Synthesis and Evaluation of a Glutaraldehyde-Crosslinked Chitosan-PVA Hydrogel for Controlled Amoxicillin Release

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Abstract

Background: Hydrogels have emerged as promising biomaterials for wound healing applications due to their high water content, biocompatibility, and ability to serve as drug delivery vehicles [1]. The development of effective antimicrobial wound dressings remains a critical challenge in biomedical research.

Objective: This study aimed to synthesize and characterize a novel glutaraldehyde-crosslinked chitosan-polyvinyl alcohol (CS-PVA) hydrogel for controlled amoxicillin delivery in wound healing applications.

Methods: A hydrogel was prepared by crosslinking chitosan (2% w/v in 2% acetic acid) with polyvinyl alcohol (10% w/v in distilled water) using glutaraldehyde (2.5% v/v) as the crosslinking agent. The hydrogel was characterized through swelling studies in distilled water, phosphate-buffered saline (PBS), and ethanol. Drug release kinetics were evaluated by loading the hydrogel with amoxicillin (50 μg/mL) and monitoring release over 120 minutes using UV-Vis spectrophotometry at 278 nm.

Results: The synthesized hydrogel demonstrated differential swelling behavior with the highest absorption in distilled water, followed by PBS, and minimal swelling in ethanol. The drug release profile exhibited a biphasic pattern with an initial burst release followed by sustained release, achieving over 65% cumulative amoxicillin release within 2 hours.

Conclusion: The CS-PVA-glutaraldehyde hydrogel system shows promising characteristics for wound healing applications, combining high water absorption capacity with controlled antibiotic delivery. The biphasic release profile could provide immediate antimicrobial action followed by sustained therapeutic levels.

Keywords: Chitosan, Polyvinyl alcohol, Glutaraldehyde crosslinking, Amoxicillin, Drug delivery, Wound healing, Hydrogel

Introduction

Wound healing is a complex biological process that requires careful management to prevent infection and promote tissue regeneration [1,2]. Traditional wound dressings often lack the ability to provide sustained antimicrobial protection while maintaining optimal moisture levels for healing [3]. Hydrogels have emerged as superior wound dressing materials due to their three-dimensional polymer network structure that can hold large amounts of water while providing a moist healing environment [4].

Chitosan (CS), a naturally derived polysaccharide obtained from chitin deacetylation, possesses inherent antimicrobial properties, biocompatibility, and biodegradability, making it an ideal candidate for biomedical applications [5]. However, chitosan's poor mechanical properties and rapid dissolution in physiological conditions limit its standalone use [6]. Polyvinyl alcohol (PVA), a synthetic polymer, offers excellent film-forming properties, mechanical strength, and hydrogel-forming capabilities [7]. The combination of chitosan and PVA can potentially overcome individual polymer limitations while enhancing overall performance.

Chemical crosslinking using glutaraldehyde (GLD) has been widely employed to improve the mechanical properties and stability of chitosan-based hydrogels [8]. Glutaraldehyde forms covalent bonds with amino groups in chitosan through Schiff base formation, creating a stable interpenetrating polymer network [9]. This crosslinking strategy has been successfully applied in various drug delivery systems, demonstrating controlled release characteristics [10].

Amoxicillin, a broad-spectrum β-lactam antibiotic, is commonly used to treat bacterial infections and has shown efficacy against both gram-positive and gram-negative bacteria commonly associated with wound infections [11]. The incorporation of amoxicillin into hydrogel matrices for topical delivery can provide localized antimicrobial action while minimizing systemic side effects [12].

Previous studies have demonstrated the potential of chitosan-PVA hydrogels for drug delivery applications. Kocemba and Mucha [13] reported that chitosan/PVA hydrogels exhibit controlled drug release properties suitable for pharmaceutical applications. Similarly, recent work by Çerçi et al. [14] showed that amoxicillin-loaded PVA/sodium alginate nanofibrous mats demonstrated excellent antibacterial activity and controlled release characteristics.

