Bioengineering Functional Copolymers: V. Synthesis, LCST, and Thermal Behavior of Poly(*N*-isopropyl acrylamide-*co*-*p*-vinylphenylboronic acid)

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ABSTRACT: New boron-containing stimuli-responsive (pH- and temperature-sensitive) copolymers were synthesized and characterized. Structure and composition of copolymers were determined by FTIR and ^{1}H-NMR spectroscopy, and elemental analysis and titration (N and B contents for NIPA and VPBA unit, respectively). By DSC and XRD measurements, it is established that the synthesized copolymers have a semicrystalline structure due to formation of intra- and/or intermolecular H-bonded supramolecular architecture. The copolymer composition–structure–property relationship indicates semicrystalline structure of copolymers with different compositions, degrees of crystallinity, and thermal and stimuli-responsive behaviors depends on the content of boron-containing monomer linkage. Results of DSC, DTA, and TGA analyses indicated that copolymers have T_g and T_m and high thermal stability. These water-soluble and temperature- and pH-sensitive amphiphilic copolymers can be used as polymeric carries for delivery of biological entities for diverse biomedical use, including boron neutron capture therapy. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 95: 573–582, 2005

Key words: *N*-isopropylacrylamide; *p*-vinylphenylboronic acid; copolymerization

INTRODUCTION

It is known that some B-containing aromatic compounds, such as *p*-carboxyphenyl boronic acid and its mono- and disubstituted derivatives, *p*-hydroxyborylphenyl-, L-carboranyl- and L-*p*-dihydroxyborylphenyl alanines, various substituted benzeneboronic acids, and some carborane derivatives are important tumor targeting agents for boron neutron capture therapy (BNCT).[1–5] BNCT is currently being evaluated as an effective treatment for cancer, including brain tumors. The evaluation of liposomes as carriers of boron compounds for BNCT has been extensively carried out. They are capable of carrying large quantities of boron compounds and may be selectively localized to the tumor. Thus, Pan et al.[6] studied B-containing folate receptor-targeted limosomes as potential delivery agents for BNCT. Shelly et al.[7] have investigated boron delivery to murine tumors with liposomes.

Hoffman et al.[8,9] described synthesis and homo- and copolymerization of *p*-vinylboronic acid and *p*-vinyl boroxole, as well as their saturated and unsaturated and diesters such as dialkyl and diallyl esters. It was shown that these boron-containing monomers are copolymerized with vinyl comonomers (styrene, vinyl acetate, methyl methacrylate, and acrylonitrile) under anhydrous conditions with free radical initiators and ionic catalysts. For the *p*-vinylboronic acid–acrylamide pair, approximate values of the monomer reactivity ratios were determined by using Fineman–Ross method ($r_1 = 1.0$ and $r_2 = 0.0$).[10] Radical polymerization of *p*-vinylboronic acid was carried out with an AIBN initiator in aqueous *t*-butyl alcohol at 30°C. Methanol (or dioxane) and water mixture solutions of synthesized polymer show polyelectrolyte effect and high thermal stability ($\sim 300°C$ by TGA).[10] Other vinyl and allyl derivatives of boron such as *B*-trivinyl(allyl)-*N*-triphenylborazines do not polymerize and diffically copolymerize with vinylic acrylic comonomers in the presence of radical initiators.[11] Synthesis of glucose-sensitive copolymers of *m*-(arylamido)phenylboronic acid with *N*-isopropylacrylamide using a radical copolymerization technique (in ethanol at 45°C and in DMF at 70°C, respectively) has been reported by Kataoka et al.[12] Recently, synthesis of pH-, temperature- and RNA-sensitive poly(*p*-vinylboronic acid-*co*-NIPA) copolymer using a radical solution copolymerization method in ethanol at 65°C has been reported.[13] It was found that two type of complexes were formed in the RNA and copolymer mixture due to the interaction of the amino and vicinal-diol groups of RNA

and boronic acid groups of copolymer in the tetrahedral anionic form.

Some aminoboron compounds have also found some utility in BNCT[14] and other forms of cancer therapy;[15] as a result, much effort has focused on the synthesis of boron-containing amino acid and peptide derivatives for possible applications as enzyme inhibitors.[16] Boronic acids, $RB(OH)_2$, and boronate esters have received considerable attention as glucose sensors for diabetes therapy.[17] They have been also found to facilitate the transport of various ribonucleosides in and out of liposomes, an important attribute in the field of drug design.[18] Boronic acid-based sensors[19,20] provide an attractive alternative to enzyme-based sensors[21] since the complexation is a reversible, equilibrium-based reaction. In addition to these applications, they provide versatile chemical reactivity that allows them to be converted into a wide range of substituent functional groups.[22] An important clinical trial of BNCT, using 4-hydroxy- borylphenyl alanine, was carried out by researchers at the Massachusetts Institute of Technology.[23] A variety of boron-containing amino acid derivatives that may carry boron to tumors by becoming incorporated in protein synthesis or metabolism within the rapidly growing tumor cell and boron delivery mechanism have been described by Hawthorne.[24] The synthetical aspects of different types of B-containing organic and inorganic compounds including B-containing derivatives of nucleic acid precursors, amino acids, and related peptides, as well as the chemistry of BNCT were described and discussed in a recently published review article.[25] Some organic boron compounds having a monomer structure and their iodized derivatives were used as X-ray contrasting agents and for the pharmaceutical preparations, as well as in the selective therapy of tumors.[26] A general synthetic method has been developed for the preparation of boron-rich macromolecular structures for BNCT and for conjugation with or inclusion in receptor-mediated delivery systems.[27] This method was used to yield precisely ordered soluble and hydrophilic oligophosphates that may by prepared with a variety of functional groups. Synthesis of boron-containing enzyme-analogue built polymers by the introduction of amino and boronic acid groups into chiral polymer cavities has been reported by Sarhan and Wulff.[28]

