Articles

Novel Thymine-Functionalized Polystyrenes for Applications in Biotechnology. Polymer Synthesis and Characterization

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ABSTRACT: Novel polystyrenes (PS) carrying thymine functional groups were synthesized by copolymerizing styrene (St) and 1-(vinylbenzyl)thymine (1-VBT) in the presence and absence of divinylbenzene (DVB) in batch free radical emulsion polymerization. Microsphere latexes were obtained with an average particle size of ≈60 nm (3.0 and 5.0 wt % $[VBT]_0$) with ≈80% conversion and ≈38 nm (10 wt % $[VBT]_0$) with 91% conversion. The final copolymer latexes were freeze-dried to obtain particles in the size range of 32−544 μm. Copolymer compositions were determined by FTIR-DRIFT, ^{1}H NMR, and elemental analysis and were found to be close to the composition of the monomer charges. XPS analysis revealed that VBT concentration on the surface of the particles was much higher (17, 24, and 36 wt %) than in the bulk. Phenol was selected as a model compound to examine adsorption onto the thymine functional groups. Hydrogen bonding between the phenolic hydroxyl group and the thymine units of soluble polymers was evidenced by ^{1}H NMR and FTIR spectroscopy. Adsorption isotherms obtained with all samples showed a good fit with Langmuir's model, supporting evidence for a monolayer chemisorption model in the heterogeneous adsorbent−phenol/hexane system investigated. 89.4% of the phenol was desorbed by adding Borax buffer solution of pH ≥ 10 to the adsorbent−phenol/hexane system. These novel copolymers have potential in biotechnology.

Introduction

The area of functional polymers is one of the most active in polymer science. Incorporating specific functional groups into polymers can influence the physical, mechanical, and rheological properties of the materials.[1] Polymer surface properties are also closely related to functional groups. By incorporating desired functional groups on the surface of and/or within polymeric microspheres, the particles can be used in a variety of applications including medical diagnostics, immunoassays, cell separation, drug delivery, and chemical analysis.[2] Several different surface groups have been bonded to polymer particles, including carboxylate, amino, aldehyde, acetalhydroxyl, and disaccharide groups.[3]

Functionalizing polymers with nucleic acid bases (DNA bases) is of particular interest because of their role in forming complexes between complementary bases through hydrogen-bonding interactions. Japanese researchers reported the synthesis of polyethylenimine carrying adenine and thymine groups by grafting carboxylic acid derivatives of the nucleic acids onto the polymer backbone,[4,5] obtaining 35−100 mol % functionality. Poly(L-lysine) was functionalized with various nucleic acids (thymine, adenine, and uracil) using a similar method, reaching 8−93 mol % base content.[6] Polyacrylate, polymethacrylate, and poly(methyl methacrylate) carrying thymine functionalities (4−100 mol %) were synthesized by free radical homopolymerization and copolymerization of various thymine-functionalized acrylic monomers.[7] These polymers were used to study photodimerization via the photosensitive nucleic acid functional groups. Higashi et al.[8] synthesized novel surface thymine-functionalized poly(methacrylic acid) (PMAA) amphiphiles by postpolymerization functionalization. They studied the interaction of the monolayers of these amphiphiles at the air−water interface with polynucleotides having the complementary nucleic acid base (adenine). They used FTIR to demonstrate that guest adenine−nucleotides bonded to the thymine moiety through a complementary hydrogen-bonding mechanism. We have not found any reports on the synthesis and characterization of cross-linked polymeric microspheres carrying nucleic acid functional groups suitable as a stationary phase for oligonucleotides purification/separation. Affinity chromatography used for the separation, purification, and analysis of oligonucleotides mostly employs inorganic solid supports. Yashima et al.[9] immobilized adenine and thymine onto porous silica gel

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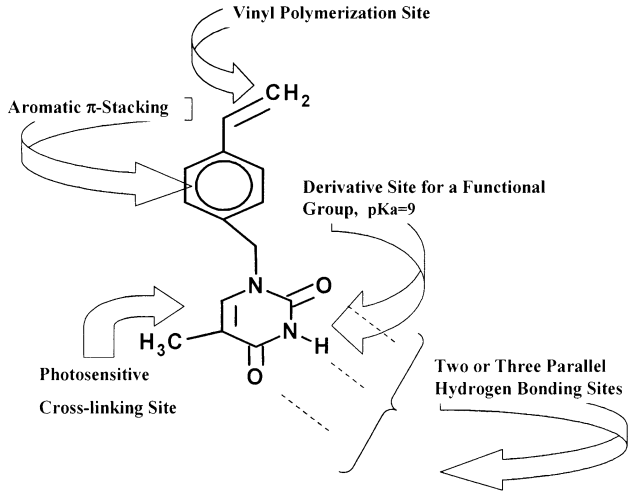

Figure 1. Versatility of 1-VBT.

stationary phase for the separation of oligomers of adenylic and uridylic acids. Other groups[10,11] have used immobilized homopolymers of thymidylic acid on Sepharose (cross-linked agarose gel) for the purification of FadR protein. Breemen et al.[12] immobilized 3-(1-thymidyl)propanoic acid on a silica HPLC column carrying aminopropyl functional groups. This column was used successfully for the separation of polyadenylic or polythymidylic deoxyribonucleotides.

1-(Vinylbenzyl)thymine (1-VBT) was introduced as a versatile monomer by Taylor et al.,[13,14] as illustrated in Figure 1. They reported the homopolymerization of 1-VBT as well as its copolymerization with a variety of monomers such as styrene (St), *n*-butyl methacrylate, methacrylic acid, diacetoneacrylamide, and carbomethoxymethyl acrylate by the emulsion free radical mechanism. However, characterization of these polymers was not reported.

