

Sustainable and bioactive chitosan/PVA hydrogels incorporating allantoin for wound healing applications

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Conventional wound dressings mainly provide passive protection and are often insufficient to maintain the bioactive conditions necessary for effective healing. Hydrogel-based materials have attracted considerable attention due to their biocompatibility, moisture-retention capacity, and structural similarity to biological tissues. In this study, bioactive PVA/chitosan hydrogel films incorporating allantoin (0.5–1.5 wt.%) were developed using a solution casting method, and evaluated in terms of morphology, tensile properties, and antioxidant activity. Morphological observations revealed that increasing allantoin concentration affected film homogeneity and transparency. The sample containing 1.0 wt.% allantoin exhibited visible particulate aggregation within the polymer matrix. Tensile analysis demonstrated that allantoin incorporation decreased tensile strength and elongation at break while increasing elastic modulus, indicating reduced polymer chain mobility and a transition toward a stiffer structure. Antioxidant activity results exhibited a concentration-dependent behavior. Among the developed samples, the 0.5 wt.% allantoin-loaded hydrogel showed the highest antioxidant activity in both DPPH and β -carotene/linoleic acid assays, whereas higher concentrations resulted in reduced performance. The results indicate that 0.5 wt.% allantoin incorporation provides an effective balance between antioxidant functionality and mechanical performance, highlighting the promising potential of the hydrogel system as a bioactive and mechanically stable material for wound healing and dermatological applications.

Keywords: Allantoin, Chitosan, Polyvinyl alcohol, Hydrogel, Wound healing.

INTRODUCTION

Wound healing is a complex biological process involving overlapping phases of hemostasis, inflammation, proliferation, and tissue remodeling. Successful healing requires a balanced microenvironment that supports tissue regeneration while minimizing microbial contamination and oxidative stress. Conventional wound dressings such as gauze mainly provide passive protection and are often insufficient to maintain the moist and bioactive conditions required for efficient wound repair. In recent years, hydrogel-based wound dressings have attracted considerable attention due to their high-water content, flexibility, oxygen permeability, and extracellular matrix-like structure, making them promising materials for advanced wound care applications [1]. Among hydrogel-forming polymers, poly (vinyl alcohol) (PVA) is widely used because of its excellent film-forming ability, mechanical stability, hydrophilicity, and biocompatibility. However, PVA alone exhibits limited biological functionality. Chitosan, a naturally derived polysaccharide, has been extensively investigated for biomedical applications owing to its biodegradability, hemostatic activity,

and intrinsic antimicrobial behavior. The combination of PVA and chitosan enables the development of composite hydrogel systems with balanced mechanical and biological properties through intermolecular hydrogen bonding interactions [2].

To further improve hydrogel functionality, bioactive compounds can be incorporated into the polymer matrix. Allantoin is a bioactive molecule known for its antioxidant, anti-inflammatory, moisturizing, analgesic activity and tissue regenerative properties [3]. In addition, allantoin has been reported to promote epithelial cell proliferation and support wound healing processes in hydrogel and scaffold systems [3]. Moreover, allantoin promotes skin, moisturization, supports the elimination of necrotic tissue, and aids in the prevention of gastric ulcers [4]. However, studies investigating the influence of allantoin concentration on the mechanical and antioxidant properties of PVA/chitosan hydrogel films remain limited.

Therefore, the present study aimed to develop allantoin-loaded PVA/chitosan hydrogel films using a solution casting method and to evaluate the effect of allantoin concentration on the morphology, tensile

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properties, and antioxidant activity of the prepared hydrogel systems for potential wound dressing applications.

MATERIALS AND METHODS

Materials

Chitosan was obtained from Sigma-Aldrich (Darmstadt, Germany). Poly (vinyl alcohol) was supplied by Zag Kimya (İstanbul, Türkiye). Glycerol was purchased from Merck (Darmstadt, Germany), while glacial acetic acid (100% anhydrous) was obtained from ISOLAB Chemicals (Germany). Allantoin ($\geq 99\%$) was provided by Sky Pharmaceutical Chemical Food Industry and Trade Ltd. (Türkiye). Butylated hydroxytoluene (BHT), α -tocopherol, β -carotene, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and linoleic acid were acquired from Sigma-Aldrich (Steinheim, Germany).