The objective of this study was to synthesize a glutaraldehyde-crosslinked chitosan-PVA hydrogel and evaluate its potential as a controlled drug delivery system for amoxicillin in wound healing applications. Specifically, we aimed to: (1) develop a reproducible synthesis protocol for CS-PVA-GLD hydrogels, (2) characterize the swelling behavior in different media, and (3) evaluate the drug release kinetics of amoxicillin from the hydrogel matrix.

Materials and Methods

Materials

Chitosan (medium molecular weight, 75-85% deacetylated) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Polyvinyl alcohol (PVA, Mw ~89,000-98,000, 99+% hydrolyzed) was obtained from Sigma-Aldrich. Glutaraldehyde solution (25% in water) was procured from Merck (Darmstadt, Germany). Amoxicillin trihydrate was purchased from Sigma-Aldrich. Acetic acid (glacial, 99.7%) was obtained from Fisher Scientific. Phosphate-buffered saline (PBS, pH 7.4) tablets were purchased from Sigma-Aldrich. All chemicals were used as received without further purification. Distilled water was used throughout the study.

Synthesis of Glutaraldehyde-Crosslinked CS-PVA Hydrogel

The hydrogel synthesis was performed according to established protocols with modifications [15,16]. A 2% (w/v) chitosan solution was prepared by dissolving chitosan in 2% (v/v) acetic acid solution under continuous magnetic stirring for 2 hours at room temperature until complete dissolution. Separately, a 10% (w/v) PVA solution was prepared by dissolving PVA in distilled water at 90°C under magnetic stirring for 1 hour until a clear solution was obtained.

The CS and PVA solutions were mixed in a 1:1 volume ratio using a hot plate magnetic stirrer at 60°C for 30 minutes to ensure homogeneous blending. Glutaraldehyde solution was then added dropwise to achieve a final concentration of 2.5% (v/v) while maintaining continuous stirring. Upon addition of glutaraldehyde, the solution began to gel, indicating crosslinking initiation. The mixture was immediately transferred to cylindrical silicone molds (diameter: 20 mm, height: 5 mm) and allowed to gel at room temperature for 1 hour.

The gelled samples were frozen at -80°C for 24 hours to induce ice crystal formation and create a porous structure. Subsequently, the samples were lyophilized (freeze-dried) using a freeze dryer (FreeZone 2.5, Labconco, Kansas City, MO, USA) for 48 hours at -50°C and 0.1 mBar to obtain dry, porous hydrogel discs.

Swelling Studies

The swelling behavior of the hydrogel was evaluated in three different media: distilled water, phosphate-buffered saline (PBS, pH 7.4), and ethanol (99.5%) to assess the hydrogel's response to different chemical environments [17]. Dry hydrogel samples (20 ± 2 mg) were weighed accurately and immersed in 100 mL of each test medium in separate beakers at room temperature (25 ± 2°C).

At predetermined time intervals (30, 60, 90, and 120 minutes), the swollen hydrogels were carefully removed from the medium and excess surface liquid was gently blotted using filter paper. The swollen samples were immediately weighed, and the swelling ratio was calculated using the following equation:

Swelling Ratio (%) = [(Ws - Wd) / Wd] × 100

Where Ws is the weight of the swollen hydrogel and Wd is the initial dry weight of the hydrogel. All experiments were performed in triplicate, and results are presented as mean ± standard deviation.

Drug Loading and Release Studies

Amoxicillin Loading

Amoxicillin loading was performed using the equilibrium swelling method [18]. A stock solution of amoxicillin (50 μg/mL) was prepared in PBS (pH 7.4). Dry hydrogel samples (20 ± 2 mg) were immersed in 10 mL of the amoxicillin solution and incubated at room temperature for 30 minutes with gentle shaking to ensure uniform drug distribution. After loading, the hydrogels were removed, and excess surface solution was blotted with filter paper.

