

Status: Currently Official on 16-Feb-2025
 Official Date: Official as of 01-May-2018
 Document Type: USP Monographs
 DocId: GUID-86B7F887-B199-430D-A403-5AF285E1792B_4_en-US
 DOI: https://doi.org/10.31003/USPNF_M9194_04_01
 DOI Ref: 03g11

© 2025 USPC
 Do not distribute

Scaffold Silk Fibroin

DEFINITION

Scaffold Silk Fibroin is a surgical scaffold device made from the silk of the *Bombyx Mori* silkworm. Raw silk filaments are comprised of a fibroin protein core that is naturally coated with the globular protein sericin. The sericin is removed from the fiber by aqueous extraction leaving the fibroin protein behind, consisting of layers of anti-parallel beta sheets, which give the fiber strength. The fiber is then assembled into a three dimensional patterned scaffold. The scaffold is a mechanically strong, flexible, single use device that can be produced in a variety of shapes, sizes, and thicknesses, and is terminally sterilized.

IMPURITIES

• NONPOLAR SOLVENT EXTRACTION

Positive control: 20 mg of mineral oil¹

Negative control: 45 mL of *n*-hexane

Sample: Scaffold Silk Fibroin

Analysis: [NOTE—Gloves should be worn throughout the procedure as natural skin oil may affect the results. Glassware preparation: Each sample or control requires one 60-mL bottle and cap and one glass Petri dish. Wash glass Petri dishes (10 cm × 2 cm) and a corresponding number of 60-mL glass jars with lids with liquid detergent and rinse well with tap water. Rinse again with deionized water. Transfer glassware in the chemical hood and rinse all glassware with hexane. Air-dry glassware in the chemical hood for at least 15 min. Transfer dried glassware to a desiccator for NLT 30 min before weighing.] Ensure the relative humidity of the room is between 30% and 80% before weighing or labeling bottles and petri dishes. Label and pre-weigh 60-mL bottles. For the *Positive control*, add 20 mg of mineral oil to a 60-mL bottle and for the *Negative control*, add 45 mL of hexane to a 60-mL bottle. For the *Sample*, use stainless steel tweezers to place one device per 60-mL bottle and cap. Re-weigh bottles and calculate the sample weight (SW) by subtracting the initial mass of the bottle from the mass of the sample-containing bottle. In the chemical hood, open the bottles and add 45 mL of *n*-hexane to each, then re-close making sure that the hexane does not come in contact with the caps. Prepare an ultrasonic water bath for sample processing by degassing it. For this, use the “degas” setting of the equipment for 10 min. Place all 60-mL bottles, prepared as above, in the water bath. Ensure that the water level is 0.5-cm below the lids of the bottles. Sonicate bottles for 5 min on the “sonication” setting (frequency of 40 KHz). Label Petri dishes so that each bottle has a correspondingly labeled Petri dish. Weigh each dish individually and record the mass (m_{initial}). Pour the hexane solution from each bottle into the corresponding pre-labeled Petri dish. Add 15 mL of *n*-hexane to each bottle, rinse then pour in the corresponding Petri dish. Repeat this rinsing step and collect the solution. The final hexane volume in each of the Petri dishes should be approximately 75 mL (45 mL from extraction + 15 mL from first rinse + 15 mL from second rinse). Leave Petri dishes uncovered in the chemical hood for 12 h to evaporate the *n*-hexane. Re-weigh each Petri dish after hexane evaporation and record mass (m_{final}).

Calculate the recovery of the *Positive control* in percentage with the following formula:

$$\text{Result} = [(m_F - m_i)/W_o] \times 100$$

m_F = final mass of Petri dish after *n*-hexane evaporation in mg

m_i = initial mass of empty Petri dish in mg

W_o = weight of oil added, mg

Calculate the concentration of organic residuals, in ppm, in each tested device:

$$\text{Result} = [(m_F - m_i)/W_s] \times F$$

m_F = final mass of Petri dish after *n*-hexane evaporation in mg

m_i = initial mass of empty Petri dish in mg

Typesetting math: 66%

W_s = sample weight, g (calculated by subtracting the initial mass of the bottle from the mass of the sample-containing bottle)

F = conversion factor for kg to g, 1000

System suitability criteria: The determined m_F for the *Negative control* should be NMT 0.2 mg and the *Positive control* recovery should be 95%–105%.

