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Role of Silk Fibroin in Modulating Thermo-Responsive Behavior of PNIPAM Hydrogels

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Abstract

Thermo-responsive hydrogels based on Poly N-Isopropylacrylamide (PNIPAM) have attracted significant attention for advanced biomedical applications due to their smart response near physiological temperature. In this study, Silk Fibroin (SF) was incorporated into PNIPAM hydrogels to develop a tunable composite system with enhanced physicochemical properties. The hydrogels were successfully synthesized via free radical polymerization, and the effect of SF content (0–15 wt%) on the thermo-responsive behavior, swelling characteristics, deswelling kinetics, and network stability was systematically investigated. The results demonstrated that the incorporation of SF led to a gradual increase in the Lower Critical Solution Temperature (LCST), shifting from 32.1 °C for pure PNIPAM to 34.6 °C for the highest SF content. Swelling studies revealed a reduction in equilibrium Swelling Ratio (SR) with increasing SF concentration at 25 °C, while maintaining controlled water retention above LCST. Deswelling analysis showed a more gradual water release behavior in SF-containing hydrogels compared to pure PNIPAM, indicating improved regulation of thermo-responsive collapse. Additionally, gel fraction results confirmed enhanced network stability and crosslinking efficiency with increasing SF content, reaching up to 90.3%. Overall, the incorporation of SF effectively modulated the structural and thermo-responsive properties of PNIPAM hydrogels, resulting in a more stable and controllable hydrogel system. These findings highlight the potential of PNIPAM/SF composite hydrogels for biomedical applications such as controlled drug delivery, tissue engineering, and smart biomaterials.

Keywords: Poly N-Isopropylacrylamide, Silk fibroin, Thermo-responsive hydrogels, Lower critical solution temperature, Swelling behavior, Gel fraction.

1 | Introduction

Thermo-responsive hydrogels have emerged as a prominent class of smart materials capable of undergoing reversible and significant physicochemical changes in response to external temperature stimuli. These materials are typically composed of three-dimensional crosslinked polymer networks that can absorb large amounts of water while maintaining structural integrity [1–5]. A defining characteristic of thermo-sensitive hydrogels is their ability to exhibit a sharp and reversible phase transition at a specific temperature, commonly

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referred to as the Lower Critical Solution Temperature (LCST). Below the LCST, hydrophilic interactions dominate, allowing polymer chains to remain in an expanded and hydrated coil conformation [6]. However, when the temperature exceeds the LCST, hydrophobic interactions become dominant, resulting in a coil-to-globule transition and subsequent collapse of the hydrogel network. A schematic representation of the LCST-driven coil-to-globule transition of Poly N-Isopropylacrylamide (PNIPAM) and the influence of Silk Fibroin (SF) on intermolecular interactions and network structure is presented in *Fig. 1*. This unique temperature-dependent behavior has positioned thermo-responsive hydrogels as highly promising candidates for a wide range of advanced applications, particularly in the biomedical field [7]. These include controlled and targeted drug delivery systems, injectable hydrogels for minimally invasive therapies, tissue engineering scaffolds, biosensors, and actuators. The ability to fine-tune the transition temperature close to physiological conditions is especially important for biomedical applications, as it enables precise control over material behavior within the human body. Among various thermo-responsive polymers, PNIPAM has been extensively investigated due to its well-defined LCST around 32 °C, which is close to human body temperature. PNIPAM exhibits a rapid and reversible phase transition in aqueous environments, making it an ideal candidate for temperature-triggered systems. Despite these advantages, several intrinsic limitations hinder the broader application of PNIPAM-based hydrogels. One of the primary challenges is their relatively poor mechanical strength, which limits their structural stability under physiological conditions. Additionally, PNIPAM is inherently non-biodegradable, raising concerns regarding long-term biocompatibility and accumulation in biological systems. Furthermore, the abrupt and sometimes uncontrollable volume phase transition behavior can lead to undesirable effects such as burst drug release, thereby reducing the efficiency of drug delivery systems [8–12]. To address these limitations, recent research has focused on the development of composite and hybrid hydrogels by integrating synthetic polymers with natural biomacromolecules. Natural polymers offer several advantages, including inherent biocompatibility, biodegradability, and the presence of functional groups that facilitate intermolecular interactions. Among these, SF has attracted considerable attention as a reinforcing and functional component in hydrogel systems. SF is a fibrous protein derived from the cocoons of the silkworm Bombyx mori [13]. It is characterized by a unique hierarchical structure composed of crystalline β-sheet domains embedded within an amorphous matrix. These β-sheet structures are responsible for the remarkable mechanical strength, elasticity, and stability of silk-based materials. In addition, SF contains a variety of functional groups, including amino, hydroxyl, and carbonyl groups, which enable the formation of hydrogen bonds and other non-covalent interactions with synthetic polymers such as PNIPAM.

