

Silk fibroin hydrogels formed by addition of ethanol

M. A. Moraes, M. F. Silva, G. M. Nogueira and M. M. Beppu*

Departamento de Termofluidodinâmica DTF/FEQ, UNICAMP, Campinas/SP, Brasil.
beppu@feq.unicamp.br

* Corresponding author.

Abstract – The present study evaluated the influence of the addition of an organic solvent (ethanol) in SF hydrogels formation. The addition of ethanol reduced the gelation time of SF in all proportions studied. Mechanical tests of compression show that the hydrogels containing only 25% of ethanol are the most resistant to compression. The morphology of the hydrogels was observed by SEM, presenting a porous and interconnected structure. The X-ray diffraction revealed a β -sheet structure. The proposed method proved to be effective in obtaining a hydrogels with good mechanical strength in a reduced time.

The study of SF hydrogels formation is important for understanding the behavior of SF metastable solution. SF gelation occurs because SF chains tend to aggregate and form a more stable structure (β -sheet). SF hydrogels can be applied in drug release [1] and also as biomaterial [2]. The aim of this study was to analyze the time of gelation of SF solution through the addition of ethanol and characterize this hydrogels.

Silk cocoons were deggumed and dissolved in $\text{CaCl}_2:\text{EtOH}:\text{H}_2\text{O}$. The solution was dialyzed for 3 days in distilled water. The final SF aqueous solution was 4 wt %. Pure grade ethanol was diluted with water at 50 vol %. The ethanol solution was added, with stirring, into the SF aqueous solution in some volume ratios: SF/EtOH 75:25, 50:50 and 25:75. The pure SF solution and SF solution with ethanol were placed in molds in thermostatic bath at 30°C until gelation occurred. The influence of ethanol addition in time of gelation was studied. Mechanical tests of compression were performed in samples of 15 mm in diameter and 10 mm in height. The hydrogels, after freeze-drying, were characterized by SEM and DRX.

The results demonstrated that the gelation time decreased significantly with the addition of ethanol. SF/EtOH hydrogels at 25:75, 50:50, 75:25 and 100:0 were obtained at 0.08, 0.25, 13.5 and 141 h, respectively. Increasing ethanol proportion in SF also decreased gelation time. The addition of ethanol induces SF chains contact and consequently the formation of a β -sheet structure in a reduced time.

The compressive strength of SF hydrogels is shown in Table 1. The hydrogel with 75% of SF presented the maximum strength to compression among the hydrogels studied. The strength to compression of these hydrogels is superior to some SF hydrogels obtained by different methods such as salt leaching and freeze-thaw in the presence of several organic solvents [3,4]. Furthermore, the compressive strength can be significantly increased by increasing SF concentration and temperature of hydrogel formation [5].

SEM micrographs are shown in Fig. 1. SF hydrogels exhibit a porous structure when freeze-dried, with connected pores. The XRD demonstrate that all analyzed SF hydrogels have a characteristic peak at $2\theta = 20^\circ$, attributed to β -sheet structure [4]. The addition of ethanol did not changed SF structure.

Table 1: Compressive strength of SF hydrogels.

Hydrogel (SF/EtOH)	Compressive strength, kPa (average \pm standard deviation)
100:0	13,91 \pm 1,85
75:25	33,19 \pm 1,58
50:50	15,16 \pm 0,50
25:75	7,05 \pm 0,30

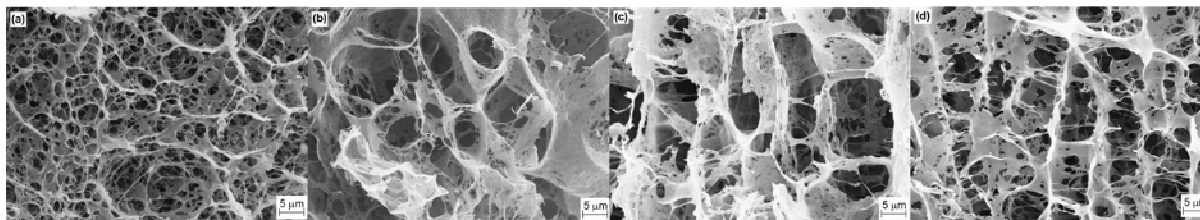


Figure 1: SEM images of freeze-dried SF hydrogels. SF/EtOH 100:0 (a); 75:25 (b); 50:50 (c); 25:75 (d).

References

- [1] J.-Y. Fang et al. Chem. Pharm. Bull. 54, 2 (2006) 156-162.
- [2] B. B. Mandal and S. C. Kundu. Biomaterials 30 (2009) 2956-2965.
- [3] U.-J. Kim et al. Biomaterials 26 (2005) 2775-2785.
- [4] Y. Tamada. Biomacromolecules 6 (2005) 3100-3106.
- [5] U.-J. Kim et al. Biomacromolecules 5 (2004) 786-792.