# Synthesis and Characterization of Polystyrene–Poly(Ethylene Oxide)–Heparin Block Copolymers

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## Synopsis

A procedure for the preparation of new block copolymers composed of a hydrophobic block of polystyrene, a hydrophilic spacer-block of poly(ethylene oxide) and a bioactive block of heparin was investigated. Polystyrene with one amino group per chain was synthesized by free radical oligomerization of styrene in dimethylformamide, using 2-aminoethanethiol as a chain transfer agent. This amino group was used in the coupling reaction with amino-telechelic poly(ethylene oxide) to produce an AB type diblock copolymer with one amino group per polystyrene (PSt)–poly(ethylene oxide) (PEO) chain. The amino-semitelechelic oligo-styrene was converted into the isocyanate-semitelechelic oligo-styrene using toluene 2,4-diisocyanate and subsequent coupling with $H_2N$–PEO–$NH_2$ afforded AB type block copolymers with terminal amino groups. The coupling of PSt–PEO–$NH_2$ with heparin was performed in a DMF–$H_2O$ mixture, first by activating the heparin carboxylic groups with EDC at pH 5.1–5.2 and subsequently reacting the activated carboxylic groups with the amino groups of the PSt–PEO–$NH_2$ at pH 7.5. Depending on the molecular weights of the diblock copolymer used 25–29% w/w heparin was incorporated. These polymers will be further evaluated for their blood-compatibility.

## INTRODUCTION

The extent of thrombus formation at blood–polymer interfaces in blood-contacting devices, such as cardiovascular prostheses and catheters, can be minimized by utilizing nonthrombogenic polymers or nonthrombogenic polymeric coatings.

Enhancement of the blood-compatibility of materials can be achieved by modification of the surface without altering the bulk properties. An example is the treatment of material surfaces by plasma polymerization of tetrafluoroethylene (TFE). Mylar, poly(ethylene teraphthalate) surfaces covered by thin plasma-polymerized films of TFE showed significantly increased blood coagulation times (using Lindholm's test) as compared to bare Mylar surfaces.[2]

Another approach was followed by Horbett and co-workers,[3] who found that hydrogel-grafted surfaces adsorbed less protein and adhered blood cells less strongly than ungrafted hydrophobic substrate polymers. This was hypothesized to be due to the low interfacial tension exhibited between a hydrogel surface and blood. Mori and co-workers[4] reported studies on poly(ethylene oxide) (PEO) chains grafted onto surfaces. It was shown that the number of adhered platelets and the amount of adsorbed plasma proteins significantly decreased with an increase in the PEO chain length ($n$), with a maximum value at $n = 100$ (water content 40–45%).

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A different method to improve the blood-compatibility of polymeric surfaces is to immobilize bioactive agents onto a polymer surface through the use of a spacer. Heparin, the most commonly used anticoagulant, has been covalently coupled to hydroxyl-bearing surfaces via an ethylene imide intermediate,[5] ionically-bound to polyethylene surfaces by prior adsorption of a cationic surfactant,[6] immobilized on a variety of cyanogen bromide-activated surfaces,[7] and recently immobilized via carboxylic groups to diaminoalkane agarose gels.[8] Heparin directly affixed to the surface does not provide optimal, solution-like anticoagulant behavior, but anticoagulant activity increases precipitously for 10-carbon unit spacer groups and longer, while heparin coupled with less than 10-carbon spacer groups demonstrates only minimal anticoagulant activity.[8] This concept was applied to polyurethane surfaces and improved the blood-compatibility of catheters positioned in the external jugular and femoral veins in the direction of the blood flow in short-term dog experiments.[9]

Block copolymer surfaces that exhibit heterogeneous microphase separated surface structures of hydrophilic and hydrophobic microdomains have shown an ability to suppress platelet adhesion[10] and morphological changes of adhering platelets.[11] Because of this heterogeneous microdomain surface structure, an "organized protein layer" is hypothesized to be formed after contact with blood and is supposed to regulate the distribution of different binding sites on a molecular level at the platelet and polymer interface, thereby influencing the adhesion and activation of platelets.[12]

We hypothesize that, through the use of an ABC type block copolymer consisting of a hydrophobic group (A), a hydrophilic spacer (B), and a bioactive agent (C), the blood-compatibility of biomaterials could be improved. The hydrophobic block can be anchored into a substrate by swelling with a proper solvent in a coating process resulting in the exposure of the hydrophilic spacer coupled with antithrombotic agents. Then, the block copolymer could be coated onto materials so that a coating of heterogeneous microphase-separated hydrophobic and hydrophilic microdomains would be exhibited. Finally, the block copolymer could be used for fabrication of blood contacting devices.