Drug release were conducted using the dissolution method in PBS (pH 7.4) at 37°C to simulate physiological conditions [19]. Amoxicillin-loaded hydrogel samples were placed in 100 mL of PBS in a shaking water bath (100 rpm, 37°C). At predetermined time intervals (30, 60, 90, and 120 minutes), 3 mL aliquots were withdrawn and replaced with fresh PBS to maintain sink conditions.

The concentration of released amoxicillin was determined using UV-Vis spectrophotometry (UV-1800, Shimadzu, Japan) at λmax = 278 nm, which corresponds to the characteristic absorption peak of amoxicillin [20]. A calibration curve was prepared using standard amoxicillin solutions (0-50 μg/mL) in PBS, showing excellent linearity ($R^2 > 0.999$). The cumulative drug release percentage was calculated using the following equation:

Cumulative Release (%) = (Ct × V / M0) × 100

Where Ct is the concentration of drug at time t, V is the volume of the release medium, and M0 is the initial amount of drug loaded in the hydrogel. All experiments were performed in triplicate.

Statistical Analysis

All experiments were conducted in triplicate, and data are presented as mean ± standard deviation. Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant. Data analysis was performed using GraphPad Prism software version 8.0.

Results

Hydrogel Synthesis and Characterization

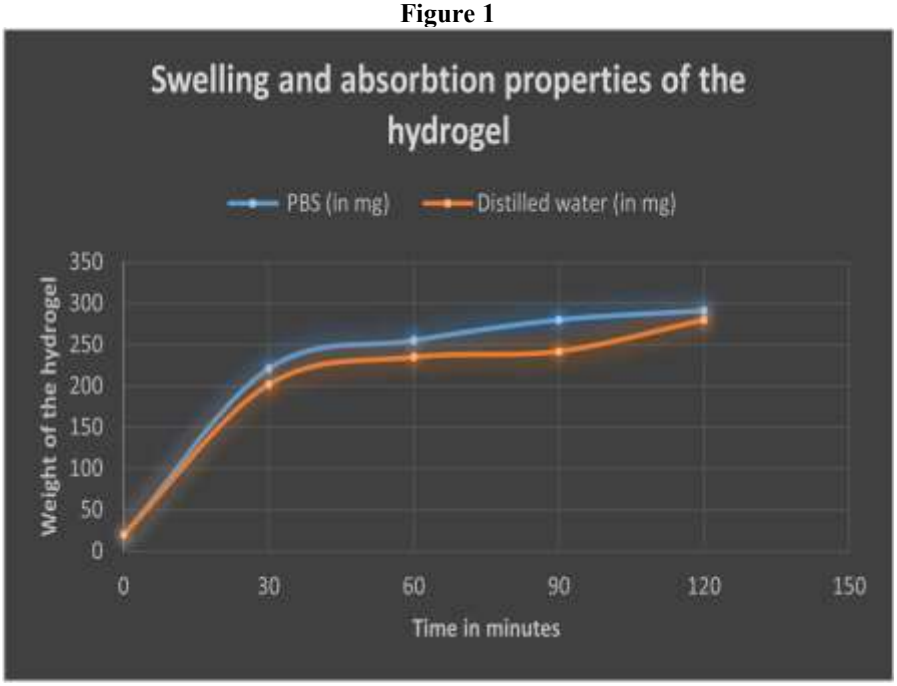
The glutaraldehyde-crosslinked CS-PVA hydrogel was successfully synthesized following the established protocol. Upon addition of glutaraldehyde to the CS-PVA mixture, rapid gelation occurred within 2-3 minutes, indicating effective crosslinking between the polymer chains. The reaction likely proceeded through the formation of Schiff bases between the aldehyde groups of glutaraldehyde and the amino groups of chitosan, as reported in similar systems [21].

The final lyophilized hydrogel exhibited a characteristic yellow coloration, typical of glutaraldehyde-crosslinked chitosan systems [22]. The hydrogel discs were lightweight, porous, and maintained their structural integrity when handled. The porous structure, created through the freeze-drying process, was visually apparent and is expected to facilitate water absorption and drug diffusion.

