



Gelation of Regenerated Fibroin Solution

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Introduction:-

- The main constituent of silk fibres is the fibrous protein called fibroin.
- Fibroin is a random block copolymer of alternating hydrophobic and hydrophilic sequences¹.
- Aqueous Regenerated Fibroin Solution (RFS) prepared from *Bombyx Mori* silk undergoes sol-gel transition when subjected to pH below its isoelectric pH of 4.0.
- RFS gels are strong, porous and biocompatible, and have potential applications as 3D porous scaffolds for tissue engineering.²



Sol Gel

Method of preparation of RSF :-

- Silk cocoons were boiled in 0.5 wt% NaHCO₃ for 1 hour and washed thoroughly with excess quantity of water.
- The silk fibroin so obtained was then dissolved in 9.3M LiBr solution to obtain a 10 wt% solution.
- This solution was dialyzed against de-ionized water for 48 hours to make the RFS.
- After dialysis the protein solution was centrifuged and the concentration of protein in the solution was determined by UV spectroscopy.

Objective:-

We are interested in developing mechanistic understanding of gelation of RFS sol and investigating the microstructure of RFS gels.

Results and Data Analysis:-

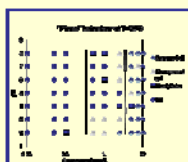


Figure 1: RFS shows four different phases at 70 °C after 30 days. Above 1 mg/ml RFS forms opaque gel, and below 0.1 it remains in solution state. At intermediate concentrations RFS precipitates or forms transparent gel.

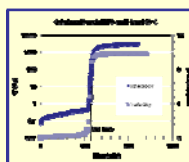


Figure 2: Storage modulus and turbidity increase rapidly and simultaneously after an initial induction period.

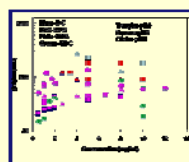


Figure 3 : Characteristic gelation time is weakly dependent on temperature and concentration below the isoelectric pH of RFS.

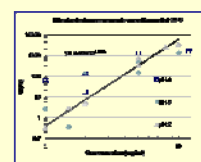


Figure 4: Opaque gels of RFS show strong concentration dependence of modulus at all pH<4.0

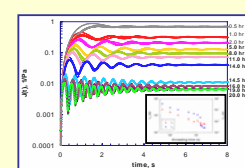


Figure 5: Creep ringing of RFS gel (7.5 mg/ml at pH and 25 °C). Lines through data show single mode fit of Jeffery model. Inset shows variation of time constants.

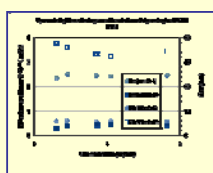


Figure 6: Two different sizes of protein moieties are observed in dialyzed silk solution. Protein aggregates of size ~ 50 nm and native protein molecule of size 8-9 nm.

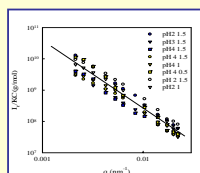


Figure 7: The solid line has slope -2.1 shows dependence of I₁/K_C on the scattering wave vector for silk protein gels formed at different conditions.

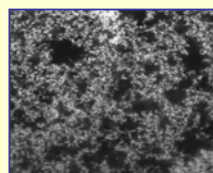


Figure 8: CSLM image of silk protein gel formed at 25°C. (C=7.5 mg/ml, pH=2)

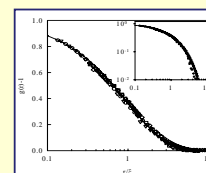


Figure 9: Pair correlation functions of gels obtained at different pH, T and C. Inset shows superposition of g(r) obtained at different concentrations at pH 3 at 20° (filled symbols) and 50°C (open symbols).

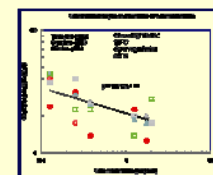


Figure 10: Correlation length shows weak dependence on concentration for gels formed at different pH and T.

Conclusions and future work:-

- Rapid gelation as probed by turbidity, rheology and CLMS suggests Nucleation and Growth mechanism for aggregation.
- The gelation time depends weakly on temperature and is independent of concentration and pH (below isoelectric point).
- Correlation length is weakly dependent on concentration storage; modulus is a strong function of concentration.
- Time course circular dichroism and microcalorimetry are planned to study the conformation change in protein during gelation.

Reference

- Jin, H. J., Kaplan, D., L., Mechanism of silk processing in insects and spiders, *Nature*, (2003), 424, 1057-1061
- Nazarov, R., Jin, H-J. and Kaplan, D., Porous 3-D Scaffolds from Regenerated Silk Fibroin, *Biomacromolecules*, (2004), 5, 718-726

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