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ORIGINAL CONTRIBUTION

B. Elmas
M.A. Onur
S. Şenel
A. Tuncel

Temperature controlled RNA isolation by *N*-isopropylacrylamide–vinylphenyl boronic acid copolymer latex

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B. Elmas · S. Şenel
Hacettepe University,
Department of Chemistry,
Ankara, Turkey

M.A. Onur
Hacettepe University,
Department of Biology,
Ankara, Turkey

A. Tuncel (✉)
Hacettepe University,
Chemical Engineering Department,
Ankara, Turkey
E-mail: atuncel@hacettepe.edu.tr
Fax: +90-312-299 21 24

Abstract Thermosensitive *N*-isopropylacrylamide-4-vinylphenylboronic acid (NIPA-co-VPBA) copolymer latex particles were used as pseudo-specific sorbent for the selective isolation of RNA by boronate affinity chromatography. In the proposed isolation procedure, RNA was adsorbed onto the latex particles via the interaction between boronic acid groups of the particles and diol groups of RNA at a low temperature (i.e. +4 °C). No significant amount of DNA was bound to the particles under the conditions that were used for RNA adsorption. This result indicated that the developed sorbent could be used effectively for selective removal of RNA from the RNA–DNA mixtures. RNA adsorption onto the latex particles significantly decreased with increasing temperature. On the other hand, NIPA-co-VPBA copolymer latex showed a thermoflocculation behaviour at temperatures higher than 30 °C. These two properties were used for temperature controlled RNA isolation by the proposed sorbent. After adsorption of RNA onto the stable latex particles at +4 °C, the particles were transferred into a desorption medium. Temperature increase in this medium resulted in both desorption of RNA from the particles and thermoflocculation of the latex suspension. Hence RNA was recovered in the clear supernatant without applying any additional separation for the removal of sorbent material. In the proposed procedure, the temperature was used as an on–off switch controlling the adsorption and desorption of RNA. The necessity of a separate medium (at a certain pH and ionic strength) for desorption was eliminated by the proposed isolation method. This behaviour allowed the desorption of RNA to a medium at any ionic strength and at any pH.

Keywords Thermosensitive latex · Isopropylacrylamide · RNA · Boronate affinity chromatography · Boronic acid · Glucose sensor · Affinity precipitation · Uniform latex particles

Introduction

Boronate-affinity chromatography has been conducted based on the complex formation between boronic acid groups of support materials and diol groups of target biomolecules. In the conventional boronate affinity chromatography applications involving the selective isolation of nucleotides, RNA, glycated proteins and glycoenzymes, boronic acid functionalized polyacrylamide, agarose and polyacrylate based gels were commonly preferred as support materials [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14].

Thermosensitive polymers have been also utilized as carriers in the immobilization or isolation of different

biomolecules such as enzymes, cells and oligonucleotides [15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33]. The interaction of boronic acid functionalized thermosensitive polymers with the diol-carrying biomolecules was investigated by different researchers [28, 29, 30, 31]. Thermosensitive copolymers of *N*-isopropylacrylamide, (NIPA) and acrylamidophenylboronic acid (AcPBA) and the terpolymers of NIPA–AcPBA–dimethylaminopropylacrylamide (DMAPA) showed an LCST change with glucose concentration [29]. A good correlation was observed between the diol complexation rate and the fraction of boronate groups in the polymers [30]. The thermosensitive terpolymer gels in the form of NIPA–AcPBA–dimethylaminopropylmethacrylamide (DMAPM) were utilized in the endothelial cell differentiation as a cell substratum [31]. Recently, NIPA based thermosensitive polymers were tried as carriers for DNA delivery into the cells [32, 33].

Uniform latex particles are another class of materials tried as carriers in the isolation or immobilization of biological agents. Modified emulsion or dispersion polymerization techniques have been applied for the synthesis of uniform latex particles carrying desired functionality depending upon the intended use [34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44]. Recently, thermosensitive latexes were promoted as "new generation carriers" in the immobilization of DNA fragments and oligonucleotides for diagnostic purposes [40, 41, 42, 43, 44]. Elaissari et al. investigated the RNA adsorption behaviour of magnetic-thermosensitive latex particles [45]. However, studies on the use of thermosensitive latexes in the chromatographic separation of nucleic acid fragments and nucleotides were limited.

In this study, boronic acid carrying thermosensitive latex particles were prepared by the dispersion copolymerization of NIPA and a boronic acid carrying comonomer, 4-vinylphenylboronic acid (VPBA). Poly(*N*-isopropylacrylamide-co-4-vinylphenylboronic acid) [poly(NIPA-co-VPBA)] latex particles were used as pseudospecific sorbent for temperature controlled RNA isolation from nucleic acid mixtures. In the proposed method, RNA was selectively adsorbed onto the latex particles at a low temperature (i.e. +4 °C). RNA adsorbed particles were transferred into the desorption medium and the temperature was increased to 37 °C. In this medium, desorption of RNA and flocculation of latex particles simultaneously occurred. Since the latex particles were precipitated by thermoflocculation, no additional operation was done for the separation of carrier material (i.e. latex particles) from the desorption solution. Hence RNA was recovered in the clear supernatant by the desorption and flocculation processes induced by the temperature elevation.

