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Preparation and Characterization of Novel Amphiphilic Hydrogels with Covalently Attached Drugs and Fluorescent Markers

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ABSTRACT: This paper describes the synthesis of styrene-based macromonomers with covalently attached model drugs (ibuprofen and naproxen) or fluorescent markers (pyrene) and their incorporation into linear or hyperbranched *p*-(chloromethyl)styrene copolymers. Alternatively the copolymers were produced by post-polymerization modification of linear or hyperbranched poly[*p*-(chloromethyl)styrene], PPCMSt, with the same compounds. The incorporation of these copolymers into amphiphilic conetworks was achieved by two methods: Williamson ether synthesis between PPCMSt and poly(ethylene glycol), PEG, with hydroxyl end groups or by nucleophilic substitution between the chloromethyl moieties in PPCMSt and the amine end groups in poly(oxyalkylenediamine), Jeffamine. The dynamic and equilibrium swelling properties were studied on representative Jeffamine hydrogels. The swelling studies showed that the conetworks absorb water quickly and reach equilibrium in 1−2 h, the equilibrium swelling ratio of gels based on linear or hyperbranched copolymer being 181−358% and 244−480%, respectively. Preliminary drug release studies in different aqueous media showed that the release kinetics and the amount of drugs released from hydrogels depend on the physical properties of drugs, the microstructure of polymer network, and the drug−polymer interaction and more particularly on the hydrolysis dynamics of ester linkage between the drug and the polymer matrix.

Introduction

The development of novel techniques for drug delivery and controlled release is part of the continuing efforts toward more efficient therapeutic strategies.[1−5] In recent years, amphiphilic hydrogels and polymer conetworks have attracted significant attention as promising materials for drug delivery and wide range of other applications due to their unique structure and properties.[6−14] Since the amphiphilic cross-linked materials often contain hydrophilic and hydrophobic chains, they are able to swell in and interact with both aqueous and organic media and under certain conditions they can absorb increased amounts of hydrophobic molecules, which then can be controllably released by application of external stimulus. That is why the creation of new hydrogels of improved binding and release capabilities continues to be extensively pursued. Often the research has been focused on conetworks, consisting of polymers with block structures, which are prepared by copolymerization of comonomers with different hydrophilic/hydrophobic balance.[15] Alternatively, amphiphilic hydrogels, formed by dendrimers[16] as the cross-linking agents, have been developed by us and other research groups.[17−19] The dendritic conetworks represent interesting substitutes of the traditional systems with their well-defined highly branched and globular domains. In our previous studies we have explored the synthesis of hydrogels based on dendritic poly(benzyl ether)s and linear poly(ethylene glycol)s, PEG.[17,18] The synthetic strategy was based on the reaction of PEG with isocyanate or epoxy end groups as the hydrophilic component and hydrophobic dendritic poly(benzyl ethers) with amino groups at the periphery. The hydrogels obtained had high degree of cross-linking and swelled in both water and organic solvents. In aqueous media these networks were also able to encapsulate pH sensitive stains or model compounds and release them over a period of time,[18b] and thus have intriguing potential for biomedical and biotechnological applications.

The hyperbranched polymers are imperfect analogues of dendrimers sharing many of their unique characteristics, including the well-defined globular shape and the presence of multiple and modifiable surface and interior functionalities.[20] These properties, combined with the much lower cost, make these macromolecules attractive dendrimer substitutes for use in biological and pharmaceutical applications.

Despite their promising potential the hyperbranched structures have been rarely used as cross-linking agents. In an interesting series, Wooley and co-workers have prepared amphiphilic networks by *in situ* cross-linking of mixtures, containing hyperbranched fluoropolymer (HBFP) and diamino-terminated PEG.[21−24] The changes in the PEG/HBFP ratio influenced not only the composition, but also the degree of cross-linking and the topology of resulting network. Recently we have shown that another factor, which strongly affects the characteristic properties of the hyperbranched conetworks, is the macromolecular architecture of the hydrophilic and hydrophobic constituents.[25]

In this paper, we describe the synthesis and characterization of novel model drug-containing *p*-chloromethylstyrene derivatives and the copolymerization of these derivatives with *p*-chloromethylstyrene to afford new copolymers with covalently attached drugs. They are further combined with PEG or poly(oxyalkylenediamine), Jeffamine, to prepare amphiphilic hydrogels via Williamson ether synthesis or nucleophilic substitution. Conetworks of different cross-linking densities and hydrophilicity are obtained by varying the amount of the two polymeric components, the architecture, and the molecular weight of the poly(styrene) derivatives.

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The substance-release properties of the amphiphilic hydrogels are evaluated and correlation of the observed phenomena with network characteristics is made, as well.

Experimental Section

Materials. Linear poly(ethylene glycol), PEG, (L-PEG 5k: M_n = 5000; M_w = 5400, M_w/M_n = 1.08) was purchased from Polymer Source, Inc. Poly(oxyalkylenediamine), Jeffamine ED-6000 (JEFF, nominal molecular weight 6,000) was obtained from Texaco Chemical Company. Sodium hydride, NaH, (95%, dry), ethanol (95%), tetrahydrofuran, THF, (99.8%), 2,2′-Azobis(isobutyronitrile), AIBN (98%), (s)-(+)-4-isobutyl-α-methylphenylacetic acid (ibuprofen) (99%), (s)-(+)-6-methyoxy-α-methyl-2-naphthaleneacetic acid, (naproxen) (98%), 1-pyrenecarboxylic acid (98%), *N*,*N*-diisopropylethylamine, DIPEA (99.5%); copper(I) chloride (≥99%), 2,2′-bipyridil (≥99%), and 18-crown-6 (≥99%) were all purchased from Aldrich. Chlorobenzene (99%) and *p*-chloromethylstyrene (90%) were obtained from Acros. Toluene (99.9%) from Fisher Scientific, K_2CO_3 (≥99%, anhydrous) from Spectrum, dichloromethane (99.9%) from J. T. Baker and chloroform (99.8%) from Mallinckrodt were used as received. *p*-Chloromethylstyrene was distilled under low pressure (0.4 mmHg, 45 °C); AIBN was purified by recrystallization in ethanol. THF was dried over benzophenone-sodium and distilled immediately before use. Acetone, toluene, and chlorobenzene were dried using molecular sieve (mesh size 5 Å) prior to use. Deionized (DI) water (18.3 MΩ) was purified by a Barnstead NANOPure ultrapure water system. NaOH (99%, Mallinckrodt), was used to prepare solutions with pH = 10.1 for the release the drugs from hydrogels.

Instrumentation. Size-exclusion chromatography (SEC) analyses were conducted in THF using a SEC line consisting of a Waters 510 pump, Waters U6K universal injector, an Applied Biosystems 785A programmable UV−vis detector, and a Viscotek Model 250 instrument for dual refractive index and viscometry detection. The separations were achieved at 40 °C across a set of three 5 μ PLgel columns (50 Å, 500 Å and a mixed C) from Polymer Laboratories at eluent flow rate of 1 mL/min. The apparent molecular weights and polydispersities of the polymers were determined by calibration with 20 monodisperse polystyrene standards and software OmniSEC Ver. 3.1 from Viscotek Corporation.

The chemical compositions of polymers, copolymers and conetworks were determined by FT-IR and NMR. The samples were analyzed in a powder/particle form by Magna 750 FT-IR spectrometer (Nicolet) using a MTEC 300 photoacoustic module in the spectral range from 500 to 4000 cm^{-1}. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ at room temperature with a Bruker Avance 300 instrument (^1H: 300 MHz).

Elemental analyses were conducted by Galbraith Laboratories (Knoxville, TN).

The UV spectroscopic measurements for the release of the drugs from the hydrogels were conducted on a DU 640B UV−vis spectrometer (Beckman) from 200 to 800 nm with a scanning speed 600 nm/min at room temperature.

The surface morphology of the conetworks formed was investigated by scanning electron microscopy (SEM) using JEOL 5800 LV SEM instrument with acceleration voltage 5−10 kV. The swollen hydrogel samples were immersed in water for 48 h and then quickly frozen with liquid nitrogen. The frozen hydrogels were subjected to cryofracturing, followed by lyophilization. The dry samples were sputter coated with palladium and gold for SEM examination.

Synthesis. *General Synthesis of p-(Chloromethyl)styrene Derivatives with Ibuprofen, Naproxen, and Pyrene.* In a 25 mL round-bottom flask, K_2CO_3 (2.5 equiv), 18-crown-6 (0.2 equiv), and a drug or a model compound (1 equiv) were added and dissolved in acetone. Then this mixture was purged with nitrogen and *p*-(chloromethyl)styrene (1.05 equiv) was added. The reaction solution was vigorously stirred under nitrogen and refluxed. The reaction process was monitored by thin layer chromatography (TLC) and was stopped after the spots of the starting reagents disappeared on the TLC plate. K_2CO_3 was filtered off and after solvent removal about 90% of crude mixture was obtained. The reaction products were purified by flash chromatography with hexane/diethyl ether mixture with increasing ether content as follows: 32:1−16:1−8:1 and 4:1. These products were characterized by ^1H NMR, ^{13}C NMR, FT-IR, and elemental analysis.

Ibuprofen-Modified p-(Chloromethyl)styrene. This was prepared from ibuprofen and *p*-chloromethylstyrene, yield 46%. R_f = 0.46 in hexane/diethyl ether (4:1). The product was a colorless liquid. ^1H NMR ($CDCl_3$): δ 0.93 (6H, d, 2CH_3), 1.54 (3H, q, CH_3), 1.87 (1H, sept. C*H*), 2.47 (2H, d, CCH_2Ph), 3.78 (1H, m, PhCH_2COO), 5.13 (2H, q, COOCH_2Ph), 5.26 (1H, d, H(Ph)C=CH_2), 5.74 (1H, q, H(Ph)C=CH_2), 6.69 (1H, m, PhC*H*=CH_2), 7.08−7.38 (8H, m, Ar*H*). ^{13}C NMR ($CDCl_3$): δ 18.40 (*C*H_3), 22.37, 22.39 (2*C*H_3), 30.18 (*C*H), 45.02 (*C*HCH$_3$), 45.13 (*C*CH_2Ph), 66.02, 66.09 (COO*C*H_2Ph), 114.13 and 114.22 (Ph*C**H*=*C*H_2), 125.52, 125.79, 126.23, 127.08, 127.22, 128.02, 128.61, 129.31, 129.33, 135.59, 136.35, 136.38, 136.46 (Ar*C*), 174.8 (Ar*C*OO). FT-IR, ν: 2958, 1740, 1514, 1466, 1379, 1332, 1168, 1093, 990, 911, 850, 799, 714 cm^{-1}. Elemental analysis: C: 81.95% (theor), 81.40% (found); H: 8.13% (theor), 8.45% (found) and O: 9.92% (theor), 10.04% (found).