On the other hand, homo- and copolymers of N-isopropylacrylamide exhibited pH and thermal sensitivity and were used in protein conjugation as cation-active polymers soluble in water and physiological medium, as well as a carrier system for DNA delivery, affinity separation of genotoxins, and as reversible bioconjugates.[29–36] Enzymatic characteristics of these biosystems were changed significantly, depending on the hydrophilicity of the functional polymer-modifier and the degree of modification. The N-isopropylacryl-

amide–containing hydrogels were synthesized by radical crosslinking copolymerization of N-isopropylacrylamide with acrylic, itaconic, and maleic acids,[37] and N-isopropylacrylamide with acrylamidolactamine[38] in the presence of N,N'-methylene-bisacrylamide in aqueous solution. A simple FTIR spectroscopic method for the determination of the lower critical temperature of N-isopropylacrylamide copolymers has been reported by Percot et al.[39] Effect of pH on the thermoreversible swelling behavior of N-isopropylacrylamide-acidic comonomers, copolymers,[40,41] and phase behavior of ionic copolymers of N-isopropylacrylamide in water were studied in detail.[42] Recently, synthesis and characterization of maleimide-terminated oligo(N-isopropylacrylamide)[43] and amphiphilic N-isopropyl acrylamide–acrylic acid random and graft copolymers,[44] 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-(dimethylamino- propyl methacrylamide)] and their alkyl bromide derivatives having a delicate balance between thermosensitive, hydrophobic, and ionic groups;[45–47] cationic stimuli-responsive acrylic acid-terminated poly(N-isopropylacrylamide), potentially useful as carrier for gene delivery; conjugates of poly(N-isopropylacrylamide) with amino acids as proddings;[48,49] new hydrogels of N-isopropylacrylamide copolymers and polylysine as membrane immobilization systems;[50] poly(N-isopropylacrylamide-co-methacrylic acid) hydrogels for temperature- and pH-responsive release of antithrombolytic agents;[51,52] poly(N-isopropyl-acrylamide-co-diallyldimethylammonium chloride) as temperature-sensitive flocculants;[53,54] synthesis and characterization of bioengineering copolymers of N-isopropylacrylamide with maleic and citraconic anhydrides and their macrobranched derivatives potentially useful as a carrier for gene delivery and conjugates with biopolymers and determination of monomer reactivity ratios for these monomer pairs have been reported.[55–57]

As evidenced from above-mentioned short reviewed analysis, growing interest and much effort have been focused on the synthesis of boron-containing low molecular weight functional compounds, biopolymers, and drugs with boron ligands and evaluation of their suitability for the bioengineering applications, including BNCT. On the other hand, considerable progress has been demonstrated in the synthesis of bioengineering polymer systems on the base of N-isopropyl-acrylamide homo- and copolymers. However, a wide range of the macromolecular engineering can be utilized for the design of more effective synthetic routes to new boron functional compounds, especially copolymerization of boron-containing monomers and chemical modification of biocompatible polymers with organoboron reactive compounds

and monomers. Unfortunately, this very important route is not effectively used at this time by many researers for the further development of new bioengineering N- and B-containing polymer systems and their suitability for BNCT.

In the present article, results of synthesis and characterization of a new generation of boron-containing biocompatible reactive macromolecules by the radical-initiated copolymerization of *N*-isopropylacrylamide (NIPA) and 4-vinylphenylboronic acid (VPBA) with 2,2′-azobisiso- butyronitrile (AIBN) as an initiator in 1,4-dioxane at 65°C under nitrogen atmosphere in a wide range of monomer ratios are described and discussed. Special attention is given to the copolymer composition–structure–properties (crystallinity, thermal, and LCST behavior) relationship of synthesized stimuli-responsive copolymers.

EXPERIMENTAL

Materials

NIPA monomer (Aldrich) was purified before use by distillation under vacuum and recrystallization from diethyl ether solution: bp 91.5°C/2 mm, m.p. 61.6°C; ^{1}H-NMR spectra, ppm (in THF with trace of DMSO-d_6): NH, 1H 7.75, CH =, 1H multiplet 6.19–6.25, CH$_2$ =, 2H two doublets 6.11–6.16 and 5.45–5.48, CH, 1H multiplet 4.02–4.03 and 2CH$_3$, 6H 1.11–1.13 in –CH(CH$_3$)$_2$, respectively. VPBA monomer (Aldrich) was purified by recryctallization from anhydridous ethanol: m.p.108.9°C (by DSC). ^{1}H-NMR spectra, ppm (in THF with trace of DMSO-d_6): CH$_2$=, 2H two quartet 5.69 and 5.73, CH=, 1H multiplet 6.69–6.76, 2OH, 2H 7.71 in –B(OH)$_2$, CH=, two doublet for 4CH=, 2H 7.83 and 2H 7.38 in *o*- and *m*-phenyl, respectively. 2,2′-Azobisisobutyronitrile (Fluka) was twice recrystallized from methanol: m.p. 102.5°C.