Experimental

Materials

N-Isopropylacrylamide (NIPA, Aldrich Chem. Co., Milwaukee, Wisc., U.S.A.) was recrystallized from hexane–acetone. The comonomer, 4-vinylphenylboronic acid (VPBA) was supplied from Aldrich Chem. Co. and used without further purification. *N*,*N*-methylenebisacrylamide (MBA, BDH Chemicals Ltd., Poole, U.K.) was used as the crosslinking agent. The copolymerization was initiated with potassium persulfate (KPS, Analar grade, BDH Chemicals Ltd.). Ribonucleic acid from baker's yeast (RNA, Cat No: R 7125, Sigma Chemical Co., St. Louis, Mo., U.S.A.) and deoxyribonucleic acid from salmon testes (DNA, Cat No: D 1626, Sigma Chemical Co.) were used in the adsorption experiments. The buffer solutions for the adsorption experiments were prepared by using *N*-[2-hydroxyethyl]piperazine-*N*'-[2-ethane sulfonic acid] (HEPES, Sigma Chem. Co.). Distilled deionized water was used in all experiments. The boronic acid content of the latex particles was determined by using a reactive dye, carmine (C.I. 75470, alum lake of carminic acid, Merck A.G., Darmstad, Germany).

Preparation of poly(NIPA-co-VPBA) copolymer latex

Typically, NIPA (0.5 g), VPBA (0.05 g) and MBA (0.021 g) were dissolved in distilled water (40 ml) in a cylindrical Pyrex reactor. The initiator, KPS (0.03 g) was dissolved in the resulting homogeneous solution. The reactor was purged with bubbling nitrogen for 5 min and sealed. Dispersion copolymerization was conducted at 70 ± 0.5 °C for 24 h in a temperature-controlled water bath shaken at 120 cpm. After completion of the polymerization period, the latex was cooled to room temperature and cleaned by serum replacement. For this purpose, the latex suspension was centrifuged at 14,000 rpm for 15 min. The supernatant was discarded and the particles were redispersed in water (40 ml) by ultrasonication for approximately 1 min. This operation was performed three times. To obtain thermosensitive poly(NIPA-co-VPBA) latex particles with different boronic acid contents, the VPBA feed concentration was varied in the copolymerization experiments.

Characterization of latex particles

Particle size was determined by transmission electron microscopy (TEM, JEOL, JEM 1200EX, Japan). An aqueous dispersion of cleaned latex particles diluted approximately 10-fold (about 0.1 ml) was spread onto a formvar-coated grid and the water was evaporated at room temperature. The specimens were examined by TEM and the fields including particles placed in the monolayer form were photographed with a magnification of 5,000×.

The boronic acid content of the latex particles was determined by the carmine method [46]. For this purpose, a concentrated HCl solution (0.1 ml) containing 5% (w/w) HCl was added to the cleaned latex sample (2 ml, solid content approximately 50 mg). Following the addition of concentrated sulfuric acid solution (10 ml), the resulting mixture was cooled to room temperature. Then, carminic acid solution (10 ml) prepared by dissolving carmine (0.092 g) in concentrated sulfuric acid (100 ml) was added. The absorbance of the final solution was measured at 550 nm in a UV–Vis spectrophotometer (Shimadzu, Kyoto, Japan). The VPBA content of latex particles was calculated by means of the calibration curve prepared by measuring the absorbances of the aqueous VPBA solutions of known concentrations.

The thermosensitive behaviour of poly(NIPA-co-VPBA) latex particles was followed using a UV–Vis spectrophotometer equipped with a heater and a temperature control system (Varian, Cary 100, U.S.A.). For this purpose, the cleaned latex was diluted to obtain a suspension having a solid content of approximately 0.1%

(w/w). The pH of this suspension was adjusted using aqueous NaOH solution to a prescribed value (i.e. 5, 7 or 9). The absorbance was recorded at 500 nm in the temperature range of 15–40 °C by increasing the temperature with a heating rate of 1 °C/min. The same procedure was also followed in the determination of the critical flocculation temperature (CFT) of poly(NIPA-co-VPBA) latex particles. For each poly(NIPA-co-VPBA) latex, these determinations were performed at pH 7.0 in the aqueous media containing NaCl at different concentrations. The temperature at which a peak was observed in the variation of absorbance with the temperature was evaluated as the CFT of the selected latex in the aqueous medium with a certain NaCl concentration [47].

Nucleic acid adsorption experiments

RNA and DNA adsorption experiments were performed in batch fashion. Typically, a certain volume of cleaned latex containing poly(NIPA-co-VPBA) particles (0.05 g) was centrifuged at +4 °C and 14,000 rpm for 10 min and the supernatant was discarded. The precipitated particles were redispersed in HEPES buffer (i.e. adsorption medium, 5 ml, pH 8.5, 0.1 M $MgCl_2$) containing RNA or DNA at a certain concentration. The adsorption medium was magnetically stirred at 250 rpm for 2 h at +4 °C. The preliminary adsorption experiments indicated that the adsorption equilibrium was established within this period. The latex suspension was centrifuged at 14,000 rpm for 10 min at +4 °C and the supernatant was isolated. In the experiments performed for the effect of temperature on the RNA adsorption, the centrifugation was conducted at a temperature identical to that of adsorption. After adsorption, the RNA concentration in the supernatant was determined by measuring the absorbance at 260 nm in a UV–Vis spectrophotometer (Shimadzu, Kyoto, Japan). The amount of RNA adsorbed onto the poly(NIPA-co-VPBA) particles (Q_{RNA}, mg RNA/g dry particles) was calculated according to Eq. (1), where C_o (mg/ml) and C_f (mg/ml) are the initial and final RNA concentrations in the adsorption medium, respectively. V (ml) and M (g) are the volume of adsorption medium and the amount of dry particles, respectively.

$$Q_{RNA} = [(C_o - C_f)]V/M \quad (1)$$

After adsorption, the DNA concentration in the supernatant was determined by Spirin's method described in detail elsewhere [48, 49]. The amount of DNA adsorbed onto the particles (Q_{DNA}, mg DNA/g dry particles) was calculated by adapting Eq. (1) for DNA.

