

# Recovery of silk fibroin from sericulture waste and formulation of a non-toxic calcium chloride based spinning solution for electrospinning

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## Abstract

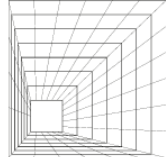
Silk fibroin is among the most attractive natural fibre-forming polymers for biomedical electrospinning, but its use is constrained at both ends of the preparation chain: sericin must be removed without hydrolysing the fibroin, and the solvent system used to dissolve it must leave residues acceptable in a product intended for human contact. This work develops and quantitatively characterises the complete feedstock preparation route from sericulture waste to a spinnable solution. A mildly alkaline degumming bath containing 1.5 g L<sup>-1</sup> of 65% laundry soap and 2.0 g L<sup>-1</sup> of sodium carbonate was applied at a liquor ratio of 40, 100 °C and 30 min; the sodium carbonate concentration corresponds to 0.019 M, which coincides with the 0.02 M protocol widely used internationally, and the theoretical fibroin yield estimated from the initial sericin content is 72–80%. The dissolving system was selected as calcium chloride / water / ethanol on the grounds of residue toxicity rather than dissolving power alone; its molar composition is 1 : 4.52 : 1.51 with a calcium chloride molality of 12.3 mol kg<sup>-1</sup> in the aqueous phase, making it a water-lean, higher ionic strength variant of the classical Ajisawa reagent (1 : 8 : 2). Spinning-solution compositions are expressed in mass fractions for the first time: fibroin 1.79–3.51 wt%, total polymer in the composite solutions 4.63–8.51 wt% and 11.0 wt% in the final formulation. Electrospinning of the pure fibroin solution was unstable and yielded droplet deposits rather than fibres; this is shown to be consistent with a polymer concentration below the entanglement threshold, and the poly(vinyl alcohol) fraction required to exceed that threshold is quantified as a carrier-to-fibroin ratio of 6.8 : 1. A national-scale resource estimate indicates that silk waste is not the limiting factor for the technology.

**Keywords:** silk fibroin; sericulture waste; degumming; calcium chloride; Ajisawa reagent; entanglement concentration; poly(vinyl alcohol); spinning solution; electrospinning

## 1. Introduction

Sericulture and silk processing generate substantial quantities of fibrous waste. On the basis of current norms, 3–3.2 kg of dry selected cocoons are consumed to produce 1 kg of raw silk, so that reeling enterprises generate more than one kilogram of assorted waste per kilogram of raw silk, including more than 0.6 kg of nine types of fibrous waste [1]. At present the bulk of this material is converted to carded silk tow and exported at comparatively low prices, so that most of the added value is created outside the producing country.

Silk fibroin, the principal component of this waste, is one of the most promising fibre-forming polymers for biomedical use. It is non-toxic and biocompatible; its degradation products are amino acids that are metabolised by cells rather than acidic by-products; it withstands sterilisation at up to 150 °C; and, uniquely among the commonly used biopolymers, its degradation rate can be regulated through the  $\beta$ -sheet fraction of its structure without altering the polymer chemistry [2–4]. Electrospun fibroin constructs have been reported for wound dressings, cell substrates, drug delivery and cosmetic films [5, 6].



Two constraints, however, govern whether such a material can move from the laboratory to production. The first is the removal of sericin, the water-soluble adhesive protein that constitutes up to 30% of silk fibre and 20–28% of fibrous waste. Sericin is allergenic and provokes an immune reaction, which restricts the use of sericin-contaminated silk in regenerative medicine; it also binds fibroin molecules together and prevents dissolution. Its removal requires an alkaline medium, but fibroin itself is sensitive to alkali, so the degumming regime must balance two opposing requirements [2, 7].

The second constraint is the dissolving system. Several systems are established — lithium bromide, sodium hydroxide, formic acid, calcium chloride — but most require a subsequent dialysis step to remove the reagent, which is difficult to scale and adds cost [4, 8]. For a product intended for contact with skin or wounds, the residue left by the solvent is therefore as important a selection criterion as its dissolving power.

