

Gelatin-Immobilized Cu(II) Metal Complex: Synthesis and Applications

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Abstract

Gelatin, resulting from collagen, a naturally occurring protein found in ligaments and tissues, is formed through the boiling of connective tissues, bones and animal skins, commonly sourced from cows. Its exceptional properties, including the ability to form strong, transparent gels and flexible films, easy digestibility, solubility in hot water and positive binding action, make gelatin a highly valuable resource in various industries such as food processing, pharmaceuticals, photography and paper production. It is regarded as non-toxic, biocompatible, biodegradable and generally safe for use. Gelatin serves as a key ingredient in the manufacturing of a wide range of products, with a notable example being hard gelatin capsules, which come in varying levels of solubility in water. In this study, a Schiff base was synthesized by modifying gelatin with salicylaldehyde in ethanol at room temperature, a mixture of gelatin-Schiff base added to appropriate copper chloride sonicated in ethanol, the mixture was poured onto glass plates and allowed to dry. Modified gelatin film was produced and characterized using FTIR spectroscopy, while its surface properties were examined through Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM). Furthermore, the modified gelatin film's potential biological activity was assessed against bacterial strains. The antibacterial screening tests revealed that the modified gelatin exhibited promising antibacterial activity against microorganisms, indicating an improvement in its antibacterial efficacy. These findings highlight the enhanced antibacterial potential of the modified gelatin film, further contributing to its versatility and potential applications in various fields. Different molecular weight distributions within the gelatin can lead to variations in film formation and subsequent surface features. Additionally, the composition of the gelatin film, including the presence of any crosslinking agents or modified functional groups, can further influence the resulting surface morphology. This study focuses on the preparation of modified gelatin films using suitable aldehyde compounds and explores their potential for pharmaceutical applications. Various techniques assessment of biological activity will be employed to investigate the properties and performance of the modified gelatin films.

Keywords: Modification, Gelatin, Capsule shell, Biological activity, Polymers, SEM, AFM

Introduction

Gelatin has several uses, especially in the food and pharmaceutical industries. It is typically derived from bovine and porcine skin and bone. Since the early 19th century, gelatin capsules have been used in the pharmaceutical industry, with virtually unaltered technology [1]. However, in recent years, some global manufacturers of gelatin capsules have introduced a shift in their application, specifically transitioning from coated capsules to intestinal capsules by modifying the shell production formulation and eliminating the coating process. Gelatin capsules are susceptible to the acidic environment of the stomach, causing the release of the internal drug contents under such conditions with low pH. However, there are instances where certain drugs, such as supplements, probiotics, or specific antibiotics, fail to function effectively in the acidic environment of the stomach or require targeted release in cases of intestinal diseases. In these scenarios, a controlled and gradual release of the drug becomes crucial. Some drugs may need to be taken in a specific body environment, while others necessitate consistent concentration within the digestive system [2,3]. The modification of pharmaceutical capsules by formulation changes or extra coatings gives

a workable answer to the issue of improved drug delivery. One such strategy is the creation of delayed-release capsules with a shell that is digestive system resistant [4]. Additionally, Gel is a fundamental substance that can be used to create nanoparticles. It has a variety of benefits as a nanoparticle material, is a naturally occurring macromolecule, is nontoxic and non-carcinogenic in origin, has a relatively low antigenicity, and has been widely utilized in parental formulations. Graft copolymers, hydrogels, porous scaffolds and microspheres are a few examples of the construction techniques for the 3 basic Gel carrier types that have been previously discussed. Gels made of poly(2-hydroxyethyl methacrylate, or poly(HEMA)), are very resistant to high temperatures, acid and alkaline hydrolysis and amine reactivity. pHEMA gels are promising materials for the development of regulated drug delivery systems due to their chemical and thermal resilience. Ibuprofen (IBU), also known as (RS)-2-[4-(2-methylpropyl) phenyl] propanoic acid, is a significant NSAID used to treat moderate pain, osteoarthritis and rheumatoid arthritis. Due to frequent GI tract adverse effects and an ulcerogenic impact, its use is frequently restricted. Gel graft copolymer nanoparticles that regulate the release of the IBU medication utilizing HEMA and styrene (Sty) monomers may be able to lessen these issues. In other words, the production, characterization, and *in vitro* release studies of Gel graft copolymer nanoparticles loaded with IBU are the subjects of this work [5-14]. This study focuses on the preparation of modified gelatin films using suitable aldehyde compounds and explores their potential for pharmaceutical applications. By modifying gelatin films with appropriate aldehyde compounds, the aim is to optimize their suitability for pharmaceutical applications. FTIR spectroscopy will be used to analyze the chemical composition and structural characteristics of the modified films, while AFM and SEM will provide insights into their surface morphology. Furthermore, the biological activity of the modified gelatin films will be evaluated, focusing on their potential for pharmaceutical use. This research endeavors to enhance the functionality and performance of gelatin films through modifications, paving the way for their application in pharmaceutical settings. The comprehensive analysis and assessment of the modified gelatin films will provide valuable insights into their potential benefits and contribute to the development of improved drug delivery systems.