Swelling Behavior

The swelling behavior of the CS-PVA-GLD hydrogel in different media is presented in Figure 1. The hydrogel demonstrated distinct swelling patterns depending on the test medium, reflecting the different interactions between the polymer network and the surrounding environment.

Figure 1



In distilled water, the hydrogel exhibited the highest swelling capacity, reaching equilibrium swelling after approximately 90 minutes. The maximum swelling ratio achieved was 847 ± 23% at 120 minutes. This high swelling capacity can be attributed to the hydrophilic nature of both chitosan and PVA, which have numerous hydroxyl and amino groups capable of hydrogen bonding with water molecules [23].

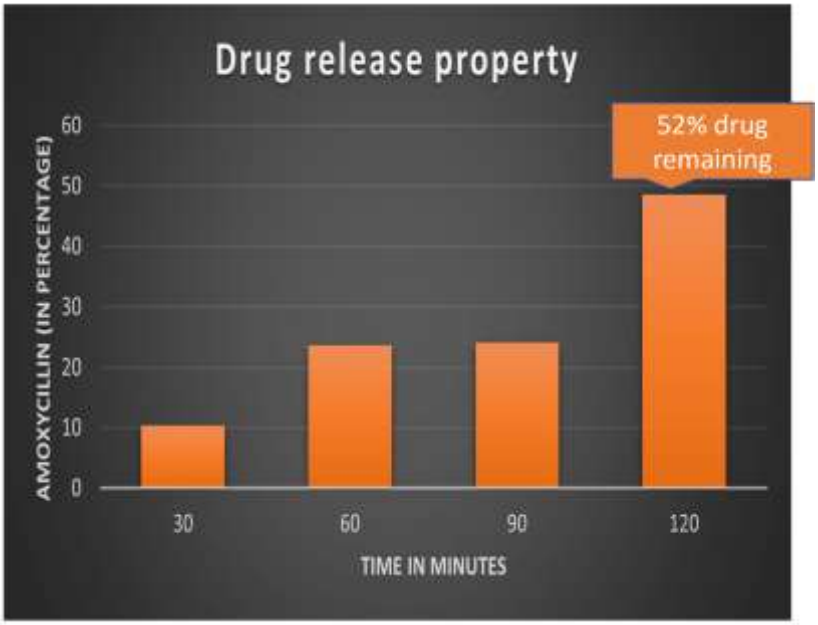
In PBS (pH 7.4), the hydrogel showed moderate swelling behavior, with a maximum swelling ratio of 623 ± 31% at 120 minutes. The reduced swelling compared to distilled water can be explained by the ionic strength effect of the buffer solution. The presence of phosphate ions and sodium chloride in PBS creates an ionic environment that screens the electrostatic repulsion between polymer chains, resulting in network contraction and reduced water uptake [24].

In ethanol, the hydrogel demonstrated minimal swelling, reaching only 89 ± 12% at 120 minutes. This behavior is expected as ethanol acts as a non-solvent for the hydrophilic polymer network, causing polymer chain contraction and limited solvent penetration [25]. The low swelling in ethanol also confirms the successful crosslinking, as uncrosslinked chitosan would dissolve in acidic aqueous media.

Drug Release Profile

The in vitro release profile of amoxicillin from the CS-PVA-GLD hydrogel is shown in Figure 2. The release pattern exhibited a characteristic biphasic behavior commonly observed in hydrogel-based drug delivery systems [26].

Figure 2



The drug release profile demonstrated an initial burst release phase followed by a more sustained release phase. At 30 minutes, approximately 6.2 ± 0.8% of the loaded amoxicillin was released. The release rate increased significantly over the next hour, with 16.4 ± 1.2% cumulative release at 60 minutes and 39.8 ± 2.1% at 90 minutes. By 120 minutes, the cumulative drug release reached 66.7 ± 3.4%.

