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Statistical Optimization of Physically Crosslinked Poly(vinyl alcohol)/Starch Hydrogelsfor Biomedical Applications

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Abstract

Hydrogels are hydrophilic polymer networks with three-dimensional architectures and potential in biomedical applications. Poly(vinyl alcohol) (PVA) is a standard base material but exhibits an inherent trade-off between mechanical strength and swelling capacity, while chemical crosslinkers can compromise biocompatibility. This study overcomes these limitations by statistically optimizing physically crosslinked PVA/starch (ST) hydrogels to achieve a balanced combination of tensile strength (TS), elongation at break (EAB), and swelling index (SI). Hydrogel membranes were prepared without chemical crosslinkers by two freeze–thaw cycles (−80 °C for 18 h; thawing 6 h) while varying PVA concentration and ST/PVA ratio. Quadratic response-surface models from a central composite design agreed well with experimental responses. Within the design space, TS, EAB, and SI ranged from 3.78–8.88 MPa, 228.8–437.7%, and 217–363%, respectively. Multi-response optimization identified 11.4 wt% PVA and 1.5 wt% ST/PVA as the best compromise, delivering TS equal 7.85 MPa, EAB equal 387.2%, and SI equal 273% with small deviations from predicted values. Scanning Electron Microscopy indicated uniform ST dispersion and ductile fracture features. Overall, this work provides a crosslinker-free and scalable formulation roadmap for tuning mechanical robustness and absorbency in PVA/ST hydrogels, supporting flexible dressings for high-mobility anatomical sites.

Keywords: Freeze–thaw, hydrogel, poly(vinyl alcohol), response surface, starch.

1. Introduction

Hydrogels are three-dimensional polymer networks with hydrophilic functional groups that absorb and retain substantial amounts of water. These soft and porous materials have attracted growing interest for biomedical applications such as tissue scaffolds, wound dressings, and drug delivery systems [1-3]. Among the various candidates, poly(vinyl alcohol) (PVA)-based hydrogels have been widely studied owing to their excellent film-forming ability, biocompatibility, and biodegradability. However, pure PVA hydrogels often have a limited swelling index (*SI*) and suboptimal mechanical properties, constraining their effectiveness in physiological environments [4].

To overcome these shortcomings, incorporating natural polysaccharides, particularly starch (ST), into PVA networks has emerged as a promising strategy to enhance the hydrophilicity, degradability, and overall gel performance [5]. ST is an abundant biodegradable and low-cost biopolymer rich in hydroxyl groups that enables the formation of strong intermolecular hydrogen bonds with PVA and improves gel cohesion [6]. Many PVA/ST films reported for wound applications rely on chemical crosslinkers (e.g., glutaraldehyde, citric acid, and borate) to stabilize the network and boost functionality. However, potential cytotoxicity and the need for post-processing raise biocompatibility and scalability concerns [7-12]. For example, citric-acid–crosslinked PVA/ST membranes optimized by response surface methodology (RSM) achieved *TS* equal 8.82 MPa and *SI* equal 343.5% [10], yet chemical crosslinkers remain a translation barrier

Physical crosslinking via freeze–thaw (F–T) cycling offers a “green” alternative in which PVA microcrystallites act as reversible junctions and facilitate scale-up [13, 14]. Despite this promise, a systematic, quantitative understanding of how PVA and ST concentration co-govern the coupled mechanical strength and *SI* responses in chemically crosslinker-free PVA/ST hydrogels is still limited. Prior studies often varied one factor at a time, focused on additive-bearing systems, or did not employ statistical design to delineate non-linear composition–property relationships. To our knowledge, no study in Vietnam has statistically optimized physically crosslinked PVA/ST hydrogels for wound care.