The incorporation of SF into PNIPAM hydrogels presents a promising strategy to enhance both the mechanical and functional properties of the resulting composite system. From a structural perspective, SF can act as a physical crosslinking agent, reinforcing the polymer network and improving its mechanical integrity. From a physicochemical standpoint, the hydrophilic nature of SF can influence the hydration behavior of PNIPAM chains, thereby affecting the LCST and swelling characteristics of the hydrogel [14–16]. Moreover, the presence of β-sheet domains can contribute to the formation of more stable and resilient network structures. Despite the growing interest in PNIPAM/SF composite hydrogels, several critical aspects remain insufficiently explored. In particular, the quantitative relationship between SF content and the thermo-responsive behavior of PNIPAM has not been systematically established. Previous studies have often focused on qualitative observations, without providing a comprehensive understanding of how variations in composition affect key parameters such as LCST, Swelling Ratio (SR), and mechanical strength. Additionally, the underlying mechanisms governing the interactions between PNIPAM chains and SF molecules such as hydrogen bonding, hydrophilic–hydrophobic balance, and network architecture require further elucidation. Another important aspect that has not been fully addressed is the tunability of the phase transition behavior. For practical applications, especially in biomedical systems, it is essential to precisely control the LCST and swelling-deswelling kinetics [17]. The incorporation of SF may provide a viable pathway for achieving such control; however, systematic experimental investigations are needed to validate this hypothesis. Therefore, the primary objective of this study is to systematically investigate the role of SF in modulating the structure and thermo-responsive properties of PNIPAM hydrogels. Specifically, this research aims to evaluate the effect of varying SF content on: 1) the LCST of the hydrogel system, 2) the swelling behavior at temperatures below

and above the LCST, and 3) the mechanical properties of the composite network. It is hypothesized that increasing the SF content will enhance the hydrophilicity and intermolecular interactions within the hydrogel, leading to a shift in LCST and improved structural stability. By providing a detailed analysis of the structure property relationships in PNIPAM/SF composite hydrogels, this study seeks to contribute to the rational design of advanced thermo-responsive biomaterials. The findings of this work are expected to offer valuable insights for the development of next-generation smart hydrogels with tunable properties for biomedical and pharmaceutical applications.

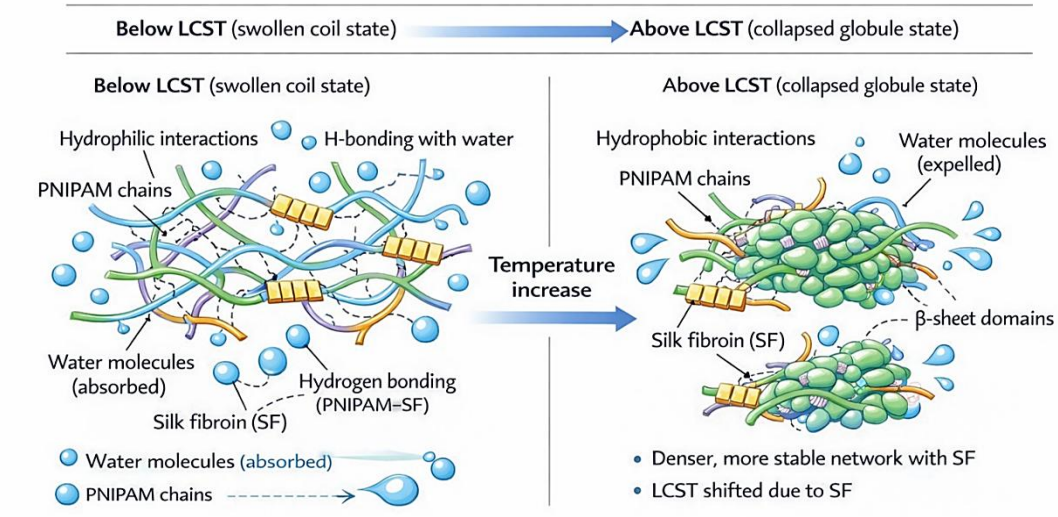

Fig. 1. Schematic illustration of LCST-triggered swelling–collapse transition in PNIPAM/silk fibroin hydrogels.

