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Synthesis of Conductive Cocoon Silk Composites

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Abstract

Herein we report on the preparation of polyaniline (PANI) composite using a cocoon by in situ chemical oxidative polymerisation. The composites thus prepared were characterised by scanning electron microscopy (SEM), thermogravimetric analysis (TGA), infrared (IR), and electron spin resonance (ESR) spectroscopy. We confirmed the PANI/cocoon composite and subsequent carbonisation allowed the formation of micro-sized wafer-shaped carbon. The polymerisation reaction occurs in a chiral reaction field of the silk surface, which can be referred to as bio-interface polymerisation.

Key words: electron spin resonance, polyaniline, secondary doping, silk.

The silk worm, Bombyx mori, cocoon looks like a composite shell. It is composed of a single continuous silk thread, whose length is approximately 1000-1500 m [14]. Also the cocoon is known as a three-dimensional nonwoven structure with multiple layers [15]. Cocoon filament is composed of two silk proteins: sericin and fibroin. The cocoon fil-
ament includes sericin (20-30 wt%) and fibroin (70-80 wt%) [14,16]. The sericin envelopes fibroin threads to glue them together and contains many hydrophilic amino acids, including serine (ca. 35%) [17,18], which brings it high hydrophilicity and sensitivity to chemical modifications [19,20]. Recently sericin biocompatibility and affinity to human skin and hair have attracted attention and been investigated for applications such as biomaterials and cosmetics [18,21-23]. Fibroin is a raw material of silk [16]. Silk fibroin has a unique sheen, hydrophilicity, strength, and its toughness is up to ten times greater than that of artificial fibres [24-28]. Due to their unique properties, silk fibroin has been applied as a membrane for enzyme immobilisation, as well as in non-woven fabric and cell scaffold material [29-31]. Besides this, it is reported that natural silk secretions after leaving the gland firstly become liquid crystal and solidify into a fibre, promoting the whole molecular alignment in the fibre [24,32]. Previously we carried out the polymerisation of aniline in the presence of sericin showing chiroptical activity [42]. The combination of PANI and cocoon or fibroin fibre can be expected to provide a unique structure and properties. However, there are only few reports on the PANI/cocoon or PANI/ Fibroin composite. We attempted not only to obtain conductive fibre but also change the morphology and improve the performance by using a natural fibre as a bio-template. Here, we examine its properties, morphology, and discuss the polymerisation mechanism of aniline monomer on the surface of cocoon filament. The synthesis of pure PANI and composites were carried out with ammonium persulfate (APS) as an oxidiser for a polymerisation reaction, as shown in Figure 1.

Aniline was purchased from Wako Chemical Ltd (Japan), and purified by distillation prior to use. (+)-10-camphorsulfonic acid (Tokyo Chemical Industry, TCI, Japan), and sulfuric acid (Wako, Japan), APS (Yoneyama Chemical Industry Co., Ltd., Japan) were used as received. Cocoon as natural material produced by a silk worm was employed.

Synthesis of pure PANI as reference

Pure PANIs as references for the composites were prepared with a general method for the synthesis of PANI (Figure 1). Distilled aniline, (+)-10-camphorsulfonic acid [42] (or sulfuric acid), and water were stirred in a vial. The mixture was cooled to 0°C and stirred for 1 h. Then ammonium persulfate (APS) was added to the mixture. After 24 h, the dark green precipitate was washed in a large amount of methanol, water, and then filtered. The polymerisation condition, recipe, and abbreviations of the results are
summarised in Table 1. The PANI thus prepared, abbreviated as PC (polyaniline/camphor sulfonic acid) and PS (polyaniline/sulfuric acid), were dried under a vacuum to obtain a black powder.

## Synthesis of PANI/cocoon silk

Aniline as a monomer, (+)-10-camphorsulfonic acid (or sulfuric acid), and a cocoon were stirred in water in a small vial. The mixture was stirred for 1 h at 0°C. Then APS in a small volume of water was added to the mixture to initiate polymerisation. After 24 h, the dark green precipitate was washed in a large volume of methanol and water, followed by filtration. The product (abbreviated as CPC and CPS, CP = cocoon polymer, C = camphor sulfonic acid, S = sulfuric acid, Table 1) was dried under a vacuum to obtain a black powder.

