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Silk-Inspired Polyurethane Containing GlyAlaGlyAla Tetrapeptide. II. Physical Properties and Structure

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ABSTRACT: Biomedical polyurethane (BPU) and silk fibroin have similarly molecular architecture in their primary and aggregate structure, both of which have amide bonds and microphase separation, and they have been employed as scaffold materials for biomedical applications. Based on this, as the featured peptide sequence of silkworm silk fibroin, GlycineAlanineGlycineAlanine (GlyAlaGlyAla) tetrapeptide was synthesized by using traditional liquid-phase peptide synthesis method with Boc-protected glycine and alanine as starting materials, and was transformed to its derivative with two amine-terminated functional groups. The derivative was introduced as a chain extender into the backbone to form the hard segment of a silk-inspired PU with urea-linkage. Related measurements show that molecular weight of the synthesized silk-inspired PU ranged from 13,000 to 15,000. Fourier transform infrared (FTIR) absorption bands of Amides I and II are at 1651 cm^{-1} and 1534 cm^{-1}, and Raman absorption band of Amide III is at 1302 cm^{-1}. UV-Vis absorption peak of the silk-inspired PU is at 266 nm. This new concept and strategy may allow the fabrication of a new class of thermoplastic polyurethane elastomers to mimic the structure and properties of silk fibroin of silkworms and spiders. Information provided by this study may be used to better understand the correlation between the natural and man-made materials.
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INTRODUCTION

Polyurethanes (PUs) were initially synthesized by Bayer and they have been known to users for several decades predominantly as elastomers. PUs are also produced in the form of foamed plastics, structural elastomers and coating elastomers, adhesives, leather-like materials and auxiliary agents. As one subclass of the polyurethane family, segmented polyurethanes have found a number of biomedical applications due to the unique combination of excellent physical, mechanical and biological properties, besides the wide ranging applications in furnishings, textiles, paper making, packaging, adhesives, and sealants.[1]

On the other hand, mankind has farmed *Bombyx mori* silkworms for thousands of years. The natural production of silk fiber looks easy for silkworms in contrast to the man-made spinning of chemical fiber commonly demanding high temperature and pressure. Silk proteins are secreted within the animal, and when necessary, the protein can be transported through a duct to assembly into fibers at the ambient temperature under the normal pressure. Actually, this life-control process is highly complex and thus impractical for mankind. Naturally occurring silkworm silk fiber has been used for applications as diverse as strings, nets, textiles, and wound dressings owing to its mechanical properties and biocompatibility.[2] Although silkworms can reproduce and make silk fiber year by year, restricted productivities of naturally producing silk fiber are not very approving for increasingly requirements.

Industrial silk-like fibers for textile use are commonly produced by the alkaline hydrolysis of polyester fibers without any component of silk fibroin to mimic the handle and shape of the natural fiber.[3,4] However, structures determine properties. Besides regeneration of silk fibroin,[5–8] to achieve the artificial production of high performance and mimetic structure of silk-like fibers, a variety of polyamino acids (known as polypeptides or proteins) and polymers based on silk fibroin model have been explored by chemical method or genetic bioengineering.

Various silk-based hybrid proteins (all-proteins) have been produced in the hope of generating proteins incorporating the attractive properties of both compounds. Tirrell et al. produced periodic polypeptides by high-cell-density fermentation of

recombinant *Escherichia coli*.[9] Werten et al. biosynthesized an amphiphilic silk-like polypeptide rendering hydrophobic surfaces hydrophilic, and hydrophilic surfaces hydrophobic.[10] Wang et al. biosynthesized and characterized the typical fibroin crystalline polypeptides of silkworm *B. mori*, and they demonstrated that the hexapeptide (GAGAGS) was the key basis to form β-sheet conformation in the silk fibroin crystalline domain.[11] Yamamoto et al. obtained a silk-like poly(amino acid) fiber at aqueous solutions.[12,13] Asakura et al. developed silk-like materials by applying genetic engineering strategies. The primary structure of these materials combined the repetitive crystalline region of silk fibroin with elastic or hydrophilic motifs.[14–16]

Although solid-phase synthesis would theoretically allow the preparation of proteins with accurate primary sequences, the high molecular masses of naturally occurring silk proteins make this approach impractical.[17] The chemical elements and even primary structure are not necessarily the decisive factor for protein properties. The secondary or higher hierarchy structure or alignment conditions may play a more important role.[18,19] Therefore, peptide/nonpeptide copolymers have been extensively investigated for biomimetic synthesis of silk-inspired biomaterials.

