Silylation of Poly-L-Lysine Hydrobromide to Improve Dissolution in Apolar Organic Solvents

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ABSTRACT: Bis(trimethylsilyl)acetamide (BSA) was used to trimethylsilylate the hydrobromide salt of the synthetic polyamide poly-L-lysine (PLL–HBr) to improve its solubility in apolar organic solvents. The resulting trimethylsilylated derivative of PLL (PLL–$SiMe_3$) was found to be soluble in methylene chloride, tetrahydrofuran, and xylenes. The PLL–$SiMe_3$ was eventually used in the formation of an interpenetrating polymer network (IPN) with ultrahigh molecular weight polyethylene via xylenes. Elemental analysis, FTIR, and NMR spectroscopic evidence supported highly efficient (81.7%) silylation for the BSA-treated PLL–$SiMe_3$ product. Spectral data also showed complete silylation at the carboxyl, *N*-terminal α-amine, and the ε-amine groups. This chemistry provides solvent processing accessibility for PLL blends or grafting reactions not possible for the commercial hydrobromide salt of PLL. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 79: 2264–2271, 2001

Key words: poly-L-lysine; silylation; solubility; poly(amino acid); derivatization

INTRODUCTION

Trimethylsilylation (silylation) has long been known to increase the apolar character of molecules by replacement of active hydrogens with the apolar trimethylsilyl group. This derivatization both reduces the possibility of hydrogen bonding and increases the apolar nature of the silylated molecule, increasing its dissolution in apolar solvents.[1] Silylation of the ε-and/or α-amino groups of poly-L-lysine hydrobromide (PLL–HBr), a synthetic poly(α-amino acid) readily soluble in aqueous media, to trimethylsilylated PLL (PLL–$SiMe_3$) was therefore utilized to facilitate PLL dissolution into a number of apolar organic solvents. Successful achievement of this objective would extend the utility of this versatile commercial polymer in copolymerization, grafting, conjugation, and blending using organic solvents currently not possible for PLL–HBr. In addition, the silyl derivatives are chemically removable, facilitating restoration of the original PLL polymer if desired. While the silylation approach has been often used with monomeric α-amino acids, its application to poly(α-amino acid)s has not been reported. The resulting trimethylsilylated (i.e., "silylated") polymer species is of a slightly larger molecular weight but more soluble in apolar organic solvents, like xylenes, due to the reduction in the number of reactive or polar sites available.[1]

Silylation has been classically used for a number of purposes including the protection of and

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solubilization of α-amino acids in peptide synthesis.[1–9] Specifically, individual α-amino acids have been silylated by many methods. Birkofer and Ritter silylated α-amino acids with hexamethyldisilazane (HMDS) and a few drops of concentrated sulfuric acid in refluxing toluene.[3] Others have used bis(trimethylsilyl)acetamide (BSA),[2,10] *N*-(trimethylsilyl)acetamide (MSA),[10] and trimethylchlorosilane (TMCS).[5] Specifically, Rogozhin and coworkers silylated the hydrochloride salt of L-lysine (Lys–HCl) with a 0.67 reaction site molar excess of trimethylsilydiethylamine (TMSDEA) in dimethylformamide (DMF).[7] The results demonstrated complete silylation of both the α- and ε-amino groups resulting in a N^{α}, N^{ε}-bis(trimethylsilyl)trimethylsilyl ester of L-lysine.

The objective of this study was to assess the effectiveness of silylating the target polymer PLL–HBr with BSA to increase its solubility in apolar solvents. Additionally, characterization was performed to verify the degree and location of silylation. The ultimate goal of this work was to form an interpenetrating polymer network (IPN) between the derivatized PLL and ultrahigh molecular weight polyethylene (UHMWPE) to be used as a model material for tribology studies on total hip replacement implants. The silylation reaction described below was used to make PLL soluble in xylenes for swelling into UHMWPE, the first step of IPN synthesis.