A third issue is rarely addressed explicitly: the concentration at which fibroin solutions can actually be obtained. Continuous fibre formation from a polymer solution requires a network of macromolecular entanglements, which is established only above a characteristic entanglement concentration; below it the jet breaks into droplets and the process degenerates into electrospraying [9, 10]. Because reported fibroin solution concentrations are low, this constraint is often binding, and it is usually circumvented by blending in a carrier polymer without stating the cost of doing so.

The novelty of the present work lies in treating the whole preparation chain as a single quantitative problem: the degumming regime is related to the internationally used protocol on a molar basis, the dissolving system is characterised through its molar composition and ionic strength and compared with the classical reagent, the resulting spinning solutions are expressed in mass fractions rather than in volumetric proportions, and the carrier fraction required to exceed the entanglement threshold is quantified.

The aim of the work is to develop and characterise quantitatively a complete route from sericulture waste to a spinnable silk fibroin solution whose residues are acceptable in products intended for human contact.

## **2. Materials and methods**

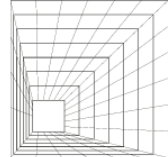
### ***2.1. Raw material and preliminary preparation***

Fibrous waste from cocoon reeling and silk samples with a comparatively low sericin content were used. Preliminary preparation comprised mechanical cleaning of the raw material from foreign matter and pupal residues; release of the cocoon bands from their ties and cutting with disc knives, which permits the waste to be loaded into boiling packets; air maceration of the sorted waste in a chamber at 26–32 °C and 75–80% relative humidity for 12–16 h; and treatment in drum openers with smooth wooden drums, which separates the bulk of the pupal shells from the fibrous mass.

### ***2.2. Degumming***

Sericin removal is possible by three routes: chemical, based on the solubility of sericin in hot alkaline or acidic aqueous solutions; biological, based on enzymatic degradation of sericin and fats; and combined, in which preliminary boiling in soap–soda solutions is followed by biological cleaning. Acidic systems were excluded because they damage fibroin and substantially reduce the strength of the resulting material.

Sericin is acidic in nature and its solubility increases in an alkaline medium, in which the carboxyl groups of the sericin macromolecules dissociate, the negative charge of the protein particles increases and they pass into solution, with partial hydrolysis of peptide bonds proceeding in parallel. Fibroin, however, is also sensitive to alkali: highly concentrated



solutions cause hydrolysis of its peptide bonds and degrade its mechanical properties. The degree of hydrolysis depends on pH, with the safe interval for fibroin being pH 4–8; the boiling liquor was therefore held at pH 9–10, which is sufficient to dissolve sericin without substantially damaging fibroin.

The bath composition and regime are given in Table 1. Expressing the sodium carbonate concentration in molar terms, with  $M(\text{Na}_2\text{CO}_3) = 105.99 \text{ g mol}^{-1}$ ,

$$c(\text{Na}_2\text{CO}_3) = 2.0 / 105.99 = 0.0189 \approx 0.019 \text{ mol L}^{-1}, \quad (1)$$

which coincides in practice with the 0.02 M protocol widely used internationally for degumming *Bombyx mori* silk [4]. The agreement indicates that the selected regime is not merely empirical but rests on a generally accepted physico-chemical basis.

Table 1. Composition and regime of the degumming bath.

| Parameter                           | Value            | Note                         |
|-------------------------------------|------------------|------------------------------|
| 65% laundry soap, $\text{g L}^{-1}$ | 1.5              | surface-active component     |
| Sodium carbonate, $\text{g L}^{-1}$ | 2.0 (0.019 M)    | creates the alkaline medium  |
| Liquor ratio                        | 40               | 40 L of bath per kg of fibre |
| Temperature, $^{\circ}\text{C}$     | 100              | water bath                   |
| Duration, min                       | 30               | with continuous agitation    |
| pH of the medium                    | 9–10             | monitored                    |
| Rinsing medium                      | distilled water  | hardness below 4%            |
| Drying                              | to constant mass | moisture controlled          |

After treatment the sample was rinsed in distilled water of hardness below 4% and dried to constant mass. Completeness of sericin removal was assessed indirectly, through the softness of the fibre, its separability into elementary filaments and its complete dissolution at the subsequent stage.