Studies of the self-assembly of a number of silk-protein-inspired copolymers containing blocks of β-sheet-forming peptides $[Gly\text{-}Ala]_n$ or Ala_n) have been reported in the last decades.[17,20] Sogah's group comprehensively studied silk-inspired multiblock copolymers, which were composed of a spacer linked to flexible blocks of short alkane or ethylene glycol chains.[21–23] Shao et al. produced silk-protein-like multiblock copolymers.[24,25] The starting materials are similar to those for typical PU synthesis. Takahara *et al* fabricated biodegradable segmented PU elastomers without nontoxic degradation products using lysine-based diisocyanate.[26] Korley et al. utilized peptidic ordering to design hierarchical polyurethane/ureas.[27]

All-non-protein materials have been adopted to synthesize silk-like biomaterials. Hammond et al. produced a series of silk-like PUs containing poly(ethylene oxide) (PEO) soft segments and 1,6-hexamethylene diisocyanate-1,4-butanediol (HDI-BDO) hard segments (without any peptide links), in which PEO soft segments were semicrystalline and reinforced the PU matrix.[28,29] Eceiza et al. molecularly engineered elastic and strong supertough polyurethanes to discover the best molecular architecture of the Spider silk to develop best mechanical performance after macromolecular alignment.[30]

Consequently, the above helpful investigations prompted us to develop a novel synthetic PU containing polypeptides composed of amino acid residues of silk fibroin. In our previous work, we presented silk-inspired polyurethane biofiber by wet-spinning of biomedical PU/silk proteins blend solution.[31] Then the silk-inspired PU containing GlyAlaGlyAla tetrapeptide was synthesized and its primary structure was qualitatively identified by means of elemental analysis (EA), ^{1}H nuclear magnetic resonance (^{1}H-NMR) spectroscopy and pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS).[32] Currently, we describe herein our efforts in investigating its physical properties and hierarchy structure of more importance. Molecular weights were determined by intrinsic viscosity ($[\eta]$) method and gel permeation chromatography (GPC), and microstructural analysis was characterized by Fourier transform infrared (FTIR) spectroscopy and ultraviolet-visible (UV-Vis) spectroscopy. Relative comparisons of these measurements of the varying polyurethanes were discussed.

EXPERIMENTAL

Starting Materials

Chloroform (Sinopharm Chemical Reagent, China, abbreviated as SCRC, 99.0%) and *N*-methylmorpholine (NMM; SCRC, 98.0%) were distilled prior to use. Tetrahydrofuran (THF; SCRC, 99.9%) were dried using sodium wire and distilled before use. *N, N′*-dimethylformamide (DMF; SCRC, 99.5%) and dimethyl sulfoxide (DMSO; SCRC, 99.0%) were used after dehydration with 4 Å molecular sieves (SCRC, Φ 3–5 mm) for 2 days. Polytetrahydrofuran (PTHF, $M_W \approx 2000$, Aladdin Reagent, China) was dried under vacuum at 60°C for 9 h before use. *N-tert*-butoxycarboxyl-L-alanine (Boc-Ala-OH; Yangzhou Baosheng Biochemical, China, 98.0%), *N-tert*-butoxycarboxyl-glycine (Boc-Gly-OH; Yangzhou Baosheng Biochemical, China, 98.0%), *N*-hydroxysuccinimide (HOSu; SCRC, 97.0%), 1,6-hexamethylene diisocyanate (HDI; Alfa Aesar, 98.0%), 4, 4′-diphenylmethane diisocyanate (MDI; Alfa Aesar, 98.0%), *N,N′*-dicyclohexyl carbodiimide (DCC; SCRC, 95.0%), and other accessorial reagents were used without further purification.

Synthesis of Silk-Inspired PU

The silk-inspired PU containing GlyAlaGlyAla tetrapeptide in this study was obtained by a two-step polymerization method. The detailed synthesis procedures and the chemical composition of the final PU were illustrated in a previous article.[32] Briefly, the specific chain extender, H-GlyAlaGlyAla-NH$(CH_2)_6$NH-AlaGlyAlaGly-H, was prepared and purified as described in the earlier literature,[25] using traditional liquid-phase peptide synthesis method. Subsequently, the classical two-step polymerization method[33] was employed to synthesize the silk-inspired PU, using the specific chain extender mentioned above. The total yield was about 30%. For comparison, the PU with a classical 1, 4-butanediol (BDO; Aladdin Reagent, China) chain extender was also synthesized in the same condition, which was denoted as BDO-extended PU. The silk-inspired PU is a poly (urethane urea), while BDO-extended PU is a poly (urethane ether). The PU products were made into granules by being dripped into a mixture of methanol-water (3/1, v/v) and then coagulated.[33] The granules were further purified by extraction in toluene, dried, and stored in granular or flaky forms. No catalyzer was used throughout the synthesis to compare the rationale.

The chemical structures of the raw materials used in the formulation of PUs are presented in Figure 1. Reaction route for the synthesis of polyurethane is shown in Figure 1. Schematic illustration of chemical structure of the silk-inspired PU containing GlyAlaGlyAla tetrapeptide is illustrated in Figure 1.