It was a desirable objective to synthesize thymine-functionalized cross-linked polystyrene (PS) microspheres for biotechnology applications. Potential applications include affinity chromatography for the separation/purification of oligonucleotides and proteins and drug binding/delivery by adsorption/desorption.

In this work, we will discuss the synthesis of novel thymine-functionalized polystyrenes (PS) by the copolymerization of styrene (St) with 1-VBT in the absence and presence of divinylbenzene (DVB) and their characterization. The novelty of this work is that, with the exception of a brief mention of copolymers prepared with the 1-(vinylbenzyl)thymine monomer by Taylor et al.,[13,14] there has been no description of well-characterized poly(styrene-*co*-(vinylbenzyl)thymine) copolymers in the literature. It will be shown that true copolymers formed in which the thymine groups are covalently bonded to the structure. This structure is expected to be advantageous for binding large molecules such as proteins, since the benzene ring spacer group between the thymine and the polymer backbone gives more flexibility to the functional groups. Indeed, in the work of Breemen et al.,[12] a propyl spacer group separated the thymine moieties from the solid support. In this paper, hydrogen bonding will be investigated using phenol, a model acid. The application of the novel copolymers in selective bioseparation of bovine serum albumin (BSA) and bovine hemoglobin (BHb) and drug binding/release will be discussed in consecutive parts of this series.

Experimental Section

Materials. 1-VBT was used as received (courtesy of Dr. L. D. Taylor, Polaroid Co., Cambridge, MA). The structure and purity were verified by ^{1}H NMR spectroscopy in deuterated benzene (C_6H_6-d_6). St (99%, Aldrich), DVB (55% isomer mixture, Aldrich), hydroquinone (HQ) (99%, Aldrich), sodium dodecyl sulfate emulsifier (99%, Sigma), potassium persulfate initiator (99.6%, Fisher), sodium bicarbonate (99.7%, Fisher), Borax (sodium tetraborate) (99%, Sigma), ammonium acetate (98%, BDH), hydrochloric acid (38%, BDH), acetic acid (99.7%, Caledon), and sodium hydroxide (97%, Caledon) were all used as received without further purification. Phenol (99+%, Fisher) was vacuum-distilled prior to use.

Tetrahydrofuran (THF) (HPLC grade, EM Science) was distilled off CaH_2 prior to use. Hexane (98+%, BDH) was further purified by distillation from Na/benzophenone. Deuterated THF (THF-d_8), deuterated chloroform ($CDCl_3$-d), and deuterated benzene (C_6H_6-d_6) (Aldrich) were used as received.

Instrumentation. Capillary hydrodynamic fractionation (CHDF-2000, Matec Applied Science) was used to measure the latex particle size distribution, calibrated against colloidal particles of known size. All final samples were freeze-dried (Refrigeration for Science, Inc.) at −100 °C under vacuum (0.013 Pa).

Optical microscopy (Zeiss Axioplan, interfaced to a high-resolution color video camera) was used to investigate the final dried copolymer particles and their dispersion in hexane for the adsorption experiments in hexane.

The molecular weight MW (M_n, M_w) and molecular weight distribution MWD (M_w/M_n) of the soluble non-cross-linked copolymer were determined by size exclusion chromatography (SEC) using a Waters system equipped with six Styragel-HR columns (10^6, 10^5, 10^4, 10^3, 500, and 100 Å pore sizes), thermostated at 35 °C, a Waters 410 DRI detector thermostated at 40 °C, a Dawn DSP multiangle laser light scattering detector (MALLS) (Wyatt Technology), and a Waters 484 UV detector. The mobile phase was THF at 1 mL/min, continuously distilled off CaH_2, and recirculated. The software Astra (Wyatt Technology) controlled the data acquisition and processing. $dn/dc = 0.183$ given for PS[15] was used for the calculations, which gave good agreement with values calculated assuming 100% mass recovery.

Fourier transform infrared spectroscopy in diffuse reflectance mode (FTIR-DRIFT) was performed using a Bruker IFS 55 infrared spectrometer equipped with a mercury−cadmium−telluride (MCT) detector cooled with liquid nitrogen. Spectra were analyzed using a Spectra-Tech Baseline Drifts accessory. The copolymer samples were extracted with hexane and dried in a vacuum desiccator before IR analysis. The samples were ground and diluted to 10 wt % in KBr and then analyzed using a microsampling cup. Measurements were run in triplicates. For quantitative analysis, FTIR spectra (standard VBT and copolymer samples) were converted according to Kubelka−Munk theory,[16] and the 1700 cm^{-1} peak was deconvoluted assuming a Lorentzian shape. Peak areas, assuming proportionality with concentration, were obtained by the Grams/32 software (Galactic Industries Co.).

The ^{1}H NMR spectrum of the 1-VBT monomer was recorded using a 400 MHz Varian DRX-500 spectrometer in C_6H_6-d_6 solvent. ^{1}H NMR spectra of hexane-extracted copolymer samples dissolved in THF-d_8 were recorded using a 500 MHz Bruker DRX-500 spectrometer (Bayer Inc., Sarnia, Canada).

Elemental Analysis (C, H, N) of the copolymer samples was carried out by Guelph Chemical Laboratories Ltd., Canada, using the "dynamic flash combustion" method and a Fisons C, H, N analyzer (model 1108).