### 2.3. Dissolving system and solution preparation

Calcium chloride was selected as the principal component of the solvent. It is a colourless crystalline substance of molar mass  $110.98 \text{ g mol}^{-1}$ , readily soluble in water and ethanol and strongly hygroscopic, registered as food additive E509. Pharmacologically it is haemostatic, detoxifying, antiallergic and anti-inflammatory and reduces capillary permeability; its non-toxicity allows the finished material to be used in medicine and cosmetology [11].

The second component was distilled water complying with GOST 6709-72 (pH 5.0–7.0; specific conductivity not exceeding  $5 \times 10^{-4} \text{ S m}^{-1}$  at  $20^{\circ}\text{C}$ ). The third was 96% ethanol (boiling point  $78.39^{\circ}\text{C}$ , relative density 0.789 and refractive index 1.3614 at  $20^{\circ}\text{C}$ ), introduced to accelerate solvent evaporation so that the fibre solidifies before reaching the collector.

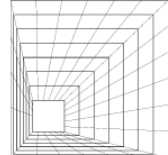
The solvent proportions were established empirically: per gram of fibroin, 23.3 g of calcium chloride, 17.1 g of distilled water and 14.6 g of ethanol. Dissolution was carried out in a water bath at  $70\text{--}80^{\circ}\text{C}$  until the fibroin had dissolved completely, the temperature being kept below  $100^{\circ}\text{C}$ ; the solutions were then filtered through six-fold medical gauze.

For quantitative characterisation the amounts of substance were computed as

$$n(\text{CaCl}_2) = 23.3 / 110.98 = 0.2099 \text{ mol}; \quad n(\text{H}_2\text{O}) = 17.1 / 18.02 = 0.9492 \text{ mol}; \quad n(\text{C}_2\text{H}_5\text{OH}) = 14.6 / 46.07 = 0.3169 \text{ mol}. \quad (2)$$

The mass fraction of fibroin in the spinning solution was computed as

$$w(\text{fibroin}) = m(\text{fibroin}) / [m(\text{fibroin}) + m(\text{solvent})] \times 100\%, \quad (3)$$



and the total polymer content of the composite solutions from the concentration of the poly(vinyl alcohol) solution (16%) and its share in the composite.

### 3. Results

#### 3.1. Degumming and fibroin yield

In the selected mildly alkaline regime the residual sericin content was reduced to a level that did not impede the subsequent dissolution step. The treated fibre became soft and separated readily into elementary filaments, which indicates that the sericin sheath binding the fibroin filaments had been destroyed.

From the initial sericin content of the waste (20–28%), the theoretical mass yield of fibroin is

$$Y = 100 - c(\text{sericin}) = 100 - (20 \dots 28) = 72 \dots 80\%. \quad (4)$$

In practice, because fats and mechanical impurities are also removed, the actual yield approaches the lower bound of this interval; a conservative value of  $Y = 75\%$  was adopted for the resource estimate below.

The overall technological sequence developed is presented as the block diagram of Fig. 1.

