CLEAR FOR LAUNCH:
Standardizing Cellulose and Pluronic Protocols for Crystal Delivery

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BACKGROUND

XFEL crystallography needs a steady stream of protein crystals delivered without clumping or settling

- Crystals suspended in a viscous “carrier” gel
- Extruded through a narrow capillary.
- Increased viscosity → continuous liquid → reduces sample consumption.

WHY CELLULOSE AND PLURONIC

- Why hydrogels?

Figure 1.1. Main characteristics of hydrogel use in XFEL.

- Hydroxyethyl cellulose (HEC) and Pluronic F-127 are hydrophilic carrier media, alongside LCP.
- Both gels are much simpler to prepare than LCP.

METHODS

HEC gels:

- Concentrations tested: 10-18% w/v
- Tested with and without heat during mixing
- Standardized mixing procedure for consistency

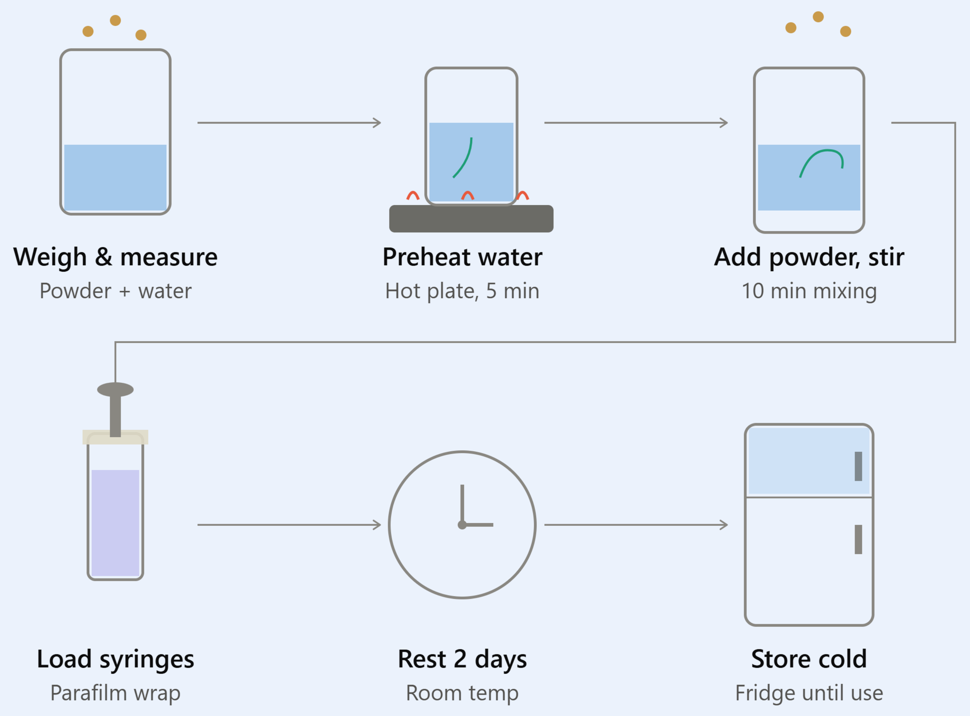

Figure 2.1 Flowchart diagram of hydroxyethyl cellulose protocol.

Pluronic F-127:

- Screened 20-40% w/v (5% steps)
- 20% did not solidify and 40% did not dissolve
- Narrowed to 25-30% w/v (1% steps)

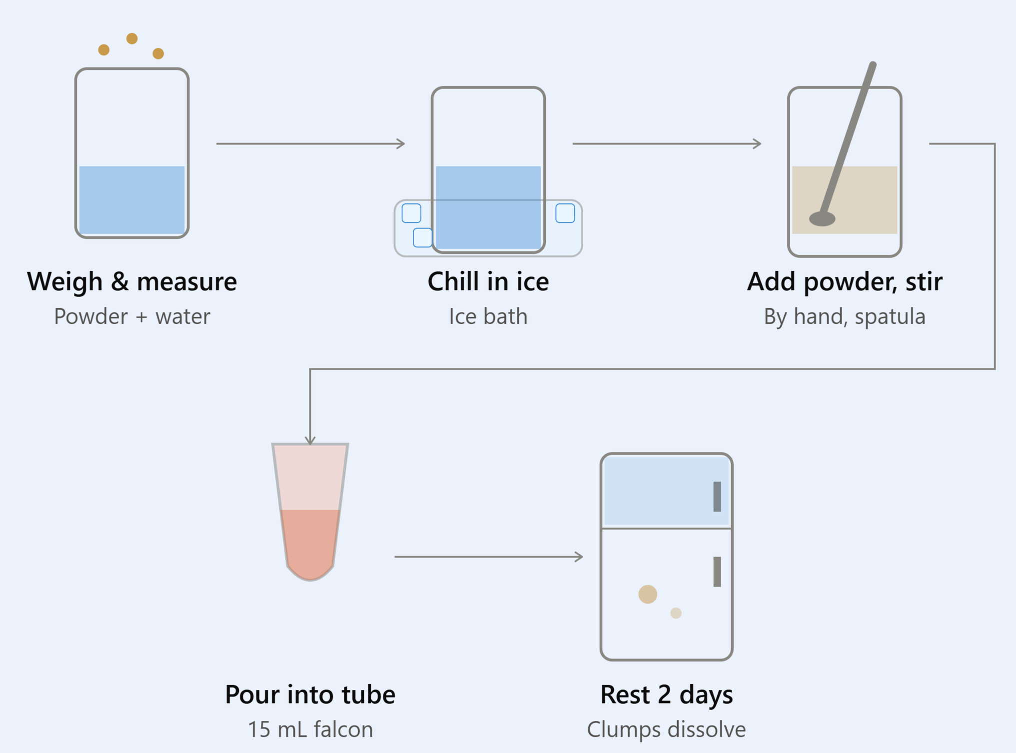

Figure 2.2 Flowchart diagram of Pluronic F-127 protocol.