## Refining of cocoon to obtain fiber

A water solution containing a cocoon was stirred in a flask for 40 minutes at 50°C. Then sodium hydrogen carbonate was added to the flask, and the mixture stirred at 90°C. After 1 h, the crude product was washed in a large volume of water at room temperature to obtain fibroin.

## Synthesis of PANI/fibroin

Distilled aniline, (+)-10-camphorsulfonic acid (or sulfuric acid), fibroin and water were stirred in a vial. The mixture was cooled to 0°C and stirred for 1 h. APS in a minimum amount of water was added dropwise to the mixture. After 24 h, the dark green precipitate was washed in a large volume of methanol and water, followed by filtration. The product (abbreviated as FPC and FPS, FP = fibroin polymer, C = camphor sulfonic acid, S = sulfuric acid) was dried under a vacuum to obtain a black powder.

## IR spectroscopy measurements

FTIR measurements of the samples, fibroin and PS, CPC, CPS, FPC and FPS of cocoon were carried out, to confirm chemical structures (Figure 2). Absorption bands originated from chemical structure of polyaniline are observed at 1562 cm⁻¹ (quinoid (Q) C = C stretching), 1475 cm⁻¹ (benzenoid (B) C = C stretching), 1300 cm⁻¹ (νBBB C = N stretching), 1240 cm⁻¹ (νC-C-N stretching) and 1121 cm⁻¹ (νC-C = N stretching) [9]. On the other hand, absorption bands corresponding to protein, amide I, II and III are observed at 1639, 1520, and 1235 cm⁻¹, respectively [33,34]. Amide I absorption represents C = O stretching of the amide group. Amide II absorption arises from N-H bending coupled to C-N stretching. Amide III contains absorptions contributed from C-N stretching and N-H in-plane bending vibration. All composites, CPC, CPS, FPC, and FPS contain absorption bands at 1639 cm⁻¹ (amide I), 1300 cm⁻¹ (C=N stretching) and 1121 cm⁻¹ (νC-C = N stretching) [9]. This result confirms formation of PANI/cocoon and PANI/fibroin composites. Here, all composites except for FPS have the IR bands at 1639 and 1520 cm⁻¹ (amide I and II) with intense absorption compared with those at 1562 and 1475 cm⁻¹ (quinoid (Q) C = C stretching and benzenoid (B) C = C stretching).

## Electrochemical property of composites

In-situ ESR measurements for the cocoon, fibroin, PS, CPC, CPS, FPC and FPS were performed (Figure 3). As prepared, PANI is in a half-protonated state (so-called emeraldine salt). Therefore as prepared, PANIs have a charge running along the backbone and can show electrical conductivity. Pure PANI and all composites (Figure 3.c-h) show

### Table 1. Preparation of PANI/Cocoon & PANI/Fibroin.

| Entry | Aniline, g | Acid       | Cocoon, g | Fibroin, g | Product, g | Recovered amount, g | Recovered amount, wt% |
|-------|------------|------------|-----------|------------|------------|----------------------|-----------------------|
| CPC   | 2.0        | (+)-CSA    | 0.2       | –          | 0.24       | 0.04                 | 16.7                  |
| CPS   | 2.0        | H₂SO₄      | 0.38      | –          | 0.43       | 0.05                 | 11.6                  |
| FPC   | 0.2        | (+)-CSA    | –         | 0.034      | 0.04       | 0.006                | 15.8                  |
| FPS   | 0.2        | H₂SO₄      | –         | 0.023      | 0.04       | 0.017                | 42.5                  |

![Figure 2. Fourier transform infrared (FTIR) spectra of (a) cocoon, (b) fibroin, (c) PS, (d) CPC, (e) CPS, (f) FPC and (g) FPS.](image)