RNA desorption experiments

Following RNA adsorption at +4 °C, the latex was centrifuged at the same temperature and the supernatant was separated from the particles. RNA adsorbed poly(NIPA-co-VPBA) particles were transferred into a desorption medium (HEPES buffer at pH 8.5, 0.1 M $MgCl_2$). The particles were redispersed in the solution at room temperature. After obtaining a stable suspension, the temperature of the desorption medium was increased to 37 °C with a heating rate of 0.3 °C/min. The entire mixture including aggregated poly(NIPA-co-VPBA) particles was kept at 37 °C for 30 min. Following complete precipitation of poly(NIPA-co-VPBA) particles by the thermoflocculation taking place in the desorption medium, a certain volume of sample (3–4 ml) was withdrawn from the clear supernatant and the absorbance was measured at 260 nm. By using the calibration curve (i.e. RNA concentration versus absorbance), the desorption yield was calculated as the ratio of desorbed amount of RNA (mg) to the amount adsorbed onto the particles (mg).

Results and discussion

Characterization of thermosensitive latex particles

Thermosensitive poly(NIPA) and poly(NIPA-co-VPBA) latex particles with different boronic acid contents were prepared by dispersion polymerization, by using the conditions in Table 1. First, two types of poly(NIPA) particles with different average sizes (NV1 and NV2) were obtained. As seen here, poly(NIPA) particles with higher size could be achieved by the use of higher NIPA feed concentration. The particles encoded as NV2 were used as control sorbent in the RNA isolation experiments to evaluate the RNA adsorption/desorption behaviour of boronic acid functionalized particles.

Boronic acid functionalized latex particles were synthesized by fixing the crosslinking agent (MBA) concentration at 2.7% mol (except particles encoded as NV5). Representative TEM photographs of poly(NIPA-co-VPBA) particles are given in Fig. 1. These photographs indicated that the copolymerization of NIPA and VPBA provided latex particles of nearly uniform size. The properties of the particles are given in Table 1. As seen here, either the size or the boronic acid content of particles increased with increasing VPBA feed concentration.

Thermosensitive behaviours of poly(NIPA-co-VPBA) latexes as followed by UV–Vis spectrophotometry are given in Fig. 2. Here, the magnitude of absorbance difference between fully shrunken and fully swollen states

Table 1 The production conditions and properties of poly (NIPA-co-VPBA) particles (C_{NIPA}: NIPA concentration in the polymerization medium, d_p: particle size, Q_{VPBA}: VPBA content of latex particles. Polymerization conditions: KPS: 30 mg, water: 40 ml, temperature: 70 °C, shaking rate: 120 cpm, time: 24 h)

Code	C_{NIPA} (mg/ml)	Feed monomer composition			d_p (nm)	Q_{VPBA} (mg/g dry particles)
		NIPA (% mol)	VPBA (% mol)	MBA (% mol)		
NV1	12.5	97.3	0.0	2.7	266	0.0
NV2	25.0	97.3	0.0	2.7	429	0.0
NV3	12.5	93.7	3.6	2.7	214	49.2
NV4	12.5	90.4	6.9	2.7	286	94.9
NV5	12.5	87.9	6.7	5.4	329	66.9
NV6	12.5	87.3	10.0	2.7	386	144.0
NV7	12.5	84.4	12.9	2.7	471	171.0

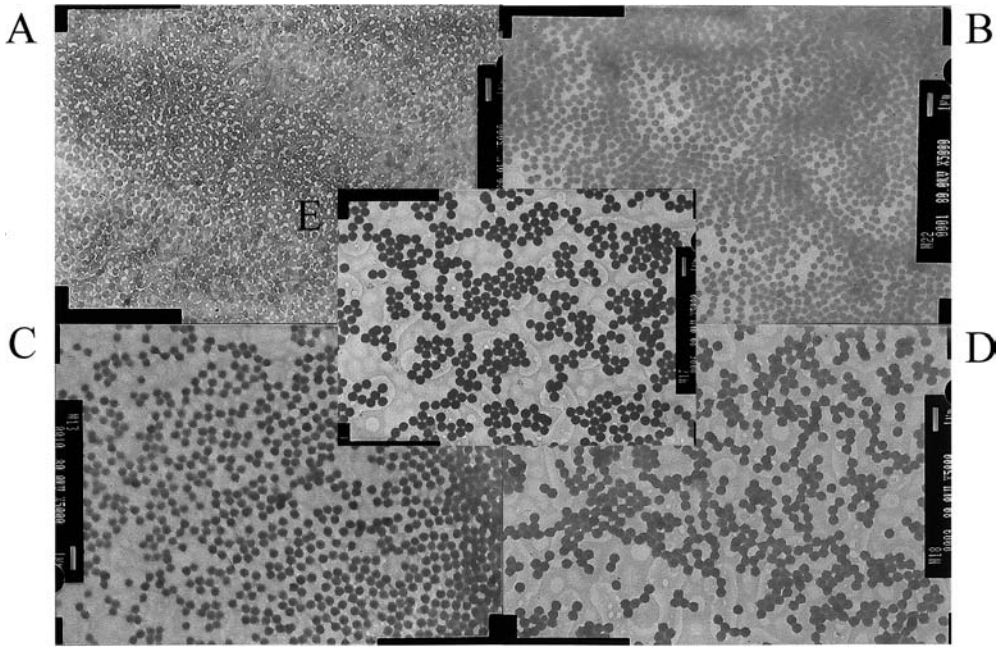

Fig. 1 TEM photographs of poly(NIPA-co-VPBA) particles prepared with different boronic acid feed concentrations. Magnification 5,000×. Polymerization code and VPBA feed concentration (mol %): **A** NV3, 3.6; **B** NV4, 6.9; **C** NV5, 6.7; **D** NV6, 10.0; **E** NV7, 12.9. MBA feed concentration: 2.7% mol for NV3, NV4, NV6 and NV7 and 5.4% mol for NV5