Physical and Structural Characterizations

Intrinsic Viscosity ($[\eta]$). Intrinsic viscosity measurement of the PU samples (Dow BPU, BDO-extended PU, and silk-inspired PU) was made using an Ubbelohde suspended level viscometer

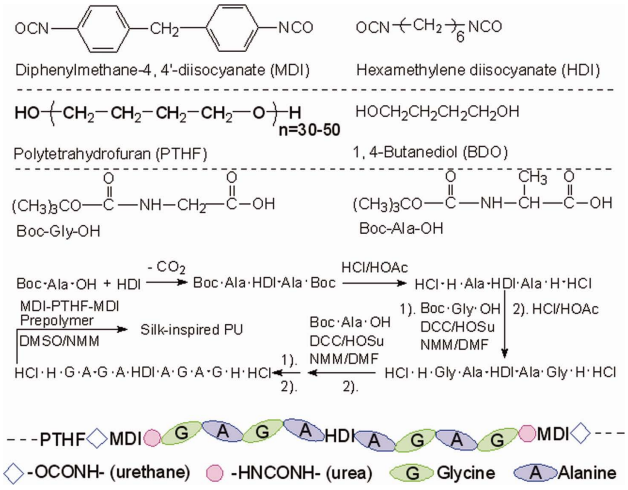

Figure 1. Starting materials, synthetic scheme, and schematic structure for the silk-inspired polyurethane containing GlyAlaGlyAla tetrapeptide. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

at 30 ± 0.1°C in a thermostated water bath using DMF as solvent. Dried PU samples were dissolved in DMF at room temperature under stirring. The specific (η_{sp}) and reduced (η_r) viscosities were calculated at six different concentrations (0.002, 0.003, 0.004, 0.006, 0.008, and 0.012 g/mL, respectively.) and converted to intrinsic viscosity ($[\eta]$) by extrapolation to infinite dilution (Figure 2). The PU/DMF solution was filtered directly into the viscometer. The viscometer was equipped with a $CaCl_2$ tube and the temperature was equilibrated for ~ 10 min prior to each flow-time measurement. For each concentration, the measurements were repeated until the relative error of five successive measurements was less than 0.1% and an average value of flow times was recorded. The viscosity-average molecular weight was calculated from the empirical Mark-Houwink equation ($[\eta] = KM_\eta^\alpha$) using the following constants: $K = 6.80\times10^{-5}$ dL/g and $\alpha = 0.86$.[34] Parallel measurements were carried out on the PU samples dissolved in THF.

Gel Permeation Chromatography. Molecular weights (M_n, M_w) and polydispersity index (M_w/M_n) were determined relative to a calibration with polystyrene standards, by using GPC (Waters 1525/2414, Waters Instrument, MA) equipped with Empower software at ambient temperature. Freshly distilled THF served as the mobile phase and was delivered at a flow rate of 1.0 mL/min. Sample concentrations were 10–12 mg/mL in freshly distilled THF, and the injection volume was 50 μL.

Fourier Transform Infrared Spectroscopy. Fourier transform infrared (FTIR) spectra were recorded with a Bruker Tensor 27 spectrometer from 4000 to 400 cm^{-1}, at 2 cm^{-1} resolution using 32 scans. The specimens of the synthesized BDO-extended PU and silk-inspired PU before coagulation were coated on potassium bromide (KBr) discs for testing at 25°C.

Raman Spectroscopy. Raman scattering spectra of the synthesized BDO-extended PU and silk-inspired PU before coagulation were collected at 25°C on a Raman microscope DXR model, equipped with the Omnic software from Thermo Fisher Scientific. The excitation laser wavelength was 532 nm using a laser power level of about 6.0 mW.

Ultraviolet-Visible Spectroscopy. UV-Vis spectra of the PU samples (Dow BPU, BDO-extended PU, and silk-inspired PU) were recorded at room temperature in the wavelength range of 350–800 nm by using an UV-Vis spectrophotometer (UV-2550, Simadzu, Japan) with a dilute solution (colorless and transparent) of 0.01% (w/v) in spectroscopic grade DMF solvent in a 1 mm thick quartz cell. The spectrophotometer was set at a scanning rate of 480 nm/min and 2 nm per scan step.

RESULTS AND DISCUSSION

Molecular Weights of the PU Samples

The PU samples obtained in this study were linear materials and thus soluble in typical solvents such as DMF or THF. Viscosity measurements were performed to evaluate the intrinsic viscosity and molecular weight of the PU samples (Dow BPU, BDO-extended PU, and silk-inspired PU). The concentration dependence of specific (η_{sp}) and reduced (η_r) viscosities for the PU samples in DMF is linear with only one deviation of the point at the lower solution concentration (Figure 2). The related data are collected in Table I. These PU samples were then analyzed by GPC, and their molecular weights were also determined (Figure 3). The values of average number molecular weight (M_n) analyzed by GPC are in approximate agreement with the values of viscosity-average molecular weight (M_η) obtained by intrinsic viscosity (Mark-Houwink equation) as listed in Table I. The values of molecular weight and intrinsic viscosity for our PU samples are in accordance with Žagar's similar research result for noncarboxylated, carboxylated Pus, and PU-ionomers[35] and Bagheri's liquid crystalline Pus.[36]