![Figure 3. Electron spin resonance (ESR) spectra of (a) cocoon, (b) fibroin, (c) PS, (d) CPC, (e) CPS, (f) FPC and (g) FPS.](image)
ESR signals, indicating unpaired electrons in these polymers. The $g$-value, the peak-to-peak magnetic field (Δ$H_{pp}$), spin concentration and electrical conductivity of the all samples are summarised in Table 2. The $g$-value can be obtained by the following formula:

$$g = \frac{h \nu}{\beta H_0}$$

where $h$ is the Plank constant ($6.63 \times 10^{-34}$ m²·kg/s), $\nu$ the resonance microwave frequency in the measurement, $\beta$ the Bohr magneton ($9.27 \times 10^{-23}$ J·T⁻¹), and $H_0$ is the resonance magnetic field. The inflection point of the ESR signal corresponds to the resonance magnetic field. The $g$-Value of the free electron is to be 2.0023. The peak-to-peak magnetic field range corresponds to Δ$H_{pp}$ values in the ESR. The $N_s$ (spin concentration) of the samples were obtained from integration of the signal compared against that of the CuSO₄ standard. The peak to peak line-width of CPC, CPS, FPC and FPS are sharpened compared with pure PANI in the case of using the same acid, implying delocalisation of the charge species [36]. The apparent spin concentration and electrical conductivity of CPC, CPS, FPC and FPS are less than those of pure PANI. However, the composite contains silk with no spin. Therefore PANI constituent in the composite is low compared with pure aniline, and the spins derived from the PANI in the entire composite can be higher than those of pure PANI. The main-chain of PANI in the composite forms a linear shape, such as an expanded coil [37] because of interaction between the main-chain of the PANI and cocoon filament or silk fibre. On the other hand, the silk fibre in the composite between the conductive main-chains can be an insulating wall for inter-chain hopping transportation of electrons in the main-chain. Therefore the composite resulted in lower electrical conductivity compared to pure PANI.

### Surface morphology of composites

The cocoon has a multi-layer structure and its surface forms a 3-D network (Figure 4.a) [15]. SEM images of the cross section of CPC and that of CPS are shown in Figure 5.a-d. Pure PANI shows a bulky structure, as shown in Figure 4.b [9]. Both PANI/cocoon samples show a surface coating of the PANI on the cocoon filament (Figure 4.c,d and Figure 4.a,c). SEM images of fibroin, PS, CPC and FPS are shown in Figure 4.

**Table 2. ESR results and electrical conductivity.**

| Polymer | $g$-value | $\Delta H_{pp}$ mT | $N_s$, spins/g | $\sigma$, S/cm | $N_s'$, spins/g |
|---------|-----------|-------------------|---------------|---------------|----------------|
| PS      | 2.00399   | 0.410             | 1.41×10⁰⁶    | 3.52          | 1.41×10²⁰      |
| CPC     | 2.00410   | 0.410             | 4.08×10¹⁵    | 2.24×10⁻²    | 2.45×10²⁰      |
| CPS     | 2.00407   | 0.381             | 6.00×10¹⁵    | 0.52          | 5.70×10²⁰      |
| FPC     | 2.00410   | 0.410             | 5.00×10¹⁵    | 3.52×10⁻⁴    | 3.16×10²⁰      |
| FPS     | 2.00411   | 0.381             | 9.25×10¹⁵    | 1.88×10⁻²    | 2.18×10²⁰      |

* $g$-value can be obtained from $g = h \nu / \beta H_0$. $h$ = Plank constant ($6.63 \times 10^{-34}$ m²·kg/s), $\nu$ = resonance microwave frequency in the measurement. $\beta$ = Bohr magneton ($9.27 \times 10^{-23}$ J·T⁻¹). $H_0$ = resonance magnetic field (inflection point of the ESR signal).

$\Delta H_{pp}$ = peak-to-peak line width.

Spin numbers of the sample.

$\sigma$ = Electrical conductivity.

$N_s$ calculated from PANI mass only.