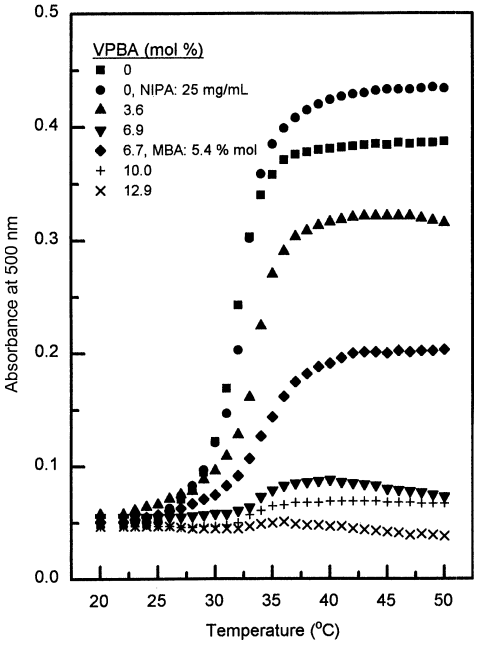

Fig. 2 Thermosensitive behaviour of poly(NIPA) and poly(NIPA-co-VPBA) latexes followed by UV–Vis spectrophotometer

of latex particles was considered as a measure of thermosensitivity. Hence the latex sample exhibiting the largest absorbance difference between fully shrunken and fully swollen states is probably the most thermosensitive one among the materials produced. As seen in Fig. 2, poly(NIPA) latexes and poly(NIPA-co-VPBA) latex with the lowest boronic acid content (i.e. 49.2 mg/g) exhibited a larger absorbance difference between upper and lower plateau values with respect to the other particles. The absorbance difference decreased with increasing VPBA content of the latex. This behaviour is probably expected since VPBA is the temperature non-responsive component of particles. Among the poly (NIPA-co-VPBA) particles obtained at a constant crosslinking agent concentration of 2.7% mol, the particles produced by keeping the VPBA feed concentration at 6.9% mol or higher did not exhibit a significant absorbance difference between fully shrunken and fully swollen states (Fig. 2). To obtain another poly(NIPA-co-VPBA) particle sample exhibiting an appreciable absorbance difference, a recipe as an alternative to NV4 was used in the experimental design. For this purpose, the VPBA feed concentration was fixed at 6.7% mol while the crosslinker concentration was doubled with respect to NV4. The use of higher crosslinking agent concentration with respect to NV4 caused a slight decrease in the boronic acid content; hence an appreciable thermosensitivity was obtained with the particles encoded as NV5 (Table 1 and Fig. 2).

Poly(NIPA-co-VPBA) particles had a critical flocculation temperature (CFT) in the salt containing aqueous medium. The absorbance–temperature curves utilized for CFT determination at different salt concentrations are exemplified for poly(NIPA-co-VPBA) particles with the highest thermosensitivity (i.e. NV3) in Fig. 3A. While the variation of absorbance with the temperature in the aqueous medium containing no salt was expressed as an S-shaped curve, the absorbance exhibited a peak point at the CFT in the aqueous media prepared with different NaCl concentrations. The decrease in the

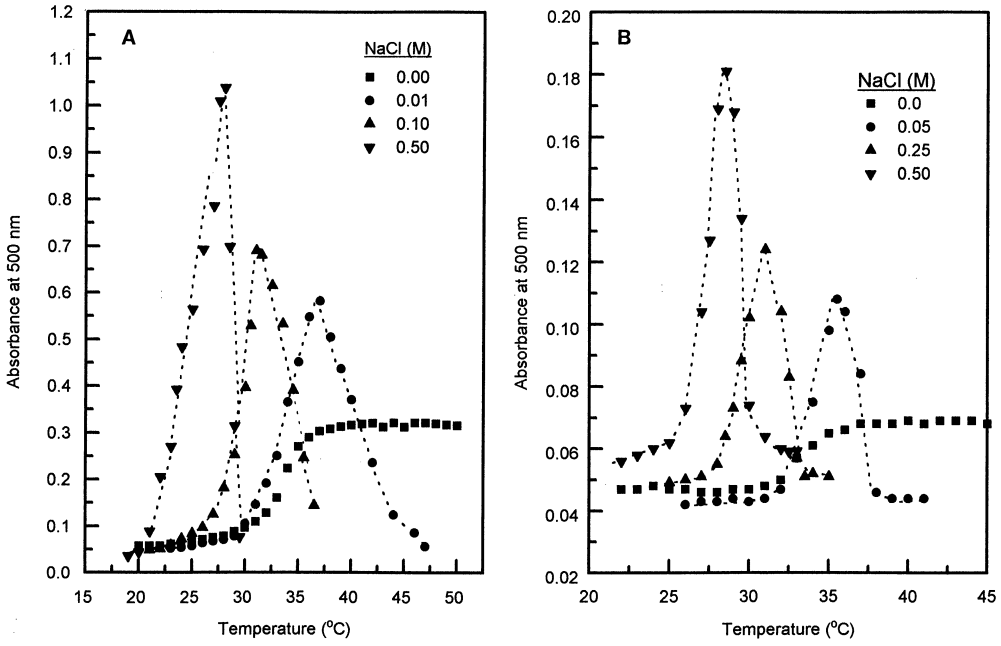

Fig. 3 Typical absorbance–temperature curves indicating the critical flocculation temperatures of poly(NIPA-co-VPBA) latexes having relatively low and high boronic acid contents. Latex type and boronic acid content: **A** NV3, 49.2 mg/g; **B** NV6, 144.0 mg/g

absorbance after the peak point originated from the flocculation of shrunken particles. As seen in Fig. 3A, the peak intensity increased with increasing salt concentration. In other words, thermosensitive behaviour of poly(NIPA-co-VPBA) particles was observed more clearly at higher salt concentration. Even for the poly (NIPA-co-VPBA) particle types providing no significant absorbance difference in pure water (i.e. NV6), clear and sharp peaks were obtained in the salt-containing media (Fig. 3B). The variation of CFT with the salt concentration is exemplified for different poly (NIPA-co-VPBA) particles in Fig. 4. As expected, the CFT decreased with increasing salt concentration in the aqueous medium. The particles with higher absorbance difference between fully shrunken and swollen states (Fig. 2) had lower CFTs at constant salt concentration.