EXPERIMENTAL

Materials

PLL–HBr (Sigma Chemical Co., St Louis, MO, 1000 g/mol molecular weight per manufacturer's determination by capillary electrophoresis) was stored at approximately −20°C and used without further purification. Prior to use, the PLL–HBr was dried under a vacuum (~15 mTorr) over either anhydrous calcium sulfate ($CaSO_4$) or phosphorus pentoxide (P_2O_5) desiccants for 24–48 h. BSA (reagent grade) and TMCS (98%) were purchased from Aldrich Chemical Co. (Milwaukee, WI) and were stored at 4°C and used as received. Tetrahydrofuran (THF, 99+%) and xylenes (99%) from either Fisher (Pittsburgh, PA) or Aldrich were refluxed over sodium metal overnight (at least 8 h) and then distilled. Acetonitrile (99%) and methylene chloride (99%) from Fisher were dried over 4–5 Å molecular sieves for at least 24 h prior to use. Deuterium oxide (D_2O, 99.9%) and deuterated chloroform ($CDCl_3$, 99.8%, without tetramethylsilane), from Cambridge Isotope Laboratories, Inc. (Andover, MA), were used as received. All glassware used in the preparation, transfer, storage, and reaction of the silylated substance were silylated with 1 m*M* TMCS in methylene chloride prior to use.

PLL Derivatization Method

Silylation reactions involving PLL–HBr in nonaqueous media were, by necessity, two-phase PLL solid dispersions in an organic solvent miscible with the silylation agent. To a clean, dry, and silylated 100-mL three-neck round-bottomed flask fitted with a Teflon® stir bar, H_2O condenser, syringe septa stopper, and nitrogen purge inlet and outlet control valves fitted with bubblers, 500 mg (500 μmol) of PLL–HBr was added under a dry ($CaSO_4$ filtered) nitrogen purge. To the flask, 6.0 mL of either acetonitrile or THF and 4.0 mL (16.2 mm) BSA were added via a cannula. The contents of the flask were stirred gently and heated with a stirred oil bath to 60–63°C. The reaction continued until the two-phase mixture became a translucent, light yellow solution, approximately 6–13 h, and then was allowed to react for an additional 0.5 h.

To purify the product, the solution was transferred, via a cannula, into a clean, dry, silylated, and nitrogen-purged 250-mL two-neck round-bottomed flask. The product was dried from THF repeatedly under a vacuum (~15 mTorr) in a 70°C water bath. Crystals, either MSA or acetamide, formed on the flask with each evaporation and were left behind as the product polymer was purified by serial dilution into 5.0 mL THF and evaporated under a vacuum. Dilution and evaporation were performed as necessary for the absence of crystal formation and the quick solidification of PLL–$SiMe_3$. The purification took approximately 20–24 h.

Polymer Characterization

Solubility of PLL–$SiMe_3$

The polymer product solubility in THF was visually monitored and was used to determine the reaction endpoint. After purification from THF, xylenes were added to produce a clear solution of the PLL–$SiMe_3$ product, and product solubility was positively verified visually.

Elemental Analysis

Galbraith Laboratories (Knoxville, TN) performed the polymer elemental analysis. Carbon, hydrogen, and nitrogen were measured in accordance with ASTM standard D 5291[11] on a LECO Model 1000 inductively coupled plasma optical emission spectroscopy (ICP–OES) CHN analyzer. Bromine was determined through use of the Scheniger burn/titration method. A burn bomb followed by ICP–OES analysis was used to determine silicon weight percent of the samples.

Infrared Spectroscopic Characterization of PLL–HBr and PLL–SiMe$_3$

Transmission FTIR spectra were collected on a Nicolet 760 MAGNA-IR spectrometer fitted with a liquid nitrogen-cooled mercury–cadmium–tellurium (MCT) detector. Dry PLL–HBr was ground into anhydrous KBr and pressed into a pellet. PLL–SiMe$_3$, being very moisture-sensitive, was dissolved into anhydrous hexanes and analyzed in a dry nitrogen-purged airtight KBr flow cell. Spectra for PLL–HBr and PLL–SiMe$_3$ were collected using 128 scans at a resolution of 2 cm^{-1}. Subtraction spectra for PLL–SiMe$_3$ were created by subtracting an anhydrous hexane-filled flow-cell reference spectrum from that of the PLL–SiMe$_3$/anhydrous hexane solution. Omnic version 3.1 software was used for scaling, automatic baseline correction, peak identification, and spectra subtraction. No other spectra alterations were performed.