2 | Materials and Methods

2.1 | Materials

N-isopropylacrylamide (NIPAM) was used as the thermo-responsive monomer for hydrogel synthesis. N, N'-Methylenebisacrylamide (MBA) was employed as the chemical crosslinking agent, while Ammonium Persulfate (APS) served as the radical initiator. All chemicals were of analytical grade and used without further purification. Deionized water was used as the solvent in all experiments. SF was extracted from Bombyx mori cocoons through a standard degumming and dissolution procedure. Briefly, silk cocoons were cut into small pieces and boiled in a Sodium Carbonate (Na_2CO_3) solution to remove sericin proteins. The degummed fibers were thoroughly rinsed with deionized water and dried at room temperature. Subsequently, the purified fibroin was dissolved in a concentrated Lithium Bromide (LiBr) solution, followed by dialysis against deionized water for 72 hours to obtain an aqueous SF solution. The final concentration of SF solution was adjusted for further use.

2.2 | Preparation of Poly N-Isopropylacrylamide/Silk Fibroin Composite Hydrogels

PNIPAM/SF composite hydrogels were synthesized via free radical polymerization. NIPAM monomer was first dissolved in deionized water under continuous magnetic stirring to obtain a homogeneous solution. SF solution was then added at different weight percentages (0, 5, 10, and 15 wt% relative to NIPAM) to investigate its effect on hydrogel properties. After complete mixing, MBA was introduced as a crosslinker, followed by the addition of APS as the initiator. The reaction mixture was purged with nitrogen gas for 15 minutes to remove dissolved oxygen and prevent premature termination of free radicals. Polymerization was carried out at a controlled temperature (25–30 °C) for several hours until stable hydrogel formation was

achieved. The obtained hydrogels were removed from the molds and immersed in deionized water for 48 hours to eliminate unreacted monomers and residual chemicals. The washing water was refreshed periodically. Finally, the samples were freeze-dried for structural and morphological characterization [18–20].

2.3 | Determination of Phase Transition Temperature

The phase transition behavior of the PNIPAM/SF composite hydrogels was evaluated by determining the LCST using a turbidity method. Hydrogel samples were first equilibrated in distilled water at room temperature and then heated gradually from 20 to 45 °C at a controlled rate (1–2 °C/min). The change in optical transparency of the samples was monitored visually during heating. The LCST was defined as the temperature at which the hydrogel transitioned from a transparent (swollen) state to an opaque (collapsed) state. This transition corresponds to the coil-to-globule collapse of PNIPAM chains due to the dominance of hydrophobic interactions over polymer–water interactions. All measurements were performed in triplicate, and the average LCST value was reported. The influence of SF content on LCST was evaluated by comparing composite hydrogels with different SF concentrations [20].

2.4 | Swelling Behavior

The swelling behavior of the synthesized PNIPAM/SF composite hydrogels was systematically investigated to evaluate their thermo-responsive characteristics and water uptake capability. Swelling studies were performed at two different temperatures, namely 25 °C (below the LCST) and 37 °C (above LCST), in order to assess the effect of temperature on hydrogel hydration and network expansion. For this purpose, pre-dried hydrogel samples were accurately weighed and recorded as Wd. The samples were then immersed in an excess amount of distilled water at the desired temperature. At predetermined time intervals (e.g., 10, 20, 30, 60, 120, and 240 minutes), the hydrogels were removed from the medium, gently blotted with filter paper to remove surface water, and immediately weighed to determine the swollen weight (Ws). The swelling process was continued until equilibrium swelling was achieved, defined as the point at which no significant change in sample weight was observed with time. The SR was calculated using the following *Eq. (1)* [19], [20]:

$$\mathrm{SR} = \mathrm{Ws} - \mathrm{Wd}/\mathrm{Wd}, \quad (1)$$

where Wd represents the initial dry weight of the hydrogel and Ws represents the swollen weight at time t. To evaluate the swelling kinetics, the SR was plotted as a function of time. The resulting curves were used to analyze the rate of water uptake, equilibrium swelling capacity, and the influence of SF content on the diffusion of water molecules within the polymer network. All measurements were conducted in triplicate, and the results were reported as mean ± standard deviation to ensure reproducibility and statistical reliability.