In this paper the results of the synthesis and characterization of a new polystyrene–poly(ethylene oxide)–heparin ABC type block copolymer, containing polystyrene as hydrophobic block, poly(ethylene oxide) as hydrophilic spacer-block and covalently bonded heparin as bioactive block, are presented.

## EXPERIMENTAL

### Materials

2-Aminoethanethiol (AESH, Aldrich Chemical Company, Inc., Milwaukee, WI), amino-telechelic poly(ethylene oxide)s, with molecular weights 500 and 4000 [$H_2N$–PEO(500 or 4000)–$NH_2$, a generous gift of Nippon Oil & Fats Company, Ltd., Ibaraki Japan], 2,2′-azobisisobutyronitrile (AIBN, Polysciences Inc., Warrington, PA), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, Sigma Chem. Co., St. Louis, MO), heparin (Hep) from

porcine mucosa, with a specific activity of 161.9 U/mg, as indicated by the manufacturer (Diosynth Inc., Chicago, IL,), tetrahydrofuran (THF) for use in high performance liquid chromatography (J. T. Baker B. V., Deventer, Holland), toluidine blue (Fluka, Buchs, Switzerland), and the solvents chloroform (Fisher Scientific Co., Fair Lawn, NJ), ethanol (E. Meck, Darmstadt, Germany), ether and hexanes (J. T. Baker Chem. Co., Phillipsburg, NJ), methanol (Mallinckrodt Inc., Paris, KY), and toluene (E. Merck) were used as received.

N,N-Dimethylformamide (DMF, J. T. Baker Chem. Co.) was distilled under nitrogen at reduced pressure, after drying over anhydrous magnesium sulphate (100 g/L), and the fraction of bp 65°C/39 mm Hg was used. Dioxane (J. T. Baker Chem. Co.) was distilled once. Styrene (St, Polysciences Inc.) was distilled under $N_2$ at reduced pressure and the fraction of bp 45°C/18 mm Hg was collected. Toluene 2,4-diisocyanate (TDI, Eastman Kodak Co., Rochester, NY) was distilled under $N_2$ at reduced pressure and the fraction of bp 120°C/10 mm Hg was used.

### Amino-Semitelechelic Oligo-Styrene ([1], PSt–$NH_2$)[13]

PSt–$NH_2$ [1] was prepared according to the procedure of Okano and co-workers.[13] DMF solutions containing 2,2′-azobisisobutyronitrile (AIBN, $5 \times 10^{-3}$ mol/L), styrene (St, 5.00 mol/L) and different amounts of 2-aminoethanethiol (AESH) as a chain transfer agent were placed into ampoules. The ampoules were then immersed in liquid $N_2$ and evacuated to $2 \times 10^{-2}$ mm Hg. After warming, clear solutions were obtained. These solutions were subsequently refrozen and the degassing operation was repeated three times. The ampoules were then sealed under reduced pressure at $2 \times 10^{-2}$ mm Hg. Reactions were carried out by placing the ampoules in a shaking water bath, maintaining the temperature at 60°C.

After the appropriate reaction time the contents of the ampoules were poured into methanol to precipitate the PSt–$NH_2$ oligomers [1]. The oligomers were collected using a sintered-glass funnel, washed several times with methanol, and subsequently dried at reduced pressure.

### Amino-Semitelechelic Polystyrene–Poly(ethylene oxide) Diblock Copolymer ([5], PSt–PEO–$NH_2$)[12,14]

PSt–PEO–$NH_2$ diblock copolymers [5] were synthesized by a procedure analogous to that used to prepare polystyrene–HEMA block copolymers (as published by Okano and co-workers[12,14]). The current procedure involves a coupling reaction of the oligomers of isocyanate-semitelechelic oligo-styrene ([3], PSt–N=C=O) and amino-telechelic poly(ethylene oxide) ([4], $H_2N$–PEO–$NH_2$). PSt–N=C=O [3] was prepared as follows: PSt–$NH_2$ ([1], 15% w/v in dioxane) was added dropwise to a solution of toluene 2,4-diisocyanate [2] in dioxane (0.3% v/v) during a period of 120 h until a final ratio of $[NH_2]/[NCO] = 0.5$ was obtained. The reaction mixture was kept at 40°C under a nitrogen blanket. A sample of PSt–N=C=O [3] was obtained after precipitation in hexane, filtration, and drying at reduced pressure. The presence of isocyanate groups in PSt–N=C=O [3] was confirmed by IR-spectroscopy.