NMR Analysis of PLL–HBr and PLL–SiMe$_3$

Free-induction decay (FID) spectra were acquired on a Varian (Palo Alto, CA) Model Unity/INOVA 300-MHz nuclear magnetic resonance spectrometer utilizing Varian's VNMR software, version 6.1b. FIDs were processed into NMR spectra using MestRe-C (Spain) NMR data processing software, version 2.02.

Sample Preparations

PLL–HBr was dissolved into deuterium oxide (D_2O) and PLL–SiMe$_3$ was dissolved into deuterated chloroform ($CDCl_3$). Concentrations of PLL–HBr in D_2O for the ^{1}H and ^{13}C spectra were 2.5and 250% (w/v), respectively. Concentrations of PLL–SiMe$_3$ in $CDCl_3$ for the ^{1}H and ^{13}C spectra were approximately 5.0 and 500% (w/v), respectively. ^{1}H spectra for the precursor PLL–HBr and PLL–SiMe$_3$ product were referenced to the solvent proton peaks of D_2O at $\delta = 4.80$ ppm and $CHCl_3$ at $\delta = 7.27$ ppm, respectively. The ^{13}C spectrum for the PLL–SiMe$_3$ product was referenced to the solvent reference peak of $CDCl_3$ at $\delta = 77.23$ ppm.[12] The absence of carbon in D_2O made referencing the ^{13}C of precursor PLL–HBr spectrum impossible.

RESULTS AND DISCUSSION

The reaction of PLL–HBr to PLL–SiMe$_3$ in acetonitrile proved problematic due to the solubility (see below) while that in THF was very effective. The procedure utilizing acetonitrile was based on that originally performed by Klebe and coworkers.[2] In that study, BSA in acetonitrile was used to silylate amides, ureas, and 22 amino acids. However, with PLL poly(α-amino acid) and following similar procedures as those of Klebe and coworkers, two insoluble solution phases were produced. This motivated the change to THF and the absence of any further insolubility problems. Hence, acetonitrile was not further investigated. Similar problems with acetonitrile have been reported, indicating that a 3:1 (v/v) ratio of acetonitrile to BSA is required for complete amino acid miscibility.[10]

We noted that BSA reactions utilizing PLL–HBr that had absorbed ambient atmospheric water prior to vacuum drying over desiccants took much longer than did reactions using initially dry PLL–HBr. "Wet" PLL–HBr that had been subsequently vacuum-dried formed a solid polymer dispersion in THF that had a much lower surface area-to-volume ratio than that of solid polymer dispersions formed by the initially dry PLL–HBr. The reduced surface area-to-volume ratio was presumed to be the cause of longer reaction times. Since this silylation reaction is two-phase, the BSA has limited exposure to silylate such PLL oligomer phases. Additionally, aggregation of insoluble precursor PLL–HBr chains hinders the reaction and dissolution of partially silylated oligomers from the solid polymer dispersion until PLL chains were highly silylated, soluble in the solution, and therefore released.

Klebe and coworkers reported reduced reaction rates with amides that exhibited low solubility in the silylation solvent.[2] Silylation of aromatic amides with BSA demonstrated slower reaction rates compared to other amides. Studies determined that the low solubility of the aromatic amides in acetonitrile was responsible for the slower reaction rate.[2] PLL–HBr appears to have little-to-no solubility in THF. Hence, the longer reaction times found in this study could also plau-

Table I Elemental Weight Percents of PLL–HBr and PLL–SiMe$_3$ by Theory and Analysis

	1 PLL–HBr Theory	2 PLL–HBr Actual	3 PLL–SiMe$_3$ Theory	4 PLL–SiMe$_3$ Actual
C	31.49%	29.92%	52.08%	50.55%
H	5.99%	5.67%	10.41%	10.25%
N	12.24%	11.45%	9.64%	9.87%
Br	41.89%	41.88%	0.00%	0.66%
Si	0.00%	0.30%	21.26%	17.39%
Total	91.61%	89.22%	93.39%	88.72%
Others[a]	8.39%	10.78%	6.61%	11.28%

[a] Includes oxygen.

sibly be attributed to combinations of low solubility in the solvent and higher molecular weight effects that reduce both molecular accessibility and diffusion and sterically hinder reactivity.