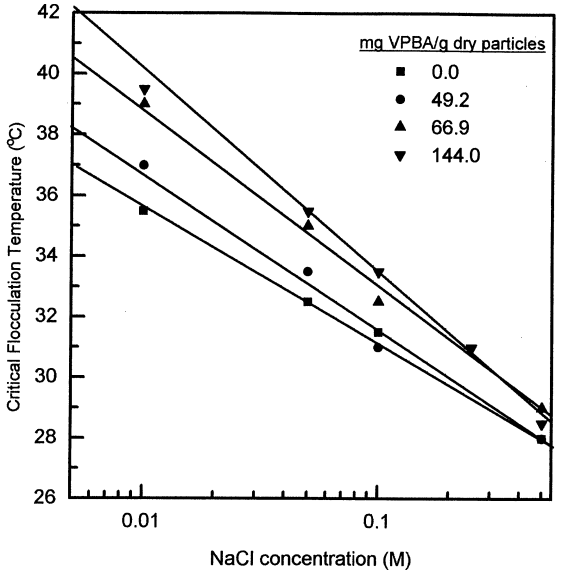

Fig. 4 The variation of critical flocculation temperature with the salt concentration for selected poly(NIPA-co-VPBA) particles

Nucleic acid adsorption onto thermosensitive latex particles

In the first group of experiments, the equilibrium adsorption behaviours of plain poly(NIPA) latex particles were investigated by using DNA or RNA as adsorbate in the separate batches. The adsorption experiments were performed at +4 °C in HEPES buffer (pH 8.5, 5 ml) containing poly(NIPA) particles (0.05 g based on dry weight) as sorbent. The selected pH was reported as an appropriate value in the chromatographic studies involving the separation nucleotides by using boronic acid carrying support materials [3, 4, 10, 11]. In the adsorption runs, the initial nucleic acid (i.e. DNA or RNA) concentration was varied between 0.5 and 3.0 mg/ml. For poly (NIPA) particles, the variation of nucleic acid adsorption with the initial nucleic acid concentration is given in Fig. 5. No detectable amount of RNA was adsorbed onto the plain poly(NIPA) particles in the selected concentration range while DNA adsorption markedly increased with increasing DNA concentration. This behaviour was used as a reference to evaluate the RNA and DNA adsorption behaviours of VPBA carrying latex particles.

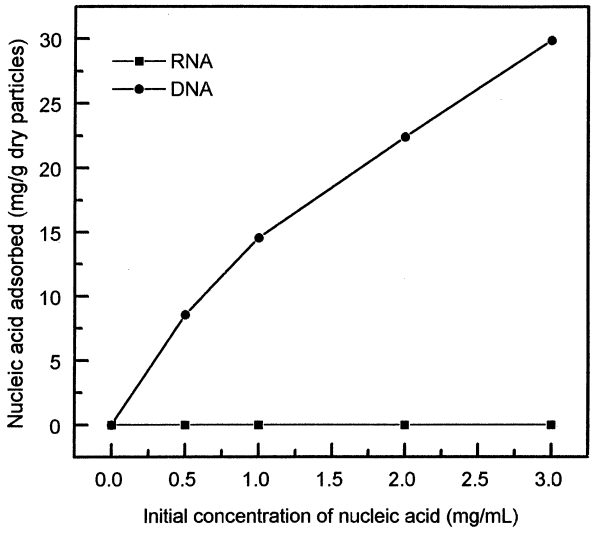

Fig. 5 The variation of nucleic acid adsorption onto the poly (NIPA) particles with the initial nucleic acid concentration

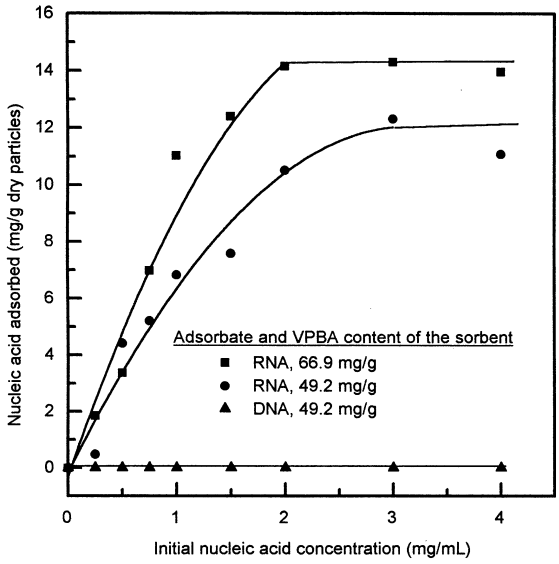

Fig. 6 The variation of nucleic acid adsorption onto the poly (NIPA-co-VPBA) particles with the initial nucleic acid concentration