Naproxen-Modified p-(Chloromethyl)styrene. This was prepared from naproxen and *p*-chloromethylstyrene, yield 55%. R_f = 0.33 in hexane/diethyl ether (4:1). The product was a white crystalline substance with melting point 40 °C. ^1H NMR ($CDCl_3$): δ 1.63 (3H, m, CHCH_3), 3.92 (1H and 3H, m, CH$_3$C*H* and CH_3O), 5.13 (2H, q, COOCH_2Ph), 5.23 (1H, m, H(Ph)C=CH_2), 5.70 (1H, q, H(Ph)C=CH_2), 6.65 (1H, m, H(Ph)C=CH_2), 7.10−7.45 (7H, m, Ar*H* and Na*H*), 7.63−7.74 (3H, m, Na*H*). ^{13}C NMR ($CDCl_3$): δ 18.44, 18.51 (*C*H_3), 45.44 (*C*HCH$_3$), 55.28 (*C*H$_3$O), 66.22 (COO*C*H_2Ph), 105.54, 114.20, 118.95, 125.57, 125.83, 125.97, 126.00, 126.26, 127.12, 128.21, 128.89, 129.28, 133.68, 135.46, 135.51, 136.33, 136.37, 137.40, 137.77 (Ph*C**H* = *C*H_2, Ar*C* and Na*C*) 157.66 (CH$_3$O−Na*C*) 174.8 (Na*C*OO). FT-IR, ν: 2980, 1734, 1606, 1506, 1485, 1393, 1267, 1233, 1177, 1034, 855 cm^{-1}. Anal. Calcd: C, 79.74; H, 6.40. Found: C, 79.44; H, 6.36.

1-Pyrenecarboxylic Acid Modified p-(Chloromethyl)styrene. This was prepared from 1-pyrenecarboxylic acid and *p*-chloromethylstyrene, yield 54%. R_f = 0.37 in hexane/diethyl ether (4:1). The product was isolated as light yellow needle crystals with melting point 101 °C. ^1H NMR ($CDCl_3$): δ 5.30 (1H, m, H(Ph)C=CH_2), 5.55 (2H, s, COOCH_2Py) 5.80, (1H, q, H(Ph)C=CH_2), 6.77 (1H, m, H(Ph)C=CH_2), 7.36−7.62 (4H, m, Ar*H*), 8.13−8.30 (7H, m, Py*H*). 8.68 (1H, d, PyH) and 9.31 (1H, d, PyH). ^{13}C NMR ($CDCl_3$): δ 66.68, 66.82 (COO*C*H_2Ph), 114.33, 114.45, 123.19, 124.09, 124.14, 124.83, 126.07, 126.20, 126.30, 126.47, 127.13, 127.72, 128.49, 128.58, 128.88, 129.50, 129.67, 130.33, 130.96, 131.27, 134.40, 136.35, 136.48, 138.00 (Ph*C**H* = *C*H_2, Ar*C* and Py*C*)167.69 (Py*C*OO). FT-IR, ν: 3050, 1710, 1596, 1507, 1387, 1252, 1230, 1195, 1134, 1088, 1043, 849, 709 cm^{-1}. Anal. Calcd: C, 86.17; H, 5.00. Found: C, 85.67; H, 4.98.

Synthesis of Linear Poly[p-(chloromethyl)styrene], LPPCMSt by Classic Free Radical Polymerization. p-(Chloromethyl)styrene (5 mmol) was mixed with AIBN at monomer to initiator ratios, [M]/[I] = 6.25−100, then chlorobenzene or toluene (1.8 mL) was added. The resulting solution was stirred in a nitrogen atmosphere in an oil-bath at 75−80 °C with chlorobenzene as the solvent or 60 °C with toluene as the solvent. The viscosity of the solution increased as polymerization proceeded and the color of solution changed to a light yellow. After 24 h the polymer was precipitated into methanol as a white powder and then filtered. The precipitate was dissolved in THF and reprecipitated into methanol. The process was repeated twice and the final product was then dried under vacuum at room temperature (yield 72−95%). The polymers had molecular weights averaging between 2.7×10^3 Da and

2.04×10^4 Da as estimated by SEC. They were further characterized by FT-IR and ^{1}H NMR. FT-IR: 3023.9, 2927.5, 2850.3, 1700.9, 1608.4, 1511.9, 1484.9, 1446.4, 1423.2, 1265.1, 836.9, 798.4, 709.7, 674.9, 609.4 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.94, 1.16, 1.46, 1.73, 4.51 (CH_2Cl), 6.50 and 7.06 (Ar*H*).

General Procedure for the Radical Homopolymerization of p-(Chloromethyl)styrene Derivatives, Containing Ibuprofen, Naproxen, and Pyrene. Modified *p*-(chloromethyl)styrene derivatives and AIBN ([M]/[I] = 12.5) were added to the solvent. The mixture solution was stirred in an oil bath at 60 °C under nitrogen atmosphere. The viscosity of solution increased and the color of solution became darker as polymerization proceeded. After 24 h, the polymer formed was precipitated into methanol as white or light yellow powder and then filtered. The precipitate was then dissolved in THF and reprecipitated into methanol. The isolated polymer was dried under vacuum at room temperature. Finally the products were characterized by ^{1}H NMR and SEC.

Homopolymer of Ibuprofen-Modified p-(Chloromethyl)styrene. This was prepared from ibuprofen-modified *p*-(chloromethyl)-styrene, AIBN as initiator, and toluene as the solvent (yield 67.5%). ^{1}H NMR ($CDCl_3$): δ 0.88 ($2CH_3$), 1.45 (CH_3), 1.86 (C*H*), 2.42 (CCH_2Ph), 3.75 ($COOCH_2Ph$), 4.32, 4.89, and 6.69 ($-CH_2-CH-$), 6.87, 7.04, and 7.17 (Ar*H*).

Homopolymer of Naproxen-Modified p-(Chloromethyl)styrene. This was prepared from naproxen-modified *p*-(chloromethyl)-styrene (yield 66.4%). ^{1}H NMR ($CDCl_3$): δ 1.46 ($CHCH_3$), 3.78 (1H and 3H, m, CH_3CH and CH_3O), 4.83 and 4.97 ($COOCH_2Ph$), 6.23 and 6.78 ($-CH_2-CH-$), 7.02, 7.31, and 7.53 (Ar*H* and Na*H*).

Homopolymer of 1-Pyrenecarboxylic Acid Modified p-(Chloromethyl)styrene. This was prepared from 1-pyrenecarboxylic acid modified *p*-(chloromethyl)styrene (yield 15%). ^{1}H NMR ($CDCl_3$): δ 5.46 ($COOCH_2Py$) 7.25−8.30 (Ar*H* and Py*H*), 8.50, 8.93, and 9.19 (Py*H*).

General Procedure for the Radical Copolymerization of p-(Chloromethyl)styrene and Its Derivatives. p-(Chloromethyl)styrene was mixed with modified *p*-(chloromethyl)styrene derivatives at different ratios [*p*-(chloromethyl)styrene:*p*-(chloromethyl)styrene derivative = 9:1, 5:1, 3:1, 1:1], and then AIBN ([M]/[I] = 12.5) and chlorobenzene or toluene were added. The reaction mixtures were processed in a similar way to the radical homopolymerization of *p*-(chloromethyl)styrene derivatives described above. The polymerization products were isolated as white powders.

Copolymer of p-(Chloromethyl)styrene and Ibuprofen-Modified p-(Chloromethyl)styrene. ^{1}H NMR ($CDCl_3$): δ 0.90, 1.15, 1.50, 1.85, 4.50(CH_2Cl), 4.97(CH_2Ib), 6.53 and 7.08 (Ar*H*).

Copolymer of p-(Chloromethyl)styrene and Naproxen-Modified p-(Chloromethyl)styrene. ^{1}H NMR ($CDCl_3$): δ 0.90, 1.12, 1.40, 1.58, 3.89 (CH_3CH and CH_3O), 4.46(CH_2Cl), 5.0(CH_2Na), 6.48 (ArH), 7.0, 7.40, and 7.64(Na*H*).

Copolymer of p-(chloromethyl)styrene and Pyrene-Modified p-(Chloromethyl)styrene. ^{1}H NMR ($CDCl_3$): δ 0.90, 1.17, 1.50, 4.36 ($PhCH_2Cl$), 5.43($PhCH_2Py$), 6.48 and 6.97 (Ar*H*), 8.10, 8.60, and 9.30 ((Py*H*).

Synthesis of Hyperbranched Poly[p-(chloromethyl)styrene], HPPCMSt, by Metal-Mediated Controlled Radical Polymerization. Hyperbranched PPCMSt was synthesized according to previously published procedure.[26] The preparation was conducted as follows: 2,2′-bipyridyl (8.4 mmol) and CuCl (4.2 mmol) were added to a cylindrical reaction vessel, followed by addition of chlorobenzene (8.0 mL) and *p*-(chloromethyl)styrene (21 mmol). Then the reaction mixture was flushed with nitrogen and placed in an oil-bath (115 °C). After ~40 min, a green solid precipitated. Polymerization went on for 4 h. Then the reaction vessel was moved out of the oil-bath and allowed to cool down for 30 min. THF (50 mL) was added to the reaction mixture, which was then stirred at room temperature overnight to complete the dissolution of the polymer and to allow for catalyst oxidization. The solution was filtered through alumina to remove the insoluble salts. The resulting clear brown liquid was concentrated and precipitated into methanol. The off-white powder precipitate was dried under vacuum. SEC analyses showed that the polymers were obtained with weight-average molecular weights between 2.4×10^3 Da and 4.5×10^4 Da. The ratio of secondary to primary benzyl chloride groups as determined by ^{1}H NMR was between 0.18 and 0.33 at monomer to catalyst ratios from 5 to 20. ^{1}H NMR ($CDCl_3$): δ 1.55, 1.90, 2.30, 2.75, 3.0 4.50 (CH_2Cl), 4.75 (C*H*Cl) and 6.50−7.41 (Ar*H*). FT-IR: 3023.5, 2930.6, 2856.1, 1699.3, 1605.5, 1511.6, 1487.8, 1445.1, 1423.7, 1266.4, 1165.0, 1017.6, 899.6, 828.6, 795.9, 706.5, 677.0, 631.9 cm^{-1}.

Modification of Linear or Hyperbranched PPCMSt with Ibuprofen, Naproxen, and 1-Pyrenecarboxylic Acid. In a 25 mL round-bottom flask, K_2CO_3 (2.5 equiv), 18-crown-6 (0.2 equiv), poly(4-vinyl benzyl chloride) (1 equiv) and drugs (ibuprofen or naproxen) or model compounds (1-pyrenecarboxylic acid) (1.05 equiv) were added and dissolved in THF. The reaction solution was vigorously stirred under nitrogen and refluxed at 70 °C for 24 h. Then K_2CO_3 was removed by filtration. The clear polymer solution was precipitated into methanol to form a white or light yellow powder. The precipitation was repeated twice and the final precipitate was dried under vacuum. Yields of 90−97% were obtained. UV−vis spectra and NMR were used to confirm the attachment and to calculate the modification degree.