Polymer Solubility

The solubility of PLL–SiMe$_3$ improved from insoluble to at least 2.5% (w/v) in THF, the reaction solvent, based on the original PLL–HBr starting weights. Solubility in xylenes, for future IPN work, was at least 2.5% (w/v), again based on the original PLL–HBr starting weights. Solubility in methylene chloride, hexanes, and deuterated chloroform ($CDCl_3$) was demonstrated by dissolution in these respective solvents but not further quantified. These concentrations represent the lower limits observed. Alhough exhaustive steps were taken to preclude water from the silylation, any water present would likely result in hydrolysis of a trimethylsilylated amine and reduce the ultimate solubility of PLL–SiMe$_3$.

Elemental Analysis of BSA Derivatized PLL–SiMe$_3$ Product

The results of elemental analysis as well as theoretical predictions for PLL–HBr are shown in Table I. The first two columns represent theoretical and measured elemental compositions, in weight percent, for the PLL–HBr starting material, respectively. Figure 1 shows the proposed structure for PLL–HBr from which theoretical compositions were calculated. The delineated α- and ε-amines are subscripted for clarity. Values for oxygen could not be measured due to matrix effects with silicon. Table I also contains theoretical and experimental elemental weight percent comparisons of PLL–SiMe$_3$ in columns 3 and 4, respectively. Figure 2 shows the proposed structure for PLL–SiMe$_3$.

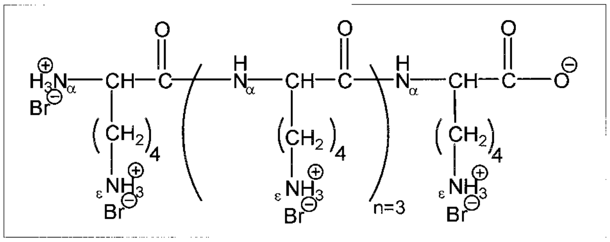

Figure 1 Structure of PLL–HBr starting material.

The PLL–HBr theoretical and actual compositional values in Table I are in close agreement. This is significant as it provides verification of both the elemental analysis technique as well as confidence in calculating theoretical expectations. An important feature of Table I is the detection of bromine in column 4, indicating incomplete silylation, trace amounts of the by-product, or both. The bromine level for the PLL product was 0.66 %. This level of bromine represents a maximum estimation for unremoved HBr of 1.6%.

The degree of silylation evidenced by the bromine and silicon levels in the sample (column 4) suggests incomplete PLL silylation. Actual silicon content (17.39%) falls between that calculated for complete derivatization (21.26%) and the absence of any silylation (0.00%), suggesting significant (81.36%) but not quantitative silylation.

Infrared Spectroscopic Analysis

Spectra from PLL–HBr and PLL–SiMe$_3$ are shown in Figure 3(a,b), respectively, for comparison. A strong background due to hexane reference solvent subtraction was complete and at some points off the absorbance scale: bands from 3005 to 2823 cm^{-1}, 1481 to 1434 cm^{-1}, and 1382 to 1386 cm^{-1} and a band at approximately 724 cm^{-1} were removed from Figure 3.

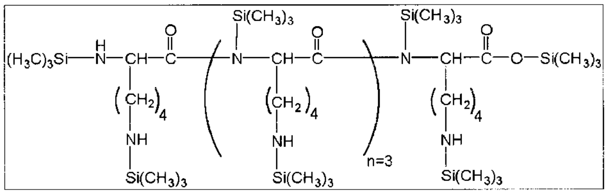

Figure 2 Structure of theoretical PLL–SiMe$_3$.