In the second group of adsorption experiments, DNA and RNA adsorption behaviours of poly(NIPA-co-VPBA) latex particles were investigated under conditions identical to those of the previous set. Poly(NIPA-co-VPBA) latexes with VPBA contents of 49.2 and 66.9 mg/g (i.e. NV3 and NV5) were tried as sorbents in these experiments. Note that the latex with the VPBA content of 49.2 mg/g provided the largest absorbance difference with change in temperature (Fig. 2). This latex (i.e. NV3) was used in both RNA and DNA adsorption experiments. The other latex, NV5 was only tried for RNA adsorption. The variation of nucleic acid adsorption onto the poly(NIPA-co-VPBA) particles with the initial nucleic acid concentration is given in Fig. 6. These runs were performed at pH 8.5 since this value was determined as the pH providing the maximum RNA adsorption in the preliminary experiments. An adsorption behaviour opposite to that of the plain poly(NIPA) latex was observed in Fig. 6. As seen here, no DNA adsorption onto the poly(NIPA-co-VPBA) particles having a VPBA content of 49.2 mg/g was observed. However, RNA adsorption exhibited a marked increase with increasing RNA concentration for both of the sorbents. Poly(NIPA-co-VPBA) particles with higher VPBA content exhibited slightly higher RNA adsorption. These results clearly indicate that poly(NIPA-co-VPBA) particles can be utilized as a pseudo-specific sorbent for selective adsorption of RNA from aqueous RNA–DNA mixtures. RNA adsorption onto the poly(NIPA-co-VPBA) latex is probably explained by the complex formation between boronate groups of the support material and the diol groups of RNA [1, 3, 4]. This conclusion is also supported by the absence of RNA adsorption onto the plain poly(NIPA) particles (Fig. 5). On the other hand, no significant DNA adsorption was observed onto the poly(NIPA-co-VPBA) particles probably due to the repulsive forces between the DNA molecules and negatively charged boronic acid groups of the support material at the studied pH.

The effect of temperature on the RNA adsorption behaviour of poly(NIPA-co-VPBA) particles is given in Fig. 7. The adsorption experiments were performed at pH 8.5 with the initial RNA concentration of 2.0 mg/ml. The sorbent concentration was fixed at 10 mg/ml in a

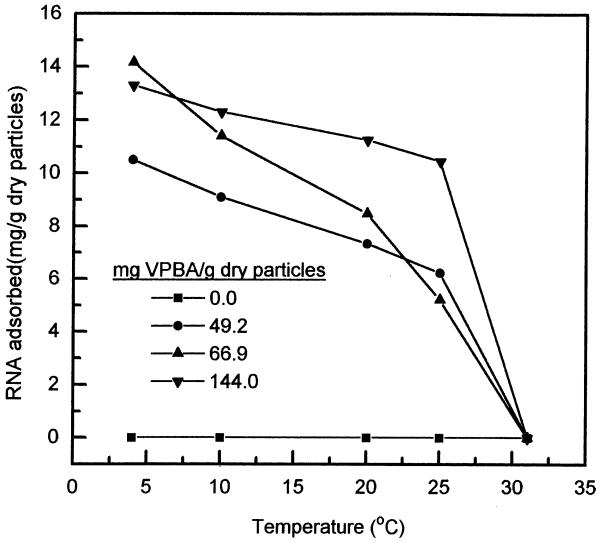

Fig. 7 The effect of temperature on the RNA adsorption behaviour of poly(NIPA-co-VPBA) particles

batch volume of 5 ml. As seen in Fig. 7, RNA adsorption onto the poly(NIPA-co-VPBA) particles markedly decreased with increasing temperature. It should be noted that no RNA adsorption was detected at temperatures higher than 30 °C with the all particles tried as sorbent. To explain the behaviour of poly(NIPA-co-VPBA) particles, a scheme was proposed as shown in Fig. 8. At low temperatures (i.e. +4 °C), thermosensitive poly(NIPA-co-VPBA) particles located in the adsorption medium probably had a tailor-made structure including a swollen-core and flexible copolymer chains carrying boronic acid groups. The slightly basic character of the adsorption medium (pH 8.5) also makes easier the ionization of boronic acid groups present on the tailored copolymer chains. Hence the diol groups in the large RNA molecules easily interact with the boronic acid groups located on the flexible copolymer chains. For this reason, RNA adsorption onto the poly(NIPA-co-VPBA) particles was higher at the lower temperatures. On increasing the temperature, the core shrinks and the copolymer chains located on the core lose their flexibility by folding and probably form a stiff layer on the surface of a shrunken core. In this case, the interaction of RNA molecules with the boronic acid groups probably becomes more difficult. Therefore the RNA adsorption capacity of the particles decreases. At constant temperature, although slightly higher RNA adsorptions were observed by using poly(NIPA-co-VPBA) particles with higher VPBA contents, RNA adsorption capacity was not directly proportional to the VPBA content of the particles. This behaviour is probably related to the molecular size of RNA.

The combination of results in Figs. 2 and 7 indicated that RNA adsorption onto the particles not exhibiting an appreciable thermoresponsive behaviour also decreased with increasing temperature (i.e. the behaviour of particles having a VPBA content of 144.0 mg/g, in Fig. 7, encoded as NV6). The thermosensitive behaviour observed by spectrophotometry is expected to be a function of the changes that occurred in the size and light transmission characteristics of the latex particles. Therefore the adsorption behaviour of NV6 should be attributed to the fact that RNA adsorption was predominantly controlled by the structural changes taking place on the particle surface with change in temperature.

