA)-I	52.2	18.3×10^{-6}
Cu(II)-poly(VP-*co*-NIPA)-II	39.4	8.7×10^{-6}
Cu(II)-poly(VP-*co*-NIPA)-III	26.1	3.1×10^{-6}
Cu(II)-poly(VP)	63.6	19.4×10^{-6}
Cu(II)-poly(NIPA)	0.0	3.7×10^{-9}

Cu(II)-poly(VP-co-NIPA)s are effective conductors. Cu(II)-copolymer-I with alternating structure is better conductor than Cu(II)-poly(VP-*co*-NIPA) with random unit composition. This fact can be explained by presentation of both the VP (preferably) and NIPA diad linkages in complexation with Cu(II) ions. Conductivity of Cu(II)-poly(VP-*co*-NIPA)s also depends on the amount of VP unit in copolymers; an increase of VP content in copolymer is increased conductivities of macrocomplexes. Both the Cu(II)-poly(VP) and Cu(II)-poly(VP-*co*-NIPA)-I have been exhibited similar higher conductivity.

CONCLUSION

Stimuli-responsive bioengineering copolymers of NIPA with VP were synthesized by complex-radical solution copolymerization, and were characterized using FTIR, ^{1}H {^{13}C} NMR (DEPT-135) and UV-spectroscopy, DSC and TGA analysis. Compositon of the copolymers synthesized in a wide range of monomer feed ratios were determined by FTIR and ^{1}H (^{13}C) NMR–DEPT-135 spectroscopy, and were achieved by comparing the absorbance values of NH (amide) and CH_2 (VP ring) groups as analytical bands and the integrals of the CH_3 and NH regions spectra of NIPA and VP units, respectively.

This work also presents synthesis and characterization of the Cu(II)-coordinated macrocomplexes of the alternating and random copolymers of VP and NIPA. Thermal behavior and electrical conductivity of the Cu(II)-macrocomplexes essentially depend on the composition and monomer unit distribution of poly(VP-*co*-NIPA)s. The most compact rigid structure is formed in Cu(II)-poly(VP-*alt*-NIPA) system, containing the alternating VP–NIPA diads. The Cu(II)-macrocomplexes exhibit crystalline structure, high thermal behavior and electrical conductivity as comparison with their Cu-free analogues. The macrocomplexes easily precipitate from aqueous solutions, and polymeric ligands have been showed some pH- and temperature sensitivity. These unique properties allow use these polymer systems for water purification from heavy metals and in bioseparation technology, and other bioengineering processes.

The monomer reactivity ratios were determined by by Fineman-Ross, Kelen-Tüdös and non-linear regression methods. It was observed that studied monomer pair has a some tendency to alternation in the chosen monomer feed ratios due to formation of intermolecular interaction through H-bonding and $N \rightarrow O{=}C$ coordination between reacted comonomers, monomer/macroradical and monomer unit linkages, which are confirmed by spectroscopy studied of structure of the copolymers with different compositions. Obtained values of the monomer reactivity ratios indicated that monomer pair relates to the better-copolymerization monomer systems. Copolymers showed some polyelectrolyte behavior and both T_s and T_g phase transitions, indicated a complexed compact structure of their macromolecules. The copolymers also show one-step decomposition and sufficiently higher thermal stability. LCST behaviour of the copolymers significantly depended on the content of VP unit in copolymer. Synthesized poly(NIPA-*co*-VP)s can be attributed to the class of temperature- and pH-sensitive bioengineering polymers useful for application in various gene- and bioengineering processes, drug delivery systems, and biomacromolecule conjugations.

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