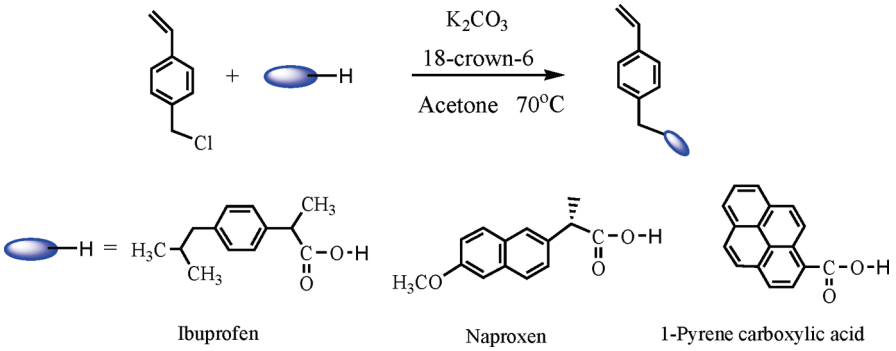
Synthesis of Hydrogels from Jeffamine and Linear or Hyperbranched PPCMSt, with or without Attached Drugs. To a test tube was added linear or hyperbranched PPCMSt with or without drugs attached, Jeffamine (400, 200, or 100 wt %, relative to PPCMSt) and dry THF (300 mg polymer/1 mL THF). The mixture was allowed to equilibrate with a magnetic stirring at 45 °C for 0.5 h before the addition of DIEA (3 molar equivalency with Jeffamine), followed by curing at 50 °C. After 24 h THF was added to swell the hydrogel and the solid part was washed with chloroform to remove the unreacted residual Jeffamine and PPCMSt fragments. Then water was used to wash out any side products. After that the hydrogel samples were quickly frozen in liquid nitrogen and dried by lyophilization for 24 h. Yields of 50−85% were calculated using the total amounts of the two original building blocks. FT-IR and swelling measurements were conducted on representative hydrogel samples.

Synthesis of Hydrogels from PEG and Linear or Hyperbranched Ibuprofen-, Naproxen- and Pyrene-Modified PPCMSt. Linear or hyperbranched modified PPCMSt and PEG were mixed at different ratios in a pear-shaped flask with two openings. Dried THF (100 mg polymer/1 mL THF) was used as the solvent to dissolve both reagents. The solution was flushed with nitrogen and NaH was added to catalyze the reaction. The mixture was stirred at room temperature under nitrogen atmosphere for 24 h, and then ethanol was added to stop the reaction and quench the excess NaH. The solution was centrifuged and the solid part was washed with ethanol and chloroform to remove the unreacted residual PEG and PPCMSt fragments. Water was used to wash out the side product-NaCl. Hydrogels were dried using lyophilization for 24−48 h. Yield (10%-90%) was calculated from the total amounts of the two original building blocks. FT-IR and swelling measurements were conducted on representative hydrogel samples.

Swelling Experiments. Before the swelling measurements were performed, all hydrogels (irregular particles) were extracted with ethanol, chloroform, and multiple DI water portions. Hydrogels were then dried in the lyophilizer. The swelling behavior of networks with different Jeffamine and PPCMSt content was investigated as a function of time. All measurements were done in triplicates.

Kinetics of Swelling. Dynamic swelling (weight) studies were conducted to elucidate the mechanism of water diffusion into the polymer networks based on PPCMSt and Jeffamine as determined by the dynamic portion of the gravimetric curve. The kinetics of swelling was investigated as follows: ~15 mg of hydrogels were placed in a vial containing 20 mL of DI water. The hydrogels were weighted periodically throughout the experiment at room temperature. The water uptake, M_t, was followed as a

Scheme 1. Synthesis of Drug/Marker-Substituted Monomers from *p*-(Chloromethyl)styrene



function of time until equilibrium was attained. M_t of the hydrogels was calculated from the average of the final weights using the formula:

$$M_t = (W_{wet} - W_{dry})/W_{dry} \times 100\%$$

Swelling Ratio. The weight swelling ratio was calculated as the ratio of the weight of the swollen hydrogels to the weight of the original dry gel according to the following procedure. The weight of the dry samples was recorded before they were immersed in a large excess of the corresponding solvent (DI water, toluene, THF, or CH_2Cl_2) at room temperature. After at least 24 h the specimens were weighted again after the solvent droplets on the surface of the samples were carefully blotted with lint-free paper. The weight swelling ratio (q) was calculated as follows:

$$q = W_{wet}/W_{dry}$$

W_{wet} and W_{dry} are the weights of swollen and dry sample, respectively.

Ibuprofen, Naproxen, and Pyrene Release Studies from the Hydrogels. Prior to the release studies, calibration curves for ibuprofen, naproxen, and 1-pyrenecarboxylic acid were created in NaOH solutions at pH = 10 to determine the corresponding molar extinction coefficients (ε) in this medium. All three substances showed good linear absorbance/concentration relationships ($R^2 > 0.99$). The absorbance intensity of ibuprofen was between 0.2 and 2.3 AU at concentrations 2.31×10^{-5} to 2.89×10^{-4} M, the absorbance intensity of naproxen was between 0.1 and 2.18 AU at 1.67×10^{-5} to 4.18×10^{-4} M, and the absorbance intensity was between 0.09 and 3.5 AU for 1-pyrenecarboxylic acid at concentrations ranging from 4.38×10^{-6} to 8.75×10^{-5} M. The ε values in NaOH solution were 8156 L mol^{-1} cm^{-1}at $\lambda = 223$ nm for ibuprofen, 4954 L mol^{-1} cm^{-1}at $\lambda = 270$ nm for naproxen and 38 851 L mol^{-1} cm^{-1}at $\lambda = 242$ nm for 1-pyrenecarboxylic acid. For all release measurements, a known amount (about 30 mg) of the dry hydrogel was preswollen overnight in DI water, and the solvent droplets on the surface of the specimens were carefully blotted with lint-free paper before their immersion in a vial with NaOH solution (15 mL). The release of the drugs from the hydrogels was studied at room temperature. At various time intervals, 3 mL of the basic solution were taken to measure the drug concentration by UV−vis. The spectra of the aliquots from the vial were recorded until there were no measurable changes in the absorbance intensity. The amount of the release percentage was calculated from the following equation:

$$\%\ \text{release} = (W_t/W_{total}) \times 100$$

Here W_t is the weight of released drugs at time t and W_{total} is the total weight of drugs being attached in the gel structure.

Results and Discussion

Incorporation of substances in cross-linked materials could be achieved by two general strategies—through covalent link formation

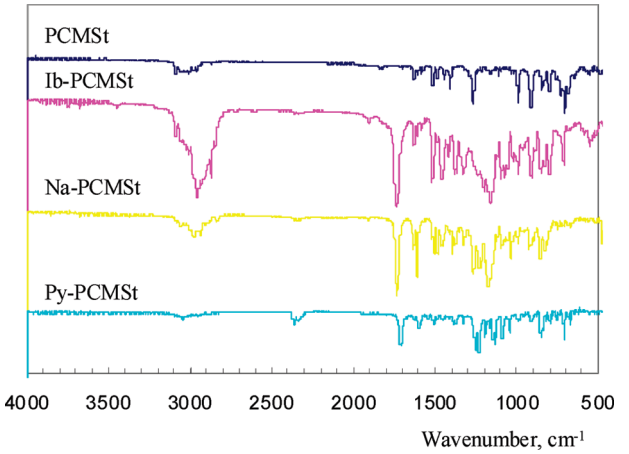


Figure 1. FT-IR spectra of *p*-(chloromethyl)styrene (PCMSt) and its ibuprofen (Ib-PCMSt), naproxen (Na-PCMSt), and pyrene (Py-PCMSt) derivatives.

or via passive binding. In turn the inclusion could be performed before or after the cross-linking operation. When a controlled release of the encapsulated substance is desired, the passive binding has certain limitations mostly associated with the partition coefficient of the substrates.[18b,27] Therefore, in this study, the incorporation and release of the model drugs and the fluorescent marker will be explored through the chemical bond formation and scission pathway. Initial pilot experiments show that the efficiency of the covalent attachment of the investigated substances into preformed and preswollen hydrogels is very low (5−10%). This method also tends to create networks with uneven substrate distribution where the surface of the hydrogel is "saturated" while the interior of the cross-linked matrix remains mostly "empty". Therefore, the adopted strategy for all subsequent experiments involves the incorporation of the desired substance before the polymer synthesis (macromonomer approach) or before the cross-linking reaction (post-polymerization modification).

Macromonomer Formation. Synthesis and Characterization of *p*-(Chloromethyl)styrene Derivatives. Three new monomers, based on *p*-(chloromethyl)styrene, PCMSt, are synthesized by reaction with drugs (ibuprofen, naproxen) or a model fluorescent compound (1-pyrenecarboxylic acid) using K_2CO_3/18-crown-6 as a catalyst, Scheme 1. After stirring the reaction mixture at reflux for 24 h no remaining drugs can be detected by TLC manifesting the completion of the reaction. The FT-IR spectra of the purified new monomers are shown in Figure 1. It is seen, that the strong peak at 1710−1740 cm^{-1} due to the carboxyl groups is observed in all of the produced monomers.

Figures 2 and 3 show the ^{1}H and ^{13}C NMR spectra of the original PCMSt and the substituted PCMSt derivatives. It is clearly seen that the $-CH_2-$ signals at 4.7 ppm (benzyl