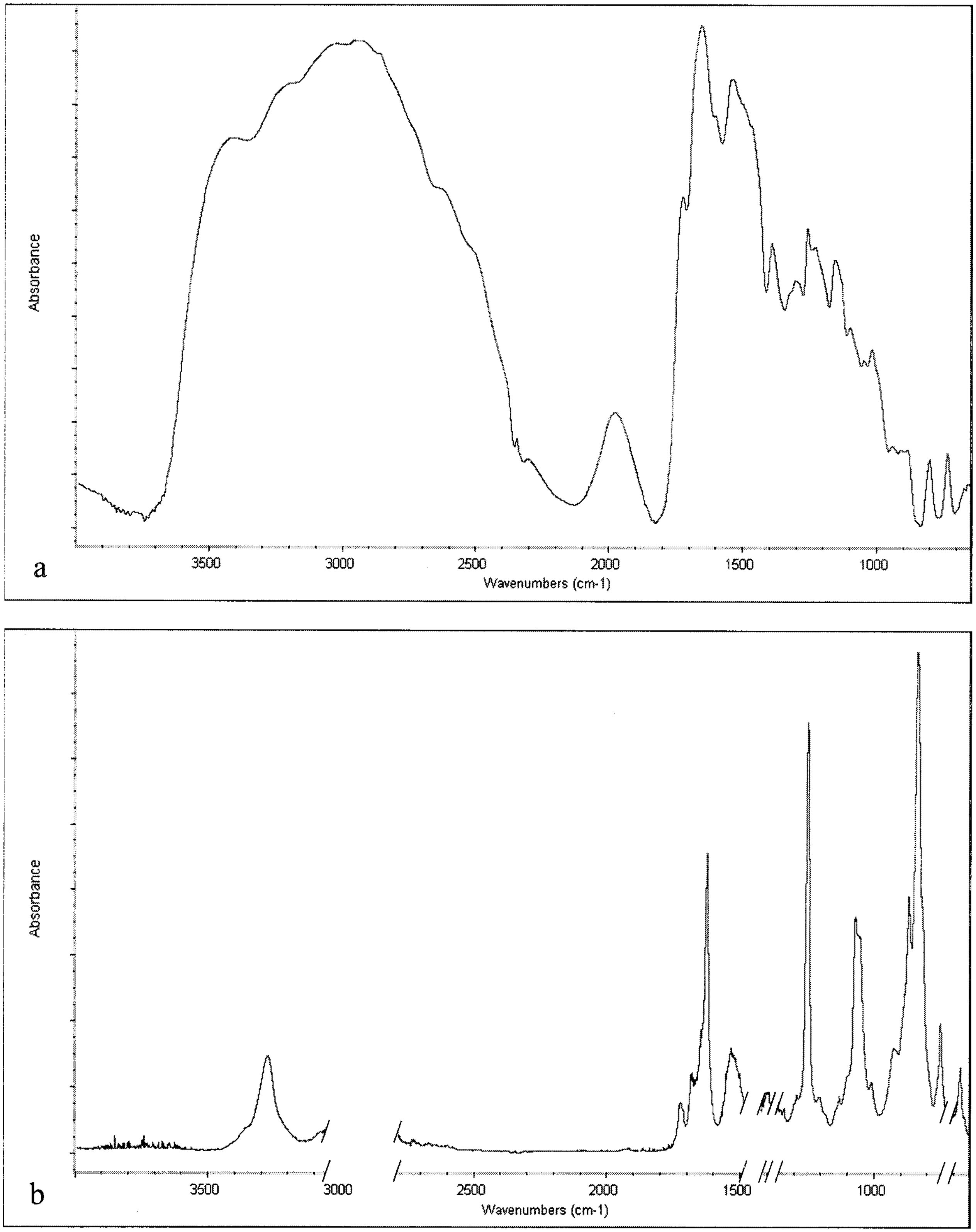

Figure 3 (a) Transmission FTIR spectra of PLL–HBr in a pressed KBr pellet and (b) transmission FTIR spectra of derivatized PLL–$SiMe_3$ in hexanes in a KBr flow cell (reference background spectra in hexanes subtracted).