Copolymerization

The copolymerizations of NIPA with VPBA using various monomer feed ratios were carried out in 1,4-dioxane at 65°C with AIBN radical initiator at a constant total concentration of monomers under the nitrogen atmosphere. Reaction conditions: $[M]_{total} = 2.78$ mol/L, $[AIBN] = 6.5 \times 10^{-3}$ 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, 1,4-dioxane, 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 and then soldered and placed in a thermostated silicone oil bath at 65 ± 0.1°C. The NIPA–VPBA copolymers were isolated from the reaction mixture by precipitation with diethyl ether and then washed
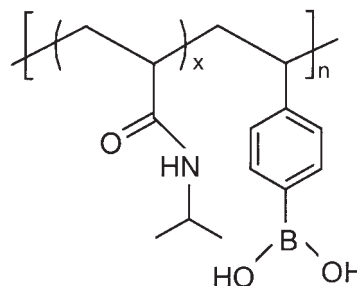
Scheme 1 Structure of poly(NIPA-*co*-VBPA).

with several portions of benzene and dried under vacuum at 40°C. The copolymer compositions were found by elemental analysis (*N* content for NIPA units); thermogravimetric (TGA) analysis (*B* content for VPBA units), and ^{1}H-NMR spectroscopy using integral area of chemical shifts of monomer functional groups for quantitative analysis.

Copolymer prepared from 70 : 30 monomer feed has the following average characteristics (Scheme 1): Monomer unit ratio ($m_1 : m_2$) : 46.2 : 53.8; Content of *N*: 4.91% and *B*: 4.41 %; intrinsic viscosity ([η]$_{in}$): 0.18 dL/g in methanol at 25 ± 0.1°C; glass-transition temperature (T_g): 53.4 °C, enthalpy (ΔH): 0.17 *mV* [by differential scanning calorimetry (DSC)] and melting point (T_m): 134.3°C, ΔH: 13.1 mJ [by DSC and differential thermal analysis (DTA)]. Fourier transform infrared (FTIR) spectra (KBr pellet), cm^{-1}: 3,850 (w-m) H-bonded OH stretching in –B(OH)$_2$, 3,750 (w) NH amide, 3,385 (versus, broad) NH stretching of *trans*-associated secondary amide, 3,100 (w) bending NH amide band, 3,080 (w) aromatic CH stretching, 2,950–2,875 (m-s), sym. and antisym. CH stretching in CH and CH$_2$ backbone and CH$_3$ side-chain groups, 1,990–1,910 (w) C=O overtones, 1,850 (w) and 1,775 (versus) two bands for *p*-disubstituted benzene, 1,730–17,00 (s) for H-bonded phenylboronic acid linkage, antisym. C=O stretching, 1,678 (w) free (non-H-bonded) C=O group, 1,650 (s) C=O stretching amide I band, 1,605 (m-s) CH stretching for CH= in phenyl ring, 1,560 (m) NH deformation-amide II band (only in associated *trans* form), 1,545 (m) free NH amide II band, 1,520 and 1,510 (m) for B–O and CH$_2$, CH$_3$ bands, 1,490–1,475 (m) CH$_2$ scissor vibration and CH$_3$ antisym. deformation, 1,420 (s) fairly strong, sharp band due to benzene ring vibration in phenyl boronic acid linkage (Ph–B), 1,375 (s) and 1,365 (s) doublet band for CH$_3$ deformation in isopropyl group, 1,345 (s) strong stretching B–O band in phenylboronic acid linkage, 1,225 (w) CH$_2$ wagging, 1,255–1,275 (w) *trans*-amide III band, 1,175 (m) broad C–N stretching or CH$_3$ rocking, 1,105 (m-s) CH$_3$ rocking, 1,018 (m) NH bending in –NH. . . O=C–, 960 (w) CH$_3$ rocking, 925 (m) OH deformation in –B(OH)$_2$, 840 (s) a single strong band for *p*-disubstituted benzene ring, 776 (w) and 750 (w)

TABLE I
^{1}H-NMR and Element Analysis Data for Determining the Composition of Poly(NIPA-*co*-VPBA)s Synthesized Using Various Initial Monomer Mixtures

Monomer Feed (mol %)		Am_1^a (NIPA unit)	Am_2^a (VPBA unit)	N (%)	Copolymer Composition (mol %)			
					^{1}H NMR Analysis		Nitrogen Analysis	
[NIPA]	[VPBA]				m_1	m_2	m_1	m_2
90	10	0.060	0.328	7.51	68.3	32.3	66.9	33.1
70	30	0.076	0.062	4.91	45.1	54.9	46.2	53.8
60	40	0.019	0.019	4.27	39.8	60.2	40.8	59.2
50	50	0.127	0.161	3.68	34.6	65.4	35.6	64.4
40	60	0.338	0.625	2.87	26.5	73.5	28.3	71.7
30	70	0.079	0.215	1.82	19.7	80.3	18.4	81.6

^{a} Integral area of chemical shift for 6H CH_3 of NIPA (isopropyl group) and for 4H phenyl of VPBA (disubstituted benzene ring) units.

CH_3 rocking coupled with skeletal modes, 650 (s) out-of-plane OH bending with stronger H-bond, 550 (w) CH bending, 525 (w) N-C=O bending, 475 (m-w) C–N–C bending. ^{1}H-NMR spectra (in DMSO-d_6 at 50°C), ppm: 2H, OH, –B(OH)$_2$ 7.84, 4H, CH= phenyl 7.63, 1H, CH 2.61 (backbone), and 2H CH_2 (backbone) 2.32 for VPBA unit; 1H, NH 6.73 (broad), 1H CH

(isopropyl) 3.56, 1H CH (backbone) 1.51, 2H CH_2 (backbone) 1.76 and 6H, CH_3 1.08 for NIPA unit.

