

Silk (Fibroin) Processing with Dyes

Standard Operating Procedure

Faculty Supervisor: Prof. Robert White, Mechanical Engineering (x72210)

Safety Office: Peter Nowak x73246 (Just dial this directly on any campus phone.)
(617)627-3246 (From off-campus or from a cell phone)

Tufts Emergency Medical Services are at x66911.

For more information on silk processing see:

Kise, M. A. Silk Fibroin Disperation. *Textile Research Journal*, Vol. 5, No. 9, 401-415 (1935)

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1. Material Requirements:

1.1 Equipment: Any approved container in cleanroom.

Note: Any chemical containers including PMP, PC, PP, PS, PVDF, PTFE and any non-water soluble polymer is permitted. Glass is also permitted.

1.1.1 Chemicals:

1.1.1.1 Fibroin (from *Bombax Mori*; [Silkworm])

1.1.1.2 DCM laser dye (4-(Dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4H-pyran

1.1.1.3 Molecule 10 – a synthesized compound similar to tris[1-phenylisoquinoline-C2, N]iridium(III), or the tris [2-phenylpyridinato-C2, N]iridium(III)

1.1.1.4 Rhodamine B – a water soluble dye (Ammonium, (o-carboxyphenyl)-6-(diethylamino)-3H-xanthen-3-ylidene)die thyl-, chloride)

1.1.1.5 Stilbene 420 – a water soluble dye(2,2'-([1,1'-biphenyl]-4,4'-diyldi-2,1-ethenediyl)bisbenzenesulfonic acid, disodium salt)

1.1.2 Hazards associated with chemicals:

1.1.2.1 Silk elicits no immune response and is considered safe for all modes of exposure including ingestion.

1.1.2.2 DCM dye is flammable and can cause irritation to skin in large quantities. Only very small quantities will be used.

1.1.2.3 Molecule 10 is also a dye, but is not flammable. It can cause irritation to skin and mucous membranes

1.1.2.4 Rhodamine B is a moderate skin and eye irritant and may be harmful if swallowed. It is not flammable.

1.1.2.5 Stilbene 420 is a low hazard in terms of skin/eye contact, inhalation and ingestion. It is not highly flammable, but may burn.

1.2 Engineering Controls:

- 1.2.1 Purified silk [fibroin] must be handled to minimize particle contamination. Filtration through 0.45 micron membranes is recommended, but only required if one's sample has *not* been centrifuged to remove host organism debris. I.e. when silk is prepared for a clean room preparation one must minimize particulate matter. The most direct method is to filter the silk solution under chaotropic conditions through a minimum of a 0.45µm filter membrane. This process will assure that debris from the silkworm is not included in clean room preparations of fibroin and is required prior to using such a substance in the clean room.
- 1.2.2 DCM, Molecule 10, Rhodamine B, and Stilbene 420 are dyes, so the "dirty spinner" should be lined with fab wipes to facilitate clean up.
 - 1.2.2.1 DCM and Molecule 10 are soluble in solvents such as isopropanol to facilitate clean up and can be disposed of in solvent waste.
 - 1.2.2.2 Rhodamine B and Stilbene 420 are water soluble and should be cleaned up using DI water. Solid waste should go in the photoresist/solvent trash. Liquid waste should go in a hazardous waste bottle labeled "Rhodamine B and Stilbene 420".
 - 1.2.2.3 All solutions of silk and the dyes to be used should be prepared before entering the clean room. Solutions should be 1-5% dye to silk by weight.