Preparation of chitosan/PVA/allantoin films

Four different composite films were developed by the casting method. Chitosan was dissolved in aqueous acetic acid solution (2 % v/v) at 50 °C with continuous stirring (600 rpm) for 2 h to obtain chitosan solution (1 % w/v). The PVA solution (5 % w/v) was prepared in distilled water at 75 °C for 12 h with stirring (250 rpm). The solutions of PVA and chitosan were mixed at 2:1 v/v ratio by mechanical stirrer (600 rpm) to obtain a homogeneous solution. Further, a specific amount of the allantoin (0, 0.50, 1.0 or 1.5 wt. % in respect to the total volume of film forming solution (100 mL) was added to the solution and stirred at 50 °C for 1.5 h (250 rpm). As plasticizer glycerol (300 μ L) was added to the mixed

solution and the resulting mixture was stirred for half an hour at room temperature. This mixture (40 mL) was then cast onto round glass petri dishes (14 $\times$ 14 cm) and dried in an oven at 50 °C for 18 h (Fig. 1.). The prepared films were labeled as CP-0A (control, without allantoin) and CPA-x, where x represents the allantoin concentration (0.5, 1.0, and 1.5%, w/w), respectively.

Tensile properties determination

The mechanical properties of the composite films were determined with ASTM D882 standard using an AGS-X Series universal testing machine (Shimadzu, Japan). The crosshead speed and load cell were 15 mm/min and 1 kN, respectively. The data was collected by testing five separate samples.

Antioxidant activity determination

The antioxidant capacities of the samples were evaluated using two complementary assays: the DPPH radical scavenging method [5, 6], and the β -carotene/linoleic acid assay [7].

For the analyses, the films were cut into small fragments and dissolved in the corresponding test solutions. Biological activity measurements were performed at four different concentrations: 25, 12.5, 6.25, and 3.125 μ g/mL. Antioxidant performance was expressed as the IC₅₀ value, representing the concentration required to achieve 50% inhibition. Butylated hydroxytoluene (BHT) and α -tocopherol (TOC) were used as reference antioxidant compounds for comparison. All measurements were conducted using a 96-well microplate reader (SpectraMax 340PC384, Molecular Devices, USA).

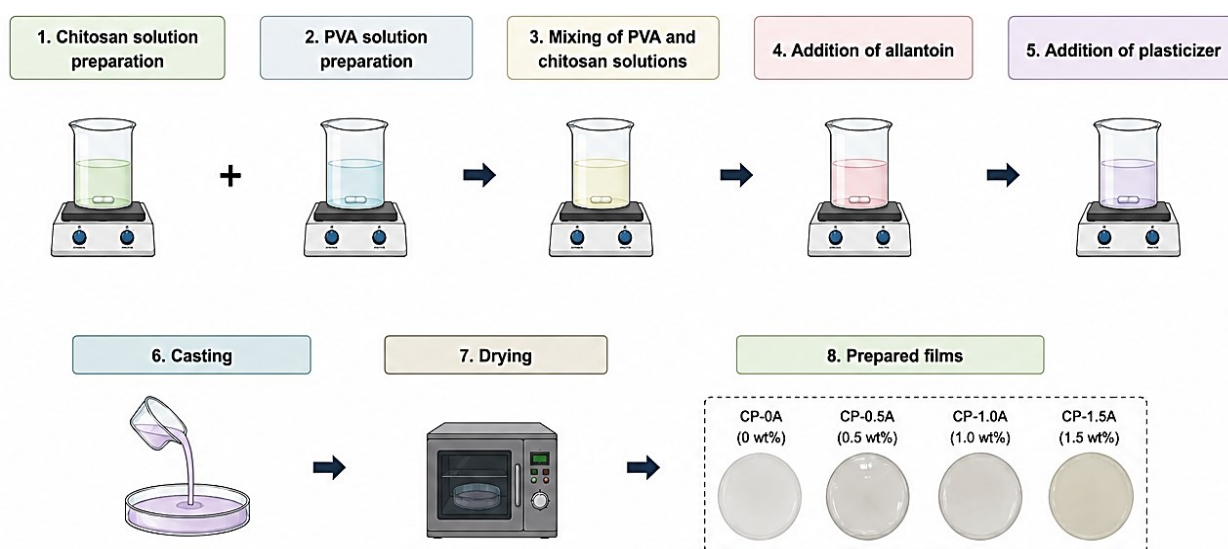


Figure 1. Schematic illustration of allantoin-loaded composite film preparation.