Characterization

FTIR spectra of the copolymers (KBr pellet) were recorded with FTIR Nicolet 510 spectrometer in the
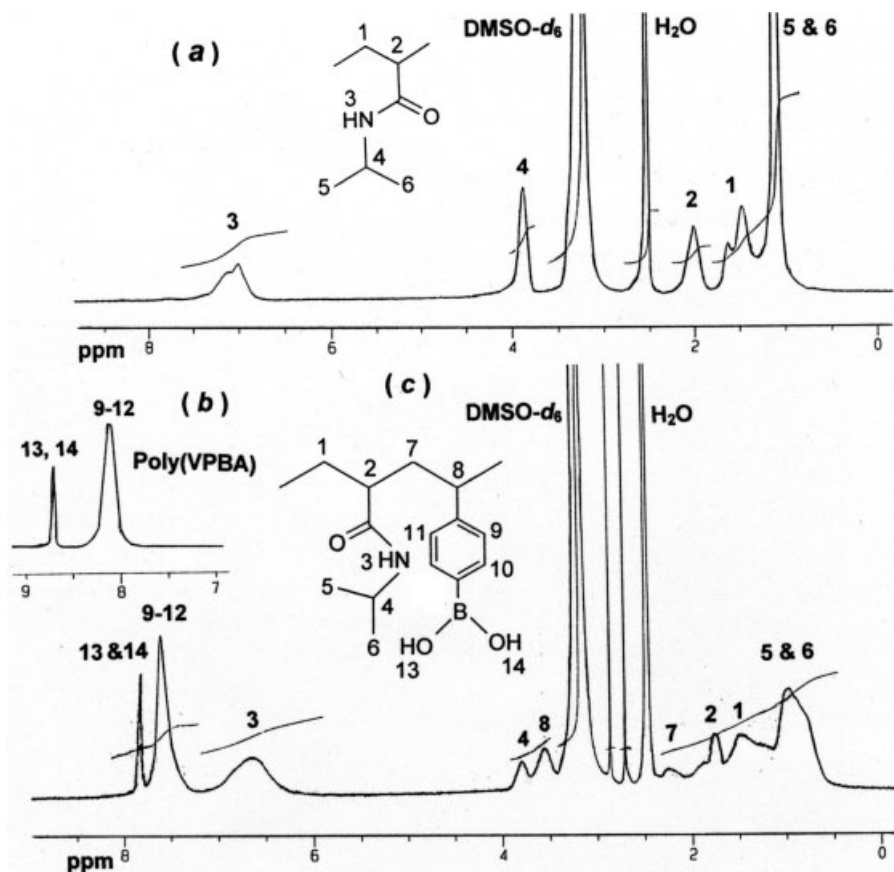

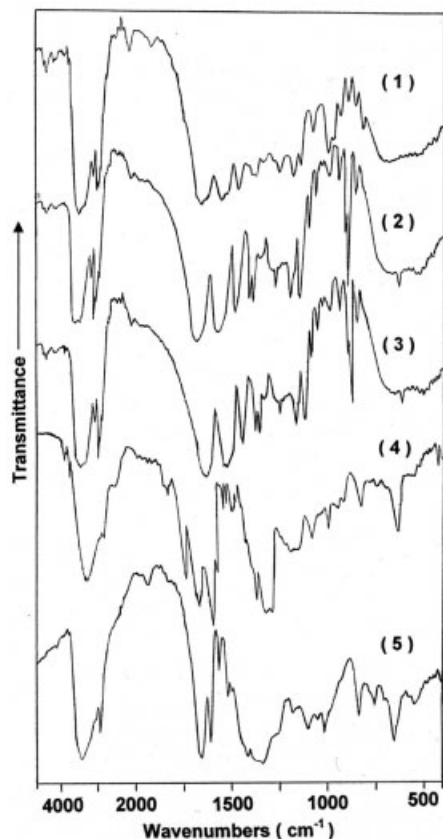**Figure 1** ^{1}H-NMR spectra of (a) NIPA and (b) VPBA and their mixtures: NIPA : VPBA = 9 : 1 (c) and 1 : 9 (d) in DMSO-d_6 at 27°C.

monomer units (m_1 and m_2); in the case of ($m_1 + m_2$) = 1, monomer unit ratios can be calculated from eqs. (1) and (2) using the following simplified form:

$$m_1/m_2 = f = n_2 Am_1(CH_3)/n_1 Am_2(\text{phenyl}) \quad (3)$$

DSC, DTA, and TGA analyses of copolymers were performed on a DuPont TA 2000 calorimeter and Setaram Labsys TG-DTA 12 Termal Analyzer, respectively, under 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 copolymers synthesized. Molar fractions (mol %) of comonomer units (m_1 and m_2) in copolymers using elemental analysis data (content of N) were calculated according to the following equations:[57]
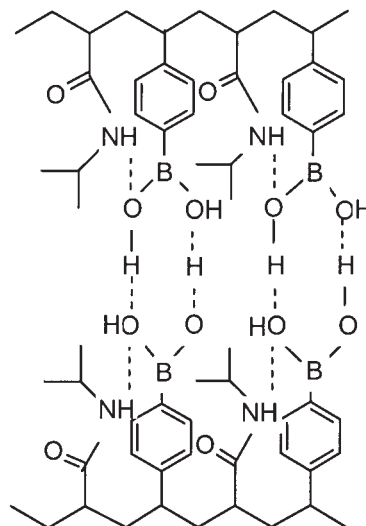
$$m_1 = M_2/[(A_N/C) - \Delta M 10^{-2}] \quad (4)$$

where M_2 is the molecular weight of VPBA unit; A_N (or A_B) is the atom weight of N (or B); C is the content of N (or B) in the copolymers (%); $\Delta M = M_1 - M_2$ (M_1 is the molecular weight of the NIPA unit).