X-ray photoelectron spectroscopy (XPS) spectra were recorded using a Kratos Axis Ultra equipped with monochromatic source at 210 W. The pass energy was 160 eV (0.7 eV steps) and 20 eV (0.1 eV steps) for the survey and high-resolution spectra, respectively. The pressure in the chamber was approximately 1×10^{-8} Torr. A takeoff angle of 90° relative to the surface plane was used to obtain all spectra. In all high-resolution spectra, the C 1s, O 1s, and N 1s peaks

were deconvoluted assuming Gaussian shape using the CasaXPS software. The atomic concentrations of C, O, and N on the copolymer surface were determined from the relative peak areas of the C 1s, O 1s, and N 1s electrons.

In the adsorption−desorption studies, phenol concentration was monitored by UV−vis spectophotometry (Philips PU 8625) at $\lambda = 270$ nm. ^{1}H NMR spectra were recorded in deuterated chloroform freshly distilled from calcium hydride, using a 400 MHz Varian DRX-500 spectrometer. Solution FTIR experiments were performed using a Bio-Rad FTS 175 C spectrometer, also in deuterated chloroform freshly distilled from calcium hydride. The IR chamber was purged with N_2 before and during sampling to eliminate interference from CO_2 and H_2O.

Procedures. a. Polymerizations. Polymerization experiments were carried out in a 500 mL kettle-shaped glass reactor under a nitrogen atmosphere. The reactor was equipped with a reflux condenser and an overhead stirrer driven by a Caframo RZR-2000 motor and operated at 300 rpm. A Tamson model 5 microprocessor controlled water bath was used to maintain a reactor temperature at 81 °C. Distilled and deionized (DDI) water, surfactant, and sodium bicarbonate were placed in the reactor and mixed, and then monomers were added; conditions and concentrations will be specified in the text and figure captions. The reaction system was purged with nitrogen for 10 min prior to the addition of the potassium persulfate initiator, and then the system was sealed. The polymerization reactions were short-stopped with a 4 wt % aqueous hydroquinone solution after 3 h. The resulting white, milky polymer latexes were immediately placed in an ice bath. 0.1 g samples taken from the final polymer latexes were suspended in 10 g of DDI water and degassed using a Fisher Scientific ultrasonic cleaner (FS-20) for particle size analysis. Total monomer conversion was determined gravimetrically.

b. Adsorption−Desorption Experiments. Batch adsorption experiments were performed in magnetically stirred beakers by adding accurately weighed amounts of copolymers (adsorbent) to solutions containing phenol (adsorbate) in hexane. Before the adsorption experiments, copolymer samples were sequentially washed with water, methanol, and hexane several times to remove any impurities or residues and dried in a vacuum desiccator overnight. Phenol concentrations were adjusted below 10 mM to avoid hydrogen bond formation between solute molecules. The heterogeneous systems were stirred for 24 h to ensure equilibrium. Phenol concentration in the adsorbed phase (q = g of phenol/g of copolymer) was calculated using the following equation: $q = [(C_0 - C)V]/W$, where V is the volume of solution, C_0 is the initial solution concentration, C is the final solution concentration, and W is the weight of the adsorbent.

After reaching adsorption equilibrium, 25 mL of an aqueous buffer was added to the phenol/polymer/hexane system; the pH values were varied between 5 and 11 (pH 5: 0.05 M ammonium acetate with acetic acid; pH 7 and 9: 0.05 M Borax with hydrochloric acid; pH = 10 and 11: 0.05 M Borax with sodium hydroxide). The three phases (hexane, water, and solid adsorbent phase) were stirred for 6 h. After equilibration, the phenol concentration in the aqueous and hexane phases was determined using UV−vis spectroscopy. The amount of phenol remaining adsorbed on the solid phase was calculated from a material balance.

Results and Discussion

Copolymer Synthesis. Nine emulsion copolymerization experiments were carried out using various initial 1-VBT concentrations ([VBT]$_0$) in the monomer charge; the conditions and resulting latexes are presented in Table 1.

In all cases, microsphere latexes had been produced. Duplicate experiments were carried out with 3 and 5 wt % VBT in the monomer charge; good reproducibility is evident from the data. It can also be seen from the data that the presence of 1 wt % DVB practically had no effect on the copolymerization. With 3 and 5 wt % VBT in the monomer charge, conversion was around 80% with number-average particle sizes of around 60 nm. Homopolystyrene synthesized under identical conditions also had about 60 nm particle size. With 10 wt % [VBT]$_0$, higher conversion (91%) and smaller particle size (38 nm) were obtained. This can be explained by more particle nucleation with higher polar comonomer content. After freeze-drying, the non-cross-linked samples, which were produced in the absence of the DVB, were soluble in solvents such as THF, benzene, toluene, and chloroform. The copolymers produced in the presence of the DVB were insoluble. The freeze-dried polymer samples, examined under an optical microscope, appeared to be flaky, with a wide particle size range (32−544 μm). Apparently, freeze-drying resulted in the generation of particle aggregates. Freeze-drying was used to protect the thymine pendant groups; high-temperature (60 °C) vacuum oven drying resulted in gelation of the polymers produced in the absence of DVB.