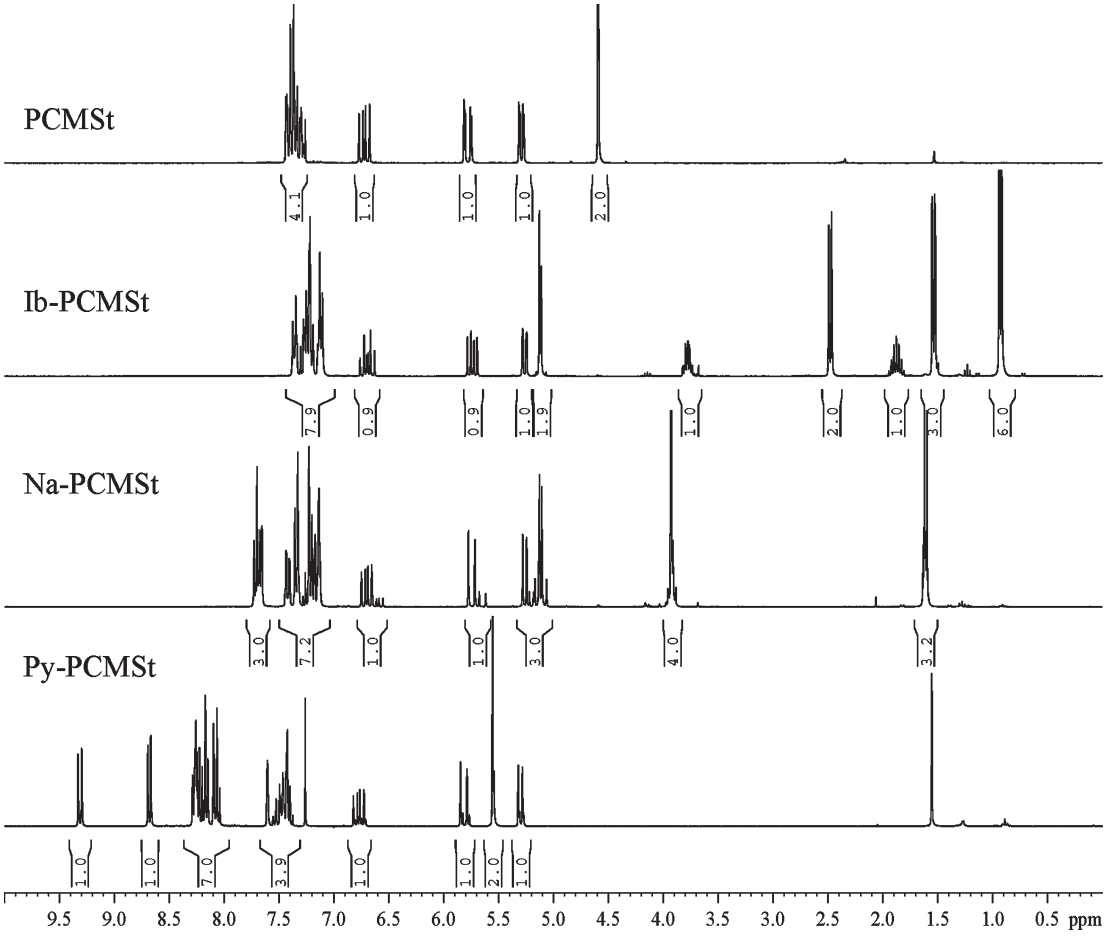


Figure 2. ^{1}H NMR of *p*-(chloromethyl) styrene, PCMSt, and its derivatives with ibuprofen (Ib-PCMSt), naproxen (Na-PCMSt), and pyrene (Py-PCMSt).

chloride moiety) shift to lower field in all three new monomers (Ib-PCMSt, Na-PCMSt, and Py-PCMSt). The integrals of all hydrogen peaks correspond to the theoretical values calculated for the chemical structures shown in Scheme 1. The attachment is also confirmed by the appearance of carboxyl group signals at $\delta = 168-175$ ppm in the ^{13}C NMR spectra, Figure 3. The chemical structure of the PCMSt derivatives and their purity are also confirmed by the elemental analysis.

Homopolymerization and Copolymerization of *p*-(Chloromethyl)styrene Derivatives. The radical homopolymerization and copolymerization of the *p*-(chloromethyl)styrene and its derivatives are initially examined using AIBN as initiator and toluene or chlorobenzene as solvents. The polymerization in chlorobenzene yields polymers with lower molecular weights and higher polydispersities. The results from the polymerizations in toluene are summarized in Table 1 and Figure 4. It should be noted that at identical reaction conditions *p*-chloromethylstyrene (PCMSt) produces polymers with the highest yield. The products also have monomodal molecular weight distribution and the lowest polydispersity. The homopolymers from Ib-PCMSt or Na-PCMSt are formed with lower yields, but have markedly higher molecular weights and polydispersities. They also have multimodal molecular weight distributions indicating the occurrence of chain transfer reactions to monomer probably through the hydrogens in the methyl groups of the drug fragments. The polymer of Py-PCMSt is formed with the lowest yield and molecular weight. This result is somewhat unexpected since under comparable reaction conditions the radical (co)polymerizations of monomers with condensed aromatic rings tend to afford polymers in good yields and with high molecular weights.[28] The low reactivity of this monomer is probably caused by the bulkiness of the attached pyrene, which disrupts favorable monomer alignment and chain propagation. SEC with UV detection tuned at the specific wavelength of the ibuprofen (223 nm), naproxen (270 nm) and pyrenecarboxylic acid (242 nm) shows that all eluting fractions contain the corresponding attached moiety, which is not cleaved during the polymerization. Therefore, the products formed are not fractionated despite their multimodal molecular weight distributions and are used directly in the network synthesis.

The copolymerization of PCMSt and derivatives proceeds at the same reaction conditions as their homopolymerization, Scheme 2.

The results obtained show that under the same reaction conditions the copolymers form with lower yields and molecular weights than the PCMSt homopolymer, Table 2. However, these parameters are notably higher for the Py-PCMSt copolymers compared with the corresponding values of the Py-PCMSt homopolymer and could serve as an indirect proof of the previously discussed causes for the low reactivity of this monomer. The copolymer composition is calculated from the integral ratio of the peak for the CH_2 group, linked to the drug (marker), and the $-CH_2Cl$ peak in ^{1}H NMR spectra. An example with Na-PCMSt is shown in Figure 5. Since the copolymerizations are driven to high conversions the actual composition of the copolymers formed is similar to the feed ratio of two monomers in the initial polymerization mixtures, Table 2.

It is also found that the reaction time increases the final yield and molecular weight of the copolymers, Table 3.

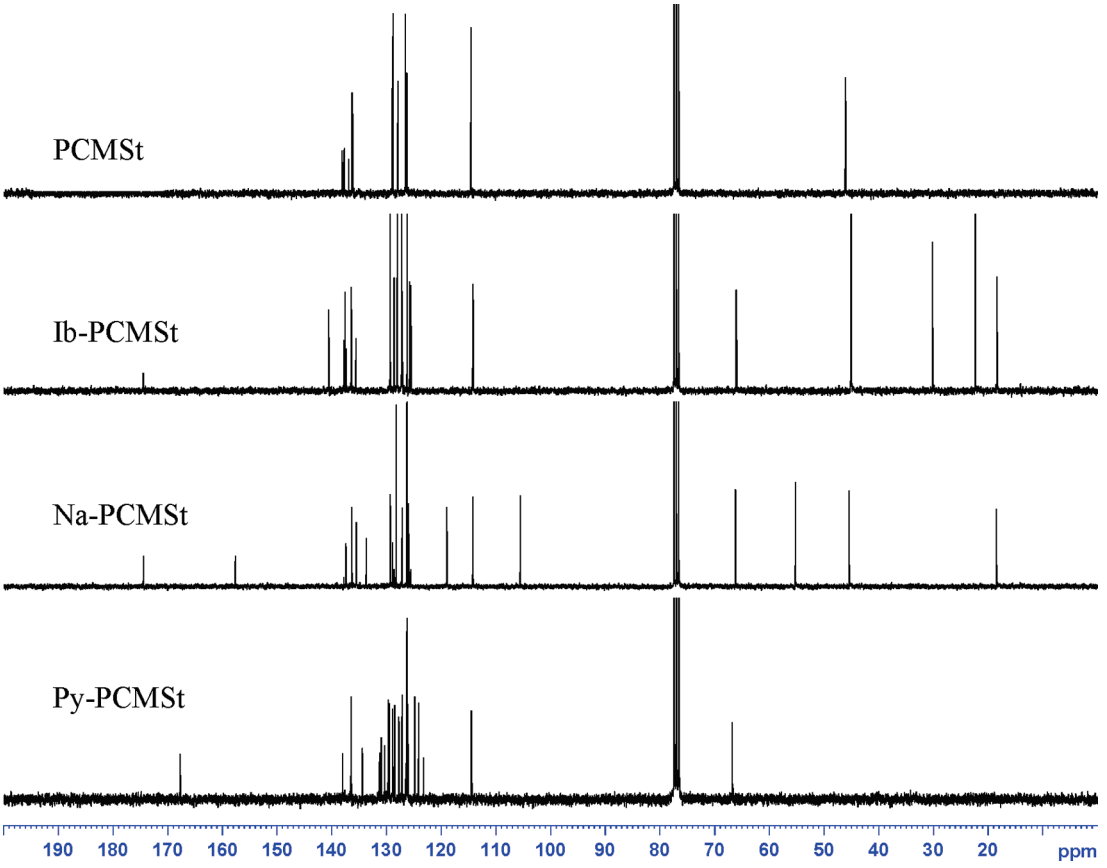


Figure 3. ^{13}C NMR of *p*-(chloromethyl)styrene, PCMSt, and its derivatives with ibuprofen (Ib-PCMSt), naproxen (Na-PCMSt), and pyrene (Py-PCMSt).

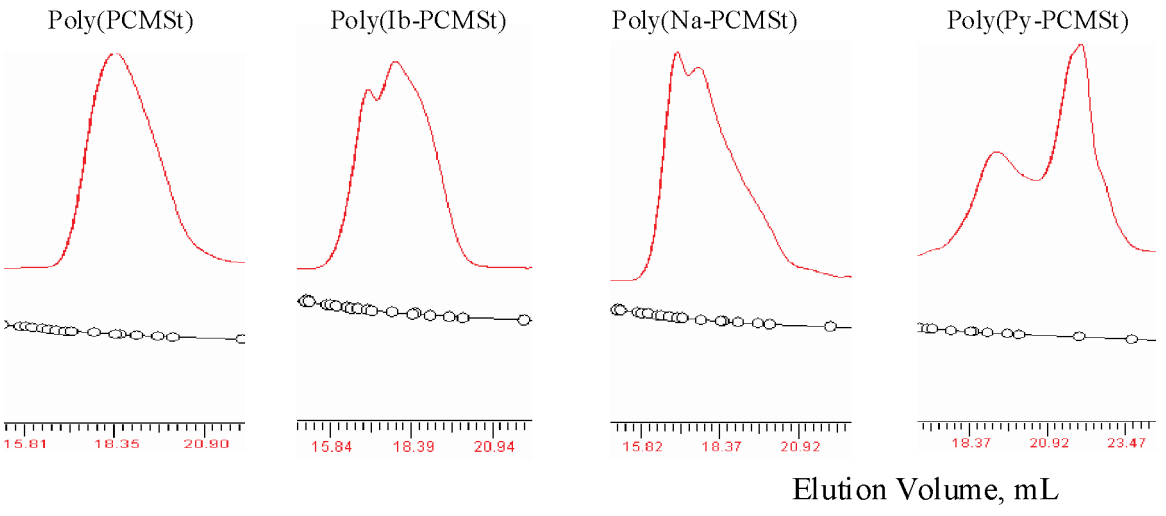


Figure 4. SEC traces of homopolymers from *p*-(chloromethyl)styrene, PCMSt and its derivatives with ibuprofen (Ib-PCMSt), naproxen (Na-PCMSt), and pyrene (Py-PCMSt). See the Experimental Section for analysis conditions.

Table 1. Yields and Molecular Weight Characteristics of Polymers formed from *p*-(Chloromethyl)styrene (PCMSt) and its Ibuprofen (Ib-PCMSt), Naproxen (Na-PCMSt), and Pyrene (Py-PCMSt) Derivatives

polymer	[M]/[I]	yield (%)	M_n	M_p[a]	M_w/M_n
poly(PCMSt)	12.5	89.9	5800	8900	1.50
poly(Ib-PCMSt)	12.5	67.5	10 400	13 700	2.16
poly(Na-PCMSt)	12.5	66.4	8700	38 000	2.57
poly(Py-PCMSt)	12.5	15.1	1200	800	2.18

[a] M_p is the apparent molecular weight at the peak maximum.