To fill this research gap, the present study hypothesizes that tuning the PVA and ST concentrations can yield hydrogels with an optimal balance between mechanical strength and swelling behavior. This study fabricates PVA/ST hydrogels by F–T without chemical crosslinkers and applies a Central Composite Design (CCD) to build response-surface models for tensile strength (*TS*),

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elongation at break (*EAB*), and swelling index (*SI*). All materials used (PVA and ST) are non-toxic and FDA-recognized, which supports their clinical relevance. By mapping the composition–property space and validating model predictions, this work establishes design rules for tunable, biocompatibility-oriented PVA/ST hydrogels and positions the platform for subsequent functionalization (e.g., antimicrobial loading) and preclinical evaluation.

2. Materials and Methods

2.1. Materials

Poly(vinyl alcohol) (PVA; grade 12A, degree of hydrolysis 97 mol%; Tianjin Fu Chen Chemical Reagents Factory, Tianjin, China) was used. The viscosity-average molecular weight of this grade is approximately 130 kDa, previously determined by dilute-solution viscometry using the Mark–Houwink–Sakurada relation [10]. Native cassava starch (Tapi Viet Thai Co., Vietnam) was used. The starch exhibited 97.7 wt% dry-basis purity and 21.64% amylose content.

2.2. Preparation of the Hydrogel

PVA powder was dissolved in distilled water at 90 °C with continuous stirring for 2 h to ensure complete solubilization. Simultaneously, ST was pre-dispersed in distilled water and added to the PVA solution. The mixture was stirred at 90 °C for 30 min to ensure uniform blending. Subsequently, the solution was allowed to cool to ambient temperature. A statistically designed experimental plan was used to systematically adjust the component ratios to optimize the PVA/ST hydrogel formulation.

Physical crosslinking was induced through F–T processing. The homogeneous polymer solution was first frozen at −80 °C for 18 h, then thawed at room temperature (25 ± 2 °C) for 6 h. In total, two freeze–thaw cycles were performed. Finally, the resulting hydrogel membranes were dried at 37 °C for 48 h to stabilize their structures and to maintain their integrity for subsequent characterization.

2.3. Design of Experiment and Statistical Analysis

A CCD was utilized to develop a response surface model and assess the primary performance attributes of the hydrogel membranes. The evaluated responses included the *TS*, *EAB*, and *SI*. Factor A (PVA concentration, wt%) varied from 8 to 12 wt%, and Factor B (ST/PVA ratio, wt% relative to PVA) varied from 1.5 to 8.5 wt%. The coded factor levels used in the CCD are listed in Table 1. Formulations were designated P*x*S*y*, where *x* and *y* denote PVA (wt%) and ST/PVA (wt% relative to PVA), respectively.

Table 1. Coded and actual values of independent variables used for central composite design

Variable (unit)	Coded factor	Coded levels				
		-α	-1	0	+1	+α
PVA (wt%)	A	8	8.6	10	11.4	12
ST/PVA (wt%)	B	0	1.5	5	8.5	10

2.4. Thickness of Hydrogel

The hydrogel thickness was measured using a digital thickness gauge (Mitutoyo 7327A, Japan) at five random positions per sample, and the average value is reported.

2.5. Tensile Strength and Elongation at Break

Mechanical properties were tested using a universal machine (Zwick/Roell BDO-FB050TN, Germany). Prior to testing, the hydrogel samples (100 mm × 10 mm) were immersed in phosphate-buffered saline (PBS, pH 7.4) to reach equilibrium swelling. After blotting the excess surface water, the samples were stretched at a 100 mm/min crosshead speed with an initial grip separation of 50 mm. The *TS* and *EAB* were calculated from the stress–strain curve.

2.6. Swelling Index

The *SI* was determined using a gravimetric method [14]. Hydrogel samples (10 mm × 10 mm) were dried in a desiccator, weighed (W_0), and then immersed in PBS at room temperature. The hydrogel was gently blotted at predetermined intervals to remove surface moisture and then weighed (W_1). The *SI* was calculated using following formula.

$$SI(\%) = \frac{W_1}{W_0} \times 100\% \qquad (1)$$

2.7. Scanning Electron Microscopy

The cross-sections of the hydrogel samples were characterized using a scanning electron microscope (SEM; JCM-7000, JEOL Ltd., Tokyo, Japan) operated at an accelerating voltage of 5 kV. Prior to imaging, the samples were coated with a thin layer of platinum via sputtering for 20 seconds to enhance conductivity.