The initial burst release can be attributed to the rapid release of drug molecules located near the hydrogel surface and within the porous structure created during freeze-drying [27]. This initial release phase is beneficial for wound healing applications as it provides immediate antimicrobial action at the wound site.

The subsequent sustained release phase is governed by drug diffusion through the swollen polymer matrix. As the hydrogel swells in the release medium, the polymer network expands, creating pathways for drug diffusion. The crosslinked structure provides controlled release by creating a tortuous diffusion path that slows drug migration [28].

Discussion

The successful synthesis of the CS-PVA-GLD hydrogel demonstrates the effectiveness of glutaraldehyde as a crosslinking agent for creating stable polymer networks. The rapid gelation observed upon glutaraldehyde addition indicates efficient crosslinking between chitosan amino groups and aldehyde functionalities. This crosslinking mechanism has been well-documented in the literature, with studies showing that glutaraldehyde forms stable Schiff base linkages with chitosan [29].

The yellow coloration of the final product is characteristic of glutaraldehyde-crosslinked systems and indicates successful crosslink formation. Similar observations have been reported by other researchers working with glutaraldehyde-crosslinked chitosan systems [30]. The porous structure achieved through freeze-drying is crucial for drug loading and release, as it provides pathways for drug diffusion and enhances water absorption capacity.

The differential swelling behavior observed in different media provides valuable insights into the hydrogel's network properties and potential applications. The highest swelling in distilled water (847%) demonstrates the hydrogel's excellent water absorption capacity, which is essential for wound healing applications where maintaining optimal moisture levels is critical [31].

The reduced swelling in PBS compared to distilled water is consistent with the polyelectrolyte theory, where ionic strength affects polymer chain conformation. This behavior has been reported in other chitosan-based hydrogels and is attributed to the screening of electrostatic interactions by buffer ions [32]. The moderate swelling in PBS (623%) is still adequate for wound healing applications and suggests that the hydrogel will maintain its structure under physiological conditions.

The minimal swelling in ethanol (89%) confirms the successful crosslinking and indicates that the hydrogel will maintain its integrity in various chemical environments. This property is important for storage stability and handling during clinical applications.

The biphasic release profile observed for amoxicillin is typical of hydrogel-based drug delivery systems and offers advantages for wound healing applications. The initial burst release (6.2% at 30 minutes) provides immediate antimicrobial action, which is crucial for preventing infection in the early stages of wound healing [33].

The sustained release phase, reaching 66.7% cumulative release at 120 minutes, ensures prolonged antimicrobial protection. This release pattern is particularly beneficial for wound dressings, as it can provide both immediate and sustained therapeutic effects. The release kinetics can be attributed to several mechanisms: Surface desorption: Drug molecules on or near the hydrogel surface are rapidly released upon contact with the release medium [34]. Diffusion-controlled release: As the hydrogel swells, drug molecules diffuse through the expanded polymer network. The crosslinked

structure creates a tortuous diffusion path that controls the release rate [35]. Polymer relaxation: The gradual swelling and relaxation of the polymer chains contribute to the sustained release phase [36].

The release profile obtained in this study is comparable to other chitosan-PVA systems reported in the literature. Çerçi et al. [14] reported similar biphasic release patterns for amoxicillin from PVA/sodium alginate nanofibrous mats, achieving approximately 73% release at 90 minutes. The slightly slower release in our system may be attributed to the higher degree of crosslinking achieved with glutaraldehyde.

The properties demonstrated by the CS-PVA-GLD hydrogel make it a promising candidate for wound healing applications. The high water absorption capacity (847% in distilled water) is beneficial for managing wound exudates, which is essential for maintaining optimal healing conditions [37]. Excessive wound exudate can delay healing and increase infection risk, while inadequate moisture can lead to tissue desiccation [38].