The attempted formation of functional hyperbranched macromolecules by self-condensing vinyl copolymerization[26] of PCMSt and its derivatives produces copolymers in very low yields (5−10%) and broad multimodal molecular weight distributions. That is why the incorporation of ibuprofen, naproxen, and pyrene in this macromolecular architecture is achieved by postpolymerization modification of preformed hyperbranched poly(PCMSt) as described in the section below.

Postpolymerization Approach. Modification of Hyperbranched Poly(PCMSt). Modified hyperbranched poly(PCMSt),

Scheme 2. Co polymerization of *p*-(Chloromethyl)styrene and Its Derivatives

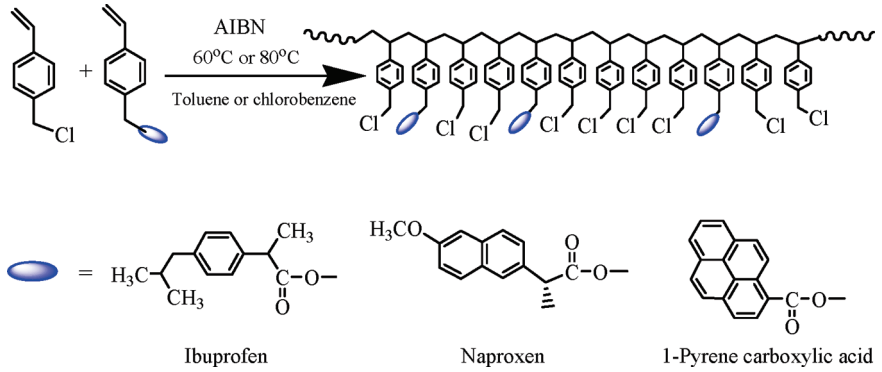


Table 2. Molecular Weights of Copolymers of *p*-(Chloromethyl)styrene (PCMSt) and Naproxen- or Pyrene-Modified PCMSt Prepared at 80 °C in Chlorobenzene at [M]/[I] = 12.5

polymer or copolymer	feed ratio of modified PCMSt (%)	content of modified PCMSt in copolymer (%)	yield (%)	M_n	M_p[a]	M_w/M_n
poly(PCMSt)	0	0	84.0	3500	4500	1.33
poly(PCMSt -*stat*-Na-PCMSt)	10.0	10.0	37.1	2300	2700	1.21
poly(PCMSt -*stat*-Na-PCMSt)	16.7	16.1	36.6	2300	2800	1.23
poly(PCMSt -*stat*-Na-PCMSt)	25.0	25.0	47.5	2300	2700	1.23
poly(PCMSt -*stat*-Na-PCMSt)	50.0	44.5	72.0	2300	2800	1.30
poly(PCMSt -*stat*-Py-PCMSt)	10.0	5.0	71.9	2400	3000	1.26
poly(PCMSt -*stat*-Py-PCMSt)	16.7	16.0	64.9	2200	2800	1.26
poly(PCMSt -*stat*-Py-PCMSt)	25.0	23.4	79.3	2100	2900	1.31
poly(PCMSt -*stat*-Py-PCMSt)	50.0	41.3	74.4	2000	2900	1.34

[a] M_p is the apparent molecular weight at the peak maximum.

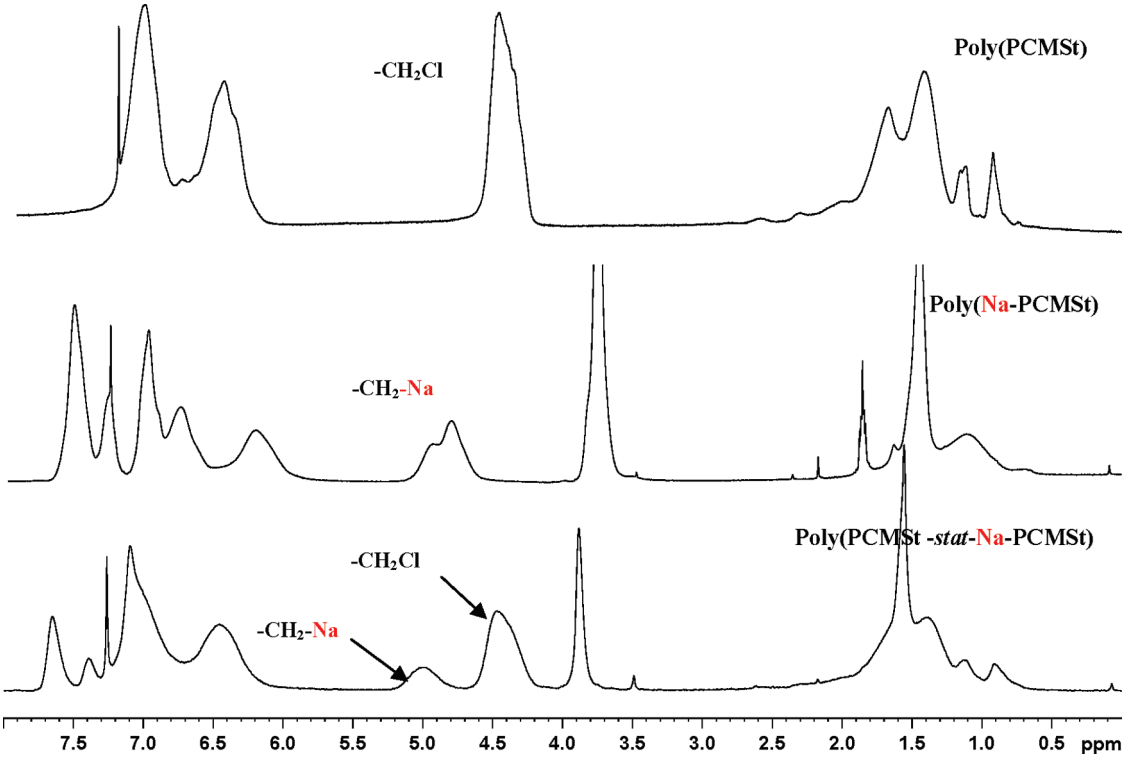


Figure 5. ^{1}H NMR spectra of homopolymers − poly(PCMSt) and poly(Na-PCMSt) and the copolymer of the same comonomers.

H-PPCMSt, is prepared by esterification under mild basic conditions as shown in Scheme 3. The degree of modification is controlled by changing the molar composition of reaction mixture. The incorporation of the corresponding moiety is confirmed by the appearance of strong UV absorbance in the SEC traces of the modification products at 312 nm (naproxen) and 332 nm (pyrene), respectively. Pure PPCMSt has no UV absorbance at these two wavelengths.

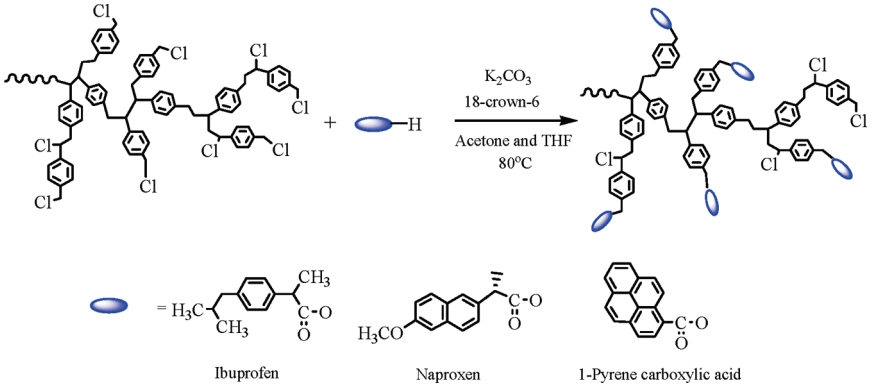
NMR is also used to verify the chemical transformations of the benzyl chloride groups and the apparent degree of substitution (DS). The values are determined by ^{1}H NMR as the ratio of the peak areas for the initial and substituted methylene protons in the 4-benzyl chloride moiety. Figure 6

Table 3. Molecular Weight Characteristics of PCMSt Copolymers Prepared at Different Reaction Times with [M]/[I] = 12.5 in Toluene at 60 °C

polymer or copolymer	reaction time (h)	feed ratio of modified PCMSt (%)	content of modified PCMSt in copolymer (5)	yield (%)	M_n	M_p[a]	M_w/M_n
poly(PCMSt)	24	0	0	84.0	3500	4500	1.33
poly(PCMSt-*stat*-Ib-PCMSt)	24	10.0	7.2	31.1	3500	4500	1.40
	72	10.0	6.9	79.9	5600	7700	1.58
poly(PCMSt-*stat*-Na-PCMSt)	24	10.0	10.0	37.1	2300	2700	1.21
	72	10.0	8.5	92.4	5200	7700	1.50
poly(PCMSt-*stat*-Py-PCMSt)	24	10.0	5.0	71.9	2400	3000	1.26
	72	10.0	8.1	93.8	6300	11 100	1.75

[a] M_p is the apparent molecular weight at the peak maximum.

Scheme 3. Attachment of Model Compounds to Hyperbranched Poly(PCMSt)



shows for example the ^{1}H NMR spectrum of hyperbranched PPCMSt and the pyrene modified product. The decrease in the relative integral intensities of the signals between 4 and 5 ppm indicate that the covalent attachment occurs both through the primary and secondary methyl chloride groups with slight preference for the primary methyl chloride moiety (1:3.63 before to 1:3.06 after Py attachment). Ibuprofen and naproxen modification of PPCMSt proceeds in a similar fashion. It should be emphasized that multimodifications of PPCMSt can also be achieved with more than one drug and the resulting macromolecules can be used directly to prepare templates and formulations, where the amount and type of drugs could be tailored to desired levels and specific applications.

Network Formation. Preparation of Amphiphilic Hydrogels from PPCMSt and Reactive Aliphatic Polyethers. Amphiphilic hydrogels are synthesized from linear or hyperbranched PPCMSt (modified or not modified) and reactive aliphatic polyethers (Jeffamine and PEG) by two reactions: Williamson ether synthesis and nucleophilic substitution as shown in Scheme 4. The reactions lead to the formation of two types of networks—type I (containing hyperbranched PPCMSt) and type II (with linear PPCMSt). The hydrogels produced by Williamson ether reaction are well characterized and extensively discussed in our previous paper.[25] That is why in following sections, only the hydrogels prepared by the nucleophilic substitution reaction will be described in detail.

Both linear and hyperbranched PPCMSt react with Jeffamine and form cross-linked structures (JEFF-L-PPCMSt and JEFF-H-PPCMSt, respectively) at different feeding ratios. At 45−50 °C with small amounts of THF, the reaction mixtures turn milky and the viscosity increases rapidly in the presence of triethylamine as catalyst. The yields range from 50 to 85% as calculated from the weight of dried gels. The decrease of the absorption bands, characteristic for primary amino groups at 3370−3400 cm^{-1}, and the appearance of the N−H bending (scissoring) absorbance at 1508 cm^{-1} in the FT-IR spectra of isolated products confirms the network formation. The coupling efficiency is evaluated at three different weight feeding ratios of the two building blocks (Jeffamine/PPCMSt = 4, 2, and 1). The yield of formed networks increases by ∼12% upon decrease in the Jeffamine amount from 80 to 50 wt %. This phenomenon is probably caused by the decreased ammonium salt formation at the Jeffamine chain ends, which would enable their more efficient participation in the cross-linking reaction. At the same reaction conditions, the yields of hydrogels synthesized from linear PPCMSt are higher than those from the hyperbranched analogue probably due to differences in reactivity and steric factors.