![Figure 4. Scanning electron microscopy (SEM) images of cocoon (a), PS (b), CPC (c) and CPS (d).](image)

![Figure 5. SEM images of the cross section of CPC (a) (b) and that of CPS (c) (d).](image)
Figure 6. PANI on the cocoon surface forms a small bulk structure whose diameter is ca. 200 nm (Figure 6.d). Both PANI/fibroin composites also show PANI deposition on the fibre (Figure 6.c-f).

**Thermal property of composites**

The thermal behaviour of the cocoon, PC (pure PANI-CSA) and CPC was examined by thermogravimetric (TG) and differential thermogravimetry (DTA) analyses. All TG curves are shown in a temperature range of 120-900°C. As shown in Figure 7, the TG curve of pure PANI showed two main steps of weight loss: the first at 170°C due to the removal of dopant CSA and discharge of oligomers, and the second in the temperature range of 310-610°C corresponds to structural decomposition of the PANI main-chain [38]. The TG curve of the pure cocoon filaments showed a two-stage weight loss profile, an initial weight loss at 250°C due to the removal of molecular fragments such as O-H CH\(_2\)-OH groups, and a second weight loss at 380-580°C due to the decomposition of the protein backbone [39]. The TG curve of the PANI/cocoon showed a four-stage weight loss profile. The initial weight loss at 170-250°C is due to the removal of dopant CSA and the discharge or decomposition of residual oligomers in the sample. The second and third weight loss in the range of 250-350°C and 350-450°C are due to the decomposition of the cocoon filament. The last weight loss at 450-700°C is attributed to the structural decomposition of PANI. The samples show a weight loss of 62.4-72.9%, except for the release of water (cocoon – 73.3%; PC – 62.4%; CPC – 72.9%).

**Morphology of carbonised samples**

Scanning electron microscopy (SEM) images of the carbonised cocoon, PC, CPC and CPS were obtained (Figure 8, see page 18). Carbonisation of the composite was conducted at 900°C under an Ar gas flow. Carbon from the pure polyaniline forms the granular structure. Carbons prepared from the composites show fibres (Figure 8.a,d) and are wafer-shaped (Figure 8.d). These characteristic shapes were derived from the decomposition steps of the cocoon filament with PANI. Especially a carbon micro-sized wafer, as seen in Figure 8.d, could be created by aggregation in the process of the carbonisation.
Possible mechanism for the formation of composites

In the polymerisation, aniline molecules were absorbed and permeated into the surface of the cocoon filament [40]. Then aniline formed aniline salt by the addition of (+)-10-camphorsulfonic acid or sulfuric acid. The aniline molecules are positioned on the filament due to the intermolecular hydrogen bonding of N-H⋯O pairs or electrostatic interaction of N′-H⋯O⋯H between aniline molecules and the cocoon main-chain (Figure 9) [41]. After the addition of APS to initiate a polymerisation reaction, the propagation process occurs along the formation of PANI-emeraldine salt. The main-chain is expanded along the protein main-chain guide because of intermolecular interactions. Then the polymer on the cocoon filament formed a linear-like expanded coil. The process improves carrier mobility in the main-chain of PANI, electrical conductivity, and co-planarity of adjacent units in PANI to extend the π-conjugated system.

Conclusions

PANI/cocoon and PANI/fibroin composites were prepared through chemical oxidative polymerisation. PANI/silk cocoon composite and subsequent carbonisation allow the formation of micro-sized wafer-shaped carbon. The interfacial polymerisation of natural silk fibre as a reaction field was performed. The SEM observation reveals polyaniline deposited on the silk fibres. Moreover IR spectroscopy measurements confirm the chemical structure of the resultant composites. Especially absorption bands due to the quinoid and benzenoid structures of the doped state of the PANI and amide bands derived from silk protein were confirmed. Radical cation in the composites as the charge species polarons was detected by using the ESR spectroscopy. Spin concentrations of the composites are in the range of \(4.08 \times 10^{-19} - 9.25 \times 10^{-19}\) spin/g. Carbonisation allowed the formation of a micro-sized wafer structure. The natural fibres have flexibility and strength, and the combination of which with synthetic conducting polymer can produce new fibre materials having both the advantages of natural fibres and synthetic metals. Carbonisation of the composite allows the formation of a unique structure derived from the original natural fibres.