The viscosity-average molecular weights of the synthesized PU samples (BDO-extended PU and silk-inspired PU) ranged from 13,000 to 15,000 thus being not comparable to those of commercial biomedical polyurethanes (e.g., Dow BPU). but they all still can be electrospun to form fibrous membranes. Another interesting finding is that the determined intrinsic and reduced viscosities of the PU samples in DMF are lower than those in THF solution (Table I and Figure 2). This is also in accordance with Žagar's results.[37]

FTIR and Raman Spectroscopy

FTIR has been used extensively to both identify and quantify chemical functionality and hierarchy structure in polymers. Figures 4 and 5 are the FTIR and Raman spectra of the BDO-extended PU and silk-inspired PU, respectively. Table II summarizes the main FTIR bands in typical PU, while Table III shows the FTIR values of amides obtained for BDO-extended PU, silk-inspired PU and controls. In our previous report,[32] relative comparisons of FTIR and Raman spectra indicating the primary structure changes have been discussed. Currently, we describe herein relative comparisons of Amide I, II, and III in BDO-extended PU, silk-inspired PU and silk.

As shown in Figures 4 and 5, the figures show no band at about 2230 cm^{-1} in FTIR, suggesting NCO group has completely reacted.[38] To investigate whether the introduction of

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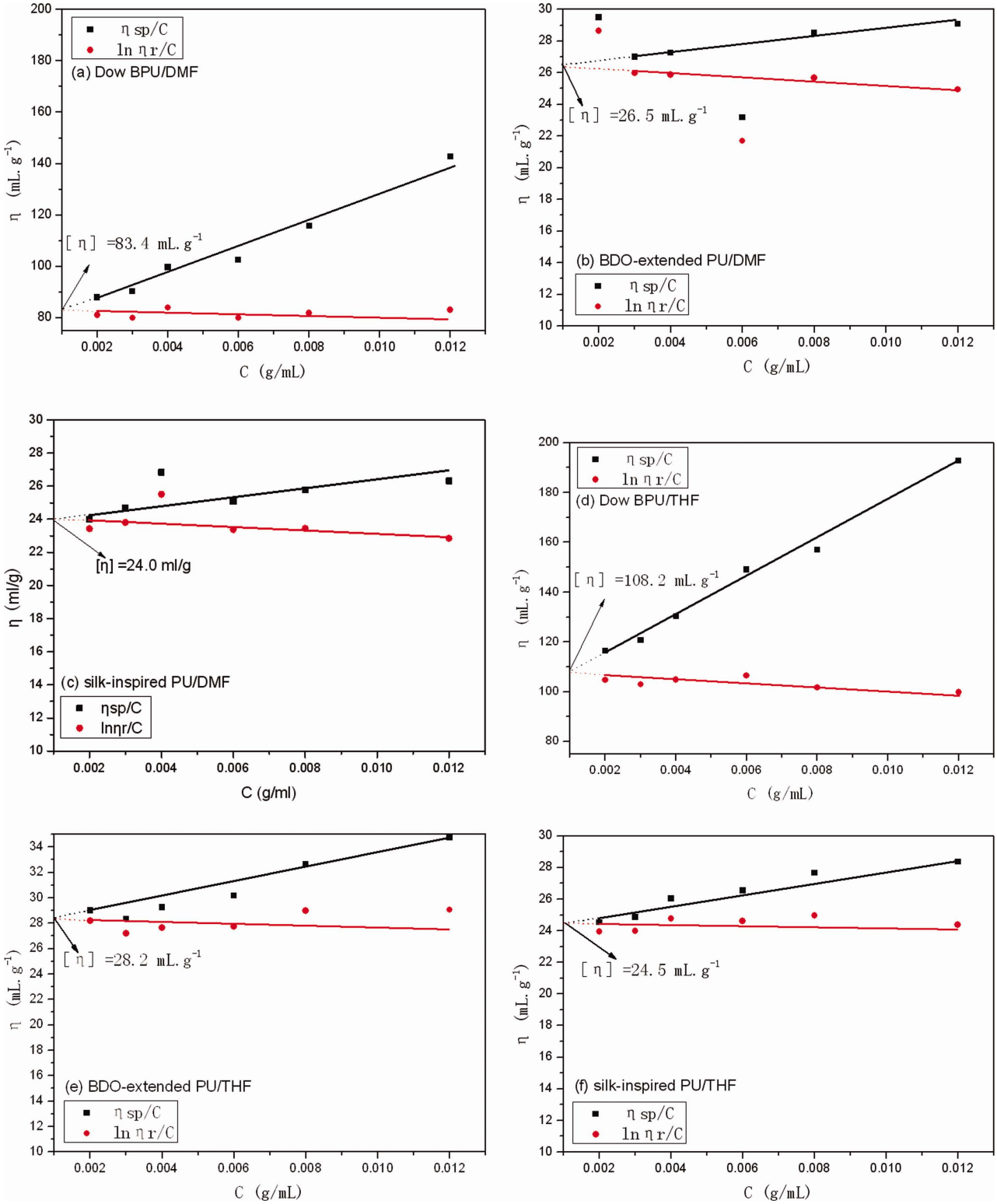