The PSt–PEO–$NH_2$ diblock copolymer [5] was synthesized by adding the PSt–N=C=O [3] dioxane solution as described above dropwise over a period of 72 h to a solution of $H_2N$–PEO–$NH_2$ [4] in dioxane (10% w/v) until the final ratio of [$NH_2$]/[NCO] groups was 2.4. This reaction was carried out at room temperature under a nitrogen atmosphere. The unreacted oligo-styrene was removed by pouring the reaction mixture into a 10-fold volume of mixture of ether and hexanes (1 : 9, v/v). The precipitated polymer was then a mixture of [4] and [5]. To remove unreacted poly(ethylene oxide) [4], the precipitate was dissolved in dioxane and reprecipitated in a methanol–water mixture (1 : 9, v/v). The resulting diblock copolymer was isolated by filtration of the reaction mixture using a sintered-glass funnel and dried at reduced pressure.

### Polystyrene–Poly(ethylene oxide)–Heparin Block Copolymer ([7], PSt–PEO–Hep)

PSt–PEO–Hep block copolymers [7] were synthesized via a coupling reaction of PSt–PEO–$NH_2$ diblock copolymer [5] with heparin ([6], Hep) using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride[15] (EDC) as a coupling agent. PSt–PEO–$NH_2$ [5] was dissolved in DMF. Small amounts of water were then added until the final ratio of DMF : $H_2O$ became 40 : 1 (v/v) and the final concentration of [5] was 4.88 g/100 mL. Hep [6] was dissolved in water. DMF was added until the final ratio of $H_2O$ : DMF was 1 : 40 (v/v) and the final concentration of [6] was 2.0 g/L. The two solutions were combined and the pH of the resulting solution was adjusted to 5.1–5.2 using a 1*N* hydrochloric acid solution. EDC was dissolved in a DMF–$H_2O$ mixture (40 : 1, v/v) and eight equal portions of this solution (32.2 g/L) were added to the combined solution at 30 min intervals. The pH was maintained at 5.1–5.2 by adding a 1*N* hydrochloric acid solution or a 1*N* sodium hydroxide solution. Thirty minutes after the last addition of EDC, the pH was adjusted to 7.5 with a 1*N* sodium hydroxide solution and the resulting solution was gently stirred for 20 h.

The polymer was precipitated by adding this solution to a 10-fold excess of vigorously stirred methanol. The polymer was collected by filtration, suspended in water to remove the unreacted Hep and EDC and then in THF to remove the unreacted PSt–PEO–$NH_2$ [5]. After filtration using a sintered-glass funnel the block copolymer was collected and dried at reduced pressure.

### Spectroscopy

Infrared (IR) spectra of films of the oligomers and block copolymers cast on sodium chloride crystals from solutions in either chloroform or dioxane were recorded on a Beckman, Microlab 620 MX infrared spectrophotometer. $^1$H-nuclear magnetic resonance (NMR) spectra were obtained using a JEOL JNM-FX 270 apparatus. The oligomers and diblock copolymers were dissolved in chloroform-$d_1$ and tetramethylsilane was added as an internal standard.

### Molecular Weights

Number-average molecular weights ($\overline{M}_n$), weight-average molecular weights ($\overline{M}_w$) and the molecular weight distributions ($\overline{M}_w/\overline{M}_n$) of PSt—$NH_2$ [1] and $\overline{M}_{n,\text{app}}$ and $\overline{M}_{w,\text{app}}$ of diblock copolymers were determined by high per-

formance liquid chromatography/low angle laser light scattering (HPLC/LALLS) (Waters Model 6000 A HPLC-system, coupled to a Chromatix KMX-6 LALLS-detector and a Waters Differential Refractometer R 401 concentration-detector), using ultra-Styragel columns with exclusion limits $10^5$, $10^4$, $10^3$, and 500 Å in series. THF was used as a solvent and the flow rate was 2.02 mL/min. Refractive index increments ($dn/dc$) were determined by a Brice Phoenix differential refractometer. The $\overline{M}_n$ of low molecular weight samples of PSt–$NH_2$ [1] and PSt–PEO–$NH_2$ [5] were determined by vapor pressure osmometry (VPO) (Hewlett-Packard 301 A) in toluene at 37°C.