Table 1. Emulsion Copolymerization of 1-VBT and St in the Presence and Absence of DVB[a]

run I.D.	[VBT]$_0$, wt %	[DVB]$_0$, wt %	conv,[b] %	mean particle size[c] S_n, nm	mean particle size[c] S_w, nm
1	3		83.1	63.2	68.7
5			81.2	56.3	62.1
3		1	83.1	65.9	74.0
7		1	79.0	63.9	65.8
2	5		79.6	62.3	68.1
6			78.0	62.7	68.7
4		1	76.1	56.8	67.3
8		1	78.7	58.8	64.3
9	10		91.0	37.7	45.5

[a] Polymerization conditions: temperature 81 °C; time 3 h; surfactant: sodium dodecyl sulfate (0.454 M); initiator: potassium persulfate (0.042 M); buffer solution: sodium bicarbonate (0.116 M, pH = 7.5). [b] Gravimetric analysis. [c] CHDF. Standard deviations (SD) = 10.5% for both S_n and S_w.

Copolymer Characterization. The molecular weight of the non-cross-linked samples was $M_n \approx 620\,000$ g/mol with $M_w/M_n \approx 2.0$ for the charges with 3.0 and 5.0 wt % [VBT]$_0$. M_n values calculated based on dn/d$c = 0.183$ for PS[15] showed good agreement with the values calculated on the basis of assuming 100% mass recovery on the SEC columns, with an average difference of 0.8%. Samples obtained with 10% VBT in the charge had extremely high molecular weight ($M_n \gg 10^6$ g/mol). The SEC traces were narrow and skewed, indicating size exclusion so they were not evaluated.

The VBT content in the soluble copolymers (samples 1, 2, 5, 6, and 9 in Table 1) was determined by FTIR-DRIFTS, ^{1}H NMR, and elemental analysis. Table 2 summarizes the results.

A representative ^{1}H NMR spectrum (sample 2 in Table 2) is shown in Figure 2.

The VBT content of the copolymers was obtained by comparing the peaks arising from the amide proton (a) at 10.2 ppm, the methylene protons (d) at 4.65 ppm, and the methine proton (i) in the VBT at 3.15 ppm, with the broad peaks arising from the aromatic protons in the VBT units (e1 and e2) and the St units (e3, e4, and e5) in the copolymer backbone between 6.3 and 7.3 ppm. ^{1}H NMR results are summarized in Table 2. Examination of the ^{1}H NMR data in Table 2 reveals that the values based on the methine (i) and methylene (d) protons are very close, while the VBT content based on the amide proton (a) is slightly lower. This difference

Table 2. Composition Analysis of the Copolymer Samples Listed in Table 1

	wt % of VBT in the copolymer bulk						wt % of VBT on the polymer surface		
		^{1}H NMR					XPS		
run I.D	FTIR	peak a	peak d	peak i	elem anal.	$[VBT]_{ave}$[a]	O/C ratio	N/C ratio	$[VBT]_{ave}$[b]
1 (soluble)	2.80	2.69	2.90	2.96	2.86	2.88	16.35	15.86	16.11
5 (soluble)	2.94	2.68	2.90	2.94	3.03	2.95	18.51	18.03	18.27
3 (cross-linked)	2.92				3.03	2.98	17.15	15.26	16.21
7 (cross-linked)	2.86				2.94	2.90	18.06	17.38	17.72
2 (soluble)	4.48	4.74	4.88	4.90	5.11	4.85	22.97	22.68	22.83
6 (soluble)	4.73	4.55	4.99	4.81	4.93	4.87	25.76	25.14	25.45
4 (cross-linked)	4.68				4.93	4.81	23.13	21.10	22.12
8 (cross-linked)	4.47				4.67	4.57	25.26	24.75	25.0
9 (soluble)	8.56	7.33	8.59	8.44	8.56	8.54	36.24	36.13	36.19

[a] Average of VBT content measured by NMR (excluding peak a), FTIR, and elemental analysis. [b] Average of surface VBT content based on O/C and N/C values.

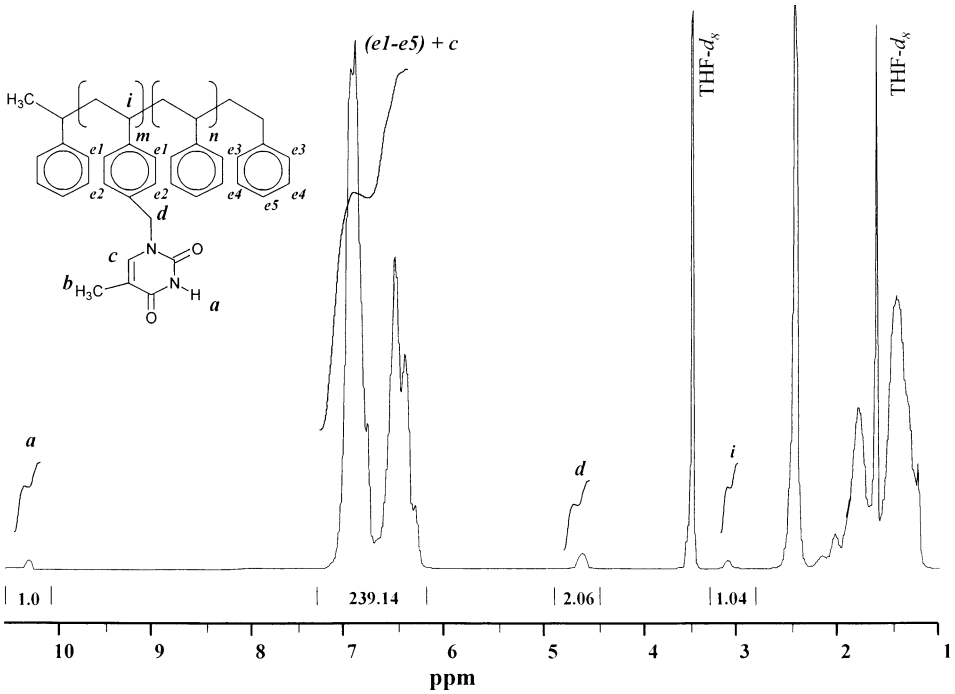

Figure 2. ^{1}H NMR spectrum of a copolymer sample in THF-d_8 ($[VBT]_{ave}$ = 4.85 wt %, sample 2 in Table 2).

likely arises from the fact that the amide peak is significantly broader than the other peaks (quadrupole broadening).[17,18] In $CDCl_3$ this peak completely disappears due to fast proton exchange.