Figure 2. Plots of η_{sp}/c and $\ln\eta_r/c$ against the concentration for varying PU solutions in *N,N′*-dimethylformamide (DMF) and in Tetrahydrofuran (THF): (a) Dow BPU/DMF; (b) BDO-extended PU/DMF; (c) silk-inspired PU/DMF; (d) Dow BPU/THF; (e) BDO-extended PU/THF; (f) silk-inspired PU/THF. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

GlyAlaGlyAla tetrapeptide leads to the formation of β-sheet elements in the triblock copolymer, secondary structure analysis had to be performed. One technique which is simple, reliable, and not influenced by the presence of the polymer backbone is FTIR spectroscopy, which was therefore used for the structure analysis of the prepared BDO-extended PU and silk-inspired

Table I. Molecular Weights and Intrinsic Viscosities of the PU Samples Estimated by GPC and Ubbelohde Viscometer

PU sample	M_n(GPC) (Daltons)	M_w(GPC) (Daltons)	Polydispersity	$[\eta]_{DMF}$ (mL/g)	M_η(DMF)	$[\eta]_{THF}$ (mL/g)
Dow BPU	97,934	194,513	1.986	83.4	56783	108.2
BDO-extended PU	14,838	29,097	2.150	26.5	14972	28.2
silk-inspired PU	13,534	27,921	1.882	24.0	13346	24.5

PU, and the Amides I and II values were compared to values reported in the literature for β-sheet structures.

FTIR absorption band of Amide I in BDO-extended PU is at 1672 cm^{-1}, while that of the silk-inspired PU is at 1651 cm^{-1}. FTIR absorbance band of Amide II in BDO-extended PU is at 1534 cm^{-1}, while that of the silk-inspired PU is at 1534 cm^{-1}. The data are very similar to those of random coil and antiparallel β-sheet of silk fibroin.

Tentative band assignments in the partial Raman spectra of PU are made from reference to earlier Raman studies and are listed in Table IV.[41] Raman spectra for the synthesized silk-inspired PU containing GAGA tetrapeptide links and the comparable BDO-extended PU shows that the band assignments includes the C=C stretching (ν_{Ar}) near 1615 cm^{-1}, the symmetric N=C=O stretching at 1434 cm^{-1} and 1487 cm^{-1}, the C—H bending ($\delta_{C\text{-}H}$) of urethane Amide III near 1302 cm^{-1}, and the urethane amide near 1181 cm^{-1}. The data are consistent with the tentative Raman band assignments for the comparable BDO-extended polyurethane.[41]

The research of Raman spectra mainly focuses on the Amide I region (1650−1680 cm^{-1}) and Amide III region (1230−1300 cm^{-1}).[42] The absorption peak of Amide I consists of the carbon stretching vibration with small contributions from the C—N—H in-plane bending and the C—N stretching vibrations. The 1645–1660 cm^{-1} component has been assigned to the α-helical conformation; the 1665–1680 cm^{-1} component has been assigned to the β-pleated-sheet conformation. Raman absorption band of amide III in BDO-extended PU is at 1312 cm^{-1}, while that of the silk-inspired PU is at 1302 cm^{-1}.

UV-Vis Absorption Spectra

The UV-Vis absorption spectra of the PU samples (Dow BPU, BDO-extended PU, and silk-inspired PU) are shown in Figure 6. Absorption peaks observed around 265 nm are attributed to the π-π^* transition of the localized benzenoid ring.[43,44] From the absorption spectra, it is concluded that there is no significant difference in the three PUs of this study. The absorption peak of BDO-extended PU (265 nm) exhibited a slight red shift in wavelength compared with that of Dow BPU (263 nm), while

