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Reconstitution Protocol

Pluronics Powder

This is a suggested procedure, please adjust according to your experimental needs. To maintain the sterility of the product, work under sterile conditions.

Protocol aim

The aim of this protocol is to provide instructions for reconstituting Pluronics Powder. Pluronics is a sterile powder that may be dissolved in water or a buffer solution of your choice. Dissolved Pluronics exhibits high viscosity at room temperature and becomes liquid when cooled.

Materials needed

- Pluronics Powder*
- Female/female Luer lock adaptor*
- Cartridges, 3cc*
- BIO X* or INKREDIBLE series* 3D Bioprinter
- Bioprinting nozzles* or needles*
- Well plate or Petri dish*
- Syringes with Luer lock connections
- Centrifuge tubes (1-50 mL)
- Spatulas/spoons
- Laboratory balance
- Reconstitution liquid (e.g. water, PBS, HBSS, cell culture medium etc.)
- Magnetic stirring plate
- Magnetic stir bar
- Ice bath (for cooling)

*The product can be purchased in the CELLINK store at *www.cellink.com/store/*.

Protocol

Step	Title	Material	Description
1	Defining gel properties		- Note the desired final concentration of Pluronics (c_P). To achieve a gel that is 3D printable at room temperature, the recommended final concentration is 40% w/v, c_P.= 40. - Record the desired volume of gel to prepare, in mL (V_L). - See Figure 1 for difference in viscosity of Pluronics gels of different concentrations.
2	Calculation		- Calculate the amount of Pluronics powder to be used. $m_P = \frac{V_L \cdot c_P}{10}$ See Table 1 with calculations for suggested c_P. Note: This equation gives a final concentration in weight/volume. However, depending on the concentration of Pluronics, the gel may swell, resulting in slightly increased final volume.
3	Preparation of reconstitution solution	- Reconstitution liquid - Centrifuge tube	- Transfer V_L of the reconstitution liquid to a centrifuge tube or container of your choice. - Cool your reconstitution liquid to ~4°C using an ice bath or fridge. Note: Since dissolved Pluronics becomes liquid at low temperature, while being a gel at room temperature, the dissolution must be performed at low temperatures when making highly concentrated Pluronics gels. See Figure 2 for temperature dependence of 20% and 40% Pluronics.
4	Preparation of Pluronics	- Spatula/spoon - Container - Pluronics Powder	- In another container, weigh up m_P of Pluronics Powder using a spatula/spoon.
5	Pluronics dissolution	- Cooled reconstitution liquid - Pluronics Powder - Magnetic stir bar	- Into the container with reconstitution liquid, add the Pluronics Powder. To reduce the formation of clumps, add the powder in increments and mix with a spatula. - Add a magnetic stir bar to the container. - Stir the mixture at high speed in an ice bath until dissolved. If clumps form, crush them with a spatula or shake the container.
6	Storage	- Pluronics gel	- Store at 4-8°C.

7	3D printing	- Pluronics gel - BIO X or INKREDIBLE series 3D Bioprinter	- For suggestions on 3D printing and using 40% Pluronics gel as a sacrificial ink, see the *Printing Protocol Pluronics 40%*.

Table 1. Suggested concentrations and the corresponding amount of Pluronics Powder used for the preparation of 3 mL Pluronics gel.

Concentration of Pluronics gel, c_P (%)	Volume of reconstitution liquid, V_L (mL)	Mass of Pluronics powder, m_P (g)
20%	3	0.6
40%	3	1.2