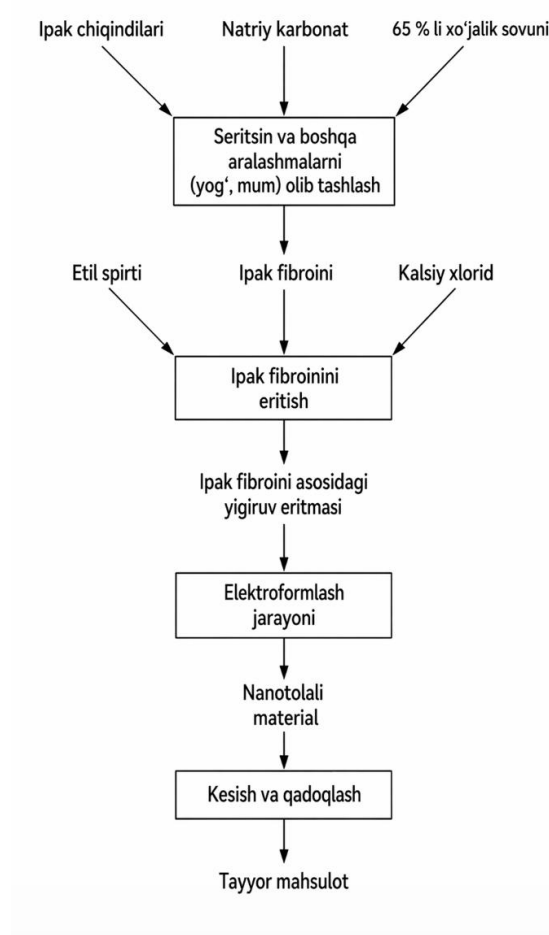
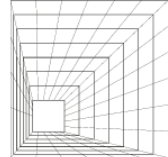


Fig. 1. Block diagram of the technology for producing nanofibrous material based on silk fibroin.

#### 3.2. Composition of the dissolving system

From Eq. (2) the molar composition of the solvent is

$$CaCl_2 : H_2O : C_2H_5OH = 1 : 4.52 : 1.51, \quad (5)$$



and its mass composition is 42.4% calcium chloride, 31.1% distilled water and 26.5% ethanol. The molality of calcium chloride in the aqueous phase is

$$b(\text{CaCl}_2) = 0.2099 / 0.0171 = 12.3 \text{ mol kg}^{-1}, \quad (6)$$

which corresponds to a very high ionic strength. Table 2 compares the selected system with the Ajisawa reagent, which is the reference calcium chloride system in the literature.

Table 2. Comparison of the selected dissolving system with the Ajisawa reagent.

| Indicator                                                           | This work       | Ajisawa reagent [8] |
|---------------------------------------------------------------------|-----------------|---------------------|
| Molar ratio $\text{CaCl}_2 : \text{H}_2\text{O} : \text{EtOH}$      | 1 : 4.52 : 1.51 | 1 : 8 : 2           |
| Mass fraction of $\text{CaCl}_2$ , %                                | 42.4            | $\approx 32$        |
| $\text{CaCl}_2$ molality in the aqueous phase, $\text{mol kg}^{-1}$ | 12.3            | $\approx 6.9$       |
| Dissolution temperature, $^{\circ}\text{C}$                         | 70–80           | 70–80               |
| Fibroin concentration attainable, wt%                               | 1.79–3.51       | 1–5                 |

The selected system is therefore a water-lean variant of the classical reagent with approximately twice its ionic strength in the aqueous phase, which increases the driving force for dissolution but also increases the quantity of salt retained in the solution.

### 3.3. Structure of the polymer and rationale for dissolution

The primary structural formula of fibroin is shown in Fig. 2. The heavy chain is built from the Gly-Ala-Gly-Ala-Gly-Ser motif, which constitutes 70% of the total protein sequence and forms antiparallel  $\beta$ -sheets linked by hydrogen bonds. These densely packed crystalline blocks are the reason for the insolubility of fibroin in water and for the requirement of a high ionic strength system to dissolve it: the calcium ion acts chaotropically, coordinating with the carbonyl groups of the peptide backbone and disrupting the hydrogen-bond network that holds the  $\beta$ -sheets together [4, 8].