RNA desorption from thermosensitive latex particles

In studies involving the isolation of nucleic acids and nucleotides, the desorption process is usually performed in a strongly basic medium (i.e. pH > 10) and/or containing salt at extremely high concentrations (approximately 1 M NaCl or $MgCl_2$) [3, 4]. Then an extensive dialysis is usually applied for the purification of desorbed biological agent. The behaviour in Fig. 7 demonstrated that RNA adsorption onto the poly (NIPA-co-VPBA) particles could be controlled by adjusting the temperature. Based on this behaviour, the desorption of RNA from poly(NIPA-co-VPBA) particles should be achieved only by elevating the temperature. This approach provides a flexibility for the properties of the desorption medium and eliminates the necessity of a special medium with certain properties. On the other hand, on elevating the temperature, poly (NIPA-co-VPBA) particles exhibited a thermoflocculation behaviour during desorption. By the precipitation of aggregated particles, the separation of sorbent material from the desorption medium was achieved during the desorption of RNA. Hence the amount of RNA

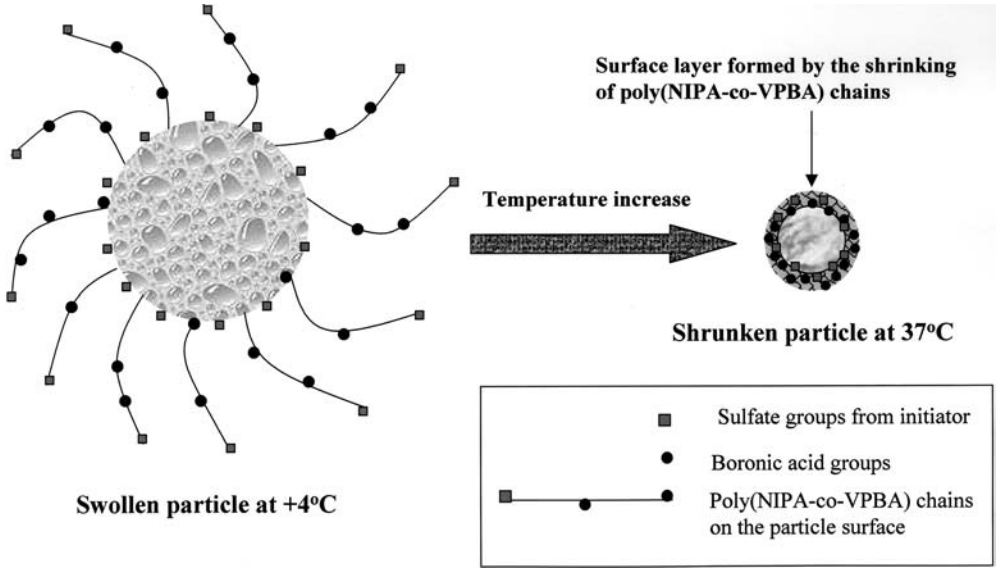

Fig. 8 Possible structural changes occurring in the poly (NIPA-co-VPBA) particles with change in temperature

desorbed was recovered in the clear supernatant. The route followed for the isolation of RNA from nucleic acid mixtures is summarized in Fig. 9. In our procedure, RNA was adsorbed onto the poly(NIPA-co-VPBA) particles at +4 °C and pH 8.5. RNA adsorbed poly (NIPA-co-VPBA) particles were separated from the adsorption medium by centrifugation conducted at the same temperature (i.e. +4 °C). The particles were redispersed in the desorption medium at any pH and ionic strength, at room temperature. For desorption of RNA and thermoflocculation of poly(NIPA-co-VPBA) particles, the temperature was elevated to 37 °C. The desorption of RNA was conducted at this temperature for 30 min in the absence of stirring. At the end of this period, the precipitation of aggregated poly(NIPA-co-VPBA) particles was completed and RNA removed from the particles was recovered in the clear supernatant. The desorption temperature (i.e. 37 °C) was selected as a sufficiently high value at which the desorption process was conducted with a satisfactory yield and the particles were aggregated. The preliminary experiments showed that no significant increase occurred in the desorption yield by increasing temperature to values higher than 37 °C. In the desorption experiments, HEPES buffer solutions with different pH values between 6.0 and 9.0 and containing different salts were used as desorption media. The variation of RNA desorption yield with pH is given in Fig. 10. Here the desorption behaviour was examined at both +4 and 37 °C in the presence of 0.1 M $MgCl_2$. As seen in this figure, the desorption yield was below 10% at +4 °C. However, desorption yields higher than 50% were obtained at 37 °C by using the same conditions. It should be noted that higher desorption yields relative to those of NaCl-containing media were observed in the presence of $MgCl_2$. This figure also indicated that pH was not an effective parameter controlling the desorption yield at 37 °C. In other words, satisfactorily high desorption yields could be obtained in the aqueous solutions with pH between 6.0 and 9.0. The use of a boronic acid carrying sorbent in the thermosensitive form allowed the desorption of selected diol carrying biomolecules (i.e. RNA) over a relatively wide pH range without a significant change in the yield occurring. The effect of salt concentration on the desorption behaviour was investigated at 37 °C by using two different salts and is given in Fig. 11. As seen here, the lowest RNA desorption was obtained in the medium containing no salt. The RNA desorption yield with 0.1 M NaCl or $MgCl_2$ was significantly higher relative to that in the