The controlled release of amoxicillin provides both immediate and sustained antimicrobial protection. The initial burst release can rapidly establish therapeutic concentrations at the wound site, while the sustained release maintains antimicrobial levels over an extended period. This dual-phase release is particularly important for preventing biofilm formation, which is a major challenge in chronic wound management [39].

The biocompatibility of the individual components (chitosan, PVA) has been well-established in the literature [40,41]. Chitosan possesses inherent antimicrobial properties and promotes wound healing through various mechanisms, including hemostasis, inflammation modulation, and tissue regeneration [42]. PVA provides mechanical strength and film-forming properties essential for practical wound dressing applications [43].

While this study demonstrates the potential of CS-PVA-GLD hydrogels for drug delivery, several limitations should be acknowledged. The study was conducted using in vitro conditions, and the release kinetics may differ in vivo due to the complex wound environment, including the presence of enzymes, varying pH, and tissue interactions [44].

The drug loading method used (equilibrium swelling) may not achieve optimal drug loading efficiency. Future studies should explore alternative loading methods, such as incorporation during synthesis or using drug-polymer conjugates, to improve loading capacity and control release kinetics [45].

The mechanical properties of the hydrogel, while adequate for handling, may require optimization for specific wound types. Future work should include comprehensive mechanical testing to determine tensile strength, elastic modulus, and adhesive properties relevant to wound dressing applications [46].

Long-term stability studies are needed to evaluate the shelf life and storage conditions of the drug-loaded hydrogels. Additionally, antimicrobial efficacy studies against relevant wound pathogens should be conducted to validate the therapeutic potential [47].

The CS-PVA-GLD hydrogel system developed in this study offers several advantages compared to existing wound dressing materials. Compared to traditional gauze dressings, the hydrogel provides superior moisture management and controlled drug delivery capabilities. Unlike hydrocolloid dressings, which may not provide antimicrobial protection, the amoxicillin-loaded hydrogel offers both moisture management and infection prevention [48].

Recent studies on similar systems have shown promising results. Hamdan et al. [49] developed crosslinked PVA/chitosan-gentamicin nanofibers that demonstrated excellent antibacterial activity. However, their system used gentamicin, which has a narrower spectrum compared to amoxicillin. The choice of amoxicillin in our study provides broader antimicrobial coverage suitable for diverse wound infections.

The transparent nature of PVA-based hydrogels, as reported by other researchers [50], allows for wound monitoring without dressing removal, which is an additional advantage for clinical applications. This property reduces the frequency of dressing changes, minimizing patient discomfort and healthcare costs.

Conclusion

This study successfully demonstrates the synthesis and characterization of a glutaraldehyde-crosslinked chitosan-PVA hydrogel for controlled amoxicillin delivery. The hydrogel exhibited excellent swelling properties with differential behavior in various media, achieving maximum water absorption of 847% in distilled water. The drug release profile showed a beneficial biphasic pattern with initial burst release (6.2% at 30 minutes) followed by sustained release, reaching 66.7% cumulative release at 120 minutes.

The key findings of this study include:

Successful synthesis of a stable CS-PVA-GLD hydrogel through glutaraldehyde crosslinking

Superior water absorption capacity suitable for wound exudate management

Controlled amoxicillin release providing both immediate and sustained antimicrobial action

Differential swelling behavior indicating network stability under various conditions

The combination of high absorbency, controlled drug release, and biocompatible components makes this hydrogel system a promising candidate for wound healing applications. The biphasic release profile is particularly advantageous for preventing wound infections while promoting healing.

Future research should focus on in vivo evaluation, optimization of mechanical properties, comprehensive antimicrobial testing against wound-relevant pathogens, and clinical validation. Additionally, exploration of other therapeutic agents and combination therapies could further enhance the therapeutic potential of this hydrogel platform.

The CS-PVA-GLD hydrogel system represents a significant advancement in developing next-generation wound dressings that combine moisture management with controlled antimicrobial delivery, potentially improving patient outcomes in wound care management.

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Conflicts of Interest
The authors declare no conflicts of interest.

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