### Amino Group Analysis

End group analysis of amino groups in PSt–$NH_2$ [1] and PSt–PEO–$NH_2$ [5] was carried out by potentiometric titration of polymer solutions in DMF with a 0.02 $N$ perchloric acid–DMF solution. The perchloric acid solution was prepared by dissolving approximately 0.84 mL of 70% perchloric acid (Fluka) in 500 mL DMF. This solution was standardized with diphenylguanidine (DPG, Janssen Chimica, Beerse, Belgium) dissolved in DMF.

### Heparin Content in Block Copolymers

The weight percentages of heparin in the different PSt–PEO–Hep block copolymers [7] were determined according to a slightly modified colorimetric assay described by Smith and co-workers.[17] This assay has also been used to determine the amount of immobilized heparin in heparin–agarose and heparin–Sepharose preparations.

A standard curve for the measurement of heparin content in PSt–PEO–Hep [7] was obtained as follows: Glass tubes were cleaned by treatment with 5% w/v potassium dichromate in nitric acid. After washing three times with double distilled water and two times with methanol the tubes were dried in an oven at 120°C. Solutions of toluidine blue [2.5 mL, 50 mg/L DMF–$H_2O$ (40 : 1, v/v)] were pipetted into clean glass tubes. Then different amounts of a standard heparin solution [0.2 g/L DMF–$H_2O$ (40 : 1)] were added to the glass tubes containing the toluidine blue solution, affording a range of 10–70 $\mu$g of heparin per tube. One tube which contained no heparin solution was used as a reference. The contents of the tubes were diluted with DMF–$H_2O$ (40 : 1) to a total volume of 5 mL. The contents were mixed using a Vortex mixer for 30 s. Hexanes (5 mL) were then added to each tube and the tubes were shaken vigorously for another 30 s. The dye depletion of toluidine blue in the DMF–$H_2O$ layers was detected in samples which were first diluted with absolute ethanol (1 : 5) by measuring the absorbance at 631 nm using a Zeiss PM6 spectrophotometer. Absorbances were measured within 60 min after mixing the heparin containing agent and the dye.

The standard curve was obtained by plotting the decrease in absorbance of the toluidine blue solution at 631 nm as a function of the amount of heparin present. The heparin content in the block copolymers was determined by adding three different amounts of a PSt–PEO-Hep solution [0.6 g/L DMF–$H_2O$ (40 : 1)] to tubes containing the toluidine blue solution [2.5 mL, 50 mg/L DMF–$H_2O$ (40 : 1)]. The same procedure as described for heparin was followed. Quantitation of the amount of heparin bound to PSt–PEO–$NH_2$ diblock copolymers [5] was achieved by monitoring the dye depletion at 631

nm in the DMF–$H_2O$ layer [which was also previously diluted with absolute ethanol (1 : 5)] and comparing the results with the standard curve obtained for heparin.

## RESULTS AND DISCUSSION

### Synthesis of Amino-Semitelechelic Oligo-Styrene [1]

PSt–$NH_2$ [1] was synthesized by a free-radical oligomerization of St, using AESH as a chain transfer agent.[13] Similar radical polymerizations to obtain specific end groups were reported by Ikada and co-workers.[18] The reaction proceeds according to the conventional telomerization mechanism as given in Scheme 1. Because the number-average molecular weights calculated from the amino group analysis (one amino group to one oligo-St) were approximately the same as those determined by HPLC or VPO, as shown in Table I, it was confirmed that, on the average, one terminal amino group was present per oligo-St chain. The molecular weights of PSt–$NH_2$ [1] can be easily controlled by varying the amounts of AESH.[14,18] This is due to the large chain transfer constant, $C_s$, of AESH as calculated from the slope of the relation between $1/P_n$ and AESH/St. We found $C_s = 1.6$ while Okano and co-workers[14] reported a $C_s$ value of 1.0. Ikada and co-workers[18] showed that no appreciable chain transfer reaction took place when methyl methacrylate was polymerized using AESH as chain transfer agent.

$^1$H-NMR spectra (phenyl groups: 6.5–7.0 ppm and PSt backbone: 1.4–2.0 ppm) and IR spectra (N—H stretch: 3340 $cm^{-1}$ and C—N stretch: 1090 $cm^{-1}$) confirmed the structure of PSt–$NH_2$ [1].