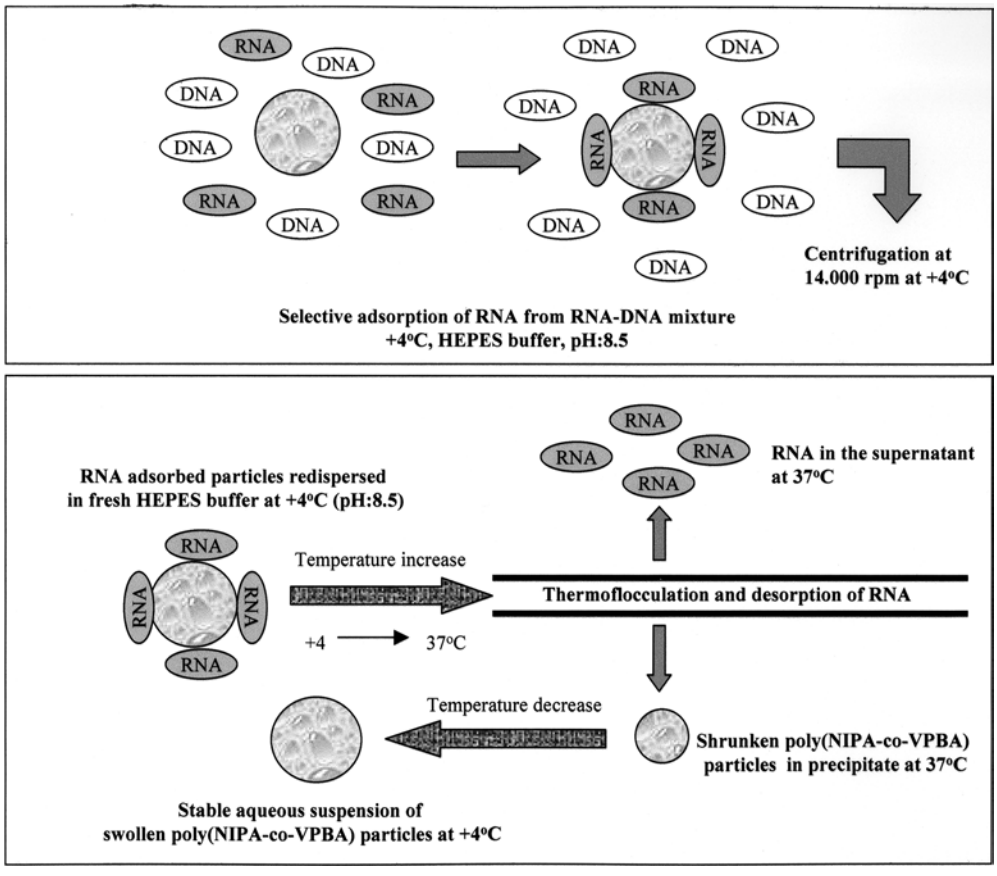

Fig. 9 A scheme showing temperature controlled RNA isolation from the nucleic acid mixtures using thermosensitive poly(NIPA-co-VPBA) particles as pseudo-specific sorbent

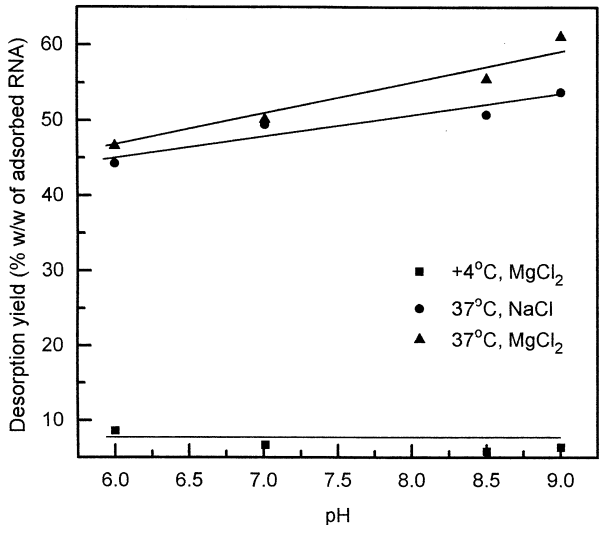

Fig. 10 The variation of RNA desorption yield with the medium pH. (Adsorption conditions: sorbent: NV3, sorbent concentration: 10 mg/ml, temperature: +4 °C, pH: 8.5, initial RNA concentration: 1.5 mg/ml, time: 2 h. Desorption conditions: sorbent concentration: 10 mg/ml, temperature: +4 or 37 °C, salt concentration: 0.1 M, time: 30 min)

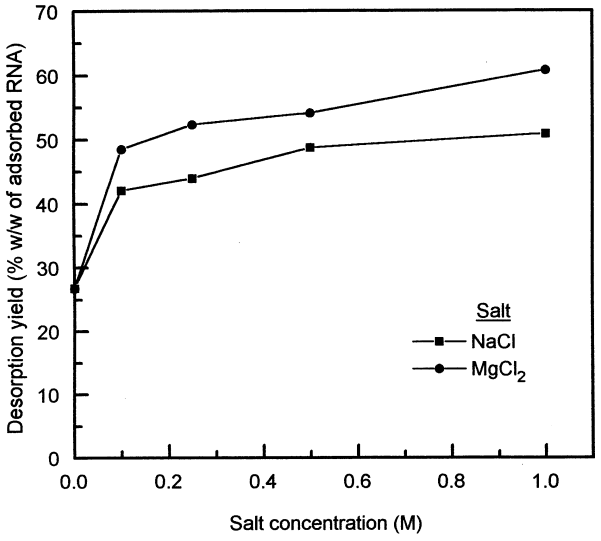

Fig. 11 The variation of RNA desorption yield with the salt concentration. (Adsorption conditions: sorbent: NV3, sorbent concentration: 10 mg/ml, temperature: +4 °C, pH: 8.5, initial RNA concentration: 1.5 mg/ml, time: 2 h. Desorption conditions: sorbent concentration: 10 mg/ml, temperature: 37 °C, pH: 8.5, time: 30 min)

absence of salt. However, a further increase in the salt concentration did not provide a significant increase in the desorption yield. The behaviours in Figs. 10 and 11 indicated that RNA desorption from poly(NIPA-co-VPBA) particles was predominantly controlled by the temperature.

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