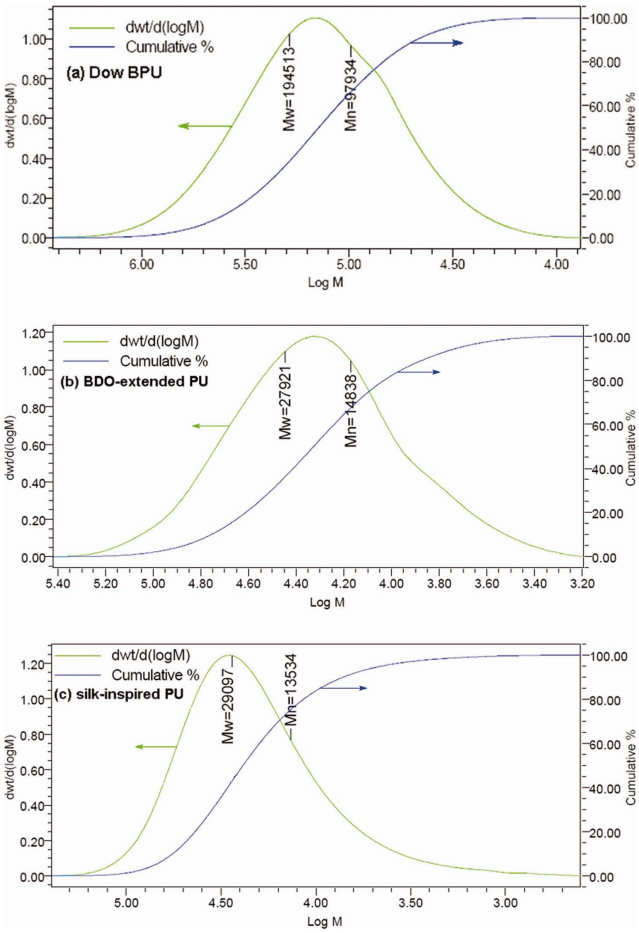

Figure 3. Gel permeation chromatography (GPC) analysis of : (a) Dow BPU; (b) BDO-extended PU; (c) silk-inspired PU. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

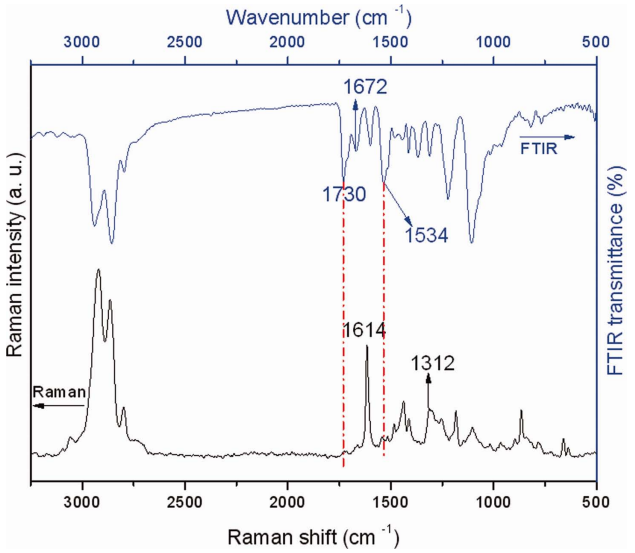

Figure 4. FTIR and Raman spectroscopy of BDO-extended PU. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

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Table II. Bands Assigned to FTIR Spectroscopy of PU[38,39]

Wavenumber (cm^{-1})	Assignment
3310	Strong vs. (N—H) bonded N—H
2930	Strong vs. (CH_2)
2860	Strong vs. (CH_2)
1730	Very strong free C=O
1702	Very strong bonded C=O
1600	Strong vs. (C=C)
1530	Very strong δ (N—H) + ν (C—N)
1410	Strong vs. (C—C) in benzene ring
1310	Strong δ (N—H) + ν (C—N), β (C—H)
1230	Strong δ (N—H) + ν (C—N)
1110	Very strong vs. (CH_2—O—CH_2) of aliphatic ether
1020	Weak β (C—H) in benzene ring
818	Weak γ (C—H) in benzene ring

Table III. FTIR Values (cm^{-1}) of Amides Obtained for BDO-Extended PU, Silk-Inspired PU and Controls

Compound	Amide I	Amide II
Random coil of silk[a]	1656	1535
Antiparallel β-sheet of silk[a]	1632 s/1685 w	1530
BDO-extended PU	1672	1534
Silk-inspired PU	1651	1534

[a]Standard FTIR values for the amide I and II bands obtained from Ref. 40

the absorption peak of silk-inspired PU (266 nm) exhibited a farther slight red shift in wavelength compared with that of BDO-extended PU (265 nm). The detailed chemical structures of the tested PU samples have been depicted in the previous report,[32] in which there is no conjugated system other than benzenoid ring. Therefore, UV-Vis absorption peaks of the three PUs are very contiguous. However, the intensity at absorption peaks (λ_{max}) gradually increases in the order of Dow BPU, BDO-extended PU, and silk-inspired PU. Compared with

Table IV. Band Assignments in the Partial Raman Spectra of PU Ref. 41

Raman shift (cm^{-1})	Assignment
2275	$\nu_{assym.}$ (N=C=O)
1732	Ester ν (C=O), urethane amide I ν (C=O)
1612	ν (Ar)
1530	ν (Ar), urethane amide II: ν (C—N) + δ (N—H)
1445	$\nu_{sym.}$ (N=C=O), δ (CH_2)
1303	δ (CH), urethane amide III
1251	Urethane amide III
1185	Urethane amide

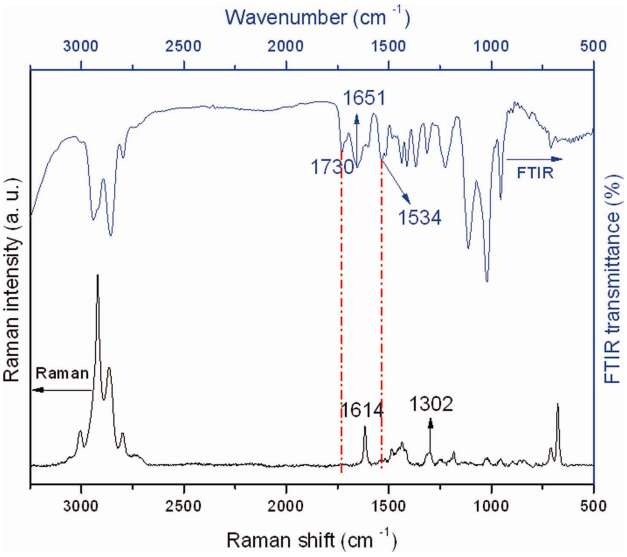

Figure 5. FTIR and Raman spectroscopy of silk-inspired PU. [Color figure can be viewed in the online issue, which is available at wileyonline library.com.]