Crystallization behaviors of copolymers were determined by Philips manual spectrogonimeter employing Cukα ($\lambda = 1.54,184$ Å) radiation over the range S° $\leq 2\theta \leq 50$°. Crystallinity degrees (χ_c) of synthesized (co)polymers were determined by area ratio method using the following equation:[58]

$$\chi_c = {_o}s^2 I_c(s)d / {_o}s^2 I(s)d \quad (5)$$

where s is the magnitude of the reciprocal-lattice vector which is given by $s = (2\sin\theta)/\lambda$ (θ is one-half the

Figure 2 FTIR spectra of (1) poly(NIPA), (5) poly(VPBA), and poly(NIPA-*co*-VPBA)s with different compositions. Content of m_2 unit in copolymer (mol %): (2) 8.2, (3) 12.7, and (4) 64.4.

4,000–400 cm^{-1} range, where 30 scans were taken at 4 cm^{-1} resolution. ^{1}H-NMR spectra were recorded on a JEOL 6X-400 (400 MHz) spectrometer with DMSO-d_6 as a solvent at 27°C.

The compositions of the copolymers synthesized using various monomer feed ratios were determined by a well-known NMR method[55] and were achieved by comparing the integrals of the CH$_3$, NH, and phenyl group regions in the spectra of NIPA and VPBA units, respectively. Molar fractions of the comonomer units (m_1 and m_2) in NIPA–VPBA copolymers using ^{1}H-NMR analysis data were calculated according to the following equations:

$$Am_1(CH_3)/A_{\text{total}} = n_1 m_1/(a_1 m_1 + b_2 m_2) \quad (1)$$

$$Am_2(\text{phenyl})/A_{\text{total}} = n_2 m_2/(a_1 m_1 + b_2 m_2) \quad (2)$$

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; A_{total} is the total area of protons in the copolymer; n_1 and n_2 are the integers of proton(s) in the functional group of the monomers; a and b are the integers of protons in the

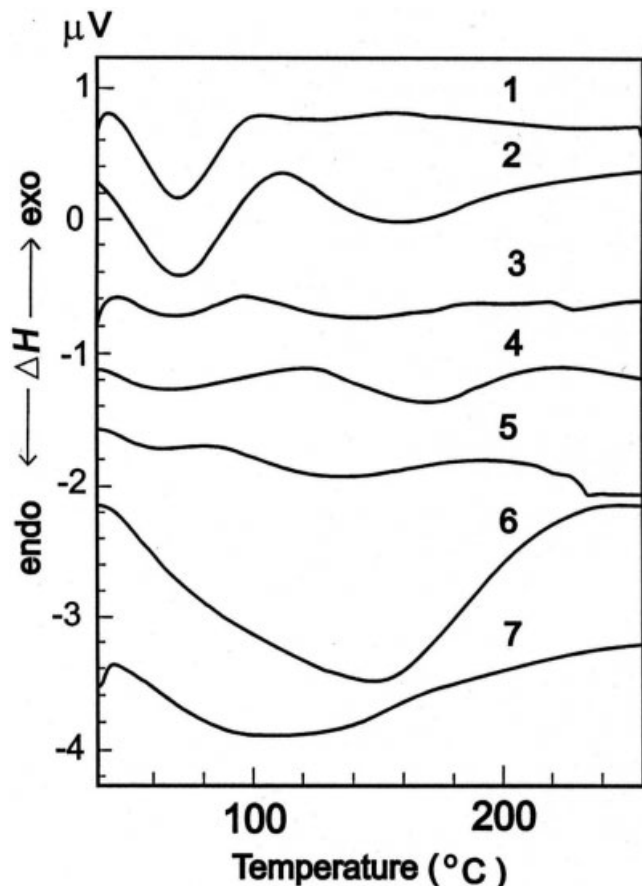**Scheme 2** Self-assembled supramacromolecular structure.

Figure 3 DSC curves of (1) poly(NIPA), (7) poly(VPBA), and poly(NIPA-*co*-VPBA) with different compositions: *f* (m_1/m_2) = (2) 2.02, (3) 0.86, (4) 0.69, (5) 0.39, and (6) 0.23.

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.

Intrinsic viscosities of the copolymers with different compositions were determined in methanol at 25 ± 0.1°C in the concentration range of 0.1–1.0 g/dL

using an Ubbelohde viscometer. Lower critical solution temperature (LCST), pH, and temperature sensitivity at pH 4.0, 5.0, and 7.4 were obtained spectrophotometrically (Jasco V-530 UV spectrophotometer) at 500 nm using acetic acid/sodium acetate (pH 4.0 and 5.0) and Na_2HPO_4/ $NaH_2PO_4.2H_2O$ (pH 7.4) buffers.

RESULTS AND DISCUSSION

Synthesis of self-assembled supramacromolecular structure

Judging by the nature of the conjugation between double bonds and functional groups, NIPA [(π (C=C) → π′(C=O)] and VPBA [(π (C=C) ← π′(CH=, benzene ring)] can be considered as electron acceptor and electron donor monomers, respectively. On the other hand, it is also possible that the formation of H-bonded complex between amide and boron containing fragment. It is important to note the known fact that the benzeneboronic acid derivatives (isostructural analogs of VPBA) can also easily form complexes with various proton-acceptor compounds through intermolecular H- bonding.[25]

Results of ^{1}H-NMR and elemental analyses of the copolymers synthesized in a wide range of monomer feed are summarized in Table I. Copolymer compositions calculated using elemental analysis data (content of N and B) were in reasonable agreement with those obtained from ^{1}H-NMR analysis using eqs. (3) and (4). As evidenced from the data shown in Table I, copolymerization of monomers in NIPA–VPBA systems has a tendency toward alternation. For the known *N*-propylacrylamide–styrene monomer pair, which can be used as a model system, monomer reactivity ratios were determined by Braun et al.[59] (r_1 = 0.058 and r_2 = 1.6).