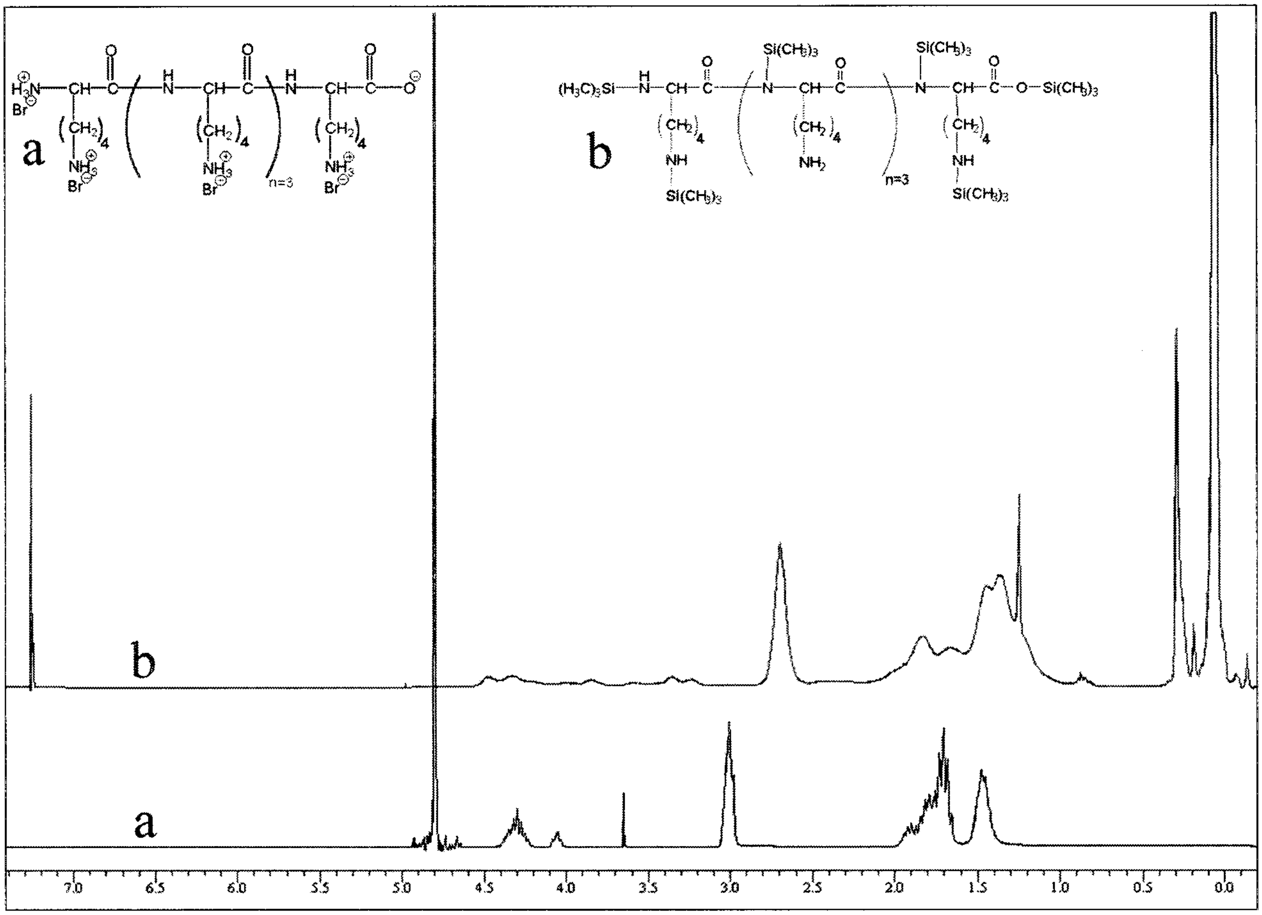

Figure 4 (a) ^{1}H-NMR spectra of PLL–HBr in D_2O; (b) ^{1}H-NMR spectra of derivatized PLL–$SiMe_3$ in $CDCl_3$.

FTIR bands unique to PLL–$SiMe_3$ in Figure 3(b) yield information related to the silylation process. The absence of a wide, strong absorption at 3600 cm^{-1}, typically associated with the carboxyl group, indicates silylation of this group. Additionally, a strong absorption at 1627 cm^{-1} is shifted but usually assigned to the characteristic amide I absorption at 1650 cm^{-1}.[13,14] This amide I band is a combination of carbonyl symmetric stretching (ν_sC═O), in-plane N—H bending (δN—H), and the coupling between these two modes. Other, more electron-withdrawing groups bonded to the nitrogen have been known to increase the absorption frequency.[14] The trimethylsilyl group is believed to have the opposite effect, reducing the amide I absorption frequency. Amide I absorption bands for PLL were reported at 1676, 1650, and 1640 cm^{-1}.[6,15,16] Significant silylation of the α-amine should shift the amide I absorption to lower frequencies. The same band was measured at 1660 cm^{-1} in Figure 3(a) for PLL–HBr, supporting this analysis and demonstrating silylation of the α-amines.