2.5 | Deswelling Behavior

The temperature-responsive deswelling behavior of the hydrogels was investigated to evaluate their dynamic response above the LCST. Initially, dried hydrogel samples were immersed in distilled water at 25 °C and allowed to reach equilibrium swelling over 24 hours. The fully swollen hydrogels were gently removed, and excess surface water was carefully blotted using filter paper to obtain the initial swollen weight (W_0). Subsequently, the samples were rapidly transferred to a water bath maintained at 37 °C (above the LCST) to induce the deswelling process. At predetermined time intervals (e.g., 1, 3, 5, 10, 15, 20, and 30 minutes), the hydrogels were removed from the bath, surface water was gently wiped, and the weight (Wt) was recorded immediately. The Deswelling Ratio (DR) was calculated using the following *Eq. (2)* [21]:

$$\mathrm{DR}\ (\%) = \mathrm{Wt}/\mathrm{W0} \times 100, \quad (2)$$

where W_0 is the initial swollen weight at 25 °C, and Wt is the weight of the hydrogel sample at time t measured at 37 °C. The deswelling kinetics were analyzed by plotting DR versus time. A faster decrease in DR indicates a higher rate of water expulsion and stronger thermo-responsive behavior. All measurements were conducted in triplicate, and the average values were reported.

2.6 | Gel Fraction Determination

The gel fraction was evaluated to determine the degree of network formation and crosslinking efficiency within the synthesized PNIPAM/SF hydrogels. This parameter provides important information regarding the proportion of insoluble, crosslinked polymer chains in the hydrogel structure and is considered a key indicator of network stability. For this purpose, pre-weighed dried hydrogel samples (W_0) were initially recorded. The samples were then immersed in an excess amount of distilled water and kept at room temperature for 48 hours. During this period, the water was replaced periodically to ensure the complete removal of unreacted monomers, soluble oligomers, and any physically unbound SF or PNIPAM chains.

After the extraction process, the samples were removed from water and dried in an oven (or freeze-dryer) until a constant weight was achieved. The final dry weight of the insoluble gel fraction was recorded as W_1.

The gel fraction was calculated using the following *Eq. (3)* [22]:

$$\text{Gel Fraction (\%)} = W1/W0 \times 100, \quad (3)$$

where W_0 represents the initial dry weight of the hydrogel before extraction, and W_1 corresponds to the dry weight after removal of soluble components.

3 | Results and Discussion

3.1 | Phase Transition Behavior and Lower Critical Solution Temperature Analysis

The thermo-responsive behavior of PNIPAM/SF composite hydrogels was first evaluated by determining the LCST using a turbidity-based method. The LCST was defined as the temperature at which the hydrogel transitioned from a transparent swollen state to an opaque collapsed state, corresponding to the coil-to-globule transition of PNIPAM chains. The results demonstrated that the incorporation of SF significantly influenced the phase transition temperature of the hydrogel system. Pure PNIPAM hydrogel exhibited an LCST of approximately 32.1 °C, which is consistent with previously reported values. However, with increasing SF content, a gradual shift in LCST was observed. Specifically, the LCST increased from 32.1 °C (0 wt% SF) to 33.0 °C (5 wt%), 33.8 °C (10 wt%), and 34.6 °C (15 wt%), indicating that SF alters the thermodynamic balance of polymer–water interactions within the hydrogel network (*Fig. 2*).

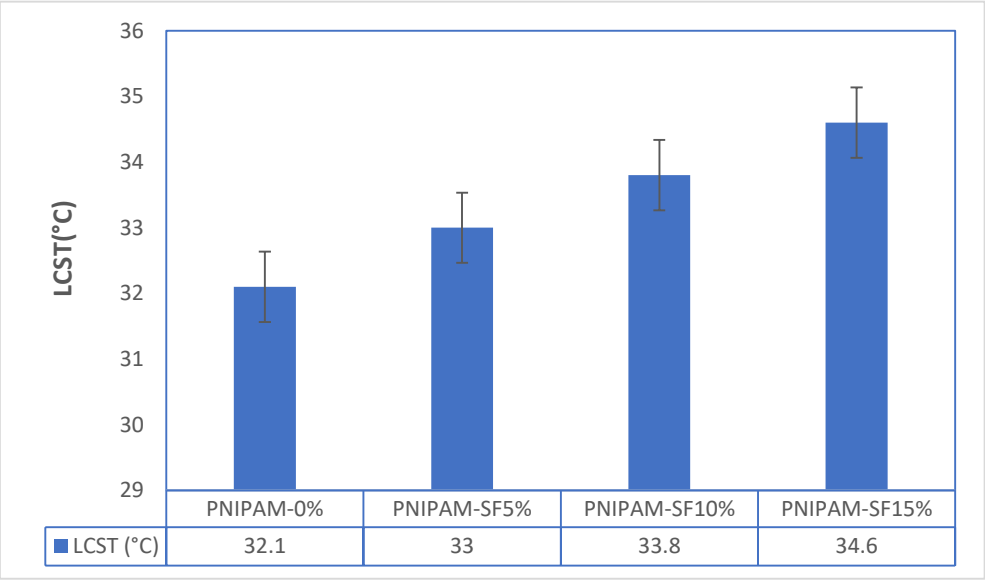

Fig 2. Effect of silk fibroin content on LCST.