### Synthesis of Amino-Semitelechelic Polystyrene–Poly(ethylene oxide) Diblock Copolymer [5]

A schematic representation of the synthesis of PSt–PEO–$NH_2$ diblock copolymers [5] is shown in Scheme 2. PSt–N=C=O [3] was prepared by a reaction of PSt–$NH_2$ [1] with TDI [2], at 40°C for 120 h using a ratio of

$$I \longrightarrow 2R^{\bullet}$$

$$R^{\bullet} + H_2NCH_2CH_2SH \longrightarrow RH + H_2NCH_2CH_2S^{\bullet}$$

$$H_2NCH_2CH_2S^{\bullet} + M \longrightarrow H_2NCH_2CH_2SM_1^{\bullet}$$

$$H_2NCH_2CH_2SM_1^{\bullet} + (n-1)M \longrightarrow H_2NCH_2CH_2SM_n^{\bullet}$$

$$H_2NCH_2CH_2SM_n^{\bullet} + H_2NCH_2CH_2SH \longrightarrow H_2NCH_2CH_2SM_nH + H_2NCH_2CH_2S^{\bullet}$$

Scheme 1. Mechanism for the free-radical oligomerization of St using AESH as chain transfer agent. I is initiator, M is styrene.

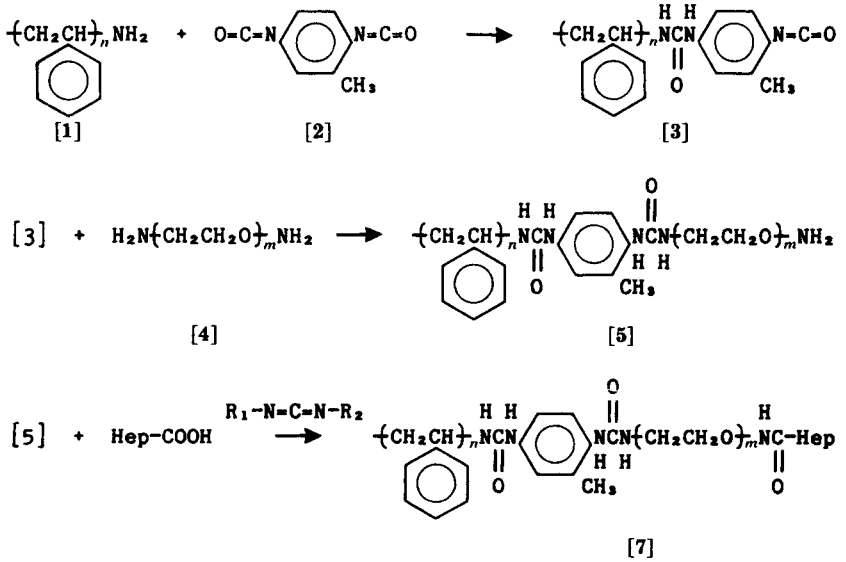
TABLE I
Preparation and Analysis of PSt–$NH_2$ [1][a]

| Code | Molar ratio AESH/St | Yield (%) | $\bar{M}_n$[b] | $\bar{M}_w/\bar{M}_n$[b] | $\bar{M}_n$[c] | $\bar{M}_n$[d] | $\bar{M}_n$[b,c]/$\bar{M}_n$[d] |
|---|---|---|---|---|---|---|---|
| P1 | 0.005 | 21 | 15700 | 2.87 | — | 15900 | 0.99 |
| P2 | 0.010 | 21 | 11900 | 2.98 | — | 10300 | 1.16 |
| P3 | 0.020 | 9 | 2800 | 1.46 | 3300 | 3500 | 0.94 |

[a] Reaction conditions: Styrene (St, 5.00 mol/L in DMF), AIBN $5 \times 10^{-3}$ mol/L and 2-aminoethanethiol, AESH. Reaction for 16 h at 60°C.
[b] $\bar{M}_n$ and $\bar{M}_w$ determined by HPLC in THF.
[c] $\bar{M}_n$ determined by VPO in toluene at 37°C.
[d] $\bar{M}_n$ determined by end group analysis.