The presence of intra- and intermolecular interactions through amide and boronic acid fragments in the studied copolymers is confirmed by ^{1}H-NMR (400 MHz) analysis of homopolymers and poly(NIPA-*co*-VPBA)s with different compositions. Comparative analysis of ^{1}H-NMR spectra of poly(NIPA), poly-

TABLE II
Effect of VPBA Unit on the Thermal Behavior and Crystallinity of the Poly(NIPA-*co*-VPBA)s

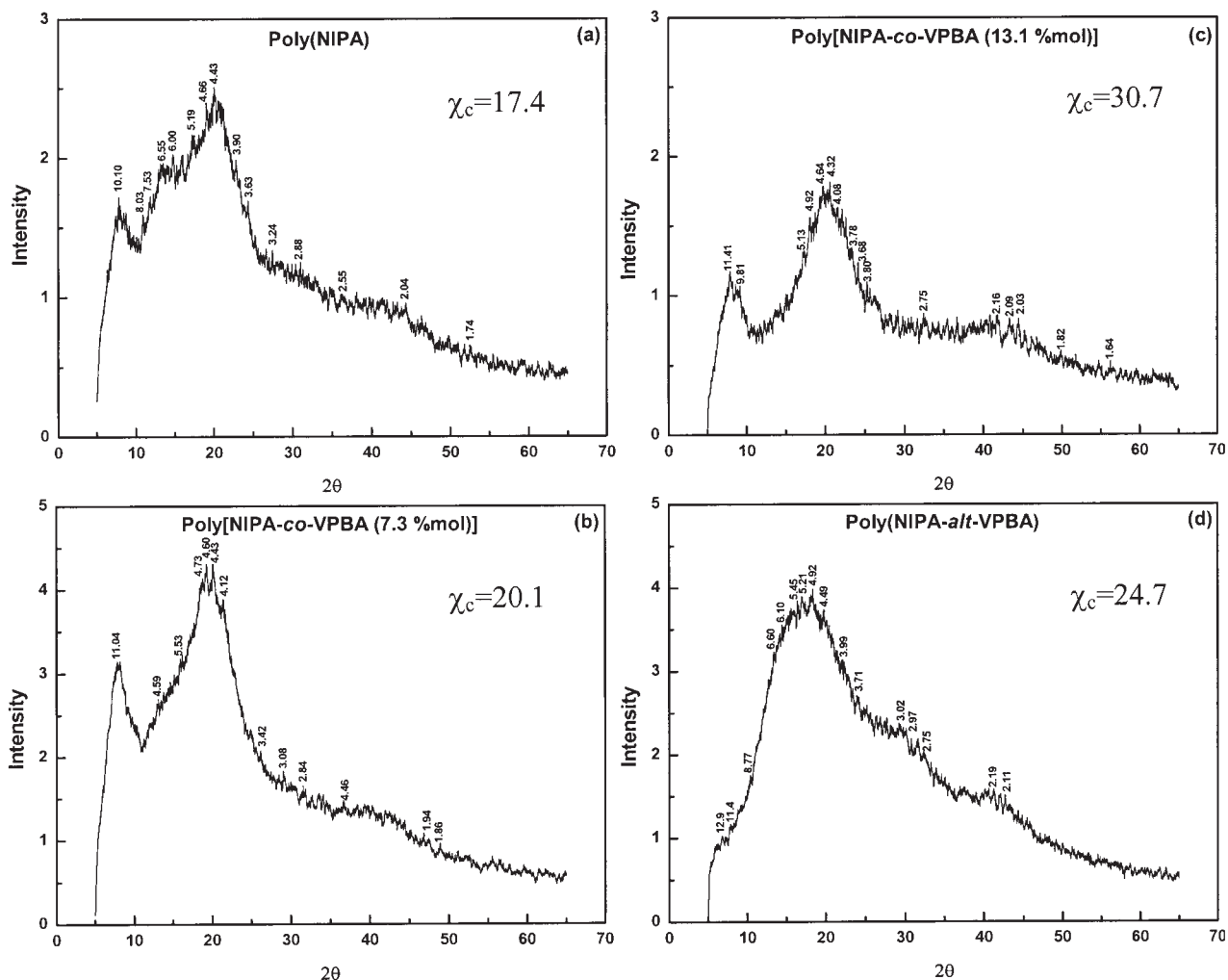| Content of VPBA Units (mol %)	DSC Analysis				Crystallinity (by XRD)		
	T_g (°C)	ΔH (J/g)	T_m (°C)	ΔH (J/g)	Area		χ_c (%)
					Cryst.	Amorph.	
Poly(NIPA)	26.9	0.37	145.5	1.96	11.7	67.2	17.4
7.3	36.3	7.35	175.9	1.69	20.3	101.1	20.1
13.1	45.8	0.56	174.0	4.09	11.5	46.6	24.7
21.7	53.8	1.75	169.4	1.55	22.6	89.7	25.2
33.1	55.3	7.66	159.3	2.78	25.4	98.6	25.8
53.8	56.1	3.70	115.6	6.12	26.7	102.1	26.1
Poly(VPBA)	59.1	3.15	197.8	2.50	33.2	108.0	30.7

Figure 4 X-ray difraction patterns of (a) poly(NIPA), (b) poly(VPBA), (c) poly[NIPA-*co*-VPBA (7.3 mol %)] and (d) poly[NIPA-*co*-VPBA (13.1 mol %)].