3. Results and Discussion

3.1. Regression Model Summary for Mechanical and Swelling Properties of Poly(vinyl alcohol)/Starch Hydrogels

According to the CCD, response-surface models were constructed to quantify how PVA concentration (factor A, wt%) and the ST/PVA ratio (factor B, wt%) affect three responses: *TS*, *EAB*, and *SI*. *TS* indexes the hydrogel's resistance to tensile loading, *EAB* its extensibility under strain, and *SI* measures water uptake relevant to wound-exudate management. As summarized in Table 2, the fitted quadratic models showed good agreement with the experimental data, with coefficients of determination in the range R^2 from 0.8331 to 0.8616. Importantly, the lack-of-fit tests were not significant ($p > 0.05$) for all responses, indicating that the residual variation is primarily attributable to experimental error rather than systematic model inadequacy. Together, these statistics confirm that the regression equations provide an adequate representation of *TS*, *EAB*, and *SI* within the investigated design space.

3.2. Response Surface Analysis of Tensile Strength and Elongation at Break

Mechanical properties are essential for evaluating hydrogel performance under physiological conditions, particularly in wound-dressing applications [15, 16]. *TS* indicates the material's resistance to external stress, while *EAB* reflects flexibility and strain tolerance. This study analyzed *TS* and *EAB* as the key mechanical responses influenced by PVA and ST/PVA concentrations. Response surface plots (Fig. 1) were generated using RSM to visualize the effects of the formulation variables. *TS* ranged from 3.78 to 8.88 MPa, and *EAB* varied from 228.8% to 437.7%, confirming broad mechanical tunability.

Experimentally, at fixed high PVA (11.4 wt%), increasing ST/PVA from 1.5 to 8.5 wt% depressed *TS* (Fig. 1a) and drove a near-linear decline in *EAB* (Fig. 1b). Mechanistically, excessive ST interferes with the development of well-organized PVA–PVA hydrogen-bonded microcrystalline domains during freeze–thaw cycling. Meanwhile, the presence of starch-rich rigid domains restricts polymer chain mobility, reducing energy dissipation under strain and promoting brittleness and premature failure[7, 17-18]. At lower PVA, a modest ST fraction can contribute to flexibility by providing a compliant polysaccharide phase; however, beyond a narrow window, imperfect compatibility and heterogeneous dispersion introduce defects that compromise stress transfer and strain accommodation.

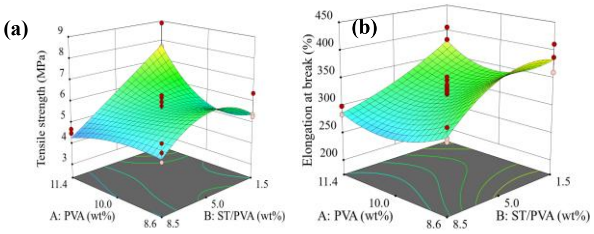

Fig. 1. Response surface analysis of the combined influence of PVA and ST/PVA concentrations on hydrogel mechanical properties: (a) tensile strength, (b) elongation at break

For benchmarking only, Delavari *et al.* (2022) reported a maximum experimentally measured *TS* of 8.82 MPa for citric-acid–crosslinked PVA/ST films optimized by RSM [10]. Within the present experimental design space, the highest experimentally observed *TS* of 8.88 MPa was obtained for the crosslinker-free PVA/ST hydrogels prepared via purely physical freeze–thaw crosslinking. This result demonstrates that freeze–thaw processing combined with composition control can deliver wound-dressing relevant mechanical performance while avoiding potentially toxic chemical crosslinkers.