$[NH_2]/[NCO] = 0.5$. The presence of isocyanate groups in the prepolymer was confirmed by IR spectroscopy (N=C=O stretch: 2270 $cm^{-1}$). The coupling with $H_2N$–PEO–$NH_2$ [4] was carried out at room temperature for 72 h using a final $[NH_2]/[NCO]$ ratio of 2.4. As shown in Table II, the molecular weights determined from the amino group analysis (one amino group to one molecule PSt–PEO), correlated well with the calculated molecular weights, except for the low molecular weight copolymer PE3. Also the compositions of the diblock copolymers determined from $^1$H-NMR spectra correlated well with the calculated compositions, except for the low molecular weight components. In this case possibly some ABA type block copolymer (calcd. St mol% 85) was formed, resulting in less than one amino group per PSt–PEO molecule chain.

The molecular weight distributions of the synthesized diblock copolymers and their precursors were determined. The HPLC plots for two diblock




Scheme 2. Schematic representation of the synthesis of polystyrene–poly(ethylene oxide)–heparin block copolymers.

TABLE II
Preparation and Analysis of PSt–PEO–$NH_2$ [5][a]

| Code | $\overline{M}_n$ PSt | $\overline{M}_n$ PEO | Yield[b] (%) | [St mol %] Calcd. | [St mol %] Found[c] | $\overline{M}_n$[d] | $\overline{M}_n$[e] | $\overline{M}_n$[f]/$\overline{M}_n$[e] |
|---|---|---|---|---|---|---|---|---|
| PE1 | 15700 | 4000 | 31 | 62 | 61 | — | 20900 | 0.94 |
| PE2 | 11900 | 4000 | 33 | 56 | 56 | — | 16300 | 0.98 |
| PE3 | 3300 | 500 | 31 | 74 | 87 | 7400 | 6600 | 0.58 |

[a] Reaction conditions: for details see experimental part.
[b] Yield of copolymer based on mol PSt.
[c] From NMR-spectroscopy.
[d] $\overline{M}_n$ determined by VPO in toluene at 37°C.
[e] $\overline{M}_n$ determined by end group analysis.
[f] $\overline{M}_n = \overline{M}_n$(PSt) + $\overline{M}_n$(PEO) calculated.

copolymers, PE1 and PE3, and the HPLC plots for their intermediate polymers (P1 and P3) are shown in Figure 1. We quantitatively determined the $\overline{M}_w/\overline{M}_n$ ratio only for the PSt blocks using available calibration curves (see Table I). For diblock copolymer PE1 (see Fig. 1), $\overline{M}_{w,\text{app}}/\overline{M}_{n,\text{app}} = 2.20$, whereas copolymer PE3 shows a less broader distribution with $\overline{M}_{w,\text{app}}/\overline{M}_{n,\text{app}} = 1.20$. For all polymers, single peaks were obtained without shoulders, although some tailing in the lower molecular weight region can be seen. These results indicate that the polymerizations and subsequent coupling reactions have followed an uniform pattern although it cannot be deduced from the HPLC plot that copolymer PE3 also may contain some ABA type block copolymer.

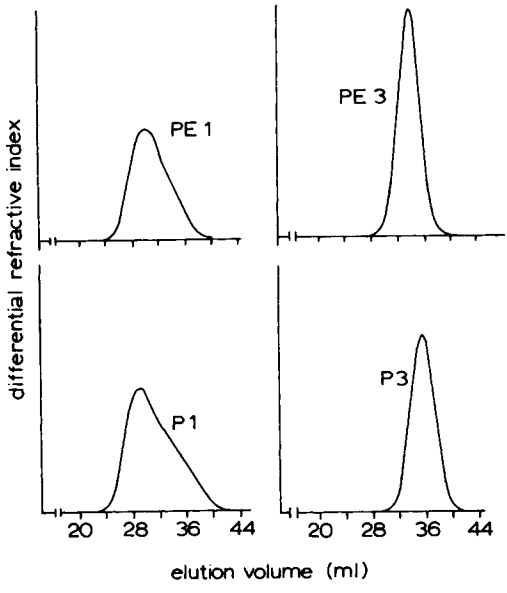



Fig. 1. HPLC plot of polystyrene (P1 and P3) and polystyrene–poly(ethylene oxide) diblock copolymers (PE1 and PE3).