absorption peak of BDO-extended PU, that of silk-inspired PU increases in the intensity, accompanied by a tiny bathochromic shift from 265 nm to 266 nm. This may be due to the auxochrome (urea). On the contrary, compared with absorption peak of BDO-extended PU, that of Dow BPU decreases in the intensity, accompanied by a tiny hypsochromic shift from 265 to 263 nm. This can be attributed to the shield of benzenoid ring by more urethane groups in Dow BPU than that in BDO-extended PU.

CONCLUSIONS

This article presents experimental results of physical and microstructural studies of silk-inspired polyurethane containing GlyAlaGlyAla tetrapeptide. The silk-inspired polyurethane has been

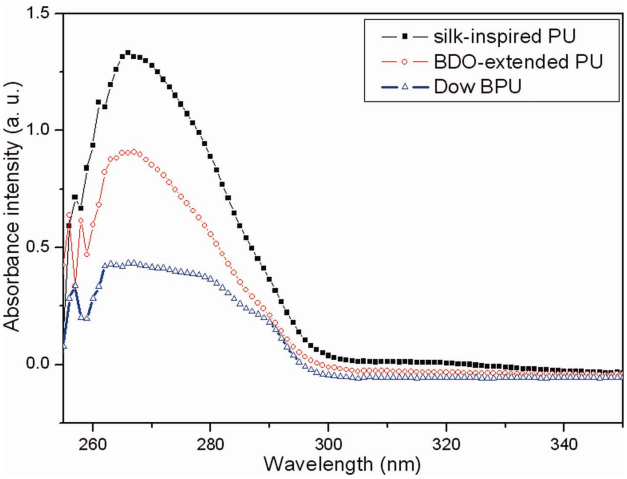

Figure 6. UV-Visible absorption spectra of silk-inspired PU, BDO-extended PU and Dow BPU in dimethylformamide solution at the concentration of 0.01% (m/v). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

synthesized via introducing a chain extender containing GlyAla-GlyAla tetrapeptide into the backbone of traditional polyurethane to form the hard segment with urea-linkage. It is soluble in a variety of solvents facilitating its processing into fibers, films, and gels that are potentially suitable for various biomedical applications. This new concept and strategy may allow the fabrication of a new class of thermoplastic polyurethane elastomers to mimic the structure and properties of silk fibroin of silkworms and spiders. Information provided by this study may be used to better understand the correlation between the natural and man-made materials.