Materials and methods

Chemicals

All reagents and solvents utilized in this study were of high purity.

Instrumentation

The infrared spectra of the synthesized compound were recorded using a Shimadzu 4800 FT-IR spectrophotometer, employing KBr pellets, within the range of 4000 to 400 cm^{-1} . SEM observations were achieved utilizing an Inspect S50 microscope (FEI Company, Czech Republic) with an accelerating voltage of 15 kV. The morphology of the gelatin surface was examined utilizing AFM on a Veeco instrument. After making wells in the culture medium with 2 different antibiotic concentrations (1×10^{-2} , 1×10^{-4} M), 50 μL of each was poured into the holes and allowed to absorb. The culture medium was then stored in the incubator at a temperature of (37 $^{\circ}\text{C}$), which is the appropriate degree for bacterial growth for a period of 24 h.

Synthesis of modified gelatin

A quantity of 2 g of gelatin (Gel) was dissolved in 20 mL of ethanol by stirring at room temperature until fully dissolved. An equivalent molar amount of salicylaldehyde and a few drops of acetic acid were added to the solution. The mixture was then stirred at reflux for 3 h, followed by cooling to room temperature. Subsequently, the solvent was evaporated to obtain the modified gelatin. The identification of the modified gelatin was confirmed through FTIR spectroscopy.

Synthesis of modified gelatin-Cu(II) complex

In a procedure, 0.4 g of gelatin-Schiff base and 0.1 g of copper chloride were combined in 10 mL of ethanol (EtOH) and subjected to ultrasonication for 1 h. Subsequently, the resulting mixture was evenly spread onto glass plates with an approximate thickness of 40 μm and left to air-dry for 24 h at a temperature of 25 $^{\circ}\text{C}$.

Results and discussion

Gelatin is a versatile material commonly employed in the production of both hard and soft capsules, offering numerous advantages. Its utilization as a capsule shell facilitates the protection of contents from light exposure, atmospheric oxygen, contamination and microbial growth. Additionally, gelatin helps to

mask undesirable taste and odor associated with certain pharmaceutical formulations. The unique properties of gelatin make it an ideal choice for ensuring the integrity and stability of encapsulated products.

In this study, a Schiff base was synthesized by modifying gelatin with salicylaldehyde, aiming to enhance its pharmaceutical applicability. The inclusion of salicylaldehyde in the gelatin structure introduces new functional groups, potentially imparting specific characteristics to the modified gelatin film. The choice of salicylaldehyde as the modifying agent is based on its known reactivity and ability to form stable Schiff base compounds. By dissolving gelatin in ethanol and subsequently adding salicylaldehyde, the modified gelatin was formed through a reflux reaction. Acetic acid was used as a catalyst to facilitate the Schiff base formation. The resulting modified gelatin was identified and confirmed using FTIR spectroscopy.

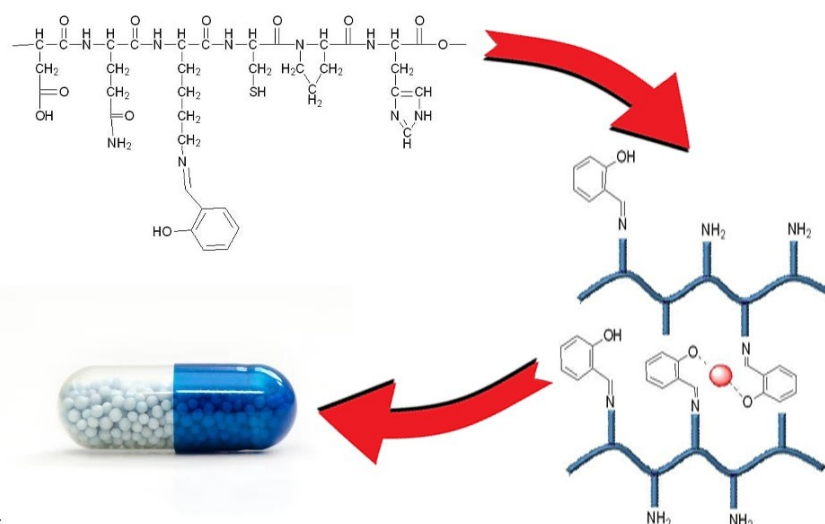


Figure 1 Structure of modified gelatin-Cu(II).