#### 3.3.1. Stoichiometry of the dissolving system

The quantity of calcium chloride used can be related to the quantity of peptide groups it must act upon. Taking the reported amino-acid composition of fibroin — glycine 43%, alanine 30%, serine 12%, tyrosine 5% and valine 2%, which together account for 92% of the sequence — the mean molar mass of an amino-acid residue in the chain (the free amino acid less one water molecule) is  $72.2 \text{ g mol}^{-1}$ . One gram of fibroin therefore contains

$$n(\text{residues}) = 1.0 / 72.2 = 13.85 \text{ mmol}, \quad (7)$$

against  $n(\text{CaCl}_2) = 209.9 \text{ mmol}$  from Eq. (2). The molar ratio is thus

$$n(\text{Ca}^{2+}) / n(\text{residues}) = 209.9 / 13.85 \approx 15.2. \quad (8)$$

Fifteen calcium ions are therefore supplied per peptide residue — and per potential carbonyl coordination site. The dissolution of fibroin in this system is consequently not a stoichiometric complexation but a bulk effect of very high ionic strength, in which the chaotropic action of  $\text{Ca}^{2+}$  shifts the equilibrium of the whole hydrogen-bond network rather than saturating particular sites. This has two practical corollaries developed in Section 4: a large excess of salt necessarily remains in the solution after dissolution, and the concentration of fibroin attainable is governed by the solubility limit of the salt system rather than by the polymer.

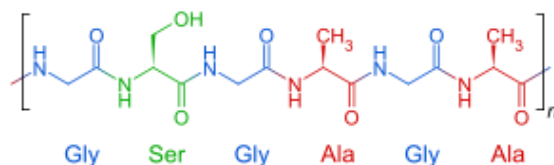
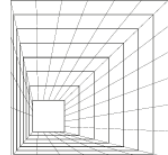


Fig. 2. Primary structural formula of fibroin.

### 3.4. Spinning-solution compositions

Electrospinning was first attempted from the pure fibroin spinning solution. The process proved highly unstable: the Taylor cone did not form steadily, jet continuity was disrupted, and droplet deposits were obtained instead of fibres. The resulting material also adhered strongly to the substrate, which made removal from the collector difficult without damage.

A second fibre-forming polymer — poly(vinyl alcohol) (PVA), Arkofil PPL grade, as a 16% aqueous solution — was therefore introduced, since electrospinning from PVA solutions is known to be highly stable. PVA is a water-soluble linear thermoplastic with film-forming ability; its glass-transition temperature is 85–90 °C, its melting point 225–230 °C and the onset of thermal degradation 160–170 °C [12, 13]. Its structural formula is shown in Fig. 3.

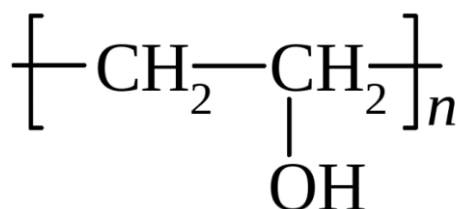
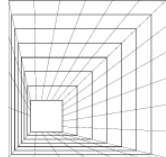


Fig. 3. Structural formula of poly(vinyl alcohol).

Composite solutions were prepared with 20%, 30% and 40% shares of the 16% PVA solution, and the fibroin mass per 5.5 g of solvent was varied at three levels. Table 3 gives the compositions expressed in mass fractions computed from Eq. (3).

Table 3. Composition of the spinning-solution samples and their polymer content.