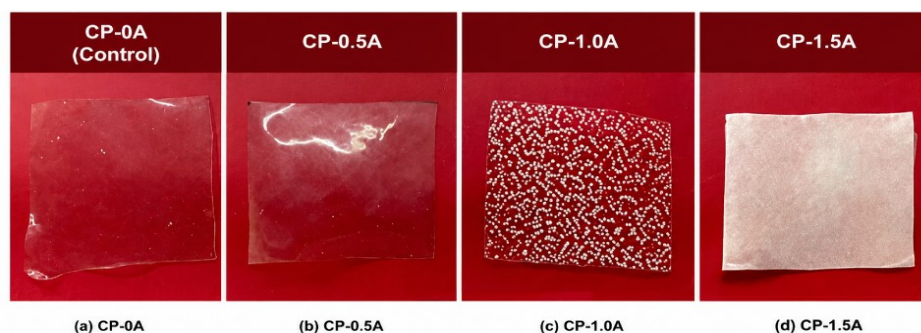


Figure 2. Photographs of chitosan/PVA hydrogel films incorporating different concentrations of allantoin. a) CP-0A (control, without allantoin), b) CP-0.5A (0.5% w/w), c) CP-1.0A (1.0% w/w), and d) CP-1.5A (1.5% w/w).

RESULTS

Morphological evaluation of films

The visual appearance of chitosan/PVA films incorporating different concentrations of allantoin were presented in Figure 2. The images demonstrated the successful formation of chitosan/PVA/allantoin films using the casting method. The control sample (CP-0A) exhibited a relatively smooth and transparent structure, indicating a homogeneous polymeric network. A similar morphology was observed for CP-0.5A, although slight surface irregularities and minor opacity changes became noticeable, suggesting initial interactions between allantoin and the polymer matrix.

In contrast, the CP-1.0A sample showed a distinctly heterogeneous appearance with visible white particulates distributed throughout the film. This observation may indicate aggregation of allantoin within the polymer network, leading to reduced structural uniformity. Such heterogeneity can be associated with limited dispersion of the bioactive compound at higher concentrations.

For the CP-1.5A sample, the film appeared opaquer and more uniform compared to CP-1.0A, although transparency was significantly reduced relative to lower concentrations. This change may reflect a partial redispersion of allantoin, resulting in a more compact but less transparent structure.

The visual observations suggest that allantoin concentration influences the morphological characteristics of the hydrogel films, affecting transparency, homogeneity, and surface structure. These changes may have implications on the functional properties of the materials.

Mechanical properties of the films

The mechanical properties of the chitosan/PVA films are presented in Table 1. The control sample (CP-0A) exhibited the highest tensile strength (40.55 N / mm²) and elongation at break (325.17 %),

indicating a flexible and mechanically robust network.

Table 1. Tensile results of synthesized compounds

Parameter	CP-0A	CP-0.5A	CP-1.5A
Tensile strength at break (N/mm ²)	40.55	30.50	23.25
Elongation at break (%)	325.17	287.50	132.65
Elastic modulus	20.91	70.00	100.26

The incorporation of allantoin (CP-0.5A and CP-1.5A) resulted in a decrease in tensile strength and elongation at break, accompanied by a significant increase in elastic modulus. This behavior can be attributed to the disruption of intermolecular hydrogen bonding between chitosan and PVA chains, as well as the formation of heterogeneous domains that restrict polymer chain mobility. It is well established that the mechanical performance of chitosan/PVA hydrogels is strongly influenced by hydrogen bonding, crystallinity, and network homogeneity [8, 9]. Furthermore, the increase in elastic modulus with higher allantoin content indicates a transition toward a stiffer and less deformable structure, consistent with reduced chain mobility. From an application perspective, an ideal wound dressing material requires a balance between mechanical strength and flexibility. In this context, the CP-0.5A formulation appears to provide a more suitable mechanical profile compared to higher allantoin concentrations.