Acceptance criteria: Hexane extractable residues should be NMT 1106 ppm per tested device.

• [ELEMENTAL IMPURITIES—LIMITS \(232\)](#) and [ELEMENTAL IMPURITIES—PROCEDURES \(233\)](#).

Nitric acid solution: 2% (v/v) nitric acid in water

Sample solution: Weigh 3–4 g of Scaffold Silk Fibroin, record the mass, then place in a 50-mL conical tube. Add 25 mL of *Nitric acid solution* to the tube. Incubate for 16 h at room temperature.

Analysis: Transfer 5 mL of the *Sample solution* to a tube that is compatible for testing. The levels of chromium, cadmium, nickel, vanadium, lead, and arsenic are determined by an inductively coupled plasma–atomic emission spectroscopy (ICP-AES) (see [Plasma Spectrochemistry \(730\)](#)) normalized to the mass of the tested device and reported in parts per million (ppm).

Acceptance criteria: Meets the requirements of the Parental Daily Dose PDE ($\mu\text{g/day}$) in [Elemental Impurities—Limits \(232\)](#).

SPECIFIC TESTS

• FIBROIN CONTENT

Digestion solution: 6 N hydrochloric acid

Reconstitution solution: 0.02 N hydrochloric acid

Borate buffer: 0.2 M sodium borate, pH 8.8 with 5 mM calcium disodium ethylenediaminetetraacetic acid (EDTA). Prepare the buffer by dissolving 124 g boric acid in 80 mL of deionized water; add 187 mg calcium disodium EDTA then titrate with 1 N sodium hydroxide to a pH of 8.8. Add deionized water to a final volume of 100 mL.

Derivatization reagent: 10 mM of 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate in acetonitrile. Vortex for 10 s at 2700 rpm. Incubate the solution for 10 min at 55°, and vortex occasionally until the powder dissolves.

Preparation of hydrolysis sample vials and tubes: Clean glass acid hydrolysis sample vials (30 × 120 mm) and hydrolysis tubes (6 × 50 mm) used for sample preparation with a phosphate-free detergent², rinse with purified water then pyrolyze at 220° for 24 h. Allow vials to cool to room temperature then add 200 μL of constant boiling 6 N hydrochloric acid³ to each hydrolysis tube along with a single phenol crystal.⁴ Cap tubes with a polytetrafluoroethyl (PTFE) cap with a chlorotrifluoroethylene (CTFE) screw type on-off valve until needed.

System suitability solution: 25 μM in water from commercially available amino acid standard⁵

Fibroin solution: Bring 2 L of water to a boil in a glass beaker, then add 4.24 g of sodium carbonate. Add 5 g of raw silk yarn⁶ and boil for 1 h. Remove yarn containing fibroin with a spatula and rinse in 2 L of water. Squeeze yarn with gloved hands to remove excess water. Rinse yarn 3 more times in 2 L of water for 20 min under agitation (use a stir bar for agitation and set the stir plate to 250 rpm). Squeeze yarn with gloved hands to remove excess water, place the fibroin yarn on a clean sheet of aluminum foil and dry for 16 h in a chemical hood. Transfer 1 mg of the fibroin yarn to a hydrolysis tube to obtain 5 mg/mL of fibroin in 6 N hydrochloric acid. Prepare three replicates. Incubate at 115° for 16 h. Allow the tubes to cool for 1 h at room temperature. Remove acid with a speed vacuum. Add 20 μL of *Reconstitution solution* to each tube and incubate at 50° for 10 min. Dilute each solution 5-fold (to 0.1 $\mu\text{g}/\mu\text{L}$) with *Reconstitution solution*.

Sericin solution: Add 1 mg of sericin⁷ per hydrolysis tube to obtain 5 mg/mL sericin in 6 N hydrochloric acid. Prepare three replicates. Incubate at 115° for 16 h. Allow the tubes to cool for 1 h at room temperature. Remove acid with a speed vacuum. Add 20 μL of *Reconstitution solution* to each tube and incubate at 50° for 10 min. Dilute each solution 5-fold (to 0.1 $\mu\text{g}/\mu\text{L}$) with *Reconstitution solution*.