3.2|Swelling Behavior of Poly N-Isopropylacrylamide/Silk Fibroin Hydrogels

The swelling behavior of PNIPAM/SF composite hydrogels was investigated at 25 °C and 37 °C to evaluate their thermo-responsive water uptake capacity. The results demonstrated a strong temperature-dependent swelling response consistent with the phase transition behavior observed at the LCST. At 25 °C (below LCST), all hydrogel samples exhibited high swelling capacity due to the hydrophilic nature of PNIPAM chains. The equilibrium SR for pure PNIPAM hydrogel was recorded as 18.5 ± 0.06 g/g. However, with increasing SF content, a gradual decrease in SR was observed, reaching 12.3 ± 0.05 g/g for the 15 wt% SF sample. At 37 °C (above LCST), a significant reduction in swelling capacity was observed for all samples due to the collapse of PNIPAM chains. The SR decreased sharply for pure PNIPAM to 4.2 ± 0.03 g/g, while composite hydrogels showed slightly higher retained water content due to the presence of SF networks (*Table 1*).

Table 1. Swelling ratio of hydrogels at different temperatures.

Sample	SF (wt%)	SR at 25 °C (g/g)	SR at 37 °C (g/g)
PNIPAM	0%	18.5 ± 0.06	4.2 ± 0.03
PNIPAM/SF-5	5%	16.2 ± 0.05	4.8 ± 0.02
PNIPAM/SF-10	10%	14.1 ± 0.04	5.3 ± 0.03
PNIPAM/SF-15	15%	12.3 ± 0.05	5.9 ± 0.02

3.3|Deswelling Kinetics of Poly N-Isopropylacrylamide/Silk Fibroin Hydrogels

The deswelling behavior of PNIPAM/SF composite hydrogels was investigated at 37 °C to evaluate their dynamic water release behavior above the LCST. The results revealed a rapid and time-dependent expulsion of water from the hydrogel network, corresponding to the collapse of PNIPAM chains from a hydrated coil structure to a compact globule state. Pure PNIPAM hydrogel exhibited a fast deswelling response, reaching a DR of 42.5 ± 1.2% within 30 minutes. In contrast, SF-containing hydrogels showed a slower and more controlled deswelling behavior. The DR values after 30 minutes were 48.3 ± 1.0% (5 wt%), 53.6 ± 1.3% (10 wt%), and 58.1 ± 1.1% (15 wt%), indicating a reduced rate of water expulsion with increasing SF content (*Table 2*).

Table 2. Deswelling behavior of hydrogels at 37 °C.

Sample	SF (wt%)	DR After 5 Min (%)	DR After 15 Min (%)	DR After 30 Min (%)
PNIPAM	0%	65.2 ± 1.5	48.7 ± 1.3	42.5 ± 1.2
PNIPAM/SF-5	5%	70.1 ± 1.2	55.4 ± 1.1	48.3 ± 1.0
PNIPAM/SF-10	10%	74.3 ± 1.4	61.2 ± 1.2	53.6 ± 1.3
PNIPAM/SF-15	15%	78.5 ± 1.3	66.8 ± 1.0	58.1 ± 1.1

3.4|Gel Fraction Analysis of Poly N-Isopropylacrylamide/Silk Fibroin Hydrogels

The gel fraction of PNIPAM/SF composite hydrogels was evaluated to determine the efficiency of network formation and the degree of crosslinking within the polymer structure. This parameter is directly related to the stability of the three-dimensional network and the proportion of insoluble polymer chains remaining after extraction. The results showed that pure PNIPAM hydrogel exhibited a gel fraction of 78.4 ± 0.8%, indicating a relatively stable but partially soluble network structure. Upon incorporation of SF, a significant increase in gel fraction was observed. The gel fraction values increased to 82.6 ± 1.1% (5 wt%), 86.9 ± 1.0% (10 wt%), and 90.3 ± 0.9% (15 wt%), respectively (*Fig. 3*).