(VPBA), and poly(NIPA-*co*-VPBA) (Fig. 1) indicated the essential changes of chemical shift values for the protons of NH (from 7.05 ppm for PNIPA to 6.73 ppm for copolymer), isopropyl CH group (from 3.87 to 3.75 pm), and phenyl group CH= in VPBA unit (from 8.17 to 7.84 ppm). Observed effect of copolymer composition on the chemical shift of protons from NH (amide group), CH= (*o*-position relatively boronic acid substituent in disubstituted benzene ring), and CH (isopropyl fragment) groups indicated that the increase of the VPBA unit or the decrease of the NIPA unit contents in the copolymer causes significant changes of chemical shifts of interacted groups, as well as chemical shift of the methine group of the isopropyl fragment in NIPA linkage, which also shows higher sensitivity to this complexation.

FTIR spectra of homo- and copolymers are showed in Figure 2. The formation of the intermolecular H-bonded complexes between NIPA and VPBA linkages in the copolymers is confirmed by the appearance of the following characteristic bands: (1) very strong broad band (NH stretching) at 3,385 cm^{-1} for *trans*-associated secondary amide group and/or for H-bonded OH groups of phenylboronic acid linkage, (2) NH amide II deformation band at 1,650 cm^{-1} (only in associated *trans*-form), (3) 1,730–1,700 cm^{-1} strong bands for H-bonded phenylboronic acid lingkage, (4) 1,018 cm^{-1} band for NH bending in –NH. . . O=C—complex, and (5) 650 cm^{-1} out-of-plane OH bending with a strong H-bond in boronic acid fragment. Copolymer is easily transferred to an ionized form by the absorption of the atmospheric water molecule with the formation of ionic –B(OH)$_3$ group. However, this group disappears after thermotreatment at 90–110°C due to the easily dehydrated boronic acid fragment. The formation of two symmetrical singlet peaks at 2.95 and 3.11 ppm in ^{1}H-NMR spectra [Fig. 1(c)] of copolymer can be related to the ionized forms of boronic acid groups.
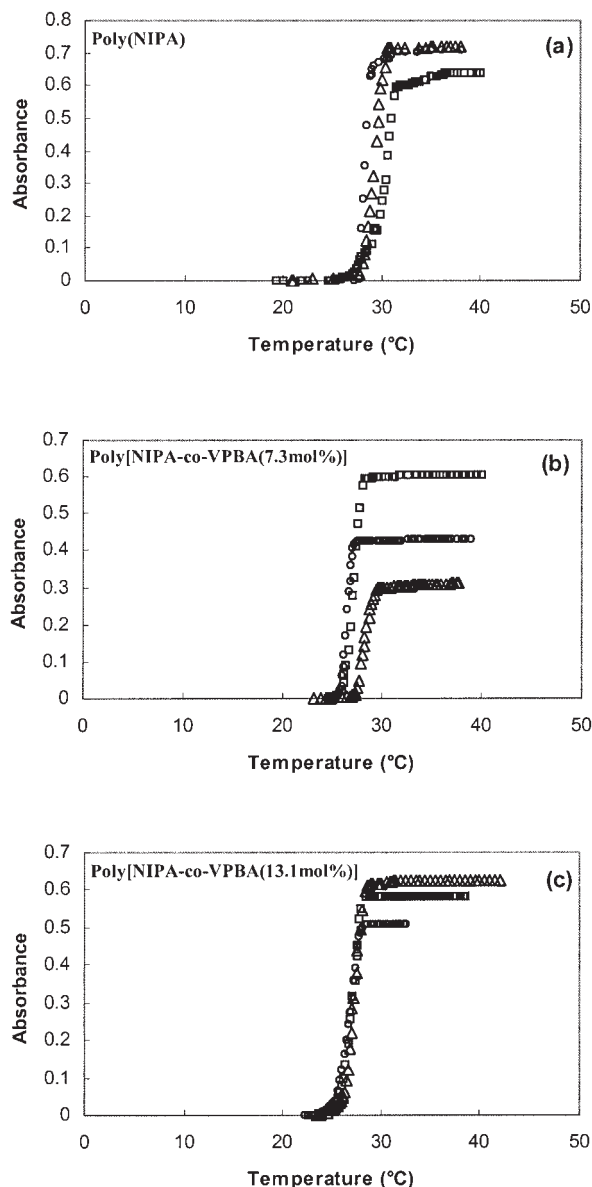
Figure 5 Temperature–absorbance plots for poly(NIPA-*co*-VPBA)s with different compositions at pH (1) 4.0, (2) 5.0, and (3) 7.4: VPBA unit content, m_2 = (a) 0.0, (b) 7.3, and (c) 13.1 mol %.

Taking into consideration these observed effects, it can be proposed that the formation of self-assembled supramacromolecular architecture is a result of a donor–acceptor type of radical copolymerization of the hydrogen-bonding monomer system (Scheme 2). The tetrahedral anionic form of the used VPBA monomer[13] is increased by its complexation with the amide group of NIPA and the probability of the formation of a supramolecular structure as shown in above presented scheme.

Copolymer structure—composition–property relationships

The results of DSC and XRD studies of the copolymer composition–thermal behavior relationship and crystallinity for the synthesized homo- and copolymers of NIPA and VPBA can also serve as an additional confirmation of the formation of the intermolecular and/or intramolecular H-bonded supramolecular structure in these systems.