The FTIR spectrum of the same sample (sample 2 in Table 2) is shown in Figure 3a. In this spectrum, the peak at 1700 cm^{-1} was assigned to the coupled vibration of secondary amide and ketone groups in the thymine moiety, which confirmed the presence of the VBT in the final copolymers.

For quantitative FTIR analysis, a calibration curve was constructed with pure 1-VBT in KBr, based on the area under the 1700 cm^{-1} peak and assuming proportionality with VBT concentration (not shown). Difference spectra were also obtained by subtracting the FTIR spectra of copolymers with lower VBT content from the spectra of those with higher VBT content. A difference spectrum of samples 2 and 1 is shown in Figure 3b. The 1700 cm^{-1} band shows very clearly in the difference spectra, as demonstrated in Figure 3b. Using the calibration curve yielded a difference of 1.67 wt % VBT between samples 2 and 1 in Table 2, which is in good agreement with the difference of 1.68% calculated from polymer composition data in Table 2. Similar agreement was obtained with all the difference spectra. The compositions of the soluble copolymers calculated on the basis of carbon, hydrogen, and nitrogen elemental analysis, also listed in Table 2, agree well with those obtained by FTIR-DRIFT and ^{1}H NMR. These results verify the formation of true copolymers. $[VBT]^a_{ave}$ data in Table 2 represent overall composition of the copolymer samples, calculated as the average of the data obtained by various analytical techniques (excluding peak a in the NMR analysis as explained above). In summary, 3 wt % VBT in the monomer charge yielded soluble copolymers with 2.88 wt % (sample 1) and 2.95 wt % (sample 5) VBT, while 5 wt % VBT in the monomer charge resulted in 4.85 wt % (sample 2) and 4.87 wt % (sample 6) VBT content in the soluble copolymers. This is slightly higher than the ideally expected 2.4 and 4 wt % at 80% conversion, indicating slightly higher reactivity for the more polar comonomer.

The cross-linked nonsoluble polymers were characterized by DRIFT-FTIR and elemental analysis. The data in Table 2 show that 3 wt % $[VBT]_0$ yielded 2.98 wt % (sample 3) and 2.90 wt % (sample 7), and 5 wt % $[VBT]_0$ yielded 4.81 wt % (sample 4) and 4.57 wt % (sample 8) VBT content in the corresponding cross-linked copolymers. These data demonstrate that the non-cross-linked