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REFERENCES

1. Christenson, E. M.; Anderson, J. M.; Hiltner, A. *Corros. Eng. Sci. Technol.* **2007**, *42*, 312.
2. Vepari, C.; Kaplan, D. L. *Prog. Polym. Sci.* **2007**, *32*, 991.
3. Raslan, W. M.; Bendak, A. *J. Appl. Polym. Sci.* **2005**, *98*, 1829.
4. Okamoto, M.; Kajiwara, K. *Text. Prog.* **1997**, *27*, 1.
5. Ayutsede, J.; Gandhi, M.; Sukigara, S.; Micklus, M.; Chen, H. -E.; Ko, F. *Polymer* **2005**, *46*, 1625.
6. Marsano, E.; Corsini, P.; Arosio, C.; Boschi, A.; Mormino, M.; Freddi, G. *Int. J. Biol. Macromol.* **2005**, *37*, 179.
7. Phillips, D. M.; Drummy, L. F.; Naik, R. R.; Long, H. C. D.; Fox, D. M.; Trulove, P. C.; Mantz, R. A. *J. Mater. Chem.* **2005**, *15*, 4206.
8. Um, I. C.; Kweon, H. Y.; Lee, K. G.; Ihm, D. W.; Lee, J. -H.; Park, Y. H. *Int. J. Biol. Macromol.* **2004**, *34*, 89.
9. Panitch, A.; Matsuki, K.; Cantor, E. J.; Cooper, S. J.; Atkins, E. D. T.; Fournier, M. J.; Mason, T. L.; Tirrell, D. A. *Macromolecules* **1997**, *30*, 42.
10. Werten, M. W. T.; Moers, A. P. H. A.; Vong, T.; Zuilhof, H.; van Hest, J. C. M.; de Wolf, F. A. *Biomacromolecules* **2008**, *9*, 1705.
11. Wang, J. -N.; Yan, S. -Q.; Lu, C. -D.; Bai, L. *Mater. Sci. Eng. C* **2009**, *29*, 1321.
12. Hachisu, M.; Ohkawa, K.; Yamamoto, H. *Macromol. Biosci.* **2003**, *3*, 92.
13. Takahashi, Y.; Hachisu, M.; Ohkawa, K.; Yamamoto, H. *Macromol. Rapid Commun.* **2002**, *23*, 540.
14. Yang, M.; Kawamura, J.; Zhu, Z.; Yamauchi, K.; Asakura, T. *Polymer* **2009**, *50*, 117.
15. Yao, J.; Asakura, T. *J. Biochem.* **2003**, *133*, 147.
16. Nagano, A.; Kikuchi, Y.; Sato, H.; Nakazawa, Y.; Asakura, T. *Macromolecules* **2009**, *42*, 8950.
17. Hardy, J. G.; Scheibel, T. R. *Biochem. Soc. Trans.* **2009**, *37*(Part 4), 677.
18. Shao, Z.; Vollrath, F. *Nature* **2002**, *418*, 741.
19. Hayashi, C. Y.; Lewis, R. V. *Science* **2000**, *287*, 1477.
20. Krishna, O. D.; Kiick, K. L. *Biopolymers. ((Pept. Sci.))* **2010**, *94*, 32.
21. Rathore, O.; Winningham, M. J.; Sogah, D. Y. *J. Polym. Sci. Part A: Polym. Chem.* **2000**, *38*, 352.
22. Rathore, O.; Sogah, D. Y. *Macromolecules* **2001**, *34*, 1477.
23. Rathore, O.; Sogah, D. Y. *J. Am. Chem. Soc.* **2001**, *123*, 5231.
24. Zhou, C.; Leng, B.; Yao, J.; Qian, J.; Chen, X.; Zhou, P.; Knight, D. P.; Shao Z. *Biomacromolecules* **2006**, *7*, 2415.
25. Yao, J.; Xiao, D.; Chen, X.; Zhou, P.; Yu, T.; Shao, Z. *Macromolecules* **2003**, *36*, 7508.
26. Takahara, A.; Hadano, M.; Yamaguchi, T.; Otsuka, H.; Kidoaki, S.; Matsuda, T. *Macromol. Symp.* **2005**, *224*, 207.
27. Johnson, J. C.; Wanasekara, N. D.; Korley, L. T. J. *Biomacromolecules* **2012**, *13*, 1279.
28. Korley, L. T. J.; Pate, B. D.; Thomas, E. L.; Hammond, P. T. *Polymer* **2006**, *47*, 3073.
29. Koc, Y.; Hammond, P. T.; Lendl, B.; Gregoriou, V. G. *Macromol. Symp.* **2004**, *205*, 191.
30. Fernández-d'Arlas, B.; Ramos, J. A.; Saralegi, A.; Corcuera, M.; Mondragon, I.; Eceiza, A. *Macromolecules* **2012**, *45*, 3436.
31. Liu, H.; Xu, W.; Zou, H.; Ke, G.; Li, W.; Ouyang, C. *Mater. Lett.* **2008**, *62*, 1949.
32. Liu, H.; Xu, W.; Zhao, S.; Huang, J.; Yang, H.; Wang, Y.; Ouyang, C. *J. Appl. Polym. Sci.* **2010**, *117*, 235.
33. Hsu, S. -H.; Tseng, H. -J. *J. Biomater. Appl.* **2004**, *19*, 135.
34. Gorna, K.; Gogolewski, S. *Polym. Degrad. Stabil.* **2003**, *79*, 475.
35. Žagar, E.; Žigon, M. *Polymer* **1999**, *40*, 2727.
36. Bagheri, M.; Pourmoazzen, Z. *React. Funct. Polym.* **2008**, *68*, 507.
37. Žagar, E.; Žigon, M. *Polymer* **2000**, *41*, 3513.
38. Zhang, S.; Ren, Z.; He, S.; Zhu, Y.; Zhu, C. *Spectrochim.ica Acta Part A: Mol. Biomol. Spectrosc.* **2007**, *66*, 188.
39. Khan, A. S.; Ahmed, Z.; Edirisinghe, M. J.; Wong, F. S. L.; Rehman, I. U. *Acta Biomater.* **2008**, *4*, 1275.
40. Ayres, L.; Adams, P. H. H. M.; Löwik, D. W. P. M.; van Hest, J. C. M. *Biomacromolecules* **2005**, *6*, 825.
41. Parnell, S.; Min, K.; Cakmak, M. *Polymer* **2003**, *44*, 5137.
42. Zhou, A. J.; Liu, H. L.; Yu, W. D.; Carr, C. M. *J. Mol. Struct.* **2012**, *1030*, 40.
43. Raghu, A. V.; Gadaginamath, G. S.; Priya, M.; Seema, P.; Jeong, H. M.; Aminabhavi, T. M. *J. Appl. Polym. Sci.* **2008**, *110*, 2315.
44. Qiu, F.; Zhang, W.; Yang, D.; Zhao, M.; Cao, G.; Li, P. *J. Appl. Polym. Sci.* **2010**, *115*, 146.