All gels have a uniform appearance and most of the swollen gels are transparent. An example of modified and unmodified JEFF-L-PPCMSt networks is shown in Figure 7.

It should be emphasized, that the incorporation of substances of biological importance via hydrolyzable linkage prior to the final formation of the hydrogels offers several notable advantages over the passive or chemical binding of the same compounds into preformed networks, the most important ones being the increased load and the homogeneous distribution across the entire gel matrix.

The evaluation of the accessibility and release of the compounds bonded in the hydrogels under different conditions will be discussed in the following section.

Swelling Behavior. *A. Dynamic Swelling Properties.* The ability to control swelling and solute diffusion through hydrogels determines their usefulness as tissue engineering scaffolds, drug delivery platforms, and enzyme immobilization templates. The polymer composition, the structure and size of the gel pores, the water content, and the nature and size of the incorporated substances jointly affect the processes of swelling and solutes diffusion.

The time dependence of the swelling ratio is shown in Figure 8 for hydrogels with different chemical composition composed of Jeffamine and linear or hyperbranched PPCMSt. Here, the water uptake at time zero corresponds to the start of swelling process. All measurements are performed at room temperature and the water uptake is monitored gravimetrically. The figure illustrates that the swelling increases with time up to a certain level, and then levels off to reach equilibrium

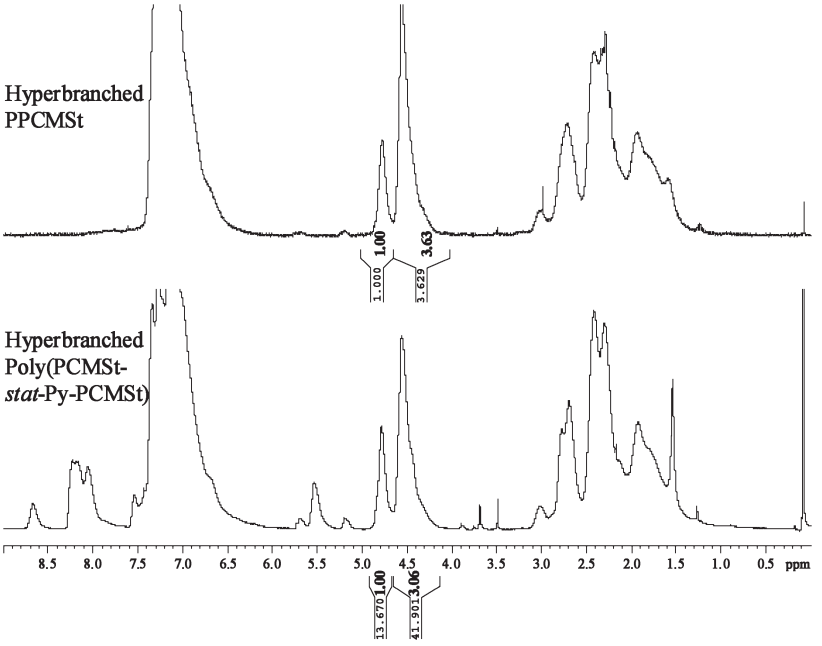
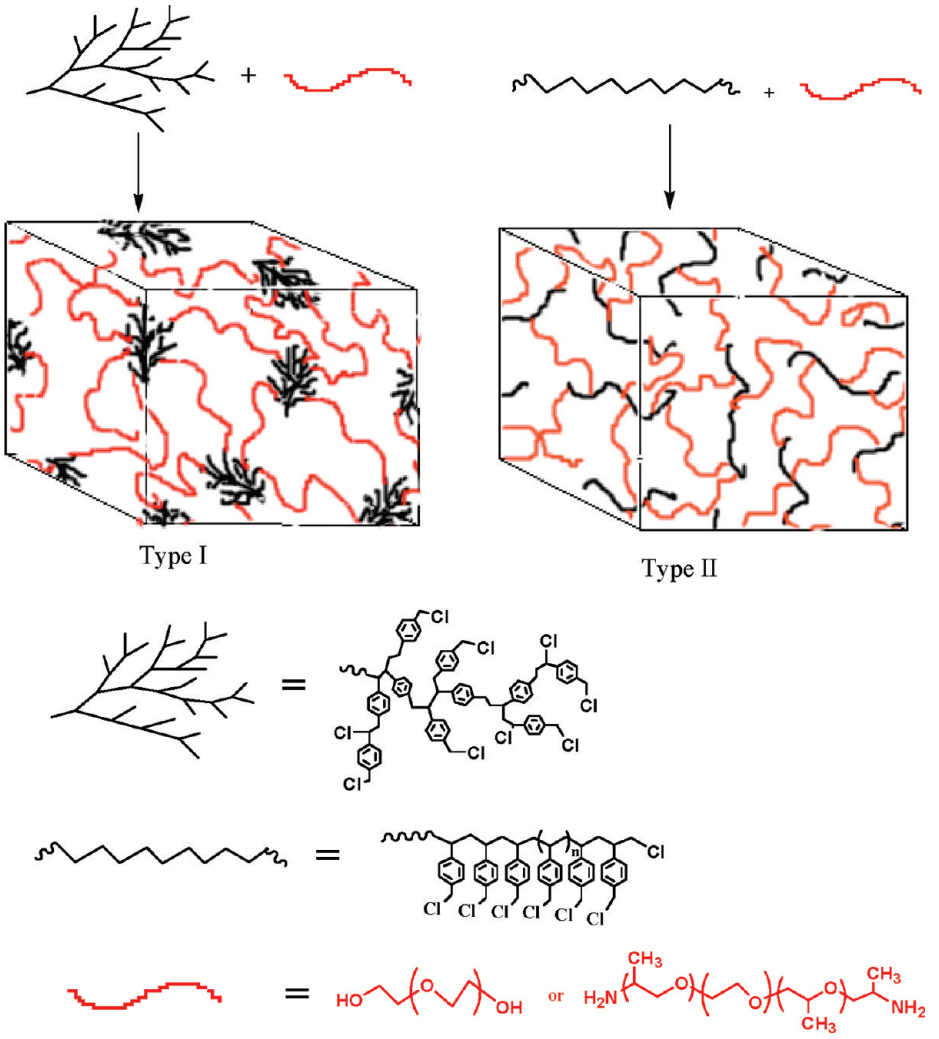


Figure 6. ^{1}H NMR ($CDCl_3$) of hyperbranched PPCMSt and its modification derivatives with 1-pyrenecarboxylic acid, poly(PCMSt-*stat*-Py-PCMSt).

Scheme 4. Syntheses of Hydrogels from Poly(PCMSt) and Polyethers



after approximately 2 h. This equilibrium swelling ratio depends naturally on the amount of hydrophilic component (Jeffamine) used in the preparation of the conetwork. The results also show that the equilibrium process strongly depends on the polymers architecture used in the hydrogel construction. Thus, cross-linked materials, built of hyperbranched PPCMSt, swell ~50% more than those from linear PPCMSt at the same feeding ratios. In that aspect the Jeffamine hydrogels show swelling behavior similar to the previously explored PPCMSt hydrogel series containing different PEG segments.[25]

The exact diffusion mechanism in these hydrogels and the influence of the specimens shape and size need further investigation.

B. Equilibrium Swelling of Hydrogels. The amount of solvent absorbed by a network is quantitatively represented by the equilibrium water weight swelling ratio (q). It is measured at room temperature in four solvents for the hydrogel family of Jeffamine and hyperbranched or linear PPCMSt. The results of swelling experiments depending on networks composition are summarized in Table 4. The values included are the average results from three separate determinations.

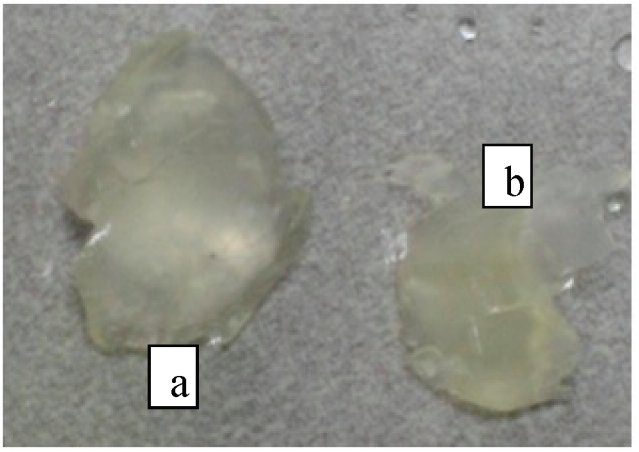


Figure 7. Photographs of swollen hydrogels from (a) Jeffamine and linear PPCMSt, Jeffamine/PPCMSt = 2, and (b) Jeffamine and pyrene-modified linear PPCMSt.

Naturally, the equilibrium swelling ratio of any hydrogel reflects the affinity of the components toward the selected media. For the networks in this study the highest swelling ratio is observed in CH_2Cl_2, a good solvent for both constituent blocks. Since Jeffamine is the only component that can dissolve in water, the swelling ratio in water for both types of hydrogels decreases with the weight percentage decrease of the hydrophilic building block. For all systems, the hydrogel swelling ratio depends also on the cross-linking density, chemical balance of two components and the porosity parameters.

There is marked difference in the swelling ratio of gels with linear and hyperbranched PPCMSt with the same JEFF content. In distinction to the values of the hyperbranched networks, the weight swelling ratios of linear PPCMSt hydrogels are significantly lower in all four solvents. The reason is not only the cross-linking density difference, caused by the different polymer architecture as described in the previous paper,[25] but also the different hydrogel morphology as revealed by scanning electron microscopy (SEM), Figure 9. All hydrogels formed from hyperbranched PPCMSt have macroporous organization, which is independent of the weight percentage of Jeffamine therein. The pore diameter is about 2−10 μm with a relatively wide pore size distribution. The morphology of hydrogels formed from linear PPCMSt does not show any macropore structure. The lack of macropores, which serve as effective solvent reservoirs, affects significantly the ability of these hydrogels to swell. It is noteworthy that both hydrogel lines, formed by the two methods, are stable and show no sign of degradation even after repeated swelling in different solvents.