The absorption peak at 1545 cm^{-1} is assigned to the amide II region, a combination of C—N stretching (νC—N) and out-of-plane N—H bending (δN—H). For monosubstituted amides, this band is usually assigned to 1550 cm^{-1}.[13,14] For PLL, the amide II band was measured at 1550 and 1540 cm^{-1}.[6,7] Again, electron-donating groups such as the trimethylsilyl decrease the absorption frequency, consistent with the conclusion that the observed 1540 cm^{-1} band in Figure 3(b) supports silylation of PLL α-amines. The strong FTIR absorption bands located at 1255 and 839 cm^{-1} and a weaker absorption at 760 cm^{-1} also are consistent with the silylation of the PLL.[1,17]

Polymer NMR Analysis

The ^{1}H- and ^{13}C-NMR spectra for the precursor PLL–HBr and PLL–$SiMe_3$ product are shown for

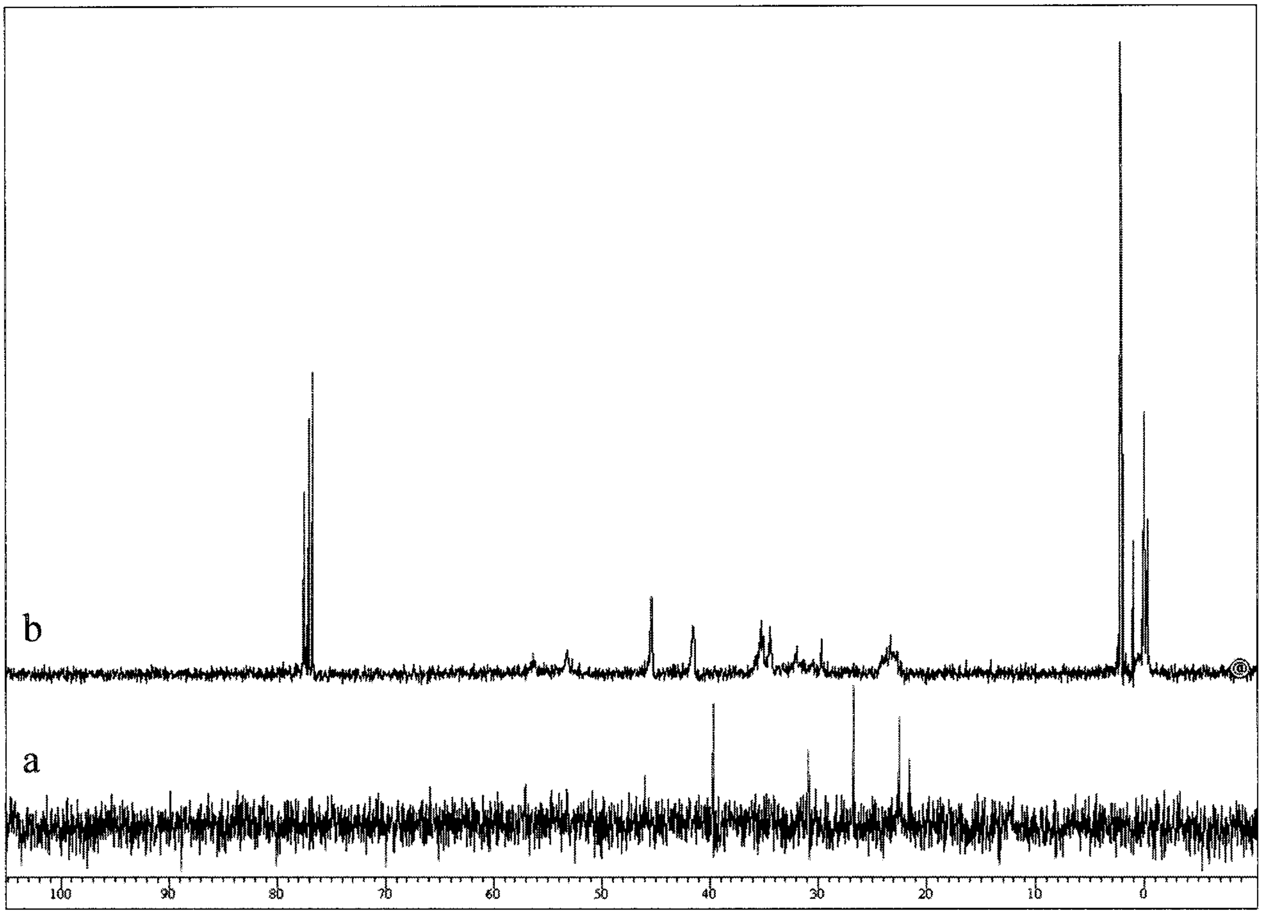

Figure 5 (a) ^{13}C-NMR spectra of PLL–HBr in D_2O; (b) ^{13}C-NMR spectra of derivatized PLL–$SiMe_3$ in $CDCl_3$.

comparison in Figures 4 and 5, respectively. In Figure 4(a), a notable feature for PLL–HBr is the absence of any proton peaks from 0.0 to 1.4 ppm. The series of peaks from 1.4 to 2.0 ppm are all associated with methylene protons. The peaks at 3.0 and 4.05 ppm can be assigned to the proton signals from the ε-amine group and *N*-terminal α-amine, respectively. The peak at 4.3 ppm is assigned to the amide of PLL.

Figure 4(b), the 1H spectrum for the PLL–$SiMe_3$ product, exhibits proton peaks from 0.1 to 0.3 ppm, demonstrating the presence of the trimethylsilyl group. The strong peak at 0.1 ppm is associated with all silylated amine and amide groups. The proton peak at 0.3 ppm is assigned to the silylated carboxyl *C*-terminal group. The highly electron-withdrawing carboxyl group diminishes electron shielding from the normally highly shielded trimethylsilyl group and shifts this peak slightly downfield from its expected 0.0 ppm position. Proton peaks from 1.0 to 2.0 ppm are attributed to various methylene protons. The peak at 2.7 ppm is assigned to the ε-amine peak shifted from