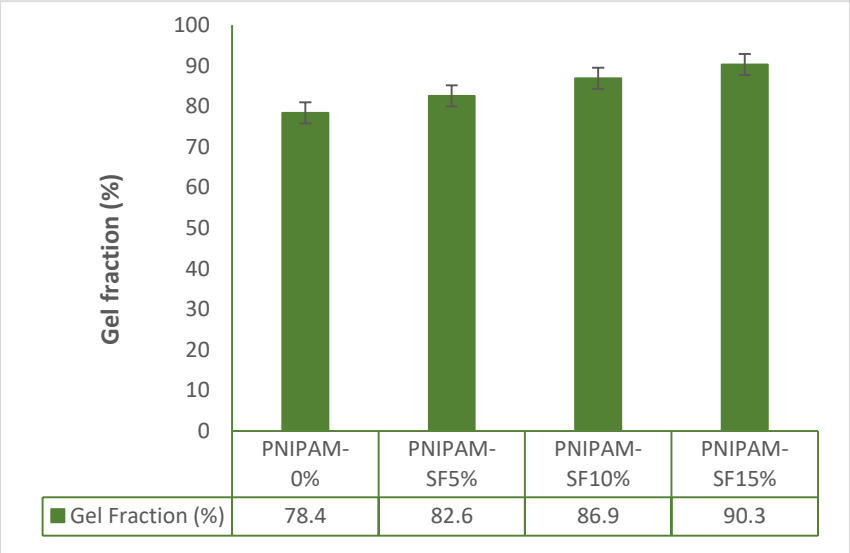

Fig 3. Gel fraction of hydrogels with different silk fibroin contents.

4 | Conclusion

In this study, PNIPAM/SF composite hydrogels were successfully synthesized via free radical polymerization, and their thermo-responsive behavior was systematically investigated. The overall results demonstrated that the incorporation of SF significantly modulates the structural and functional properties of LCST-based PNIPAM hydrogels, leading to a more tunable and application-oriented material system. The LCST analysis revealed a gradual increase in phase transition temperature with increasing SF content, shifting from 32.1 °C for pure PNIPAM to 34.6 °C for the highest SF concentration (15 wt%). This behavior was attributed to enhanced hydrophilic interactions and hydrogen bonding between SF and PNIPAM chains, which stabilize the hydrated state and delay the coil-to-globule transition. Swelling experiments further confirmed that SF plays a crucial role in regulating water uptake capacity. While pure PNIPAM exhibited a high SR at 25 °C, the incorporation of SF resulted in a controlled reduction in swelling behavior due to increased network density and additional physical crosslinking. At temperatures above LCST, all hydrogels showed a significant decrease in swelling capacity; however, SF-containing samples retained slightly higher water content, indicating improved structural stability. The deswelling kinetics analysis demonstrated that SF effectively modulates the rate of water expulsion from the hydrogel network. Pure PNIPAM hydrogels exhibited rapid collapse above LCST, whereas composite systems showed a more gradual and controlled deswelling behavior. This effect is beneficial for applications requiring sustained and regulated release profiles. Furthermore, gel fraction analysis confirmed that the presence of SF significantly enhances the structural integrity of the hydrogel network. The increase in gel fraction with higher SF content suggests improved crosslinking efficiency and reduced soluble polymer fractions, which can be attributed to strong intermolecular interactions and the reinforcing effect of β-sheet crystalline domains in SF. Overall, the results collectively indicate that SF acts not only as a reinforcing biopolymer but also as a functional modulator of thermo-responsive behavior in PNIPAM hydrogels. By adjusting the SF content, it is possible to fine-tune LCST, swelling capacity, deswelling kinetics, and network stability, thereby enabling the design of hydrogels with tailored properties. These findings highlight the potential of PNIPAM/SF composite hydrogels as promising candidates for advanced biomedical applications, particularly in controlled drug delivery systems, tissue engineering scaffolds, and injectable biomaterials. Future work may focus on in vitro and in vivo evaluations, as well as the incorporation of bioactive molecules to further enhance functional performance.

Authors' Contributions

The author solely conducted the research and prepared the manuscript and has approved its final version.

Data Availability

The data are available from the corresponding author upon reasonable request.

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Conflict of Interest

There are no competing interests to declare.

Consent for Publication

The author confirms consent for the publication of this work

Ethics Approval and Consent to Participate

This article does not include experiments involving humans or animals.

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