Both gels:

- ~15 min active mixing
- Minimum 2-day rest before use
- Extrusion tested via HPLC pump + high viscosity capillary extruder

RESULTS

HEC

- No heat → solid layer at the bottom, loose at the top (poor homogeneity)
- With heat → more homogeneous, but higher concentrations solidified faster.
- Heating is more homogeneous, and it is recommended to use 12% for future user experiments.

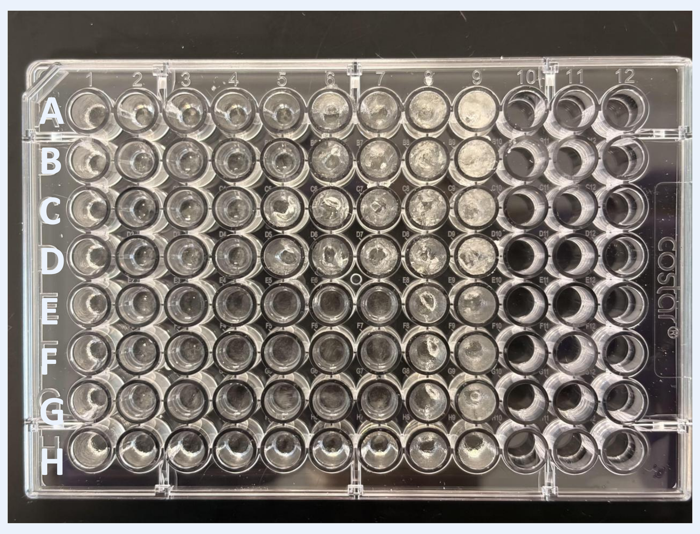

Figure 3.1 Image of both heated and nonheated HEC gels ranging from 10-18% (left to right) concentration on a 96-well plate. Rows A-D are heated HEC, and rows E-H are non-heated.

Pluronic F-127

- Faster, more complete dissolution with revised protocol

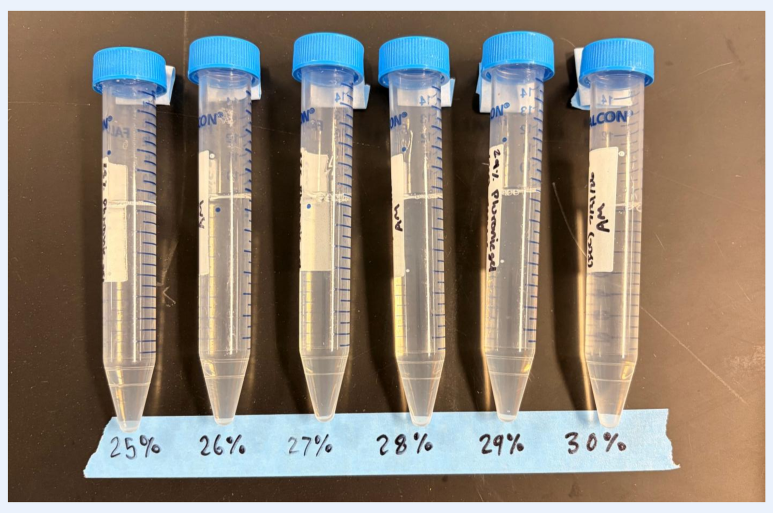

Figure 3.2 Image of Pluronic F-127 gels ranging from 25-30% concentration.

Extrusion

- Both gels extruded successfully through the high-viscosity extruder.
- There was a slight problem with the gel strands curling while they were being extruded at lower flow rates.

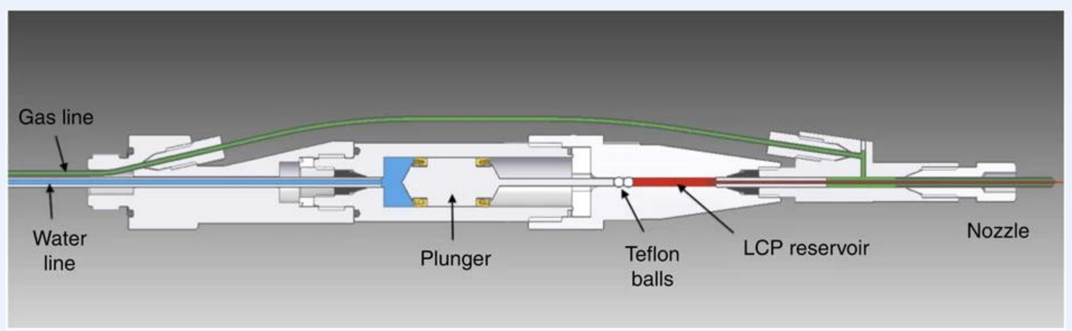

Figure 3.3. Diagram of a high-viscosity extruder.

DISCUSSION

- Recommendation: 11-13% HEC → most homogeneous, fewest trapped bubbles, and viscous.
- Pluronic was homogeneous between 25-30% but looser compared to the cellulose.
- Limitations: evaluation was mostly qualitative

Next steps:

- Quantify viscosity via viscometer
- Test in XFEL

CONCLUSION

- Standardized HEC and Pluronic F-127 protocols improved observed batch consistency and homogeneity
- Recommended 11-13% HEC with heat and 25-30% Pluronic as the starting condition for future users.
- Both gels are far simpler to prepare than LCP.

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