The dissimilarity in the swelling behavior of PEG and Jeffamine gels even at close interjunction lengths (the molecular weights of both polyethers are between 5 and 6 kDa) is very obvious. The weight swelling ratio of the Jeffamine hydrogels is more than two times lower than that of the PEG conetworks discussed in our previous paper[25] (Tables 4 and 5). One of the contributing factors is the lower affinity of Jeffamine to these four solvents, the main probable reason being the presence of poly(propylene oxide) in this copolymer. This different composition would ultimately lead to differences in the hydrogels morphology. The hydrogels, formed by the Williamson ether reaction contain also numerous

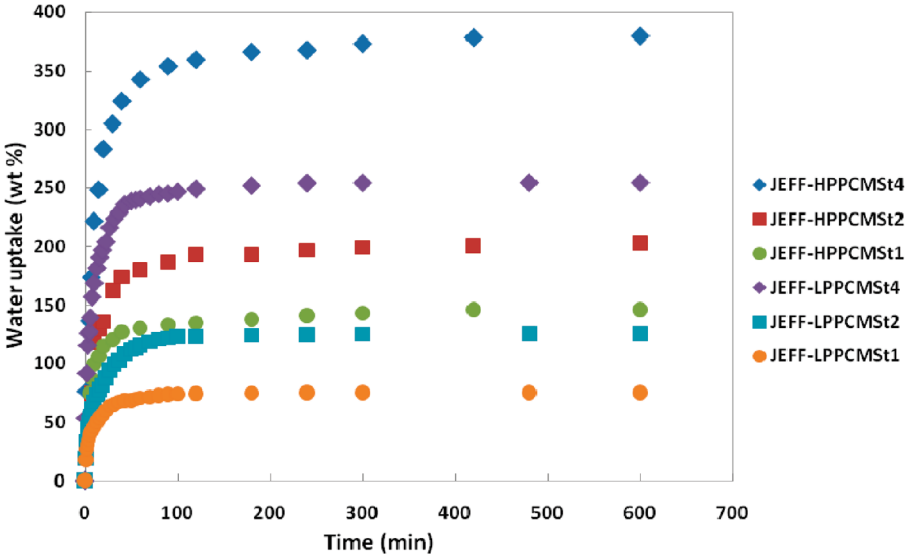


Figure 8. Time dependence of the swelling process, measured at room temperature for amphiphilic hydrogels from Jeffamine (JEFF) and hyperbranched (HPPCMSt) or linear (LPPCMST) poly[*p*-(chloromethyl)styrene]. The numbers signify the Jeffamine to PPCMSt ratio.

Table 4. Yields and Weight Swelling Ratios of Hydrogels Formed by Reaction of Jeffamine (JEFF, M_n = 6000) and HPPCMSt (Hyperbranched Polymer: M_n = 5000, M_w = 16 500) or LPPCMSt (Linear Polymer: M_n = 10 100, M_w = 16 800)[a]

			weight swelling ratios			
hydrogel	JEFF wt %	yield (%)	DI water	toluene	THF	CH_2Cl_2
JEFF-HPPCMSt4	80	52.6	4.80 ± 0.05	8.39 ± 0.24	10.03 ± 0.32	17.48 ± 0.16
JEFF-HPPCMSt2	67	65.5	3.04 ± 0.04	7.15 ± 0.06	9.38 ± 0.29	15.86 ± 0.11
JEFF-HPPCMSt1	50	75.5	2.44 ± 0.08	8.83 ± 0.15	10.98 ± 0.11	18.89 ± 0.32
JEFF-LPPCMSt4	80	70.1	3.58 ± 0.02	3.32 ± 0.05	3.81 ± 0.02	7.32 ± 0.06
JEFF-LPPCMSt2	67	74.3	2.27 ± 0.02	3.90 ± 0.04	4.28 ± 0.10	8.08 ± 0.07
JEFF-LPPCMSt1	50	82.6	1.81 ± 0.01	3.15 ± 0.05	3.88 ± 0.06	6.98 ± 0.04

[a] The numbers signify the Jeffamine to PPCMSt ratio.

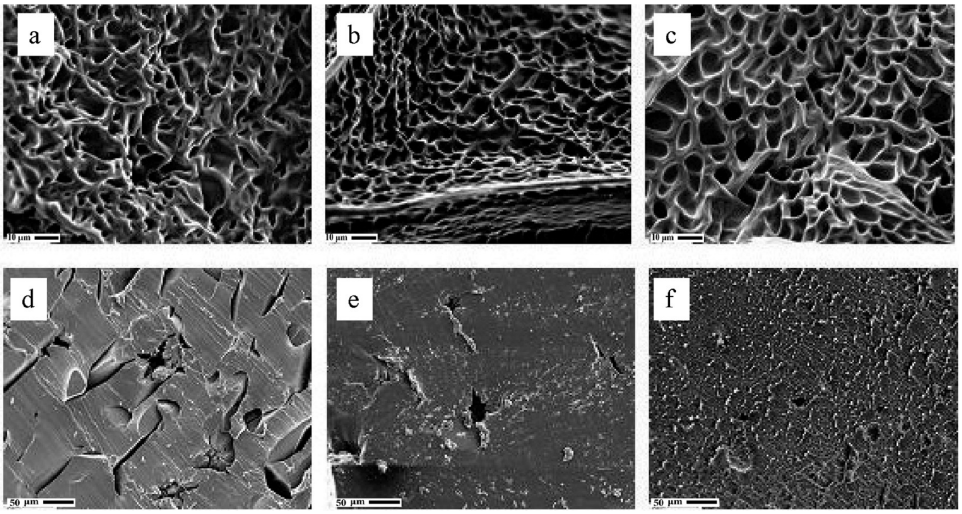


Figure 9. SEM micrographs of Jeffamine based hydrogels: (a) JEFF-HHPCMSt4; (b) JEFF-HPPCMSt2, (c) JEFF-HPPCMSt1, (d) JEFF-LPPCMSt4, (e) JEFF-LPPCMSt2, (f) JEFF-LPPCMSt1.

Table 5. Yields and Weight Swelling Ratios of Hydrogels Series,[25] Formed by Reaction of Linear PEG (L PEG: M_w = 5400) and Hyperbranched PPCMSt (HPPCMSt: M_n = 5000; M_w = 16 500) or Linear PPCMSt (LPPCMSt: M_n = 10 080; M_w = 16 800)[a]

			weight swelling ratios			
hydrogel	PEG wt %	yield (%)	DI water	toluene	THF	CH_2Cl_2
L PEG5k-HPPCMSt4	80	87.7	19.2 ± 0.2	16.3 ± 0.1	17.4 ± 0.1	39.0 ± 0.3
L PEG5k-HPPCMSt2	67	90.7	16.6 ± 0.4	17.0 ± 0.1	17.5 ± 0.1	37.2 ± 0.7
L PEG5k-HPPCMSt1	50	90.6	16.0 ± 0.4	19.3 ± 0.7	18.4 ± 0.1	36.6 ± 1.1
L PEG5k-LPPCMSt4	80	93.8	14.7 ± 0.1	14.8 ± 0.1	15.6 ± 0.1	37.7 ± 0.1
L PEG5k-LPPCMSt2	67	97.9	10.0 ± 0.1	14.6 ± 0.1	14.0 ± 0.8	34.1 ± 0.9
L PEG5k-LPPCMSt1	50	98.9	9.6 ± 0.1	19.3 ± 0.1	18.5 ± 0.4	41.0 ± 0.3

[a] The numbers signify the PEG to PPCMSt ratio.

Table 6. Yields and Weight Swelling Ratios of Hydrogels Formed by Reaction of Jeffamine (JEFF, M_n = 6000) and Modified PPCMSt

			weight swelling ratios			
hydrogel	JEFF wt %	yield (%)	DI water	toluene	THF	CH_2Cl_2
JEFF-HPPCMSt2[a]	67	65.5	3.04 ± 0.04	7.15 ± 0.06	9.38 ± 0.29	15.86 ± 0.11
JEFF-HPPCMSt-Ib	67	69.2	2.88 ± 0.01	3.77 ± 0.03	5.51 ± 0.02	11.06 ± 0.07
JEFF-HPPCMSt-Na	67	69.5	2.85 ± 0.01	4.58 ± 0.02	5.70 ± 0.04	11.35 ± 0.04
JEFF-HPPCMSt-Py	67	72.4	2.93 ± 0.01	4.17 ± 0.03	5.29 ± 0.12	10.78 ± 0.08
JEFF-LPPCMSt2[a]	67	74.3	2.27 ± 0.02	3.90 ± 0.04	4.28 ± 0.10	8.08 ± 0.07
JEFF-LPPCMSt-Ib	67	73.1	3.94 ± 0.01	3.10 ± 0.06	3.21 ± 0.03	6.06 ± 0.01
JEFF-LPPCMSt-Na	67	74.3	3.34 ± 0.03	2.84 ± 0.01	3.14 ± 0.01	5.68 ± 0.05
JEFF-LPPCMSt-Py	67	83.6	3.44 ± 0.01	2.81 ± 0.01	3.26 ± 0.02	6.08 ± 0.05

[a] The numbers signify the Jeffamine to PPCMSt ratio.

micropores, while their analogues derived from Jeffamine have few pores, Figure 9. It is not clear at this point, however, whether the cross-linking chemistry or the fabrication technology are the contributing factors for the different morphologies observed.

The effect of drug attachment on the equilibrium water content in hydrogel at the similar polymer composition is investigated as well. Comparing the data in Tables 4 and 6, it is evident that the yields are higher for networks of types I and II, when the PPCMSt used is modified with Ib, Na, or Py. The equilibrium weight swelling ratio for the hydrogels with modified hydrophobic segments is generally lower in all solvents. The results in aqueous medium could be probably explained by the increased overall hydrophobicity of the resulting networks. The SEM micrographs also reveal the absence of interconnected porous structure for both types of

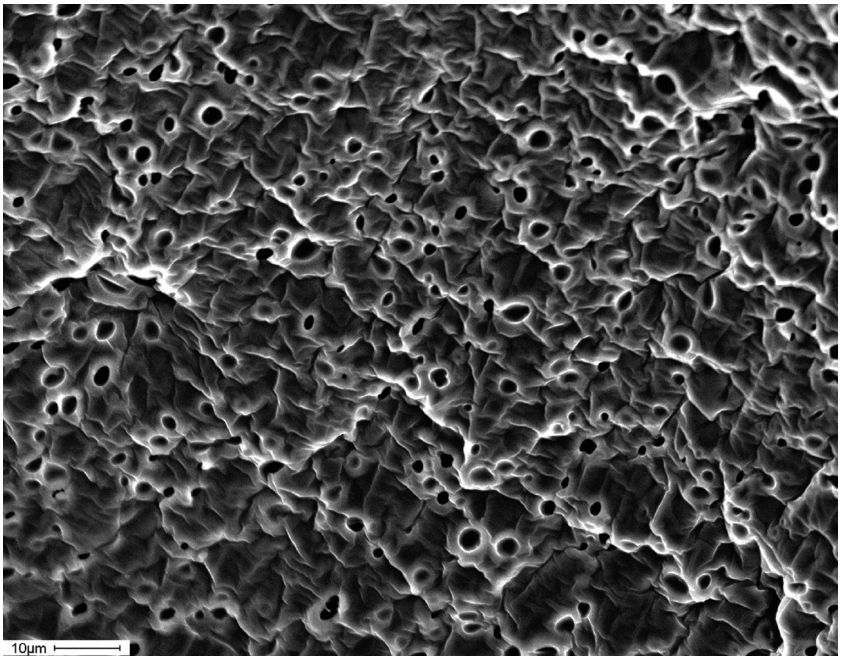


Figure 10. SEM micrographs of Jeffamine-based hydrogel with incorporated naproxen moieties, JEFF-LPPCMSt-Na (see also Table 6). Magnification 1000×.