Standard solutions: Prepare amino acid standards at the following concentrations: 600, 500, 400, 300, 200, 100, 50, 10, 5, and 1 μM in water from commercially available amino acid standard.⁸

Sample solutions: Add 1 mg of Scaffold Silk Fibroin per hydrolysis tube to obtain 5 mg/mL of Scaffold Silk Fibroin in 6 N hydrochloric acid. Prepare six replicates. Incubate at 115° for 16 h. Allow the tubes to cool for 1 h at room temperature. Remove acid with a speed vacuum. Add 20 μL of *Reconstitution solution* to each tube and incubate at 50° for 10 min. Dilute each solution 5-fold (to 0.1 $\mu\text{g}/\mu\text{L}$) with *Reconstitution solution*.

Amino acid derivatization: *Standard solutions* and reconstituted *Fibroin solution*, *Sericin solution*, and *Sample solutions* are derivatized. *Borate buffer* (70 μL) is added to 12- × 32-mm screw neck total recovery vials (the number of vials needed is equal to the number of Standards, samples, fibroin and sericin solutions, and one blank solution). An aliquot of 10 μL of each amino acid standard (one standard sample for each concentration) and each control (triplicates of *Fibroin solution* and *Sericin solution*) and sample (one sample per finished Scaffold Silk Fibroin) are added to the respective vials. The vials are vortexed for 5 s at 2700 rpm, followed by the addition of 20 μL of *Derivatization reagent*. The vials are capped and vortexed again for 5 s. The vials are then heated for 10 min at 55°. A blank is also prepared using 10 μL of *Reconstitution solution* instead of amino acid standard.

Solution A: Prepare a mixture of acetonitrile, formic acid, and 100 mM ammonium formate (10:6:84). Dilute this mixture with water (9:9).

Typesetting math: 66% acetonitrile and formic acid (98:2)

Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	99.9	0.1
0.54	99.9	0.1
5.74	90.9	9.1
7.74	78.8	21.2
8.04	40.4	59.6
8.64	40.4	59.6
8.73	99.9	0.1
9.5	99.9	0.1

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

- Mode: LC
- Detector: UV 260 nm
- Column: 2.1-mm × 10-cm; L1 with particle size 1.7 μm and pore size 130 Å
- Column temperature: 55°
- Flow rate: 0.7 mL/min
- Injection volume: 1 μL
- Run time: 10 min

System suitability

- Sample: System suitability solution
- Suitability requirements
 - Plate count: NLT 2000 for all the amino acids
 - Tailing factor: NMT 2
 - Resolution: NLT 2
 - Signal-to-noise ratio: NLT 10 for all the amino acids

Analysis

- Samples: Fibroin solution, Sericin solution, Standard solutions, and Sample solutions
- Create a calibration curve for each amino acid by integrating the peak area obtained from the chromatograph for each concentration of Standard solution then plot the integrated peak area as a function of concentration. Run each replicate of Fibroin solution and Sericin solution. Perform one run for each of the six Sample solutions. Determine the concentration of each constituent amino acid of each control solution and Sample solution from the calibration curves of the amino acid standards.
- A mathematical algorithm development software is used to set up a matrix based calculation for fibroin purity.⁹ The algorithm uses 7 out of the 16 detected amino acids: glycine (Gly), alanine (Ala), tyrosine (Tyr), serine (Ser), asparagine (Asx), glutamate/glutamic acid (Glx), and threonine (Thr). [NOTE—The 7 amino acids were selected because the magnitude difference in weight percent of each found in sericin compared to fibroin is at least 2-fold.]
- Convert the fibroin and sericin reference data into a [7 × 2] matrix ([Matrix A](#)), with 7 (columns) representing each of the selected amino acids and 2 (rows) representing the number of Reference Standards analyzed. Represent the amino acid composition of the silk samples being analyzed as a [7 × 6] matrix ([Matrix B](#)), where 7 (columns) represent each of the selected amino acids and 6 (rows) for each of the total number of samples analyzed. Specifically,

Matrix A =
$$\begin{bmatrix} Gly & Ala & Tyr & Ser & Glx & Asx & Thr \\ Gly & Ala & Tyr & Ser & Glx & Asx & Thr \end{bmatrix}$$