Table 2. Summary of regression models

Response	Lack-of-fit test result (*p-value*)	Coefficient of determination R^2	Significant terms	Equation in terms of coded factors
TS	Insignificant (0.2025)	0.8331	B, AB, A^2, B^2, A^2B, AB^2	$5.66 - 0.0790A - 0.3253B - 0.7175AB + 0.4278A^2 - 0.5213B^2 - 0.7039\,A^2B + 0.5465AB^2$
EAB	Insignificant (0.7993)	0.8367	A, B, A^2, B^2, AB^2	$317.55 - 26.72A - 35.4B - 3.00AB + 33.05A^2 - 11.73B^2 - 7.85A^2B + 21.5AB^2$
SI	Insignificant (0.0563)	0.8616	A, B, A^2, B^2	$324.68 + 6.53A + 25.70B + 0.0417AB + 6.75A^2 - 25.96B^2$

3.3. Response Surface Analysis of Swelling Index

The swelling index is a critical parameter that reflects the hydrogel's ability to retain moisture and manage wound exudates [19]. In this study, the *SI* ranged from 217% to 363%, with most samples exhibited swelling indices exceeding 260%, indicating a high absorbency level favorable for wound exudate management [11] (Fig. 2).

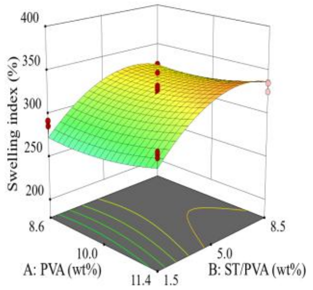

Fig. 2. Response surface analysis of PVA and ST/PVA concentrations influencing the swelling index of the hydrogel

The effects of PVA and ST/PVA concentrations on *SI* were analyzed from the experimental dataset and the fitted surface (Fig. 2). Within 8.6–11.4 wt% PVA, *SI* changed only slightly, suggesting that increasing polymer density primarily tightens the physically crosslinked network and limits water diffusion rather than increasing accessible –OH sites. In contrast, raising ST/PVA from 1.5 to 8.5 wt% produced a non-linear *SI* response: moderate *ST* increased *SI* due to its highly hydrophilic amylopectin-rich structure, which promotes water uptake and increases free volume [20]. At the upper ST levels, *SI* decreased slightly, likely due to aggregation or phase heterogeneity that reduces effective water–polymer interactions.

Compared with a chemically crosslinked benchmark (Delavari *et al.*, 2022; experimentally reported maximum *SI* of 343.5% for citric-acid–crosslinked PVA/ST films) [10], the present crosslinker-free hydrogels exhibited a higher maximum experimentally observed *SI* of 363% within the investigated formulation range. The hydrogels also reached equilibrium swelling within 60 min, which is suitable for managing sudden exudate surges in heavily draining wounds. These results confirm that physically crosslinked PVA/ST systems can match or surpass the absorptivity of chemically modified counterparts, providing a safer and more sustainable platform.

3.4. Regression Model Evaluation, Morphology, and Potential Applications of Poly(vinyl alcohol)/starch Hydrogel

To directly compare model calculations with experimental results, a multi-response desirability function implemented in Design-Expert was employed to identify a compromise optimum, rather than the absolute *TS* maximum, by maximizing *TS* while simultaneously maintaining high *EAB* and *SI* within the investigated design space. The model predicted an optimal composition of 11.4 wt% PVA and 1.5 wt% ST/PVA. This formulation was subsequently fabricated and tested under identical experimental conditions. As summarized in Table 3, the experimentally measured responses (*TS* = 7.85 MPa, *EAB* = 387.2%, *SI* = 273%) showed excellent agreement with the model predictions, with absolute deviations of 0.9%, 1.9%, and 4.6%, respectively, thereby confirming the predictive reliability of the regression models within the studied design space.