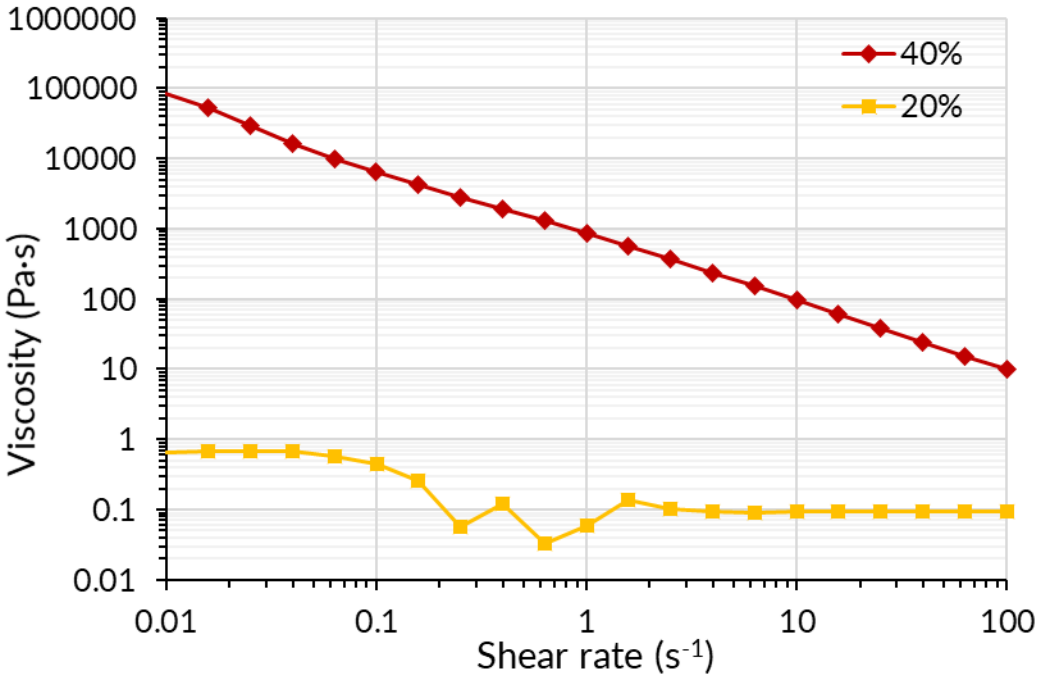

Figure 1. Viscosity of Pluronics dissolved in deionized water at 20% and 40% over a shear rate range of 0.01 to 100 s^{-1}, 25°C.

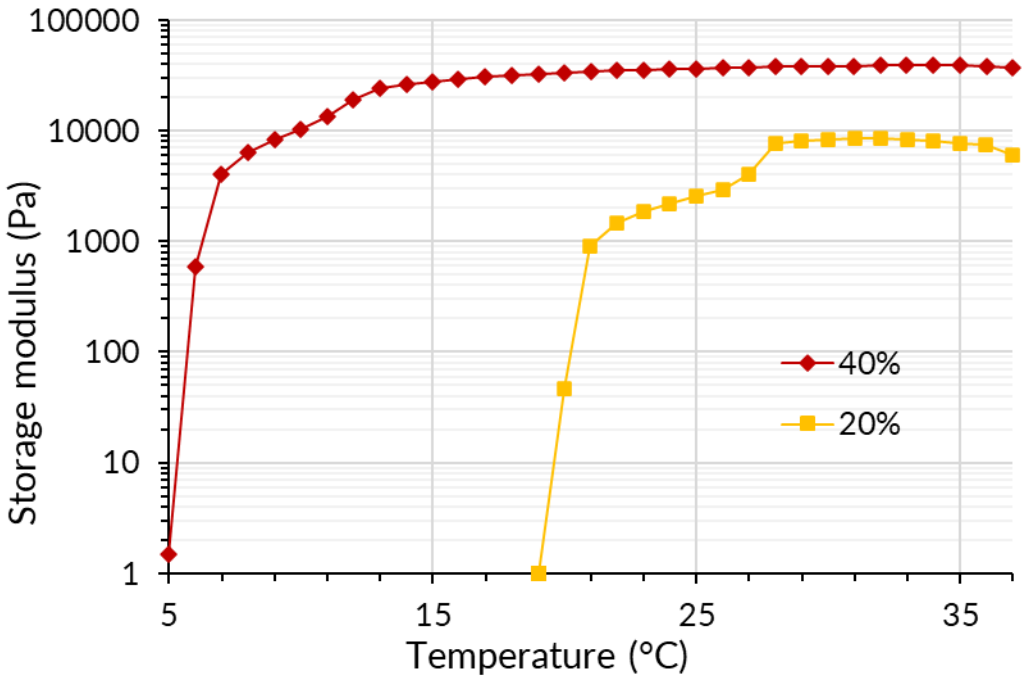

Figure 2. Storage modulus of Pluronics dissolved in deionized water at 20% and 40% concentration, over a temperature range of 5-37°C.