Figure 8. SEM images of carbonised cocoon (a), carbonised PC (b), carbonised CPC (c) and carbonised CPS (d).

Figure 9. Plausible forms and polymerisation mechanism on the cocoon fibre for obtaining linear polyaniline.

Editorial note

SEM observations were performed with a JEOL JSM-7000F (Japan). The surfaces of shell membrane and composites were sputtered with gold by a JEOL JFC-1500 (Japan) prior to observations of the surface structure with SEM. IR absorption spectroscopy measurements were carried out using a Thermo Scientific NICOLET iS5 (USA) with the KBr method. ESR measurements were carried out by a Bruker EMX-T ESR spectrometer (USA). Electrical conductivity was measured by four-probe method using Mitsubishi Chemical Analytech LORES-TA-GP MCP-T610 (Japan). TG and DTG were conducted by a Seiko Instruments Inc. EXSTAR7000 (Japan).
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DRESDEN-BASED SCIENTISTS ESTABLISH WORLD’S LARGEST RESEARCH PLATFORM FOR FIBRE-BASED HIGH-TECH MATERIALS

An interdisciplinary team consisting of 500 scientists, engineers and technicians from five Dresden-based research institutes is set to jointly tackle a range of topical issues in the fields of fibre research and textiles technology.

The Research Centre for High-Performance Fibres, Structures and Textile Machine Development – or “HP Fibre Structures” – bundles the unique knowledge and expertise Dresden possesses in its role as an outstanding location for research into high-performance fibre- and textile-based materials. The aim of HP Fibre Structures is to develop additive-generatively manufactured, function-integrating textile concepts which can be realized using bespoke or series-ready manufacturing techniques depending on the application involved.

The Dresden-based research centre will blaze a trail for technologies which facilitate research, development and the transfer of results in the fields of materials science, multi-material design, lightweight engineering, sensor/actuator technology and mechanical engineering. Over the next few years, participating scientists aim to design and implement continuous research chains which extend from basic and applied research to the realization of competitive products. The potential offered by near-net-shape materials and structures based on bionic principles in the aforementioned fields is huge, with applications already identified in sectors such as energy and mobility, lightweight engineering, healthcare, architecture/construction and digital communication.

HP Fibre Structures will officially be founded by the Institute of Textile Machinery and High Performance Material Technology (ITM), the Institute of Lightweight Engineering and Polymer Technology (ILK, both TU Dresden), the Leibniz Institute of Polymer Research Dresden (IPF), the Fraunhofer Institute for Material and Beam Technology Dresden (IWS) and the Fraunhofer Institute for Ceramic Technologies and Systems (IKTS) on October 28, 2016. Prof. Hans Müller-Steinhagen, Rector of TU Dresden, welcomes the new initiative: “All of the institutes involved in this new research centre form part of either TU Dresden or the DRESDEN concept network. This highlights once again that the DRESDEN concept has developed into an extremely dynamic scientific network. In particular, it demonstrates that intensive communication and the resultant trust between network members provides a platform for cooperation and research of the highest order.”

The founding of HP Fibre Structures means than Dresden is now home to the world’s largest platform for research into novel, multi-scale, configurable fibre-based material systems for high-tech applications. The heads of the participating organizations – Prof. Brigitte Voit (IPF), Prof. Chokri Cherif (ITM), Prof. Hubert Jäger (ILK), Prof. Eckhard Beyer (IWS) and Prof. Alexander Michaelis (IKTS) – eagerly anticipate this new opportunity to engage in large-scale joint research projects.

The overall portfolio of services offered by the participating research institutes ranges from project planning, design, drive and control technology, material development, the simulation of structures and processes, technology development, measuring technology, material characterization and prototype manufacturing to the development of robust processes, structural testing and recycling.

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