The synthesis of the Schiff base modified gelatin provides an opportunity to explore its potential benefits in pharmaceutical applications. The addition of salicylaldehyde may introduce desirable properties to the gelatin, such as improved drug compatibility, controlled release, or enhanced bioactivity. Further investigations and characterization techniques, including SEM and AFM, can provide valuable insights into the morphology and surface characteristics of the modified gelatin film.

The Schiff base modification of gelatin expands its versatility and opens up new avenues for its utilization in pharmaceutical formulations. By tailoring the gelatin structure through this modification, it may be possible to optimize its performance in terms of drug delivery, stability and overall efficacy. Future studies can focus on evaluating the specific attributes and applications of the Schiff base modified gelatin in pharmaceutical formulations, taking into account factors such as release kinetics, biocompatibility and targeted drug delivery (**Figure 1**).

Characterization of modified gelatin

FTIR spectroscopy

Schiff base synthesis is a common organic reaction used in the preparation of compounds with biological and industrial applications. The synthesis involves the condensation of an aldehyde or ketone with a primary amine to form an imine, also known as a Schiff base. After the synthesis of the Schiff base, it is essential to characterize the compound to confirm its structure and purity. Fourier-transform infrared spectroscopy (FTIR) is a powerful technique used for this purpose. In FTIR, a beam of infrared radiation is passed through the sample, and the absorption of the radiation is measured. The resulting spectrum provides information about the functional groups present in the compound. Schiff bases typically show characteristic absorption peaks in the 1600 - 1700 cm^{-1} region, corresponding to the C = N stretching vibration. The FTIR spectrum of the Schiff base can be compared to reference spectra to confirm the identity of the compound. The presence of other peaks in the spectrum, such as those corresponding to C-H or N-H stretching vibrations, can provide information about the structure of the Schiff base and any impurities present. The intensity and position of the peaks can also give insight into the purity and

composition of the compound. Overall, the characterization of the Schiff base by FTIR is an essential step in ensuring the quality and usefulness of the compound in further applications.

In this work, it was synthesized the Schiff base compound from the reaction of amine group attached to the gelatin polymer with the aldehyde group of salicylaldehyde. FTIR spectrum of the product shows the disappearance of NH_2 bands at about 3276 cm^{-1} and appearance of new bands at 1602 cm^{-1} which belongs to azomethine ($\text{C}=\text{N}$) group (**Figure 2**).

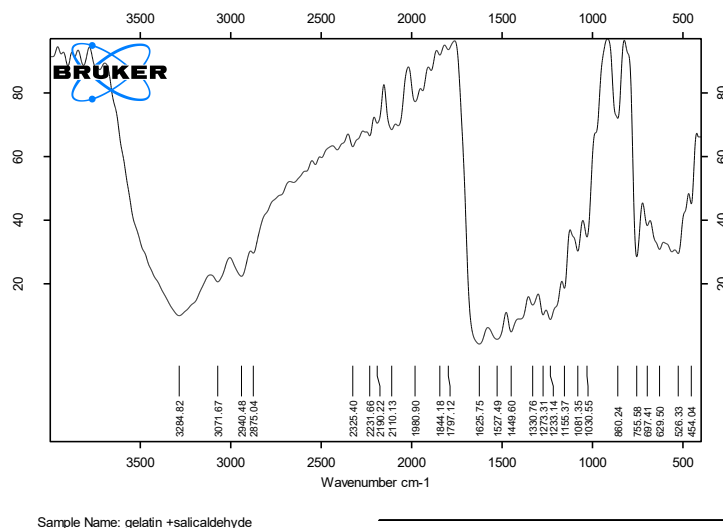


Figure 2 FTIR spectrum of modified gelatin.