It should be noted that the CP-1.0A sample was not included in the tensile analysis. As observed in the morphological evaluation (Figure 2), this formulation exhibited pronounced heterogeneity and visible particulate domains, suggesting phase separation and poor dispersion of allantoin within the polymer matrix. Such structural inhomogeneity can compromise film integrity and reproducibility during tensile testing, leading to unreliable mechanical data. Similar limitations have been

reported in hydrogel systems where non-uniform distribution of components adversely affects structural integrity and mechanical performance [10].

Antioxidant activity of the films

Oxidative stress arises from an imbalance between the production of reactive oxygen species (ROS) and the capacity of the biological system's antioxidant defenses to neutralize them [11]. ROS are regulated by a complex antioxidant defense system that maintains their levels within an appropriate range, enabling their normal physiological functions while limiting excessive oxidative damage that may contribute to the development of various diseases [12]. Elevated ROS levels can lead to metabolic disturbances, loss of cellular integrity, altered gene expression, and impaired enzymatic activity [13]. In infected wounds, excessive ROS and free radicals further damage tissues and inflammatory cells, thereby delaying the healing process and contributing to chronic wounds [14]. Compounds exhibiting strong free radical scavenging activity have been reported to enhance and accelerate wound repair [15].

The antioxidant activity results varied by the allantoin concentration (Table 2). The control sample exhibited the highest IC₅₀ values, indicating minimal activity that can be attributed to the chitosan containing the amino groups, whereas 0.5 wt.% allantoin significantly enhanced antioxidant performance, suggesting effective radical scavenging at low concentrations. Increasing the concentration of allantoin to 1 wt.% and 1.5 wt.% resulted in decline in antioxidant activity. This reduction may be attributed to concentration-dependent physicochemical changes, such as intermolecular interactions or aggregation, limiting the accessibility of active sites. Similar behavior has been reported in hydrogel-based systems, where antioxidant efficiency depends on network structure and the distribution of functional components [16]. Additionally, studies on allantoin-containing biomaterials indicate that its activity depends on its interaction with the surrounding matrix and effective availability within the system [4].

In conclusion, allantoin exhibits a concentration-dependent dual effect, where lower dose of incorporation enhances antioxidant capacity, whereas higher concentrations may reduce functional efficiency, highlighting the need for optimized formulation.

Table 2. Antioxidant activity results of the synthesized compounds

Compounds antioxidant activity	β-Carotene- linoleic acid (IC ₅₀ μM/mL)	DPPH [•] assay (IC ₅₀ μM/mL)
CP-0A	75.11±0.27	60.44±0.35
CP-0.5A	30.36±0.28	21.19±1.01
CP-1.0A	64.13±1.21	55.07±0.44
CP-1.5A	46.04±0.09	34.31±0.20
α-TOC	4.67±0.20	12.79±0.65
BHT	2.84±0.65	55.22±0.84

CONCLUSION

In this study, chitosan/PVA-based hydrogel films incorporating different concentrations of allantoin were successfully prepared and evaluated in terms of their morphological, mechanical, and antioxidant properties. The results demonstrated that allantoin concentration plays a critical role in determining the overall performance of the hydrogel system.

Morphological observations revealed that increasing allantoin content affects the structural homogeneity of the films, with higher concentrations leading to visible heterogeneity and reduced transparency. These structural changes were found to be closely associated with variations in mechanical behavior. Specifically, the incorporation of allantoin resulted in decreased tensile strength and elongation at break, accompanied by an increase in elastic modulus, indicating a transition toward a stiffer but less flexible network. Antioxidant analysis further indicated a non-linear concentration-dependent behavior, with the 0.5 wt.% allantoin formulation exhibiting the highest antioxidant activity.

The findings highlight that an optimal balance between structural integrity, mechanical performance, and antioxidant activity can be achieved at lowest allantoin concentration. Among the tested formulations, CP-0.5A demonstrated the most favorable combination of properties, suggesting its potential suitability for wound healing applications and skin regeneration. These results emphasize the importance of formulation optimization in the development of bioactive hydrogel systems. Further studies should be realized on the anti-inflammatory, antimicrobial and cytocompatibility properties of the fabricated films.

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