$^1$H-NMR spectra (phenyl groups: 6.5–7.0 ppm, methylene–ether PEO backbone: 3.4–3.8 ppm and PSt backbone: 1.4–2.0 ppm) confirmed the structure of PSt–PEO–$NH_2$ diblock copolymers [5]. Copolymer formation was indicated by the disappearance of the isocyanate IR band (2270 $cm^{-1}$) of PSt–N=C=O [3] and appearance of the covalent urea linkage (—NH—CO—NH—: 1660 $cm^{-1}$) between the PSt and PEO (C—O—C stretch: 1115 $cm^{-1}$) chains.

## Synthesis of Polystyrene–Poly(ethylene oxide)–Heparin Block Copolymer [7]

PSt–PEO–Hep [7] was synthesized by a coupling reaction of PSt–PEO–$NH_2$ [5] with heparin [6] using EDC[15] as a coupling agent in a DMF–$H_2O$ (40 : 1, v/v) mixture. EDC activates carboxylic groups which are present in heparin. These activated carboxylic groups react exclusively with the amino groups of the PSt–PEO–$NH_2$ [5] AB type block copolymers to yield ABC type block copolymers, shown in Table III. It was shown that depending on the molecular weight and composition of the diblock copolymers 25–29% w/w heparin was coupled. Taking into account that the $\bar{M}_n$ of this heparin as determined by Hennink and co-workers[19] is 11000, it is possible to calculate the average-number of PSt–PEO chains coupled to one heparin molecule from the results in Table III. It was determined that approximately 2 PSt–PEO (PE2, $\bar{M}_{n,app}$ = 16300) chains per heparin molecule chain and approximately 4 PSt–PEO (PE3, $\bar{M}_{n,app}$ = 6900) chains per heparin molecule chain were coupled.

In the coupling reaction of PSt–PEO–$NH_2$ [5] and Hep [6], heparin carboxylic groups are modified and consequently the heparin activity may change. As pointed out by Danishefsky and co-workers[20] and Sederel and co-workers,[21] modification of heparin carboxylic groups leads to a decreased anticoagulant activity. To neutralize thrombin activity, heparin molecules must contain binding regions for both AT III and thrombin. Thus heparin modification or linking may alter the formation or exposure of these binding sites, resulting in reduced bioactivity. As stated by Ebert and Kim,[8] irrespective of the anticoagulant mechanisms involved with immobilized heparins, the anticoagulant activity greatly increases as the immobilized heparin molecules are removed form the surface environment to a bulk-like plasma environment via spacer groups. The studies for these materials as being possible compounds for the improvement of the blood-compatibility are now under investigation.

TABLE III
Preparation and Analysis of PSt–PEO–Hep [7][a]

| Code | PSt–PEO–$NH_2$ | (g) | Hep (mg) | EDC (mg) | Yield (g) | Hep in block [w/w %, (s.e.m.)] |
|---|---|---|---|---|---|---|
| PEH1 | PE1 | 2.03 | 180 | 66 | 0.62 | — |
| PEH2 | PE2 | 2.63 | 376 | 132 | 1.19 | 25 (±4) |
| PEH3 | PE3 | 2.53 | 737 | 264 | 1.37 | 29 (±2) |

[a] Reaction conditions: for details see experimental part.

## CONCLUSIONS

Polystyrene with one amino group per molecular chain was synthesized by free radical oligomerization of styrene in DMF, using 2-aminoethanethiol as chain transfer agent.[13] This amino group was used in the coupling reaction with amino-telechelic poly(ethylene oxide) [4] to produce an AB type diblock copolymer with one amino group per PSt–PEO molecular chain. The amino-semitelechelic oligo-styrene [1] was converted into the isocyanate-semitelechelic oligo-styrene [3] using toluene 2,4-diisocyanate [2] and a subsequent coupling with $H_2N$–PEO–$NH_2$ [4] afforded AB type block copolymers with terminal amino groups.

The coupling of heparin [6] to PSt–PEO–$NH_2$ [5] was performed in a DMF–$H_2O$ mixture, first by activating the heparin carboxylic groups with EDC[15] at pH 5.1–5.2 and subsequently reacting the activated carboxylic groups with the amino groups of the PSt–PEO–$NH_2$ [5] at pH 7.5. The yield of coupled heparin was 25 to 29% w/w depending on molecular weight of diblock copolymers. This new procedure enables the preparation of block copolymers composed of a hydrophobic block of polystyrene, a hydrophilic spacer-block of poly(ethylene oxide) and a bioactive block of heparin. These polymers will be further evaluated as possible compounds for the improvement of the blood-compatibility of polymeric surfaces.

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