Scanning electron microscopy (SEM)

SEM was employed to investigate the surface morphology of the modified gelatin. **Figures 3 - 5** display the SEM images capturing the surface characteristics of the modified gelatin film. The obtained surface morphologies provide visual confirmation of the successful crosslinking achieved through the utilization of salicylaldehyde. However, it is crucial to note that, at the highest concentration of gelatin, the Gel matrix exhibited significant heterogeneity. This heterogeneity is evident from the considerable variation observed in the distribution of pore sizes. Such variations can be attributed to the insufficient availability of salicylaldehyde to facilitate complete crosslinking reactions. Nevertheless, based on the average particle size, this modified gelatin was selected for further encapsulation studies [15-17].

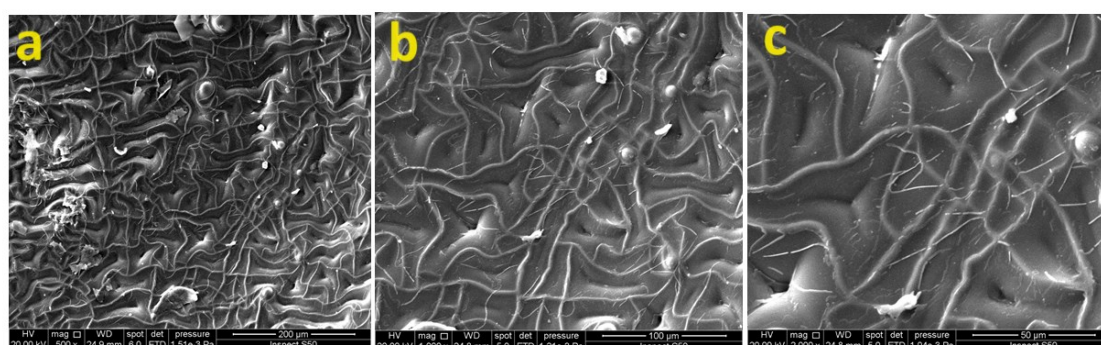


Figure 3 SEM image for modified gelatin at 200, 100 and 50 μm .

The SEM analysis of the modified gelatin film offers valuable insights into its surface characteristics and structural features. The observed morphological variations provide evidence of the successful incorporation of salicylaldehyde as a crosslinking agent. However, the presence of heterogeneity in the Gel matrix, particularly at higher gelatin concentrations, suggests the need for careful optimization of the crosslinking process. Future investigations can explore strategies to enhance the uniformity and pore size distribution of the modified gelatin film. Considering the average particle size, the chosen modified gelatin demonstrates potential suitability for subsequent encapsulation studies. These findings pave the way for

further exploration of the modified gelatin's encapsulation capabilities, with a focus on evaluating its performance in terms of controlled release, stability and compatibility with specific active ingredients.

Atomic force microscopy (AFM)

The surface topography of the modified gelatin was assessed using AFM, as depicted in **Figure 6**. The AFM analysis revealed distinct variations in the surface morphology of the modified gelatin. These differences in morphologies can be attributed to several factors, including the solubility of the polymer, evaporation of the solvent, overall thickness of the film, molecular weight of the gelatin, and surface composition [18-20].

The AFM imaging allows for a high-resolution examination of the modified gelatin's surface, providing valuable insights into its structural characteristics and topographical features. The observed variations in surface topography highlight the influence of multiple factors on the final morphology of the modified gelatin film. Parameters such as the solubility of the gelatin in the solvent used during film preparation, the rate of solvent evaporation, and the resulting film thickness can all contribute to the observed differences in surface structure.

Moreover, the molecular weight of the gelatin used in the modification process can also impact the surface topography. Different molecular weight distributions within the gelatin can lead to variations in film formation and subsequent surface features. Additionally, the composition of the gelatin film, including the presence of any crosslinking agents or modified functional groups, can further influence the resulting surface morphology.

By examining the modified gelatin through AFM, a comprehensive understanding of its surface topography can be gained. This knowledge aids in optimizing the fabrication process and further tailoring the properties of the modified gelatin for specific applications. Future studies can explore the correlation between the observed surface topography and the performance of the modified gelatin in terms of its mechanical strength, drug release behavior and bioactivity [21-25].