| Sample | Fibroin, g | 16% PVA share, % | PVA, wt% | Fibroin, wt% | Total polymer, wt% | PVA / fibroin |
|--------|------------|------------------|----------|--------------|--------------------|---------------|
| 1.1    | 0.10       | 20               | 3.2      | 1.43         | 4.63               | 2.2           |
| 1.2    | 0.10       | 30               | 4.8      | 1.25         | 6.05               | 3.8           |
| 1.3    | 0.10       | 40               | 6.4      | 1.07         | 7.47               | 6.0           |
| 2.1    | 0.15       | 20               | 3.2      | 2.12         | 5.32               | 1.5           |
| 2.2    | 0.15       | 30               | 4.8      | 1.86         | 6.66               | 2.6           |
| 2.3    | 0.15       | 40               | 6.4      | 1.59         | 7.99               | 4.0           |
| 3.1    | 0.20       | 20               | 3.2      | 2.81         | 6.01               | 1.1           |
| 3.2    | 0.20       | 30               | 4.8      | 2.46         | 7.26               | 2.0           |
| 3.3    | 0.20       | 40               | 6.4      | 2.11         | 8.51               | 3.0           |
| Final  | 0.20       | 60               | 9.6      | 1.40         | 11.00              | 6.8           |



All samples were homogeneous, free of mechanical inclusions and free of sediment; after filtration no visible gel particles were observed.

### 3.5. Resource estimate

To assess the resource constraint of the technology, the waste generated per tonne of raw silk was taken as the basis, together with the raw-material consumption norm established at a later stage of the work, namely 1 g of fibroin per 100 units of finished product (Table 4).

Table 4. Fibroin resource associated with the production of one tonne of raw silk.

| Indicator                 | Basis of calculation                     | Value                   |
|---------------------------|------------------------------------------|-------------------------|
| Raw silk production       | basis                                    | 1000 kg                 |
| Fibrous waste generated   | $\geq 0.6$ kg per kg of raw silk         | $\geq 600$ kg           |
| Theoretical fibroin yield | $600 \times 0.75$                        | $\approx 450$ kg        |
| Fibroin consumption norm  | 1 g per 100 units                        | 0.01 g per unit         |
| Notional product volume   | $450\,000 \text{ g} \div 0.01 \text{ g}$ | $4.5 \times 10^7$ units |

The calculation is conditional and serves only to assess the raw-material constraint. Its outcome is that the available quantity of waste exceeds by several orders of magnitude the quantity of fibroin required for nanofibrous material production.

## 4. Discussion

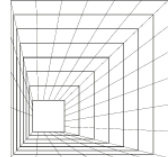
The decisive variable in the degumming step is the degree of alkalinity. Although an alkaline medium is necessary to dissolve sericin, increasing its concentration hydrolyses the peptide bonds of fibroin. The coincidence of the selected 0.019 M sodium carbonate concentration with the 0.02 M protocol used internationally is therefore not accidental: this concentration falls within the interval that provides alkalinity sufficient for complete sericin dissolution without appreciably reducing the molecular weight of fibroin [4, 7].

The advantage of combining sodium carbonate with laundry soap lies in the division of functions. The carbonate creates the alkaline medium and effects the hydrolytic dissolution of sericin, while the soap acts as a surfactant that emulsifies fatty substances and prevents the re-deposition of dissolved sericin on the fibre surface. Both reagents are inexpensive, widely available and non-toxic, which matters for industrial implementation.

In selecting the dissolving system, two requirements are in conflict. On the one hand the system must be able to break the hydrogen bonds between the crystalline blocks of fibroin; on the other its residues must not exert a toxic effect in the finished material. Lithium bromide and formic acid satisfy the first requirement well but require an additional dialysis or neutralisation step for a product intended for medical or cosmetic use. Calcium chloride, by contrast, performs a useful function even when retained in the fibre, since it is haemostatic and anti-inflammatory. This reasoning — selection by residue acceptability rather than by dissolving power — is what distinguishes the present formulation from those optimised purely for yield.

The failure of the pure fibroin solution to form fibres is explained quantitatively by the concentration analysis. Continuous fibre formation requires that the polymer concentration exceed the entanglement concentration  $c_e$ , and in practice a concentration of at least  $2\text{--}2.5 c_e$  is needed for bead-free fibres [9, 10]. The pure fibroin solutions in this work reached only 1.79–3.51 wt%, which was insufficient to establish an entanglement network: the jet broke up through capillary instability, that is, electrospraying occurred instead of electrospinning.