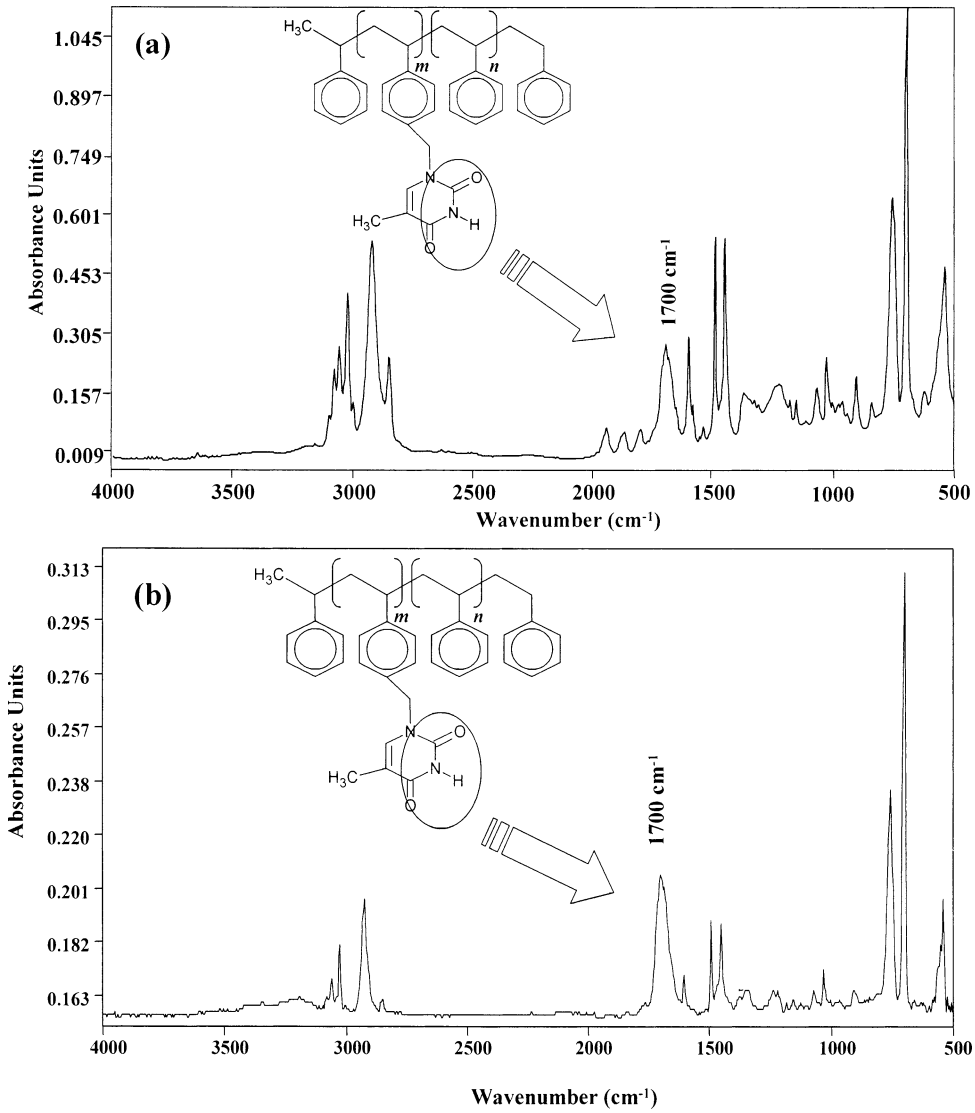

Figure 3. (a) FTIR spectrum of a copolymer sample (4.85 wt % VBT, sample 2 in Table 2, 10 wt % in KBr). (b) A representative FTIR difference spectrum (Table 2, sample 2 (4.85 wt % VBT), and sample 1 (2.88 wt % VBT), both diluted to 10 wt % in KBr).

and cross-linked copolymers prepared under similar conditions have practically identical VBT content. The analysis discussed above demonstrates that the copolymerization conditions selected yielded true copolymers reproducibly.

XPS was used to characterize the surface composition of the copolymers. XPS survey spectra of the copolymer samples showed peaks corresponding to O 1s and N 1s electrons, representing the thymine moieties on the copolymer surface, together with C 1s signals. The chemical composition of the surface of the copolymers was further characterized by C 1s, O 1s, and N 1s high-resolution spectra, as shown in Figure 4.

Figure 4a shows the high-resolution C 1s spectrum of sample 2 in Table 2. Five types of carbon atoms can be identified as follows: C═C at 284.9 eV, C−C and C−H carbons at 285 eV, C−N carbons at 286.2 eV, and C═O carbons at 287.6 eV. In the high-resolution O 1s spectra one peak representing the C═O groups in the thymine moiety was observed for the O 1s electrons at 532 eV, as shown in Figure 4b. In the high-resolution N 1s spectra, the peak at 400.6 eV in Figure 4c was assigned to the N−C and N−H nitrogens. These results confirm the presence of the thymine moieties on the copolymer surface.

Quantitative XPS analysis was carried out by relating the O 1s and N 1s peaks to the total C 1s peak. Good agreement was observed between composition data based on O/C and N/C ratios, as shown in Table 2; $[VBT]^{b}_{ave}$ represents the average value based on these two methods. Examination of the results in Table 2 reveals that higher VBT content was found on the copolymer surfaces than in the copolymer bulk. This can be rationalized on the basis of the relative hydrophilicity of the VBT monomer compared to St, so the former will be enriched in the surface of the particle interfacing the aqueous phase during emulsion copolymerization. Both cross-linked and non-cross-linked copolymer made with 3 wt % $[VBT]_0$ showed about 16−18 wt % VBT on their surface, while those made with 5 wt % $[VBT]_0$ had 22−25 wt % VBT on the surface. Thus, the non-cross-linked and cross-linked copolymers can be considered equivalent in terms of thymine functionality on the surface as well as in the bulk.

Table 2 includes a soluble sample made with 10 wt % $[VBT]_0$. Analysis of this copolymer sample showed 8.54 wt % VBT in the bulk and 36.19 wt % VBT on the surface. This was the highest VBT loading achieved under the specified polymerization conditions due to the

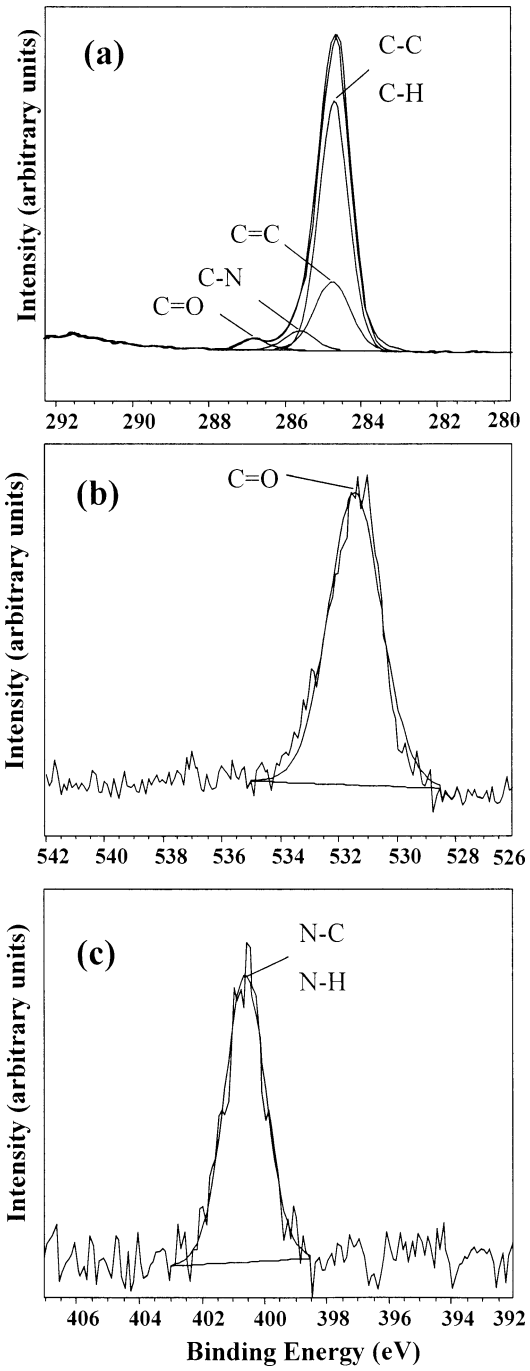

Figure 4. XPS high-resolution spectra of sample 2 (4.85 wt % VBT). (a) XPS high-resolution C 1s. (b) XPS high-resolution O 1s. (c) XPS high-resolution N 1s.

poor solubility of VBT. This soluble copolymer was also included in the adsorption studies.