Table 3. Evaluation results of the regression models

Responses	Optimized design point		
	Predicted	Actual value	Percent error (%)
TS (MPa)	7.78	7.85	0.9
EAB (%)	379.9	387.2	1.9
SI (%)	286.3	273	4.6

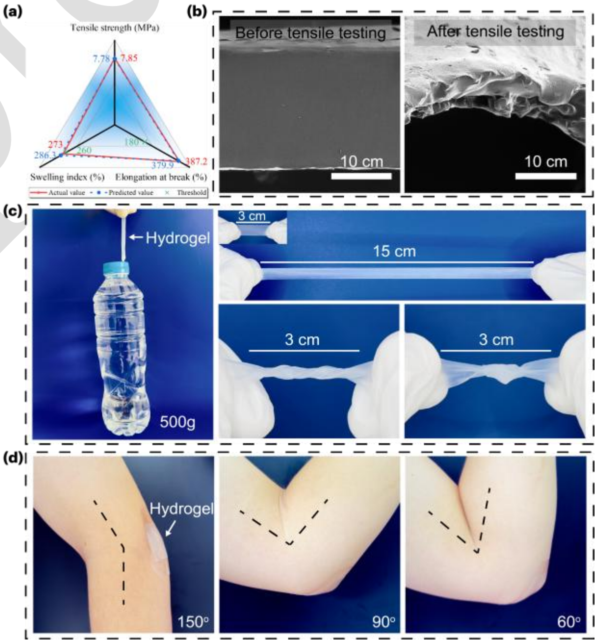

Fig. 3. Mechanical performance and flexibility of the optimized PVA/ST hydrogel (PVA/ST-Opt). (a) Predicted vs. actual *TS*, *EAB*, and *SI* confirming model validity; (b) SEM cross-sections before and after tensile testing showing uniform ST dispersion and ductile fracture; (c) Demonstration of mechanical robustness under 500 g loading, elongation, twisting, and knotting; (d) Conformability of PVA/ST-Opt on the human elbow at different bending angles, highlighting suitability for flexible wound dressings

Morphological observations corroborated these statistical findings. Scanning Electron Microscopy (SEM) micrographs (Fig. 3b) revealed uniform ST dispersion in the PVA matrix, indicating good interfacial compatibility and the absence of phase separation. Post-tensile images displayed elongated fibrils and folded fracture surfaces, features characteristic of ductile, energy-dissipating structures, consistent with the high measured *EAB* and toughness.

Macroscopic testing further verified the hydrogel's robustness. The membrane supported a 500 g load and elongated by nearly 400% without rupture (Fig. 3c). It retained structural integrity under twisting and knotting, demonstrating exceptional flexibility and resilience. When applied to the human elbow at bending angles of 60°, 90°, and 150° (Fig. 3d), the hydrogel conformed smoothly and remained stable during motion, confirming its suitability for dynamic joint dressings [16, 21,22].

Overall, the physically crosslinked PVA/ST hydrogel achieves a balanced profile of strength, extensibility, and absorbency comparable to chemically crosslinked systems but without cytotoxic reagents. The regression-based framework establishes a quantitative structure–property–performance model, supporting green and scalable development of biocompatible wound-dressing hydrogels.

4. Conclusions

This study delivers two key outcomes for crosslinker-free PVA/ST hydrogels intended for biomedical applications. (i) Physically crosslinked (freeze–thaw) PVA/ST hydrogels are shown to achieve wound-dressing–relevant mechanical robustness and swelling performance without chemical crosslinkers, offering a greener and potentially safer alternative to chemically crosslinked systems. (ii) A quantitative composition–property optimization framework based on CCD/RSM is established, enabling simultaneous prediction and optimization of *TS*, *EAB*, and *SI* as coupled responses. By varying PVA (8.6–11.4 wt%) and ST/PVA (1.5–8.5 wt%) under a freeze–thaw regimen, response-surface models effectively captured the composition–property relationships, enabling reliable prediction and optimization. The optimal hydrogel (11.4 wt% PVA, 1.5 wt% ST/PVA) exhibited a balanced profile of *TS* (7.85 MPa), *EAB* (387.2%), and *SI* (273%), together with uniform morphology and high mechanical flexibility under load and joint bending. Overall, the study provides both an experimentally verified crosslinker-free hydrogel platform and a statistical design roadmap for tailoring performance, supporting scalable development of flexible dressings for dynamic or high-mobility anatomical sites.

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