Figure 3 shows the DSC scans of copolymers prepared from the different monomer feed compositions, as well as DSC curves of homopolymers, poly(NIPA) and poly(VPBA), synthesized in the similar conditions as in copolymer synthesis. These results indicate that the intensity and position of higher temperature endopeaks, which are associated with the melting point (T_m), significantly depend on the monomer unit ratios in the copolymers, and especially on the degree of hydrogen bonding between NIPA and VPBA linkages. It is known that the high melting points of polymers are associated with many factors, including inter- and intramolecular structural regularity and rigidity of macromolecules.[60] The lower temperature endoeffects on the DSC curves, associated with the glass transition temperature (T_g), change insignificantly with an appreciable increase in VPBA unit concentration in copolymers. This observed phenomenon indicates that the mechanism of glass transition is similar in these copolymers and homopolymers.

The values of T_g, T_m, and ΔH (enthalpy) for the copolymers are presented in Table II. It is shown that the increase of VPBA-unit content in copolymers increases the value of T_g. The results of DSC analysis of copolymers with different compositions indicate the formation of a semicrystalline supramolecular structure on account of intramolecular H-bonded complexes formed between alternating fragments of macromolecules. This observed fact is also confirmed by X-ray powder diffraction analysis of copolymers. As evidenced from XRD patterns, illustrated in Figure 4, copolymers consist of a predominantly amorphous phase with some crystallinity (χ_c = 20–26%), the degree of which depends on the content of VPBA in copolymer.

Temperature and pH sensitivity of copolymers

One of the important properties of bioengineering polymers consists in exhibiting a first-order phase transition caused by a small change in external conditions such as temperature and pH of medium, solvent composition, electric field, etc. Most results were obtained for polyacrylamide as a solvent-sensitive polymer,[61,62] temperature- and pH-sensitive poly-(NIPA),[63–67] and various amphiphic copolymers of NIPA.[39–47,55–57] These stimuli-responsive polymers have been a subject of many extensive investigations in the field of modern biotechnology, especially in the fields of cell and enzyme immobilization, protein purification, controlled drug delivery, and gene delivery.

TABLE III
LCST Behavior of Poly(NIPA-*co*-VPBA)s with Different Compositions

Copolymers	LCST Values (°C) at Different pH Media		
	4.0	5.0	7.4
Poly(NIPA)	27.8	27.8	28.3
Poly[NIPA-*co*-VPBA (3.2 mol %)]	26.6	26.9	27.8
Poly[NIPA-*co*-VPBA (7.3 mol %)]	25.9	26.2	27.5
Poly[NIPA-*co*-VPBA (13.1 mol %)]	25.6	25.8	26.3
Poly[NIPA-*co*-VPBA (21.7 mol %)]	Not observed	Not observed	Not observed

Synthesized B-containing copolymers as a new generation of intelligent polymers also show temperature and pH sensitivity in aqueous solutions. These important parameters, including LCST at different pH media, were obtained for the copolymers with various compositions using a well-known UV spectroscopy method.[68] The effect of composition (VPBA-unit content) on the LCST values of poly(NIPA-*co*-VPBA)s is studied in aqueous solutions having different pH values. LCST values for these copolymers determined by UV spectroscopy from the temperature–absorbance plots, are shown in Figure 5. Results of these measurements are presented in Table III. As seen in these data, copolymers show pH and temperature sensitivity at relatively lower contents of VPBA in copolymer (around 3.2–13.1 mol %). Copolymers containing VPBA unit content higher than $\sim$ 20 mol % do not show LCST behavior. At relatively lower VPBA unit content in poly[NIPA-*co*-VPBA (< 20 mol %)]s, probability of coil-to-globule transition is increased due to decreasing ionization of boronic acid linkage. For the copolymer containing more than 20 mol % of the VPBA units, the compact structure is not formed and therefore the coil-to-globular conformational change is not observed. This phenomenon can be explained by the increase in the ionic strength of the aqueous medium and formation of strong H-bonding in the studied copolymers. It is observed that LCST values of the copolymers depend on the pH of medium and the content of the VPBA unit (Table III). Increases of pH from 4.0 to 7.4 and VPBA content from 3.2 to 13.1 mol % resulted in visible decreases in the LCST value of the copolymers compared with poly(NIPA).

CONCLUSIONS

This work presents the synthesis and characterization of novel N- and B-containing stimuli-responsive copolymers of NIPA with VPBA. The formation of the intermolecular N→B coordinated and H-bonded supramolecular structure in these systems is conformed by FTIR and ^{1}H-NMR spectroscopy, DSC, and XRD analyses. The results of the copolymer composition–thermal behavior–crystallinity relationship studies indicate the formation of a semicrystalline supramolecular structure on account of intra- and intermolecular H-bonded complexes between alternating fragments of macromolecules. These copolymers have a predominantly amorphous structure with some crystallinity (χ_c = 20–26%), the degree of which depends on the content of VPBA in the copolymer. Synthesized copolymers, which have relatively lower contents of VPBA unit, show pH and temperature sensitivity; the probability of coil-to-globule transition of these copolymers increases due to the decrease in the ionization of boronic acid linkage. For the copolymer containing more than 20 mol % of the VPBA units, the compact structure is not formed and therefore the coil-to-globular conformational change is not observed. Increases of pH from 4.0 to 7.4 and VPBA content from 3.2 to 13.1 mol % resulted in visible decreases in the LCST values of the copolymers compared with poly(NIPA) due to the increase in the ionic strength of the aqueous medium and formation of strong H-bonding in the studied copolymers.

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