Adsorption of Phenol, a Model Weak Acid onto VBT−PS Copolymers. Phenol was used as a model compound to study the hydrogen-bonding capability of the thymine functional groups. The interaction between the phenolic hydroxyl group (hydrogen bond donor) and the thymine units (hydrogen bond acceptor) was characterized by ^{1}H NMR and FTIR spectroscopy in deuterated chloroform with soluble non-cross-linked copolymer samples. Figure 5 shows the ^{1}H NMR spectra of phenol in the presence and absence of copolymer (sample 6 in Table 2). Hydrogen bonding is evidenced in Figure 5c by a downfield shift of 0.31 ppm in the phenol hydroxyl proton at 5.11 ppm (Figure 5b) and by

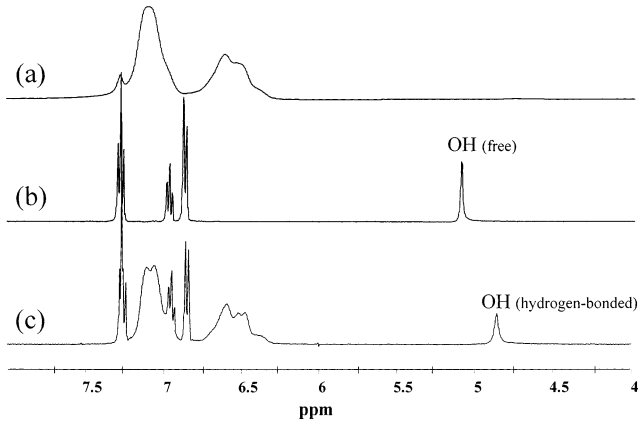

Figure 5. ^{1}H NMR spectra in chloroform-*d* of (a) sample 6, 4.87 wt % VBT; (b) [phenol] = 5 mM; and (c) sample 6, 4.87 wt % VBT + $[\text{phenol}]_0$ = 5 mM.

the broadening of the signal as well. The same 0.31 ppm shift and signal broadening were consistently observed with all soluble copolymer samples. No shift was observed in the aromatic proton resonances at 6.3−7.3 ppm, so stacking interactions or hydrogen bonding with the π electrons of the styrenic backbone of the adsorbent did not occur.[19,20]

Figure 6 shows FTIR spectra of phenol (5 mM) in the absence and presence of soluble copolymer samples (samples 5, 6, and 9 in Table 2) in chloroform-*d*.

In the FTIR spectra of phenol in the absence of copolymer, a single peak is observed at 3624 cm^{-1} (dashed lines in Figures 6a, 8b, and 6c). This peak corresponds to the OH groups of free phenol molecules. When the concentration of phenol exceeded 10 mM, another peak arose at 3508 cm^{-1} (not shown). The 3508 cm^{-1} peak is attributed to the intermolecular hydroxyl/hydroxyl self-association between phenol molecules.[21] To avoid having solute molecule self-association, the concentration of phenol was kept at 5 mM. In the presence of copolymer 5 (Figure 6a, 2.95 wt % VBT), the intensity of the 3624 cm^{-1}peak decreased, and a broad peak arose at a lower frequency of 3470 cm^{-1}. This lower frequency peak is attributed to phenol molecules hydrogen bonded (adsorbed) to the thymine functionality of the copolymer. As the VBT content increased in the copolymers (Figure 6b,c), the intensity of the 3470 cm^{-1} increased but its position did not change. Thus, with increasing VBT content more phenol molecules adsorb via hydrogen bonding to the thymine sites.

Adsorption experiments were conducted in hexane, a non-hydrogen-bonding and nonpolar solvent. None of the copolymer samples (cross-linked or non-cross-linked) dissolved or swelled in the hexane solvent, so the systems were heterogeneous. Stirring the polymer particles in hexane for 24 h led to a particle size range of 2−8 μm. Figure 7 shows a representative linearized Langmuir plot of the equilibrium adsorption isotherm for sample 6 in Table 2, containing 4.87 wt % VBT, at 25 °C.

The good fit to the Langmuir model indicates that phenol is chemisorbed to the thymine units in the heterogeneous adsorbent−phenol/hexane system investigated, most likely via hydrogen bonding. Similarly, a good fit was obtained for all copolymer samples (cross-linked and non-cross-linked) at various temperatures (5, 10, 15, 25, and 35 °C).