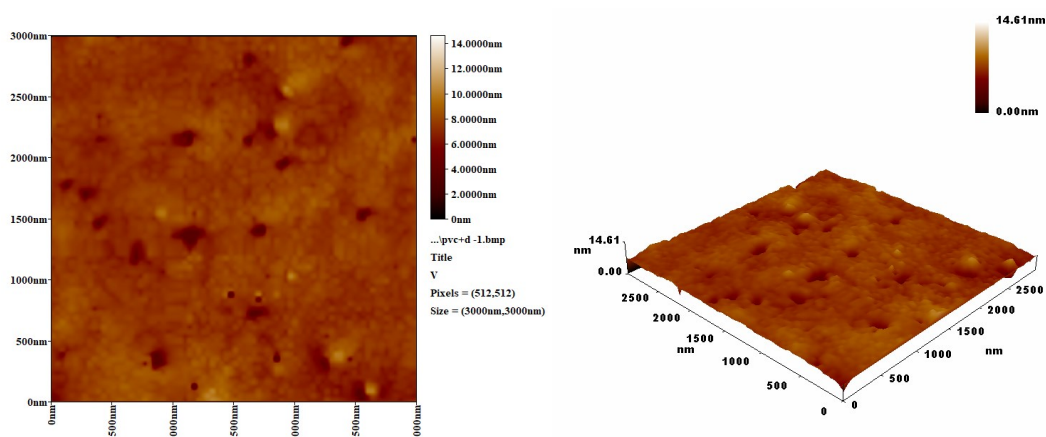


Figure 6 AFM image of modified gelatin.

Biological activity

The biological activity of the modified gelatin was assessed *in vitro* to evaluate its antibacterial and antifungal potential using the zone inhibition technique. The selected microorganisms for testing included *Bacillus Pumilus* (+) as a representative bacterium and *Candida albicans* as a representative fungus. The results of the antibacterial activity testing revealed that the modified gelatin exhibited a higher killing rate against *Bacillus Pumilus* (+). The zone inhibition technique demonstrated a significant inhibitory effect of the modified gelatin on the growth of this bacterium. This finding suggests that the modified gelatin possesses potent antibacterial properties, potentially making it a promising candidate for applications where antibacterial activity is desired.

On the other hand, the antifungal activity of the modified gelatin showed a relatively weaker killing rate against the tested fungi, particularly *Candida albicans*. While the modified gelatin demonstrated some inhibitory effect on fungal growth, it was less effective compared to its antibacterial activity. Further investigations are necessary to explore the reasons behind the observed disparity in activity between bacteria and fungi. Possible factors influencing the antifungal activity of the modified gelatin may include

differences in cell wall structure, susceptibility to antimicrobial agents, or variations in the mode of action of the modified gelatin against different types of microorganisms.

These results suggest that the modified gelatin has a stronger potential as an antibacterial agent compared to its antifungal properties. The antibacterial activity observed in this study supports the notion that the modified gelatin may find applications in areas where bacterial inhibition is desired, such as in wound dressings or antimicrobial coatings.

Generally, the modified gelatin displayed notable antibacterial activity against *Bacillus Pumilus* (+) while exhibiting relatively weaker antifungal activity against *Candida albicans*. These findings highlight the potential of the modified gelatin as an antibacterial agent, opening up possibilities for its incorporation into various biomedical and pharmaceutical applications. Further research and development are warranted to optimize the antifungal activity and explore the modified gelatin's broader spectrum of antimicrobial properties.

Conclusions

In conclusion, gelatin is a versatile additive widely utilized in the fields of medicine, food and cosmetics. However, its potential as an excipient, particularly for hard-shell capsules, remains largely unexplored. Through the modification of gelatin, we successfully produced hard-shell capsules that met the necessary requirements for pharmaceutical use. The modified gelatin was subjected to comprehensive characterization, including analysis via FTIR spectroscopy to examine its chemical composition, as well as SEM and AFM to investigate its surface morphology. These analyses provided valuable insights into the structural properties of the modified gelatin film, aiding in its optimization for specific applications. Furthermore, the modified gelatin film was assessed for its biological activity against bacterial strains. Antibacterial screening tests revealed that the modified gelatin exhibited promising antibacterial properties, showcasing its potential for improved antimicrobial activity against microorganisms.

The findings of this study highlight the potential of gelatin as an excipient for hard-shell capsules and emphasize the importance of modifying gelatin to enhance its functionality and performance. The characterization techniques employed, such as FTIR spectroscopy, SEM and AFM, provided valuable information for understanding the modified gelatin's properties.

Acknowledgments

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