Introducing poly(vinyl alcohol) raises the total polymer concentration to 4.63–8.51 wt% in the composite solutions and to 11.0 wt% in the final formulation, and provides entanglement



density through intermolecular hydrogen bonding. The cost of this intervention can now be stated: in the final formulation the PVA-to-fibroin ratio is 6.8 : 1, so that fibroin constitutes only about 13% of the polymer in the finished web. Any biological benefit attributed to fibroin must be weighed against this dilution, and reducing the carrier fraction — by raising the attainable fibroin concentration or by increasing its molecular weight — is the natural direction for further work.

The resource estimate has a clear implication for development priorities. Because the fibroin consumption per unit of product is 0.01 g and its share in the unit cost is below 1%, the raw material is not the limiting factor for this technology. The binding constraint is the throughput of the electrospinning process itself, which points towards multi-emitter spinning heads and semi-industrial installations as the priority for scale-up rather than towards raw-material sourcing.

The study has several limitations. First, the completeness of sericin removal was assessed indirectly; quantitative confirmation would require gravimetric measurement of the mass loss during degumming (theoretically 20–28%), analysis of the amide I (1600–1700 cm<sup>-1</sup>), amide II (1500–1600 cm<sup>-1</sup>) and amide III (1220–1300 cm<sup>-1</sup>) absorption bands by infrared spectroscopy, and electron-microscopic inspection of the fibre surface. Second, the average molecular weight of the fibroin was not measured, although the entanglement concentration is inversely related to it; this measurement would permit the minimum concentration required for carrier-free spinning to be estimated theoretically. Third, the residual calcium chloride content of the finished material was not determined, and for medical applications this quantity would have to be specified and controlled. Fourth, the regime was developed for raw material with a comparatively low sericin content; waste containing more than 28% sericin may require a longer boiling time, with the proviso that the pH must not be allowed to exceed 10.

## 5. Conclusions

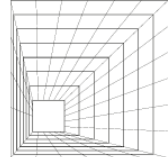
A complete route from sericulture waste to a spinnable silk fibroin solution was developed and characterised quantitatively, and its technological sequence was formalised as a block diagram. The degumming regime was justified physico-chemically: 1.5 g L<sup>-1</sup> of 65% laundry soap and 2.0 g L<sup>-1</sup> (0.019 M) of sodium carbonate at a liquor ratio of 40, 100 °C, 30 min and pH 9–10. The selected carbonate concentration was shown to coincide with the 0.02 M protocol used internationally. The theoretical fibroin yield from the initial sericin content is 72–80%.

The dissolving system was characterised through its molar composition, CaCl<sub>2</sub> : H<sub>2</sub>O : C<sub>2</sub>H<sub>5</sub>OH = 1 : 4.52 : 1.51, its mass composition 42.4 : 31.1 : 26.5, and its calcium chloride molality of 12.3 mol kg<sup>-1</sup> in the aqueous phase — a water-lean, higher ionic strength variant of the classical Ajisawa reagent.

Spinning-solution compositions were expressed in mass fractions: fibroin 1.79–3.51 wt%, total polymer 4.63–8.51 wt% in the composite solutions and 11.0 wt% in the final formulation. The instability of electrospinning from the pure fibroin solution was attributed to a polymer concentration below the entanglement threshold, and the carrier fraction required to exceed that threshold was quantified as a PVA-to-fibroin ratio of 6.8 : 1, corresponding to approximately 13% fibroin in the polymer of the finished web.

A resource estimate showed that approximately 450 kg of fibroin is associated with the fibrous waste from one tonne of raw silk, so that the raw material is not the limiting factor; the binding constraint on scale-up is the throughput of the electrospinning process.

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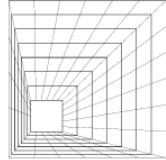


**Declaration of competing interest.** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Data availability.** The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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