Figure 8 shows adsorption and desorption plots with a copolymer sample having 4.87 wt % VBT (sample 6,

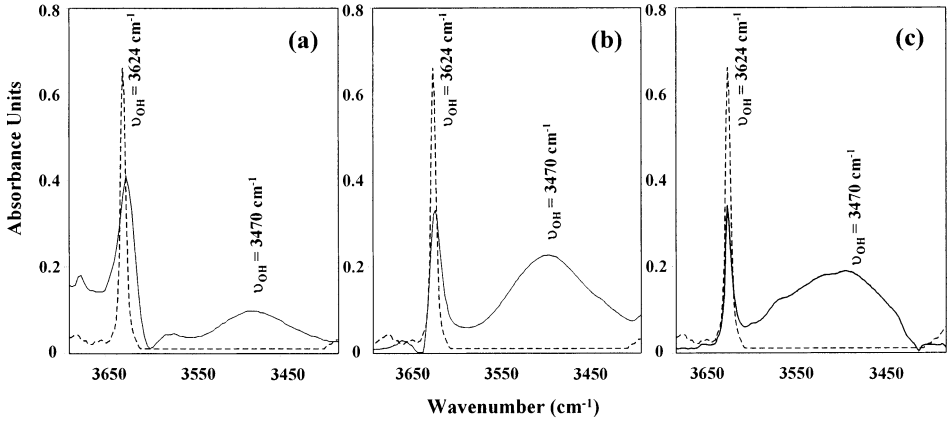

Figure 6. FTIR spectra of phenol (5 mM) in the presence (solid line) and absence (dashed line) of copolymer in chloroform-*d*: (a) sample 5, 2.95 wt % VBT; (b) sample 6, 4.87 wt % VBT; (c) sample 9, 8.54 wt % VBT.

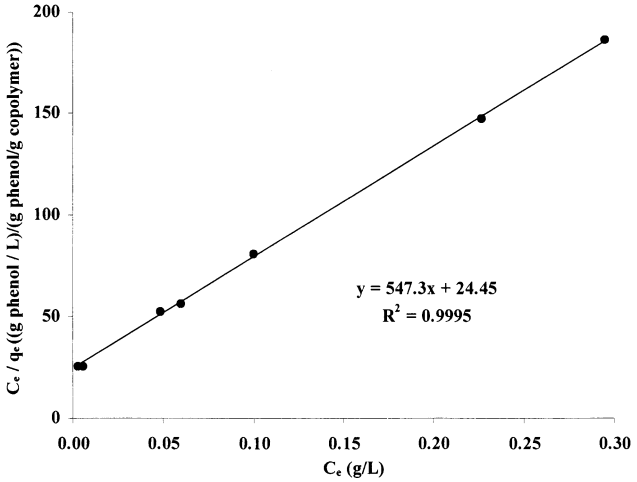

Figure 7. Langmuir isotherm of the adsorption of phenol (0.25−6 mM) from hexane onto thymine-functionalized PS (sample 6 in Table 2, 4.87 wt % VBT) at 25 °C.

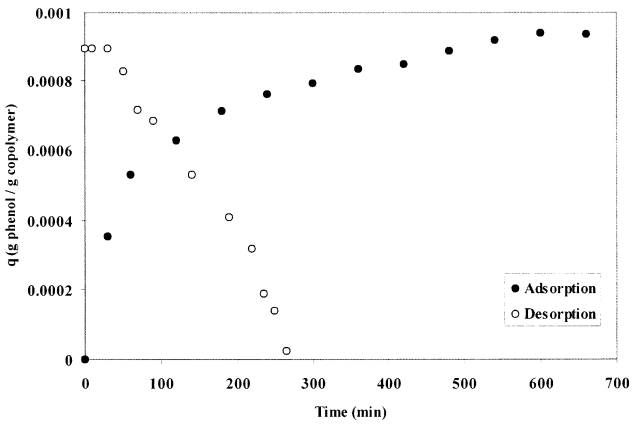

Figure 8. Adsorption of phenol (2 mM) from hexane onto thymine-functionalized PS (sample 6 in Table 2; 4.87 wt % VBT) and desorption by Borax buffer solution (pH_{buffer} = 10, 25 °C).

Table 2). The thymine functional groups were saturated in about 10 h.

Desorption by adding Borax buffer solution of pH = 10 and 11 to the adsorbent−phenol/hexane system was much faster and completed in about 4.5 h. The pK_a of phenol is 9.89; at pH ≥ 10, 89.4% of the phenol was desorbed into the aqueous phase, while 7.2% and 3.4% remained in the adsorbed and hexane phases, respectively. At lower pH, the partition was nearly constant at an average of 48.7% of phenol in the adsorbed phase, 31% in the aqueous phase, and 20.3% in the hexane phase.

Conclusions

In summary, novel cross-linked and non-cross-linked thymine-functionalized polystyrenes were synthesized by emulsion copolymerization with 3, 5, and 10 wt % VBT in the monomer charge. In the presence of DVB, cross-linked copolymers were produced. Compositional analysis of the polymers was carried out using ^{1}H NMR (soluble polymers only), FTIR-DRIFT, elemental analysis, and XPS. Copolymer compositions close to the monomer charge compositions were obtained, and the presence of DVB did not have an effect on the composition. XPS showed considerably higher VBT content on the surface of the copolymer particles than in the bulk. Adsorption via hydrogen bonding was demonstrated using phenol as a model compound. Hydrogen bonding was evidenced by ^{1}H NMR and FTIR spectroscopy. Adsorption data in hexane fit well with the Langmuir model, indicating monolayer chemisorption. Desorption was achieved by deprotonation using aqueous buffer solutions of controlled pH. These new copolymers have potential use in biotechnology applications; such potential applications include affinity chromatography for the separation/purification of oligonucleotides and proteins and drug binding/delivery by adsorption/desorption. Consecutive parts of these series of papers will present representative examples.

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