its original 3.0 ppm position in PLL–HBr seen in Figure 4(a). This shift upfield to a more shielded position would be expected from derivatization with an electron-donating trimethylsilyl group as well as the change from a primary amine to a secondary amine. Significantly, the proton peak at 4.3 ppm reveals incomplete silylation of the amide group.

Figure 5(a) shows the ^{13}C spectra for the PLL–HBr starting material. Absence of any ^{13}C peaks from 0.0 to 20.0 ppm and the inability to reference the spectrum to a standard peak makes interpretation difficult except for comparison with the PLL–$SiMe_3$ product.

Significant features for the PLL–$SiMe_3$ product [Fig. 5(b)] spectra include peaks at or very near $\delta = 0.00$ ppm. This area of the spectrum represents highly electron shielded species such as trimethylsilyl or tetramethylsilane (often doped into

NMR solvents as a $\delta = 0.00$ ppm internal reference).[12] Solvents were chosen without this reference to clearly show this peak as a prime indicator for silylation of the PLL–$SiMe_3$.

A consideration of all the polymer characterization information permits us to conclude that PLL–HBr can be converted to an organically soluble PLL–$SiMe_3$ derivative. The structure in Figure 2 is proposed as the product of the silylation of PLL–HBr with BSA. It is proposed that the *C*-terminal carboxyl, the *N*-terminal α-amine, and the ε-amine groups were completely silylated and the amides were partially silylated.

CONCLUSIONS

PLL–$SiMe_3$ was synthesized using BSA in THF. Elemental, FTIR, and both ^{1}H- and ^{13}C-NMR analyses demonstrated an almost complete removal of HBr and an 81.4 % overall degree of silylation. The spectral data demonstrated complete silylation at the *C*-terminal carboxyl, *N*-terminal α-amine, and ε-amine groups. However, the amide group was found to be incompletely silylated. A solution of PLL–$SiMe_3$ in xylenes is suitable for preparing an IPN with apolar polymers, including UHMWPE.

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