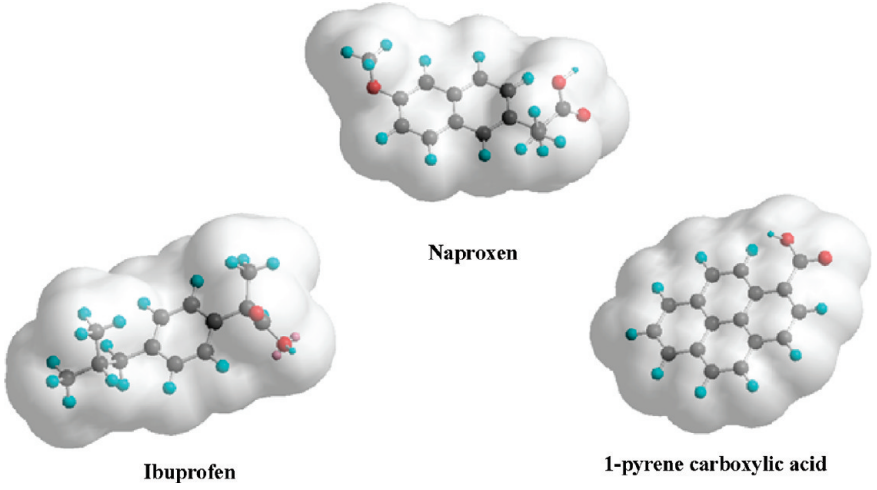


Figure 11. Size and shape of ibuprofen, naproxen, and 1-pyrenecarboxylic acid.

modified networks. An example with naproxen is shown in Figure 10. Thus, it is reasonable to assume that the decrease in the q values for the modified hydrogels in the four solvents is also probably due to the morphological changes in the network matrix.

Release Behavior of JEFF-PPCMSt Hydrogels. The potential use of hydrogels based on Jeffamine and PPCMSt in biomedical and biotechnological applications is evaluated with ibuprofen, naproxen and 1-pyrenecarboxylic acid as model compounds with increasing hydrophobicity. The solvent accessible surfaces of these compounds, modeled by CambridgeSoft Chem3D software (map property: hydrophobicity) are shown in Figure 11. It is seen that Ip and Na have similar size and elongated shape, while Py is ovoid and more compact.

To understand how the diffusion process of the drugs is influenced by the structure of the polymer network, the cumulative release profile of these substances is investigated in basic medium (pH = 10.1). It should be noted that the conditions are chosen for accelerated bond scission and by no means are intended to mimic physiological conditions. Release examples with modified JEFF-HPPCMSt hydrogels are shown in Figure 12. The data show that the molecules of the model compounds are able to slowly diffuse through the swollen networks and the release is completed in about 3 days. The release mechanism and the total amount released in one portion are different for the compounds investigated. At similar attachment levels to PPCMSt, the hydrogels with ibuprofen and naproxen release ~7−8 mg drug/g dry gel, while the pyrene gel releases only 0.32 mg Py/g dry gel. This result is somewhat unexpected since the mesh (pore) sizes in these networks are much larger than hydrodynamic radius of the released substances and therefore their diffusion should not experience significant interference from the swollen hydrogel matrices. In this particular case the difference is most probably caused by the (in)solubility of the pyrene carboxylate in water. Thus, the more hydrophobic pyrene moiety strongly interacts with the hydrophobic PPCMSt domains in the conetwork and shows very low tendency to migrate into the aqueous medium surrounding the swollen gel particles.

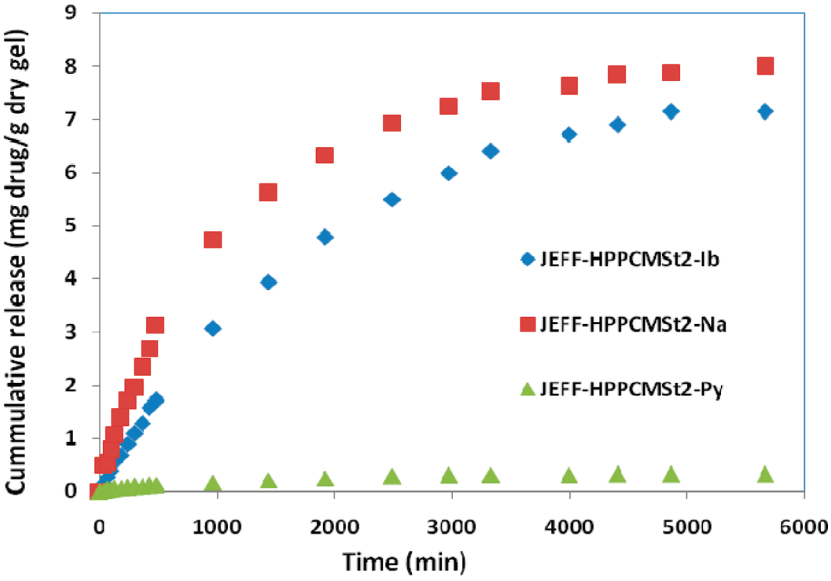


Figure 12. Cumulative release of ibuprofen (Ib), naproxen (Na), and pyrene carboxylate (Py) from type I hydrogels (67 wt % of Jeffamine), investigated in basic medium (pH = 10.1).

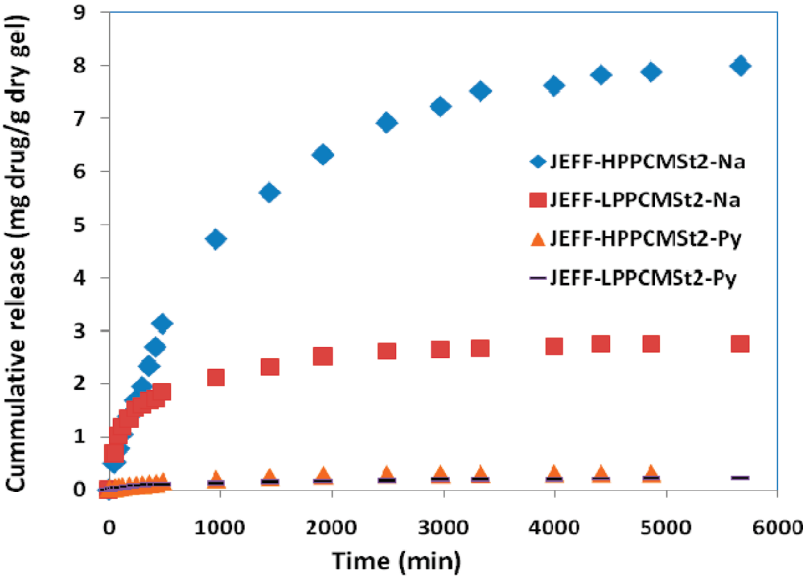


Figure 13. Cumulative release from networks of type I and II with 67 wt % of Jeffamine, containing covalently attached naproxen (Na) and pyrene (Py).

The influence of the macromolecular architecture of the hydrophobic PPCMSt components (type I and type II Jeffamine-based networks) on the release profile of naproxen and pyrene is shown in Figure 13. It can be seen that the Na amounts released from hyperbranched PPCMSt containing hydrogels are almost four times larger than those from the hydrogel of the linear polymer. It is also noteworthy that the drug release from the network JEFF-LPPCMSt levels of after 1 day while with the gel JEFF-HPPCMSt the process continues for more than 3 days, Figure 13. These results indicate that the drug diffusion process is not only affected by the molecular size of the drugs, but is also influenced by the structure of polymer network. In that aspect one should take into account that at equivalent molecular weight the globular hyperbranched polymers are more compact and occupy less volume in the expanded framework of the swollen hydrogels. Consequently the surface contact and the interactions with the penetrating medium will differ significantly from the same processes in the hydrogels containing the modified hydrophobic linear segments. Since the rate of release depends also on the rate of bond scission (chemical or enzymatic[29]) the amount of the released covalently linked substance will be significantly different even at similar initial attachment levels.

The total substance load in hydrogels with different macromolecular architectures and the amount of the substance released from these hydrogels are shown in Table 7. The cooperative influence of all previously discussed factors is obvious. The released amounts from JEFF-HPPCMSt hydrogels are higher than those released from JEFF-LPPCMSt hydrogels after the interaction with two consecutive fresh portions of basic aqueous medium. Despite the fact that the gels are immersed in fresh basic solution after the release profile in the starting medium levels off, the amount of the released substances is consistently lower than the initial burst (Table 7, second portion release). Thus, the lower release is not caused by decreased concentration gradient. It is most probably due to the lack of interconnected pores

Table 7. Total Drug Loading and Equilibrium Release from Hydrogels with Different Macromolecular Architecture

		first portion release		second portion release	
hydrogel[a]	drug bonded (mg/g dry gel)	amount (mg/g dry gel)	% release	amount (mg/g dry gel)	% release
JEFF-HPPCMSt2-Ib	33.3	8.01	24.1	4.48	13.5
JEFF-HPPCMSt2-Na	33.3	7.63	22.9	4.41	13.2
JEFF-HPPCMSt2-Py	25.3	0.34	1.3	0.27	1.0
JEFF-LPPCMSt2-Ib	33.3	3.20	9.6	2.02	6.1
JEFF-LPPCMSt2-Na	19.2	2.10	11.0	1.10	5.8
JEFF-LPPCMSt2-Py	33.3	0.23	0.7	0.12	0.4

[a] The numbers signify the Jeffamine to PPCMSt ratio.

(Figure 10) in the modified conetworks, which prevents the efficient access of the swelling medium to all hydrolyzable attachment sites in the hydrogel matrix.

The results obtained show that the release process includes several interrelated phenomena: (1) penetration of the medium into the network matrix and swelling, affected by the hydrophilic/hydrophobic balance of the hydrogel, the cross-linking density and the morphology (pore structure and organization); (2) hydrolysis of the ester linkages (only the deattached substances can be released under suitable conditions); (3) dissolution of the cleaved molecules in the surrounding aqueous media (i.e., the amount of drugs that can be spectroscopically detected outside the gels); (4) the residual interaction of the detached compounds with their original carriers (hydrophobic/hydrophobic or $\pi-\pi$ interactions) and (5) diffusion process through the swollen gel as a consequence of the preceding events (1−4).

Conclusions

New monomers with attached model compounds (drugs and fluorescent marker) are successfully synthesized and incorporated into copolymers with *p*-(chloromethyl)styrene. The new copolymers are formed with controllable molecular weight and chemical compositions and are used for the preparation of new amphiphilic conetworks with PEG and Jeffamine. The incorporation of macromolecules with different types of architecture into the hydrogels has a pronounced effect on their degree of swelling, morphology, and the diffusion processes into and out of the cross-linked systems. The hydrogels, which include different amounts of bonded model substances (ibuprofen, naproxen and pyrenecarboxylic acid), are able to release them in aqueous media under basic conditions. The multiple-step release process depends on the hydrolysis of ester linkage, the microstructure of hydrogels, the physical properties of the attached compounds, as well as their interaction with the surrounding conetwork matrix.

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