1.3 Personal Protective Equipment: Protective eyewear and nitrile gloves are required.

2.0 Procedure: Spin coating Silk//Dye thin films

2.1 Prepare solutions of Silk/Dye in 1.5 mL Eppendorf tubes before entering clean room

- 2.1.1 0.5 mL of solution is sufficient for spin coating on a standard glass slide
 - 2.1.1.1 Solution should be 1-5% dye by weight relative to the weight of the silk.
- 2.1.2 When spin coating silk be sure to use the 'dirty' spin coater (SU-8 spin coater)
 - 2.1.2.1 Line the spin coater with fab wipes to facilitate clean up
- 2.1.3 See Laurell Spinner SOP for more information on spin coating
- 2.1.4 To spin coat Silk solutions use glass slide adapter for spin coater
- 2.1.5 Start at 200 rpm for 3 s
- 2.1.6 Go to 500 rpm in 3 s
- 2.1.7 Spin at 500 rpm for 2 s
- 2.1.8 Ramp to 500-5000 rpm in 3 s

- 2.1.9 Then spin at 500 – 5000 rpm for 10 – 45 s depending on the film thickness desired. 150 nm is optimum thickness**
- 2.2 When finished clean exposed materials with water and fab wipes to remove Silk and dye residue from tools. Then clean with isopropanol and fab wipes to further clean any dye residue. Dispose of fab wipes in solvent waste**

3.0 Storage:

- 3.1** Fibroin should be stored in a clean room approved container under refrigeration when not in use. Lack of refrigeration will result in silk self-assembling over the course of days to weeks into a gel.
- 3.2** The dyes should be stored at room temperature away from oxidizing agents in tightly closed containers. (These molecules will not be stored in the clean room).

4.0 Waste Disposal:

4.1 Fibroin [silk]

- 4.1.1** Solid waste for Silk should go directly into uncontaminated trash unless other hazardous chemicals have been used in conjunction with the fibroin [silk]. Fibroin [silk] is a protein and poses no health or environmental issues and readily biodegrade over time.
- 4.1.2** Silk containing dyes should be disposed of in the solvent/photoresist waste trash can.

4.2 Liquid waste:

- 4.2.1** Liquid waste containing silk, DCM and Molecule 10 can be mixed with the mixed solvent waste.
- 4.2.2** Liquid waste containing silk, Rhodamine B, and Stilbene 420 should be disposed of in a HDPE or glass waste bottle and tagged with a hazardous waste tag listing the chemical components.

5.0 Accident Procedures:

- 5.1 Contact:** Read MSDS prior to working with any chemical to familiarize yourself with the symptoms of exposure and recommendations for treatment.

5.1.1 Silk:

- 5.1.1.1** Skin contact: Wash exposed area with water
- 5.1.1.2** Eye contact: Wash eyes with water
- 5.1.1.3** Ingestion: No adverse effects should result. Contact health professional if any symptoms persist.
- 5.1.1.4** Inhalation: Remove exposed individual from area and contact health professional if necessary.

5.1.2 DCM, Molecule 10, Rhodamine B and Stilbene 420:

- 5.1.2.1** Skin contact: Wash exposed area with water
- 5.1.2.2** Eye contact: Wash eyes with water
- 5.1.2.3** Ingestion: Give large amounts of water to drink. Contact health professional.
- 5.1.2.4** Inhalation: Remove exposed individual from area and contact health professional if necessary.

5.2 Spill:

- 5.2.1 If a small, contained spill occurs, such as inside the hood, wipe it up with chemical wipes and dispose of in the appropriate trash container.
- 5.2.2 If a large spill occurs that you are not comfortable cleaning up:
 - 5.2.2.1 If it is a large **Fibroin [silk], DCM, Molecule 10, Rhodamine B, or Stilbene 420** spill, Wipe spill up with absorbent material and dispose of in appropriate disposal container. Note that only small mL quantities will be used so large spills are very unlikely.

If at any time you feel a situation is dangerous, do not hesitate to call the safety office (x73246, Peter Nowak) or the faculty supervisor (x72210, Robert White).

Report all accidents (injuries, major spills, fires) to the safety office at x73246 (Peter Nowak) and the faculty supervisor at x72210 (Robert White). For emergencies, call Tufts Emergency Services at x66911.